Extractives from *Samadera madagascariensis* & *Toddaliopsis bremekampii*

By

Dashnie Naidoo
BSc, BSc (Hons), M.Sc (Natal)

Submitted in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy
in the
School of Chemistry,
University of KwaZulu-Natal
South Africa

2004

ஓம்

To my

Mum & Dad

" You are my everything "

Preface

The experimental work described in this thesis was carried out in the School of Chemistry, University of KwaZulu-Natal, South Africa, under the supervision of Professor D.A. Mulholland.

This study represents original work by the author and has not been submitted in any other form to another university. Where use was made of the work of others, it has been duly acknowledged in the text.

Signed:

D Naidoo

....

D. Naidoo
M.Sc (Natal)

I hereby certify that the above statement is correct.

Signed:

DA Mulholland

....

Professor D.A. Mulholland
Ph.D. (Natal)
Professor of Organic Chemistry,
University of KwaZulu-Natal

Acknowledgements

I thank my supervisor Professor Dulcie Mulholland for all her support, guidance and friendship over the years. You have truly been an inspiration.

To Dr. Phil Coombes, I am grateful for your assistance throughout my research. Thanks to Mr. Dilip Jagjivan for the NMR techniques and spectra, Mr. Bret Parel for the GC/MS techniques, and Mr. Ernest Makhaza for keeping a neat working environment.

To Dr. A.J.J. van den Berg and Dr. C.J. Beukelman, thank you for giving me the opportunity to visit your research group to learn the bioassay techniques. Special thanks to Dr. S.B.A. Halkes for all the laboratory assistance. Thank you Ir. H.C. Quarles van Ufford for the toxicity determinations.

Thank you to the botanists Dr. Milijaona Randrianarivelojosia and Dr. Neil Crouch for timeously providing me with plant material and information.

Thanks to Mr. John Hill at Kent Mass Spectrometry and Dr. Philip Boshoff at the Cape Technikon for the high resolution mass spectra.

Thank you Miss Taryn Toach and Miss Desree Govender for assisting me during my research.

Thank you to Dr. Neil Koorbanally, Dr. Peter Cheplogoi and the Natural Products Research Group for all your assistance and friendship. Special thanks go to Miss Taryn Toach, Miss Fathima Ismail, Miss Ursulla Harripersadh and Miss Raylene Kemm for making my research here a happy one.

I also like to thank all staff of the School of Pure and Applied Chemistry, both academic and technical, who have in some way assisted me.

I thank the photographers and internet sites for the photographs of various plants included in this work. Acknowledgements have been included in the text at the appropriate places.

I acknowledge the support of the NRF for their financial assistance.

I thank my family, Julius, Senba, Bruce, Dean, Cyril, Phelan and Kassandra, for their support.

List of Abbreviations

^{1}H NMR spectroscopy	-	proton nuclear magnetic resonance spectroscopy
^{13}C NMR spectroscopy	-	carbon-13 nuclear magnetic resonance spectroscopy
COSY	-	correlated spectroscopy
NOESY	-	nuclear Overhauser effect spectroscopy
HMBC	-	heteronuclear multiple bond coherence
HSQC	-	heteronuclear single quantum coherence
DEPT	-	distortionless enhancement by polarisation transfer
MS	-	mass spectrometry
HRMS	-	high resolution mass spectrometry
EIMS	-	electron impact mass spectrometry
CIMS	-	chemical ionization mass spectrometry
IR	-	infra-red
UV	-	ultraviolet absorption
Mp	-	melting point
t.l.c	-	thin layer chromatography
s	-	singlet
bs	-	broad singlet
d	-	doublet
bd	-	broad doublet
m	-	multiplet
dd	-	doublet of doublets
ddd	-	doublet of double doublets
Hz	-	Hertz
c	-	concentration
p-TSA	-	*para*-toluene sulphonic acid
NADPH	-	nicotinamide adenine dinucleotide phosphate
MPO	-	myeloperoxidase
TMS	-	tetramethylsilane
ROS	-	reactive oxygen species
PMNs	-	polymorphonuclear leukocytes
DPPH	-	α,α-diphenyl-β-picrylhydrazyl
PBS	-	Phosphate buffered saline
HBSS	-	Hanks Buffered Salt Solution
Bz	-	benzyl
OH	-	hydroxy
Me	-	methyl
OMe	-	methoxy
OAc	-	acetoxy
Et	-	ethyl
Ph	-	phenyl
sp.	-	species
IPP	-	*iso*pentenyl pyrophosphate
DMAPP	-	dimethylallyl pyrophosphate
GPP	-	geranyl pyrophosphate
FPP	-	farnesyl pyrophosphate
GGPP	-	geranylgeranyl pyrophosphate
FFPP	-	farnesylfarnesyl pyrophosphate
NADH	-	nicotinamide adenine dinucleotide

PAGE NO.

List of Tables

Chapter 2:

Chapter 5:

PAGE NO.

Chapter 6:

List of Schemes

Chapter 2:

Chapter 3:

Chapter 4:

PAGE NO.

List of Figures

Chapter 5:

Chapter 6:

Abstract

This work involves the isolation, structural elucidation and the biological screening of compounds from the Madagascan *Samadera madagascariensis* and *Toddaliopsis bremekampii* from South Africa.

Samadera madagascariensis Jussieu (locally known as "fatriana") belongs to the Simaroubaceae family and is endemic to Madagascar. The leaves of *Samadera madagascariensis* are used in Madagascar for the treatment of stomach aches and dysentry, and the juice of the fresh leaves is used to treat wounds and burns. *Samadera madagascariensis* leaves were investigated in this work for the presence of quassinoids and triterpenoids. Eighteen compounds, including seven quassinoids: samaderine A (i), the novel 5β,6-dihydrosamaderine A (**ii**), the novel 2-chlorosamaderine A (**iii**), the novel samaderine DN (**iv**), samaderine B (**v**), cedronin (**vi**) and the novel 3,4β-dihydrosamaderine C (**vii**), and eleven triterpenoids: the novel protosamaderine A (**viii**), 1,2-dihydrobruceajavanin A (**ix**), chisocheton compound A (**x**), the novel protosamaderine B (**xi**), the novel protosamaderine C (**xii**), the novel protosamaderine D (**xiii**), the novel protosamaderine E (**xiv**), the novel protosamaderine F (**xv**), the novel protosamaderine G (**xvi**), the novel protosamaderine H (**xvii**) and the novel protosamaderine I (**xviii**) were isolated from the *Samadera madagascariensis* leaves. The quassinoids isolated in this work are of the C_{18} and C_{19} classes, and only five of the C_{18} type have been published previously. Quassinoids exhibit a range of biological activities such as antileukemic, antiviral, antimalarial, anti-inflammatory, antifeedant and amoebicidal. 2-Chlorosamaderine A (**iii**), cedronin (**vi**) and 3,4β-dihydrosamaderine C (**vii**) were found to have anticarcinogenic properties. The triterpenoids isolated in this work are of the protolimonoid type and protolimonoids are known to exhibit anticarcinogenic and antifungal properties.

Toddaliopsis bremekampii I. Verd. belongs to the Rutaceae family. Members of the *Toddaliopsis* genus are found in the warmer regions of southern Africa. The phytochemistry of this species and genus has not been investigated previously, however, other members of the Rutaceae have yielded a wide range of alkaloids and other compounds. The leaves and branches of *Toddaliopsis bremekampii* were investigated in this work and yielded four novel acridone alkaloids: 1,2,3-trimethoxyacridone (i), 1,2,3-trimethoxy-10-acetoxymethylacridone (ii), 1-hydroxy-2,3-dimethoxy-10-acetoxymethylacridone (**iii**) and 1,2,3-trimethoxy-10-methoxy methylacridone (**iv**). Acridone alkaloids are known to have antineoplastic and antitumour properties. These alkaloids were active in the chemiluminescence assay, which showed the suppression of the reactive oxygen species generated by polymorphonuclear leukocytes (PMNs) in humans. The PMNs play a key role in the host's defence. However, the reactive oxygen species generated as a result of this defence mechanism cause tissue damage.

Foreword

Part I of this thesis contains the written text and part II the spectra. Where a spectrum is referred to in the text, a spectrum reference page number for part II will be provided in bold print within square brackets. Example, " the ^{1}H NMR spectrum of samaderine A (**i**) **[5]**." The ^{1}H NMR spectrum for samaderine A (**i**) is therefore available on page 5 of part II.

Part I consists of eight chapters. Chapter one is a general introduction into medicinal plants and their natural products. Chapters two, three and four are introductions to the types of compounds isolated in this work. Chapter two is an introduction to alkaloids, chapter three is an introduction to triterpenoids and chapter four is an introduction to quassinoids. These chapters include the classification, biosynthesis and biological activities of these classes of compounds.

Chapters five and six discusses the two plant species investigated in this work. These chapters include the structural elucidation and biological activities of the compounds isolated. Chapter seven is an account of the experimental procedures carried out in this work and also includes the physical data of the compounds isolated. Chapter eight is the conclusion.

TABLE OF CONTENTS PAGE NO.

PART II : SPECTRA

PART I

Chapter 1

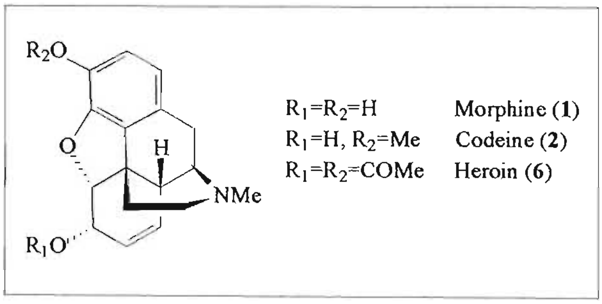
General Introduction

CHAPTER 1
General Introduction

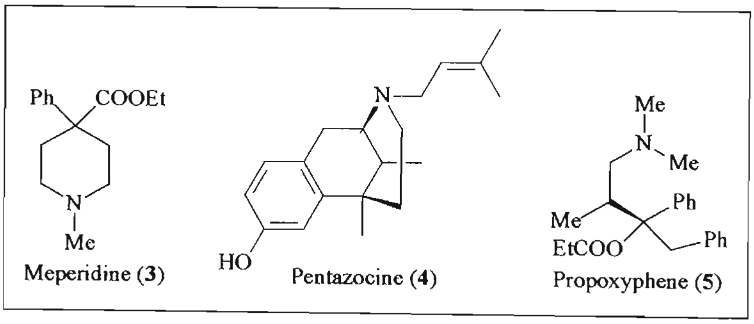
Introduction

Medicinal plants throughout the world have been used since ancient times for healing purposes. Various types of plants i.e. trees, shrubs, herbs and grasses etc. have been used extensively to cure illnesses and diseases,[1] for therapeutic purposes, and for relieving stress,[2] fevers, pains and infections.[3] Natural product chemistry involves the isolation and identification of the biologically active compounds naturally occurring in the plant organisms widely spread across the diverse climatic regions of the world. The pharmaceutical industry uses these biologically active molecules as lead compounds in the development of new drugs. The research in this work was undertaken in the hope of finding novel biologically active compounds. South African and Madagascan plant species have been investigated in this work.

Papaver somniferum (the opium poppy) known from antiquity for its narcotic syrup, contains alkaloids such as the analgesics morphine (**1**), codeine (**2**). These natural products were later used as model compounds for synthesizing further analgesic drugs such as meperidine (**3**), pentazocine (**4**) and propoxyphene (**5**).[4] Heroin (**6**), the poisonous drug to man was also produced from morphine (**1**).



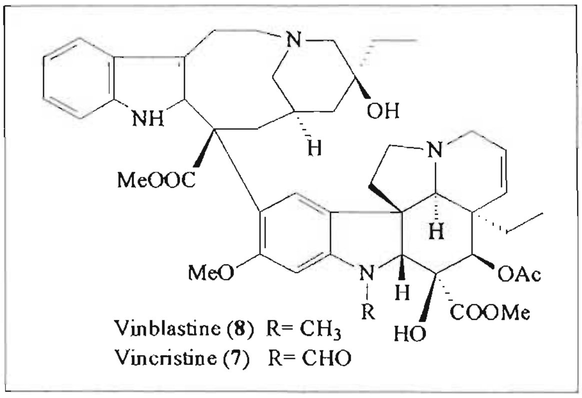
Papaver somniferum
(http://www-ang.kfunigraz.ac.at/~katzer/engl/generic_frame.html?Papa_som.html)



Meperidine (3) Pentazocine (4) Propoxyphene (5)

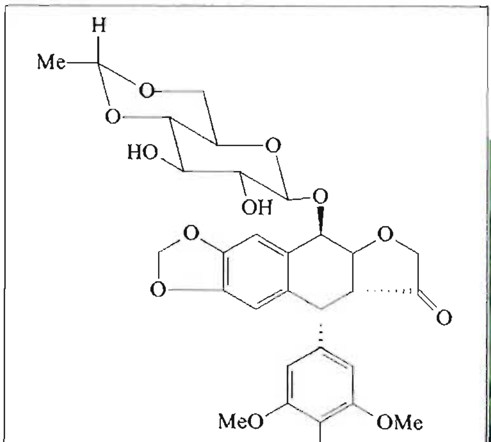
Catharanthus roseus (the Madagascan periwinkle) contains vincristine (**7**) and vinblastine (**8**) which are anticancer agents. Vinblastine (**8**) is used to treat Hodgkin's disease, breast and testicular cancer. Vincristine (**7**) is used in combination with other drugs in cancer chemotherapy.[5]

Catharanthus roseus
(Photograph by Victory Cranberry)



Vinblastine (**8**) R= CH_3
Vincristine (**7**) R= CHO

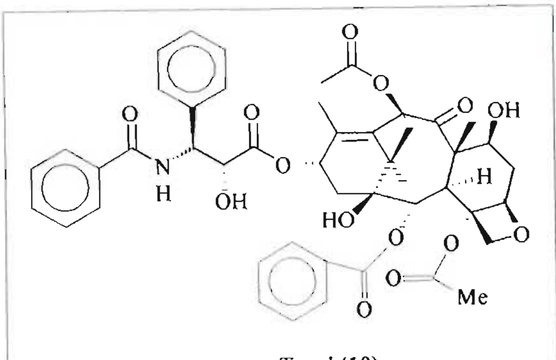
Podophyllum peltatum (the Mayapple) yields the podophyllum resin which is modified to give etoposide (**9**), a drug useful in the treatment of testicular carcinomas, non-lymphocytic leukemias and non-Hodgkin's lymphomas.[6]



Etoposide (**9**)

Podophyllum peltatum
(www.nichegdn.com/.../images/ fullsize/podophyllum.jpg)

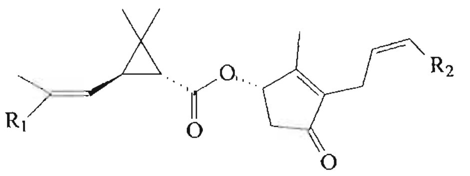
The natural product paclitaxel, which is sold as taxol (**10**) by Bristol-Myers Squibb,[7] from *Taxus brevifolia* (Pacific yew), is active against human breast, ovarian and lung cancers.[8]



Taxol (**10**)

Taxus brevifolia
(http://www.nearctica.com/trees/conifer/taxus/Tbrevi.htm)

Natural products are not only sources for drugs, but also for insecticides[6] and spices.[9] Pyrethrin I (**11**) and II (**12**) from *Chrysanthemum cinerariaefolium* (Pyrethrum) are natural pesticides.[6]



R_1	R_2	
Me	$CH{=}CH_2$	Pyrethrin I (**11**)
CO_2Me	$CH{=}CH_2$	Pyrethrin II (**12**)

Chrysanthemum cinerariaefolium
(www.agri.pref.hokkaido.jp/ .../hata2/image/pyre2.jpg)

Research in Natural Products is therefore important for the development of new and better drugs. In this work, a South African and a Madagascan plant species have yielded novel compounds such as quassinoids, triterpenoids and alkaloids. Some of these compounds are active in the chemiluminescence assay in that they suppress the reactive oxygen species (ROS) that are generated by the polymorphonuclear leukocytes (PMNs) in humans. The PMNs play a key role in the host's defence and the ROS, which are generated as a result of this defence mechanism, cause tissue damage. Some of the quassinoids have been screened at the National Cancer Institute in the USA, and were found to be active. The compounds isolated in this work will be further tested by members of the newly established South African Drug Discovery Platform for antimalarial and other activities.

References

1. Lambert, J., Srivastava, J. and Vietmeyer, N., Medicinal plants. Rescuing a global heritage, The World Bank, Washington D.C., 1997, 29-46.
2. http://quickchange.com/ginsengstore/ (23/11/2004)
3. National Research Council., Neem: A tree for solving global problems, National Academy Press: Washington D.C., 1992, ix.
4. http://library.thinkquest.org/C007974/1_1pop.htm (23/11/2004)
5. Dewick, P.M., Medicinal natural products: a biosynthetic approach, John Wiley & Sons: Chichester, second edition, 2002, 291-403.
6. Hussar, D.A., *American Pharmacy*, 1984, **NS24 (3)**, 31.
7. http://www.taxol.com/ (23/11/2004)
8. Dewick, P.M., Medicinal natural products: a biosynthetic approach, John Wiley & Sons: Chichester, second edition, 2002, 167-289.
9. Balandrin, M.K., Klocke, J.A., Wurtele, E.S. and Bollinger, W.H., Natural plant chemicals: sources of industrial and medicinal materials, 1985, *Science*, **228**, 1154-1160.

Chapter 2

Introduction to Alkaloids

CHAPTER 2
Introduction to Alkaloids

2.1. Introduction

Alkaloids are nitrogenous secondary metabolites isolated mainly from plants. They are also found in frogs, ants, butterflies, marine bacteria, sponges, fungi, spiders and beetles. Species of the Rutaceae produce alkaloids, coumarins, volatile oils, flavonoids and limonoids.

Alkaloids follow the amino acid biosynthetic pathway and have been used in solving taxonomic questions within the Rutaceae family. Alkaloids exhibit a range of biological activities such as antimicrobial,[1,2] uterotonic,[3] antifungal[1] and cytotoxic activities.[1] Acridone alkaloids are the type of alkaloids isolated in this work.

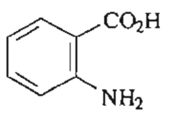
2.2. Classification

Alkaloids are classified according to the origin of the nitrogen in the structure. True alkaloids are formed from amino acid precursors and the nitrogen forms part of a heterocyclic ring. Pseudoalkaloids are formed from precursors other than amino acids and the nitrogen also forms part of a heterocyclic ring. The types of alkaloids isolated from species of the Rutaceae are classified into the 1-benzyltetrahydroisoquinolines, aporphines, berberines, protopines, benzophenanthrines, 2-quinolones, 3-dimethylallyl-2-quinolones, 2-isopropyl-2,3-dihydrofuroquinolines, furoquinolines, furoquinolones, pyranoquinolones, acridones, 4-quinolones, quinolines, quinazolines, canthin-6-ones, carbazoles, imidazoles, oxazoles, amines and amides.

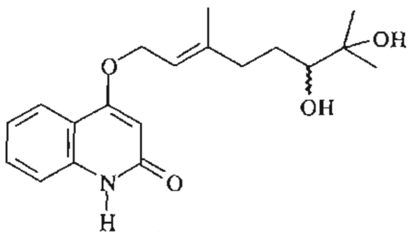
2.2.1. Alkaloids derived from anthranilic acid

Alkaloids derived from anthranilic acid (**1**) include quinolines, acridones, pyranoquinolines, dihydrofuroquinolines, furoquinolines, quinolones and carbazoles.

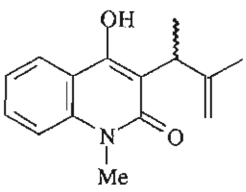
Bucharaine (**2**) (*Haplophyllum* sp.) is an example of a 2-quinolone type alkaloid, as are ravenoline (**3**) (*Ravenia spectabilis*) and dubinine (**4**) (*Haplophyllum* sp.). Dictamnine (**5**) (*Zanthoxylum tsihanimposa*) is a furoquinoline. The rutalinium ion (**6**) (*Ruta* sp.) is a linear pyranoquinoline[3,4,5] and haplamine (**7**) (*Haplophyllum*) is an angular pyranoquinolone.[3]



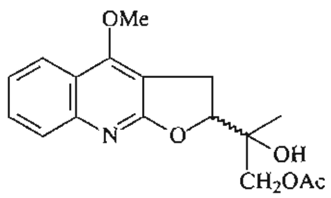
Anthranilic acid (1)



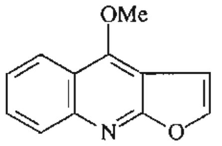
Bucharaine (2)



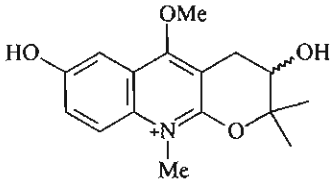
Ravenoline (3)



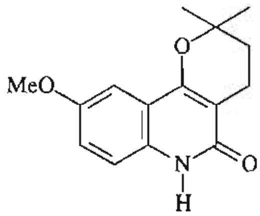
Dubinine (4)



Dictamnine (5)

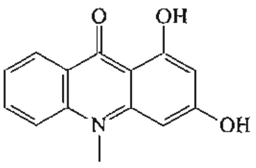


Rutalinium ion (6)

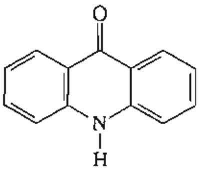


Haplamine (7)

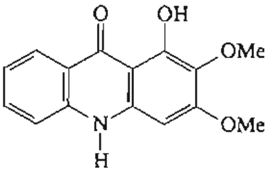
Acridone alkaloids, *e.g.* rutacridone (**8**), have been found in the genus *Ruta*. Acridone alkaloids are confined to the Rutaceae. The species *Euodia alata, Euodia xanthoxyloides, Melicope fareana* and *Acronychia baueri* were the original sources of the acridan-9-one alkaloids. The genera *Diphasia, Teclea* and *Vepris* contain simple acridone alkaloids. Acridones have also been found in the genera *Glycosmis, Atalantia* and *Thamnosma*. The structures of acridone alkaloids range from simple to complex as seen below for compounds (**8**) to (**50**).[6]



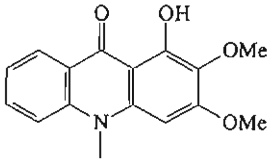
1,3-Dihydroxy-10-methylacridone (**8**)
Citrus tamurana, Citrus natsudaidai



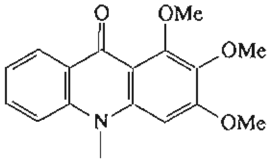
Acridone (**9**)
Thamnosma montana



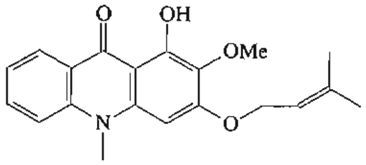
Xanthoxoline (10)
Evodia xanthoxyloides



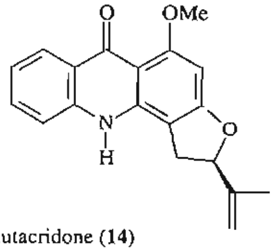
Arborinine (11)
Glycosmis arborea, Fagara leprieurii, Evodia xanthoxyloides, Ruta graveolens



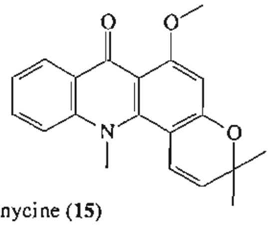
1,2,3-Trimethoxy-10-methylacridone (12)
Evodia alata, Vepris bilocularis, Melicope leratii



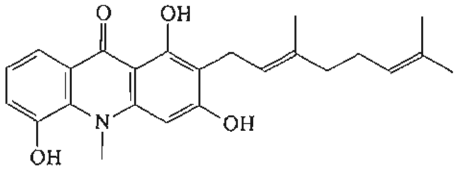
Evoprenine (13)
Evodia alata



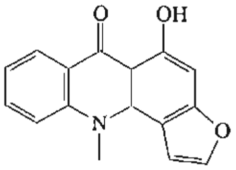
Rutacridone (14)
Ruta graveolens, Ruta chalepensis



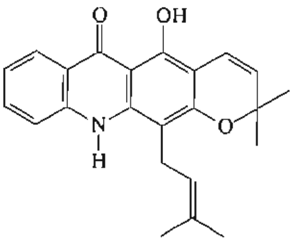
Acronycine (15)
Acronychia baueri, Melicope leptococca, Sarcomelicope argyrophylla



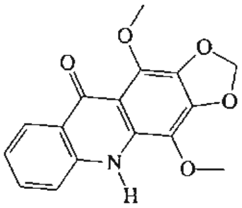
Glycocitrine III (16)
Glycosmis pentaphylla



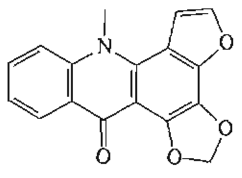
Furofoline (17)
Ruta graveolens, Glycosmis citrifolia



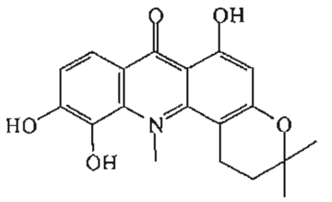
Buxifoliadine D (18)
Severinia buxifolia



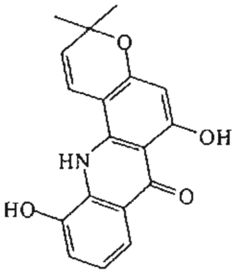
Xanthevodine (19)
Evodia xanthoxyloides, Acronychia baueri, Melicope leratii



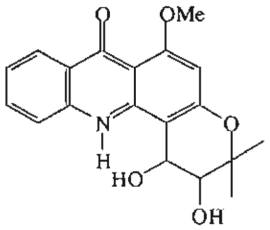
Chaloridone (20)
Ruta chalepensis



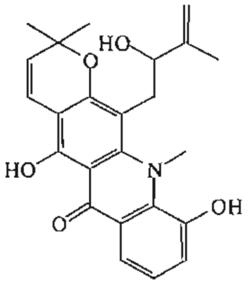
Citracridone III (**21**)
Citrus yuko



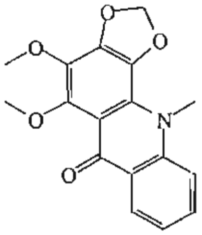
Atalaphyllidine (**22**)
Atalantia monophylla



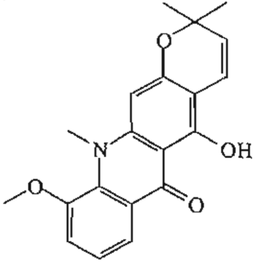
1,2-Dihydro-1,2-dihydroxy-N-demethylacronycine (**23**)
Sarcomelicope dogniensis



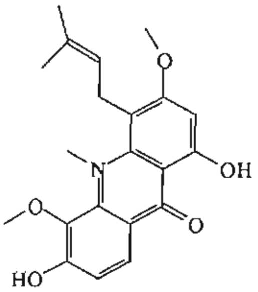
4-(2-Hydroxy-3-methyl-3-butenyl)yukocitrine (**24**)
Bosistoa transversa



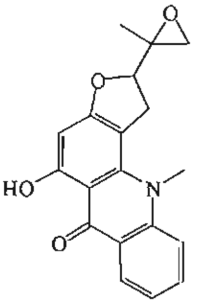
Melicopine (**25**)
Melicope fareana,
Evodia alata, Acronychia baueri,
Teclea natalensis, Bauerella simplicifolia



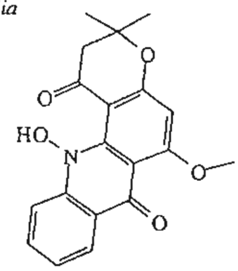
Junosidine (**26**)
Citrus junos



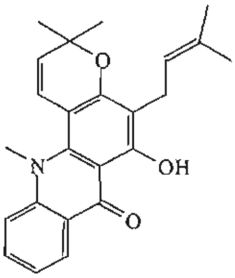
Grandisinine (**27**)
Citrus grandis f. *hakunikuyu*



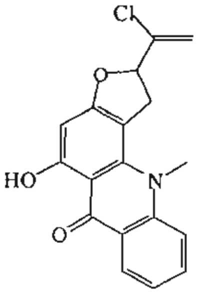
Rutacridone epoxide (**28**)
Ruta graveolens



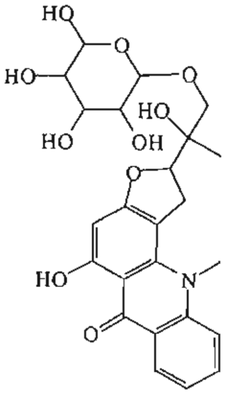
N-Demethyl-1,2-dihydro-N-hydroxy-1-oxoacronycine (**29**)
Sarcomelicope dogniensis



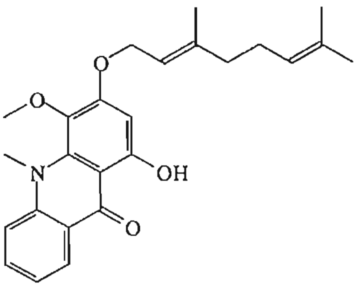
Severifoline (**30**)
Severinia buxifolia



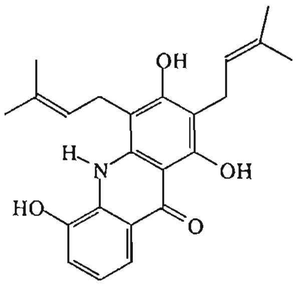
Alkaloid A6 (**31**)
several Ruta spp.



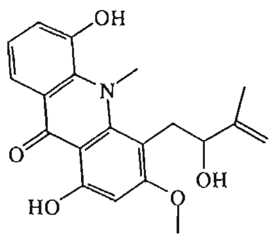
Gravacridonediol glucoside (**32**)
Ruta graveolens



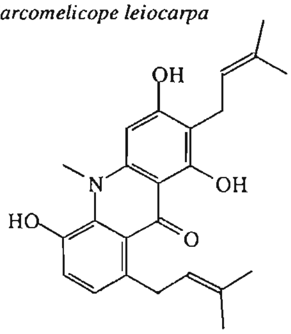
1-Hydroxy-3-geranyloxy-4-methoxy-10-methylacridone (**33**)
Sarcomelicope leiocarpa



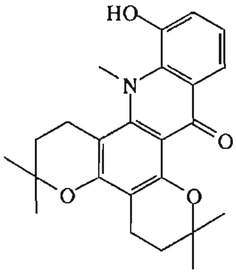
Atalaphylline (**34**)
Atalantia monophylla



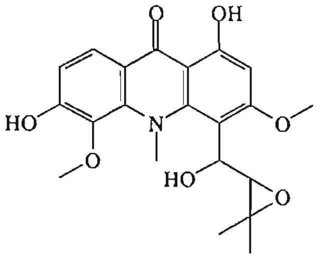
Marshmine (**35**)
Citrus paradisi



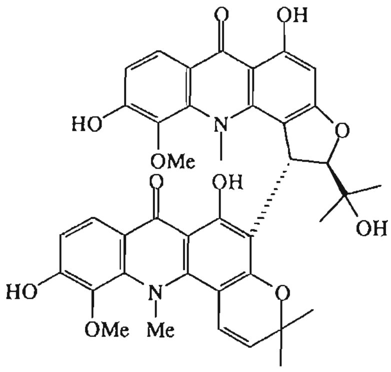
1,3,5-Trihydroxy-9-methyl-2,8-diprenylacridone (**36**)
Swinglea glutinosa



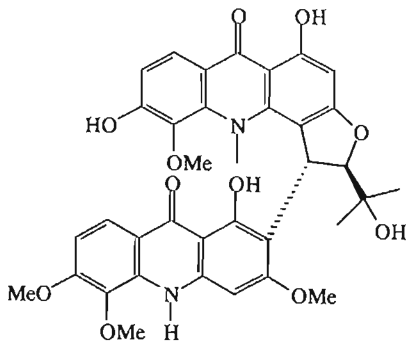
N-Methylbicycloatalaphylline (**37**)
Atalantia monophylla



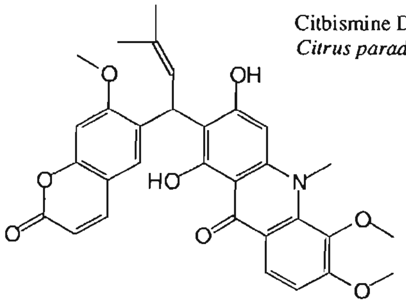
Margrapine A (**38**)
Citrus paradisi



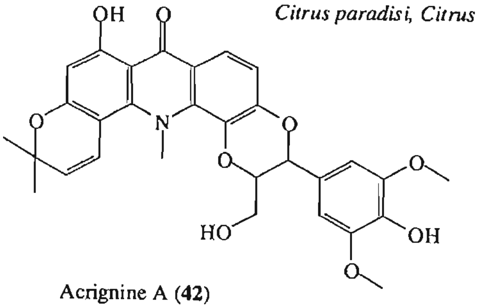
Citbismine D (**39**)
Citrus paradisi



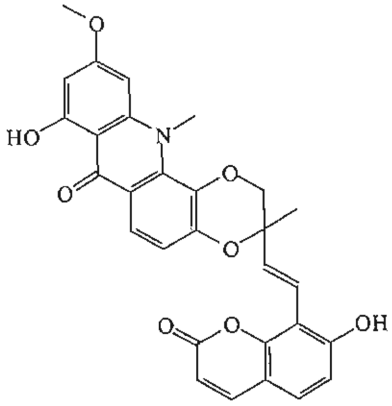
Citbismine B (**40**)
Citrus paradisi, Citrus grandis



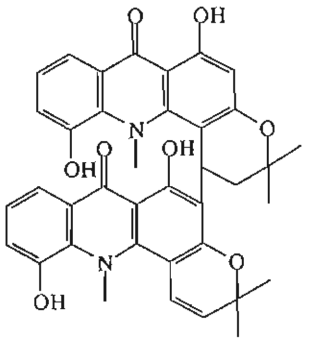
Acrimarine A (**41**)
Citrus funadoko



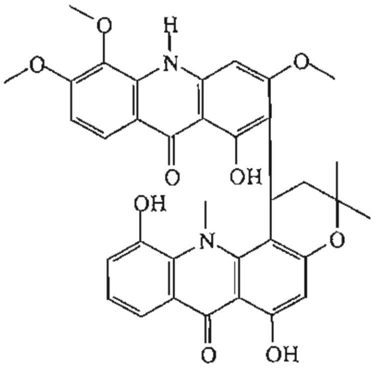
Acrignine A (**42**)
Citrus grandis f. *Hirado, Citrus yuko*



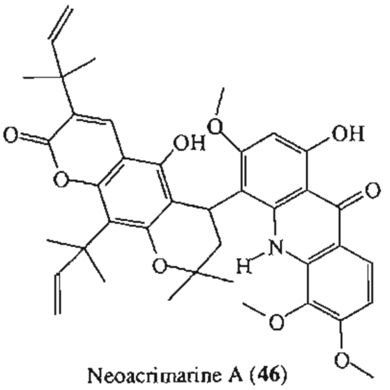
Dioxinoacrimarine A (**43**)
Citrus paradisi, Citrus tangerina



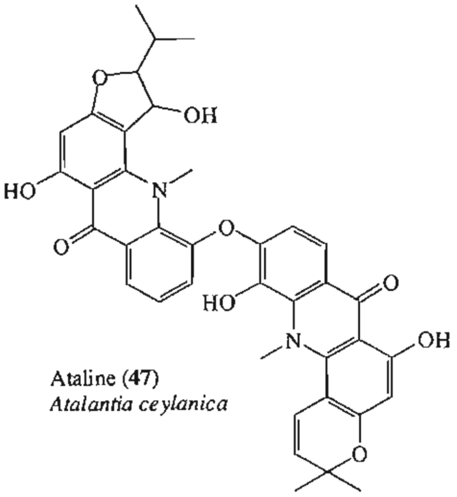
Bis(5-hydroxynoracronycine) (**44**)
Citrus paradisi



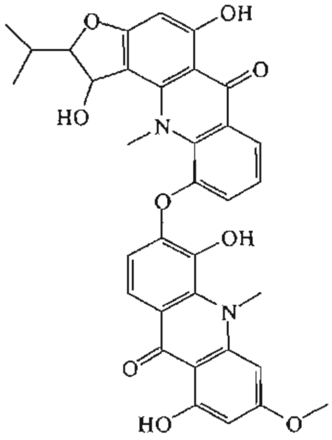
Buntanbismine (**45**)
Citrus grandis f. *buntan*



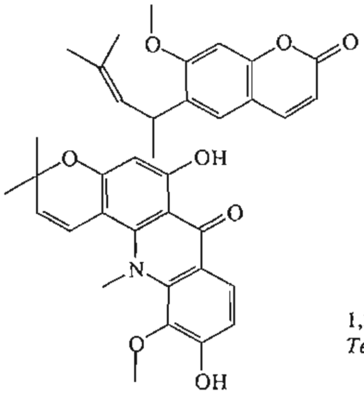
Neoacrimarine A (**46**)
Citrus paradisii



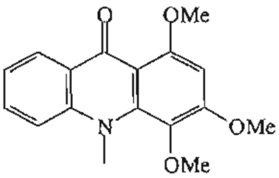
Ataline (**47**)
Atalantia ceylanica



Atalanine (**48**)
Atalantia ceylanica



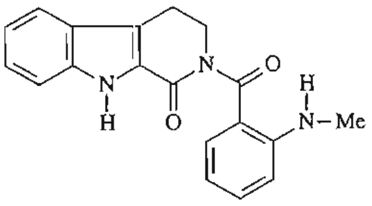
Acrimarine J (**49**)
Citrus paradisii, Citrus tangerina

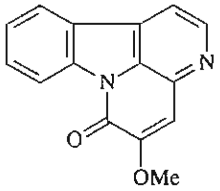


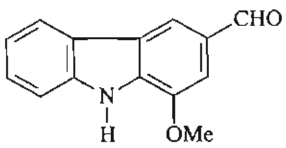
1,3,4-Trimethoxy-10-methylacridone (**50**)
Teclea boiviniana, Acronychia baueri

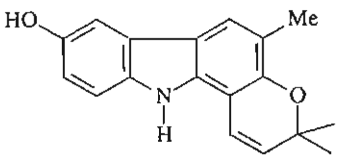
In all of the acridone alkaloids isolated previously, there was only one type of N-substituent i.e. N-Me.

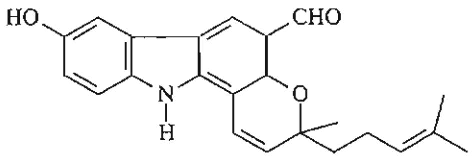
Rhetsinine (**51**) (*Evodia rutaecarpa*) is an alkaloid with a simple β-indoloquinazoline skeleton and a unique tetracyclic amine structure.[3,4] The alkaloid 5-methoxycanthin-6-one (**52**) has been found in *Zanthoxylum elephantiasis* and *Zanthoxylum caribeum*.[3] Murrayanine (**53**) (*Clausena heptaphylla*) is the simplest alkaloid of the C_{13} class. Koenine (**54**) is a C_{18} class alkaloid and murrayacinine (**55**) is a C_{23} class alkaloid, both being isolated from *Murraya koenigii*.


Rhetsinine (**51**)


5-Methoxycanthin-6-one (**52**)


Murrayanine (**53**)

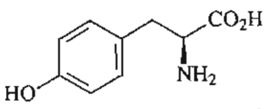

Koenine (**54**)


Murrayacinine (**55**)

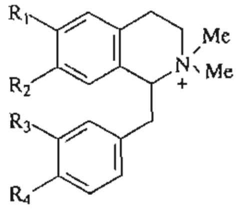
2.2.2. Alkaloids derived from tyrosine and phenylalanine

These alkaloids include the aporphines, protoberberines and protopines.

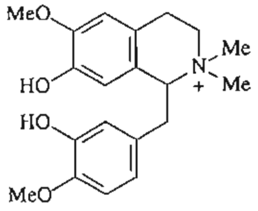
The 1-benzyltetrahydroisoquinoline (**56**) alkaloids such as tembetarine (**57**) (*Zanthoxylum*) have unmodified benzylisoquinoline skeletons.[5] The aporphine (**58**) alkaloids such as magnoflorine (**59**), occur in several *Zanthoxylum* species. Tetrahydroprotoberberine (**60**) and protoberberine (**61**) type alkaloids are tetracyclic alkaloids derived from benzylisoquinolines by condensation with a one-carbon unit (the berberine bridge). The alkaloid berberine (**62**) (*Zanthoxylum*, *Phellodendron*) is a derivative of tetrahydroprotoberberine (**60**). Protopine (**63**) is the simplest alkaloid of this group. Alkaloids possessing a benzophenanthridine ring, such as decarine (**64**) have been found in the genus *Zanthoxylum*.



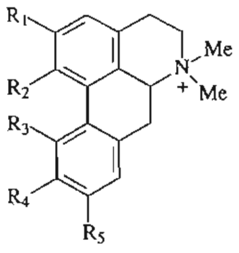
L- Tyrosine



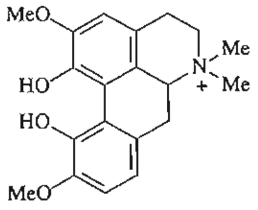
1- Benzyltetrahydroisoquinoline (**56**)



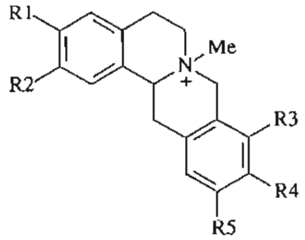
Tembetarine (**57**)



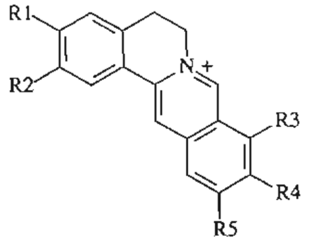
Aporphines (**58**)



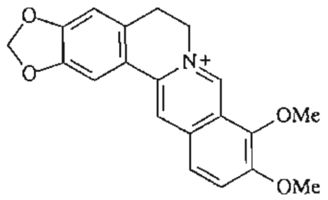
Magnoflorine (**59**)



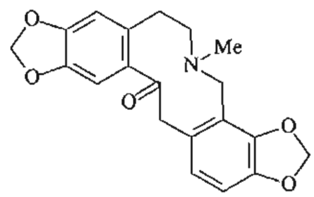
Tetrahydroprotoberbeines (**60**)



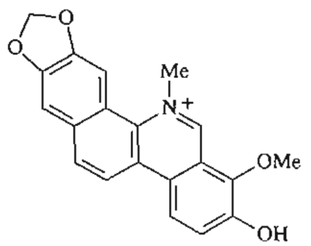
Protoberberines (**61**)



Berberine (**62**)



Protopine (**63**)

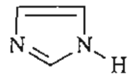


Decarine (**64**)

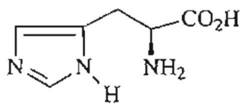
R_1,R_2,R_3,R_4 and R_5 are substituents
H, OH, OMe, O-Me-O etc.

2.2.3. Alkaloids derived from histidine

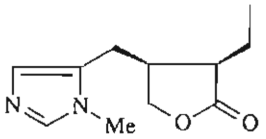
These alkaloids have the imidazole (**65**) structure as the basic skeleton. The precursor of alkaloids in this group is likely to be *L*-histidine (**66**). The imidazole alkaloid, pilocarpine (**67**) (*Pilocarpus microphyllus*), used for the treatment of glaucoma, and pilosine (**68**) (*Pilocarpus microphyllus*) incorporate a phenylpropane $C_6.C_3$ unit.[3,4]



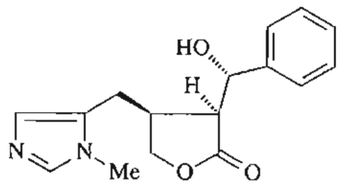
Imidazole (**65**)



L-Histidine (**66**)



Pilocarpine (**67**)

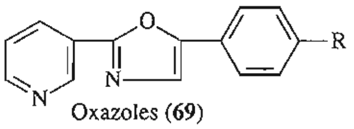


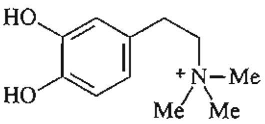
Pilosine (**68**)

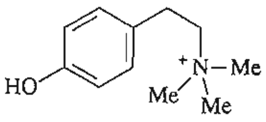
2.2.4. Oxazoles and miscellaneous alkaloids

The oxazoles (**69**) are a small group probably derived from nicotinic acid, tyramine and mevalonic acid. Halfordinol (**70**) (*Halfordia scleroxyla*, *Aegle marmelos*) is the simplest alkaloid of this group.

Simple amines derived from tyramine have been isolated from the leaf and fruit of *Citrus reticulata* and *C. sinensis*. Coryneine (**71**) and candicine (**72**) are N,N,N-trimethyl derivatives that have been isolated from the genus *Zanthoxylum*. There are two groups of Rutaceae amides. The one group of Rutaceae amides is formed by the condensation of tyramine with either benzoic or cinnamic acid and occurs in three sub-families of the Rutaceae. The other group has an isobutylamine group incorporated, and is found in *Zanthoxylum* and *Euodia*. In feruloylputrescine (**73**) (*Citrus paradisii*), the isobutylamine unit is replaced by 1,4-diamino-*n*-butane.^{3}


Oxazoles (69)
Halfordinol (70) R = H


Coryneine (71)


Candicine (72)

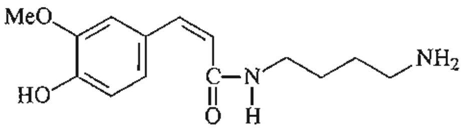

Feruloylputrescine (73)

Table 2.1. Distribution of classes of alkaloids in the genera of the Rutaceae[3,7]

Sub-family	Tribe	Sub-tribe	Genus	No. of taxa investigated	No. of taxa having alkaloids	classes of alkaloids found																
						I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII
Rutoideae	Zanthoxyleae	Eoudiinae	*Zanthoxylum*	64	56	50	2	4	22				2			4	8				10	9
			Geijera	3	2			1	1			1										
			Euodia	19	11	3			5				2	1		1						2
			Orixa	1	1	1		1	1						1							
			Melicope	10	3			1	3				1									
			Pentaceras	1	1												1					
			Pelea	6	1			1	1	1												
		Lu	*Lunasia*	1	1			1	1	1	1				1							
		De	*Decatropis*	1	1				1													
		Ch	*Medicosma*	1	1				1													
			Choisya	3	3			1	3													
			Platydesma	2	2			1	2					1								
		Pv	*Pitaria*	1	1				1													
	Ruteae	Ru	*Boenninghausenia*	1	1				1													
			Ruta	10	3			1	2		1		1		2	1						
			Haplophyllum	17	15		6	4	14	1	3	2		1	3							
			Thamnosma	1	1				1				1									
		Di	*Dictamnus*	8	3			2	3	2					1							
	Boronieae	Bo	*Boronia*	12	1				1					1								
		Er	*Eriostemon*	14	6			1	5													
			Phebalium	10	1				1													
			Geleznowia	1	1				1													
		Ne	*Chorilaena*	1	1				1													
	Cusparieae	Pi	*Pilocarpus*	6	6														6			
			Esenbeckia	2	2				2													
		Cu	*Euxylophora*	1	1											1						
			Angostura	1	1				1					1	1							
			Ravenia	1	1		1	1	1	1			1									
			Monnieria	1	1				1				1									
Dt	Dc	Di	*Dictyoloma*	1	1																1	
St	Sa	Sp	*Spathelia*	2	1		1					1										
Toddalioideae	Toddalieae	Ph	*Phellodendron*	5	5	5															2	
		Pt	*Helietta*	2	2				2	1												
			Balfourodendron	1	1			1	1	1	1		1		1							
			Ptelea	9	4			2	3	2												
		Or	*Oricia*	1	1							1										
			Diphasia	1	1				1				1									
		Toddaliinae	*Araliopsis*	1	1			1	1													
			Casimiroa	2	1		1	1	1						1				1			1
			Vepris	3	2				2				2	1							1	
			Toddalia	1	1	1			1													
			Acronychia	7	3				1	1			3	1								
			Halfordia	3	3		2		2											3		
			Hortia	2	2				1							2						
			Skimmia	6	5			2	5													
		Am	*Teclea*	7	7				4				4								1	
Aurantioideae	Cl	Ci	*Glycosmis*	4	3				2				1			1		1			1	
			Murraya	3	2				1									1				
			Clausena	7	3													2				1
	Citreae	Citrinae	*Hesperethusa*	1	1		1															
			Atalantia	3	2								2									
			Pamburus	1	1																	1
			Severinia	1	1																	1
			Poncirus	1	1				1													
			Citrus	44	7	1		1	3					1							2	1
		Ba	*Aegle*	1	1				1											1		1
			Swinglea	1	1																	1
			Afraegle	1	1				1													
			Aeglopsis	1	1															1		

The numbers in the table refer to the classes of alkaloids listed below. **I** Isoquinolines (1-Benzyltetrahydroisoquinolines, Aporphines, Berberines, Protopines, Benzophenanthrines), **II** 2-Quinolones, **III** 3-Dimethylallyl-2-quinolones and 2-Isopropyl-2,3-dihydrofuroquinolines, **IV** Furoquinolines, **V** Furoquinolones, **VI** Linear pyranoquinolones, **VII** Angular pyranoquinolones, **VIII** Acridones, **IX** 4-Quinolones, **X** Quinolines and 4-quinolones, **XI** Quinazolines, **XII** Canthin-6-ones, **XIII** Carbazoles, **XIV** Imidazoles, **XV** Oxazoles, **XVI** Amines and **XVII** Amides.

The two letters in the table represent the following in the respective columns: LU = Lunasiinae, De = Decatropidinae, Ch = Choisyinae, Pi = Pitavinae, Ru = Rutinae, Di = Dictamninae, Bo = Boroniinae, Er = Eriostemoninae, Ne = Nematolepidinae, Pi = Pilocarpinae, Cu = Cuspariinae, Dt = Dictyolomatoideae, Dc = Dictyolomateae, Di = Dictyolominae, St = Spathelioideae, Sa = Spathelieae, Sp = Spatheliinae, Ph = Phellodendrinae, Pt = Pteleinae, Or = Oriciinae, Am = Amyridinae, Cl = Clauseneae, Ci = Clauseninae and Ba = Balsamocitrinae

Table 2.1 shows the overall distribution of alkaloids in the Rutaceae family. *Zanthoxylum* has been investigated the most, and 56 of the 64 taxa have been found to contain alkaloids, most of which were the isoquinolines. The Toddaliinae sub-tribe has yielded furoquinolines, acridones, benzophenanthridines, β-indolo-quinazolines, imidazoles and oxazoles.[3] The furoquinolines have been found consistently throughout the Rutaceae and in every genera of the Toddaliinae sub-tribe.

Acridones have been previously found in fourteen genera namely, *Zanthoxylum*, *Euodia*, *Melicope*, *Ruta*, *Thamnosma*, *Ravenia*, *Monnieria*, *Balfourodendron*, *Diphasia*, *Vepris*, *Acronychia*, *Teclea*, *Glycosmis* and *Atalantia*.

From the 44 taxa of the *Citrus* genus investigated, only 7 taxa were found to have alkaloids present. No alkaloids have previously been reported from the following genera; *Cneoridium*, *Acradenia*, *Philotheca*, *Barosma*, *Coleonema*, *Empleurum*, *Empleuridium*, *Merrillia*, *Microcitrus*, *Eremocitrus*, *Fortunella Feronia and Toddaliopsis*.

2.3. Biosynthesis

The nitrogen group originates from an amino acid and the carbon skeleton of the amino acid precursor is retained in the alkaloid structure, although the carboxylic acid carbon of the amino acid is often lost through decarboxylation. The amino acid precursors involved in alkaloid biosynthesis are ornithine, lysine, nicotinic acid, tyrosine, tryptophan, anthranilic acid, histidine and phenylalanine.

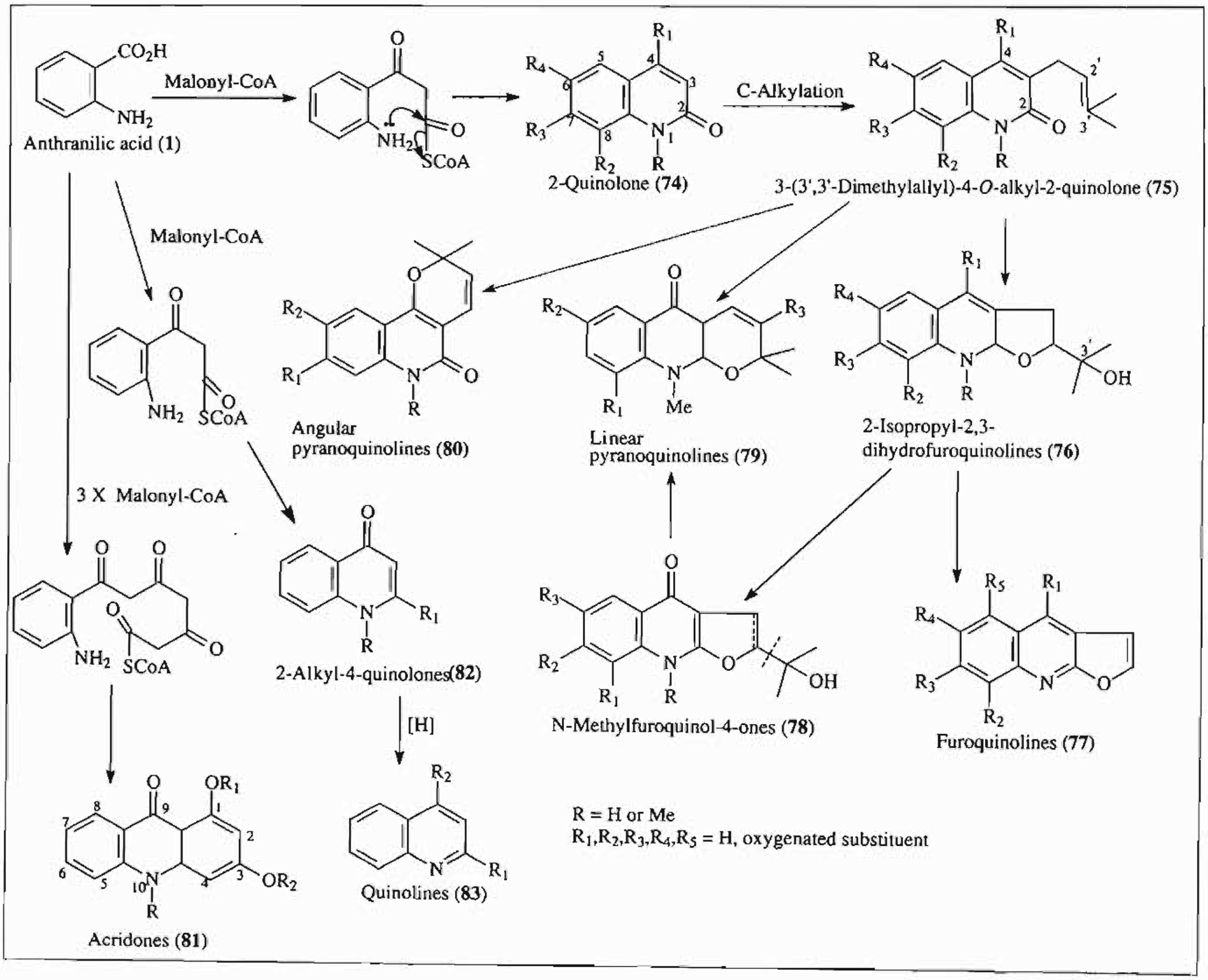
2.3.1. Alkaloids derived from anthranilic acid

Alkaloids with the quinoline ring system are formed by condensation of anthranilic acid (**1**) with a 2-carbon (acetate) unit (scheme 2.1). Usually the 2-quinolone (**74**) type alkaloids are formed. Alkylation *via* dimethylallyl diphosphate yields the 3-(3',3'-dimethylallyl)-4-*O*-alkyl-2-quinolone-type skeleton (**75**), which undergoes oxidative cyclisation between C-2 and C-2', followed by the formation of an allylic carbocation to give rise to 2-isopropyl-2,3-dihydrofuroquinolines (**76**).

2-Isopropyl-2,3-dihydrofuroquinolines (**76**) lose the isopropyl group to give furoquinolines (**77**), by a mechanism involving either oxidation at C-3' followed by decarboxylation or direct hydride ion intervention.[3,4] Further modification of the furoquinoline (**77**) pathway allows for the formation of N-methylfuroquinol-4-ones (**78**). A number of N-methylfuroquinol-4-one (**78**) alkaloids based on the furan ring system of (**76**) and (**77**) have been isolated. However, oxidative cyclisation between C-2 and C-3' of (**75**) gives rise to the linear pyranoquinolines (**79**), while the angular pyranoquinolines (**80**) form by cyclisation only when an unsubstituted C-4 is present.

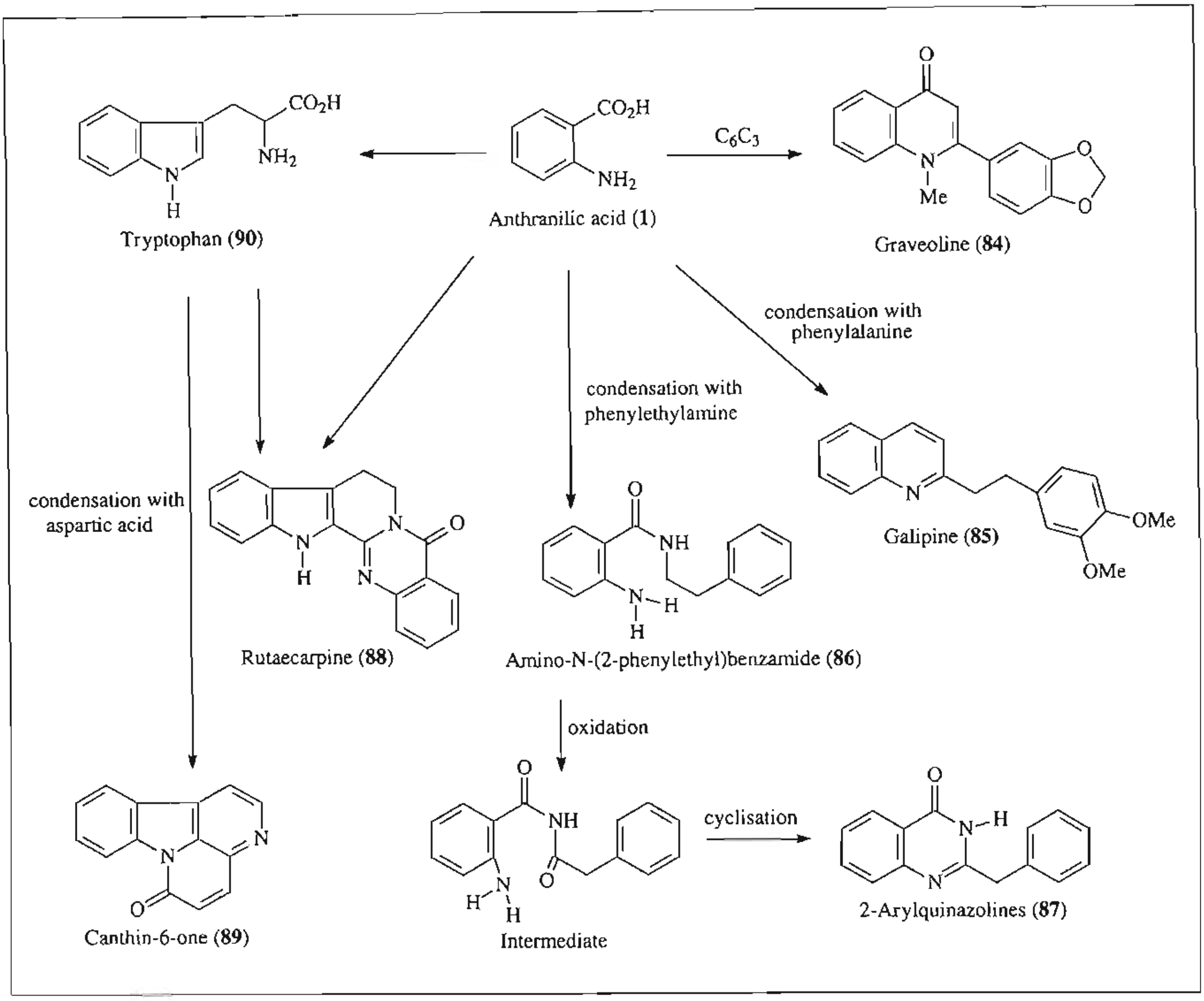
Acridones (**81**) are condensation products of anthranilic acid (**1**) with three acetate units. A polyketide chain attached to the carboxylic acid group of the amino acid is an intermediate product. Subsequent cyclisation of the polyketide chain leads to the 1,3-oxygenation pattern found in almost all acridones (**81**).

The small group of 2-alkyl-4-quinolones (**82**) and quinolines (**83**) may be formed by the condensation of anthranilic acid (**1**) and acetate.[3,4] In the 4-quinolone series, 2-alkyl and 2-aryl substituents are common but the 3- and 5- oxygenated substitution patterns are very rare.[7] The proposed biosynthetic pathway of the Rutaceae alkaloids (types **74** to **83**) is given in scheme 2.1.



Scheme 2.1. Proposed biosynthesis of Rutaceae alkaloids derived from anthranilic acid[3,4]

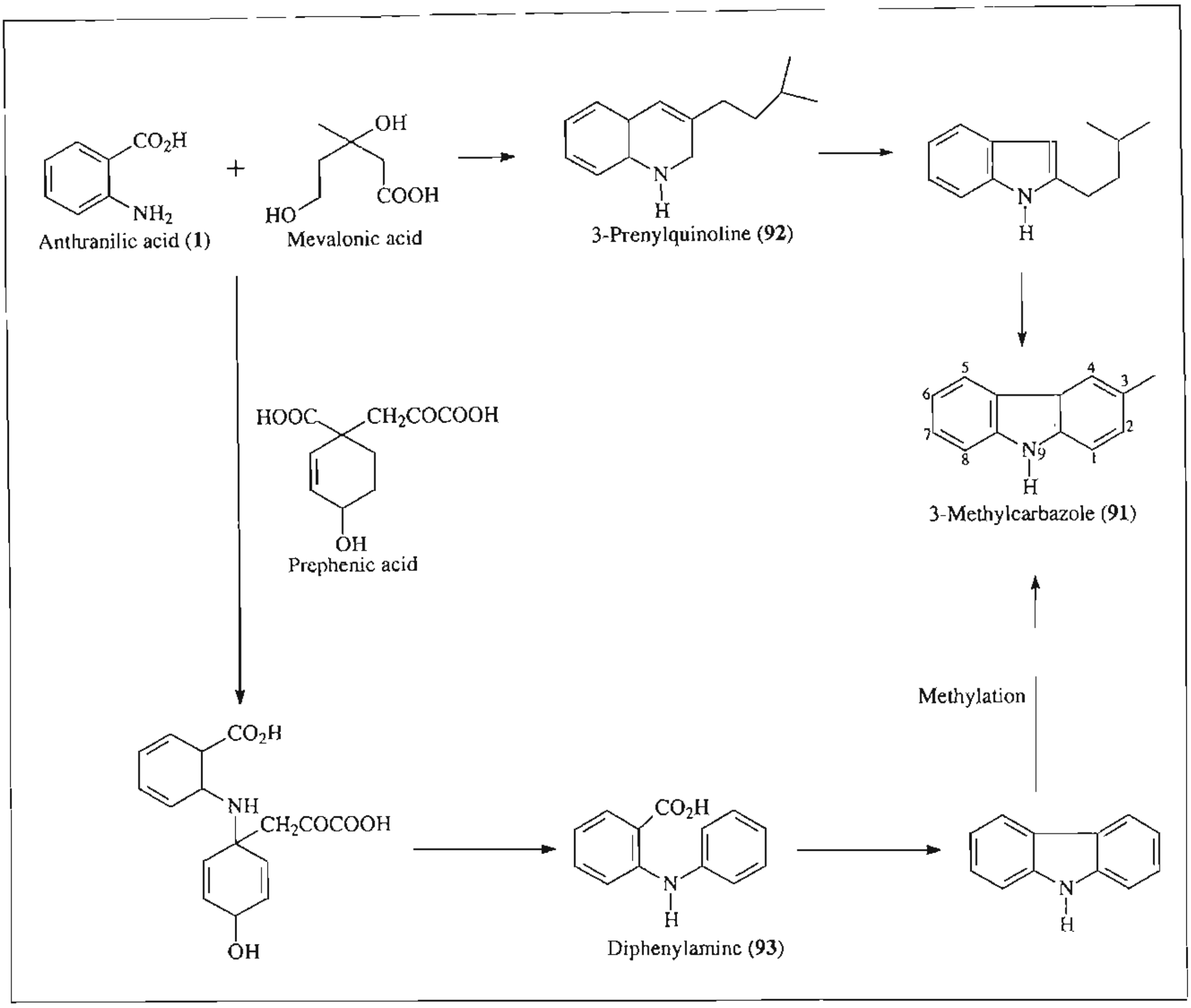
Other alkaloids of this group are formed by condensation of anthranilic acid (**1**) with aromatic amino acids or their derivatives (scheme 2.2).



Scheme 2.2. Alkaloids of the Rutaceae incorporating anthranilic acid[3,4]

In graveoline (**84**) (*Ruta graveolens*), the non-anthranilate portion of the 2-aryl-4-quinolone is derived from the decarboxylation and deamination of phenylalanine. Galipine (**85**), an alkaloid with the quinoline and phenyl ring systems separated by a carbon chain, is thought to be formed by condensation of anthranilic acid (**1**) with phenylalanine. The 2-arylquinazolines (**87**) are formed *via* phenylethylamine from a similar pathway as observed for graveoline (**84**). The β-indolo-quinazoline type *e.g.* rutaecarpine (**88**) are formed from the direct condensation of anthranilic acid and tryptophan. Canthin-6-one (**89**) is derived from tryptophan (**90**) and an aldehyde of a non-aromatic amino acid such as aspartic acid.

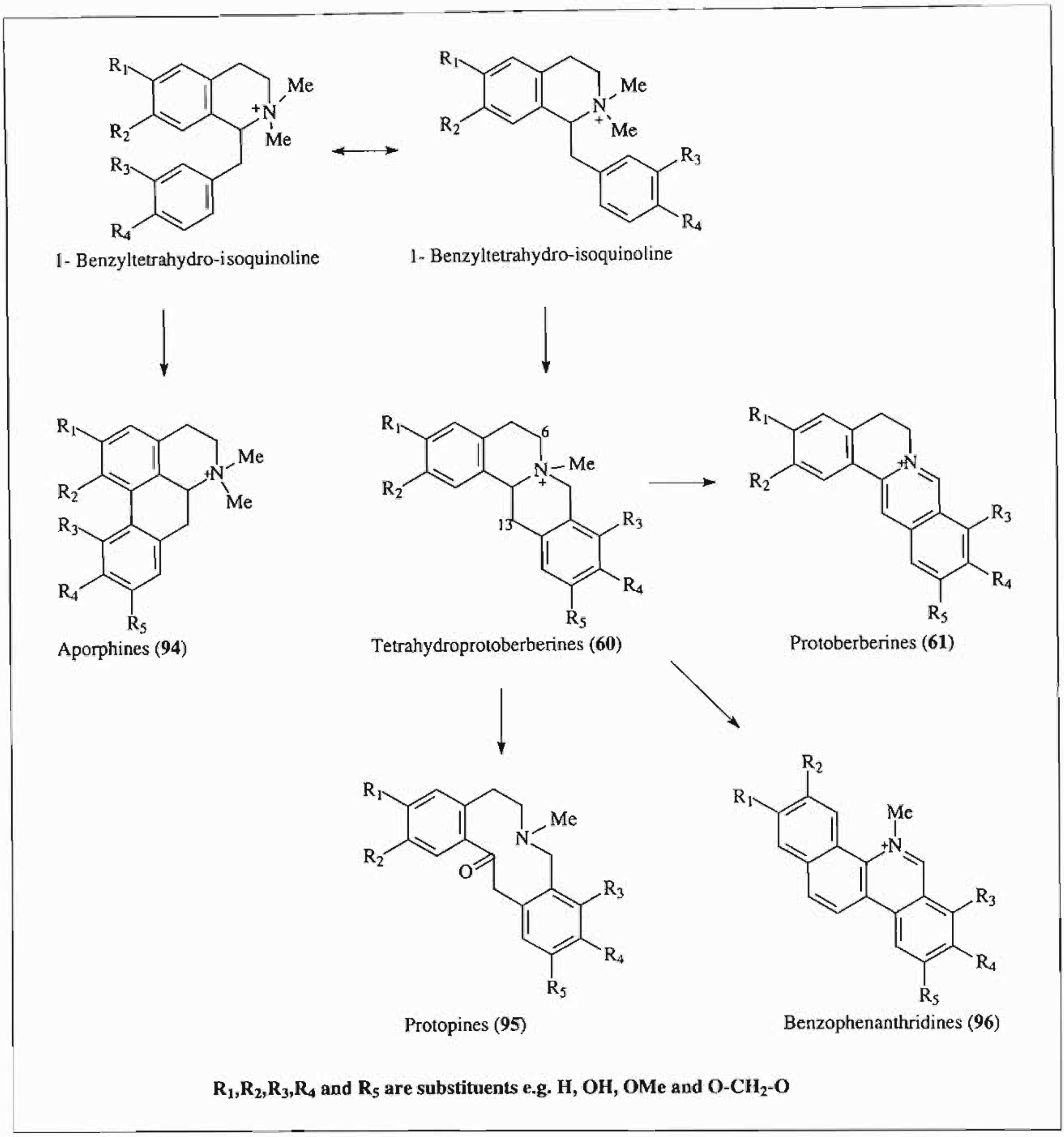
The carbazole (**91**) series is another group thought to originate from anthranilic acid (**1**). The biogenesis either involves 3-prenylquinoline (**92**) or diphenylamine (**93**) (scheme 2.3). Carbazoles are subdivided into three classes C_{13}, C_{18} and C_{23}.



Scheme 2.3. Proposed biosynthesis of carbazoles[3,4]

2.3.2. Alkaloids derived from tyrosine and phenylalanine

The aporphine (**94**) type alkaloids contain the tetracyclic ring system formed by phenolic oxidative coupling of a benzylisoquinoline skeleton.[8] Protopines (**95**) are tricyclic bases formed by oxidative ring fission of protoberberine N-methyl salts. The benzophenanthridine (**96**) group is derived from tetrahydroprotoberberine (**60**) precursors by oxidation of the C-6 to nitrogen bond followed by cyclisation of C-6 on to position C-13 (scheme 2.4).

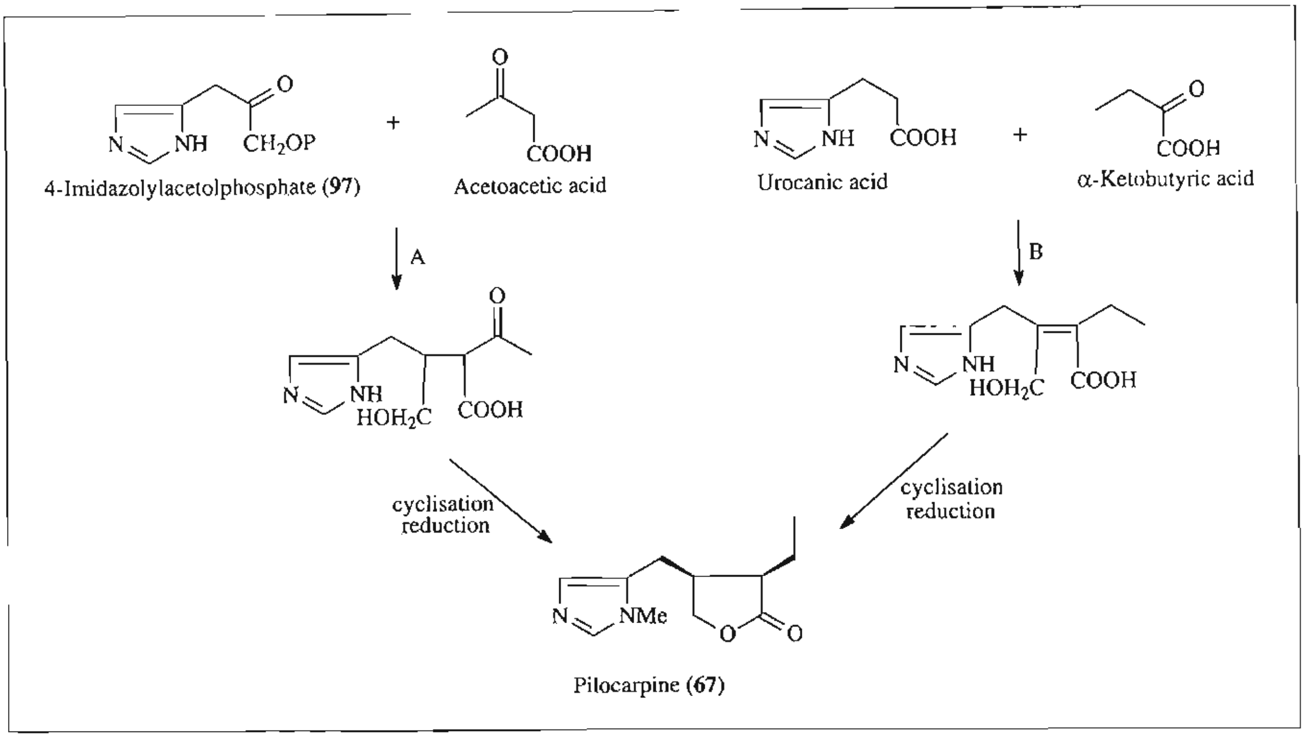


R_1,R_2,R_3,R_4 and R_5 are substituents e.g. H, OH, OMe and $O\text{-}CH_2\text{-}O$

Scheme 2.4. Alkaloids derived from tyrosine and phenylalanine

2.3.3. Alkaloids derived from histidine

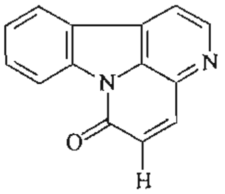
There are two possible pathways for the biosynthesis of pilocarpine (**67**) as shown in scheme 2.5. Pathway A assumes the combination of 4-imidazolylacetolphosphate (**97**) and either two molecules of acetate or one molecule of acetoacetic acid. In pathway B, a histidyl analogue of cinnamic acid and α-ketobutyric acid, a metabolite of threonine, are the precursors.[9]



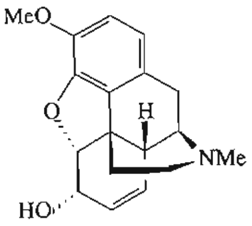
Scheme 2.5. Proposed biosynthesis of pilocarpine (25)[9]

2.4. Biological activity

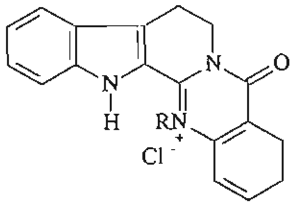
Alkaloids have been used traditionally as a result of their physiological activities in animals and humans. The benzylisoquinoline alkaloids have a variety of physiological properties. Berberine (**62**) displays antimicrobial activity against eye and intestinal infections. Codeine (**98**) is used as a narcotic analgesic and antitussive, and morphine is used as a narcotic analgesic. Rutaecarpine hydrochloride (**99**) (*Evodia rutaecarpa*) and dehydroevodiamine chloride (**100**) (*Evodia rutaecarpa*) show uterotonic activity.[3] Canthin-6-one (**89**), chelerythrine chloride (**101**) and berberine chloride (**102**) from *Fagara xanthoxyloides* have showed antimicrobial activity against oral flora.[2] Sanguinarine (**103**) is used as an antimicrobial agent in oral hygiene.[1] Emetine (**104**) is an anti-amoebic and colchicine is used as a microtubule disrupter and gout suppressant.



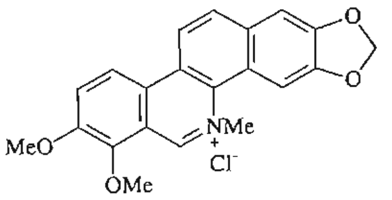
Canthin-6-one (**89**)



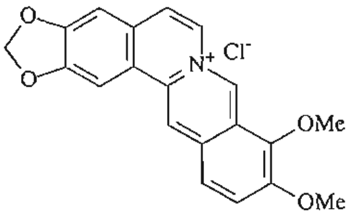
Codeine (**98**)



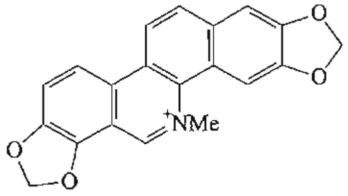
Rutaecarpine hydrochloride (R = H) (**99**)
Dehyroevodiamine chloride (R = Me) (**100**)



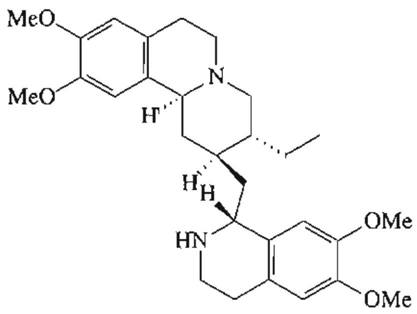
Chelerythrine chloride (**101**)



Berberine chloride (**102**)

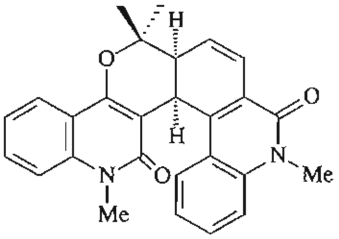


Sanguinarine (**103**)

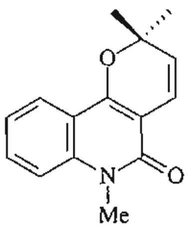


Emetine (**104**)

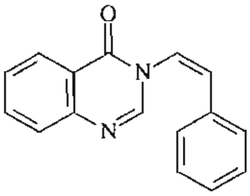
Melicobisquinolinone B (**105**) (*Melicope ptelefolia*) and N-methylflindersine (**106**) (*Melicope ptelefolia*) inhibit the mycelial growth of the fungus *Cladosporium cucumerinum* at nanomolar concentrations.[1] (*Z*)-Bogorin (**107**) (*Glycosmis cf. chlorosperma*) exhibits antifungal properties against *Cladosporum herbarium* and cytotoxic activity against *Artemia salina*.[1]



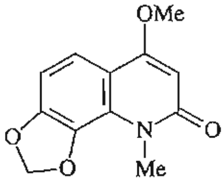
Melicobisquinolinone B (**105**)



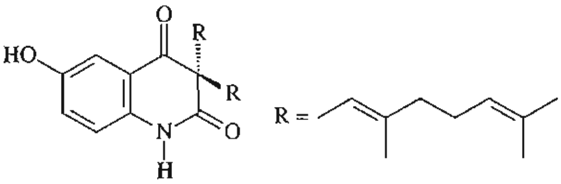
N-Methylflindersine (**106**)



(Z)-Bogorin (**107**)

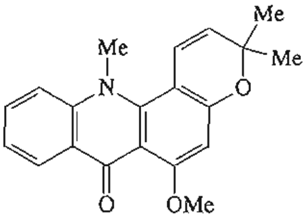


Casimiroine (**108**)



Severibuxine (**109**)

Casimiroine (**108**) (*Casimiroa edulis* seeds) shows antimutagenic and chemopreventative activity against cancer cells in mouse mammary gland cultures by inhibiting DMBA-induced lesions.[1] Severibuxine (**109**) (*Atalantia buxifolia*) shows cytotoxic activity against P-388 murine leukaemia cell lines.[1] Acronycine (**110**) is the acridone alkaloid best known to exhibit antitumor activity.



Acronycine (**110**)

Research into alkaloid chemistry provides the pharmaceutical industry with sources for new and better drugs.

2.5. References

1. Michael, J.P., Quinoline, quinazoline and acridone alkaloids, *Nat. Prod. Rep.*, 2000, **17**, 603-619.
2. Odebiyi, O.O. and Sofowora, E.A., Antimicrobial alkaloids from a Nigerian chewing stick (*Fagara zanthoxyloides*), *Planta Med.*, 1979, **36**, 204-207.
3. Waterman, P.G., Alkaloids of the Rutaceae: their distribution and systematic significance, *Biochem. Syst. Ecol.*, 1975, **3**, 149-160.
4. Dewick, P.M., Medicinal natural products, a biosynthetic approach, John Wiley and Sons, New York, 1998, 120.
5. Southon, I.W. and Buckingham, J., *Dictionary of Alkaloids,* Chapman and Hall, London, New York, 1989, 1-1161.
6. Dictionary of Natural Products, Version 10:1, Chapman and Hall Electronic Publishing Division, London, 2004.
7. Grundon, M., Chemistry and chemical taxonomy of the Rutales, Annual Proceedings of the Phytochemical Society of Europe, Ed. Waterman, P.G. and Grundon, M.G., Academic Press London, 1983, **22**, 9-30.
8. Herbert, R.B., The biosynthesis of secondary metabolites, Chapman and Hall, second edition, London, 1989, 120.
9. Bell, E.A. and Charlwood, B.V., Secondary plant products, Encyclopedia of plant physiology new series, Springer-Verlag, Berlin, Heidelberg, New York, 1980, **8**, 160-166.

Chapter 3

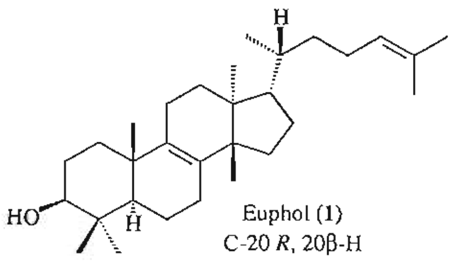
Introduction to Triterpenoids

CHAPTER 3
Introduction to Triterpenoids

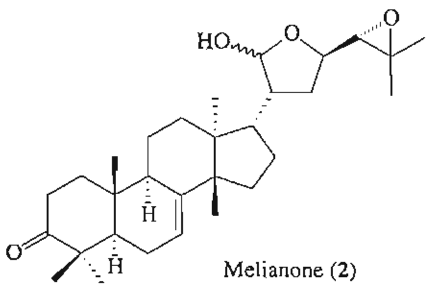
3.1. Introduction and Classification

Triterpenoids are terpenoid type compounds with a C_{30} skeleton. Terpenoids are compounds built up from C_5 isoprene units and are derived from mevalonate as the initial presursor.[1] Terpenoids are classified into hemiterpenoids (C_5 skeleton), monoterpenoids (C_{10} skeleton), sesquiterpenoids (C_{15} skeleton), diterpenoids (C_{20} skeleton), sesterterpenoids (C_{25} skeleton), triterpenoids (C_{30} skeleton) and tetraterpenoids (C_{40} skeleton).

Only the triterpenoids will be discussed since only this class of terpenoids was isolated in this work. Triterpenoids are naturally occurring compounds with a C_{30} skeleton and are present as secondary metabolites in plants. Triterpenoids range from structures where the eight carbon side chain remains intact *e.g.* euphol (**1**) to those structures where the side chain has cyclised to form a ring *e.g.* melianone (**2**).

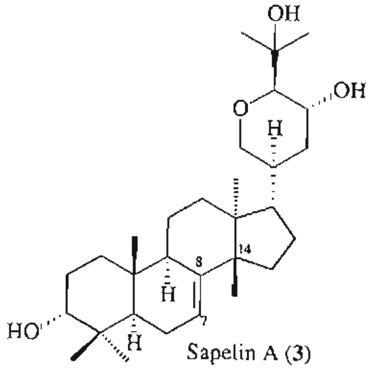


Euphol (1)
C-20 *R*, 20β-H

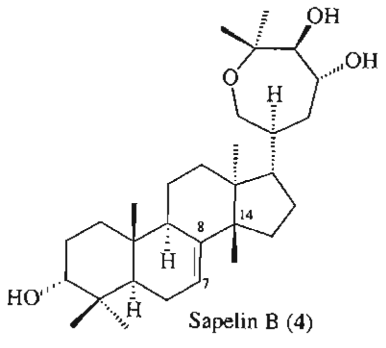


Melianone (2)

Protolimonoids, the class of triterpenoids isolated in this work, are triterpenoids in which the side chain is oxidised and loss of the four terminal carbon atoms often results in the formation of cyclic structures. These compounds are thought to be precursors of limonoids, hence the name protolimonoids. Protolimonoids are divided into two groups (Ia and Ib). The group Ia protolimonoids have a β-methyl group at C-14 and a Δ^7-double bond and group Ib protolimonoids have undergone the apo-euphol rearrangement to form a Δ^{14}-double bond with the β-methyl group at C-8, and an oxygenated substituent at C-7α. The group Ia protolimonoids include melianone (**2**) (*Turrea obtusifolia, Entandrophragma caudatum*), sapelin A (**3**) (*Entandrophragma cylindricum, Entandrophragma utile*) and sapelin B (**4**) (*Entandrophragma cylindricum*).

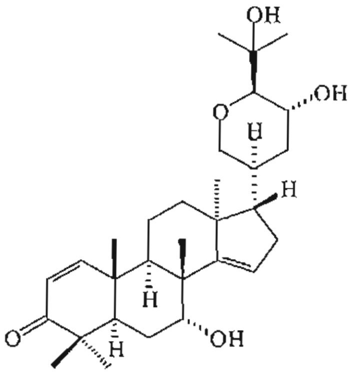


Sapelin A (3)

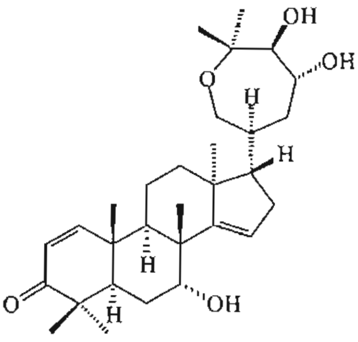


Sapelin B (4)

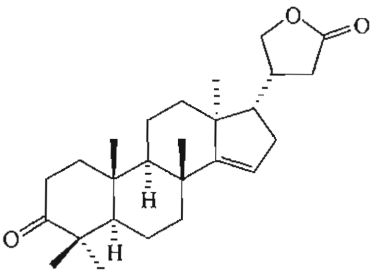
The group Ib protolimonoids include sapelin C (**5**) (*Entandrophragma cylindricum*), sapelin E (**6**) (*Entandrophragma cylindricum*) and turranolide (**7**) (*Turraea robusta*).



Sapelin C (5)



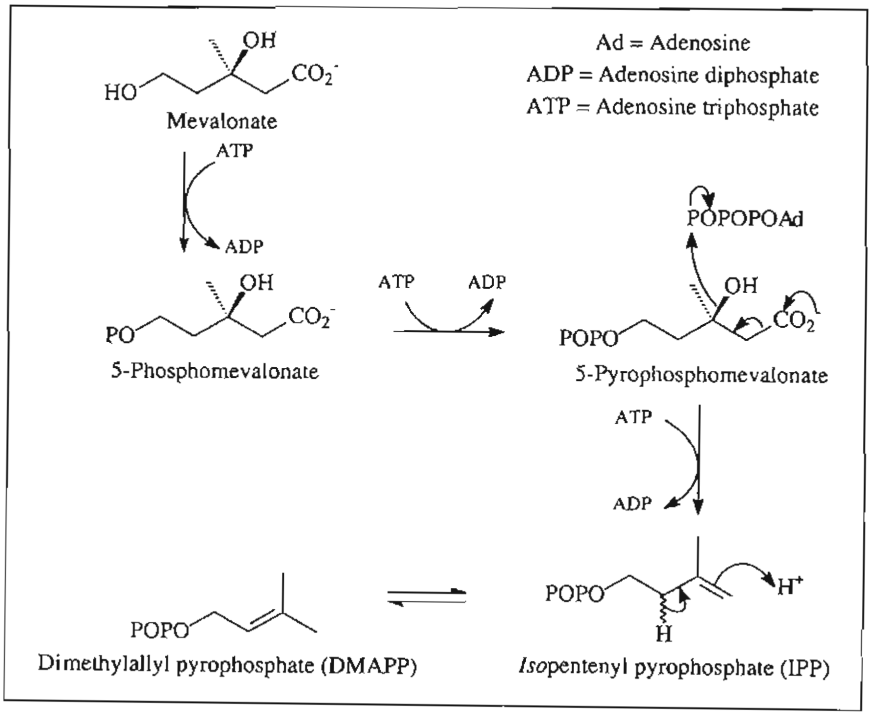
Sapelin E (6)



Turranolide (7)

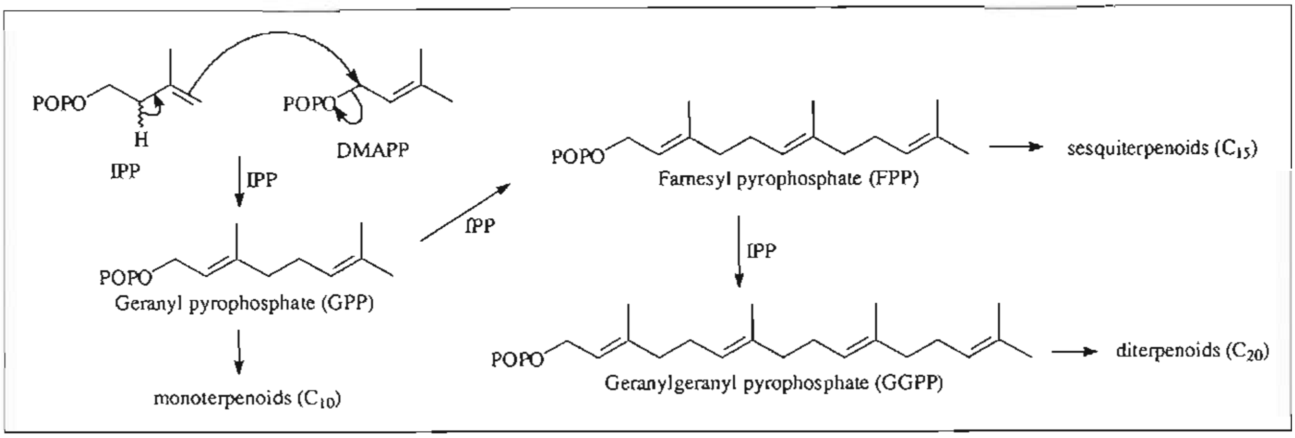
3.2. Biosynthesis

Mevalonate undergoes a series of enzyme-catalysed phosphorylation reactions to form *iso*pentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) (Scheme 3.1).[2]



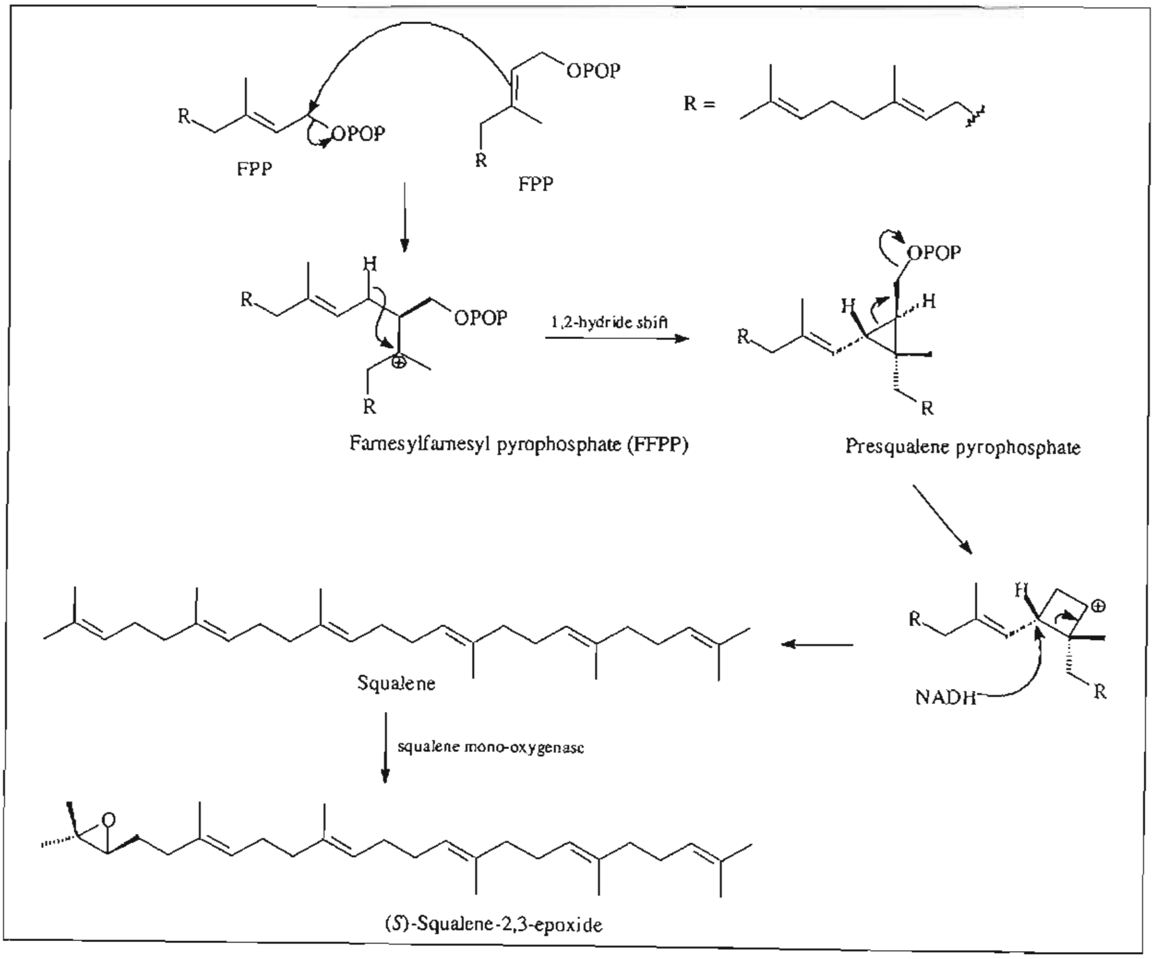
Scheme 3.1. Formation of IPP and DMAPP from mevalonate[2]

IPP and DMAPP undergo condensation to form geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). These compounds are precursors for monotriterpenoids, sesquiterpenoids and diterpenoids (scheme 3.2).[2]



Scheme 3.2. Formation of GPP (monoterpenoid), FPP (sesquiterpenoid) and GGPP (diterpenoid)[2]

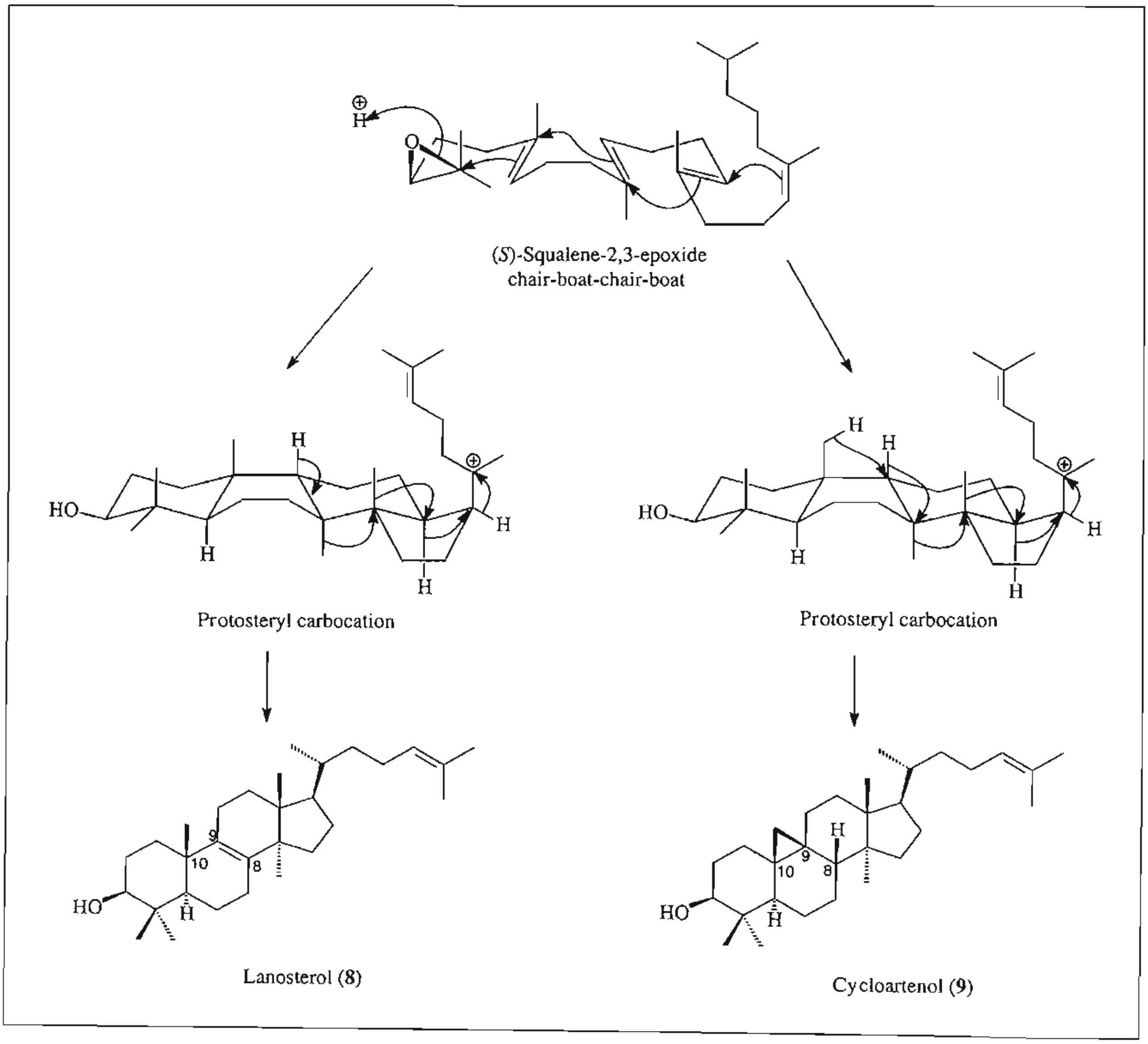
Dimerisation of two FPP molecules forms farnesylfarnesyl pyrophosphate (FFPP) which undergoes a 1,2-hydride shift to form presqualene pyrophosphate. This then reacts with nicotinamide adenine dinucleotide (NADH) to form squalene, which undergoes enzymatic oxidation to form (*S*)-squalene-2,3-epoxide (scheme 3.3).[2] Cyclisation of (*S*)-squalene-2,3-epoxide leads to the formation of triterpenoids (C_{30} skeleton).



Scheme 3.3. Formation of (*S*)-squalene-2,3-epoxide[2]

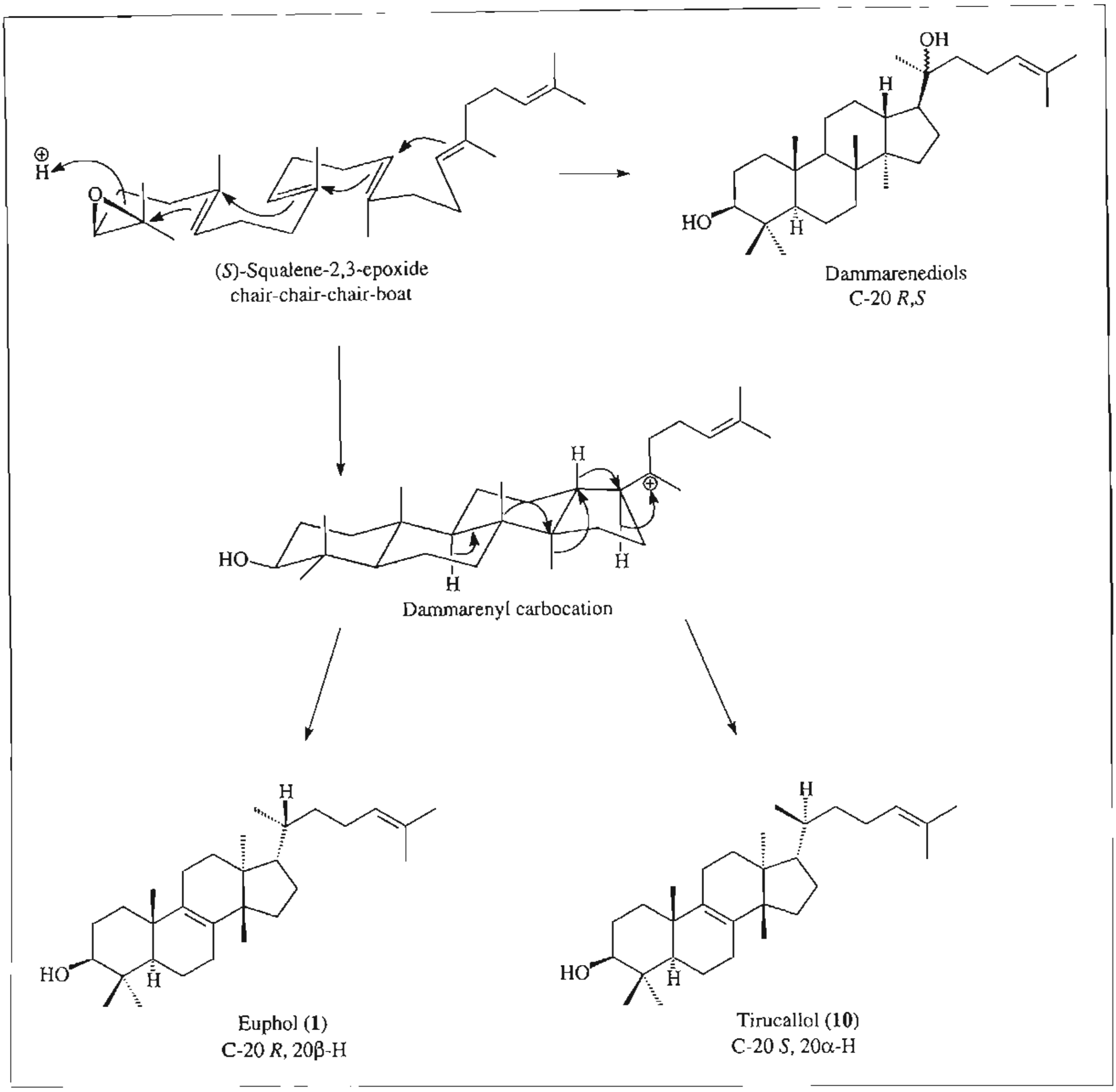
The different triterpenoid structures arise as a result of (*S*)-squalene-2,3-epoxide having a number of conformations on the enzymatic surface.[3,4] Wagner-Meerwein 1,2-hydride shifts and the different cationic intermediates result in the different types of triterpenoids.[5]

Cyclisation of (*S*)-squalene-2,3-epoxide in the chair-boat-chair-boat conformation affords the protosteryl carbocation. The protosteryl carbocation can either form lanosterol (**8**) by the loss of H-9, or H-9 can migrate to C-8, thereby initiating the 9,10-cyclopropane ring formation of cycloartenol (**9**) (scheme 3.4).[6]



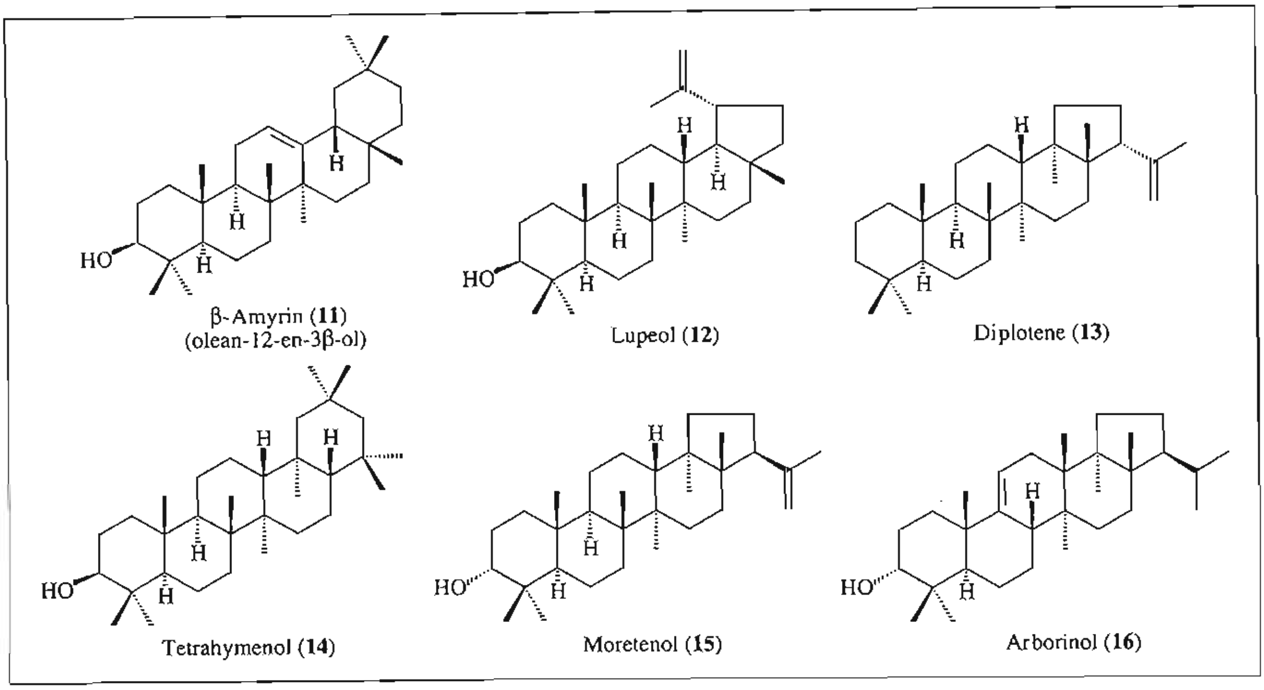
Scheme 3.4. Formation of lanosterol (8) and cycloartenol (9)[6]

Cyclisation of (*S*)-squalene-2,3-epoxide in the chair-chair-chair-boat conformation affords the C-20 epimeric dammarenediols, and the dammarenyl carbocation, which undergoes methyl migration and 1,2-hydride shifts to form the C-20 epimers euphol (**1**) and tirucallol (**10**) (scheme 3.5).[6] These compounds, (**1**) and (**10**), are important precursors to limonoids.

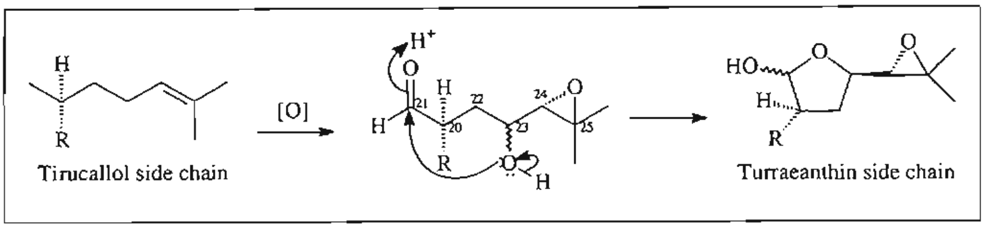


Scheme 3.5. Formation of euphol (1) and tirucallol (10)[6]

The chair-chair-chair-boat cyclisation of (*S*)-squalene-2,3-epoxide also affords pentacyclic triterpenoids such as β-amyrin (**11**) and lupeol (**12**). The chair-chair-chair-chair conformation of (*S*)-squalene-2,3-epoxide affords diplotene (**13**) and tetrahymenol (**14**).[7] The chair-chair-chair-chair-boat conformation of (*S*)-squalene-2,3-epoxide affords moretenol (**15**)[8] and the chair-boat-chair-chair-boat conformation affords arborinol (**16**).[9]

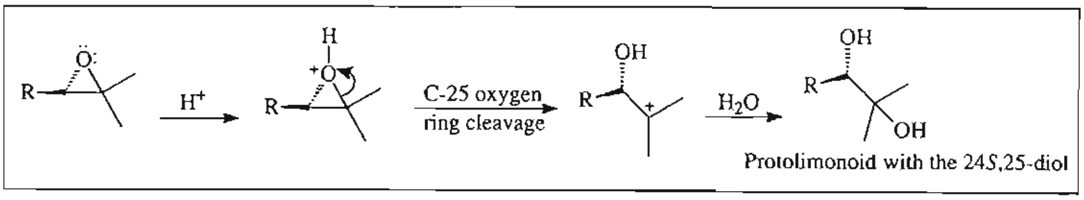


Functionalisation and/or cyclisation of the side chain of euphol/tirucallol precursors gives rise to a class of triterpenoids which have become known as the protolimonoids. Oxidation of the side chain results in the formation of an aldehyde group at C-21, a hydroxy group at C-23 and a 24,25-epoxide ring. This side chain is then cyclised to form the turraeanthin side chain, which is present in some of the protolimonoids isolated in this work (Scheme 3.6).[10]



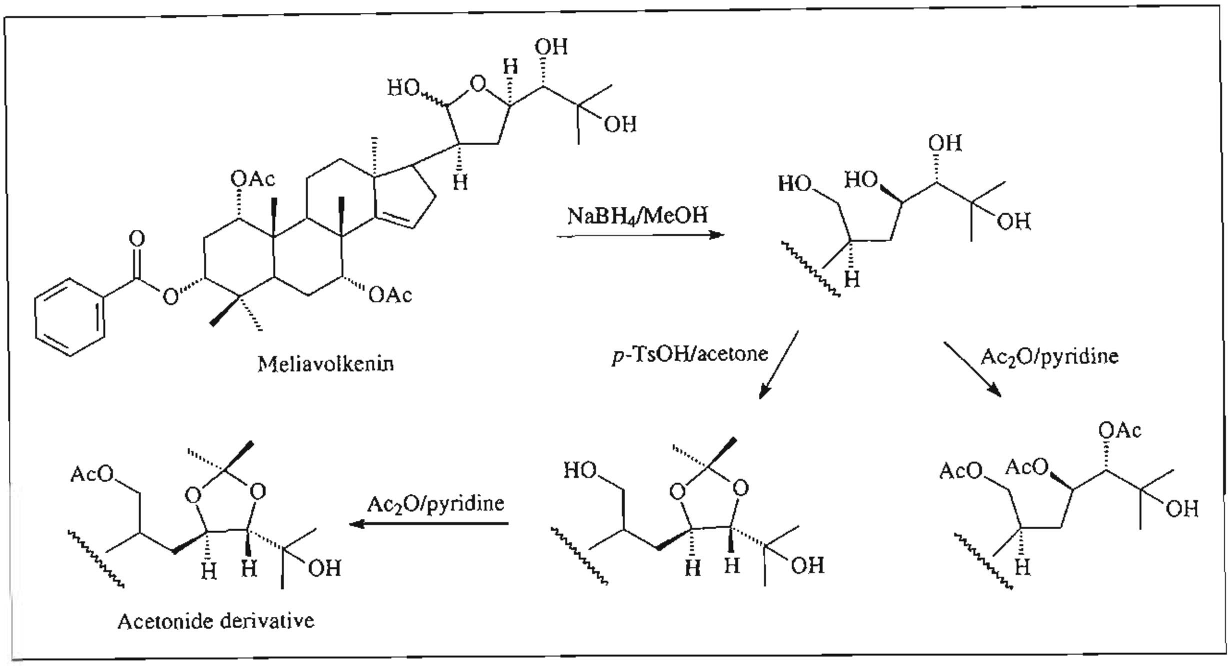
Scheme 3.6. Formation of the turraeanthin side chain[10]

Opening of the 24,25-epoxide ring results in the formation of the side chain found in other protolimonoids isolated in this work. The stereochemistry of the 24,25-epoxide ring that characterises compounds such as melianol is securely established.[11] A number of workers have reported that acid catalysed ring opening of the epoxide precursor leads exclusively to the 24*S*,25-diol.[12] This is explained by the assumption that this ring opening involves formation of a carbocation at C-25, as the C-25 tertiary carbocation would be more stable than its C-24 secondary counterpart. Ring cleavage thus occurs at the C-25-oxygen bond, with retention of the 24*S* configuration at C-24 (Scheme 3.7).



Scheme 3.7. Formation of the 24*S*,25-diol of the protolimonoids

Only a few protolimonoids with the 24,25-diol have previously been reported with established C-24 stereochemistry.[13,14,15,16,17,18] All of these protolimonoids have been reported to have the 24*S* configuration. Merrien and Polonsky[15] reported melianodiol which has the 24*S* configuration. This is expected since melianodiol was obtained previously by acid catalysed opening of the melianone epoxide by Lavie *et al.*.[19] Omobuwajo *et al.*[16] reported gentinone C with the 24*S* configuration, the structure of which was confirmed by acid catalysed opening of the epoxide of gentinone A. Zeng *et al.*[14] reported meliavolkenin with the 24*S* configuration. The stereochemistry was determined by reducing meliavolkenin and forming an acetonide derivative (scheme 3.8), which was analysed using the results of NOESY experiments.

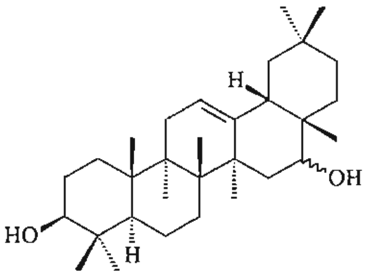


Scheme 3.8. Formation of the acetonide derivatives from meliavolkenin[14]

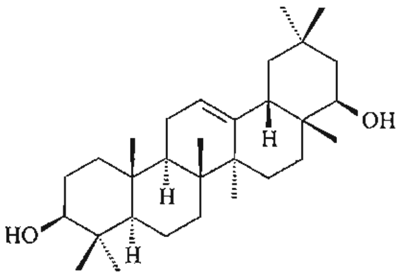
The acetonyl methyl proton resonances of the acetonide derivative occurred as a singlet at δ_H 1.42, which correlated to the oxymethine resonances at δ_H 4.03 (t, J = 8 Hz) and δ_H 3.54 (t, J = 8 Hz) in the NOESY NMR spectrum. This indicated that the diol at C-23 and C-24 was *threo* (the common substituents on the two stereogenic carbons are on opposite sides of the molecule), since two separated singlets are observed for *erythro* diols. Mulholland *et al.*[17] reported holstinones A-C, all of which were reported to have the 24*S* configuration, and Hisham *et al.*[18] also reported a protolimonoid with the 24*S* configuration. All these reports showed the H-24 proton resonance to occur at ~ δ_H 3.2 for H-24 for the 24*S* configuration. Puripattanavong *et al.*[13] reported the isolation of both C-24 epimeric diols, melianodiol and *epi*-melianodiol from *Aglaia andamanica*. The presence of the C-24 epimer was deduced only by the observed difference in the chemical shifts of the H-24 proton (δ_H 3.15 vs 3.64) and the C-24 carbon resonances (δ_C 75.24 vs 77.68) in the NMR spectra.

3.3. Biological Activity

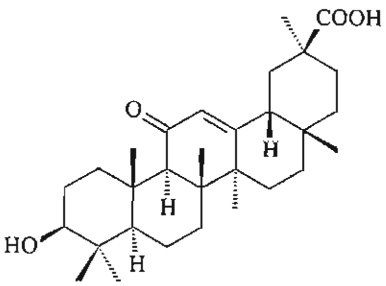
The biological activities of triterpenoids include cytotoxic and antifungal activities. Some pentacyclic and tetracyclic triterpenoids exhibit anticarcinogenic properties. The pentacyclic triterpenoid *epi*maniladiol (**17**) showed activity against the HEC-1-A, CAMA-1, ME-180, u-87MG, CALAU-1 and SK-OV-3 human cancer cell lines.[20] The 16β-epimer, maniladiol (**18**), showed cytotoxicity against the ME-180 and CAMA-1 cell lines, whereas sophoradiol (**19**) (no C-16 hydroxy group) only showed cytotoxicity against ME-180 cell lines.[20] Glycyrrhetinic acid (**20**) showed activity against SK-OV-3 and 11-oxo-β-amyrin was active against CAMA-1 cell lines.[20] These results showed the importance of the hydroxy group's position and orientation in 12-oleanenes. Compounds with hydroxy groups at both C-3 and C-16 are more active than compounds which only have the C-3 hydroxy group, and compounds containing a hydroxy group at C-16α exhibit the highest activity against the human cancer cell lines.[20]



Maniladiol (**18**)
*Epi*maniladiol (**17**)

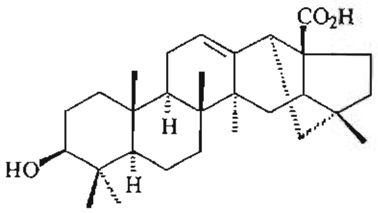


Sophoradiol (**19**)



Glycyrrhetinic acid (**20**)

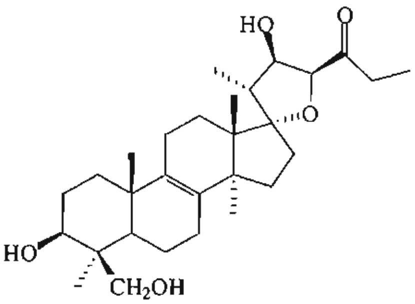
The hexacyclic nortriterpene pfaffic acid (**21**) (*Pfaffia paniculata*) exhibits growth inhibitory properties against cultured tumor cells such as melanoma (B-16), Hela (S-3) and Lewis Lung carcinoma cells.[21]



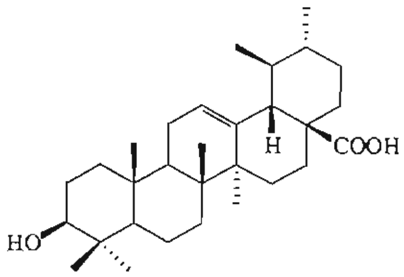
Pfaffic acid (**21**)

In vitro antitumor studies on lanosterol oligosaccharides (*Eucomis bicolor, Scilla peruviana, Chionodoxa gigantean, Chionodoxa luciliae*) showed their inhibitory action on the tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) - stimulated 32P, preventing it from being incorporated into the HeLa cell phospholipids. The antitumor properties of these compounds are enhanced by a C-16β hydroxy substituent.[22]

22β-Hydroxy-15-deoxoeucosterol (**22**) (*Ledebouria zebrina*) exhibited antifungal properties against the fungus *Fusarium moliniforme.*[23] Ursolic acid (**23**) (*Prunella vulgaris, Psychotria serpens* and *Hyptis capitata*) showed cytotoxicity against the lymphocytic leukemia cells P-388 and L-1210 and the human lung carcinoma A-549 cell line.[24]



22ß-Hydroxy-15-deoxoeucosterol (**22**)



Ursolic acid (**23**)

Triterpenoids are important anticarcinogenic agents and work in this field will continue in the hope of finding new drugs to combat these diseases.

3.4. References

1. Banthorpe, D.V. and Charlwood, B.V., Methods in plant biochemistry, Terpenoids, Academic Press, London, 1991, **7**, 26.
2. http://www.chem.qmw.ac.uk/iubmb/enzyme/reaction/terp/terp.html (23/11/2004)
3. Stork, G. and Burgstahler, A.W., The stereochemistry of polyene cyclization, *J. Am. Chem. Soc.*, 1955, **77**, 5068-5077.
4. Ruzicka, L., History of the isoprene rule: Faraday lecture, *Proc. Chem. Soc.*, 1959, 341-360.
5. Floss, H.G., Onderka, D.K. and Carroll, M., Stereochemistry of the 3-deoxy-D-arabinoheptulosonate 7-phosphate synthetase reaction and the chorismate synthetase reaction, *J. Biol. Chem.*, 1972, **247**, 736-744.
6. Dewick, P.M. Medicinal Natural Products: a Biosynthetic Approach., John Wiley and Sons, London, second edition, 2002, 167-289.
7. Caspi, E.J., Grieg, J.B., Zander, J.M. and Mandelbaum A., Incorporation of deuterium from deuterium oxide into tetrahymanol biosynthesised from squalene, *Chem. Comm.*, 1969, 28-29.
8. Galbraith, M.N., Miller, C.J., Rawson, J.W.L., Ritchie, E., Shannon, J.S. and Taylor, W.C., Moretenol and other triterpenes from *Ficus Macrophylla* Desf., *Aust. J. Chem.*, 1965, **18**, 226-239.
9. Kennard, O., Riva di Sanseverino, L., Vorbruggen, H. and Djerassi, C., Terpenoids. LVI. Complete structure of the triterpene arborinol, *Tetrahedron Letters*, 1965, 3433-3438.
10. Bevan, C.W.L., Ekong, D.E.U., Halsall, T.G. and Toft, P., West African Timbers. Part XX. The structure of turraeanthin, an oxygenated tetracyclic triterpene monoacetate, *J. Chem. Soc. (C)*, 1967, 820-828.
11. Nakanishi, T., Inada, A., Nishi, M., Miki, T., Hino, R. and Fujiwara, T., The structure of a new natural apotirucallane-type triterpene and the stereochemistry of the related terpenes. X-ray and ^{13}C NMR spectral analyses, *Chem. Letters*, 1986, 69-72.
12. Itokawa, H., Kishi, E., Morita, H. and Takeya, K., Cytotoxic quassinoids and tirucallane-type triterpenes from the woods of *Eurycoma longifolia*, *Chem. Pharm. Bull.*, 1992, **40**, 1053-1055.
13. Puripattanavong, J., Weber, S., Brecht, V. and Frahm, A.W., Phytochemical investigation of *Aglaia andamanica*, *Planta Med.*, 2000, **66**, 740-745.
14. Zeng, L., Gu, Z.-M., Chang, C.-J., Wood, K.V. and McLaughlin, J.L., Meliavolkenin, a new bioactive triterpenoid from *Melia volkensii* (Meliaceae), *Bioorg. Med. Chem.*, 1995, **3**, 383-390.
15. Merrien, A. and Polonsky, J., The natural occurrence of melianodiol and its diacetate in *Samadera madagascariensis* (Simaroubaceae): Model experiments on melianodiol directed towards simarolide, *Chem. Comm.*, 1971, 261-263.
16. Omobuwajo, O.R., Martin, M.-T., Perromat, G., Sévenet, T., Païs, M. and Awang, K., Apotirucallane triterpenes from *Aglaia argentea*, *J. Nat. Prod.*, 1996, **59**, 614-617.
17. Mulholland, D.A., Monkhe, T.V., Taylor, D.A.H. and Rajab, M.S., Triterpenoids from *Turraea holstii*, *Phytochemistry*, 1999, **52**, 123-126.
18. Hisham, A., Ajitha, Bai M.D., JayaKumar, G., Nair, M.S. and Fujimoto, Y., Triterpenoids from *Dysoxylum malabaricum*, *Phytochemistry*, 2001, **56**, 331-334.

19. Lavie, D., Jain, M.K. and Shpan-Gabrielith, S.R., A locust phagorepellent from two melia species, *Chem. Comm.*, 1967, 910-911.

20. Mahato, S.B, Nandy, A.K. and Roy, G., Triterpenoids, *Phytochemistry*. 1992, **31**, 2199-2249.

21. Takemoto, T., Nishimoto, N., Nakai, S., Takagai, N., Hayashi, S., Odasima, S. and Wada, Y., Pfaffic acid, A novel nortriterpene from *Pfaffia paniculata* Kuntze, *Tetrahedron Letters*, 1983, **24**, 1057-1060.

22. Mimaki, Y., Nishino, H., Ori, K., Kuroda, M., Muthsui, T. and Sashida, Y., Lanosterol oligosaccharides from the plants of the subfamily Scilloideae and their antitumor-promoter activity. *Chem. Pharm. Bull.*, 1994, **42**, 327-332.

23. Moodley, N., The Chemical Investigation of *Ledebouria zebrina* and *Scilla natalensis*, MSc Dissertation, University of Natal, South Africa, 2001, 40-77.

24. Lee, K.H., Lin, Y.M., Wu, T.S., Zhang, D.C., Yamagishi, T., Hayashni, T., Hall, I.H., Chang, J.J., Wu, R.Y., Yang, T.H., The cytotoxic principles of *Prunella vulgaris*, *Psychotria serpens*, and *Hyptis capitata*: ursolic acid and related derivatives, *Planta Medica*, 1988, **54**, 308-311.

Chapter 4

Introduction to Quassinoids

CHAPTER 4
Introduction to Quassinoids

4.1. Introduction

Quassinoids are bitter-tasting terpenoid compounds, which are characteristic of the Simaroubaceae family. Species of the Simaroubaceae also produce tryptophan alkaloids such as β-carbolines, together with canthinones, anthraquinones, flavonoids and limonoids. Quassinoids are classified according to their basic C_{20}, C_{25}, C_{18} and C_{19} skeleta,[1] this classification is discussed in section 4.2. The proposed quassinoid biogenesis from triterpenoid precursors is discussed in section 4.3.

The bark of *Quassia amara* L. and *Picraena excelsa* Lindt. are used by the Brazilians for the treatment of diarrhoea, dysentery, dyspepsia, blennorhagia, stomach aches, anaemia, liver disorders and gastrointestinal disorders.[2] In Europe and the U.S. the bark is used as a herbal medicine for stomach and digestive problems, as a laxative, amebicide and anthelminthic.[2] Various quassinoids isolated from the *Quassia amara* bark by the Japanese were found to have anti-ulcer activity. The water extract of the bark showed *in vitro* antiviral properties against MT-2-lymphoblastoid cells infected with HIV.[2] The bark was found to be an active larvicide.[2] The *in vivo* antifertility properties of the bark tested in rats were also demonstrated.[2] Tea made from *Quassia amara* is said to destroy the appetite for alcohol.[3] The West Indian natives leave water standing overnight in cups made from *Quassia amara* wood to take as a bitter tonic for soothing the stomach.[3] *Quassia amara* has also been reported to have antiparasitic, antibacterial, antiviral, antiplasmodial, anti-inflammatory and anticancer activities.[4]

Quassia amara
(Photograph by Gerald D. Carr)

Picraena excelsa
(http://www.xs4all.nl/~margjos/nlkassi.htm)

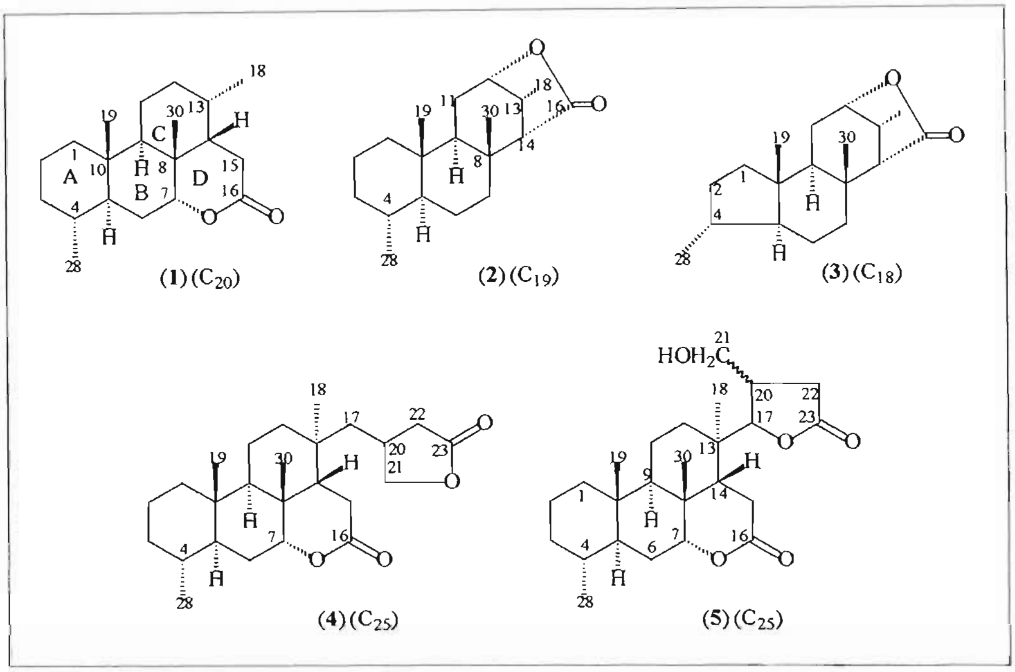
Eurycoma longifolia
(Photograph by Kazuo Yamasaki)

Eurycoma longifolia Jack, also known as "Tongkat ali", has been used by the Malaysian villagers to improve blood circulation and cure skin diseases. Recently the plant has been reported as an aphrodisiac to boost the male sex drive.[5]

Quassinoids have been reported to display a wide variety of biological activities, which include herbal and homeopathic remedies for soothing the stomach, treatment of thrush and mouth fungal disorders, sinus, eczema,[6] anorexia nervosa, fever, blennorrhagia, malaria and liver disorders,[7] flavourants in foods, liquors and tonic wines.[8] They are also reported as effective pesticides.[8,9] These biological activities are further discussed in section 4.4.

4.2. Classification

The five basic skeleta (**1**-**5**) of the quassinoids[1] are shown below.

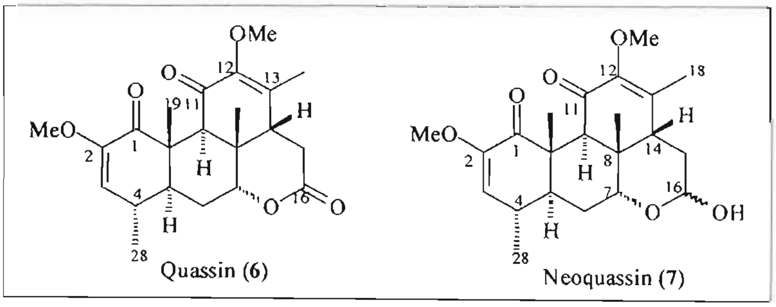


Skeletal types (**1**), (**2**), (**3**), (**4**) and (**5**) of quassinoids[1]

4.2.1. C_{20} quassinoids

The first quassinoids were isolated in 1950 from the wood of *Quassia amara.*[10] These were the C_{20} quassinoids quassin (**6**) and neoquassin (**7**) whose structures were characterised only in 1962 by Valenta and co-workers.[11,12]

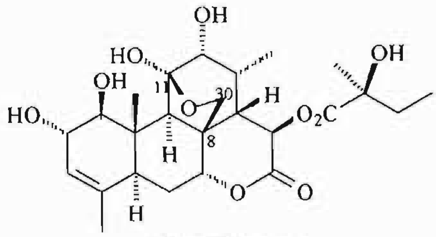
Quassia amara
(Photograph by Gerald D. Carr)



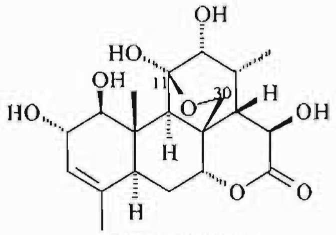
Quassin (**6**) has a δ-lactone, and neoquassin (**7**) has the corresponding hemiacetal.

In 1964 the structures of glaucarubin (**8**) and glaucarubol (**9**),[13,14] from the seeds of *Simarouba glauca* C.DC., were established.

Simarouba glauca
(Photograph by Dr. Edward F. Gilman)



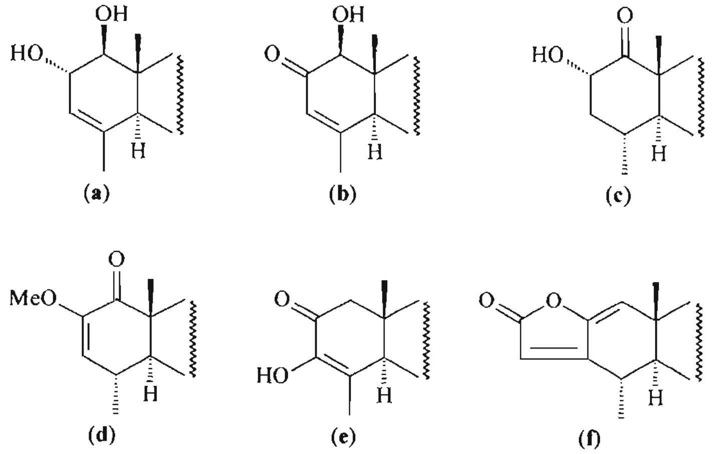
Glaucarubin (**8**)



Glaucarubol (**9**)

These quassinoids, (**8**) and (**9**), are different from quassinoids (**6**) and (**7**) in that the methyl group at C-8 has been oxidised to a hydroxymethyl group, which has then formed a hemiketal linkage to a carbonyl group at C-11.

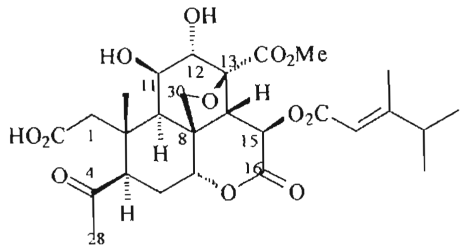
Polonsky[1] subclassified the C_{20} quassinoids on the basis of variations in the structure of ring A as shown below. Polonsky[1] also noted that ring C may possess a methyl or hydroxymethyl group at position 30, and ring D may have a hydroxy group at C-15 which is generally esterified with small fatty acids.



Variations in ring A of the C_{20} quassinoid structure[1]

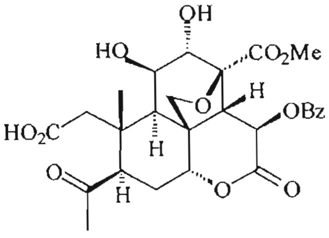
Bruceanic acid A (**10**), isolated in 1975 from the Ethiopian *Brucea antidysenterica* Mill,[15] was the first ring A-*seco* C_{20} quassinoid found.

Brucea antidysenterica
(http://mailbox.univie.ac.at/Christian.Puff/Simen2001_Report_Plates.htm)

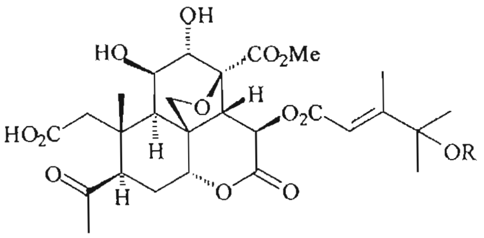


Bruceanic acid A (**10**)

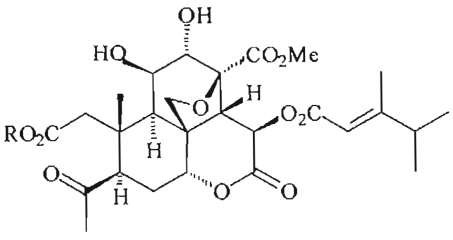
Bruceanic acids B (**11**), C (**12**) and D (**13**) and the methyl ester of bruceanic acid A (**14**) were also isolated from *Brucea antidysenterica*.[16]



Bruceanic acid B (**11**)

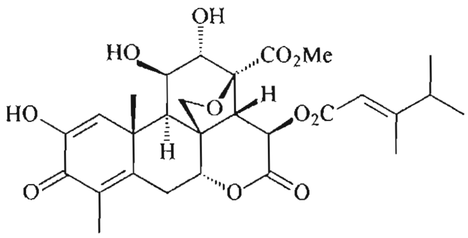


R = H Bruceanic acid C (**12**)
R = Ac Bruceanic acid D (**13**)

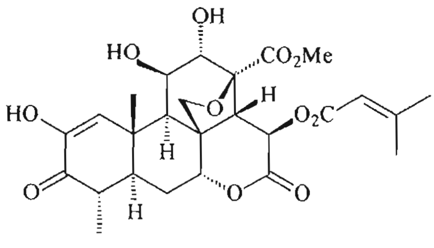


R = Me Bruceanic acid A methyl ester (**14**)

In 1975 dehydrobruceantin (**15**) was reported from *Brucea antidysenterica*.[15] In 1979 the bruceosin (**16**) type compounds were isolated.[17,18,19,20,21,22,23]



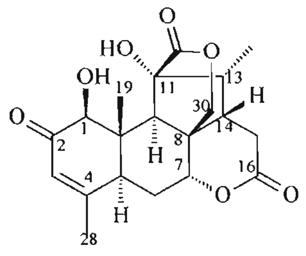
Dehydrobruceantin (**15**)



Bruceosin (**16**)

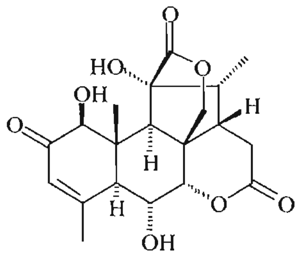
Shinjudilactone (**17**) isolated from *Ailanthus altissima* Swingle[24,25] is a C_{20} quassinoid with rearrangement of ring C and a 12,30-lactone linkage.

Ailanthus altissima
(Photograph by Gerald D. Carr)

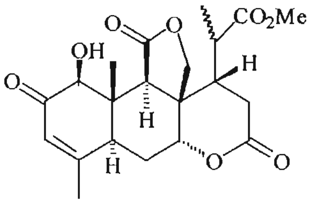


Shinjudilactone (**17**)

Ailantinol D (**18**) (*Ailanthus altissima*) is similar to shinjudilactone (**17**), the difference being the C-6 hydroxy group in ailantinol D (**18**). Ailantinol A (**19**) (*Ailanthus altissima*) undergoes further C-11, C-12 bond cleavage to form the *seco*-ring D.[26]

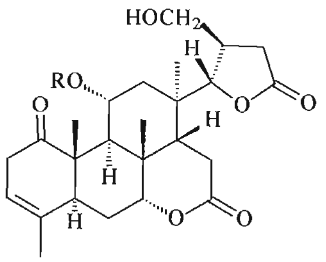


Ailantinol D (**18**)

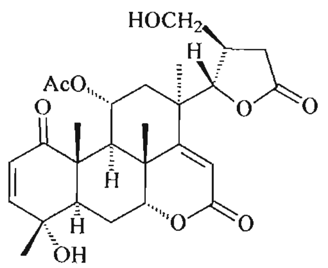


Ailantinol A (**19**)

Simarinolide (**20**), its deacetyl analogue (**21**),[1] and guanepolide (**22**) were reported from the fruits of *Simaba multiflora* A.Juss.[27]



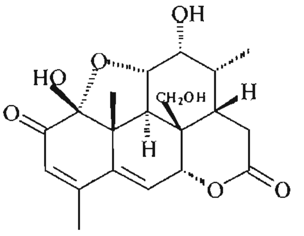
R = Ac Simarinolide (**20**)
R = H (**21**)



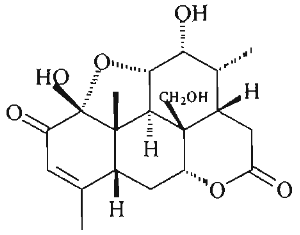
Guanepolide (**22**)

Karinolide (**23**), from *Simaba multiflora* A.Juss,[28] and shinjulactone F (**24**), from *Ailanthus altissima* Swingle,[29] possess a hydroxymethyl group at C-30 and a 1,11-hemiketal.

Ailanthus altissima
(Photographs by Gerald D. Carr)



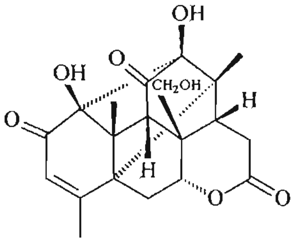
Karinolide (23)



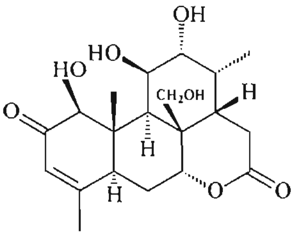
Shinjulactone F (24)

Shinjulactone C (**25**), from *Ailanthus altissima*,[24] does not possess the 1,11-hemiketal since the steric constraints caused by the presence of the C-1, C-12 and C-5, C-13 bonds prevents the hemiketal from forming. Shinjulactone G (**26**) was also isolated from *Ailanthus altissima*.[30]

Ailanthus altissima
(Photographs by Gerald D. Carr)

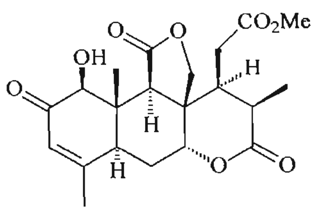


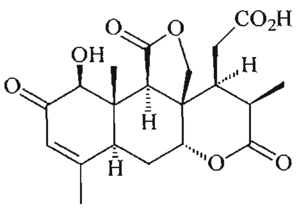
Shinjulactone C (25)

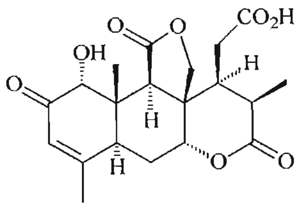


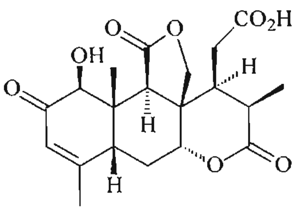
Shinjulactone G (26)

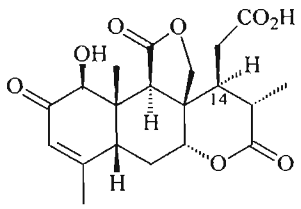
Vilmorinine B (**27**), vilmorinine C (**28**), the 1-epimer vilmorinine D (**29**), the 5-epimer vilmorinine E (**30**), and 5,15-diepimeric vilmorinine F (**31**) from *Ailanthus vilmoriana* Dode have the unusual H-14α and have lost C-20.[31] Vilmorinine A (**32**), however, has the H-14β and C-20 present but has the unusual 1,7-ether bridge.

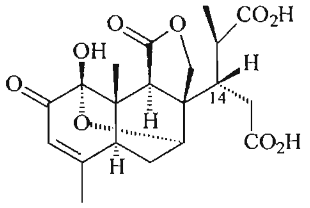

Vilmorinine B (**27**)


Vilmorinine C (**28**)

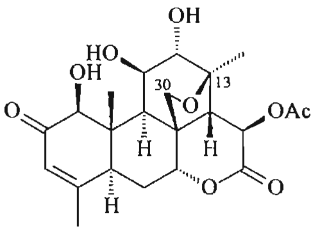

Vilmorinine D (**29**)

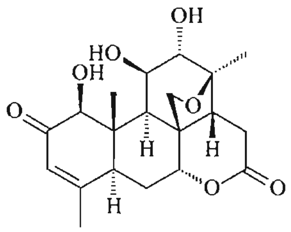

Vilmorinine E (**30**)

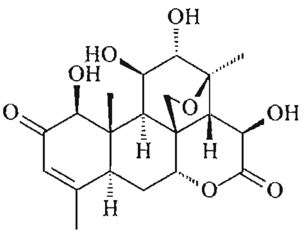

Vilmorinine F (**31**)

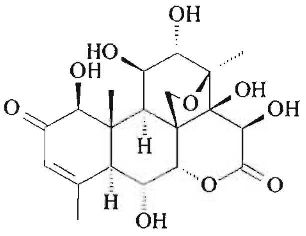

Vilmorinine A (**32**)

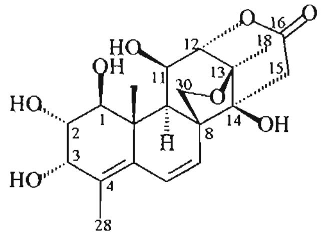
Quassia indica Gaertn. yielded samaderines X (**33**), Y (**34**), Z (**35**) and indaquassin X (**36**), which have the 13,30-ether bridge.[32] Indaquassin B (**37**) from *Quassia indica* Gaertn.[33] and cedronolactone D (**38**) from *Simaba cedron* Planch[34] are C_{20} quassinoids in which a lactone ring has formed including C-14 and C-12 rather than C-14 and C-7.

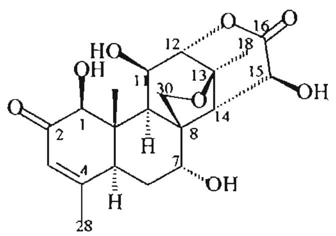

Samaderine X (**33**)


Samaderine Y (**34**)


Samaderine Z (**35**)


Indaquassin X (**36**)


Indaquassin B (**37**)

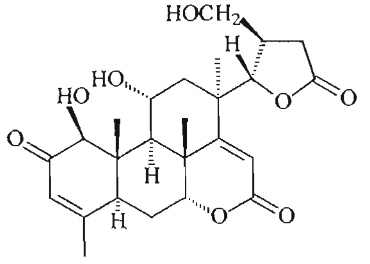

Cedronolactone D (**38**)


Quassia indica
(Photograph by G. E. Schatz)

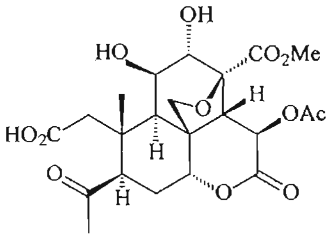

Simaba cedron
(http://www.nybg.org/bsci/res/bahia/Sim-cedr.html)

Soulameolide (**39**) was isolated from the trunk bark of the New Caledonian species *Soulamea tomentosa* Brogn. *et* Gris.[35] Bruceaketolic acid (**40**) was isolated from *Brucea javanica* (L.) Merr.[36]

Brucea javanica
(http://www.pharmanet.co.kr/dir/index5/555m29.htm)

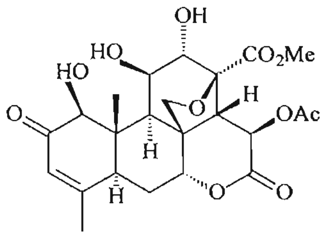


Soulameolide (39)

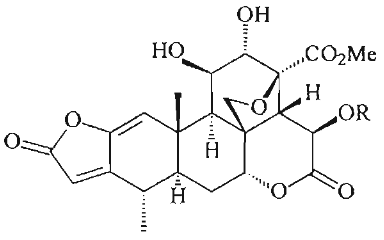


Bruceaketolic acid (**40**)

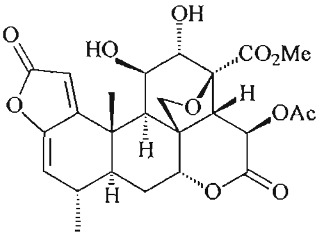
In 1982 isobrucein B (**41**) was isolated from the French Guianan species *Picrolemma pseudocoffea* Ducke.[15] *P. pseudocoffea* also yielded the 15-deacetyl analogue of sergeolide (**42**)[37] and neosergeolide (**43**), in which the butenolide ring is attached to the A ring at C-1, C-2.[38]



Isobrucein B (**41**)

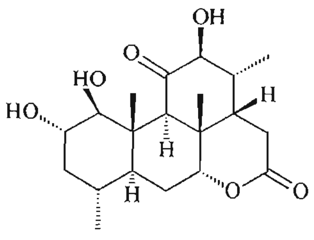


R = Ac Sergeolide (**44**)
R = H 15-Deacetyl sergeolide (**42**)

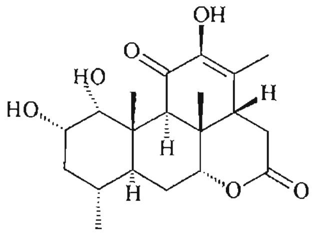


Neosergeolide (**43**)

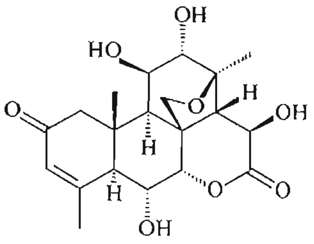
Chaparrolide (**45**) and castelanolide (**46**) were isolated from *Castela nicholsoni* Torr *et* A.Gray[39] and bruceine G (**47**) was isolated from *Brucea sumatrana* Roxb.[40]



Chaparrolide (**45**)

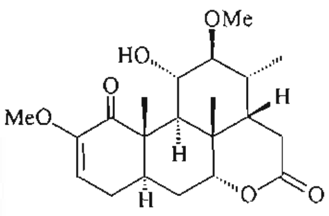


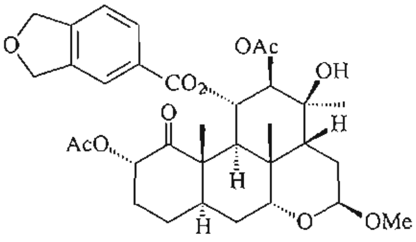
Castelanolide (**46**)

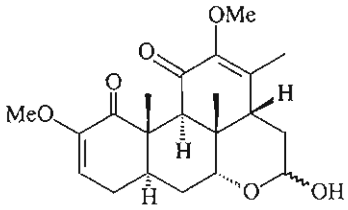


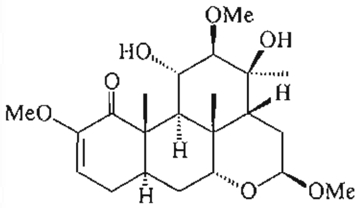
Bruceine G (**47**)

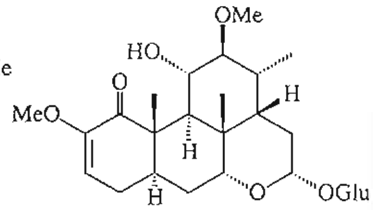
Picrasma javanica Blume yielded the javanicins A (**48**), B (**49**), C (**50**) and D (**51**) and the glycoside javanicinoside A (**52**).[41,42]


Javanicin A (**48**)

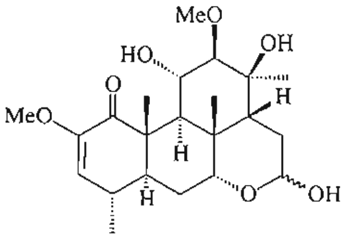

Javanicin D (**51**)

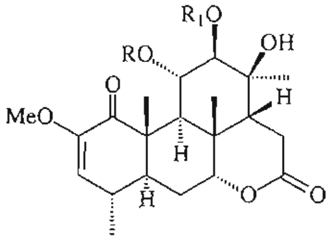

Javanicin B (**49**)

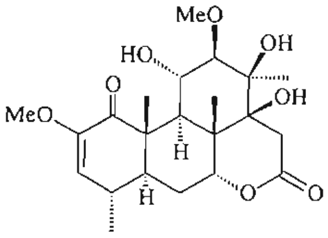

Javanicin C (**50**)

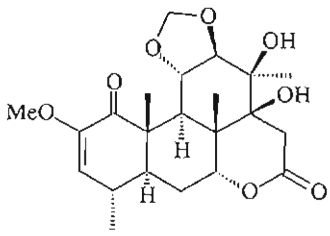

Javanicinoside A (**52**)

Nigakihemiacetal A (**53**)[43] and nigakilactones E (**54**), F (**55**) and H (**56**) (*Picrasma ailanthoides* Planchon)[44] and picrasin F (**57**) (*Picrasma quassioides* Bennet) all have the C-13 hydroxy group.


Nigakihemiacetal A (**53**)


R = Ac R$_1$ = Me Nigakilactone E (**54**)
R = H R$_1$ = Me Nigakilactone F (**55**)

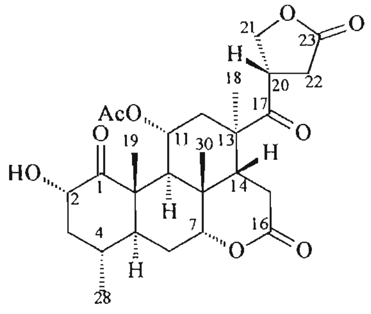

Nigakilactone H (**56**)


Picrasin F (**57**)

4.2.2. C$_{25}$ quassinoids

Simarolide (**58**), isolated from the bark of *Simarouba amara* Aubl.,[45] is a C$_{25}$ quassinoid similar to quassin (**6**), but has an additional five carbon atom sidechain containing a ketone at C-17 and a γ-lactone ring.

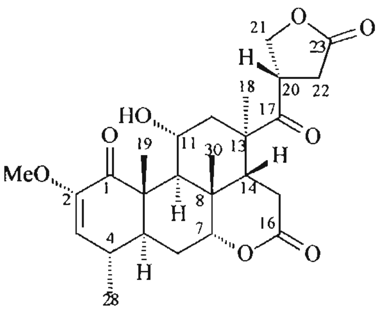
Simarouba amara
(Photograph by Harri Lorenzi www.plantarum.com.br)



Simarolide (**58**)

C_{25} quassinoids type (**5**) (page 39) differ from C_{25} quassinoids type (**4**) by the mode of formation of the γ-lactone.[1] In type (**4**) the γ-lactone is formed between C-21 and C-23 and C-17 is not involved. In type (**5**) C_{25} quassinoids, C-17 is involved in the γ-lactone and C-21 is external to the ring. In 1970 the second C_{25} quassinoid, picrasin A (**59**), was isolated from the wood of *Picrasma quassioides* Bennet.[46] The same compound was isolated from *Picrasma ailanthoides* Planchon[47] and was then named nigakilactone G.

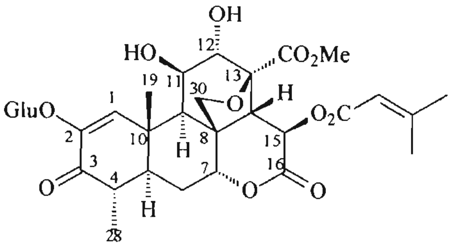
Picrasma quassiodes
(Photograph by Fa C. Esveld)



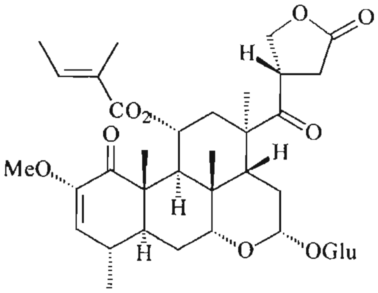
Picrasin A (**59**)

Picrasma ailanthoides
(NC State University Images by Erv Evans)

Bruceoside A (**60**) was the first quassinoid glycoside to be fully described.[17,48] The Indonesian species *Picrasma javanica* Blume[49] yielded the C_{25} quassinoid glucoside, javanicinoside E (**61**).

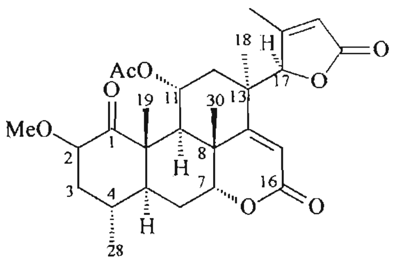


Bruceoside A (**60**)

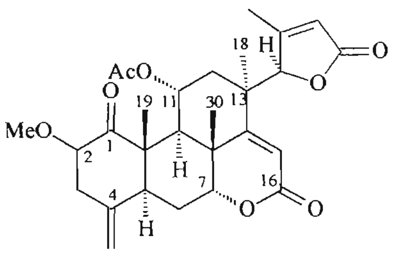


Javanicinoside E (**61**)

Odyendane (**62**) and odyendene (**63**), isolated in 1985 from the stem bark of *Odyendea gabonensis* Engler,[50] were the first type (**5**) C_{25} quassinoids with the *S* configuration at C-17 to be reported.

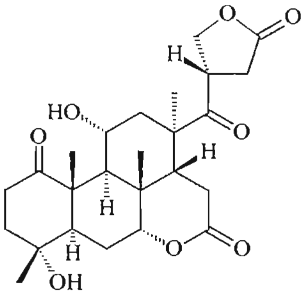


Odyendane (**62**)

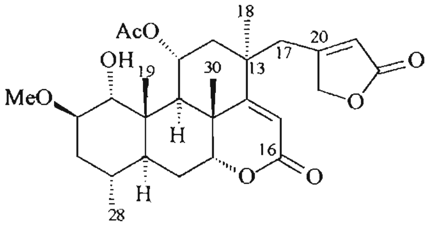


Odyendene (**63**)

Klaineanolide A (**64**), from the root bark of the Central African species *Hannoa klaineana* Pierre *et* Engler,[51] has the unique characteristic of an equatorially oriented hydroxy substituent at C-4. A novel C_{25} quassinoid, cedphiline (**65**), was isolated from *Cedrelopsis grevei* (Ptaeroxylaceae).[52] This is the first report of a quassinoid outside the Simaroubaceae family, which indicates a possible link between the two families.



Klaineanolide A (**64**)

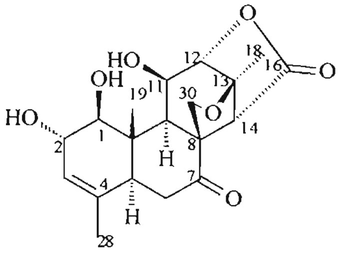


Cedphiline (**65**)

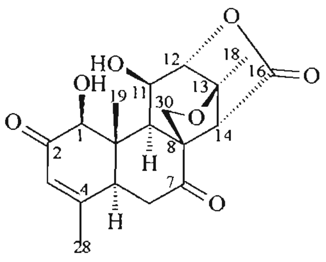
4.2.3. C_{19} quassinoids

The first quassinoids with a C_{19} skeleton to be isolated were samaderine C (**66**) and samaderine B (**67**) from *Samadera indica* Gaert.[53]

Samadera indica
(Photograph by Lani Stemmermann)



Samaderine C (**66**)

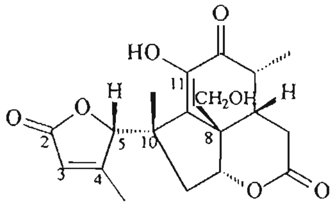


Samaderine B (**67**)

The C_{19} quassinoids such as (**66**) and (**67**) have an ether linkage between the C-8 hydroxymethyl group and C-13 rather than C-11, and the 7,14-δ-lactone has been replaced by a γ-lactone between C-14 and C-12.

Shinjulactone B (**68**) isolated from *Ailanthus altissima* Swingle[54] represents a further type of C_{19} quassinoid.

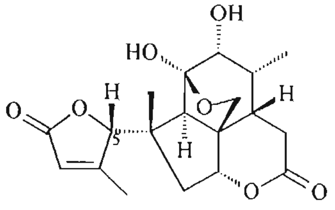
Ailanthus altissima
(Photograph by William S. Justice)



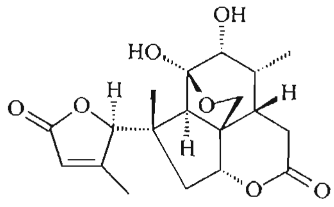
Sbinjulactone B (**68**)

Also in this group are ailanquassin A (**69**) from *Ailanthus malabarica* DC.[55] and cedronolactones B (**70**) and C (**71**) from *Simaba cedron* Planchon.

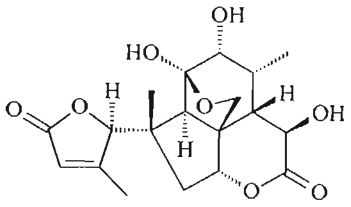
Ailanthus altissima
(Photographs by Gerald D. Carr)



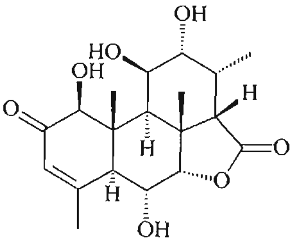
Ailanquassin A (**69**)



Cedronolactone B (**70**)



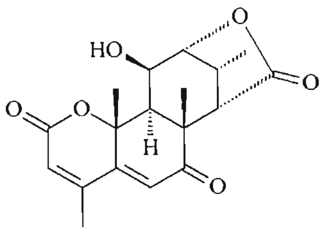
Cedronolactone C (**71**)



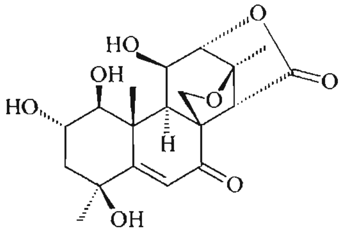
Longilactone (**72**)

Longlilactone (**72**)[56] and two related compounds[57,58] containing the 7,14-γ-lactone ring were isolated from *Eurycoma longifolia* Jack. Recently, *Eurycoma longifolia* roots yielded eurycolactone C (**73**).[59]

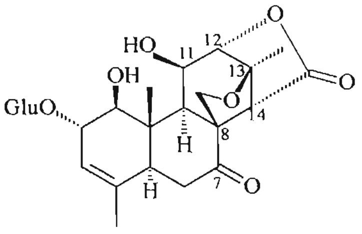
Quassia indica yielded indaquassin A (**74**),[33] the only C_{19} quassinoid to be reported with an axial 4β-hydroxy substituent, and 2-*O*-glucosylsamaderine C (**75**), the C_{19} quassinoid glycoside.[32]



Eurycolactone C (73)



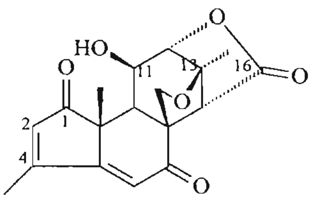
Indaquassin A (74)



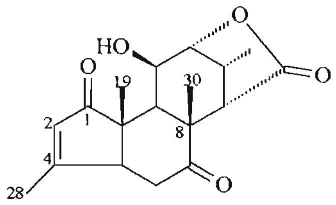
2-*O*-Glucosylsamaderine C (75)

4.2.4. C_{18} quassinoids

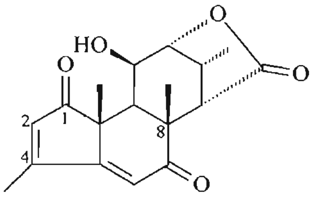
The C_{18} quassinoids (**3**) differ from C_{19} quassinoids (**2**) by the loss of one carbon atom from ring A.[60] Only five C_{18} quassinoids have been isolated. Samaderine A (**76**) was isolated first from *Samadera indica* Gaertn in 1962,[61] and its structure was established in 1978.[62] Laurycolactone A (**77**), and B (**78**) were isolated from *Eurycoma longifolia* Jack[63] (Vietnamese Simaroubaceae). Recently, *Eurycoma longifolia* roots yielded eurycolactone A (**79**) and B (**80**),[59] which are closely related to the quassinoids isolated in this work.



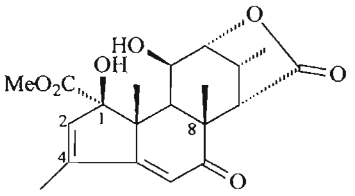
Samaderine A (76)



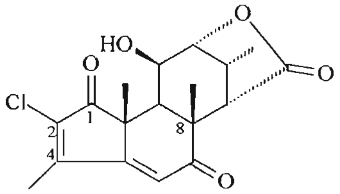
Laurycolactone A (77)



Laurycolactone B (78)



Eurycolactone A (79)



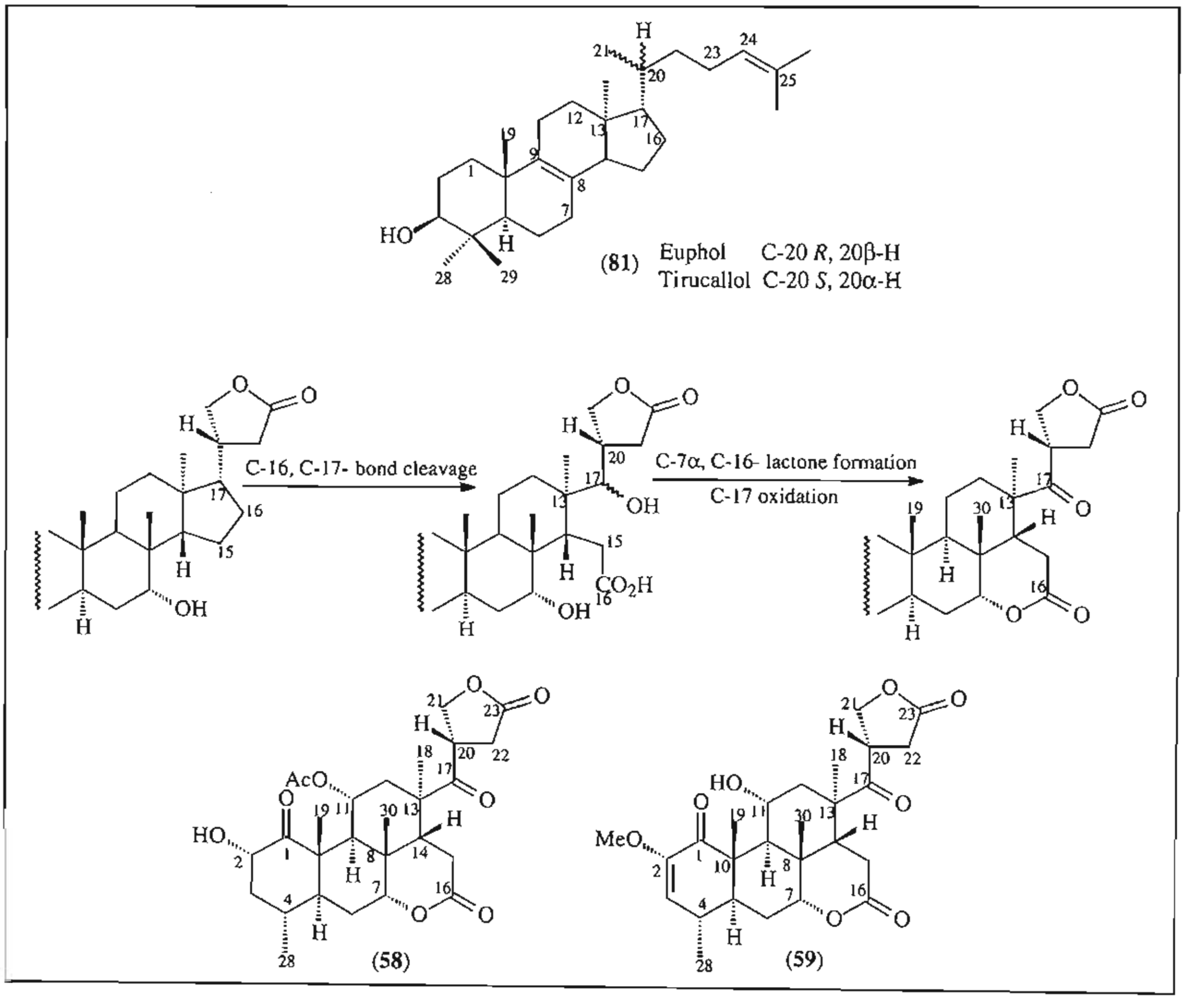
Eurycolactone B (80)

Eurycoma longifolia
(http://www.pasakbumi.com/trees.htm)

4.3. Biosynthesis

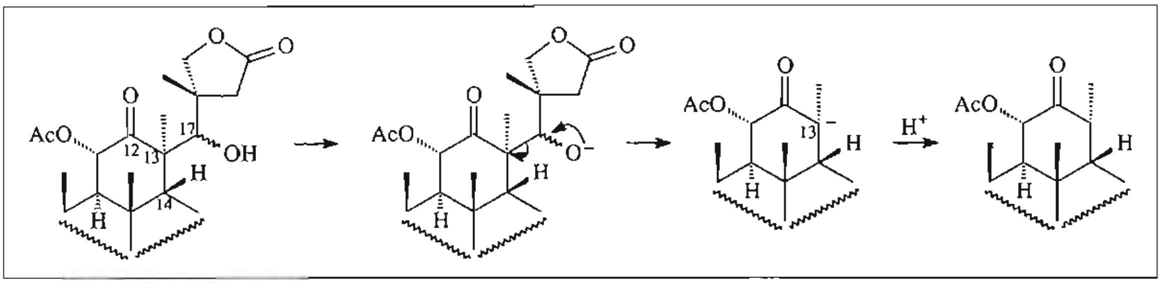
By 1964 it became apparent that quassinoids originated from triterpenoid precursors.[14,64,65] Quassinoids follow the same biosynthetic pathway as the protolimonoids discussed in chapter 3. The C_{25} quassinoids such as simarolide (**58**) and picrasin A (**59**) are proposed to arise from apo-euphol/tirucallol precursors (**81**). Since these quassinoids are *S* at C-20, it would appear to favour the tirucallol rather than the euphol origin.

The proposed biosynthesis of simarolide (**58**) and picrasin A (**59**) is shown in scheme 4.1.[65] : one methyl group at C-4 and four carbon atoms at the end of the side chain are removed and C-20 to C-23 are converted to a γ-lactone ring. The apo change is necessary to establish the C-8β methyl group. It is proposed that triterpenoids with this γ-lactone ring as the side chain, undergo oxidative ring D expansion and opening, and the C-16 carboxylic acid group forms a lactone ring with the 7α-hydroxy group. Oxidation of the C-17 hydroxy group then results in the basic C_{25} skeletons of simarolide (**58**) and picrasin A (**59**).



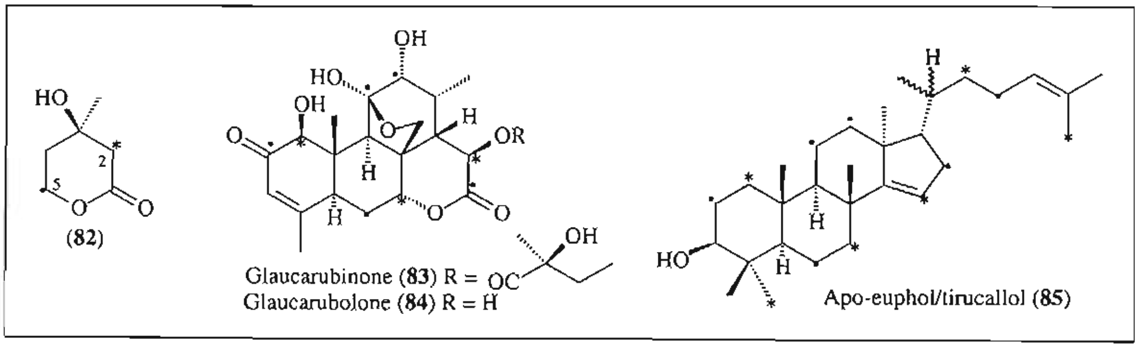
Scheme 4.1. Proposed formation of simarolide (58) and picrasin A (59) [14,64,65]

The C_{25} quassinoids are considered to be intermediates in the biosynthesis of the other groups, C_{20} and C_{19}, in which the C-13, C-17-bond is cleaved if the 12,17-dione is present or by a retro-aldol reaction of the 17-ol-12-one (scheme 4.2).[46] The loss of C-16 is also required to form C_{19} quassinoids.



Scheme 4.2. Biosynthesis of C_{20} quassinoids by a retro-aldol reaction of the 17-ol-12-one[46]

The proposed quassinoid biosynthetic pathway was experimentally verified using labelled mevalonate precursors.[66,67,68,69] Both the 2-^{14}C-(2*) and 5-^{14}C-(5$^{\bullet}$)-mevalonic acid lactone (MVA) **(82)** were incorporated into glaucarubinone **(83)** and glaucarubolone **(84)** *via* viable seeds of *Simarouba glauca*. The expected labelling patterns in the proposed apo-euphol/tirucallol triterpenoid precursor **(85)** and in glaucarubolone **(84)** are indicated respectively by the symbols* and $^{\bullet}$. Hydrolysis of the radioactive C-15 ester glaucarubinone, isolated from both 2-^{14}C and 5-^{14}C MVA incorporation, showed that the acid residue, derived from isoleucine, was inactive thereby suggesting that labelling only occurred in the C_{20} skeleton.[68]



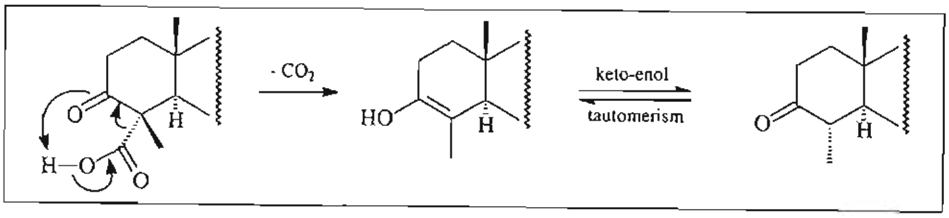
(82)

Glaucarubinone (83) R = OC

Glaucarubolone (84) R = H

Apo-euphol/tirucallol (85)

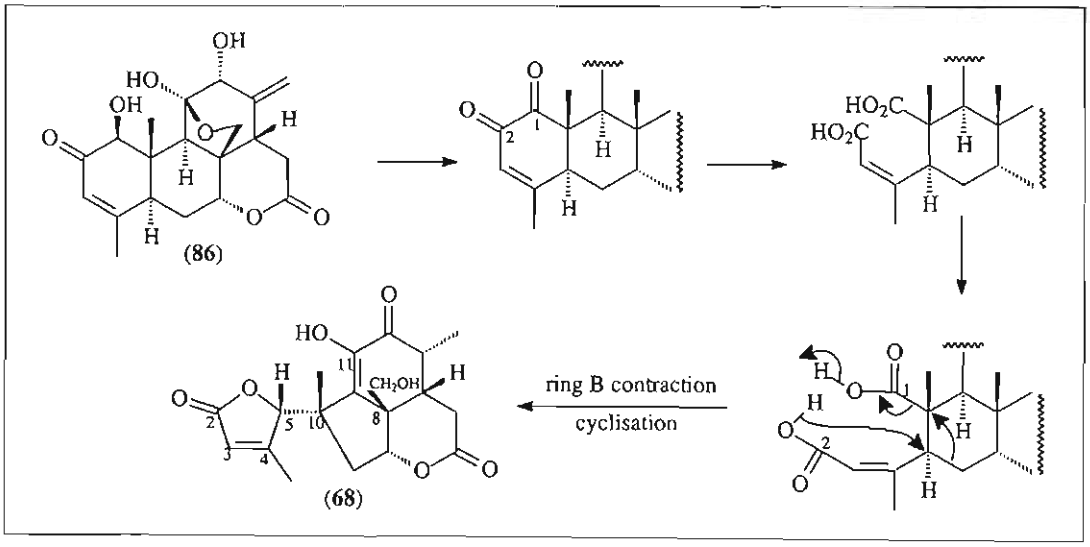
Kuhn-Roth oxidation of 2-^{14}C glaucarubolone only produced inactive acetic acid, establishing that the three methyl groups at C-4, C-10 and C-13 were unlabelled and that the methyl group at C-4 was derived from the methyl group of MVA, and thus from the β axial methyl group in the triterpenoid precursor.[65] It was suggested that the loss of a methyl group at C-4 arises from the formation of a 3-keto triterpenoid precursor common to all quassinoids followed by decarboxylation of the resulting β-keto-acid (scheme 4.3).[65] The six membered transition state required for this transformation is achieved more easily *via* a C-4 equatorial carboxyl residue than a C-4 axial carboxyl residue.



Scheme 4.3. Loss of C-4 equatorial methyl group by decarboxylation[65]

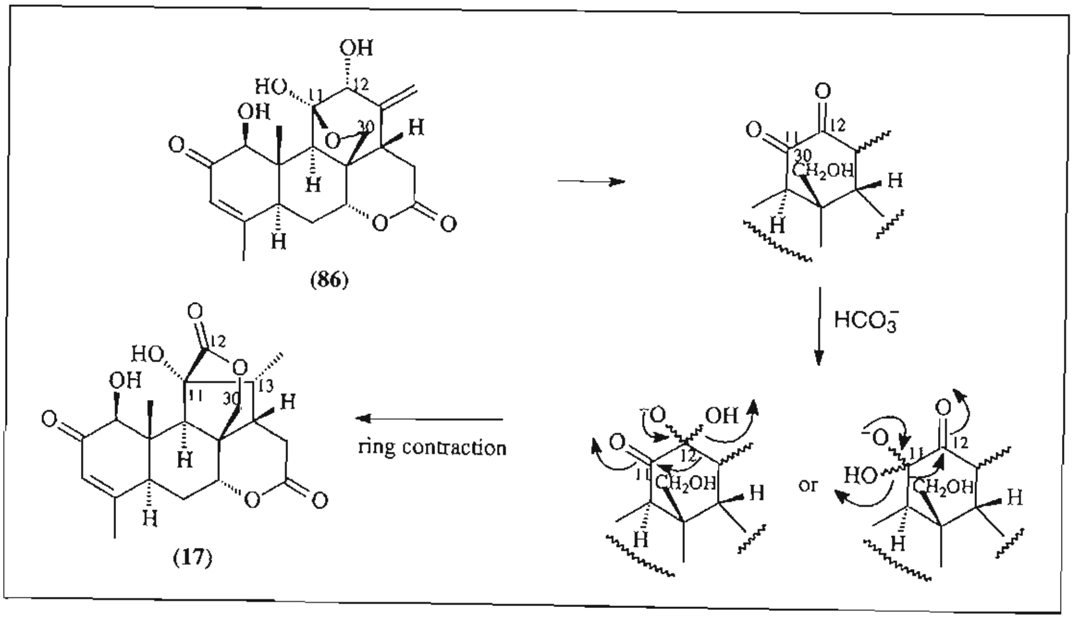
Further degradative studies showed that C-1 and C-15 were labelled by (2*), and C-12 and C-16 by (5*) MVA respectively, and the presence of five labelled carbon atoms in 5-^{14}C glaucarubolone proved the triterpenoidal origin of quassinoids.[65]

The unusual *R* configuration at C-5 in compounds such as shinjulactone B **(68)** arises from C-1, C-2-bond cleavage of the 1,2-diketo precursor, followed by nucleophilic attack at C-5, decarboxylation and ring B contraction (scheme 4.4).[54]



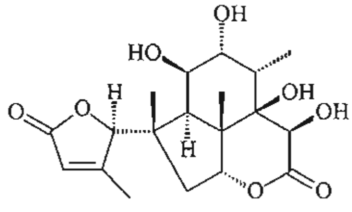
Scheme 4.4. Formation of the *R* configuration of the C_{19} quassinoid shinjulactone B (68)[54]

Ailanthone **(86)** may be converted into the C_{20} quassinoid shinjudilactone **(17)** by treatment of the intermediate diketone with $NaHCO_3$ (scheme 4.5).[25]

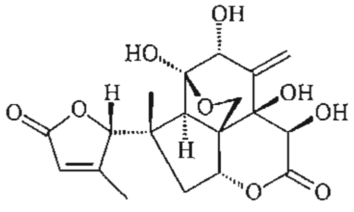


Scheme 4.5. Proposed mechanism for conversion of shinjudilactone (17) from ailanthone (86)[25]

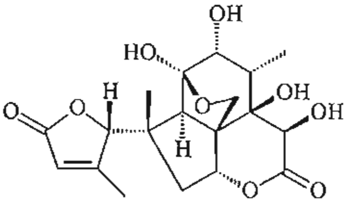
The C_{25} quassinoids, eurylactones A (**87**) and B (**88**) (*Eurycoma longifolia*),[70] polyandrol (**89**) and its C-5 epimers (**90**), (**91**) (*Castela polyandra*)[71,72] can only arise if the C-10 decarboxylation and C-5, C-10 bond migration steps occur first, producing a carbocation intermediate which can then undergo nucleophilic attack from either side to result in both the *R* and *S* configurations (Scheme 4.6).



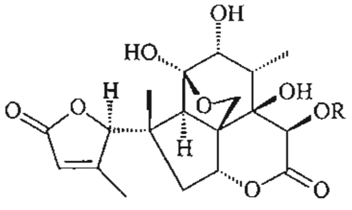
Eurylactone A (**87**)



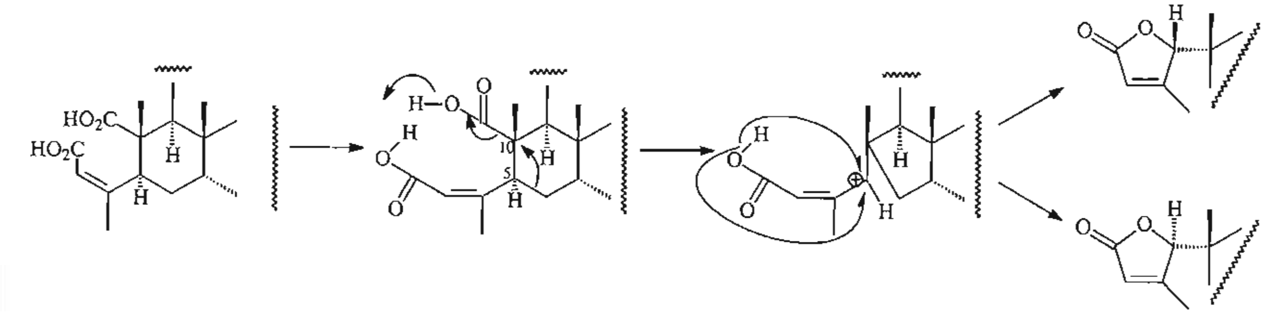
Eurylactone B (**88**)



Polyandrol (**89**)

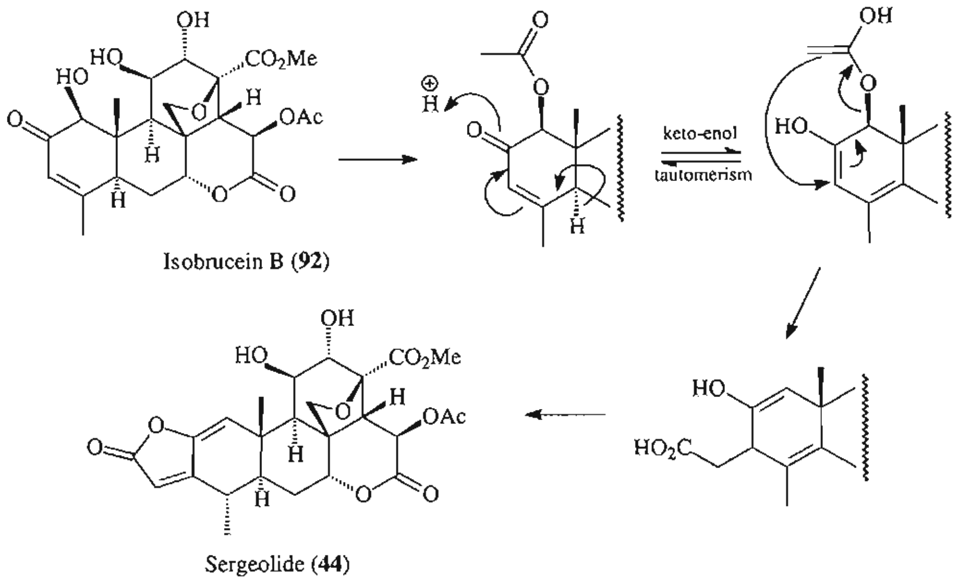


R = H (**90**)
R = Ac (**91**)



Scheme 4.6. Formation of both *R* and *S* configurations

Sergeolide (**44**) is a different type of C_{20} quassinoid where a lactone ring is attached to ring A. Sergeolide (**44**) can be formed *via* a "Claisen-type" rearrangement, double bond migration and lactonisation (Scheme 4.7).[73]



Isobrucein B (**92**)

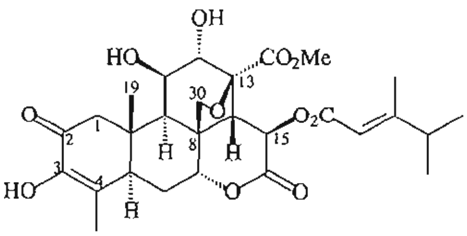
Sergeolide (**44**)

Scheme 4.7. Formation of sergeolide (44) by "Claisen-like" rearrangement.[73]

4.4. Biological activity

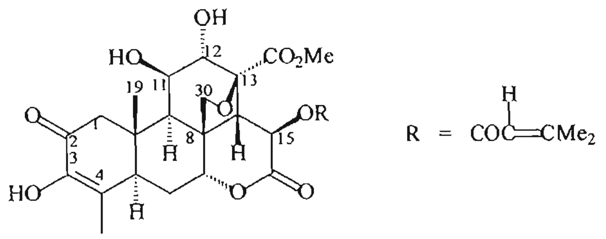
Quassinoids have a wide range of biological activities such as antileukemic, antiviral, antimalarial, anti-inflammatory, antifeedant and amoebicidal activities.

Certain quassinoids display *in vivo* antileukemic activity. Bruceantin (**93**) is one such compound which is active against the P-388 lymphocytic leukemia cell line.[1] It shows activity over a wide dose range and is active against solid tumours.[1,15,74,75,76]

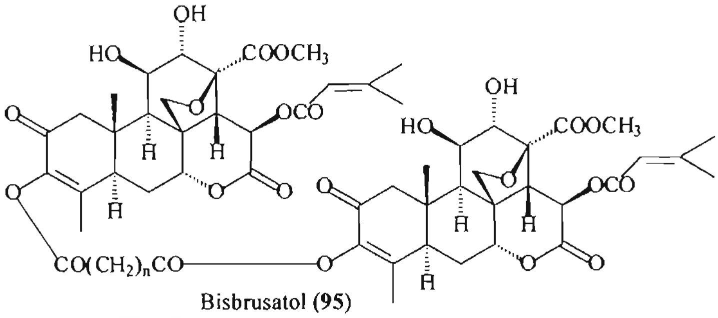


Bruceantin (**93**)

The esters of brusatol (**94**), bisbrusatol (**95**) and bruceantin (**93**) have the ability to suppress DNA and protein synthesis in P-388 lymphocytic leukemia cells. These active compounds were also shown to inhibit DNA polymerase activity and purine synthesis.[77]



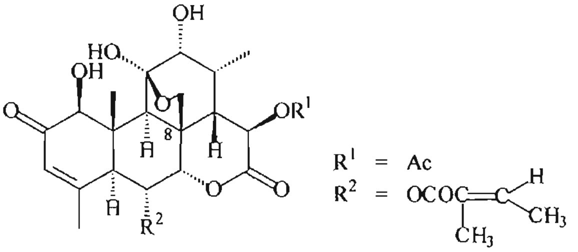
Brusatol (**94**)



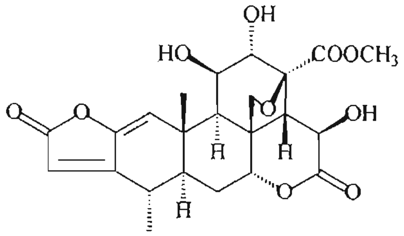
Bisbrusatol (**95**)

Quassinoids which possess the α, β-unsaturated ketol group at position 1 and 2, or a diosphenol group at position 2 and 3 in ring A, such as bruceantin (**93**), display antineoplastic activity in the NIH murine lymphocytic leukemia P-388 system.[78]

Undulatone (**96**)[79] is another cytotoxic quassinoid isolated from *Hannoa undulata*. 15-Deacetylsergeolide (**97**), isolated from the leaf extract of *Picrolemma pseudocoffea*,[37] displays potent antileukemic activity.

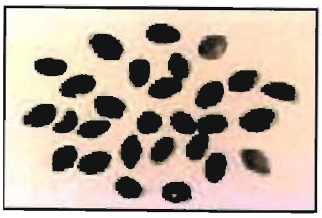


Undulatone (**96**)



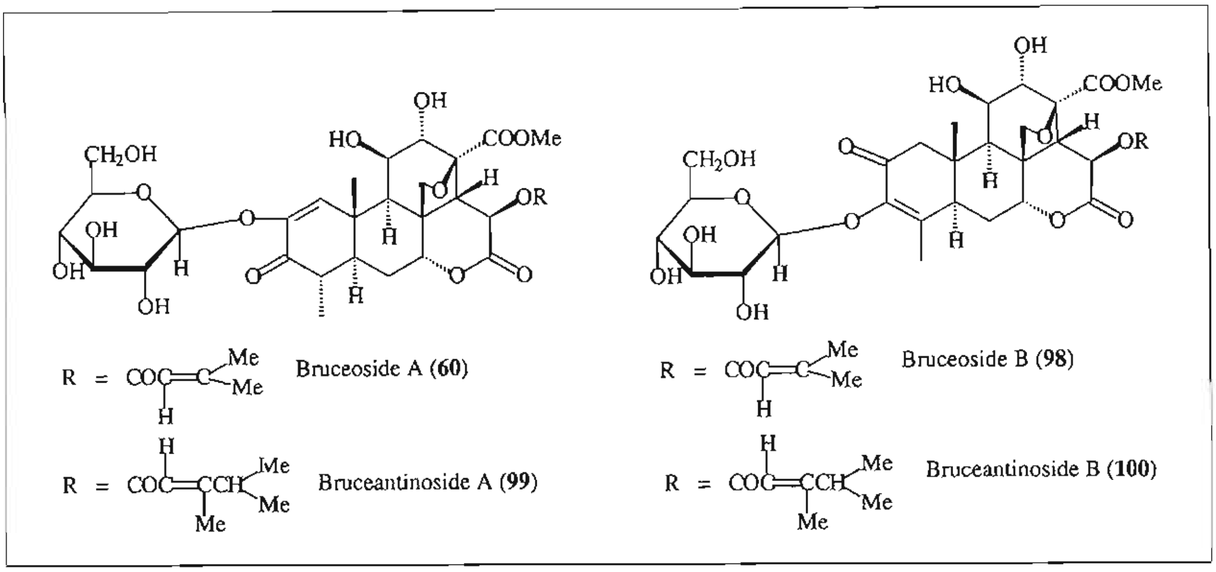
15-Deacetylsergeolide (**97**)

The quassinoid glucosides, bruceoside A (**60**) and bruceoside B (**98**) from *Brucea javanica* seeds,[17] and bruceantinoside A (**99**) and bruceantinoside B (**100**) from *Brucea antidysenterica* wood display significant antileukemic activity.[19]



***Brucea javanica* seeds**
http://www.pharmanet.co.kr/dir/index5/555m29.htm

Brucea antidysenterica
http://mailbox.univie.ac.at/Christian.Puff/Simon2001_Report_Plates.htm



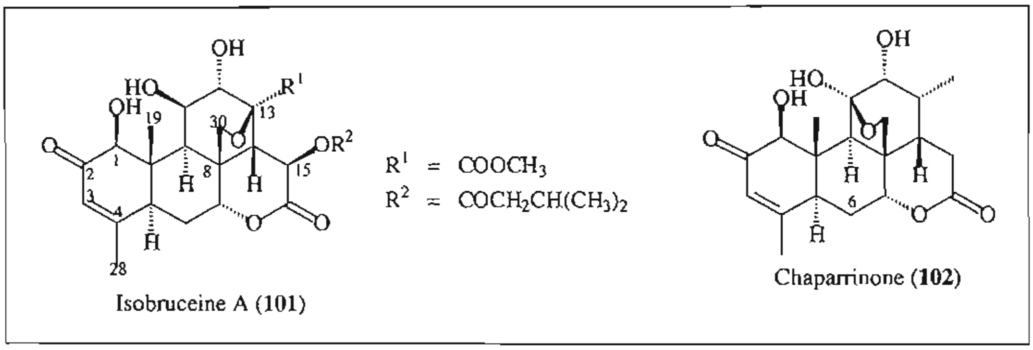
Bruceoside A (**60**)

Bruceoside B (**98**)

Bruceantinoside A (**99**)

Bruceantinoside B (**100**)

Certain quassinoids such as isobruceine A (**101**) and chaparrinone (**102**) display *in vitro* antiviral activity, for example, against the oncogenic virus *Rous sarcoma*.[1]



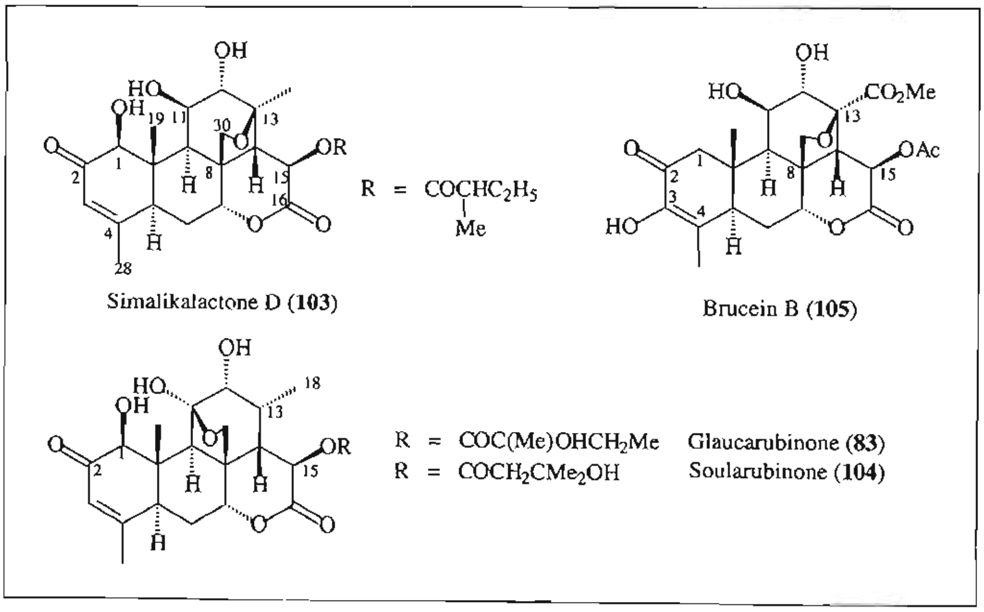
$R^1 = COOCH_3$

$R^2 = COCH_2CH(CH_3)_2$

Isobruceine A (101)

Chaparrinone (**102**)

Certain quassinoids display *in vitro* antimalarial activity.[80] Simalikalactone D (**103**) is the most active compound. Glaucarubinone (**83**) and soularubinone (**104**) are also effective.



$R = COCH(Me)C_2H_5$

Simalikalactone D (**103**)

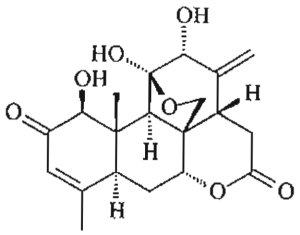
Brucein B (**105**)

$R = COC(Me)OHCH_2Me$ Glaucarubinone (**83**)

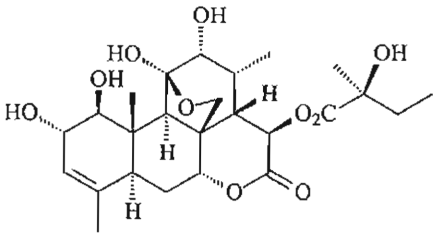
$R = COCH_2CMe_2OH$ Soularubinone (**104**)

Simalikalactone D (**103**) is a potent insect antifeedant.[1] Glaucarubinone (**83**) and brucein B (**105**) show insecticidal properties.[1]

Extracts of species such as *Castela nicholsoni* and *Chaparro amargoso* (castamargina) have been used in Mexico and China to treat fevers, dysentery and amoebiasis. Ailanthone (**86**)[1] and glaucarubin (**8**)[1] were shown to be effective amoebicides.

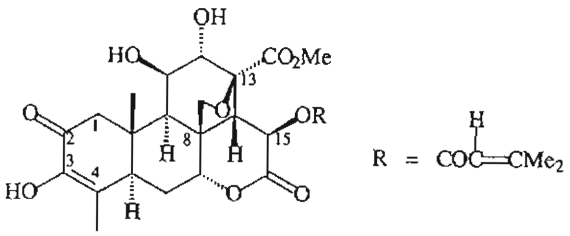


Ailanthone (**86**)



Glaucarubin (**8**)

Brusatol (**94**) and related compounds were found to be potent inhibitors of induced inflammation and arthritis in rodents.[81] The action of these anti-inflammatory quassinoids is to stabilise lysosomal membranes, thereby reducing the release of hydrolytic enzymes that cause damage to the surrounding tissues.[81]



Brusatol (**94**)

The amaroid drug consisting of quassinoids from *Quassia amara* L. is used to increase the appetite and aid in digestion.[2]

Research in this field continues in the hope of finding more quassinoids of biological importance.

4.5. References

1. Polonsky J., Quassinoid Bitter Principles II, *Progress in the Chemistry of Organic Natural Products*, 1985, **47**, 221-264.
2. http://www.raintree-health.co.uk/plants/amargo.html (23/11/2004)
3. http://www.egregore.com/herbs/BitterWood.htm (2003)
4. http://www.nutraceuticalalliance.com/h120.htm (2003)
5. http://www.news24.co.za/News24/Offbeat/Weird/0,4190,2-16-140_1054039,00.html (2003)
6. http://www.ocnsignal.com/yangtextq.shtml (2003)
7. http://www.tropilab.com/quassia-ama.html (23/11/2004)
8. http://electrocomm.tripod.com/quassia.html (23/11/2004)
9. http://www.tinasherbs.com/hlistiq.htm (2003)
10. London, E., Robertson, A. and Worthington, H., Quassin and neoquassin I, *J. Chem. Soc.*, 1950, 3431.
11. Valenta, Z., Papadopoulos, S. and Podešva, C., Quassin and neoquassin, *Tetrahedron*, 1961, **15**, 100-110.
12. Valenta, Z., Gray, A.H., Orr, D.E., Papadopoulos, S. and Podešva, C., The stereochemistry of quassin, *Tetrahedron*, 1962, **18**, 1433-1441.
13. Ham, E.A., Schafer, H.M., Denkewalter, R.C. and Brink, N.G., Structural studies on glaucarubin from *Simarouba glauca*, *J. Am. Chem. Soc.*, 1954, **76**, 6066-6068.
14. Polonsky, J., Fouquey, C.L. and Gaudemer, A., Etude de la glaucarubine II. – Structure du glaucarubol et de la glaucarubine, *Bull. Soc. Chim. France*, 1964, 1827. In Polonsky, J., Quassinoid Bitter Principles, *Progress in the Chemistry of Organic Natural Products*, 1973, **30**, 101-150.
15. Kupchan, S.M., Britton, R.W., Lacadie, J.A., Ziegler, M.F. and Sigel, C.W., The isolation and structural elucidation of bruceantin and bruceantinol, new potent antileukemic quassinoids from *Brucea antidysenterica*, *J. Org. Chem.*, 1975, **40**, 648-654.
16. Toyota, T., Fukamiya, N., Okano, M., Tagahara, K., Chang, J.-J. and Lee, K.-H., Antitumour agents, 118. The isolation and characterization of bruceanic acid A, its methyl ester, and the new bruceanic acids B, C, and D, from *Brucea antidysenterica*, *J. Nat. Prod.*, 1990, **53**, 1526-1532.
17. Lee, K.H., Imakura, Y., Sumida, Y., Wu, R.Y., Hall, J.H. and Huang, H.-C., Antitumour agents. 33. Isolation and structural elucidation of bruceoside-A and -B, novel antileukemic quassinoid glycosides, and brucein-D and -E from *Brucea javanica*, *J. Org. Chem.*, 1979, **44**, 2180-2185.
18. Li, X., and Tso, S.-H., Studies on the anticancer principles in the seed of Yadanzi (*Brucea javanica*). III. Isolation and structural elucidation of bruceoside A, bruceoside B, bruceine E, and bruceine F, *Chung Ts'ao Yao*, 1980, **11**, 530-532, C.A., 1981, **95**, 280c.
19. Okano, M., Lee, K.H., Hall, I.H. and Boettner, F.E., Antitumour agents. 39. Bruceantinoside-A and -B, novel antileukemic quassinoid glucosides from *Brucea antidysenterica*, *J. Nat. Prod.*, 1981, **44**, 470-474.

20. Sakaki, T., Yoshimura, S., Ishibashi, M., Tsuyuki, T., Takahashi, T., Honda, T. and Nakanishi, T., New quassinoid glycosides, yadanziosides A-H, from *Brucea javanica*, *Chem. Pharm. Bull.*, 1984, **32**, 4702-4705.

21. Sakaki, T., Yoshimura, S., Ishibashi, M., Tsuyuki, T., Takahashi, T., Honda, T. and Nakanishi, T., Structures of new quassinoid glycosides, yadanziosides A, B, C, D, E, G, H, and new quassinoids, dehydrobrusatol and dehydrobruceantinol from *Brucea javanica* (L.) Merr, *Bull. Chem. Soc. Jpn.*, 1985, **58**, 2680-2686.

22. Sakaki, T., Yoshimura, S., Tsuyuki, T., Takahashi, T., Honda, T. and Nakanishi, T., Structures of yadanziosides K, M, N, and O, new quassinoid glycosides from *Brucea javanica* (L.) Merr, *Bull. Chem. Soc. Jpn.*, 1986, **59**, 3541-3546.

23. Yoshimura, S., Sakaki, T., Ishibashi, M., Tsuyuki, T., Takahashi, T. and Honda, T., Constituents of seeds of *Brucea javanica*. Structures of new bitter principles, yadanziolides A, B, C, yadanziosides F, I, J, and L, *Bull. Chem. Soc. Jpn.*, 1985, **58**, 2673-2679.

24. Ishibashi, M., Tsuyuki, T., Murae, T., Hirota, H., Takahashi, T., Itai, A. and Iitaka, Y., Constituents of the root bark of *Ailanthus altissima* Swingle. Isolation and x-ray crystal structures of shinjudilactone and shinjulactone C and conversion of ailanthone into shinjudilactone, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 3683-3693.

25. Ishibashi, M., Murae, T., Hirota, H., Naora, H., Tsuyuki, T., Takahashi, T., Itai, A. and Iitaka, Y., Shinjudilactone, a new bitter principle from *Ailanthus altissima* Swingle, *Chem. Letters*, 1981, 1597-1598.

26. Takeya, K., Kobata, H., Ozeki, A., Morita, H. and Itokawa, H., A new quassinoid from *Ailanthus vilmoriniana*, *J. Nat. Prod.*, 1997, **60**, 642-644.

27. Polonsky, J., Varon, Z., Prangé, T., Pascard, C. and Moretti, C., Structures of simarinolide and guanepolide (X-Ray Analysis), new quassinoids from *Simaba* cf *Orinocensis*, *Tetrahedron Letters*, 1981, **22**, 3605-3608.

28. Polonsky, J., Gallas, J., Varenne, J., Prangé, T., Pascard, C., Jacquemin, H. and Moretti, C., Isolation and structure (x-ray analysis) of karinolide, a new quassinoid from *Simaba multiflora*, *Tetrahedron Letters*, 1982, **23**, 869-872.

29. Ishibashi, M., Yoshimura, S., Tsuyuki, T., Takahashi, T., Itai, A. and Iitaka, Y., Structure determination of bitter principles of *Ailanthus altissima*. Structures of shinjulactones F, I, J, and K, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 2885-2892.

30. Ishibashi, M., Yoshimura, S., Tsuyuki, T., Takahashi, T. and Matsushita, K., Shinjulactones G and H, new bitter principles of *Ailanthus altissima* Swingle, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 2013-2014.

31. Takeya, K., Kobata, H., Ozeki, A., Morita, H. and Itokawa, H., Quassinoids from *Ailanthus vilmoriniana*, *Phytochemistry*, 1998, **48**, 565-568.

32. Kitagawa, I., Mahmud, T., Yokota, K., Nakagawa, S., Mayumi, T., Kobayashi, M. and Shibuya, H., Indonesian Medicinal Plants XVII. Characterization of quassinoids from the stems of *Quassia indica*, *Chem. Pharm. Bull.*, 1996, **44**, 2009-2014.

33. Koike, K. and Ohmoto, T., Indaquassins A and B, two novel quassinoids from the bark of *Quassia indica* (0.6kg), *Phytochemistry*, 1993, **34**, 505-509.

34. Ozeki, A., Hitotsuyanagi, Y., Hashimoto, E., Itokawa, H., Takeya, K. and Alves, S. de M., Cytotoxic quassinoids from *Simaba cedron*, *J. Nat. Prod.*, 1998, **61**, 776-780.

35. Polonsky, J., Tri, M.V., Prangé, T., Pascard, C. and Sevenet, T., Isolation and structure (x-ray analysis) of a new C_{25} quassinoid soulameolide from *Soulamea tomentosa*, *J. Chem. Soc. Chem. Comm.*, 1979, 641-642.

36. Lin, L., Zhang, J., Chen, Z., Xu, R., *Huaxue Xuebao*, 1982, **40**, 73-78.

37. Polonsky, J., Bhatnagar, S. and Moretti, C., 15-deacetylsergeolide, a potent antileukemic quassinoid from *Picrolemma pseudocoffea*, *J. Nat. Prod.*, 1984, **47**, 994-996.

38. Zukerman-Schpector, J. and Castellano, E.E., A new quassinoid isolated from *Picrolemma pseudocoffea*, *Acta Cryst.*, 1994, C**50**, 794-797.

39. Mitchell, R.E., Stöcklin, W., Stefanović, M. and Geissman, T.A., Chaparrolide and castelanolide, new bitter principles from *Castela nicholsoni*, *Phytochemistry*, 1971, **10**, 411-417.

40. Duncan, G.R. and Henderson, D.B., Bruceines from *Brucea sumatrana*: The structure of bruceine G, *Experientia*, 1968, **24**, 768-769.

41. Ohmoto, K., Koike, K., Mitsunaga, K., Fukuda, H. and Kagei, K., Studies on the constituents of Indonesian *Picrasma javanica*. III. Structures of new quassinoids, Javanicins A, C and D, *Chem. Pharm. Bull.*, 1989, **37**, 2991-2994.

42. Koike, K. and Ohmoto, T., Quassinoids from *Picrasma javanica*, *Phytochemistry*, 1990, **29**, 2617-2621.

43. Murae, T., Sugie, A., Moriyama, Y., Tsuyuki, T., Takahashi, T., Mass spectra of the bitter principles from *Picrasma ailanthoides* Planchon, *Organic Mass Spectrometry*, 1974, **8**, 297-301.

44. Daido, M., Fukamiya, N., Okano, M., Picrasinol C, A new quassinoid, and its related compounds from the stem wood of *Picrasma ailanthoides*, *J. Nat. Prod.*,1992, **55**, 1643-1647.

45. Polonsky, J., The structure of simarolide, the bitter principle of *Simarouba amara*, *Proc. Chem. Soc.*, 1964, 292-293.

46. Hikino, H., Ohta, T. and Takemoto, T., Picrasins, simaroubolides of Japanese *Quassia* tree *Picrasma quassioides*, *Phytochemistry*, 1975, **14**, 2473-2481.

47. Murae, T., Tsuyuki, T., Ikeda, T., Nishihama, T., Masuda, S., Takahashi, T., Bitter principles of *Picrasma ailanthoides* Planchon nigakihemiacetals A, B and C, and nigakilactones G, H and I, *Tetrahedron*, 1971, **27**, 5147-5155.

48. Lee, K-H., Imakura, Y. and Huang, H-C., Bruceoside-A, a novel antileukaemic quassinoid glycoside from *Brucea javanica*, *J. Chem. Soc. Chem. Comm.*, 1977, 69-70.

49. Ishi, K., Koike, K. and Ohmoto, T., Javanicinosides D-H, quassinoid glucosides from *Picrasma javanica*, *Phytochemistry*, 1991, **30**, 4099-4103.

50. Forgacs, P., Provost, J., Touche, A., Guenard, D., Thal, C. and Guilhem, J., Structures de l' odyendane deux nouveaux quassinoides d'*odyendea gabonensis* (Pierre) Engl. Simaroubacees, *Tetrahedron Letters*, 1985, **26**, 3457-3460.

51. Vanhaelen-Fastré, R., Luyengi, L., Vanhaelen, M., Declercq, J.P. and Van Meerssche, M., Two quassinoids and two coumarinolignoids from *Hannoa klaineana* roots, *Phytochemistry*, 1987, **26**, 317-319.

52. Mulholland, D.A., Naidoo, D., Randrianarivelojosia, M., Cheplogoi, P.K. and Coombes, P.H., "Secondary metabolites from *Cedrelopsis grevei* (Ptaeroxylaceae)", *Phytochemistry*, 2003, **64**, 631-635.

53. Zylber, J. and Polonsky, J., Constituents of *Samadera indica* bark. Structure of samaderine B and of samaderine C, *Bull. Soc. Chim. France*, 1964, 2016-2019.

54. Furono, T., Ishibashi, M., Naora, H., Murae, T., Hirota, H., Tsuyuki, T., Takahashi, T., Itai, A. and Iitaka, Y., Structure determination of bitter principles of *Ailanthus altissima*. Structures of shinjulactones B, D, and E, *Bull. Chem. Soc. Jpn.*, 1984, **57**, 2484-2489.

55. Aono, H., Koike, K., Kaneko, J. and Ohmoto, T., Alkaloids and quassinoids from *Ailanthus malabarica*, *Phytochemistry*, 1994, **37**, 579-584.

56. Morita, H., Kishi, E., Takeya, K., Itokawa, H. and Tanaka, O., New quassinoids from the roots of *Eurycoma longifolia*, *Chemistry Letters*, 1990, 749-752.

57. Itokawa, H., Qin, X-R., Morita, H., Takeya, K. and Iitaka, Y., Novel quassinoids from *Eurycoma longifolia*, *Chem. Pharm. Bull.*, 1993, **41**, 403-405.

58. Morita, H., Kishi, E., Takeya, K., Itokawa, H. and Iitaka, Y., Highly oxygenated quassinoids from *Eurycoma longifolia*, *Phytochemistry*, 1993, **33**, 691-696.

59. Ang, H.H., Hitotsuyanagi, Y., Takeya, K., Eurycolactones A-C, novel quassinoids from *Eurycoma longifolia*, *Tetrahedron Letters*, 2000, **41**, 6849-6853.

60. Gibbons, S., Craven, L., Dunlop, C., Gray, A.I., Hartley, T.G. and Waterman, P.G., The secondary metabolites of aff. *Samadera* SAC-2825: An Australian Simaroubaceae with unusual chemistry, *Phytochemistry*, 1997, **44**, 1109-1114.

61. Polonsky, J., Zylber, J. and Wijesekera, R.O.B., Constituents of the bark of *samadera indica*, *Bull. Soc. Chim. France*, 1962, 1715-1721.

62. Onan, K.D. and McPhail, A.T., Crystal and molecular structure of samaderine A, a C_{18} quassinoid from *Samadera indica*, *J. Chem. Res. (S)*, 1978, 14, *(M)*, 0401-0431.

63. Nguyên-Ngoc-Suong, Bhatnagar, S., Polonsky, J., Vuilhorgne, M., Prangé, T. and Pascard, C., Structure of laurycolactone A and B, new C_{18}-quassinoids from *Eurycoma longifolia* and revised structure of eurycomalactone (x-ray analysis), *Tetrahedron Letters*, 1982, **23**, 5159-5162.

64. Connolly, J.D., Overton, K.H. and Polonsky, J., The chemistry and biochemistry of the limonoids and quassinoids, *Progress in Phytochemistry*, 1970, **2**, 385-455.

65. Polonsky, J., Quassinoid Bitter Principles, *Progress in the Chemistry of Organic Natural Products*, 1973, **30**, 101-150.

66. Moron, J., Rondest, J. and Polonsky, J., Sur la biosynthèse des constituants amers des Simarubacées, *Experientia*, 1966, **22**, 511. In Polonsky, J., Quassinoid Bitter Principles, *Progress in the Chemistry of Organic Natural Products*, 1973, **30**, 101-150.

67. Moron, J. and Polonsky, J., Sur l'origine triterpénique des constituents amers des Simarubacées, *Tetrahedron Letters*, 1968, 385. In Polonsky, J., Quassinoid Bitter Principles, *Progress in the Chemistry of Organic Natural Products*, 1973, **30**, 101-150.

68. Moron, J. and Polonsky, J., Incorporation spécifique de la L-[U-^{14}C] isoleucine dans le groupement hydroxy-2-methyl-2-butyroyle de la glaucarubinone, *European J. Biochem.*, 1968, **3**, 488. In Polonsky, J., Quassinoid Bitter Principles, *Progress in the Chemistry of Organic Natural Products*, 1973, **30**, 101-150.

69. Moron, J., Merrien, M.-A. and Polonsky, J., Sur la biosynthèse des quassinoides de *Simaruba glauca* (Simarubaceae), *Phytochemistry*, 1971, **10**, 585-592.

70. Itokawa, H., Qin, X.-R., Morita, H. and Takeya, K., C_{18} and C_{19} quassinoids from *Eurycoma longifolia*, *J. Nat. Prod.*, 1993, **56**, 1766-1771.

71. Grieco, P.A., VanderRoest, J.M., Piñeiro-Nuñez, M.M., Campaigne, E.E. and Carmack, M., Polyandrol, a C_{19} quassinoid from *Castela polyandra*, *Phytochemistry*, 1995, **38**, 1463-1465.

72. Grieco, P.A., Haddad, J., Piñeiro-Nuñez, M.M., and Huffman, J.C., Quassinoids from the twigs and thorns of *Castela polyandra*, *Phytochemistry*, 1999, **50**, 637-645.

73. Moretti, C., Polonsky, J., Vuilhorgne, M. and Prangé, T., Isolation and structure of sergeolide, a potent cytotoxic quassinoid from *Picrolemma pseudocoffea*, *Tetrahedron Letters*, 1982, **23**, 647-650.

74. Bedikian, A.Y., Valdivieso, M., Bodey, G.P., Murphy, W.K. and Freireich, E.J., Initial clinical studies with bruceantin, *Cancer Treat. Rep.*, 1979, **63**, 1843. In Polonsky J., Quassinoid Bitter Principles II, *Progress in the Chemistry of Organic Natural Products*, 1985, **47**, 221-264.

75. Fong, K.L.L., Ho, D.H.W., Benjamin, R.S., Brown, N.S., Bedikian, A.Y., Yap, B.S., Wiseman, C.L., Kramer, W. and Bodey, G.P., Clinical pharmacology of bruceantin by radioimmunoassay, *Pharmacol.*, 1982, **9**, 169. In Polonsky J., Quassinoid Bitter Principles II, *Progress in the Chemistry of Organic Natural Products*, 1985, **47**, 221-264.

76. Liesmann, J., Belt, R.J., Haas, C.D. and Hoogstraten, B., Phase I study on bruceantin administered on a weekly schedule, *Cancer Treat. Rep.*, 1981, 65, 883. In Polonsky J., Quassinoid Bitter Principles II, *Progress in the Chemistry of Organic Natural Products*, 1985, **47**, 221-264.

77. Hall, I.H., Liou, Y.F., Okano, M. and Lee, K.H., Antitumour agents XLVI: *In Vitro* effects of esters of brusatol, bisbrusatol, and related compounds on nucleic acid and protein synthesis of P-388 lymphocytic leukemia cells, *J. Pharm. Sci.*, 1982, **71**, 345-348.

78. Wall, M.E. and Wani, M.C., Plant antitumor agents. 17. Structural requirements for antineoplastic activity in quassinoids, *J. Med. Chem.*, 1978, **21**, 1186.

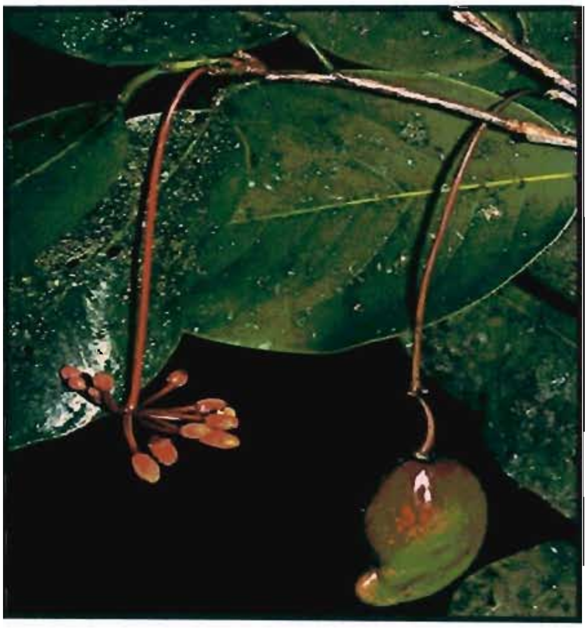
79. Wani, M.C., Taylor, H.L., Thompson, J.B., Wall, M.E., McPhail, A.T. and Onan, K.D., Plant antitumour agents. XV. Isolation and x-ray crystal structure of a new antileukaemic quassinoid undulatone from *Hannoa undulata*, *Tetrahedron*, 1979, **35**, 17-24.

80. Trager, W. and Polonsky, J., Antimalarial activity of quassinoids against chloroquine-resistant *Plasmodium falciparum in vitro*, *Am. J. Trop. Med. Hyg.*, 1981, **30**, 531-537.

81. Hall, I.H., Lee, K.H., Imakura, Y., Okano, M. and Johnson, A., Anti-inflammatory agents III: structure-activity relationships of brusatol and related quassinoids. *J. Pharm. Sci.*, 1983, **72**, 1282-1284.

Chapter 5

Extractives from *Samadera madagascariensis* leaves



Photograph by Dr. Harison Rabarison

CHAPTER 5
Extractives from *Samadera madagascariensis* leaves

5.1. Introduction

Samadera madagascariensis Jussieu is a Madagascan member of the Simaroubaceae family. Capuron regarded *S. madagascariensis* as synonymous with *S. indica* Gaertn. on botanical grounds,[1] while Nooteboom considered it synonymous with *Quassia indica* (Gaertn.) Nooteboom,[2] on botanical grounds. However, no publication on *Q. indica* has referred to the synonymy with either *S. madagascariensis* or *S. indica*.

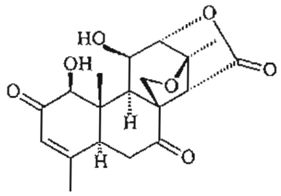
S. madagascariensis is locally known as "fatriana" in Madagascar.[3] The Madagascans use the rootbark as a febrifuge,[4] and the leaves are used for stomache aches and treatment of dysentery.[5] The locals use the juice of fresh leaves to treat wounds and burns.[5]

Previous work on the stem bark of *S. madagascariensis* in these laboratories has provided evidence that *S. madagascariensis, S. indica* and *Q. indica* can be considered synonymous with each other on chemical grounds.[6] Since the only reported investigation on the leaves of *S. madagascariensis* dates from 1971,[7] it was decided to extend the investigation[6] to that of the leaves as well, to provide further evidence of this synonymy.

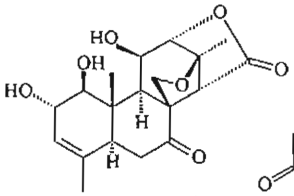
Background on *Samadera* and *Quassia*

Samadera comprises *S. indica*,[8] from Sri Lanka, Java and India, and the Australian *S. bidwillii* (Hook.f.) Oliv.[9] *Quassia* comprises the Indonesian *Q. indica*, the Congolese *Q. africana* (Baill), the Guyanan *Q. multiflora* (A.Juss.) Nooteboom, the Australian *Q. bidwillii* and *Q. amara* L. from Central America. Previous work published on *Q. bidwillii* and *S. bidwillii*, regarded these two species as being different from each other.[9,10]

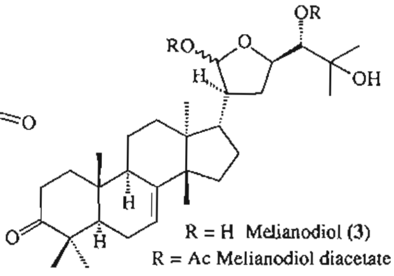
S. madagascariensis leaves have previously yielded the quassinoids samaderines B (**1**) and C (**2**), the protolimonoid melianodiol (**3**) and melianol diacetate (**4**).[7] *S. indica* has yielded samaderines B (**1**), C (**2**), D (**5**), 3,4-dihydrosamaderine B (**6**), cedronin (**7**) and cedronolin (**8**).[11,12] Samaderine A (**9**)[13] and samaderine E (**10**) have also been isolated.[14,15] Other compounds isolated from this species include the ketosteroid 5α-stigmastan-3-one (**11**)[13] from the peel, the taraxane triterpenoid taraxerone (**12**)[13] and 19-hydroxyolean-3-one derivative (**13**)[16] from the bark, and the canthinone alkaloid indacanthinone (**14**) from the wood.[17] *S. bidwillii* has yielded a range of metabolites, including the limonoid limonin (**15**), the quassinoid 2'-acetylglaucarubin (**16**), three alkaloids (**17-19**) and seven lignans (**20-26**).[9]



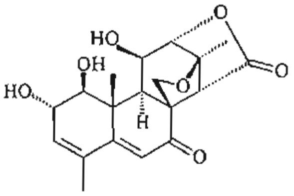
Samaderine B (**1**)



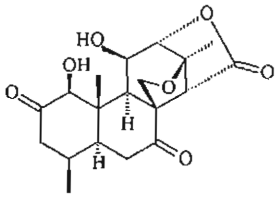
Samaderine C (**2**)



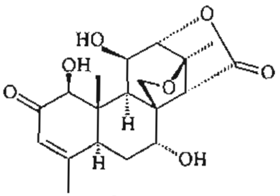
R = H Melianodiol (**3**)
R = Ac Melianodiol diacetate (**4**)



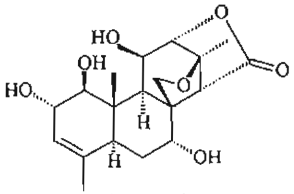
Samaderine D (**5**)



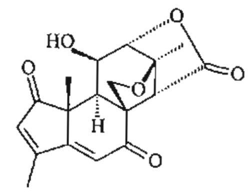
3,4α-Dihydrosamaderine B (**6**)



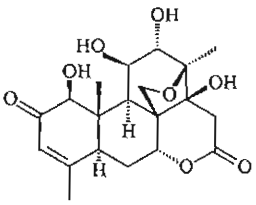
Cedronin (**7**)



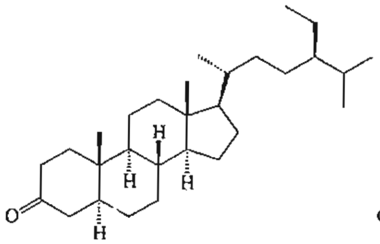
Cedronolin (**8**)



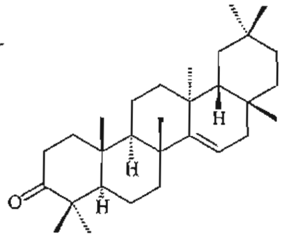
Samaderine A (**9**)



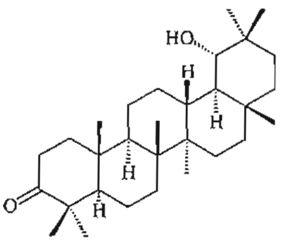
Samaderine E (**10**)



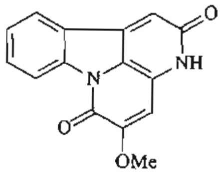
5α-Stigmastan-3-one (**11**)



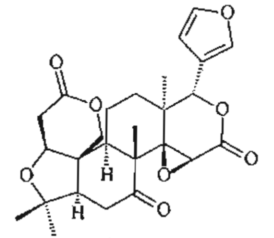
Taraxerone (**12**)



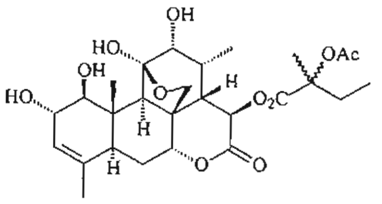
19α-Hydroxyolean-3-one (**13**)



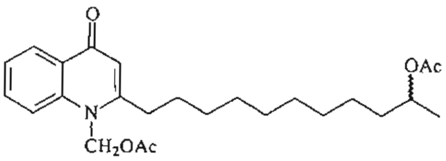
Indacanthinone (**14**)



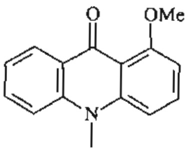
Limonin (**15**)



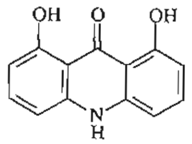
2'-Acetylglaucarubin (**16**)



2-{10'ξ-Acetoxyundecanyl}-1-acetoxymethyl-4-quinolone (**17**)

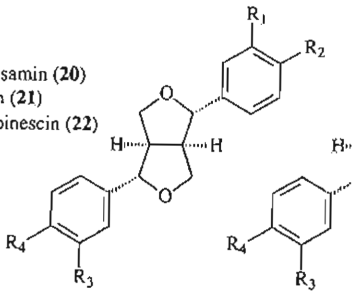


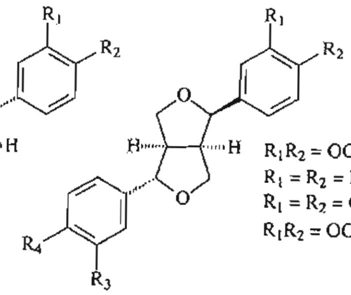
1-Methoxy-10-methylacridan-9-one (**18**)



1,8-Dihydroxyacridan-9-one (**19**)

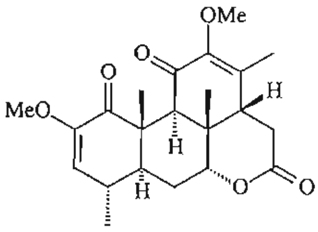
$R_1R_2 = OCH_2O$ $R_3R_4 = OCH_2O$ Sesamin (**20**)
$R_1 = R_2 = R_3 = R_4 = OMe$ Eudesmin (**21**)
$R_1 = R_2 = OMe$ $R_3R_4 = OCH_2O$ Spinescin (**22**)



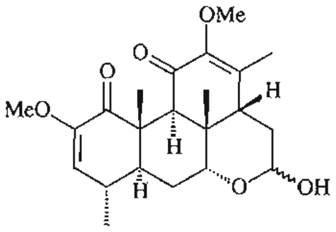


$R_1R_2 = OCH_2O$ $R_3R_4 = OCH_2O$ Episesamin (**23**)
$R_1 = R_2 = R_3 = R_4 = OMe$ Epieudesmin (**24**)
$R_1 = R_2 = OMe$ $R_3R_4 = OCH_2O$ Fargesin (**25**)
$R_1R_2 = OCH_2O$ $R_3 = R_4 = OMe$ Neofargesin (**26**)

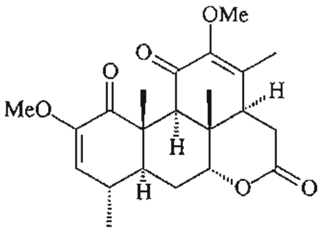
Q. amara has yielded quassin (**27**), neoquassin (**28**) and isoquassin (**29**).[18,19,20,21] A specimen from Costa Rica yielded quassimarin (**30**) and simalikalactone D (**31**).[22] The *Q. amara* wood has yielded paraine (**32**), isoparaine (**33**) and dihydronorneoquassin (**34**).[23] Other quassinoids have also been isolated (**35-39**).[24] The Brazilian *Q. amara* wood has yielded stigmast-4-en-3-one (**40**) and β-sitosterol (**41**).[25] Carboline (**42, 43**) and canthinone alkaloids (**44-48**) have also been reported from the wood.[26,27,28]



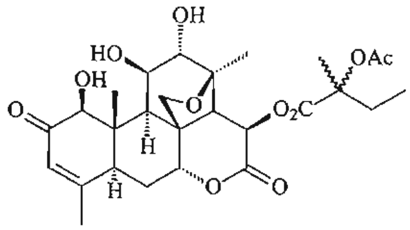
Quassin (**27**)



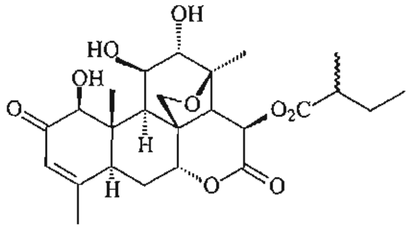
Neoquassin (**28**)



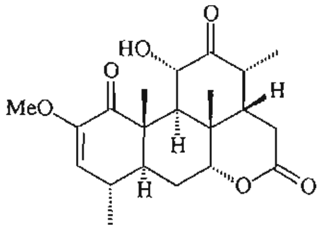
Isoquassin (**29**)



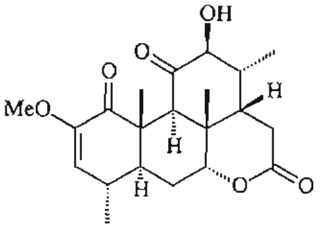
Quassimarin (**30**)



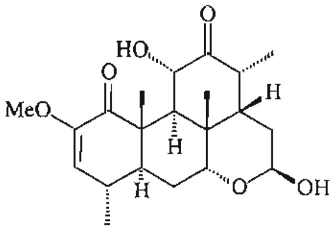
Simalikalactone D (**31**)



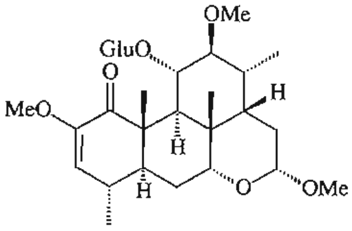
Paraine (**32**)



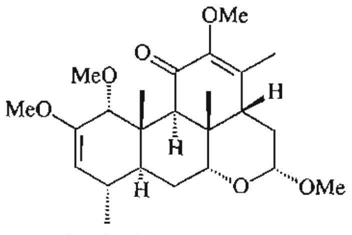
Isoparaine (**33**)



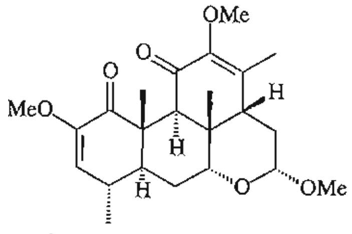
Dihydronorneoquassin (**34**)



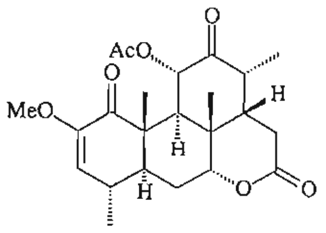
11α-*O*-(β-D-Glucopyranosyl)-16α-*O*-methylneoquassin (**35**)



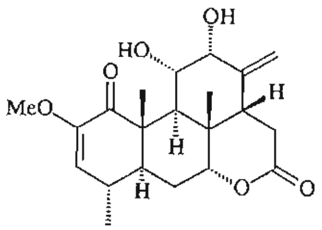
1α-*O*-Methylquassin (**36**)



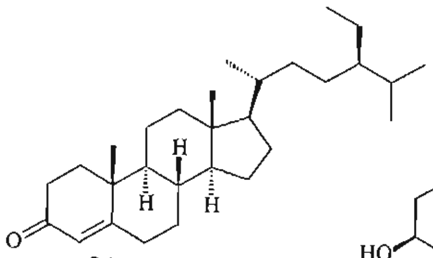
16α-*O*-Methylneoquassin (**37**)



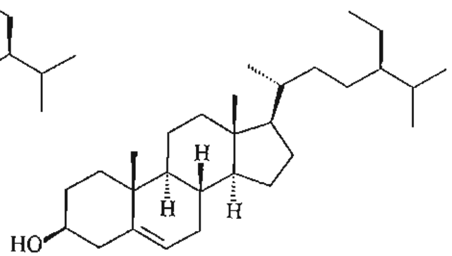
11-Acetylparaine (**38**)



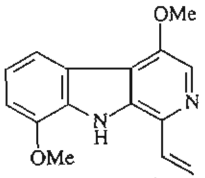
12α-Hydroxy-13,18-dehydroparaine (**39**)

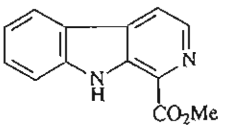


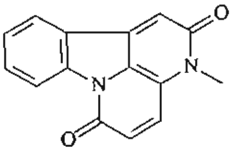
Stigmast-4-en-3-one (**40**)

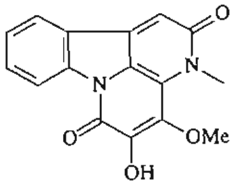


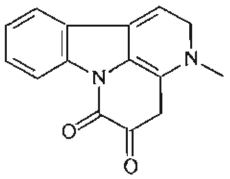
β-Sitosterol (**41**)

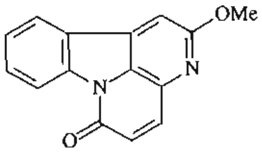

1-Vinyl-4,8-dimethoxy-β-carboline (**42**)

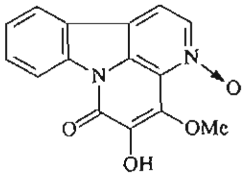

1-Methoxycarbonyl-β-carboline (**43**)

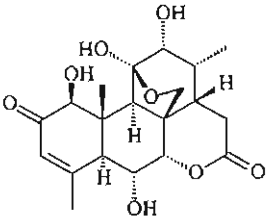

3-Methylcanthin-2,6-dione (**44**)

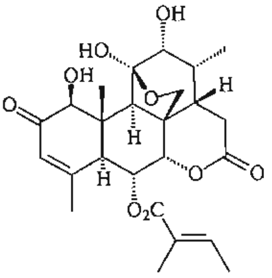

3-Methyl-4-methoxy-5-hydroxycanthin-2,6-dione (**45**)

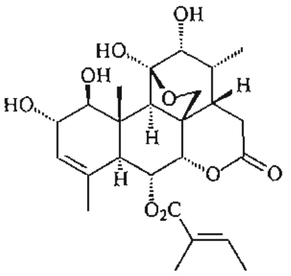

3-Methylcanthin-5,6-dione (**46**)

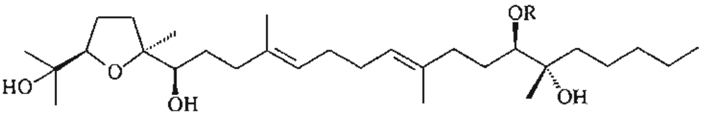

2-Methoxycanthin-6-one (**47**)

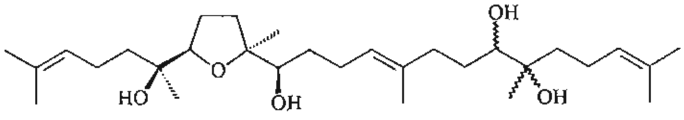

4-Methoxy-5-hydroxycanthin-6-one-3-N-oxide (**48**)

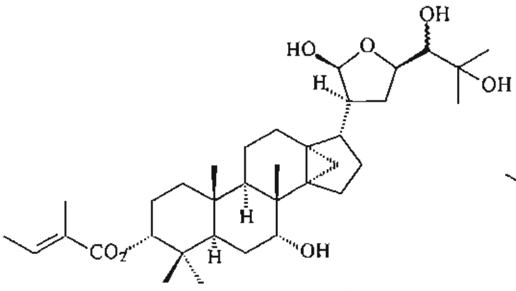

6α-Hydroxychaparrinone (**49**)

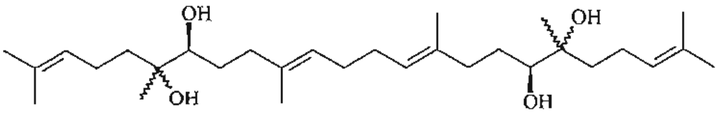

6α-Tigloyloxychaparrinone (**50**)


6α-Tigloyloxychaparrin (**51**)


R = H Quassiol A (**53**)
R = H Quassiol B (**54**)

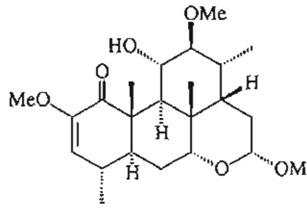

Quassiol C (**55**)

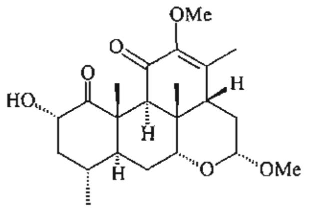

Glabretal type triterpenoid (**52**)

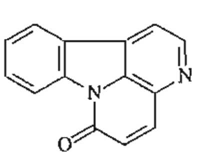

Quassiol D (**56**)

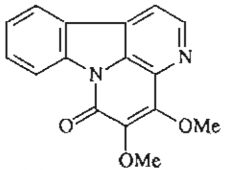
Q. multiflora root has yielded the quassinoids 6α-hydroxychaparrinone (**49**), 6α-tigloyloxychaparrinone (**50**) and 6α-tigloyloxychaparrin (**51**).[29] The glabretal type triterpenoid (**52**)[30] and the squalene triterpenoids quassiols A-D (**53-56**)[31,32] have also been reported from the *Q. multiflora* root. *Q. africana* has yielded simalikalactones A (**57**), B (**58**), C (**32**), D (**31**)[33,34] and simalikahemiacetal A (**28**). Simalikalactone A (**57**) and simalikalactone B (**58**) have also been isolated, as nigakilactone B[34] and picrasin B[35] respectively, from *Picrasma quassioides* Bennet, and simalikahemiacetal A was identified as neoquassin (**28**).

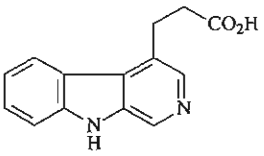
The *Q. africana* rootbark yielded quassin (**27**), and canthinone and carboline alkaloids (**59**, **60**, **61**).[36] The canthinone analogue (**62**) and the thiocanthinone derivative (**63**) were also isolated.[37] *Q. bidwillii* contains the quassinoid chapparinone (**64**) which is known for its insecticidal properties.[10]

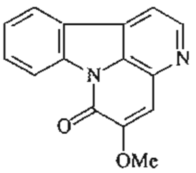

Similakalactone A / Nigakilactone B (**57**)

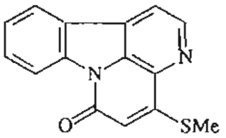

Simalikalactone B / Picrasin B (**58**)

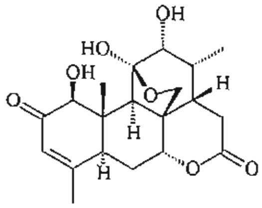

Canthin-6-one (**59**)

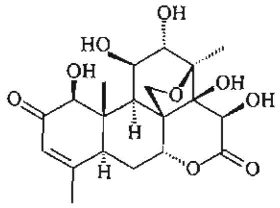

4,5-Dimethoxycanthin-6-one (**60**)

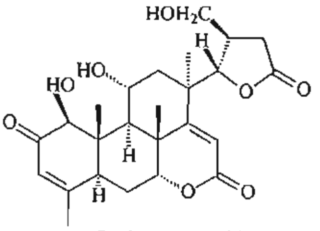

β-Carboline-1-propionic acid (**61**)

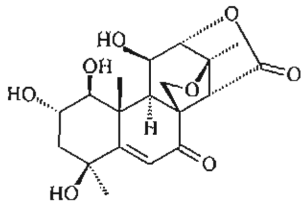

5-Methoxycanthin-6-one (**62**)

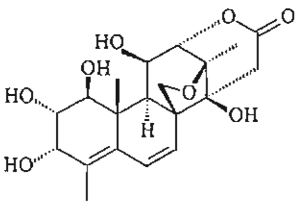

4-Methylthiocanthin-6-one (**63**)

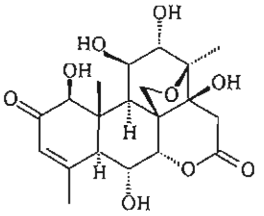

Chapparinone (**64**)

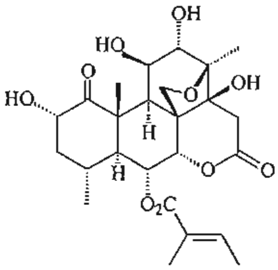

Brucein D (**65**)

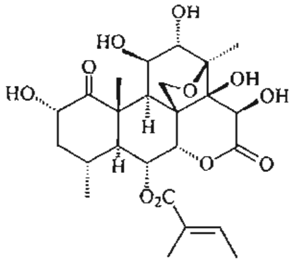

Soulameolide (**66**)

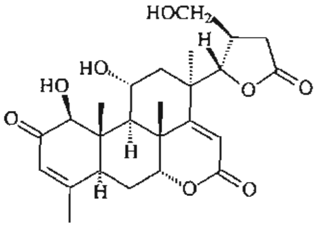

Indaquassin A (**67**)

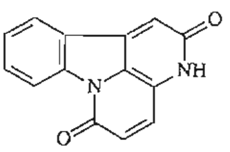

Indaquassin B (**68**)


Indaquassin C (**69**)


Indaquassin D (**70**)

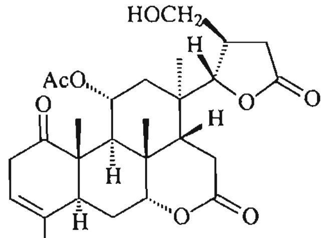

Indaquassin E (**71**)


Indaquassin F (**72**)

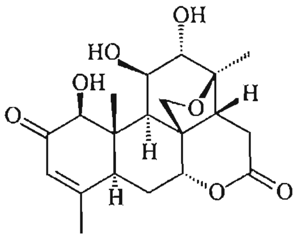

Canthin-2,6-dione (**73**)

The bark of the Indonesian *Q. indica* has yielded samaderines B (**1**), C (**2**), D (**5**), E (**10**), 3,4-dihydrosamaderine B (**6**), cedronin (**7**), brucein D (**65**), soulameolide (**66**), indaquassins A (**67**), B (**68**), C (**69**), D (**70**), E (**71**), F (**72**) and the canthinone alkaloid (**73**).[38]

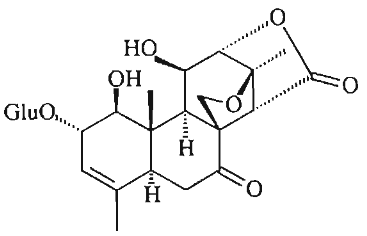
Q. indica stems have also yielded samaderines B (**1**), C (**2**) and E (**8**), indaquassin C (**69**), simarinolide (**74**), samaderine Y (**75**) (which was found to be identical to ailanquassin B),[39] 2-*O*-glucosylsamaderine C (**76**), samaderines X (**77**) and Z (**78**) and indaquassin X (**79**)[40] *Q. indica* extracts display antimalarial,[40] antirheumatic,[41] anti-inflammatory, antipyretic,[38] insecticidal[41] and cytotoxic properties.



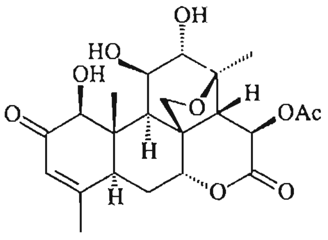
Simarinolide (**74**)



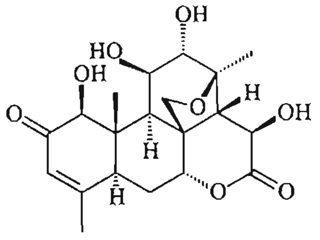
Samaderine Y (**75**)



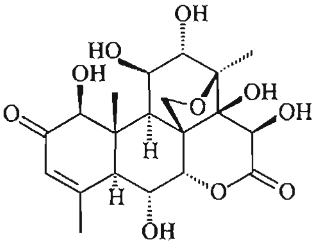
2-*O*-Glucosylsamaderine C (**76**)



Samaderine X (**77**)



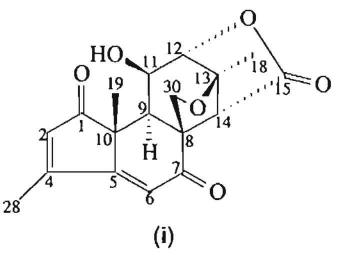
Samaderine Z (**78**)



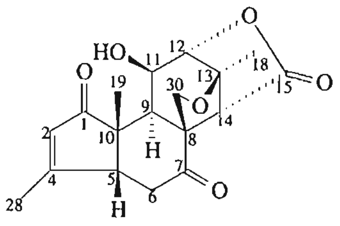
Indaquassin X (**79**)

5.2. Results and Discussion

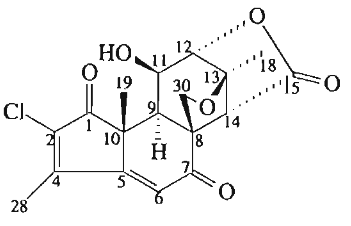
Investigation of the leaves of *Samadera madagascariensis* collected from Foulpointe, in the rainy eastern part of Madagascar, yielded eighteen compounds. The hexane extract yielded four triterpenoids, protosamaderine A (**viii**), chisocheton compound A (**x**), protosamaderine B (**xi**) and protosamaderine C (**xii**). The dichloromethane extract yielded one triterpenoid, 1,2-dihydrobruceajavanin A (**ix**). The ethyl acetate extract yielded two quassinoids, samaderine A (**i**) and 2-chlorosamaderine A (**iii**). The methanol extract yielded five quassinoids, 5β,6-dihydrosamaderine A (**ii**), samaderine DN (**iv**), samaderine B (**v**), cedronin (**vi**), 3,4β-dihydrosamaderine C (**vii**), and six triterpenoids, protosamaderine D (**xiii**), protosamaderine E (**xiv**), protosamaderine F (**xv**), protosamaderine G (**xvi**), protosamaderine H (**xvii**), protosamaderine I (**xviii**).



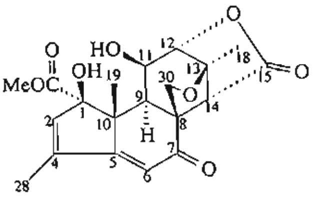
(**i**)



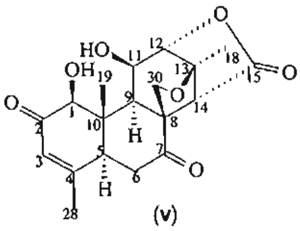
(**ii**)



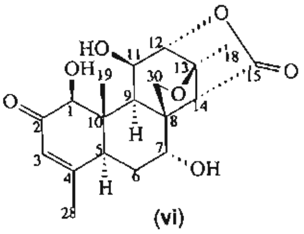
(**iii**)



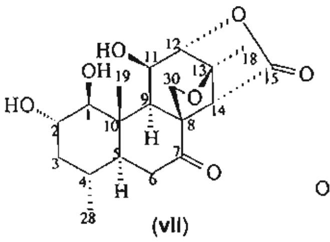
(iv)



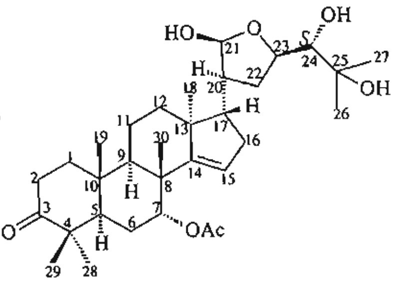
(v)



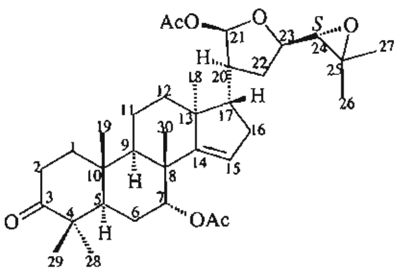
(vi)



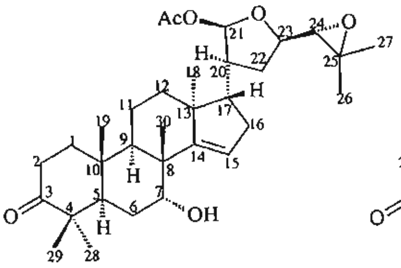
(vii)



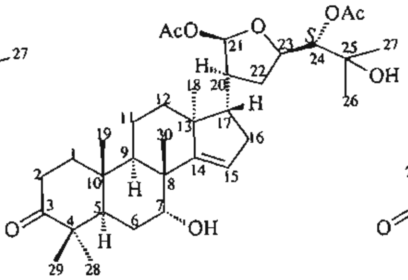
(viii)



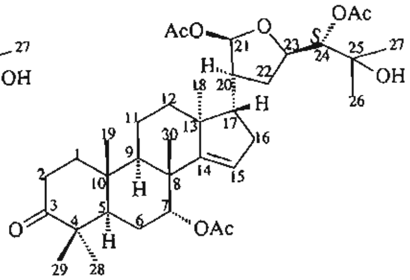
(ix)



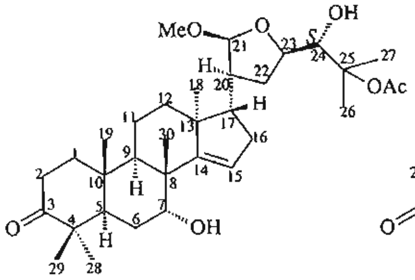
(x)



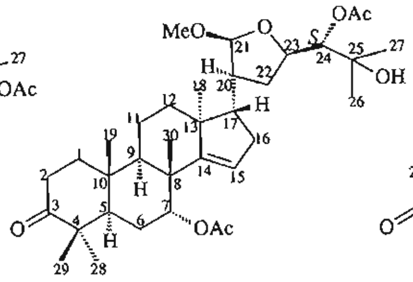
(xi)



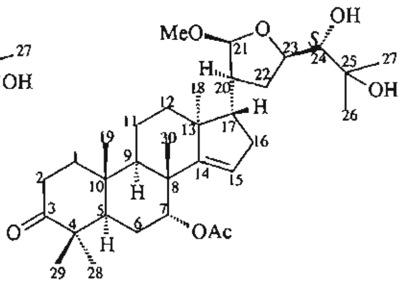
(xii)



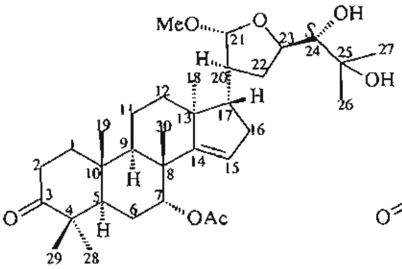
(xiii)



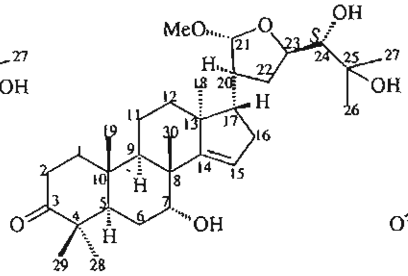
(xiv)



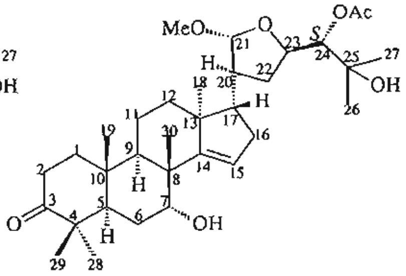
(xv)



(xvi)



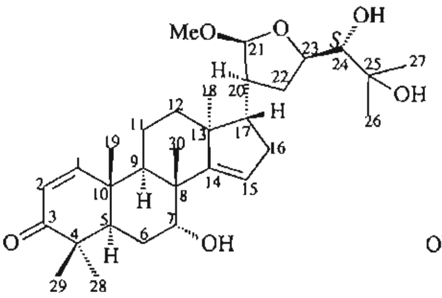
(xvii)

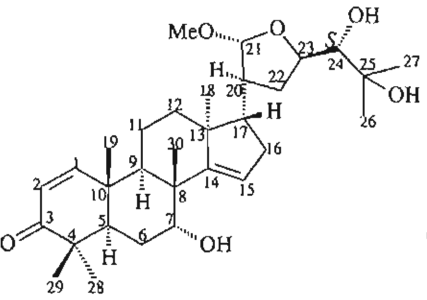


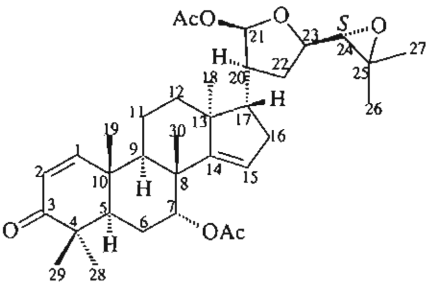
(xviii)

Compound (**i**) was isolated previously from *S. indica*, and compounds (**v**) and (**vi**) from both *S. indica* and *Q. indica*. 5β,6-dihydrosamaderine A (**ii**), 2-chlorosamaderine A (**iii**), samaderine DN (**iv**), 3,4β-samaderine C (**vii**), protosamaderine A (**viii**), protosamaderine B (**xi**), protosamaderine C (**xii**), protosamaderine D (**xiii**), protosamaderine E (**xiv**), protosamaderine F (**xv**), protosamaderine G (**xvi**), protosamaderine H (major component) (**xvii**) and protosamaderine I (**xviii**) (major component) have not been reported previously.

The triterpenoids (**viii, xi - xviii**) isolated in this work are related to holstinones B (**80**) and C (**81**) previously reported from *Turraea holstii* (Meliaceae),[42] whereas the triterpenoids (**ix - x**) isolated in this work are related to bruceajavanin A (**82**) previously reported from *Brucea javanica* (Simaroubaceae).[43] Triterpenoids (**viii, xi - xviii**) differ from holstinones B (**80**) and C (**81**) by the oxygenated substituents on C-7, C-21, C-24 and C-25 and by the absence of the $\Delta^{1,2}$-double bond. Triterpenoids (**ix - x**) differ from bruceajavanin A (**82**) by the oxygenated substituent on C-7 and by the absence of the $\Delta^{1,2}$-double bond.

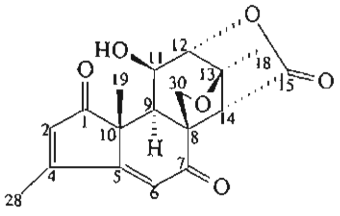

Holstinone B (**80**)


Holstinone C (**81**)


Bruceajavanin A (**82**)

It was also observed in this work that the leaves contain more triterpenoids than quassinoids compared to the stem bark of the same species.

5.3.1. Structural elucidation of Compound i: Samaderine A (i)


(**i**)

The mass spectrum **[4]** of compound (i) showed a molecular ion [M$^+$] peak at *m/z* 330.1106 corresponding to the molecular formula $C_{18}H_{18}O_6$, which requires 330.1103. The peaks at *m/z* 315.0870 [M-15]$^+$ and *m/z* 312.1018 [M-18]$^+$ were due to the loss of a methyl group and the element of water respectively. The IR spectrum **[3]** of compound (**i**) showed an O-H stretching band at 3487 cm^{-1}, and C=O stretching bands at 1793, 1717 and 1661 cm^{-1}.

The molecular formula indicated that compound (*i*) was a C_{18} quassinoid, and this was supported by the presence of only three methyl group proton resonances at δ_H 1.58 (s), 1.71 (s) and 2.21 (s) in the ^{1}H NMR spectrum **[5]**.

Further inspection of the ^{13}C NMR **[7]** and ADEPT **[8]** spectra showed the presence of three carbonyl groups, due to the C-1 (δ_C 203.52) and C-7 (δ_C 193.83) carbonyl groups and a lactone carbonyl group (δ_C 171.10), and, in addition, two trisubstituted double bonds (C-2, δ_C 134.09; C-4, δ_C 163.32; C-5, δ_C 168.80; C-6, δ_C 116.80). Four oxygenated carbons were noted: one fully substituted (C-13, δ_C 89.21), two oxymethine (C-12, δ_C 81.78; C-11, δ_C 69.03) and one oxymethylene carbon (C-30, δ_C 76.13). A literature survey conducted on this basis revealed that compound **(i)** might be samaderine A, previously isolated from *Samadera indica*.[14] However, no complete assignment of the ^{13}C NMR data for samaderine A has been reported previously.

The characteristic pair of doublets at δ_H 4.14 and 4.80 (J = 8.97 Hz) were assigned to the two oxymethylene H-30 proton resonances. The H-30a resonance (δ_H 4.14) showed a correlation to the proton resonance at δ_H 3.43 (s) in the NOESY NMR spectrum **[12]**, which was assigned to H-14. This H-14 resonance was seen to correlate to the methine carbon resonance at δ_C 58.03 (C-14) in the HSQC spectrum **[9]**, and showed a correlation to the carbonyl carbon resonance at δ_C 193.83 in the HMBC NMR spectrum **[10]**, which was assigned to C-7. The H-30b resonance (δ_H 4.80) showed a correlation to the methyl proton resonance at δ_H 1.71 (s) in the NOESY NMR spectrum **[12]**, which was assigned to 3H-19. The 3H-19 resonance showed a correlation to the carbonyl carbon resonance at δ_C 203.52 in the HMBC NMR spectrum **[10]**, which was assigned to C-1. The 3H-19 resonance also showed a correlation to the fully substituted double bond carbon at δ_C 168.80 in the HMBC NMR spectrum **[10]**, which was assigned to C-5. Therefore the remaining fully substituted double bond carbon resonance at δ_C 163.32 was assigned to C-4, and this was supported by the correlation to the downfield methyl proton resonance at δ_H 2.21 (s) in the HMBC NMR spectrum **[10]**, which was assigned to 3H-28. Both the 3H-28 and 3H-19 resonances showed correlations to the fully substituted carbon resonance at δ_C 48.17 in the HMBC NMR spectrum **[10]**, which was assigned to C-10. The H-30a resonance showed a correlation to the fully substituted carbon resonance at δ_C 57.43 in the HMBC NMR spectrum **[10]**, which was assigned to C-8. The C-8 resonance showed a correlation to the olefinic proton resonance at δ_H 6.00 (m) in the HMBC NMR spectrum **[10]**, which was assigned to H-6, and this resonance correlated to the olefinic carbon resonance at δ_C 116.80 (C-6) in the HSQC **[9]** spectrum. Therefore the remaining olefinic carbon resonance at δ_C 134.09 was assigned to C-2 and this was seen to correlate to the proton resonance at δ_H 6.22 (bs) (H-2) in the HSQC spectrum.

Both the H-30 proton resonances showed correlations to the methine carbon resonance at δ_C 40.21 in the HMBC NMR spectrum **[10]**, which was assigned to C-9. The HSQC **[9]** spectrum showed a correlation between the C-9 resonance and the proton resonance at δ_H 2.25 (d, J = 4.40 Hz), which was assigned to H-9. The H-9 resonance showed a correlation to the proton resonance at δ_H 4.80 (m) in the COSY NMR spectrum **[11]**, which was superimposed with the H-30b resonance, and was assigned to H-11. The H-11 resonance was seen to correlate with the oxymethine carbon resonance at δ_C 69.03 (C-11) in the HSQC **[9]** spectrum.

The COSY spectrum **[11]** showed a correlation between the H-11 resonance and the oxymethine proton resonance at δ_H 4.28 (d, *J* = 3.66), which was assigned to H-12, and this correlated to the oxymethine carbon resonance at δ_C 81.78 in the HSQC spectrum **[9]**, which was assigned to C-12. The C-12 resonance showed a correlation to the methyl proton resonance at δ_H 1.58 (s) in the HMBC NMR spectrum **[10]**, which was assigned to 3H-18. This 3H-18 resonance correlated to the methyl carbon resonance at δ_C 20.92 (C-18) in the HSQC spectrum **[9]**, and showed a correlation to the fully substituted oxygenated carbon resonance at δ_C 89.21 in the HMBC NMR spectrum **[10]**, which was assigned to C-13. The lactone carbon resonance at δ_C 171.10 was assigned to C-15. The stereochemistry at C-11 was assigned as a result of the NOESY correlation between the C-11 hydroxy group proton resonance and the 3H-18 resonance, which is only possible when the hydroxy group is β orientated.

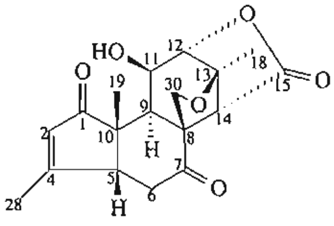
The NMR data for samaderine A (**i**) is shown in table 5.1.

Table 5.1. : NMR data for samaderine A (i)

C	δ^{13}C / ppm (CDCl$_3$)	δ^{1}H / ppm (CDCl$_3$)	HMBC (C→H)	COSY	NOESY
1	203.52 (C)	-	19		
2	134.09 (CH)	6.22 (bs)	6, 28	28	28
4	163.32 (C)	-	2, 6, 28		
5	168.80 (C)	-	19, 28		
6	116.80 (CH)	6.00 (s)			28
7	193.83 (C)	-	14		
8	57.43 (C)	-	6, 14, 30a		
9	40.21 (CH)	2.25 (d, 4.40)	12, 14, 19, 30a, 30b	11	11
10	48.17 (C)	-	2, 6, 19, 28		
11	69.03 (CH)	4.80 *		9, 12, 11-OH	9
11-OH		3.58 (bd)		11	18
12	81.78 (CH)	4.28 (d, 3.66)	14, 18	11	18, 30b
13	89.21 (C)	-	11, 12, 14, 18, 30b		
14	58.03 (CH)	3.43 (s)	11, 12, 18, 30b		18, 30a
15	171.10 (C)	-	11, 12, 14, 30b		
18	20.92 (CH$_3$)	1.58 (s)	12, 14		12, 14
19	21.42 (CH$_3$)	1.71 (s)			30b
28	13.71 (CH$_3$)	2.21 (d, 1.28)			2, 6
30	76.13 (CH$_2$)	a) 4.14 (d, 8.97)		a) 30b	a)14, 30b
		b) 4.80 (d, 8.97)		b) 30a	b)12, 19, 30a

* H-11 is superimposed with H-30b, H-11 multiplicity could not be determined.

5.3.2. Structural elucidation of compound ii: 5β, 6-Dihydrosamaderine A (ii)



(ii)

The mass spectrum **[18]** of compound (**ii**) showed a molecular ion [M^+] peak at *m/z* 332.1262, which corresponds to the molecular formula $C_{18}H_{20}O_6$. The peaks at *m/z* 317.1023 and *m/z* 315.1223 were due to the loss of a methyl group and a hydroxyl group respectively, while the peak at *m/z* 288.1359 was due to the loss of carbon dioxide. The IR spectrum **[17]** of compound (**ii**) showed an O-H stretching band at 3489 cm^{-1}, and C=O stretching bands at 1785, 1701 and 1623 cm^{-1}.

Inspection of the ^1H **[19]** and ^{13}C NMR **[20]** spectra of compound (**ii**) showed them to be very similar to those of compound (**i**), except that the fully substituted double bond C-5 resonance at δ_C 168.80 and the methine double bond C-6 resonance at δ_C 116.80 in the ^{13}C NMR spectrum **[20]** of compound (i) were replaced by a methine carbon resonance and a methylene carbon resonance at δ_C 53.06 and 41.12 respectively in the ^{13}C NMR spectrum **[20]** of compound (ii).

Further inspection of the 2D NMR spectra showed that the H-5 resonance at δ_H 2.61 (dd, *J* = 6.41, 12.64 Hz) showed correlations to the two methylene C-6 proton resonances at δ_H 2.31 and 2.79 in the COSY NMR spectrum **[24]**. The H-5 resonance also showed correlations to the 3H-19 (δ_H 1.47), 3H-28 (δ_H 2.06) and the two C-6 proton resonances in the NOESY NMR spectrum **[25]**. The correlation between the H-5 resonance and the 3H-19 resonance indicated that H-5 was β orientated, and this was shown to be possible with the use of molecular models. This indicated that compound (**ii**) is 5β, 6-dihydrosamaderine A, which has not been reported previously.

Compound (**ii**) is the only C_{18} quassinoid without the $\Delta^{5,6}$-double bond to be reported. An interesting observation is that all reported C_{19} quassinoids have an α orientated H-5, whereas this C_{18} quassinoid has a β orientated H-5.

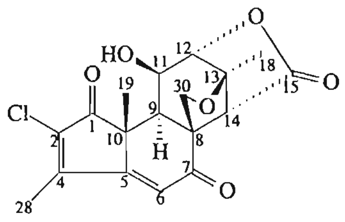
The NMR data for 5β, 6-dihydrosamaderine A (**ii**) is shown in table 5.2.

Table 5.2. : NMR data for 5β, 6-dihydrosamaderine A (ii)

C	$\delta^{13}C$ / ppm ($CDCl_3$)	$\delta^{1}H$ / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	209.15 (C)	-	19, 28, 29		
2	127.85 (CH)	5.93 (bs)	5, 28	28	28
4	175.86 (C)	-	2, 5, 6α, 28		
5	53.06 (CH)	2.61 (dd, 6.41, 12.64)	2, 6α, 6β, 9, 19	6α, 6β	6α, 6β, 19, 28
6	41.12 (CH_2)	α) 2.31 (dd, 12.64, 13.83)	5	α) 5	α) 5, 6β, 9, 28
		β) 2.79 (dd, 6.41, 13.83)		β) 5	β) 5, 6α, 28
7	206.34 (C)	-	6α, 6β, 11		
8	56.43 (C)	-	6β, 9, 11, 30a		
9	39.31 (CH)	2.15 (d, 4.40)	11, 12, 19, 30a, 30b	30a, 30b	6α, 28
10	48.91 (C)	-	2, 6β, 9, 19		
11	69.09 (CH)	4.63 *	9	12, 11-OH	9, 12
11-OH	-	3.45 (bd)		11	
12	83.07 (CH)	4.29 (d, 3.66)	11, 18	11	14, 18
13	89.05 (C)	-	11, 14, 18, 30b		
14	59.59 (CH)	2.96 (s)	9, 14, 18, 30b		18, 30a
15	171.35 (C)	-	12		
18	20.72 (CH_3)	1.54 (s)	11, 12		12, 14
19	21.17 (CH_3)	1.47 (s)	5, 9		5, 30b
28	17.01 (CH_3)	2.06 (s)		2	2, 5, 6α, 6β, 9
30	74.72 (CH_2)	a) 4.40 (d, 9.16)	9	a) 9, 30b	a) 14, 30b
		b) 4.63 (d, 9.16)		b) 9, 30a	b) 19, 30a

* H-11 is superimposed with H-30b, H-11 multiplicity could not be determined.

5.3.3. Structural elucidation of Compound iii: 2-Chlorosamaderine A (iii)



(iii)

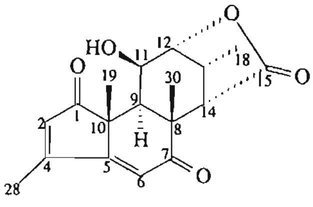
The mass spectrum **[31]** of this compound (**iii**) showed a molecular ion [M^+] peak at *m/z* 365.0794, which corresponds to the molecular formula $C_{18}H_{17}ClO_6$. The presence of a chlorine atom was indicated by the $[M+2]^+$ peak at *m/z* 367, which was one third of the intensity of the molecular ion peak. The peak at *m/z* 347 $[M-18]^+$ was due to the loss of the element of water. The IR spectrum **[30]** of compound (**iii**) showed an O-H stretching band at 3488 cm^{-1}, and C=O stretching bands at 1795, 1733 and 1672 cm^{-1}.

Inspection of the ^1H **[32]** and ^{13}C NMR **[33]** spectra of compound (iii) showed them to be very similar to those of compound (**i**), except that the resonance assigned to H-2 in compound (**i**) had disappeared. The resonances at δ_C 203.52, 134.09, 163.32, 168.80 and 48.17, which were assigned to C-1, C-2, C-4, C-5 and C-10 respectively in compound (**i**), had shifted to δ_C 195.55, 138.28, 156.61, 165.08 and 47.51 respectively in compound (**iii**). An important observation here was the change of the C-2 resonance from a methine carbon resonance to a fully substituted carbon resonance.

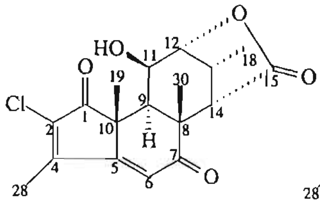
Eurycolactone B and laurycolactone B have both been previously isolated from *Eurycoma longifolia* Jack (Simaroubaceae).[44] The proton and carbon resonances in the ^{1}H and ^{13}C NMR spectra of ring A in eurycolactone B had showed the same changes in chemical shifts, relative to laurycolactone B as are observed for compound (**iii**) relative to compound (**i**). This provides further evidence that compound (**iii**) is 2-chlorosamaderine A, which has not been reported previously. These shifts are detailed for comparison in the table below. The NMR data for 2-chlorosamaderine A (**iii**) is shown in table 5.3.

Table i: ^{13}C NMR data of ring A for laurycolactone B, eurycolactone B, compound (i) and compound (iii)

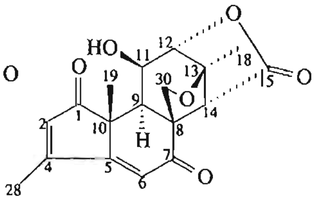
C	Laurycolactone B[44]	Eurycolactone B[44]	Compound (i)	Compound (iii)
1	205.19	197.50	203.52	195.55
2	133.36	136.43	134.09	138.28
4	164.51	158.17	163.32	156.61
5	166.65	162.86	168.80	165.08
10	49.42	48.93	48.17	47.51



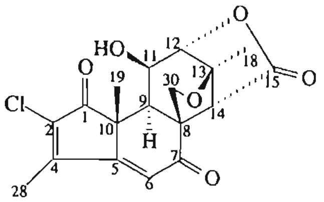
Laurycolactone B



Eurycolactone B



(i)



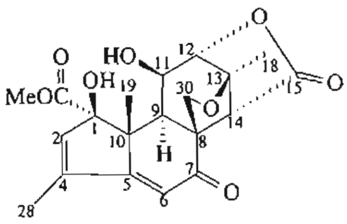
(iii)

Table 5.3. : NMR data for 2-chlorosamaderine A (iii)

C	δ^{13}C / ppm ($CDCl_3$)	δ^{1}H / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	195.55 (C)	-	19, 28		
2	138.28 (C)	-	28		
4	156.61 (C)	-	6, 28		
5	165.08 (C)	-	19, 28		
6	116.75 (CH)	6.05 (s)			28
7	193.10 (C)	-	14		
8	57.67 (C)	-	6, 11, 14		
9	40.07 (CH)	2.28 (d, 4.58)	12, 14, 19, 30a, 30b	11	11
10	47.51 (C)	-	6, 9, 19		
11	68.93 (CH)	4.79 *		9, 12, 11-OH	9, 12
11-OH	-	3.59 (bd)		11	
12	81.63 (CH)	4.29 (d, 4.76)	14, 18	11	11, 18
13	89.24 (C)	-	11, 14, 18, 30b		
14	58.03 (CH)	3.44 (s)	18, 30b		18, 30a
15	170.90 (C)	-			
18	20.88 (CH_3)	1.59 (s)	12, 14		12, 14
19	21.34 (CH_3)	1.75 (s)			30a, 30b
28	11.84 (CH_3)	2.23 (s)			6
30	76.05 (CH_2)	a) 4.15 (d, 9.07)		a) 30b	a) 14, 19, 30b
		b) 4.81 (d, 9.07)		b) 30a	b) 19, 30a

* H-11 is superimposed with H-30b, H-11 multiplicity could not be determined.

5.3.4. Structural elucidation of compound iv: Samaderine DN (iv)



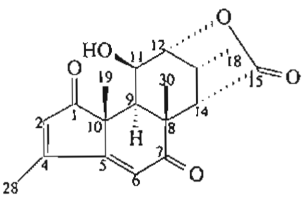
(iv)

The mass spectrum **[44]** of compound (**iv**) showed a molecular ion [M^+] peak at *m/z* 390.1313, which corresponds to the molecular formula $C_{20}H_{22}O_8$. The peak at *m/z* 331.1187 $[M-59]^+$ indicated the loss of a carbomethoxy fragment. The IR spectrum **[43]** of compound (**iv**) showed an O-H stretching band at 3457 cm^{-1}, and C=O stretching bands at 1788, 1730 and 1655 cm^{-1}.

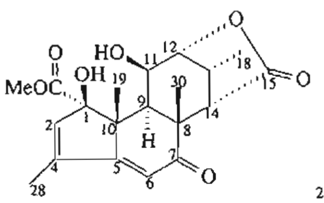
Inspection of the ^1H **[45]** and ^{13}C NMR **[46]** spectra of compound (**iv**) showed them to be similar to those of compound (i), except for the presence of a methoxy group proton resonance at δ_H 3.76 (bs) in the ^1H NMR spectrum **[45]**, and its corresponding carbon resonance (δ_C 53.91) in the ^{13}C NMR spectrum **[46]**. Minor resonances of a similar compound were also seen in the spectra.

The C-1 carbonyl resonance at δ_C 203.52 in compound (i) had disappeared in the ^{13}C NMR spectrum [46] of compound (**iv**). The carbon resonance at δ_C 88.64, which showed a correlation to the 3H-19 resonance (δ_H 1.75) in the HMBC NMR spectrum **[49]** was assigned to C-1. The C-1 hydroxy group proton resonance could not be seen in the NMR spectra. The methoxy group proton resonance (δ_H 3.76) showed a correlation to the carbonyl carbon resonance at δ_C 174.57 in the HMBC NMR spectrum **[49]**, which was assigned to the ester carbonyl carbon attached to C-1. The methoxy group proton resonance showed a correlation to the H-9 resonance (δ_H 2.42) in the NOESY NMR spectrum **[51]**, which confirmed the α orientation of the ester. Except for the substituents at C-1, the rest of compound (**iv**) is the same as compound (**i**).

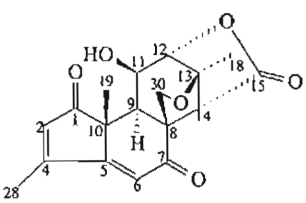
Eurycolactone A and laurycolactone B have both been previously isolated from *Eurycoma longifolia* Jack.[44] The proton and carbon resonances in the ^1H and ^{13}C NMR spectra of ring A in eurycolactone A had showed the same changes in chemical shifts, relative to laurycolactone B, as are observed for compound (**iv**) relative to compound (**i**). These shifts are detailed for comparison in table ii below.



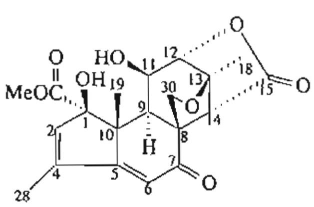
Laurycolactone B



Eurycolactone A



(i)



(iv)

Table ii: ^{13}C NMR data of ring A for laurycolactone B, eurycolactone A, compound (i) and compound (iv)

C	Laurycolactone B[44]	Eurycolactone A[44]	Compound (i)	Compound (iv)
1	205.19	90.29	203.52	88.64
2	133.36	142.01	134.09	142.02
4	164.51	142.32	163.32	142.41
5	166.65	173.52	168.80	175.68
8	48.14	47.38	57.43	56.69
10	49.42	56.66	48.17	54.92

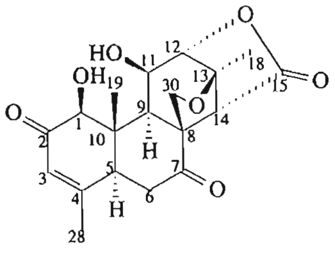
This confirms the structure of compound (**iv**) as samaderine DN, which has not been reported previously. The NMR data for samaderine DN (**iv**) is shown in table 5.4.

Table 5.4. : NMR data for samaderine DN (iv)

C	δ^{13}C / ppm ($CDCl_3$)	δ^{1}H / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	88.64 (C)	-			
2	142.02 (CH)	5.99 (d, 1.28)	28	28	28
4	142.41 (C)	-	6		
5	175.68 (C)	-	19, 28		
6	115.31 (CH)	5.88 (s)			28
7	192.49 (C)	-	14		
8	56.69 (C)	-	6, 14		
9	41.09 (CH)	2.42 (d, 3.66)	11, 12, 14, 19, 30a, 30b	11	OC$\underline{H}_3$, 11, 12
10	54.92 (C)	-	6, 19		
11	70.04 (CH)	4.16 *		9	9, 18
12	81.03 (CH)	4.16 *	14, 18		9, 18
13	89.28 (C)	-	14, 18, 30b		
14	58.21 (CH)	3.53 (s)	18, 30b		18, 30a
15	171.22 (C)				
18	21.01 (CH_3)	1.60 (s)	14		11, 12, 14
19	23.03 (CH_3)	1.75 (s)			30b
28	12.37 (CH_3)	1.91 (d, 1.28)		2	2, 6
30	75.94 (CH_2)	a) 4.07 (d, 9.16)		a) 30b	a) 14, 30b
		b) 4.72 (d, 9.16)		b) 30a	b) 19, 30a
O$\underline{C}H_3$	53.91 (CH_3)	3.76 (s)			9
$\underline{C}O_2$ CH_3	174.57 (C)	-	OC$\underline{H}_3$		

* The H-11 and H-12 resonances are superimposed, multiplicity could not be determined.

5.3.5. Structural elucidation of compound v: Samaderine B (v)



(**v**)

The positive CI mass spectrum **[54]** of compound (**v**) showed a highest peak $[M+H]^+$ at *m/z* 363.1445 indicating a molecular formula of $C_{19}H_{22}O_7$ for the molecule. The peak at *m/z* 345 $[M+H-18]^+$ indicated the loss of the element of water. The IR spectrum **[53]** of compound (**v**) showed an O-H stretching band at 3455 cm^{-1}, and C=O stretching bands at 1788, 1715 and 1672 cm^{-1}.

Inspection of the ^1H **[55]** and ^{13}C NMR **[56]** spectra showed the presence of nineteen carbon resonances in the ^{13}C NMR spectrum **[56]**, and three methyl resonances at δ_H 1.55 (s), 1.29 (s) and 1.92 (s) in the ^1H NMR spectrum **[55]**. This suggested that compound (**v**) was a C_{19} quassinoid. Minor resonances of another compound were also seen in the spectra. Inspection of the ^{13}C NMR spectrum **[56]** showed the presence of three carbonyl groups (C-7, δ_C 203.37; C-2, δ_C 196.46; C-15, δ_C 171.94), including a lactone carbonyl group, and, in addition, a trisubstituted double bond (C-4, δ_C 160.82; C-3, δ_C 124.41). Five oxygenated carbon resonances were noted, comprising one fully substituted (C-13, δ_C 87.67), three oxymethine (C-1, δ_C 80.70; C-11, δ_C 70.10; C-12, δ_C 83.74) and one oxymethylene (C-30, δ_C 75.48) carbons.

A literature survey conducted on this basis revealed that compound (**v**) might be samaderine B, previously isolated from *Samadera indica*,[11,12] from the bark[45] and stem[40] of *Quassia indica*, and very recently in these laboratories from the stem bark of *Samadera madagascariensis*.[6] A comparison of the ^{13}C NMR data confirmed that compound (**v**) is samaderine B.

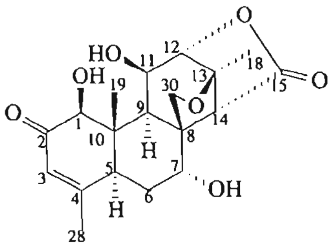
The NMR data for samaderine B (**v**) is shown in table 5.5.

Samaderine B (**v**) has been reported to exhibit inhibitory activities (IC_{50} 0.21 μM, IC_{90} 0.69 μM) against the cultured malarial parasite *Plasmodium falciparum* of a chloroquine-resistant K1 strain in human erythrocytes.[40] Samaderine B (**v**) has also been reported to exhibit *in vitro* cytotoxic activities (IC_{50} 0.07 μM) against KB cells, and inhibitory activity in the *in vitro* endothelial cell-neutrophil leukocyte adhesion assay. Samaderine B (**v**) also showed anti-inflammatory activity in Sprague-Dawley rats.[40]

Table 5.5. : NMR data for samaderine B (v)

C	$\delta^{13}C$ / ppm ($CDCl_3$)	$\delta^{13}C$ / ppm ($CDCl_3$) Lit.[6]	$\delta^{1}H$ / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	80.70 (CH)	80.70	4.13 (s)	19		5, 9, 11
1-OH	-	-	4.52 (bs)			
2	196.46 (C)	196.48	-	1, 1-OH		
3	124.41 (CH)	124.39	6.12 (s)	28		28
4	160.82 (C)	160.85	-	5, 28		
5	47.54 (CH)	47.51	2.94 (m)	19, 28	6α, 6β	1, 6β, 9, 28
6	38.93 (CH_2)	38.93	β) 2.46 (dd,15,38,18.50)		β) 5, 6α	β) 5, 6α, 19
			α) 2.98 (m)		α) 5, 6β	α) 6β, 28
7	203.37 (C)	203.38	-	5, 6α, 6β, 14		
8	60.52 (C)	60.52	-	9, 11, 14		
9	49.42 (CH)	49.40	2.17 (d ,3.48)	1, 14, 19, 30a, 30b	11	1, 5, 11
10	46.90 (C)	46.89	-	1, 1-OH, 5, 6β, 9, 19		
11	70.10 (CH)	70.09	4.76 (m)	9, 11-OH	9, 12	1, 9, 12
11-OH	-	-	3.54 (d)			
12	83.74 (CH)	83.74	4.31 (d, 3.48)	14, 18	11	11, 18
13	87.67 (C)	87.66	-	14, 18, 30b		
14	56.27 (CH)	56.25	3.60 (s)	9, 18, 30b		18, 30a
15	171.94 (C)	171.94	-			
18	20.63 (CH_3)	20.61	1.55 (s)	14		12, 14
19	10.58 (CH_3)	10.58	1.29 (s)	1, 9		6β, 30b
28	21.90 (CH_3)	21.89	1.92 (s)			3, 5, 6α, 6β
30	75.48 (CH_2)	75.45	a) 3.81 (d, 8.24)	9	a) 30b	a) 14, 30b
			b) 4.82 (d, 8.24)		b) 30a	b) 19, 30a

5.3.6. Structural elucidation of compound vi: Cedronin (vi)



(vi)

The mass spectrum **[63]** of compound **(vi)** showed a molecular ion [M^+] peak at *m/z* 364.1527 corresponding to the molecular formula $C_{19}H_{24}O_7$. The peaks at *m/z* 349 and 331 indicated the loss of a methyl group fragment and the loss of a methyl group and the element of water respectively. The IR spectrum **[62]** of compound **(vi)** showed an O-H stretching band at 3468 cm^{-1}, and a broad C=O stretching band at 1733 cm^{-1}.

Inspection of the NMR spectra showed nineteen carbon resonances in the ^{13}C NMR spectrum **[65]**, and three methyl group proton resonances at δ_H 1.52 (s), 1.23 (s) and 1.93 (s) in the ^1H NMR spectrum **[64]** which suggested that compound **(vi)** was a C_{19} quassinoid. Further inspection of the ^{13}C NMR **[65]** and ADEPT **[66]** spectra showed the presence of two carbonyl groups, due to the C-2 carbonyl group (δ_C 197.60) and a lactone carbonyl group (δ_C 174.24), and, in addition, a trisubstituted double bond (C-4, δ_C 164.84; C-3, δ_C 124.06). Six oxygenated carbon resonances were noted, comprising, one fully substituted (C-13, δ_C 87.48), four oxymethine (C-1, δ_C 82.79; C-7, δ_C 71.25; C-11, δ_C 70.16; C-12, δ_C

84.96) and one oxymethylene carbon resonances (C-30, δ_C 74.90).

A literature survey conducted on this basis revealed that compound (**vi**) might be cedronin, previously isolated from *Simaba cedron* Planch.[46] However, no assignment of the ^{13}C NMR data for cedronin has been reported.

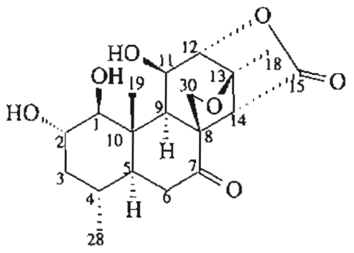
The molar mass of compound (**vi**) showed a difference, relative to compound (**v**) of two extra protons. The NMR spectra of compound (**vi**) were very similar to those of compound (**v**), except that the C-7 carbonyl carbon resonance at δ_C 203.37 in compound (**v**) was replaced by the oxymethine carbon resonance at δ_C 71.25 in compound (**vi**). The C-7 carbon resonance showed a correlation to the H-14 proton resonance at δ_H 2.66 (s) in the HMBC NMR spectrum **[68]**, and the H-7 proton resonance at δ_H 3.97 (s) showed correlations to the two C-6 methylene proton resonances at δ_H 1.58 and 2.17 in the COSY NMR spectrum **[69]**. The H-7 proton resonance also showed correlations to the H-14 and the H-30a (δ_H 3.71) proton resonances in the NOESY NMR spectrum **[70]**, which indicated that the H-7 proton was β orientated and this confirmed the α orientation of the H-7 hydroxy group.

The NMR data for cedronin (**vi**) is shown in table 5.6.

Table 5.6. : NMR data for cedronin (vi)

C	δ^{13}C / ppm ($CDCl_3$)	δ^1H / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	82.79 (CH)	4.09 (bs)	19	1-OH	1-OH, 5, 9, 11
1-OH		4.41 (bs)		1	1, 11-OH
2	197.60 (C)	-	1, 1-OH		
3	124.06 (CH)	6.06 (s)	28	5, 28	28
4	164.84 (C)	-	6α, 28		
5	42.48 (CH)	3.22 (bd, 12.27)	6α, 19, 28	3, 6β, 28	1, 9
6	29.06 (CH_2)	α) 2.17 (m)		α) 6β, 7	α) 6β, 7, 28
		β) 1.58 (m)		β) 5, 6α, 7, 9	β) 6α, 7
7	71.25 (CH)	3.97 (bs)	14	6β, 6α	6β, 6α, 14, 30a
8	54.37 (C)	-	9, 14, 30b		
9	44.07 (CH)	2.34 (d, 4.76)	1, 7, 12, 14, 19, 30a, 30b	6β, 11	1, 5, 11
10	47.97 (C)	-	1, 1-OH, 6β, 9, 19		
11	70.16 (CH)	4.82 (bs)	12	9, 12, 11-OH	1, 9, 12
11-OH		3.38 (bs)		11	1-OH
12	84.96 (CH)	4.32 (d, 3.11)	14, 18	11	11, 18
13	87.48 (C)	-	14, 18, 30b		
14	59.71 (CH)	2.66 (s)	18, 30b		7, 18, 30a
15	174.24 (C)	-	12, 14, 30b		
18	20.98 (CH_3)	1.52 (s)	12, 14		12, 14
19	11.39 (CH_3)	1.23 (s)	1, 9		30b
28	22.73 (CH_3)	1.93 (s)		3, 5	3, 6α
30	74.90 (CH_2)	a) 3.71 (d, 8.97)	7, 9	a) 30b	a) 7, 14, 30b
		b) 4.64 (d, 8.97)		b) 30a	b) 19, 30a

5.3.7. Structural elucidation of compound vii: 3, 4β-Dihydrosamaderine C (vii)



(vii)

The mass spectrum **[76]** of compound (**vii**) showed a highest peak $[M+H]^+$ at *m/z* 367.1758, indicating a molecular formula $C_{19}H_{26}O_7$ for the molecule. The peaks at *m/z* 349 and 331 indicated the loss of the elements of water. The IR spectrum **[75]** of compound (**vii**) showed an O-H stretching band at 3428 cm^{-1}, and a broad C=O stretching band at 1736 cm^{-1}.

Inspection of the ^1H **[77]** and ^{13}C NMR **[78]** spectra of compound (**vii**) showed them to be very similar to those of compound (**vi**). The carbonyl carbon resonance at δ_C 205.17 in the ^{13}C NMR spectrum **[78]** of compound (**vii**) showed correlations to the H-14 resonance at δ_H 3.55 (s) and to the two methylene C-6 proton resonances at δ_H 2.18 and 2.70 in the HMBC NMR spectra **[81-82]**. This carbonyl resonance (δ_C 205.17) was therefore assigned to C-7. Minor resonances of another compound were seen in the spectra.

In addition, the oxymethine carbon resonance at δ_C 70.90 correlated to the proton resonance at δ_H 3.83 in the HSQC spectrum **[80]** of compound (**vii**). This proton resonance (δ_H 3.83) showed correlations to the H-1 and two H-3 proton resonances at δ_H 3.30, 1.14 and 1.97 in the COSY NMR spectrum **[83]** respectively, and was assigned to H-2. The oxymethine carbon resonance (δ_C 70.90) was therefore assigned to C-2. The fully substituted double bond C-4 resonance at δ_C 164.84 and the methine double bond C-3 resonance at δ_C 124.06 in the ^{13}C NMR spectrum **[78]** of compound (**vi**) were replaced by a methine carbon resonance and a methylene carbon resonance at δ_C 29.76 and δ_C 41.50 respectively in the ^{13}C NMR spectrum **[78]** of compound (**vii**), indicating reduction of the double bond.

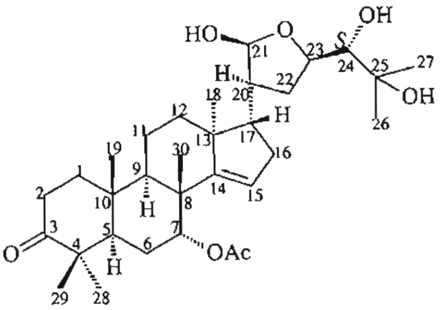
The H-5 and H-9 protons are α orientated from biosynthetic grounds and were seen to be correlated to each other in the NOESY NMR spectrum [**84**], as expected. Since the H-9 resonance at δ_H 1.83 (d, J = 4.58) showed correlations to the H-1 and H-11 (δ_H 4.61) resonances in the NOESY NMR spectrum **[84]**, the hydroxy groups at C-1 and C-11 were assigned the β orientation. The J_{H1-H2} value of 8.79 *Hz* indicated an axial/axial interaction for H-1/ H-2. Therefore H-2 was assigned the β orientation and the hydroxy group at C-2 was assigned the α orientation. This suggested that the 2-keto, 7α-hydroxy substitution pattern of compound **(vi)** has been reversed in compound **(vii)** to give the 2α-hydroxy, 7-keto structure that characterises samaderine C, previously isolated from *Samadera indica*[11] and *Quassia indica.*[40] However, no double bond is observed in compound (**vii**).

This indicated that compound (**vii**) is indeed 3, 4β-dihydrosamaderine C, which has not been reported previously. The NMR data for 3, 4β-dihydrosamaderine C (**vii**) is shown in table 5.7.

Table 5.7. : NMR data for 3, 4β-dihydrosamaderine C (vii)

C	δ^{13}C / ppm (CDCl$_3$)	Samaderine C δ^{13}C / ppm (pyridine-d$_5$) Lit.[40]	δ^{1}H / ppm (CDCl$_3$)	HMBC (C→H)	COSY	NOESY
1	81.77 (CH)	81.5	3.30 (d, 8.79)	3β, 19	2	2, 5, 9, 11
2	70.90 (CH)	72.8	3.83 (m)	3β, 1	1, 3α, 3β	1, 3β, 19
3	41.50 (CH$_2$)	127.0	α) 1.14 (m)	28	α) 2, 3β	α) 1, 3β, 5, 28
			β) 1.97 (m)	1	β) 2, 3α	β) 2, 3α, 28
4	29.76 (CH)	133.0	1.56 (m)	3β, 6β, 28	28	5, 28
5	50.80 (CH)	47.9	1.31 (m)	6β, 9, 19, 28	6β, 6α	1, 3α, 4, 9, 28
6	40.16 (CH$_2$)	39.6	α) 2.70 (dd, 4.58, 17.95)		α) 5, 6β	α) 6β, 28
			β) 2.18 (dd, 4.58, 15.75)		β) 5, 6α	β) 6α, 19
7	205.17 (C)	206.1	-	6β, 6α, 14		
8	60.24 (C)	61.6	-	6α, 9, 14		
9	50.25 (CH)	50.5	1.83 (d, 4.58)	1, 12, 14, 19, 30b	11	1, 5, 11
10	42.42 (C)	43.9	-	1, 5, 6α, 9, 19		
11	70.39 (CH)	71.2	4.61 (bs)	9	9, 12, 11-OH	1, 9, 12, 11-OH
11-OH			3.96 (bs)		11	11
12	83.53 (CH)	85.1	4.28 (d, 3.66)	14, 18, 11-OH	11	11, 18
13	87.76 (C)	87.8	-	14, 18, 30b		
14	56.66 (C	57.1	3.55 (s)	9, 18, 30b		18, 30a
15	172.13 (C)	172.8	-	12		
18	20.64 (CH$_3$)	20.2	1.53 (s)	14		12, 14
19	11.98 (CH$_3$)	11.1	1.35 (s)	1, 9		2, 6β, 30b
28	18.92 (CH$_3$)	20.7	0.85 (d, 6.59)	3β	4	3α, 3β, 4, 5, 6α
30	75.80 (CH$_2$)	76.3	a) 3.83 (d, 8.24)	9	a) 30b	a) 14, 30b
			b) 4.88 (d, 8.24)		b) 30a	b) 19, 30a

5.3.8. Structural elucidation of Compound viii: Protosamaderine A (viii)



(viii)

The low resolution mass spectrum **[90]** of compound (**viii**) showed a molecular ion [M$^+$] peak at *m/z* 546, corresponding to the molecular formula $C_{32}H_{50}O_7$. The peak at *m/z* 492 [M-54]$^+$ corresponded to the loss of elements of three water molecules and the peak at *m/z* 432 [M-114]$^+$ corresponded to the loss of the elements of three water molecules together with one acetic acid molecule. The IR spectrum **[89]** of compound (**viii**) showed an O-H stretching band at 3436 cm^{-1}, and a broad C=O stretching band at 1717 cm^{-1}.

Inspection of the ^{13}C NMR spectrum **[92]** showed the presence of some minor peaks paired with the major peaks, indicating a mixture of epimers. This is usually observed for a five membered hemiacetal ring, in which equilibrium at a rapid rate in solution results in two C-21 epimers.[47] The major peaks have been assigned in this work. Seven methyl group proton resonances were present in the ^{1}H NMR spectrum **[91]** at δ_H 0.98 (6H) (s), 1.01 (s), 1.02 (s), 1.11 (s), 1.23 (s) and 1.27 (s). One of the two resonances at δ_H 0.98 (s) and the resonance at δ_H 1.01 (s) were assigned to 3H-28 and 3H-29 by the correlations in the HMBC NMR spectra **[95-96]** to the C-3 ketone carbonyl carbon resonance at δ_C 216.85, and to the fully substituted carbon resonance at δ_C 46.96, which was assigned to C-4. These two methyl group proton resonances showed correlations in the HMBC NMR spectra **[95-96]** to the methine carbon resonance at δ_C 48.31, which was assigned to C-5. The C-5 resonance showed a correlation with the methyl group proton resonance at δ_H 1.02 (s) in the HMBC NMR spectra **[95-96]**, which was thus assigned to 3H-19. The C-19 resonance (δ_C 15.07) showed a correlation with the methine proton resonance at δ_H 1.99 in the HMBC NMR spectra **[95-96]**, which was assigned to H-9. The corresponding C-9 resonance at δ_C 42.72 showed a correlation with the methyl proton resonance at δ_H 1.11 (s) in the HMBC NMR spectra **[95-96]**, which was assigned to 3H-30.

The 3H-30 resonance showed correlations in the HMBC NMR spectra **[95-96]** with the double bond carbon resonance at δ_C 159.13 and the oxymethine carbon resonance at δ_C 74.95, which were assigned to C-14 and C-7 respectively. The H-7 resonance at δ_H 5.19 (dd, J = 1.65, 3.94 Hz) showed a correlation in the HMBC NMR spectra **[95-96]** with the acetoxy carbonyl carbon resonance at δ_C 170.19, indicating that the acetoxy group was at C-7. The correlation between the H-7 and 3H-30 resonances in the NOESY NMR spectra **[98-99]** confirmed that H-7 was β orientated and the acetoxy group was therefore α orientated, as expected on biosynthetic grounds (page 26).

The C-14 resonance showed a correlation in the HMBC NMR spectra **[95-96]** with the methyl proton resonance at δ_H 0.98 (s), which was assigned to 3H-18. The remaining two methyl group proton resonances at δ_H 1.23 (s) and 1.27 (s), assigned to 3H-26 and 3H-27, showed correlations in the HMBC NMR spectra **[95-96]** to the fully substituted carbon resonance at δ_C 73.00 and to the oxymethine carbon resonance at δ_C 75.03, which were assigned to C-25 and C-24 of the side chain respectively.

The H-24 resonance δ_H 3.17 (bs) showed a correlation to the proton resonance at δ_H 4.44 (m) in the COSY NMR spectrum **[97]**, which was assigned to H-23. The C-24 resonance showed a correlation in the HMBC NMR spectra **[95-96]** with one of the methylene proton resonances at δ_H 1.95 (m), which was assigned to H-22β. The H-22α resonance at δ_H 1.83 (m) showed a correlation in the HMBC NMR spectra **[95-96]** with the hemiacetal carbon resonance at δ_C 97.00, which was assigned to C-21 as expected for this type of triterpenoid. The COSY spectrum **[97]** showed a correlation between the H-21 resonance at δ_H 5.26 and the proton resonance at δ_H 2.10, which was assigned to H-20. H-20 is α orientated on biosynthetic grounds (page 31).

The correlation between the H-21 and H-20 proton resonances in the NOESY NMR spectrum **[98-99]** indicated that H-21 was α orientated in the major epimer, and the hydroxy group at C-21 was therefore β orientated. The stereochemistry at C-24 was assigned by comparison to literature data (page 32). According to Puripattanavong *et al.*,[48] the H-24 proton resonances for the 24*S* and 24*R* epimers occur at δ_H 3.15 and 3.64 for compounds with the 21*S*,24,25-trihydroxy sidechains. Since previous reports[49,50] show that the H-24 proton resonance occurs at ~ δ_H 3.2 for the 24*S* configuration, it is proposed that compound **(viii)** has the *S* configuration at C-24. This can be explained by the mechanism shown in page 31, where the ring opening of the epoxide, as in compounds **(ix)** and **(x)**, results in the retention of the 24*S* configuration. Although the stereochemistry at C-24 has been deduced by comparing chemical shift values with those of similar compounds in literature, this stereochemistry can be confirmed by x-ray anaylsis.

Compound **(viii)** is a Δ^{14} protolimonoid, 21*S*,23*R*-epoxy-7α-acetoxy-21*S*,24*S*,25-trihydroxyapotirucalla-14-en-3-one. Compound **(viii)** has not been reported previously and was given the common name protosamaderine A **(viii)**. The NMR data for protosamaderine A **(viii)** is shown in table 5.8.

Table 5.8. : NMR data for protosamaderine A (viii) (The major component)

C	$\delta^{13}C$ / ppm (CDCl$_3$)	δ^1H / ppm (CDCl$_3$)	HMBC (C→H)	COSY	NOESY
1	38.82 (CH$_2$)	α) 1.47 (m)	19, 2β	α) 1β, 2α, 2β	α) 1β
		β) 1.89 (m)		β) 1α, 2α, 2β	β) 1α, 19
2	33.98 (CH$_2$)	α) 2.38 (m)	1α, 1β	α) 2β, 1α, 1β	α) 2β
		β) 2.56 (m)		β) 2α, 1α, 1β	β) 2α, 19, 29
3	216.85 (C)	-	1α, 1β, 2α, 2β, 28, 29		
4	46.96 (C)	-	5, 6β, 28, 29		
5	48.31 (CH)	1.79 (m)	1β, 6α, 6β, 7, 9, 19, 28, 29	6α, 6β	9, 28
6	24.23 (CH$_2$)	α) 1.67 (m)	5	α) 5, 6β, 7	α) 6β, 7, 28
		β) 1.83 (m)		β) 5, 6α, 7	β) 6α, 7, 19, 29, 30
7	74.95 (CH)	5.19 (bs)	5, 6α, 9, 30	6α, 6β	6α, 6β, 15, 30
8	41.96 (C)	-	6α, 9, 11α, 30		
9	42.72 (CH)	1.99 (m)	1α, 7, 19, 30	11β, 11α	5, 18
10	37.02 (C)	-	1α, 2α, 2β, 5, 6α, 6β, 9, 11α, 19		
11	16.43 (CH$_2$)	α) 1.67 (m)	9	α) 9, 11β, 12β, 12α	α) 18
		β) 1.56 (m)		β) 9, 11α, 12β, 12α	β) 19
12	33.51 (CH$_2$)	α) 1.75 (m)	18	α) 11β, 11α, 12β	α) 12β, 21
		β) 1.56 (m)		β) 11β, 11α, 12α	β) 12α, 26, 27
13	46.40 (C)	-	12α, 15, 18, 20		
14	159.13 (C)	-	9, 12α, 16α, 16β, 18, 30		
15	118.79 (CH)	5.26 *	16α, 16β	16α, 16β	7, 16β, 30
16	35.07 (CH$_2$)	α) 1.99 (m)	15	α) 15, 16β, 17	α) 16β, 18
		β) 2.06 (m)		β) 15, 16α, 17	β) 15, 16α, 17
17	52.83 (CH)	1.89 (m)	16α, 18, 21	16α, 16β, 20	16β
18	20.15 (CH$_3$)	0.98 (s)	12β		11α, 16α, 20, 21, 22α, 22β
19	15.07 (CH$_3$)	1.02 (s)	1α, 1β, 9		1β, 6β, 11β, 30
20	44.90 (CH)	2.10 (m)		17, 21, 22α, 22β	18, 21, 22α, 22β
21	97.00 (CH)	5.26 *	17, 22α	20	12α, 18, 20
22	29.90 (CH$_2$)	α) 1.83 (m)		α) 20, 22β, 23	α) 18, 20, 23
		β) 1.95 (m)		β) 20, 22α, 23	β) 18, 20
23	78.55 (CH)	4.44 (m)	17, 21	22α, 22β, 24	22α, 24, 26, 27
24	75.03 (CH)	3.17 (bs)	22β, 23, 26, 27	23	23, 26, 27
25	73.00 (C)	-	26, 27		
♣26	26.72 (CH$_3$)	1.27 (s)	27		12β, 27
♣27	26.68 (CH$_3$)	1.23 (s)	26		12β, 26
28	25.79 (CH$_3$)	0.98 (s)	5, 29		5, 6α, 29
29	21.05 (CH$_3$)	1.01 (s)	5, 28		6β, 28
30	26.96 (CH$_3$)	1.11 (s)	9		6β, 7, 15, 19
7-O$\underline{C}$OCH$_3$	170.19 (C)	-	7, 7-OCOC$\underline{H}_3$		
7-OCO$\underline{C}$H$_3$	21.20 (CH$_3$)	1.93 (s)			

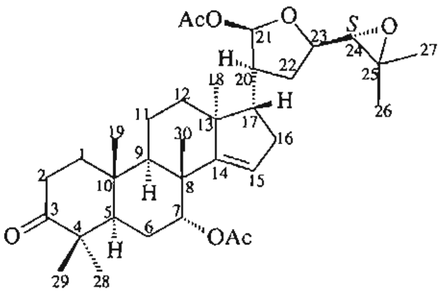
♣ interchangeable resonances

* resonances superimposed

Structural elucidation of Compounds (ix) to (xviii)

Compounds (**ix**) to (**xviii**) were found to be closely related to compound (**viii**) and to each other, differing only in the nature and location of a number of methoxy and acetoxy substituents on a common skeleton. The ^1H and ^{13}C NMR spectra of all ten compounds showed the presence of seven quaternary methyl groups, a 14,15-trisubstituted double bond, an acetal linkage (C-21), together with three other oxymethine carbons (C-7, C-23, C-24), a fully substituted oxygenated carbon (C-25), and a ketone carbonyl carbon (C-3). Thus, the structural elucidations of compounds (**ix**) to (**xviii**) concentrate only on the specific differences between them. For several of these compounds the molecular ion was not detected in the mass spectrum. This agrees with previous reports on the mass spectra of the related holstinones where the molecular ion was not detected but fragment ions resulting from the loss of water, methanol or acetic acid were seen.[42]

5.3.9. Structural elucidation of Compound ix: 1,2-Dihydrobruceajavanin A (ix)



(**ix**)

The IR spectrum [**101**] of compound (**Ix**) showed a broad C=O stretching band at 1737 cm^{-1}.

Inspection of the ^1H [**103**] and ^{13}C NMR [**104**] spectra of compound (**ix**) showed them to be very similar to those of compound (**viii**), however, the spectra of compound (**ix**) are pure with no paired peaks as observed for compound (**viii**). This indicated that no hemiacetal ring was present, but rather an acetal. Acetals may be isolated in a pure form as the ring does not open and re-close in solution. Inspection of the 2D NMR spectra showed that an additional acetoxy carbonyl carbon resonance at δ_C 170.20 showed a correlation in the HMBC NMR spectra [**108-109**] to the H-21 proton resonance at δ_H 6.22, indicating that the acetoxy group was attached at C-21 (δ_C 96.59). The C-25 and C-24 carbon resonances had shifted from δ_C 73.00 and 75.03 in the ^{13}C NMR spectrum [**92**] of compound (**viii**) respectively to δ_C 57.16 and 66.69 in the ^{13}C NMR spectrum [**104**] of compound (**ix**) respectively. This indicated the presence of the 24,25-epoxide ring.

The C-21 resonance at δ_C 96.59 showed very little change to that observed in compound (**viii**). The correlation between the H-7 (δ_H 5.20) and the 3H-30 (δ_H 1.11) resonances in the NOESY NMR spectra [**111-112**] confirmed that H-7 was β orientated and the acetoxy group was therefore α orientated as expected for these protolimonoids on biosynthetic grounds.

As observed in compound **(viii)**, the H-21 proton resonance at δ_H 6.22 showed a correlation with the H-20 resonance (δ_H 2.31) in the NOESY NMR spectra **[111-112]**, indicating that H-21 was α orientated and the acetoxy group at C-21 was therefore β orientated.

Inspection of literature showed that compound **(ix)** is the known 1,2-dihydrobruceajavanin A previously isolated from *Brucea javanica* (Simaroubaceae).[43]

From the NMR data of compound **(ix)** the molecular formula was deduced to be $C_{34}H_{50}O_7$, which requires 570. The low resolution mass spectrum **[102]** of compound **(ix)** showed peaks at *m/z* 510 $[M-60]^+$ and 450 $[M-120]^+$, corresponding to the loss of elements of one and two molecules of acetic acid respectively.

1,2-Dihydrobruceajavanin A **(ix)** has been reported to exhibit inhibitory activities (IC_{50} 2.5 μM, IC_{90} 4.3 μM) against the cultured malarial parasite *Plasmodium falciparum* of a chloroquine-resistant K1 strain in human erythrocytes.[43]

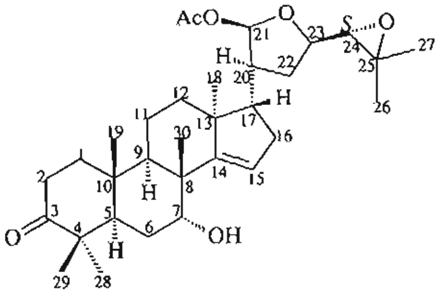
The NMR data for 1,2-dihydrobruceajavanin A **(ix)** is shown in table 5.9.

Table 5.9. : NMR data for 1,2-dihydrobruceajavanin A (ix)

C	$\delta^{13}C$ / ppm ($CDCl_3$)	$\delta^{13}C$ / ppm ($CDCl_3$) Lit.[43]	$\delta^{1}H$ / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	38.65 (CH_2)	38.6	α) 1.46 (m)	2α, 2β, 5, 9, 19	α) 1β, 2α, 2β	α) 1β, 2α
			β) 1.87 (m)		β) 1α, 2α, 2β	β) 1α, 19, 29
2	33.92 (CH_2)	33.9	α) 2.39 (m)		α) 2β, 1α, 1β	α) 1α, 2β
			β) 2.55 (m)		β) 2α, 1α, 1β	β) 2α, 19, 29
3	216.69 (C)	216.6	-	1β, 2α, 2β, 28, 29		
4	46.88 (C)	46.8	-	5, 6β, 28, 29		
5	48.19 (CH)	48.2	1.80 (m)	1α, 1β, 6α, 6β, 7, 9, 19, 28, 29	6α, 6β	6α, 9, 28
6	24.22 (CH_2)	24.2	α) 1.66 (m)	5	α) 5, 6β, 7	α) 5, 7
			β) 1.87 (m)		β) 5, 6α, 7	β) 7, 29, 30
7	74.85 (CH)	74.8	5.20 (bs)	5, 6α, 9, 30	6α, 6β	6α, 6β, 15, 30
8	41.92 (C)	41.9	-	6α, 7, 9, 11α, 30		
9	42.42 (CH)	42.4	2.04 (m)	1α, 7, 12α, 19, 30	11β, 11α	5, 11α, 18
10	36.99 (C)	37.1	-	1α, 1β, 2α, 5, 6α, 6β, 9, 11α, 19		
11	16.38 (CH_2)	16.4	α) 1.66 (m)	9, 12β	α) 9, 11β, 12β, 12α	α) 9, 11β, 12α, 18
			β) 1.53 (m)		β) 9, 11α, 12β, 12α	β) 11α, 19
12	32.75 (CH_2)	32.7	α) 1.58 (m)	18	α) 11β, 11α, 12β	α) 11α, 12β, 18, 21
			β) 1.26 (m)		β) 11β, 11α, 12α	β) 12α, 30
13	46.44 (C)	46.4	-	12α, 15, 18, 17		
14	159.16 (C)	161.5	-	9, 12α, 16α, 16β, 18, 30		
15	118.50 (CH)	119.6	5.26 (bs)	16α, 16β	16α, 16β	7, 16α, 16β, 30
16	35.14 (CH_2)	35.1	α) 2.03 (m)	15	α) 15, 16β, 17	α) 15, 16β
			β) 2.10 (m)		β) 15, 16α, 17	β) 15, 16α, 17
17	52.52 (CH)	52.5	1.87 (m)	15, 16α, 18, 22β	16α, 16β, 20	16β
18	19.82 (CH_3)	19.8	0.98 (s)	17		9, 11α, 12α, 20, 21, 22β
19	15.02 (CH_3)	15.0	1.01 (s)	1α, 1β, 5, 9, 11β		1β, 2β, 11β, 30
20	44.30 (CH)	44.3	2.31 (m)	21, 22β, 22α	17, 21, 22β, 22α	18, 21, 22α, 23
21	96.59 (CH)	96.6	6.22 (d, 4.03)	21-$OCOC\underline{H}_3$, 23	20	12α, 18, 20
22	31.34 (CH_2)	31.3	α) 2.03 (m)	20, 21	α) 20, 22β, 23	α) 20, 22β, 23
			β) 1.64 (m)		β) 20, 22α, 23	β) 18, 22α, 24
23	79.75 (CH)	79.7	3.88 (m)	21, 22β, 22α, 24	22β, 22α, 24	20, 22α, 24, 27
24	66.69 (CH)	66.7	2.64 (d, 7.51)	22β, 23, 26, 27	23	22β, 23, 26
25	57.16 (C)	57.1	-	24, 26, 27		
♣26	24.88 (CH_3)	19.3	1.30 (s)	24, 27		24, 27
♣27	19.30 (CH_3)	24.9	1.26 (s)	26		23, 26
28	25.85 (CH_3)	21.0	0.98 (s)	5, 29		5
29	21.03 (CH_3)	25.8	1.00 (s)	5, 28		1β, 2β, 6β
30	27.05 (CH_3)	27.0	1.11 (s)	9		6β, 7, 12β, 15, 19
7-$O\underline{C}OCH_3$	170.03 (C)	170.0	-	7, 7-$OCOC\underline{H}_3$		
7-$OCO\underline{C}H_3$	21.18 (CH_3)	21.1	1.92 (s)			
21-$O\underline{C}OCH_3$	170.20 (C)	170.2	-	21, 21-$OCOC\underline{H}_3$		
21-$OCO\underline{C}H_3$	21.50 (CH_3)	21.4	2.03 (s)			

♣ interchangeable resonances

5.3.10. Structural elucidation of Compound x: Chisocheton compound A (x)



(x)

The low resolution mass spectrum **[115]** of compound (**x**) showed a molecular ion [M$^+$] peak at *m/z* 528, corresponding to the molecular formula $C_{32}H_{48}O_6$. The peak at *m/z* 510 $[M-18]^+$ indicated the loss of the element of one water molecule and the peak at *m/z* 468 $[M-60]^+$ indicated the loss of the element of one acetic acid molecule. The IR spectrum **[114]** of compound (**x**) showed an O-H stretching band at 3458 cm^{-1}, and C=O stretching bands at 1741 and 1705 cm^{-1}.

Inspection of the ^{1}H **[116]** and ^{13}C NMR **[117]** spectra of compound (**x**) showed them to be very similar to those of compound (**ix**), except that the oxymethine C-7 resonance had shifted from δ_C 74.85 in compound (**ix**) to δ_C 71.92 in compound (**x**), and the H-7 proton resonance shifted from δ_H 5.20 in compound (**ix**) to δ_H 3.94 in compound (**x**), indicating that C-7 no longer had a acetoxy group attached to it. Inspection of the 2D NMR spectra showed that the acetoxy carbonyl carbon resonance at δ_C 169.96 showed a correlation in the HMBC NMR spectra **[121-123]** to the H-21 proton resonance at δ_H 6.23, indicating that the acetoxy group was attached at C-21 (δ_C 96.56).

The H-21 (δ_H 6.23) proton resonance, showed a correlation with the H-20 (δ_H 2.32) resonance in the NOESY NMR spectra **[126-127]**, indicating that H-21 was α orientated and the acetoxy group at C-21 was therefore β orientated. The correlations of the H-20 and H-23 (δ_H 3.89) proton resonances with one of the H-22 resonance (δ_H 2.06), and the correlation of the H-24 resonance (δ_H 2.65) with the other H-22 resonance (δ_H 1.68) in the NOESY NMR spectra **[126-127]** confirmed that the C-23, C-24 bond was β orientated as expected.

The stereochemistry at C-24 is the same as in compound (**ix**) as 24*S* since C-24, H-24 and C-25 in compound (**x**) have similar chemical shifts as observed for compound (**ix**). The same coupling constant for H-24 is observed in the ^{1}H NMR spectra for both compound (**ix**) and compound (**x**).

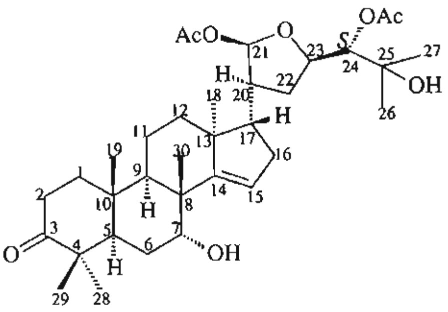
Inspection of the literature revealed that compound (**x**) is the known chisocheton compound A previously isolated from *Chisocheton paniculatus* (Meliaceae).[51] The NMR data for chisocheton compound A (**x**) is shown in table 5.10.

Table 5.10. : NMR data for chisocheton compound A (x)

C	δ^{13}C / ppm (CDCl$_3$)	δ^{13}C / ppm (CDCl$_3$) Lit.[51]	δ^{1}H / ppm (CDCl$_3$)	HMBC (C→H)	COSY	NOESY
1	38.43 (CH$_2$)	38.5	α) 1.49 (m)	2α, 9, 19	α) 1β, 2α, 2β	α) 1β, 9
			β) 1.82 (m)		β) 1α, 2α, 2β	β) 1α, 11α, 19
2	33.90 (CH$_2$)	33.9	α) 2.42 (m)	1β	α) 2β, 1α, 1β	α) 2β
			β) 2.50 (m)		β) 2α, 1α, 1β	β) 2α, 19
3	217.28 (C)	217.2	-	1β, 2α, 2β, 28, 29		
4	46.88 (C)	46.9	-	5, 28, 29		
5	46.58 (CH)	46.5	2.02 (m)	19, 28, 29	6α, 6β	28
6	24.76 (CH$_2$)	24.9	α) 1.75 (m)	5	α) 5, 6β, 7	α) 7, 28
			β) 1.78 (m)		β) 5, 6α, 7	β) 7, 19, 29, 30
7	71.92 (CH)	71.9	3.94 (bs)	5, 6β, 30	6α, 6β	6α, 6β, 15, 30
8	44.03 (C)	44.0	-	6α, 9, 30		
9	40.76 (CH)	40.8	1.97 (m)	1α, 7, 11β, 19, 30	11β, 11α	1α, 18
10	37.16 (C)	37.1	-	1β, 2α, 5, 6β, 9, 19		
11	16.24 (CH$_2$)	16.3	α) 1.67 (m)	9	α) 9, 11β, 12β, 12α	α) 1β, 11β
			β) 1.54 (m)		β) 9, 11α, 12β, 12α	β) 11α, 12 β, 19, 30
12	32.28 (CH$_2$)	32.3	α) 1.56 (m)	18	α) 11β, 11α, 12β	α) 12β, 18
			β) 1.25 (m)		β) 11β, 11α, 12α	β) 11β, 12α, 17, 22α, 30
13	46.47 (C)	46.5	-	11β, 15, 17, 18		
14	161.58 (C)	161.5	-	18, 30		
15	119.59 (CH)	119.6	5.48 (bs)		16α, 16β	7, 16α, 16β, 30
16	35.06 (CH$_2$)	35.1	α) 2.18 (m)	15, 17	α) 15, 16β, 17	α) 15, 18
			β) 2.20 (m)		β) 15, 16α, 17	β) 15, 17
17	52.58 (CH)	52.6	1.89 (m)	15, 18	16α, 16β, 20	12β, 16β, 22β
18	19.68 (CH$_3$)	19.7	1.01 (s)	17		9, 12α, 16α, 20, 21
19	14.92 (CH$_3$)	14.9	0.98 (s)	1β, 5, 9		1β, 2β, 6β, 11β, 29, 30
20	44.18 (CH)	44.2	2.32 (m)	17, 21, 22β	17, 21, 22β, 22α	18, 21, 21-OCOC$\underline{\text{H}}_3$, 22α
21	96.56 (CH)	96.6	6.23 (d, 4.03)	22α	20	18, 20, 22β
22	31.36 (CH$_2$)	31.3	α) 2.06 (m)	21	α) 20, 22β, 23	α) 12β, 20, 22β, 23
			β) 1.68 (m)		β) 20, 22α, 23	β) 17, 22α, 24
23	79.73 (CH)	79.7	3.89 (m)	21, 24	22β, 22α, 24	22α, 24, 27
24	66.68 (CH)	66.7	2.65 (d, 7.51)	23, 26, 27	23	22β, 23, 26
25	57.16 (C)	57.1	-	24, 26, 27		
*26	24.90 (CH$_3$)	24.9	1.31 (s)	24, 27		23, 24, 27
*27	19.33 (CH$_3$)	19.3	1.27 (s)	26		23, 24, 26
28	26.21 (CH$_3$)	26.2	1.07 (s)	5, 29		5, 6α, 29
29	21.15 (CH$_3$)	21.1	1.02 (s)	5, 28		6β, 19, 28
30	27.24 (CH$_3$)	27.2	1.07 (s)			6β, 7, 11β, 12β, 15, 19
21-O$\underline{\text{C}}$OCH$_3$	169.96 (C)	170.0	-	21, 21-OCOC$\underline{\text{H}}_3$		
21-OCO$\underline{\text{C}}$H$_3$	21.50 (CH$_3$)	21.5	1.93 (s)			20

* interchangeable resonances

5.3.11. Structural elucidation of Compound xi: Protosamaderine B (xi)



(xi)

The IR spectrum **[129]** of compound (**xi**) showed an O-H stretching band at 3490 cm^{-1}, and a broad C=O stretching band at 1738 cm^{-1}.

Inspection of the NMR spectra showed the presence of minor impurity peaks that have been crossed out. The ^{1}H **[131]** and ^{13}C NMR **[132]** spectra of compound (**xi**) were very similar to those of compound (**x**), except that the oxymethine C-24 and the fully substituted C-25 resonances had shifted from δ_C 66.68 and 57.16 respectively in compound (**x**) to δ_C 77.43 and 72.04 respectively in compound (**xi**). The H-24 proton resonance shifted from δ_H 2.65 in compound (**x**) to δ_H 4.75 in compound (**xi**). Inspection of the 2D NMR spectra showed that the additional acetoxy carbonyl resonance at δ_C 170.71 showed a HMBC **[136-137]** correlation to the H-24 proton resonance at δ_H 4.75, indicating that the acetoxy group was attached at C-24. This suggested that the 24,25-epoxide ring had opened resulting in the 24,25-diol followed by subsequent acetylation of the secondary hydroxy group at C-24.

As previously observed, the correlation between the H-20 (δ_H 2.30) and H-21 (δ_H 6.06) proton resonances in the NOESY NMR spectra **[139-140]** indicated that H-21 was α orientated and the C-21 acetoxy group was therefore β orientated. The correlation between the C-21 and C-24 acetoxy group proton resonances in the NOESY NMR spectra **[139-140]** confirmed that the C-21 acetoxy group was β orientated and H-21 was therefore α orientated.

The stereochemistry at C-24 has been assigned by comparison to literature.[49] The H-24 proton resonance occurs at the similar chemical shift and has the similar coupling constant as that reported by Zeng *et al.* for the diacetate of meliavolkenin which has a C-21β acetoxy group and a *S* configuration at C-24.

From the NMR data of compound (**xi**) the molecular formula was deduced to be $C_{34}H_{52}O_8$, which requires 588. The low resolution mass spectrum **[130]** of compound (**xi**) showed peaks at *m/z* 528 [M-60]$^+$ and 468 [M-120]$^+$, corresponding to the loss of the elements of one and two molecules of acetic acid respectively.

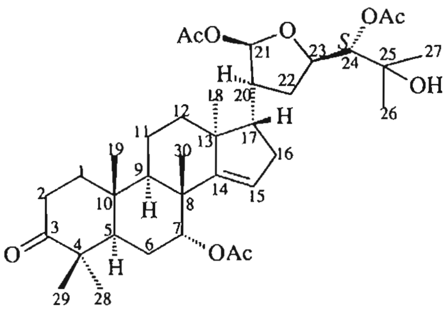
Compound (**xi**) is the 21*S*,23*R*-epoxy-21*S*,24*S*-diacetoxy-7,25-dihydroxyapotirucalla-14-en-3-one. Compound (**xi**) has not been reported previously and was given the common name protosamaderine B (**xi**). The NMR data for protosamaderine B (**xi**) is shown in table 5.11.

Table 5.11. : NMR data for protosamaderine B (xi)

C	$\delta^{13}C$ / ppm ($CDCl_3$)	$\delta^{1}H$ / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	38.43 (CH_2)	α) 1.47 (m)	2α, 2β, 19	α) 1β, 2α, 2β	α) 1β, 9
		β) 1.80 (m)		β) 1α, 2α, 2β	β) 1α, 11α, 19
2	33.89 (CH_2)	α) 2.40 (m)	1β	α) 2β, 1α, 1β	α) 2β
		β) 2.47 (m)		β) 2α, 1α, 1β	β) 2α, 19
3	217.29 (C)	-	1β, 2α, 2β, 28, 29		
4	46.59 (C)	-	5, 6α, 6β, 28, 29		
5	46.45 (CH)	2.03 (m)	1α, 1β, 7, 19, 28, 29	6α, 6β	9, 28
6	24.73 (CH_2)	α) 1.77 (m)	5	α) 5, 6β, 7	α) 7, 28, 29
		β) 1.80 (m)		β) 5, 6α, 7	β) 7, 19, 29, 30
7	71.89 (CH)	3.93 (bs)	30	6α, 6β	6α, 6β, 7-OH, 15, 30
7-OH	-	2.71 (bs)			7
8	44.00 (C)	-	9, 11β, 11α, 30		
9	40.75 (CH)	1.97 (m)	1α, 7, 12α, 19, 30	11β, 11α	1α, 5, 18
10	37.13 (C)	-	1α, 1β, 5, 9, 19		
11	16.22 (CH_2)	α) 1.69 (m)	9	α) 9, 11β, 12β, 12α	α) 1α, 11β, 18
		β) 1.52 (m)		β) 9, 11α, 12β, 12α	β) 11α, 19, 30
12	32.40 (CH_2)	α) 1.57 (m)	11α, 18	α) 11β, 11α, 12β	α) 12β, 18, 21
		β) 1.30 (m)		β) 11β, 11α, 12α	β) 12α, 17, 30
13	46.87 (C)	-	12α, 15, 18		
14	161.42 (C)	-	12α, 16α, 16β, 18, 30		
15	119.67 (CH)	5.47 (bs)	16α, 16β	16α, 16β	7, 16α, 16β, 30
16	34.99 (CH_2)	α) 2.12 (m)	15	α) 15, 16β, 17	α) 15, 17, 18
		β) 2.17 (m)		β) 15, 16α, 17	β) 15, 17
17	52.40 (CH)	1.85 (m)	15, 16α, 16β, 18, 20, 22β	16α, 16β, 20	12β, 16α, 16β
18	19.69 (CH_3)	0.99 (s)			9, 11α, 12α, 16α, 20, 21
19	14.90 (CH_3)	0.97 (s)	1α, 1β, 5, 9		1β, 2 β, 6β, 11β, 29, 30
20	43.88 (CH)	2.30 (m)	17, 21, 22β, 22α	17, 21, 22β	18, 21, 22α, 23
21	95.71 (CH)	6.06 (d, 4.21)	17, 22α, 23	20	12α, 18, 20
22	31.64 (CH_2)	α) 1.95 (m)	20, 21, 24	α) 22β, 23	α) 20, 22β, 23
		β) 1.61 (m)		β) 20, 22α, 23	β) 22α, 24
23	78.16 (CH)	4.45 (m)	21	22β, 22α, 24	20, 22α, 24, 26
24	77.43 (CH)	4.75 (d, 4.21)	22β, 23, 26, 27	23	22β, 23, 26, 27
25	72.04 (C)	-	24, 26, 27		
*26	27.21 (CH_3)	1.26 (s)	24, 27		23, 24, 27
*27	26.73 (CH_3)	1.15 (s)	24, 26		24, 26
28	26.18 (CH_3)	1.07 (s)	29		5, 6α, 29
29	21.13 (CH_3)	1.01 (s)	28		6β, 19, 28
30	27.18 (CH_3)	1.07 (s)			6β, 7, 11β, 12β, 15, 19
21-O$\underline{C}$OCH$_3$	170.25 (C)	-	21, 21-OCOC$\underline{H}_3$		
21-OCO$\underline{C}$H$_3$	21.32 (CH_3)	2.05 (s)			24-OCOC$\underline{H}_3$
24-O$\underline{C}$OCH$_3$	170.71 (C)	-	24, 24-OCOC$\underline{H}_3$		
24-OCO$\underline{C}$H$_3$	20.83 (CH_3)	2.13 (s)			21-OCOC$\underline{H}_3$

* interchangeable resonances

5.3.12. Structural elucidation of Compound xii: Protosamaderine C (xii)



(xii)

The IR spectrum **[142]** of compound (**xii**) showed an O-H stretching band at 3480 cm^{-1}, and a broad C=O stretching band at 1736 cm^{-1}.

Inspection of the ^{1}H **[144]** and ^{13}C NMR **[145]** spectra of compound (**xii**) showed them to be very similar to those of compound (**xi**), except that the oxymethine C-7 resonance had shifted from δ_C 71.89 in compound (**xi**) to δ_C 74.82 in compound (**xii**), just as in compound (**ix**) (C-7, δ_C 74.85) in the ^{13}C NMR spectra. The H-7 proton resonance shifted from δ_H 3.93 in compound (**xi**) to δ_H 5.22 in compound (**xii**), just as in compound (**ix**) (H-7, δ_H 5.20) in the ^{1}H NMR spectra. Inspection of the 2D NMR spectra showed that an additional acetoxy carbonyl resonance at δ_C 170.15 showed a correlation to the H-7 proton resonance at δ_H 5.22 in the HMBC NMR spectra **[149-151]**, indicating that the acetoxy group was attached at C-7α.

Again the correlation between the H-20 (δ_H 2.26) and H-21 (δ_H 6.04) proton resonances in the NOESY NMR spectra **[153-154]** indicated that H-21 was α orientated and the C-21 acetoxy group was β orientated. The stereochemistry at C-24 has been assigned in the same way as for compound (**xi**) since the same chemical shift and coupling constant for H-24 was observed in the NMR spectra for both compounds (**xi**) and (**xii**).

From the NMR data of compound (**xii**) the molecular formula was deduced to be $C_{36}H_{54}O_9$, which requires a mass of 630 g/mol. The low resolution mass spectrum **[143]** of compound (**xii**) showed peaks at *m/z* 510 [M-120]$^+$ and 450 [M-180]$^+$, corresponding to the loss of the elements of two and three molecules of acetic acid respectively in accordance with the proposed structure.

Compound (**xii**) is the 7-acetoxy analogue of compound (**xi**), 21*S*,23*R*-epoxy-25-hydroxy-7,21*S*,24*S*-triacetoxyapotirucalla-14-en-3-one. Compound (**xii**) has not been reported previously and was given the common name protosamaderine C (**xii**). The NMR data for protosamaderine C (**xii**) is shown in table 5.12.

Table 5.12. : NMR data for protosamaderine C (xii)

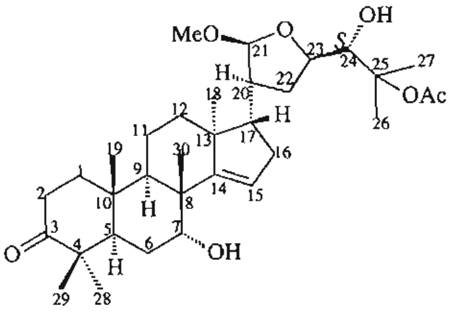
C	δ^{13}C / ppm ($CDCl_3$)	δ^{1}H / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	38.65 (CH_2)	α) 1.45 (m)	2α, 2β, 5, 9, 19	α) 1β, 2α, 2β	α) 1β, 28
		β) 1.86 (m)		β) 1α, 2α, 2β	β) 1α, 11α, 19
2	33.89 (CH_2)	α) 2.39 (m)	1α, 1β	α) 2β, 1α, 1β	α) 2β
		β) 2.54 (m)		β) 2α, 1α, 1β	β) 2α, 19
3	216.73 (C)	-	1β, 2α, 2β, 28, 29		
4	46.86 (C)	-	2α, 5, 6α, 28, 29		
5	48.17 (CH)	1.80 (m)	1β, 6 α, 6β, 7, 9, 19, 28, 29	6 α, 6β	28
6	24.18 (CH_2)	α) 1.64 (m)	5	α) 5, 6β, 7	α) 6β, 7, 29
		β) 1.86 (m)		β) 5, 6 α, 7	β) 6α, 7, 19, 29, 30
7	74.82 (CH)	5.22 (bs)	5, 6α, 7-$OCOC\underline{H}_3$, 9, 30	6 α, 6β	6α, 6β, 15, 30
8	41.89 (C)	-	6α, 9, 11α, 15, 30		
9	42.42 (CH)	1.97 (m)	1α, 7, 12α, 19, 30	11β, 11α	18
10	36.97 (C)	-	1α, 1β, 2α, 2β, 5, 6α, 6β, 9, 11α, 19		
11	16.37 (CH_2)	α) 1.64 (m)	9, 12 α	α) 9, 11β, 12β, 12α	α) 1β, 11β, 18
		β) 1.47 (m)		β) 9, 11α, 12β, 12α	β) 11α, 19
12	32.85 (CH_2)	α) 1.52 (m)	17, 18	α) 11β, 11α, 12β	α) 12β, 21, 22α
		β) 1.28 (m)		β) 11β, 11α, 12α	β) 12α, 17
13	46.45 (C)	-	11β, 12α, 15, 16β, 18		
14	159.02 (C)	-	9, 12α, 16α, 16β, 18, 30		
15	118.58 (CH)	5.27 (bs)	16α, 16β	16α, 16β	7, 30
16	35.03 (CH_2)	α) 1.99 (m)	15, 17	α) 15, 16β, 17	α) 16β, 18
		β) 2.09 (m)		β) 15, 16α, 17	β) 16α, 22α
17	52.35 (CH)	1.82 (m)	15, 16 α, 16β, 18, 21, 22β	16α, 16β, 20	12β
18	19.81 (CH_3)	0.97 (s)	12β, 17		9, 11α, 16α, 20, 21
19	14.99 (CH_3)	1.00 (s)	1α, 1β, 5, 9		1β, 2β, 6β, 11β, 30
20	43.96 (CH)	2.26 (m)	17, 21, 22β, 22α	17, 21, 22β, 22α	18, 21
21	95.78 (CH)	6.04 (bs)	21-$OCOC\underline{H}_3$, 22α, 23	20	12α, 18, 20
22	31.58 (CH_2)	α) 1.92 (m)	20, 21, 23, 24	α) 20, 22β, 23	α) 12α, 16β, 22β, 23
		β) 1.52 (m)		β) 20, 22α, 23	β) 22α, 24
23	78.18 (CH)	4.43 (m)	17, 21	22β, 22α, 24	22α, 24, 26
24	77.39 (CH)	4.74 (bs)	22β, 23, 24-$OCOC\underline{H}_3$, 26, 27	23	22β, 23, 26, 27
25	72.05 (C)	-	24, 26, 27		
♣26	27.13 (CH_3)	1.25 (s)	24, 27		23, 24, 27
♣27	26.71 (CH_3)	1.14 (s)	24, 26		24, 26
28	20.80 (CH_3)	0.99 (s)	5, 29		1α, 5, 29
29	25.81 (CH_3)	0.97 (s)	5, 28		6α, 6β, 28
30	27.03 (CH_3)	1.10 (s)	7, 9		6β, 7, 15, 19
7-$O\underline{C}OCH_3$	170.15 (C)	-	7, 7-$OCOC\underline{H}_3$		
7-$OCO\underline{C}H_3$	21.15 (CH_3)	1.91 (s)			
21-$O\underline{C}OCH_3$	170.35 (C)	-	21, 21-$OCOC\underline{H}_3$		
21-$OCO\underline{C}H_3$	21.31 (CH_3)	2.04 (s)			
24-$O\underline{C}OCH_3$	170.70 (C)	-	24, 24-$OCOC\underline{H}_3$		
24-$OCO\underline{C}H_3$	21.00 (CH_3)	2.12 (s)			

♣ interchangeable resonances

5.3.13. Introductory note to Compounds (xiii) to (xviii)

Compounds (**xiii**) to (**xviii**) were all found to have methoxy groups at C-21. Compounds (**ix**) to (**xii**) were all acetals with acetoxy groups at C-21. These compounds were isolated as pure compounds due to the fact that acetals do not ring open and close in solution as do compounds containing hemiacetal ring structures. The NMR spectra of compounds (**xiii**) to (**xviii**) indicated that they were C-21 acetals with methoxy groups at C-21. However, these were not isolated in their pure form but as mixtures of C-21 epimers. This suggests that the hydroxy groups at C-21 may have been methylated during chromatographic separation and they may be artefacts of the separation processes.

5.3.14. Structural elucidation of Compound xiii: Protosamaderine D (xiii)



(xiii)

The IR spectrum **[156]** of compound (**xiii**) showed an O-H stretching band at 3425 cm^{-1}, and a broad C=O stretching band at 1707 cm^{-1}.

Compound (**xiii**) was not isolated pure, and some peaks in the ^{13}C NMR spectrum **[159]** are paired due to the presence of a mixture of epimers. The peaks due to the major epimer are given. The ^{1}H **[158]** and ^{13}C NMR **[159]** spectra of compound (**xiii**) were very similar to those of compound (**xi**), except that the oxymethine C-21 carbon resonance had shifted from δ_C 95.71 in compound (**xi**) to δ_C 104.58 in compound (**xiii**) in the ^{13}C NMR spectra, and the H-21 proton resonance had shifted from δ_H 6.06 in compound (**xi**) to δ_H 4.75 in compound (**xiii**), with only one acetoxy group observed in the ^{1}H NMR spectrum **[158]**. The H-24 proton resonance had shifted from δ_H 4.75 in compound (**xi**) to δ_H 3.15 in compound (**xiii**), as observed for compound (**viii**) (δ_H 3.15) in the ^{1}H NMR spectra. This indicated that the acetoxy group at C-24 was replaced by a hydroxy group.

Inspection of the ADEPT NMR spectrum **[160]** showed the presence of a methoxy group carbon resonance at δ_C 55.17, which correlated to the proton resonance at δ_H 3.35 in the HSQC NMR spectra **[161-162]**. A second less intense methoxy group carbon resonance due to the methoxy group carbon of the minor epimer at δ_C 54.08 was present in the ADEPT NMR spectrum **[160]**, which correlated to the proton resonance at δ_H 3.29 in the HSQC NMR spectra **[161-162]**. The methoxy group carbon resonance (δ_C 55.17) showed a correlation to the H-21 proton resonance at δ_H 4.75 in the HMBC NMR spectra **[163-165]**, indicating that the methoxy group was attached at C-21. However, the sum of the integrals for the proton resonances due to the methoxy group protons was not correct relative to the other proton resonances in the original ^{1}H NMR spectrum **[158]** of compound (**xiii**), as shown in figure 5.1 below. The sum of the methoxy group proton resonances integral did not give the 3:1 ratio when compared to the H-24 or H-21 proton resonances.

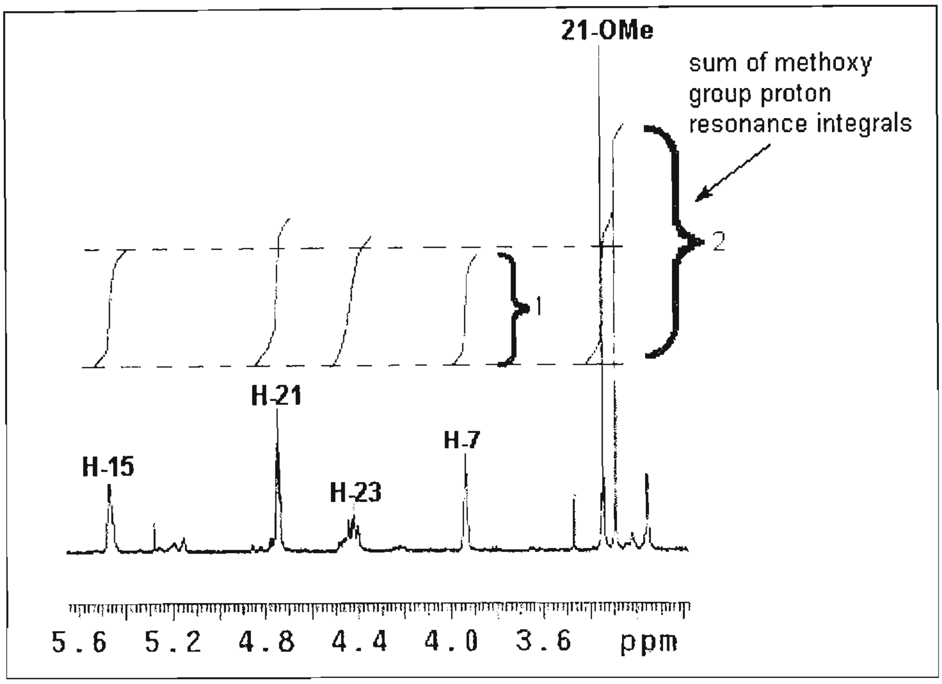


Figure 5.1. Partial ^{1}H NMR spectrum of Compound (xiii) (relaxation time = 0.0 sec)

It was then realised that a relaxation time of zero was used when running the ^{1}H NMR spectrum **[158]** of compound (**xiii**), and by increasing the relaxation time the correct integrals could be obtained.[52] The ^{1}H NMR spectrum of compound (**xiii**) (a weaker sample) was run again but this time the relaxation time was increased to 1.5 seconds and the correct integral ratio was observed, as shown in figure 5.2 below.

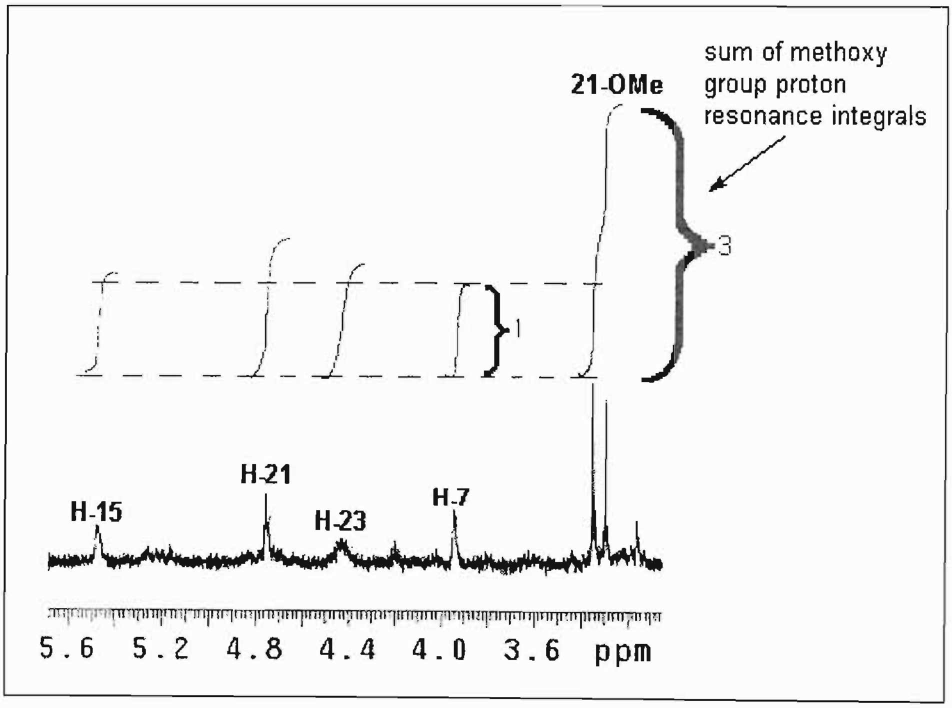


Figure 5.2. Partial ^{1}H NMR spectrum of Compound (xiii) (relaxation time = 1.5 sec)

It is presumed that increasing the relaxation times for compounds (**xiv**) to (**xviii**) will also give the correct integral ratios. However, the experiment could not be repeated for compounds (**xiv**) to (**xviii**) since very little samples were available.

The acetoxy group proton and carbon resonances did not correlate to any other resonance in the ^{1}H or ^{13}C NMR spectra. All other proton and carbon resonances occurred at similar chemical shifts as previously observed for compound (**xi**). The acetoxy group was tentatively placed at C-25 since the other oxygenated carbons have been accounted for.

The H-21 α orientation and the β orientation of the C-21 methoxy group were assigned as a result of the correlation between the H-20 (δ_H 2.17) and H-21 proton resonances in the NOESY NMR spectra **[168-169]**. The stereochemistry at C-24 has been assigned by comparison to literature.[49] The H-24 proton resonance occurs at the similar chemical shift as that reported by Zeng *et al.* for the monomethoxylate of meliavolkenin which has a C-21β methoxy group and a *S* configuration at C-24.

Although it was not possible to assign ^{13}C NMR resonances for the minor epimers, it was possible to assign the C-21 and its methoxy group carbon resonances for the minor epimer as it was clearly visible at δ_C 104.51 and 54.08 respectively.

From the NMR data of compound (**xiii**) the molecular formula was deduced to be $C_{33}H_{52}O_7$, which requires 560. The low resolution mass spectrum **[157]** of compound (**xiii**) showed peaks at *m/z* 542 $[M-18]^+$ and 524 $[M-36]^+$, corresponding to the loss of the elements of one and two molecules of water respectively.

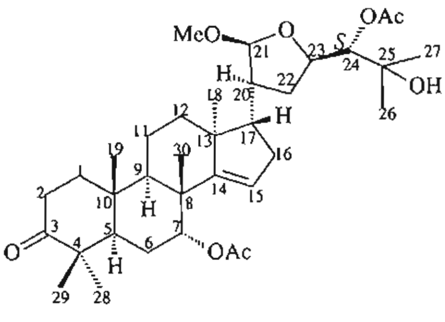
Compound (**xiii**) is the 21*S*,23*R*-epoxy-25-acetoxy-7,24*S*-dihydroxy-21*S*-methoxyapotirucalla-14-en-3-one. Compound (**xiii**) has not been reported previously and was given the common name protosamaderine D (**xiii**). The NMR data for protosamaderine D (**xiii**) is shown in table 5.13.

Table 5.13. : NMR data for protosamaderine D (xiii) (The major component)

C	$\delta^{13}C$ / ppm ($CDCl_3$)	$\delta^{1}H$ / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	38.54 (CH_2)	α) 1.49 (m)	2α, 19	α) 1β, 2α, 2β	α) 1β, 9
		β) 1.85 (m)		β) 1α, 2α, 2β	β) 1α
2	33.96 (CH_2)	α) 2.41 (m)		α) 2β, 1α, 1β	α) 2β
		β) 2.51 (m)		β) 2α, 1α, 1β	β) 2α, 19
3	217.38 (C)	-	28, 29		
4	46.94 (C)	-	28, 29		
5	46.54 (CH)	2.04 (m)	6β, 19, 28, 29	6α, 6β	28
6	24.74 (CH_2)	α) 1.78 (m)		α) 5, 6β, 7	α) 7, 28, 29
		β) 1.81 (m)		β) 5, 6α, 7	β) 7, 19, 28, 29, 30
7	71.93 (CH)	3.93 (bs)	30	6α, 6β	6α, 6β, 15, 30
8	44.03 (C)	-	30		
9	40.96 (CH)	2.00 (m)	19, 30	11β, 11α	1α, 18
10	37.17 (C)	-	1β, 2α, 5, 9, 19		
11	16.34 (CH_2)	α, β) 1.71 (m)		α, β) 9, 12β, 12α	
12	32.67 (CH_2)	α) 1.59 (m)	18	α) 11β, 11α, 12β	β) 18, 21
		β) 1.51 (m)		β) 11β, 11α, 12α	α) 21, 30
13	52.14 (C)	-	18		
14	161.41 (C)	-	18, 30		
15	119.96 (CH)	5.47 (bs)		16α, 16β	7, 16α, 16β, 30
16	34.98 (CH_2)	α) 2.10 (m)		α) 15, 16β, 17	α) 15, 17, 21
		β) 2.14 (m)		β) 15, 16α, 17	β) 15, 21, 22α
17	52.44 (CH)	1.97 (m)	18	16α, 16β, 20	16α
18	20.01 (CH_3)	1.00 (s)			9, 12α, 20, 21
19	14.96 (CH_3)	0.99 (s)	9		2β, 6β, 29, 30
20	44.54 (CH)	2.17 (m)	17	17, 21, 22β, 22α	18, 21
21	104.58 (CH)	4.75 (bs)	21-OCH_3	20	12α, 12β, 16α, 16β, 18, 20, 21-OCH_3, 23
22	31.32 (CH_2)	α) 1.89 (m)	21	α) 20, 22β, 23	α) 16β, 23
		β) 1.50 (m)		β) 20, 22α, 23	β) 24
23	78.81 (CH)	4.43 (m)	21	22β, 22α, 24	21, 22α, 24, 26, 27
24	76.47 (CH)	3.15 (bs)	26, 27	23	22β, 23, 26, 27
25	72.91 (C)	-	26, 27		
♣26	26.40 (CH_3)	1.26 (s)	27		23, 24
♣27	26.34 (CH_3)	1.25 (s)	26		23, 24
28	26.18 (CH_3)	1.08 (s)	29		5, 6α, 6β, 29
29	21.15 (CH_3)	1.03 (s)	28		6α, 6β, 19, 28
30	27.08 (CH_3)	1.07 (s)			6β, 7, 12β, 15, 19
21-OCH_3	55.17 (CH_3)	3.35 (s)	21		21
25-O$\underline{C}$OCH_3	171.18 (C)		25-OCO$\underline{CH_3}$		
25-OCO$\underline{C}H_3$	20.86 (CH_3)	2.14 (s)			

♣ interchangeable resonances
The 2H-11 resonances are superimposed

5.3.15. Structural elucidation of Compound xiv: Protosamaderine E (xiv)



(xiv)

The IR spectrum **[171]** of compound (**xiv**) showed an O-H stretching band at 3456 cm^{-1}, and a broad C=O stretching band at 1724 cm^{-1}.

Inspection of the ^{1}H **[173]** and ^{13}C NMR [**174**] spectra of compound (**xiv**) showed them to be very similar to those of compound (**xii**) and compound (**xiii**). Except that the oxymethine C-7 and C-24 resonances had shifted from δ_C 71.93 and 76.47 respectively in compound (**xiii**) to δ_C 74.99 and 77.21 respectively in compound (**xiv**), just as in compound (**xii**) (C-7, δ_C 74.82 and C-24, δ_C 77.39) in the ^{13}C NMR spectra. The H-7 and H-24 proton resonances shifted from δ_H 3.93 and δ_H 3.15 respectively in compound (**xiii**) to δ_H 5.19 and δ_H 4.75 respectively in compound (**xiv**), just as in compound (**xii**) (H-7, δ_H 5.22 and H-24, δ_H 4.74) in the ^{1}H NMR spectra.

Inspection of the 2D NMR spectra showed that the acetoxy carbonyl carbon resonances at δ_C 170.18 and 171.16 showed correlations to the H-7 and H-24 proton resonances respectively in the HMBC NMR spectra **[177-179]**, indicating that the acetoxy groups were attached at C-7α and C-24 respectively.

The H-21 (δ_H 4.74) α orientation and the β orientation of the C-21 methoxy group were assigned as a result of the correlation between the H-20 (δ_H 2.07) and H-21 proton resonances in the NOESY NMR spectra **[182-183]**, as observed previously. The H-24 proton resonance occurs at the same chemical shift as observed for compounds (**xi**) and (**xii**), which indicated the *S* configuration at C-24.[49]

From the NMR data of compound (**xiv**) the molecular formula was deduced to be $C_{35}H_{54}O_8$, which requires 602. The low resolution mass spectrum **[172]** of compound (**xiv**) showed a peak at *m/z* 542 [M-60]$^+$, corresponding to the loss of the element of one acetic acid molecule. The peak at *m/z* 510 [M-92]$^+$ was due to the loss of the elements of one acetic acid molecule together with one methanol molecule.

Compound (**xiv**) is the 21*S*,23*R*-epoxy-7,24*S*-diacetoxy-25-hydroxy-21*S*-methoxyapotirucalla-14-en-3-one. Compound (**xiv**) has not been reported previously and was given the common name protosamaderine E (**xiv**). The NMR data for protosamaderine E (**xiv**) is shown in table 5.14.

Table 5.14. : NMR data for protosamaderine E (xiv)

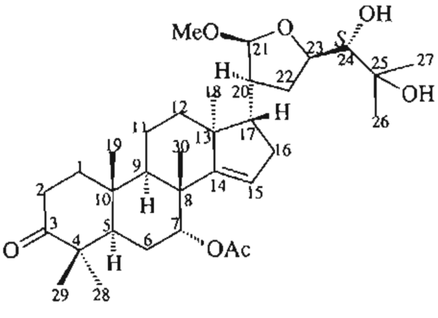
C	$\delta^{13}C$ / ppm ($CDCl_3$)	$\delta^{1}H$ / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	38.82 (CH_2)	α) 1.49 (m)	19	α) 1β, 2α, 2β	α) 1β, 9
		β) 1.89 (m)		β) 1α, 2α, 2β	β) 1α, 19
2	34.00 (CH_2)	α) 2.39 (m)		α) 2β, 1α, 1β	α) 2β
		β) 2.55 (m)		β) 2α, 1α, 1β	β) 2α, 19
3	216.84 (C)	-	28, 29		
4	46.96 (C)	-	5, 28, 29		
5	48.27 (CH)	1.84 (m)	19, 28, 29	6α, 6β	6α, 6β, 9, 28
6	24.24 (CH_2)	α) 1.66 (m)		α) 5, 6β, 7	α) 5, 7, 29
		β) 1.69 (m)		β) 5, 6α, 7	β) 5, 7
7	74.99 (CH)	5.19 (bs)	30	6α, 6β	6α, 6β, 15, 30
8	41.95 (C)	-	30		
9	42.73 (CH)	1.98 (m)	19, 30	11β, 11α	1α, 5, 18
10	37.02 (C)	-	5, 19		
11	16.56 (CH_2)	α) 1.56 (m)		α) 9, 11β, 12β, 12α	
		β) 1.50 (m)		β) 9, 11α, 12β, 12α	β) 19, 30
12	33.09 (CH_2)	α) 1.60 (m)	18	α) 11β, 11α, 12β	α) 21
		β) 1.56 (m)		β) 11β, 11α, 12α	
13	46.50 (C)	-	18		
14	159.08 (C)	-	18, 30		
15	118.85 (CH)	5.25 (bs)		16α, 16β	7, 16α, 16β, 30
16	35.02 (CH_2)	α) 1.91 (m)		α) 15, 16β, 17	α) 15, 16β, 18
		β) 2.03 (m)		β) 15, 16α, 17	β) 15, 16α, 17
17	52.10 (CH)	1.87 (m)	18	16α, 16β, 20	16β, 20
18	20.20 (CH_3)	0.97 (s)	16β		9, 16α, 20, 21
19	15.09 (CH_3)	1.02 (s)	5		1β, 2β, 11β, 30
20	44.59 (CH)	2.07 (m)	22α	17, 21, 22β, 22α	17, 18, 21
21	103.74 (CH)	4.74 (bs) •	21-OCH_3, 22α	20	12α, 18, 20, 21-OCH_3, 23
22	31.66 (CH_2)	α) 1.77 (m)	21, 24	α) 20, 22β, 23	α) 22β, 23
		β) 1.54 (m)		β) 20, 22α, 23	β) 22α, 24
23	77.43 (CH)	4.43 (m)	21, 24	22β, 22α, 24	21, 22α, 24, 26
24	77.21 (CH)	4.75 (bs) •	26, 27	23	22β, 23, 26, 27
25	72.55 (C)	-	26, 27		
♣26	27.52 (CH_3)	1.31 (s)	27		23, 24, 27
♣27	26.72 (CH_3)	1.17 (s)	26		24, 26
28	21.04 (CH_3)	1.01 (s)	5, 29		5, 29
29	25.83 (CH_3)	0.99 (s)	28		6α, 28
30	26.95 (CH_3)	1.11 (s)			7, 11β, 15, 19
$7\text{-O}\underline{\text{C}}\text{OCH}_3$	170.18 (C)	-	$7\text{-OCOC}\underline{\text{H}}_3$		
$7\text{-OCO}\underline{\text{C}}\text{H}_3$	21.20 (CH_3)	1.92 (s)			
21-OCH_3	54.08 (CH_3)	3.29 (s)	21		21
$24\text{-O}\underline{\text{C}}\text{OCH}_3$	171.16 (C)	-	24, $24\text{-OCOC}\underline{\text{H}}_3$		
$24\text{-OCO}\underline{\text{C}}\text{H}_3$	20.85 (CH_3)	2.14 (s)			

♣ interchangeable resonances

• resonances superimposed

The 2H-11 resonances were assigned from the COSY NMR spectrum

5.3.16. Structural elucidation of Compound xv: Protosamaderine F (xv)



(xv)

The IR spectrum of **[185]** compound **(xv)** showed an O-H stretching band at 3450 cm^{-1}, and a broad C=O stretching band at 1718 cm^{-1}.

Inspection of the ^{13}C NMR spectrum showed the presence of peaks due to the minor epimer indicated on the spectrum. The ^{1}H **[187]** and ^{13}C NMR **[189]** spectra of compound **(xv)** showed them to be very similar to those of compound **(xiv)**, except that the H-24 resonance had shifted from δ_H 4.74 in compound **(xiv)** to δ_H 3.15 in compound **(xv)** in the ^{1}H NMR spectra. However, no change was observed for the C-24 resonance in the ^{13}C NMR spectra. These observations in the ^{1}H **[187]** and ^{13}C NMR **[189]** spectra indicated that the acetoxy group at C-24 was replaced by a hydroxy group. The C-24 hydroxy group proton resonance occurred at δ_H 2.77 and showed correlations in the COSY NMR spectrum **[195]** to the H-24 proton resonance. The presence of the C-24 hydroxy group proton resonance was confirmed by its disappearance on addition of D_2O to the sample.

As observed previously in compound **(xiv)**, the methoxy group was attached at C-21β (δ_C 104.65) by observed correlations between the H-20 (δ_H 2.12) and H-21 (δ_H 4.73) resonances in the NOESY NMR spectra **[196-197]**. The stereochemistry at C-24 has been assigned by comparison to literature.[49] The H-24 proton resonance occurs at the similar chemical shift as that reported by Zeng *et al.* for the monomethoxylate of meliavolkenin which has a C-21β methoxy group and a *S* configuration at C-24.

From the NMR data of compound **(xv)** the molecular formula was deduced to be $C_{33}H_{52}O_7$, which requires 560. The low resolution mass spectrum **[186]** of compound **(xv)** showed a peak at *m/z* 542 [M-18]$^+$, corresponding to the loss of the element of one water molecule.

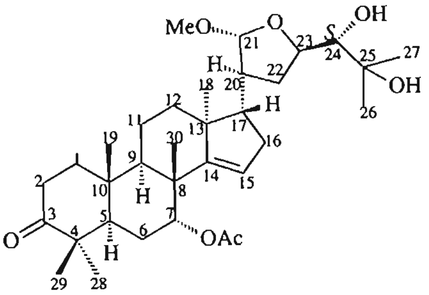
Compound **(xv)** is the 24-deacetyl analogue of compound **(xiv)**, 21*S*,23*R*-epoxy-7-acetoxy-24*S*,25-dihydroxy-21*S*-methoxyapotirucalla-14-en-3-one. Compound **(xv)** has not been reported previously and was given the common name protosamaderine F **(xv)**. The NMR data for protosamaderine F **(xv)** is shown in table 5.15.

Table 5.15. : NMR data for protosamaderine F (xv) (The major component)

C	$\delta^{13}C$ / ppm ($CDCl_3$)	$\delta^{1}H$ / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	38.81 (CH_2)	α) 1.48 (m)	2α, 2β, 5, 19	α) 1β, 2α, 2β	α) 1β, 9
		β) 1.87 (m)		β) 1α, 2α, 2β	β) 1α, 19
2	33.98 (CH_2)	α) 2.40 (m)	1α	α) 2β, 1α, 1β	α) 2β
		β) 2.56 (m)		β) 2α, 1α, 1β	β) 2α, 19
3	216.83 (C)	-	2α, 2β, 28, 29		
4	46.95 (C)	-	5, 28, 29		
5	48.27 (CH)	1.81 (m)	6β, 7, 9, 19, 28, 29	6 α, 6β	6α, 9, 28
6	24.23 (CH_2)	α) 1.67 (m)	5	α) 5, 6β, 7	α) 5, 6β, 7, 28
		β) 1.84 (m)		β) 5, 6α, 7	β) 6α, 7, 19, 28, 29, 30
7	75.00 (CH)	5.19 (bs)	6α, 30	6α, 6β	6α, 6β, 15, 30
8	41.93 (C)	-	6α, 9, 15, 30		
9	42.69 (CH)	2.01 (m)	1α, 7, 12β, 19, 30	11β, 11α	1α, 5, 18
10	37.02 (C)	-	1α, 1β, 2α, 5, 6α, 9, 19		
11	16.55 (CH_2)	α) 1.67 (m)	9, 12 β	α) 9, 11β, 12β, 12α	
		β) 1.57 (m)		β) 9, 11α, 12β, 12α	β) 19
12	33.16 (CH_2)	α) 1.55 (m)	18	α) 11β, 11α, 12β	α) 21
		β) 1.48 (m)		β) 11β, 11α, 12α	β) 30
13	46.38 (C)	-	12β, 15, 18		
14	158.96 (C)	-	15, 16α, 18, 30		
15	118.89 (CH)	5.24 (bs)	17	16β	7, 16α, 16β, 30
16	35.07 (CH_2)	α) 1.96 (m)	15, 17	α) 16β, 17	α) 15, 18
		β) 2.06 (m)		β) 15, 16α, 17	β) 15, 17
17	52.40 (CH)	1.93 (m)	15, 18	16α, 16β	16β, 20
18	20.24 (CH_3)	0.98 (s)	15, 17		9, 16α, 20, 21, $7\text{-OCOC}\underline{\text{H}}_3$
19	15.07 (CH_3)	1.01 (s)	1α, 1β, 5, 9		1β, 2β, 6β, 11β, 30
20	44.68 (CH)	2.12 (m)	21	21, 22α, 22β	17, 18, 21, 22α, 22β
21	104.65 (CH)	4.73 (d, 4.21)	21-OCH_3, 22β	20	12α, 18, 20, 21-OCH_3
22	31.35 (CH_2)	α) 1.86 (m)	21	α) 20, 22β, 23	α) 20, 23
		β) 1.93 (m)		β) 20, 22α, 23	β) 20, 24
23	78.76 (CH)	4.41 (m)	21	22α, 22β	22α, 24, 26, 27
24	76.53 (CH)	3.15 (d, 6.04)	26, 27	24-OH	22β, 23, 26, 27
24-OH	-	2.77 (bd)		24	
25	72.91 (C)	-	26, 27		
♣26	26.39 (CH_3)	1.25 (s)	27		23, 24
♣27	26.31 (CH_3)	1.24 (s)	26		23, 24
•28	25.82 (CH_3)	0.98 (s)	5, 29		5, 6α, 6β, 29
•29	21.03 (CH_3)	1.01 (s)	5, 28		6β, 28
30	26.92 (CH_3)	1.11 (s)	7, 9		6β, 7, 12β, 15, 19
$7\text{-O}\underline{\text{C}}\text{OCH}_3$	170.18 (C)	-	7, $7\text{-OCOC}\underline{\text{H}}_3$		
$7\text{-OCO}\underline{\text{C}}\text{H}_3$	21.19 (CH_3)	1.93 (s)			18
21-OCH_3	55.16 (CH_3)	3.34 (s)	21		21

♣,• interchangeable resonances

5.3.17. Structural elucidation of Compound xvi: Protosamaderine G (xvi)



(xvi)

The IR spectrum **[199]** of compound (**xvi**) showed an O-H stretching band at 3391 cm^{-1}, and a broad C=O stretching band at 1724 cm^{-1}.

The H-23 multiplet had shifted from δ_H 4.41 in compound (**xv**) to δ_H 4.23 in compound (**xvi**) in the ^{1}H NMR spectra, while the ^{13}C NMR spectra showed the shift of the C-21, C-23, C-24 and C-25 resonances from δ_C 104.65, 78.76, 76.53 and 72.91, respectively, in compound (**xv**) to 109.50, 76.93, 75.39 and 73.07, respectively, in compound (**xvi**). As in compound (**xv**), the methoxy group was attached at C-21 and the acetoxy group was attached at C-7.

The stereochemistry at C-21 could not be confirmed directly by the observation of correlations between the C-21 methoxy resonance and either the H-20 (δ_H 2.29) or H-23 (δ_H 4.23) proton resonances in the NOESY NMR spectra **[208-209]**. However, the absence of the correlations between the H-21 (δ_H 4.78) and the H-20 resonances, noted previously for the C-21β methoxy group, provided indirect proof for the C-21α methoxy group. In addition, the C-17 resonance at δ_C 57.60, which is also different from that observed previously for compounds (**viii**) to (**xv**) (δ_C ~ 52) with the β orientated ester at C-21, supported this theory. In addition, in compounds (**xiii**), (**xiv**) and (**xv**), which all possess a C-21β methoxy group, the C-21 resonance occurs consistently at ~ δ_C 103 – 105, whereas the carbon resonance in compound (**xvi**) occurs significantly further downfield at δ_C 109.50.

It is therefore proposed that the change in stereochemistry at C-21 causes a change in the chemical shifts of the ring carbon resonances, the C-17 resonance, and the H-23 proton resonance. However, no change was observed for the 3H-26 and 3H-27 methyl proton resonances (δ_H 1.24, 1.28). Holstinones B and C were reported previously as being C-21 epimers of each other.[42] After examining the series of protolimonoids isolated in this work, it became clear from NMR data that holstinones B and C are not epimeric to each other and their structures therefore need to be re-examined. The stereochemistry at C-24 has also been assigned by comparison to literature.[49] The H-24 proton resonance occurs at the similar chemical shift as that reported by Zeng *et al.* for the monomethoxylate of meliavolkenin which has a C-21α methoxy group and a *S* configuration at C-24. The small downfield shift of the H-24 proton resonance has been observed for this compound (**xvi**) and that in literature[49] with the C-21α methoxy group, compared to that with the C-21β methoxy group.

From the NMR data of compound (**xvi**) the molecular formula was deduced to be $C_{33}H_{52}O_7$, which requires 560. The low resolution mass spectrum **[200]** of compound (**xvi**) showed a peak at *m/z* 542 $[M-18]^+$, corresponding to the loss of the element of one water molecule.

Compound (**xvi**) is the C-21 epimer of compound (**xv**), 21*R*,23*R*-epoxy-7-acetoxy-24*S*,25-dihydroxy-21*R*-methoxyapotirucalla-14-en-3-one. Compound (**xvi**) has not been reported previously and was given the common name protosamaderine G (**xvi**). The NMR data for protosamaderine G (**xvi**) is shown in table 5.16.

Table 5.16. : NMR data for protosamaderine G (xvi) (The major component)

C	δ^{13}C / ppm (CDCl$_3$)	δ^{1}H / ppm (CDCl$_3$)	HMBC (C→H)	COSY	NOESY
1	38.81 (CH$_2$)	α) 1.49 (m)	19, 2β	α) 1β, 2α, 2β	α) 1β, 9
		β) 1.91 (m)		β) 1α, 2α, 2β	β) 1α, 19
2	33.98 (CH$_2$)	α) 2.40 (m)		α) 2β, 1α, 1β	α) 2β
		β) 2.55 (m)		β) 2α, 1α, 1β	β) 2α, 19
3	216.86 (C)	-	2α, 2β, 28, 29		
4	46.95 (C)	-	6α, 6β, 28, 29		
5	48.20 (CH)	1.81 (m)	1β, 6β, 7, 9, 19, 28, 29	6α, 6β	9, 28, 29
6	24.23 (CH$_2$)	α) 1.70 (m)	5	α) 5, 6β, 7	α) 6β, 7
		β) 1.85 (m)		β) 5, 6α, 7	β) 6α, 7, 19, 28, 29, 30
7	74.95 (CH)	5.19 (bs)	5, 30	6α, 6β	6α, 6β, 15, 30
8	41.96 (C)	-	6α, 9, 30		
9	42.73 (CH)	2.09 (m)	7, 19, 30	11α, β	1α, 5, 18
10	36.98 (C)	-	1β, 6α, 9, 19		
11	16.47 (CH$_2$)	α, β) 1.55 (m)	9	α, β) 9, 12α, β,	α, β) 19
12	33.66 (CH$_2$)	α) 1.77 (m)	18	α) 11α, β, 12β	
		β) 1.48 (m)		β) 11α, β, 12α	β) 30
13	46.73 (C)	-	15, 18		
14	159.26 (C)	-	12α, 12β, 16α, 16β, 18, 30		
15	118.51 (CH)	5.23 (bs)	16α, 16β	16α, 16β	7, 16α, 16β, 30
16	34.75 (CH$_2$)	α) 2.02 (m)	15	α) 15, 16β, 17	α) 15, 16β, 18
		β) 2.13 (m)		β) 15, 16α, 17	β) 15, 16α
17	57.60 (CH)	1.68 (m)	15, 18, 21, 22β	16α, 16β, 20	21, 22β
18	19.74 (CH$_3$)	1.00 (s)	17		9, 16α, 20
19	15.11 (CH$_3$)	1.02 (s)	1α, 1β, 9		1β, 2β, 6β, 11, 30
20	45.89 (CH)	2.29 (m)	22 β	17, 21, 22β, 22α	18, 22α, 23
21	109.50 (CH)	4.78 (d, 3.66)	17, 21-OCH$_3$, 22α	20	17, 21-OCH$_3$, 22β
22	33.27 (CH$_2$)	α) 1.88 (m)	17	α) 20, 22β, 23	α) 20, 23
		β) 1.77 (m)		β) 20, 22α, 23	β) 17, 21
23	76.93 (CH)	4.23 (m)	21, 22β	22α, 22β, 24	20, 22α, 24, 26
24	75.39 (CH)	3.22 (bs)	22β, 23, 26, 27	23	23, 27
25	73.07 (C)	-	26, 27		
♣26	26.46 (CH$_3$)	1.28 (s)	27		23, 27
♣27	26.35 (CH$_3$)	1.24 (s)	26		24, 26
•28	25.84 (CH$_3$)	0.99 (s)	5, 29		5, 6β, 29
•29	21.20 (CH$_3$)	1.01 (s)	5, 28		5, 6β, 28
30	26.89 (CH$_3$)	1.11 (s)	9		6β, 7, 12β, 15, 19
7-O$\underline{C}$OCH$_3$	170.23 (C)	-	7, 7-OCOC$\underline{H}_3$		
7-OCO$\underline{C}$H$_3$	21.03 (CH$_3$)	1.93 (s)			
21-OCH$_3$	55.63 (CH$_3$)	3.34 (s)	21		21

♣,• interchangeable resonances

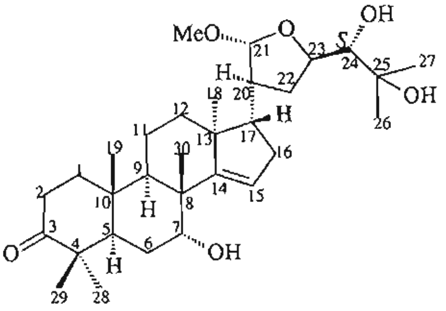
• The 2H-11 resonances are superimposed

5.3.18. Notes on the C-21 stereochemistry

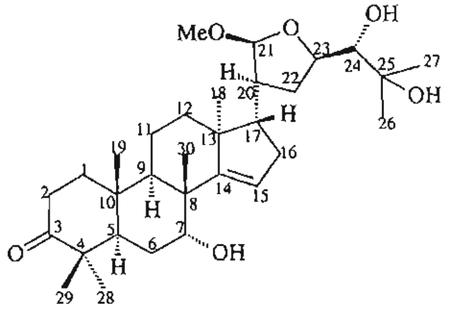
The significant difference of ~ 5 ppm between the C-21 carbon resonances of the C-21 epimeric compounds (**xiii**), (**xiv**) and (**xv**) (β methoxy group, δ_C ~ 103 – 105), and (**xvi**) (α methoxy group, δ_C ~ 109), suggested that this value might serve as a simple diagnostic tool for the determination of the stereochemistry at C-21 for triterpenoids of this type, replacing the time-consuming NOESY NMR analyses previously employed.

No other C-21 epimeric pairs of compounds, which would have provided further evidence for this conjecture, were isolated in this work. However, as mentioned previously, the epimeric C-21 resonances in melianone (δ_C 97.98 and 102.00) and melianol (δ_C 98.02 and 102.03),[53] display the same ~ 5 ppm difference, which result from the C-21β hydroxy and C-21α hydroxy epimeric carbons respectively.

5.3.19. Structural elucidation of Compound xvii: Protosamaderine H (xvii)



(**xvii**)



(**xviia**)

The IR spectrum **[211]** of compound (**xvii**) showed an O-H stretching band at 3458 cm^{-1}, and a C=O stretching band at 1702 cm^{-1}.

Initial inspection of the ^{13}C NMR spectrum **[214]** showed the presence of fifty one resonances. However, the spectrum showed the presence of the two epimeric C-21 carbon resonances at δ_C 104.56 and 109.46 for the minor C-21β methoxy group and the major C-21α methoxy group respectively. Closer analysis of the remaining resonances revealed thirty eight of these to be nineteen pairs of very narrowly separated resonances of identical multiplicity, and eleven others. The major peaks have been assigned in this work to compound (**xvii**). The minor peaks observed in this set of spectra correspond to the structure (**xviia**).

The ^{1}H NMR spectrum **[213]** of compound (**xvii**) was similar to that of compound (**xvi**), with the absence of the C-7 acetoxy group, indicated by the upfield shift of the H-7 resonance from δ_H 5.19 in compound (**xvi**) to δ_H 3.93 in compound (**xvii**), as observed in compounds (**x**), (**xi**) and (**xiii**).

As in compound (**xvi**), the H-23 resonance occurred at δ_H 4.24, and the shift in the carbon resonances of the ring attached at C-17 were also observed in compound (**xvii**). Just as in compound (**xvi**), the absence of the correlation between the H-21 (δ_H 4.77) and the H-20 (δ_H 2.31) resonances in the NOESY NMR spectra **[222-223]**, indirectly indicated that the methoxy group at C-21 was α orientated. The H-24 proton resonance occurs at the same chemical shift as observed for compound (**xvi**), which indicated the *S* configuration at C-24 with the α orientated hydroxy group.

From the NMR data of compound (**xvii**) the molecular formula was deduced to be $C_{31}H_{50}O_5$, which requires 518. The low resolution mass spectrum **[212]** of compound (**xvii**) showed a peak at *m/z* 518, corresponding to the molecular formula $C_{31}H_{50}O_5$. It would have been easier to separate this compound by acetylation or derivatisation, however, this was not possible since too small a quantity of sample was available.

Compound (**xvii**) is the 7-deacetyl analogue of compound (**xvi**), 21*R*,23*R*-epoxy-7,24*S*,25-trihydroxy-21*R*-methoxyapotirucalla-14-en-3-one, whereas the minor compound (**xviia**) is the C-21 epimer of compound (**xvii**), 21*S*,23*R*-epoxy-7,24*S*,25-trihydroxy-21*S*-methoxyapotirucalla-14-en-3-one.

Compound (**xvii**) has not been reported previously and was given the common name protosamaderine H (**xvii**). The NMR data for protosamaderine H (**xvii**) is shown in table 5.17, with the resonances for the minor epimer shown within brackets.

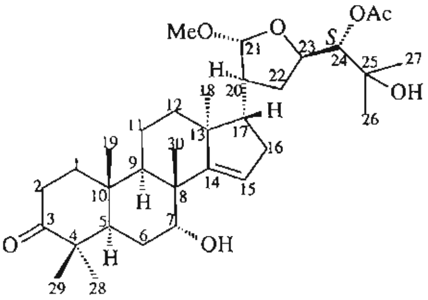
Table 5.17. : NMR data for protosamaderine H (xvii) (major component in mixture)

C	$\delta^{13}C$ / ppm ($CDCl_3$)	$\delta^{1}H$ / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	38.52 (38.47) (CH_2)	α) 1.48 (m)	2β, 9, 19	α) 1β, 2α, 2β	α) 1β, 9, 28
		β) 1.83 (m)		β) 1α, 2α, 2β	β) 1α, 19
2	33.94 (CH_2)	α) 2.41 (m)	1α, 1β	α) 2β, 1α, 1β	α) 2β
		β) 2.51 (m)		β) 2α, 1α, 1β	β) 2α, 19
3	217.43 (217.40) (C)	-	1β, 2α, 2β, 28, 29		
4	46.91 (46.86) (C)	-	5, 6α, 6β, 28, 29		
5	46.46 (CH)	2.07 (m)	1β, 7, 9, 19, 28, 29	6α, 6β	6α, 6β, 28, 29
6	24.73 (CH_2)	α) 1.73 (m)	5	α) 5, 6β, 7	α) 5, 6β, 7, 28, 29
		β) 1.80 (m)		β) 5, 6α, 7	β) 5, 6α, 7, 19, 28, 29, 30
7	71.93 (CH)	3.93 (bs)	30	6α, 6β	6α, 6β, 15, 30
8	44.52 (44.04) (C)	-	6α, 9, 11α, 30		
9	40.98 (40.94) (CH)	2.03 (m)	7, 11β, 12β, 12α, 19, 30	11β, 11α	1α, 18
10	37.14 (C)	-	1β, 2α, 5, 6β, 9, 19		
11	16.25 (16.34) (CH_2)	α) 1.65 (m)	9	α) 9, 11β, 12β, 12α	
		β) 1.53 (m)		β) 9, 11α, 12β, 12α	β) 19, 30
12	32.58 (32.65) (CH_2)	α) 1.77 (m)	17, 18	α) 11β, 11α, 12β	α) 12β, 21
		β) 1.50 (m)		β) 11β, 11α, 12α	β) 12α, 30
13	46.51 (C)	-	12α, 15, 16β, 18		
14	161.69 (161.39) (C)	-	12β, 12α, 16α, 16β, 18, 30		
15	119.54 (119.95) (CH)	5.46 (bs)	16α, 16β	16α, 16β	7, 16α, 16β, 30
16	34.72 (34.98) (CH_2)	α) 2.10 (m)	15, 17	α) 15, 16β, 17	α) 15, 16β, 18'
		β) 2.17 (m)		β) 15, 16α, 17	β) 15, 16α
17	57.53 (52.42) (CH)	1.70 (m)	12α, 15, 18, 21	16α, 16β, 20	
18	19.49 (20.00) (CH_3)	1.03 (1.00) (s)	12α, 17		9, 16α, 20, 21
19	14.98 (14.94) (CH_3)	0.98 (s)	1α, 1β, 5, 9		1β, 2β, 6β, 11β, 30
20	45.82 (CH)	2.31 (m)	17, 21, 22β, 22α	17, 21, 22β, 22α	18, 21, 22α, 23
21	109.46 (104.56) (CH)	4.77 (d, 3.66)	17, 21-OCH_3, 22α	20	12α, 18, 20, 21-OCH_3,
22	33.70 (CH_2)	α) 1.88 (m)		α) 20, 22β, 23	α) 20, 22β, 23
		β) 1.77 (m)		β) 20, 22α, 23	β) 21, 22α
23	76.92 (78.79) (CH)	4.24 (m)	21, 22β	22β, 22α	20, 22α, 24, 26
24	75.35 (76.92) (CH)	3.22 (bs)	22β, 23, 26, 27		23, 26
25	73.05 (72.91) (C)	-	26, 27		
+26	26.46 (CH_3)	1.28 (s)	27		23, 24
+27	26.38 (26.34) (CH_3)	1.24 (s)	26		
28	26.20 (26.17) (CH_3)	1.08 (s)	5, 29		1α, 6α, 6β
29	21.14 (CH_3)	1.03 (s)	2, 28		6α, 6β
30	27.04 (27.07) (CH_3)	1.07 (s)	9		6β, 7, 11β, 12β, 15, 19
21-OCH_3	55.62 (CH_3)	3.33 (s)	21		21

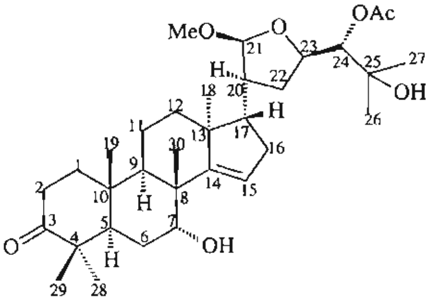
(The ^{13}C values shown in brackets in the table belong to the minor component of the mixture.)

+ interchangeable resonances

5.3.20. Structural elucidation of Compound xviii: Protosamaderine I (xviii)



(xviii)



(xviiia)

The IR spectrum **[225]** of compound (**xviii**) showed an O-H stretching band at 3396 cm^{-1}, and C=O stretching bands at 1738 and 1713 cm^{-1}.

The ^{13}C NMR spectrum **[228]** of compound (**xviii**) showed the same doubling of some peaks as observed in the ^{13}C NMR spectrum **[214]** of compound (**xvii**). The C-21 carbon resonances at δ_C 108.86 (major) and 103.65 (minor) and the C-17 carbon resonances at δ_C 52.15 (minor) and 57.50 (major) were again observed as in compound (**xvii**), which suggested a mixture of two C-21 epimeric compounds. The major peaks have been assigned in this work to compound (**xviii**), with the minor peaks corresponding to the structure (**xviiia**).

The ^{1}H NMR spectrum **[227]** of compound (**xviii**) was similar to that of compound (**xvii**), except with the additional C-24 acetoxy group indicated by the downfield shift of the H-24 resonance from δ_H 3.22 in compound (**xvii**) to δ_H 4.86 in compound (**xviii**), as observed in compounds (**xi**), (**xii**) and (**xiv**). The absence of the correlation between the H-21 (δ_H 4.82) and the H-20 (δ_H 2.30) resonances in the NOESY NMR spectra **[236-237]** again proved indirectly that the C-21 methoxy group was α orientated. The stereochemistry at C-24 has also been assigned by comparison to literature.[49]

From the NMR data of compound (**xviii**) the molecular formula was deduced to be $C_{33}H_{52}O_7$, which requires 560. The low resolution mass spectrum **[226]** of compound (**xviii**) showed peaks at *m/z* 542 $[M-18]^+$ and 528 $[M-32]^+$, corresponding to the loss of the elements of one water molecule and one methanol molecule respectively.

Compound (**xviii**) is the 24-acetyl analogue of compound (**xvii**), 21*R*,23*R*-epoxy-24*S*-acetoxy-7,25-dihydroxy-21*R*-methoxyapotirucalla-14-en-3-one, whereas the minor compound (**xviiia**) is the C-21 epimer of compound (**xviii**), 21*S*,23*R*-epoxy-24*S*-acetoxy-7,25-dihydroxy-21*S*-methoxyapotirucalla-14-en-3-one.

Compound (**xviii**) has not been reported previously and was given the common name protosamaderine I (**xviii**). The NMR data for protosamaderine I (**xviii**) is shown in table 5.18.

Table 5.18. : NMR data for protosamaderine I (xviii) (major component in mixture)

C	δ^{13}C / ppm (CDCl$_3$)	δ^{1}H / ppm (CDCl$_3$)	HMBC (C→H)	COSY	NOESY
1	38.54 (CH$_2$)	α) 1.51 (m)	19	α) 1β, 2α, 2β	α) 1β, 9
		β) 1.83 (m)		β) 1α, 2α, 2β	β) 1α, 19
2	33.94 (34.02) (CH$_2$)	α) 2.42 (m)		α) 2β, 1α, 1β	α) 2β
		β) 2.49 (m)		β) 2α, 1α, 1β	β) 2α, 19
3	217.37 (C)	-	2α, 28, 29		
4	46.92 (C)	-	28, 29		
5	46.45 (46.54) (CH)	2.05 (m)	19, 28, 29	6α, 6β	6α, 28
6	24.77 (CH$_2$)	α) 1.73 (m)		α) 5, 6β, 7	α) 5, 6β, 7, 28, 29
		β) 1.79 (m)		β) 5, 6α, 7	β) 6α, 7, 19, 28, 29, 30
7	71.95 (CH)	3.93 (bs)	30	6α, 6β	6α, 6β, 15, 30
8	44.06 (C)	-	6β, 9, 30		
9	40.97 (CH)	2.02 (m)	19, 30	11α	1α, 18
10	37.16 (C)	-	1β, 5, 9, 19		
11	16.25 (16.34) (CH$_2$)	α) 1.67 (m)		α) 9, 11β	α) 11β
		β) 1.49 (m)		β) 11α	β) 11α, 30
12	32.57 (32.61) (CH$_2$)	α) 1.93 (m)	18	α) 12β	α) 12β
		β) 1.25 (m)		β) 12α	β) 12α, 17
13	45.60 (C)	-			
14	161.93 (161.52) (C)	-	18, 30		
15	119.33 (119.90) (CH)	5.45 (bs)		16α, 16β	7, 16α, 16β, 30
16	34.77 (34.98) (CH$_2$)	α) 2.08 (m)		α) 15, 16β, 17	α) 15, 22α
		β) 2.11 (m)		β) 15, 16α, 17	β) 15
17	57.50 (52.15) (CH)	1.62 (m)	18, 21	16α, 16β, 20	12β, 21
18	19.43 (19.98) (CH$_3$)	1.07 (s)			9, 20
19	15.01 (14.97) (CH$_3$)	0.99 (s)	1β, 9		1β, 2β, 30
20	44.50 (CH)	2.30 (m)		17, 21, 22α	18, 22α, 23
21	108.86 (103.65) (CH)	4.82 (d, 3.85)	21-OCH$_3$, 22α	20	17, 21-OCH$_3$
22	31.69 (CH$_2$)	α) 1.89 (m)		α) 20, 23	α) 16α, 20, 23
		β) 1.56 (m)		β) 23	
23	75.90 (CH)	4.43 (m)	21	22β, 22α, 24	20, 22α, 24, 26
24	75.85 (CH)	4.86 (d, 2.01)	26, 27	23	23, 26, 27
25	72.64 (72.54) (C)	-	26, 27		
*26	26.64 (26.71) (CH$_3$)	1.34 (s)	27		23, 24, 27
*27	27.47 (27.52) (CH$_3$)	1.23 (s)	26		24, 26
28	26.23 (26.20) (CH$_3$)	1.17 (s)	29		5
29	21.14 (CH$_3$)	1.03 (s)	5, 28		
30	27.06 (27.12) (CH$_3$)	1.08 (s)	9		7, 11β, 15, 19
21-OCH$_3$	55.75 (54.09) (CH$_3$)	3.36 (s)	21		21
$24\text{-O}\underline{\text{C}}\text{OCH}_3$	170.97 (C)	-	$24, 24\text{-OCOC}\underline{\text{H}}_3$		
$24\text{-OCO}\underline{\text{C}}\text{H}_3$	20.84 (CH$_3$)	2.17 (s)			

(The ^{13}C values shown in brackets in the table belong to the minor component of the mixture.)

* interchangeable resonances

5.4. Biological activity of the quassinoids: anti-tumour screening

5.4.1. Introduction

The quassinoids isolated in this work were tested *in vitro* on the sixty human tumor cell lines at the National Cancer Institute in the United States of America. The nine subpanels include leukemia, non-small cell lung cancer, colon cancer, central nervous system (CNS) cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer.

The GI 50 value is the sample concentration that inhibits 50% of the cancer cell growth. The TGI value is the sample concentration that inhibits total growth of the cancer cells, i.e. no cancer cell growth. The LC 50 value is the lowest sample concentration that kills 50% of the cancer cell population. Therefore the LC 50 parameter is the most important response parameter of the three.

The dose-response curves (Appendix, pages 162, 164, 166, 168, 170) are created by plotting the percentage growth (PG) against the $\log_{10}$ of the sample concentration. The horizontal lines are drawn at the PG values of +50, 0 and –50. The sample concentrations corresponding to the points where the curves cross these lines are the GI 50, TGI and LC 50 values respectively.

The mean graphs (Appendix, pages 161, 163, 165, 167, 169) show the mean of the GI 50, TGI and LC 50 values for each of the cell lines. The bars extending to the right represent the sensitivity of the cell line to the test sample in excess of the average sensitivity of all the cell lines tested. The bars extending to the left imply that the sensitivity of the cell line is less than the mean.

5.4.2. Results

Samaderine A (**i**), 5β,6-dihydrosamaderine A (**ii**), 2-chlorosamaderine A (**iii**), cedronin (**vi**) and 3,4β-dihydrosamaderine C (**vii**), were tested for their anticarcinogenic properties. The GI 50, TGI and LC 50 values for these compounds are shown in tables iii – vii below and are expressed in g/ml.

Table iii: National Cancer Institute *In Vitro* Testing Results for Samaderine A (i)

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.172	0.940	0.877	0.882	0.981	0.931	0.886	92	92	105	99	93	>1.00E-04	>1.00E-04	>1.00E-04
HL-60(TB)	0.261	1.651	1.727	1.492	1.555	1.616	1.486	105	89	93	97	88	>1.00E-04	>1.00E-04	>1.00E-04
K-562	0.146	1.391	1.364	1.317	1.301	1.361	1.225	98	94	93	98	87	>1.00E-04	>1.00E-04	>1.00E-04
MOLT-4	0.140	0.850	0.919	0.810	0.831	0.866	0.799	110	94	97	102	93	>1.00E-04	>1.00E-04	>1.00E-04
RPMI-8226	0.261	0.898	0.833	0.865	0.855	0.865	0.764	90	95	93	95	79	>1.00E-04	>1.00E-04	>1.00E-04
SR	0.166	0.522	0.492	0.574	0.643	0.470	0.491	91	115	134	85	91	>1.00E-04	>1.00E-04	>1.00E-04
Non-Small Cell Lung Cancer															
A549/ATCC	0.142	0.687	0.765	0.710	0.670	0.729	0.620	114	104	97	108	88	>1.00E-04	>1.00E-04	>1.00E-04
EKVX	0.879	1.519	1.524	1.532	1.461	1.486	1.484	101	102	91	95	95	>1.00E-04	>1.00E-04	>1.00E-04
HOP-62	0.707	1.492	1.538	1.510	1.467	1.513	1.401	106	102	97	103	88	>1.00E-04	>1.00E-04	>1.00E-04
HOP-92	0.575	0.811	0.923	0.857	0.793	0.842	0.816	147	119	92	113	103	>1.00E-04	>1.00E-04	>1.00E-04
NCI-H322M	0.972	1.962	1.937	1.882	1.902	1.863	1.591	97	92	94	90	62	>1.00E-04	>1.00E-04	>1.00E-04
NCI-H460	0.184	1.678	1.694	1.726	1.689	1.773	1.606	101	103	101	106	95	>1.00E-04	>1.00E-04	>1.00E-04
NCI-H522	0.262	0.685	0.739	0.715	0.731	0.739	0.671	113	107	111	113	97	>1.00E-04	>1.00E-04	>1.00E-04
Colon Cancer															
COLO 205	0.444	2.632	2.737	2.665	2.573	2.556	2.486	105	102	97	97	93	>1.00E-04	>1.00E-04	>1.00E-04
HCC-2998	0.328	0.801	0.788	0.819	0.782	0.813	0.743	97	104	96	102	88	>1.00E-04	>1.00E-04	>1.00E-04
HCT-116	0.194	0.804	0.813	0.788	0.766	0.757	0.756	101	97	94	92	92	>1.00E-04	>1.00E-04	>1.00E-04
HCT-15	0.729	0.915	0.921	0.886	0.860	0.915	0.825	103	84	70	100	51	>1.00E-04	>1.00E-04	>1.00E-04
HT29	0.163	0.941	1.034	1.014	0.887	0.967	1.017	112	109	93	103	110	>1.00E-04	>1.00E-04	>1.00E-04
KM12	0.241	1.627	1.516	1.484	1.435	1.444	1.236	92	90	86	87	72	>1.00E-04	>1.00E-04	>1.00E-04
SW-620	0.412	1.554	1.499	1.458	1.472	1.435	1.378	95	92	93	90	85	>1.00E-04	>1.00E-04	>1.00E-04
CNS Cancer															
SF-268	0.511	1.485	1.503	1.474	1.469	1.456	1.413	102	99	98	97	93	>1.00E-04	>1.00E-04	>1.00E-04
SF-295	0.510	1.393	1.401	1.349	1.341	1.352	1.262	101	95	94	95	85	>1.00E-04	>1.00E-04	>1.00E-04
SF-539	0.703	1.465	1.465	1.431	1.316	1.370	1.328	100	96	80	87	82	>1.00E-04	>1.00E-04	>1.00E-04
SNB-19	0.280	2.249	2.271	2.287	2.211	2.254	2.072	101	102	98	100	91	>1.00E-04	>1.00E-04	>1.00E-04
SNB-75	0.367	0.539	0.602	0.535	0.566	0.557	0.566	136	97	115	110	116	>1.00E-04	>1.00E-04	>1.00E-04
U251	0.607	1.272	1.277	1.212	1.206	1.280	1.266	101	91	90	101	99	>1.00E-04	>1.00E-04	>1.00E-04
Melanoma															
LOX IMVI	0.470	1.250	1.301	1.254	1.165	1.225	1.164	106	100	89	97	89	>1.00E-04	>1.00E-04	>1.00E-04
MALME-3M	0.979	1.935	1.878	1.512	1.755	1.826	1.767	94	58	81	89	82	>1.00E-04	>1.00E-04	>1.00E-04
M14	0.268	0.938	0.988	0.987	0.895	0.913	0.805	108	108	94	96	79	>1.00E-04	>1.00E-04	>1.00E-04
SK-MEL-2	0.404	0.798	0.819	0.800	0.782	0.765	0.732	105	100	96	97	83	>1.00E-04	>1.00E-04	>1.00E-04
SK-MEL-28	0.276	1.942	2.038	2.024	1.950	2.018	1.962	106	105	103	105	101	>1.00E-04	>1.00E-04	>1.00E-04
SK-MEL-5	1.936	2.752	2.438	2.371	2.132	1.878	1.670	62	53	24	-3	-14	1.30E-07	7.74E-06	>1.00E-04
UACC-62	0.957	2.037	1.949	1.951	1.863	1.776	1.537	92	92	84	76	54	>1.00E-04	>1.00E-04	>1.00E-04
Ovarian Cancer															
IGROV1	0.233	0.736	0.805	0.812	0.753	0.789	0.572	114	115	103	110	67	>1.00E-04	>1.00E-04	>1.00E-04
OVCAR-3	0.642	1.390	1.360	1.438	1.300	1.429	1.318	96	106	88	104	90	>1.00E-04	>1.00E-04	>1.00E-04
OVCAR-4	0.467	1.779	1.959	1.843	1.836	1.911	1.792	114	105	104	110	101	>1.00E-04	>1.00E-04	>1.00E-04
OVCAR-5	0.375	0.785	0.744	0.765	0.725	0.811	0.787	90	95	85	106	101	>1.00E-04	>1.00E-04	>1.00E-04
OVCAR-8	0.111	0.928	0.988	0.978	0.975	0.917	0.855	107	106	106	99	91	>1.00E-04	>1.00E-04	>1.00E-04
SK-OV-3	0.510	1.112	1.155	1.169	1.118	1.170	1.137	107	109	101	110	104	>1.00E-04	>1.00E-04	>1.00E-04
Renal Cancer															
786-0	0.427	1.318	1.330	1.383	1.313	1.366	1.327	101	107	99	105	101	>1.00E-04	>1.00E-04	>1.00E-04
A498	1.345	2.260	2.529	2.393	1.760	0.929	0.933	129	115	45	-31	-31	8.58E-07	3.93E-06	>1.00E-04
ACHN	0.661	0.862	0.881	0.850	0.839	0.879	0.845	109	94	88	108	91	>1.00E-04	>1.00E-04	>1.00E-04
CAKI-1	0.749	2.235	2.271	2.178	2.149	2.138	1.959	102	96	94	94	81	>1.00E-04	>1.00E-04	>1.00E-04
SN12C	0.982	1.854	1.725	1.727	1.668	1.635	1.448	85	85	79	75	53	>1.00E-04	>1.00E-04	>1.00E-04
TK-10	0.894	1.891	1.866	1.630	1.760	1.836	1.797	98	74	87	95	91	>1.00E-04	>1.00E-04	>1.00E-04
Prostate Cancer															
PC-3	0.232	0.934	0.989	0.964	0.935	0.913	0.889	108	104	100	97	94	>1.00E-04	>1.00E-04	>1.00E-04
DU-145	0.443	1.071	1.071	1.029	0.944	0.892	0.918	100	93	80	71	76	>1.00E-04	>1.00E-04	>1.00E-04
Breast Cancer															
MCF7	0.265	1.532	1.563	1.536	1.515	1.476	1.403	102	100	99	96	90	>1.00E-04	>1.00E-04	>1.00E-04
NCI/ADR-RES	0.327	0.790	0.797	0.775	0.771	0.815	0.776	101	97	96	105	97	>1.00E-04	>1.00E-04	>1.00E-04
MDA-MB-231/ATCC	0.470	0.956	0.923	0.899	0.821	0.800	0.813	93	88	72	68	71	>1.00E-04	>1.00E-04	>1.00E-04
HS 578T	0.658	1.606	1.424	1.547	1.436	1.293	1.210	81	94	82	67	58	>1.00E-04	>1.00E-04	>1.00E-04
MDA-MB-435	0.546	1.873	1.880	1.833	1.863	1.854	1.782	101	97	99	99	93	>1.00E-04	>1.00E-04	>1.00E-04
BT-549	0.738	1.296	1.284	1.217	1.181	1.194	1.134	98	86	79	82	71	>1.00E-04	>1.00E-04	>1.00E-04
T-47D	0.664	2.884	2.923	2.987	2.739	2.935	2.980	102	105	93	102	104	>1.00E-04	>1.00E-04	>1.00E-04

Table iv: National Cancer Institute *In Vitro* Testing Results for 5β,6-Dihydrosamaderine A (ii)

			Log10 Concentration												
	Time		Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.172	0.815	0.788	0.861	0.852	0.867	0.172	96	107	106	108	.	3.45E-05	>1.00E-04	>1.00E-04
HL-60(TB)	0.261	1.827	1.781	1.693	1.592	1.511	1.290	97	91	85	80	66	>1.00E-04	>1.00E-04	>1.00E-04
K-562	0.146	1.405	1.369	1.357	1.468	1.385	1.267	97	96	105	98	89	>1.00E-04	>1.00E-04	>1.00E-04
MOLT-4	0.140	0.790	0.806	0.878	0.865	0.760	0.776	102	114	112	95	98	>1.00E-04	>1.00E-04	>1.00E-04
RPMI-8226	0.261	0.782	0.734	0.754	0.747	0.760	0.602	91	95	93	96	65	>1.00E-04	>1.00E-04	>1.00E-04
SR	0.166	0.529	0.498	0.518	0.434	0.396	0.290	91	97	74	63	34	2.86E-05	>1.00E-04	>1.00E-04
Non-Small Cell Lung Cancer															
A549/ATCC	0.142	0.713	0.693	0.692	0.716	0.745	0.687	96	96	101	106	95	>1.00E-04	>1.00E-04	>1.00E-04
EKVX	0.879	1.479	1.509	1.529	1.442	1.467	1.304	105	108	94	98	71	>1.00E-04	>1.00E-04	>1.00E-04
HOP-62	0.707	1.501	1.451	1.469	1.450	1.494	1.285	94	96	94	99	73	>1.00E-04	>1.00E-04	>1.00E-04
HOP-92	0.575	0.799	0.766	0.746	0.744	0.753	0.671	85	76	75	79	43	6.27E-05	>1.00E-04	>1.00E-04
NCI-H226	1.093	1.328	1.334	1.258	1.263	1.270	1.110	103	70	72	75	7	2.34E-05	>1.00E-04	>1.00E-04
NCI-H322M	0.972	1.886	1.897	1.885	1.904	1.866	1.616	101	100	102	98	70	>1.00E-04	>1.00E-04	>1.00E-04
NCI-H460	0.184	1.784	1.740	1.914	1.818	1.843	1.791	97	108	102	104	100	>1.00E-04	>1.00E-04	>1.00E-04
NCI-H522	0.262	0.753	0.718	0.747	0.691	0.736	0.660	93	99	87	97	81	>1.00E-04	>1.00E-04	>1.00E-04
Colon Cancer															
COLO 205	0.444	2.646	2.589	2.681	2.625	2.670	2.505	97	102	99	101	94	>1.00E-04	>1.00E-04	>1.00E-04
HCC-2998	0.328	0.783	0.768	0.748	0.743	0.745	0.626	97	92	91	92	65	>1.00E-04	>1.00E-04	>1.00E-04
HCT-116	0.194	0.846	0.804	0.673	0.790	0.783	0.655	94	73	91	90	71	>1.00E-04	>1.00E-04	>1.00E-04
HCT-15	0.729	0.962	0.853	0.863	0.847	0.845	0.774	58	63	55	54	21	1.36E-05	>1.00E-04	>1.00E-04
HT29	0.163	0.921	0.892	0.885	0.826	0.871	0.764	96	95	87	93	79	>1.00E-04	>1.00E-04	>1.00E-04
KM12	0.241	1.441	1.455	1.460	1.406	1.373	1.343	105	102	97	94	92	>1.00E-04	>1.00E-04	>1.00E-04
SW-620	0.412	1.472	1.311	1.347	1.364	1.272	1.276	85	88	90	81	81	>1.00E-04	>1.00E-04	>1.00E-04
CNS Cancer															
SF-268	0.511	1.553	1.537	1.559	1.511	1.494	1.401	98	101	96	94	85	>1.00E-04	>1.00E-04	>1.00E-04
SF-295	0.510	1.499	1.460	1.498	1.483	1.556	1.450	98	100	98	106	95	>1.00E-04	>1.00E-04	>1.00E-04
SF-539	0.703	1.541	1.464	1.503	1.391	1.386	1.288	91	95	82	81	70	>1.00E-04	>1.00E-04	>1.00E-04
SNB-19	0.280	2.378	2.292	2.360	2.371	2.449	2.251	96	99	100	103	94	>1.00E-04	>1.00E-04	>1.00E-04
SNB-75	0.367	0.509	0.430	0.462	0.437	0.449	0.410	44	67	49	57	30	.	>1.00E-04	>1.00E-04
U251	0.607	1.241	1.277	1.209	1.232	1.197	1.207	106	95	99	93	79	>1.00E-04	>1.00E-04	>1.00E-04
Melanoma															
LOX IMVI	0.470	1.276	1.257	1.221	1.252	1.116	1.072	98	92	97	80	75	>1.00E-04	>1.00E-04	>1.00E-04
MALME-3M	0.979	1.925	1.877	1.895	1.830	1.877	1.687	94	95	90	94	75	>1.00E-04	>1.00E-04	>1.00E-04
M14	0.288	0.974	0.988	0.921	0.886	1.068	0.701	102	92	87	114	60	>1.00E-04	>1.00E-04	>1.00E-04
SK-MEL-2	0.404	0.841	0.755	0.876	0.737	0.872	0.738	89	108	90	107	76	>1.00E-04	>1.00E-04	>1.00E-04
SK-MEL-28	0.276	2.001	1.915	1.946	1.926	2.022	1.737	95	97	96	101	85	>1.00E-04	>1.00E-04	>1.00E-04
SK-MEL-5	1.536	2.731	2.629	2.447	1.614	1.650	1.234	67	64	-6	-15	-36	1.59E-07	6.14E-07	>1.00E-04
UACC-62	0.957	1.886	1.955	1.968	1.540	1.914	1.648	96	99	55	92	67	>1.00E-04	>1.00E-04	>1.00E-04
Ovarian Cancer															
IGROV1	0.233	0.819	0.788	0.808	0.807	0.816	0.622	95	98	98	99	66	>1.00E-04	>1.00E-04	>1.00E-04
OVCAR-3	0.642	1.347	0.985	1.269	1.226	1.260	1.208	49	89	83	88	80	.	>1.00E-04	>1.00E-04
OVCAR-4	0.457	1.667	1.580	1.657	1.697	1.774	1.588	93	100	102	109	93	>1.00E-04	>1.00E-04	>1.00E-04
OVCAR-5	0.579	0.800	0.742	0.744	0.725	0.724	0.558	86	87	82	82	66	>1.00E-04	>1.00E-04	>1.00E-04
OVCAR-8	0.211	0.909	0.884	1.026	0.945	1.005	0.936	97	115	105	112	103	>1.00E-04	>1.00E-04	>1.00E-04
SK-OV-3	0.510	1.125	1.135	1.166	1.154	1.252	1.131	102	107	105	121	101	>1.00E-04	>1.00E-04	>1.00E-04
Renal Cancer															
786-0	0.427	1.352	1.321	1.397	1.358	1.326	1.222	97	105	101	97	86	>1.00E-04	>1.00E-04	>1.00E-04
A498	1.345	2.100	2.282	2.275	2.070	2.267	1.579	124	123	96	122	31	5.19E-05	>1.00E-04	>1.00E-04
ACHN	0.661	0.874	0.855	0.840	0.817	0.797	0.645	91	84	73	64	-2	1.62E-05	9.20E-05	>1.00E-04
CAKI-1	0.749	2.280	2.225	2.256	2.253	2.163	1.967	96	98	98	92	81	>1.00E-04	>1.00E-04	>1.00E-04
SN12C	0.982	1.837	1.787	1.762	1.797	1.737	1.558	94	91	95	88	67	>1.00E-04	>1.00E-04	>1.00E-04
TK-10	0.894	1.927	1.873	1.882	1.850	1.878	1.896	95	96	93	95	97	>1.00E-04	>1.00E-04	>1.00E-04
UO-31	0.197	0.781	0.804	0.735	0.716	0.825	0.808	104	92	89	108	105	>1.00E-04	>1.00E-04	>1.00E-04
Prostate Cancer															
PC-3	0.232	0.925	0.910	0.832	0.845	0.875	0.770	98	87	89	93	78	>1.00E-04	>1.00E-04	>1.00E-04
DU-145	0.443	1.180	1.234	1.255	1.148	1.182	0.989	107	110	96	100	74	>1.00E-04	>1.00E-04	>1.00E-04
Breast Cancer															
MCF7	0.265	1.598	1.512	1.617	1.513	1.595	1.354	94	101	94	100	82	>1.00E-04	>1.00E-04	>1.00E-04
NCI/ADR-RES	0.327	0.816	0.773	0.776	0.754	0.727	0.667	91	92	87	82	69	>1.00E-04	>1.00E-04	>1.00E-04
MDA-MB-231/ATCC	0.470	0.913	0.888	0.884	0.887	0.867	0.815	94	93	94	89	78	>1.00E-04	>1.00E-04	>1.00E-04
HS 578T	0.658	1.266	1.370	1.215	1.188	1.074	0.849	113	89	84	66	30	2.84E-05	>1.00E-04	>1.00E-04
MDA-MB-435	0.546	1.872	1.864	1.952	1.913	1.980	1.908	99	106	103	108	103	>1.00E-04	>1.00E-04	>1.00E-04
BT-549	0.738	1.152	1.105	1.155	1.131	1.182	1.093	89	101	95	107	86	>1.00E-04	>1.00E-04	>1.00E-04
T-47D	0.664	2.932	3.015	2.893	3.047	3.106	3.000	104	98	105	108	103	>1.00E-04	>1.00E-04	>1.00E-04

Table v: National Cancer Institute *In Vitro* Testing Results for 2-Chlorosamaderine A (iii)

			Log10 Concentration													
	Time		Mean Optical Densities					Percent Growth								
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.172	0.980	1.008	0.983	0.947	0.918	0.208	103	100	96	92	4	3.03E-05	>1.00E-04	>1.00E-04
HL-60(TB)	0.261	1.753	1.789	1.768	1.785	1.572	0.494	102	101	102	88	16	3.34E-05	>1.00E-04	>1.00E-04
K-562	0.146	1.527	1.625	1.658	1.632	1.559	0.382	107	110	108	102	17	4.11E-05	>1.00E-04	>1.00E-04
MOLT-4	0.140	0.915	0.905	0.983	1.023	0.964	0.384	99	109	114	106	31	5.65E-05	>1.00E-04	>1.00E-04
RPMI-8226	0.261	0.941	0.966	0.946	0.881	0.811	0.197	104	101	91	81	-25	1.96E-05	5.83E-05	>1.00E-04
SR	0.166	0.573	0.560	0.577	0.519	0.476	0.216	97	101	87	76	12	2.56E-05	>1.00E-04	>1.00E-04
Non-Small Cell Lung Cancer															
A549/ATCC	0.142	0.787	0.805	0.812	0.815	0.817	0.254	103	104	104	105	17	4.22E-05	>1.00E-04	>1.00E-04
EKVX	0.879	1.899	1.864	1.825	1.709	1.694	1.310	97	93	81	80	42	6.24E-05	>1.00E-04	>1.00E-04
HOP-62	0.707	1.642	1.630	1.631	1.614	1.646	0.877	99	99	97	100	18	4.10E-05	>1.00E-04	>1.00E-04
HOP-92	0.575	0.894	0.928	0.921	0.906	0.745	0.667	110	108	104	53	29	1.36E-05	>1.00E-04	>1.00E-04
NCI-H226	1.093	1.425	1.437	1.423	1.390	1.397	1.057	104	99	90	91	-3	2.74E-05	9.23E-05	>1.00E-04
NCI-H23	0.310	0.524	0.535	0.504	0.515	0.487	0.278	105	91	96	82	-10	2.24E-05	7.71E-05	>1.00E-04
NCI-H322M	0.972	1.976	2.005	1.970	1.946	1.954	1.368	103	99	97	98	39	6.52E-05	>1.00E-04	>1.00E-04
NCI-H460	0.184	1.894	1.880	1.972	1.928	2.010	0.858	99	105	102	107	39	6.96E-05	>1.00E-04	>1.00E-04
NCI-H522	0.262	0.916	0.911	0.913	0.887	0.870	0.299	99	100	96	93	6	3.10E-05	>1.00E-04	>1.00E-04
Colon Cancer															
COLO 205	0.444	2.709	2.726	2.711	2.745	2.738	0.706	101	100	102	101	12	3.73E-05	>1.00E-04	>1.00E-04
HCC-2998	0.328	0.981	0.970	0.592	0.995	0.958	0.338	98	102	102	97	1	3.09E-05	>1.00E-04	>1.00E-04
HCT-116	0.194	0.938	0.956	0.957	0.910	0.939	0.154	102	103	96	100	-21	2.60E-05	6.75E-05	>1.00E-04
HCT-15	0.729	1.034	1.021	1.002	1.018	0.998	0.291	96	89	95	88	-60	1.81E-05	3.93E-05	8.54E-05
HT29	0.163	1.038	1.046	1.056	0.989	0.992	0.254	101	102	94	95	10	3.39E-05	>1.00E-04	>1.00E-04
KM12	0.241	1.523	1.554	1.526	1.563	1.520	0.398	102	100	103	100	12	3.70E-05	>1.00E-04	>1.00E-04
SW-620	0.412	1.386	1.293	1.372	1.621	1.582	0.471	90	99	124	120	6	4.12E-05	>1.00E-04	>1.00E-04
CNS Cancer															
SF-268	0.511	1.585	1.563	1.630	1.596	1.633	0.842	98	104	101	104	31	5.48E-05	>1.00E-04	>1.00E-04
SF-295	0.510	1.536	1.511	1.542	1.564	1.562	0.680	98	101	103	103	17	4.05E-05	>1.00E-04	>1.00E-04
SF-539	0.703	1.853	1.762	1.678	1.588	1.556	0.385	92	85	77	74	-45	1.55E-05	4.18E-05	>1.00E-04
SNB-19	0.280	2.372	2.372	2.409	2.349	2.422	0.617	100	102	99	102	16	4.05E-05	>1.00E-04	>1.00E-04
SNB-75	0.367	0.572	0.561	0.553	0.584	0.601	0.455	95	94	105	114	43	7.99E-05	>1.00E-04	>1.00E-04
U251	0.607	1.349	1.410	1.349	1.364	1.341	0.466	108	100	102	99	-20	2.58E-05	6.80E-05	>1.00E-04
Melanoma															
LOX IMVI	0.470	1.391	1.436	1.390	1.386	1.364	0.253	105	100	99	97	-46	2.13E-05	4.75E-05	>1.00E-04
MALME-3M	0.979	1.962	1.951	1.976	1.949	1.960	1.355	99	101	99	100	39	6.52E-05	>1.00E-04	>1.00E-04
M14	0.288	1.154	1.130	1.085	1.052	1.078	0.154	97	92	89	91	-47	1.99E-05	4.59E-05	>1.00E-04
SK-MEL-2	0.404	1.010	1.019	1.059	0.984	0.964	0.467	101	108	96	92	10	3.29E-05	>1.00E-04	>1.00E-04
SK-MEL-28	0.276	2.125	2.112	2.049	2.071	2.017	0.477	99	95	97	94	11	3.39E-05	>1.00E-04	>1.00E-04
SK-MEL-5	1.936	2.652	2.560	2.828	2.614	2.574	1.123	87	125	55	85	-42	1.59E-05	4.78E-05	>1.00E-04
UACC-62	0.957	2.117	2.153	2.091	2.117	2.085	0.873	103	98	100	97	-9	2.75E-05	8.26E-05	>1.00E-04
Ovarian Cancer															
IGROV1	0.233	1.025	1.016	1.028	1.004	1.012	0.371	99	100	97	98	17	3.94E-05	>1.00E-04	>1.00E-04
OVCAR-3	0.642	1.569	1.325	1.298	1.296	1.352	0.462	94	50	90	98	-28	2.39E-05	5.98E-05	>1.00E-04
OVCAR-4	0.467	1.844	1.807	1.868	1.884	1.552	1.185	97	102	103	105	52	>1.00E-04	>1.00E-04	>1.00E-04
OVCAR-5	0.379	0.876	0.919	0.901	0.633	0.528	0.337	108	105	52	30	-11	1.25E-06	5.33E-05	>1.00E-04
OVCAR-8	0.111	0.965	0.996	1.024	1.023	1.048	0.669	104	107	107	110	58	>1.00E-04	>1.00E-04	>1.00E-04
SK-OV-3	0.510	1.154	1.193	1.318	1.285	1.361	1.032	106	125	120	135	81	>1.00E-04	>1.00E-04	>1.00E-04
Renal Cancer															
786-0	0.427	1.529	1.477	1.497	1.488	1.533	0.469	95	97	96	100	4	3.32E-05	>1.00E-04	>1.00E-04
A498	1.345	1.999	2.083	2.116	1.922	2.033	0.290	113	118	88	105	-78	2.00E-05	3.74E-05	7.00E-05
ACHN	0.661	0.988	0.978	0.973	0.973	0.979	0.414	97	95	95	97	-37	2.24E-05	5.27E-05	>1.00E-04
CAKI-1	0.749	2.361	2.403	2.384	2.362	2.340	0.971	103	101	100	99	14	3.74E-05	>1.00E-04	>1.00E-04
RXF 393	0.201	0.516	0.513	0.563	0.535	0.516	0.318	99	115	106	100	37	6.24E-05	>1.00E-04	>1.00E-04
SN12C	0.982	1.950	1.948	1.905	1.909	1.917	0.811	100	95	96	97	-17	2.56E-05	7.03E-05	>1.00E-04
TK-10	0.894	1.939	1.974	1.968	1.983	2.028	1.401	103	105	104	108	49	9.44E-05	>1.00E-04	>1.00E-04
UO-31	0.197	0.975	0.921	0.897	1.059	1.070	0.496	93	90	111	112	38	6.97E-05	>1.00E-04	>1.00E-04
Prostate Cancer															
PC-3	0.232	1.037	1.026	0.981	1.009	0.989	0.355	99	93	97	94	15	3.62E-05	>1.00E-04	>1.00E-04
DU-145	0.443	1.377	1.456	1.307	1.366	1.334	0.485	108	93	99	95	4	3.16E-05	>1.00E-04	>1.00E-04
Breast Cancer															
MCF7	0.265	1.681	1.643	1.682	1.671	1.739	0.841	97	100	99	104	41	7.13E-05	>1.00E-04	>1.00E-04
NCI/ADR-RES	0.327	0.947	0.964	0.987	0.908	0.953	0.262	103	106	94	101	-20	2.64E-05	6.83E-05	>1.00E-04
MDA-MB-231/ATCC	0.470	1.030	1.017	1.024	1.019	1.017	0.524	98	99	98	98	10	3.48E-05	>1.00E-04	>1.00E-04
HS 578T	0.658	1.618	1.483	1.450	1.354	1.358	0.419	86	83	73	73	-36	1.62E-05	4.65E-05	>1.00E-04
MDA-MB-435	0.546	2.033	2.005	1.999	2.024	2.016	0.417	98	98	99	99	-24	2.50E-05	6.40E-05	>1.00E-04
BT-549	0.738	1.347	1.244	1.328	1.278	1.334	0.772	83	97	89	98	6	3.30E-05	>1.00E-04	>1.00E-04
T-47D	0.664	3.039	3.083	3.003	3.030	3.075	1.307	102	98	100	102	27	4.92E-05	>1.00E-04	>1.00E-04

Table vi: National Cancer Institute *In Vitro* Testing Results for Cedronin (vi)

Panel/Cell Line	Time Zero	Ctrl	Mean Optical Densities (Log10 Concentration) -8.0	-7.0	-6.0	-5.0	-4.0	Percent Growth -8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.228	1.055	1.038	1.224	1.263	0.442	0.477	98	120	125	27	30	5.79E-06	>1.00E-04	>1.00E-04
HL-60(TB)	0.293	1.474	1.439	1.478	1.401	0.576	0.295	97	100	94	24	.	4.24E-06	>1.00E-04	>1.00E-04
K-562	0.238	1.697	1.175	1.767	1.541	0.765	0.242	64	105	89	36	.	5.48E-06	>1.00E-04	>1.00E-04
MOLT-4	0.250	0.619	0.502	0.385	0.398	0.235	0.149	70	37	40	-6	-40	3.98E-08	7.34E-06	>1.00E-04
RPMI-8226	0.529	2.016	2.092	2.193	2.086	1.419	0.801	105	112	105	60	18	1.73E-05	>1.00E-04	>1.00E-04
SR	0.267	0.599	0.560	0.522	0.416	0.239	0.076	88	77	45	-10	-72	6.88E-07	6.46E-06	4.42E-05
Non-Small Cell Lung Cancer															
A549/ATCC	0.334	1.426	1.474	1.480	1.404	0.696	0.237	104	105	98	33	-29	5.49E-06	3.40E-05	>1.00E-04
EKVX	0.392	0.760	0.765	0.816	0.809	0.602	0.254	101	115	113	57	-35	1.19E-05	4.16E-05	>1.00E-04
HOP-62	0.705	1.782	1.802	1.843	1.758	1.104	0.503	102	106	98	37	-25	6.12E-06	3.66E-05	>1.00E-04
HOP-92	0.966	1.422	1.525	1.449	1.428	1.190	1.357	122	106	101	49	86	.	>1.00E-04	>1.00E-04
NCI-H226	0.792	1.236	1.218	1.249	1.175	0.878	0.476	96	103	86	19	-40	3.48E-06	2.12E-05	>1.00E-04
NCI-H23	0.402	1.134	1.154	1.200	1.143	0.592	0.112	103	109	101	26	-71	4.80E-06	1.86E-05	6.11E-05
NCI-H322M	0.895	1.744	1.694	1.754	1.760	1.399	0.626	94	101	102	59	-30	1.27E-05	4.60E-05	>1.00E-04
NCI-H460	0.252	1.364	1.384	1.356	1.269	0.625	0.231	102	99	91	34	-9	5.20E-06	6.27E-05	>1.00E-04
NCI-H522	0.678	1.266	1.236	1.297	1.263	0.909	0.394	95	105	99	39	-42	6.64E-06	3.05E-05	>1.00E-04
Colon Cancer															
COLO 205	0.685	2.928	2.862	2.882	2.763	1.919	0.637	97	98	93	55	-7	1.20E-05	7.71E-05	>1.00E-04
HCT-116	0.186	0.980	0.925	0.980	0.941	0.359	0.085	93	100	95	22	-55	4.11E-06	1.93E-05	8.71E-05
HCT-15	0.381	1.182	1.174	1.219	1.105	0.608	0.197	99	105	90	28	-48	4.47E-06	2.34E-05	>1.00E-04
HT29	0.142	1.580	1.596	1.547	1.429	0.834	0.328	101	98	89	48	13	9.02E-06	>1.00E-04	>1.00E-04
KM12	0.420	2.065	2.074	2.013	1.890	1.140	0.248	100	97	89	44	-40	7.41E-06	3.36E-05	>1.00E-04
SW-620	0.105	0.773	0.651	0.683	0.640	0.368	0.103	81	86	80	39	-2	5.41E-06	8.99E-05	>1.00E-04
CNS Cancer															
SF-268	0.727	1.660	1.679	1.658	1.657	1.268	0.563	102	100	100	58	-23	1.26E-05	5.25E-05	>1.00E-04
SF-295	0.555	1.680	1.695	1.664	1.651	1.083	0.411	101	98	97	45	-31	8.02E-06	3.91E-05	>1.00E-04
SF-539	0.612	1.481	1.529	1.554	1.504	1.002	0.368	105	108	103	45	-37	8.15E-06	3.55E-05	>1.00E-04
SNB-19	0.435	2.628	2.565	2.600	2.497	1.424	0.527	97	99	94	45	4	7.94E-06	>1.00E-04	>1.00E-04
SNB-75	0.387	0.874	0.906	0.948	0.921	0.731	0.438	107	115	110	71	10	2.20E-05	>1.00E-04	>1.00E-04
U251	0.228	0.956	0.949	0.993	0.942	0.497	0.144	99	105	98	37	-37	6.10E-06	3.16E-05	>1.00E-04
Melanoma															
LOX IMVI	0.371	1.414	1.457	1.447	1.261	0.345	0.043	104	103	85	-7	-89	2.41E-06	8.40E-06	3.37E-05
MALME-3M	0.816	1.527	1.508	1.507	1.415	1.175	0.509	97	97	84	51	-38	1.01E-05	3.74E-05	>1.00E-04
M14	0.316	0.855	0.863	0.868	0.862	0.414	0.066	101	102	101	18	-78	4.14E-06	1.54E-05	5.07E-05
SK-MEL-2	0.645	1.154	1.169	1.168	1.095	0.852	0.349	103	103	88	41	-46	6.36E-06	2.95E-05	>1.00E-04
SK-MEL-28	0.192	2.082	1.397	2.074	1.963	1.008	0.357	55	100	94	43	9	7.32E-06	>1.00E-04	>1.00E-04
SK-MEL-5	0.553	2.440	2.361	2.288	2.539	1.058	0.249	96	92	105	27	-55	5.06E-06	2.12E-05	5.67E-05
UACC-257	0.748	1.430	1.432	1.497	1.437	1.033	0.492	97	107	98	42	-34	9.57E-06	3.88E-05	>1.00E-04
UACC-62	0.683	1.548	1.562	1.659	1.515	1.073	0.386	102	113	96	45	-44	8.02E-06	3.23E-05	>1.00E-04
Ovarian Cancer															
IGROV1	0.509	1.252	1.345	1.377	1.289	0.939	0.356	112	117	105	58	-30	1.23E-05	4.58E-05	>1.00E-04
OVCAR-3	0.501	1.241	1.238	1.307	1.147	1.083	0.402	100	109	87	73	-20	1.95E-05	6.29E-05	>1.00E-04
OVCAR-4	0.285	1.323	1.354	1.355	1.320	0.881	0.441	103	104	100	57	15	1.50E-05	>1.00E-04	>1.00E-04
OVCAR-5	0.373	0.841	0.867	0.877	0.881	0.732	0.339	106	108	109	77	-9	2.05E-05	7.83E-05	>1.00E-04
OVCAR-8	0.367	0.995	1.055	1.022	1.017	0.569	0.192	110	104	103	32	-48	5.57E-06	2.52E-05	>1.00E-04
SK-OV-3	0.830	2.935	2.924	2.980	2.784	2.282	0.820	99	102	93	64	-1	1.65E-05	9.56E-05	>1.00E-04
Renal Cancer															
786-0	0.327	1.091	1.100	1.124	1.001	0.590	0.182	101	104	88	34	-44	5.13E-06	2.74E-05	>1.00E-04
A498	1.275	1.792	1.781	1.853	1.760	1.672	0.924	98	112	94	77	-28	1.80E-05	5.44E-05	>1.00E-04
ACHN	0.403	0.839	0.869	0.844	0.814	0.529	0.142	107	101	94	29	-65	4.75E-06	2.03E-05	6.94E-05
CAKI-1	0.653	2.146	2.196	2.200	2.122	1.417	0.533	103	104	98	51	-18	1.04E-05	5.44E-05	>1.00E-04
RXF 393	0.672	0.819	0.843	0.844	0.760	0.598	0.279	116	117	60	-11	-59	1.36E-06	6.98E-06	6.61E-05
SN12C	0.459	1.082	1.107	1.126	1.076	0.744	0.251	104	107	99	46	-43	8.32E-05	3.27E-05	>1.00E-04
TK-10	0.961	1.802	1.786	1.889	1.841	1.445	0.658	98	110	105	58	-32	1.21E-05	4.43E-05	>1.00E-04
UO-31	0.419	1.293	1.364	1.364	1.237	0.879	0.290	108	108	94	53	-31	1.07E-05	4.27E-05	>1.00E-04
Prostate Cancer															
PC-3	0.724	2.193	2.190	2.276	2.193	1.772	1.029	100	106	100	71	21	2.64E-05	>1.00E-04	>1.00E-04
DU-145	0.320	1.015	1.042	1.097	0.927	0.522	0.227	104	112	87	29	-23	4.36E-06	3.16E-05	>1.00E-04
Breast Cancer															
MCF7	0.231	0.654	0.678	0.611	0.647	0.305	0.154	106	90	98	17	-34	3.95E-06	2.19E-05	>1.00E-04
NCI/ADR-RES	0.666	1.736	1.775	1.780	1.768	1.464	0.532	104	104	103	75	-20	1.82E-05	6.13E-05	>1.00E-04
MDA-MB-231/ATCC	0.630	0.929	0.930	1.016	0.868	0.842	0.383	100	129	80	71	-39	1.54E-05	4.39E-05	>1.00E-04
HS 578T	0.453	1.011	1.026	1.073	1.064	0.773	0.370	103	111	110	57	-18	1.25E-05	5.72E-05	>1.00E-04
MDA-MB-435	0.504	1.849	1.895	1.925	1.865	1.042	0.388	103	106	101	40	-23	6.86E-06	4.30E-05	>1.00E-04
BT-549	0.535	0.875	0.891	0.920	0.879	0.717	0.372	104	113	101	53	-30	1.10E-05	4.34E-05	>1.00E-04
T-47D	1.163	3.018	2.992	3.037	2.708	2.487	0.984	99	101	83	71	-15	1.76E-05	6.65E-05	>1.00E-04

Table vii: National Cancer Institute *In Vitro* Testing Results for 3,4β-Dihydrosamaderine C (vii)

Panel/Cell Line	Time Zero	Ctrl	Log10 Concentration Mean Optical Densities -8.0	-7.0	-6.0	-5.0	-4.0	Percent Growth -8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.172	0.815	0.847	0.794	0.785	0.150	0.115	105	97	55	-13	-31	2.62E-06	7.61E-06	>1.00E-04
HL-60(TB)	0.261	1.827	1.782	1.670	1.521	0.441	0.367	97	90	80	11	7	2.76E-06	>1.00E-04	>1.00E-04
K-562	0.146	1.405	1.400	1.419	1.232	0.281	0.163	100	102	86	11	1	3.02E-06	>1.00E-04	>1.00E-04
MOLT-4	0.140	0.790	0.776	0.709	0.665	0.200	0.129	98	88	81	9	-8	2.69E-06	3.45E-05	>1.00E-04
RPMI-8226	0.261	0.782	0.733	0.826	0.760	0.147	0.234	91	109	96	-44	-49	2.13E-06	4.86E-06	>1.00E-04
SR	0.166	0.529	0.483	0.505	0.475	0.124	0.090	87	93	86	-25	-46	2.11E-06	5.93E-06	>1.00E-04
Non-Small Cell Lung Cancer															
A549/ATCC	0.142	0.713	0.715	0.706	0.676	0.191	0.103	100	99	93	9	-27	3.25E-06	1.73E-05	>1.00E-04
EKVX	0.879	1.479	1.403	1.411	1.449	0.912	0.502	87	85	95	6	-63	3.19E-06	1.30E-05	>1.00E-04
HOP-62	0.707	1.501	1.524	1.504	1.344	0.632	0.347	103	100	80	-11	-51	2.15E-06	7.63E-06	9.49E-05
HOP-92	0.575	0.799	0.833	0.816	0.764	0.618	0.367	115	107	84	19	-36	3.36E-06	2.22E-05	>1.00E-04
NCI-H226	1.093	1.328	1.270	1.201	1.136	0.639	0.352	75	46	10	-42	-68	7.16E-08	1.55E-06	2.09E-05
NCI-H322M	0.972	1.886	1.862	1.845	1.775	0.925	0.608	98	95	82	-4	-38	2.58E-06	9.08E-06	>1.00E-04
NCI-H460	0.184	1.784	1.751	1.825	1.849	0.771	0.387	98	103	104	37	13	6.35E-06	>1.00E-04	>1.00E-04
NCI-H522	0.262	0.753	0.790	0.790	0.793	0.720	0.149	108	108	108	93	-43	2.08E-05	4.82E-05	>1.00E-04
Colon Cancer															
COLO 205	0.444	2.646	2.745	2.657	2.589	1.328	0.504	104	100	97	40	3	6.73E-06	>1.00E-04	>1.00E-04
HCC-2998	0.328	0.783	0.773	0.755	0.697	0.267	0.020	98	94	81	-19	-94	2.05E-06	6.51E-06	2.61E-05
HCT-116	0.194	0.846	0.858	0.813	0.648	0.274	-0.048	102	95	70	12	-100	2.20E-06	1.29E-05	3.59E-05
HCT-15	0.729	0.942	0.917	0.908	0.811	0.318	0.083	88	84	38	-56	-89	5.56E-07	2.54E-06	8.55E-06
HT29	0.163	0.921	0.962	0.896	0.771	0.423	0.102	105	97	80	34	-37	4.55E-06	3.01E-05	>1.00E-04
KM12	0.241	1.441	1.434	1.358	1.268	0.526	0.196	99	93	86	24	-19	3.76E-06	3.63E-05	>1.00E-04
SW-620	0.412	1.472	1.483	1.355	1.544	0.622	0.142	101	89	107	20	-66	4.50E-06	1.71E-05	6.56E-05
CNS Cancer															
SF-268	0.511	1.553	1.525	1.515	1.483	0.986	0.455	97	96	93	47	-11	8.43E-06	6.45E-05	>1.00E-04
SF-295	0.510	1.499	1.433	1.393	1.265	0.587	0.262	93	89	76	8	-49	2.42E-06	1.37E-05	>1.00E-04
SF-539	0.703	1.541	1.462	1.460	1.317	0.423	0.213	91	90	73	-40	-70	1.61E-06	4.44E-06	2.18E-05
SNB-19	0.280	2.378	2.376	2.392	2.282	0.842	0.350	100	101	95	27	5	4.59E-06	>1.00E-04	>1.00E-04
SNB-75	0.367	0.509	0.549	0.511	0.487	0.404	0.183	128	101	84	26	-50	3.83E-06	2.18E-05	9.92E-05
U251	0.607	1.241	1.287	1.186	1.056	0.284	0.172	107	91	71	-53	-72	1.47E-06	3.72E-06	9.42E-06
Melanoma															
LOX IMVI	0.470	1.276	1.291	1.218	0.931	0.147	0.009	102	93	57	-69	-98	1.14E-06	2.84E-06	7.09E-06
MALME-3M	0.979	1.925	1.868	1.809	1.622	0.845	0.582	54	87	68	-14	-41	1.65E-06	6.79E-06	>1.00E-04
M14	0.288	0.574	0.959	0.972	0.778	0.150	0.023	98	100	71	-48	-92	1.51E-06	3.96E-06	1.10E-05
SK-MEL-2	0.404	0.841	0.868	0.841	0.855	0.802	0.160	102	100	105	91	-60	1.87E-05	3.99E-05	8.54E-05
SK-MEL-28	0.275	2.001	2.092	2.047	1.874	0.926	0.302	105	103	93	32	2	5.96E-06	>1.00E-04	>1.00E-04
SK-MEL-5	1.936	2.731	2.444	2.457	2.348	0.809	0.204	64	66	52	-52	-89	1.04E-06	2.96E-06	8.42E-06
UACC-62	0.957	1.996	1.905	1.873	1.697	0.853	0.365	91	89	71	-7	-52	1.87E-06	8.19E-06	6.10E-05
Ovarian Cancer															
IGROV1	0.233	0.819	0.834	0.889	0.854	0.785	0.128	103	112	106	94	-45	2.07E-05	4.74E-05	>1.00E-04
OVCAR-3	0.642	1.347	1.373	1.390	1.169	0.652	0.342	104	106	75	3	-47	2.20E-06	1.14E-05	>1.00E-04
OVCAR-4	0.487	1.667	1.855	1.776	1.750	1.248	0.563	116	109	105	65	8	1.84E-05	>1.00E-04	>1.00E-04
OVCAR-5	0.379	0.800	0.763	0.753	0.704	0.504	0.229	91	91	77	30	-40	3.72E-06	2.67E-05	>1.00E-04
OVCAR-8	0.111	0.905	0.987	0.951	0.934	0.667	0.295	110	105	103	70	23	2.65E-05	>1.00E-04	>1.00E-04
SK-OV-3	0.510	1.125	1.141	1.151	1.130	0.748	0.498	103	101	101	39	-2	6.57E-06	8.76E-05	>1.00E-04
Renal Cancer															
786-0	0.427	1.352	1.319	1.366	1.219	0.494	0.196	96	101	86	7	-54	2.85E-06	1.31E-05	8.54E-05
A498	1.345	2.100	2.058	2.331	1.519	0.710	0.564	94	131	23	-47	-58	5.60E-07	2.12E-06	1.81E-05
ACHN	0.661	0.874	0.882	0.842	0.743	0.341	0.048	104	85	39	-48	-93	5.62E-07	2.77E-06	1.09E-05
CAKI-1	0.749	2.280	2.304	2.304	2.071	1.018	0.526	102	102	86	18	-30	3.38E-06	2.36E-05	>1.00E-04
SN12C	0.982	1.837	1.718	1.683	1.551	0.901	0.413	86	82	66	-8	-58	1.66E-06	7.76E-06	5.91E-05
TK-10	0.894	1.927	1.845	1.796	1.742	1.128	0.622	92	87	82	23	-30	3.47E-06	2.67E-05	>1.00E-04
UO-31	0.197	0.781	0.965	0.963	0.706	0.620	0.202	132	131	87	72	-48	1.54E-05	3.99E-05	>1.00E-04
Prostate Cancer															
PC-3	0.232	0.925	0.957	0.944	0.926	0.545	0.185	105	103	101	45	-20	8.17E-06	4.90E-05	>1.00E-04
DU-145	0.443	1.180	1.213	1.175	0.981	0.363	0.263	104	99	73	-18	-63	1.79E-06	6.33E-06	5.08E-05
Breast Cancer															
MCF7	0.265	1.598	1.586	1.597	1.491	0.368	0.116	99	100	92	8	-56	3.15E-06	1.32E-05	7.99E-05
NCI/ADR-RES	0.327	0.816	0.820	0.789	0.766	0.561	0.183	101	94	90	48	-44	8.87E-06	3.32E-05	>1.00E-04
MDA-MB-231/ATCC	0.470	0.913	0.881	0.875	0.791	0.516	0.248	93	91	72	10	-47	2.30E-06	1.51E-05	>1.00E-04
HS 578T	0.658	1.286	1.306	1.467	1.455	1.012	0.451	103	129	127	56	-32	1.18E-05	4.38E-05	>1.00E-04
MDA-MB-435	0.546	1.872	1.891	1.931	1.758	0.663	0.365	101	104	91	9	-33	3.17E-06	1.62E-05	>1.00E-04
BT-549	0.738	1.152	1.129	1.118	1.131	0.727	0.399	95	92	95	-2	-46	2.92E-06	9.63E-06	>1.00E-04
T-47D	0.664	2.932	2.962	3.006	2.769	1.890	0.715	101	103	93	54	2	1.20E-05	>1.00E-04	>1.00E-04

A better than mean response to the LC 50 parameter for 2-chlorosamaderine A (**iii**) was obtained for only two of the cell lines, HCT-15 (colon cancer) (LC 50: 8.54x10^{-5} g/ml) and A498 (renal cancer) (LC 50: 7.00x10^{-5} g/ml) (Appendix, pages 165, 166).

A better than mean response to the LC 50 parameter for cedronin (**vi**) was obtained for six of the cell lines, SR (leukemia) (LC 50: 4.42x10^{-5} g/ml), NCI-H23 (non-small cell lung cancer) (LC 50: 6.11x10^{-5} g/ml), LOX IMVI (melanoma) (LC 50: 3.37x10^{-5} g/ml), M14 (melanoma) (LC 50: 5.07x10^{-5} g/ml), ACHN (renal cancer) (LC 50: 6.94x10^{-5} g/ml) and RXF 393 (renal cancer) (LC 50: 6.61x10^{-5} g/ml) (Appendix, pages 167, 168).

A better than mean response to the LC 50 parameter for 3,4β-dihydrosamaderine C (**vii**) was obtained for twelve of the cell lines, NCI-H226 (non-small cell lung cancer) (LC 50: 2.09x10^{-5} g/ml), HCC-2998 (colon cancer) (LC 50: 2.61x10^{-5} g/ml), HCT-116 (colon cancer) (LC 50: 3.59x10^{-5} g/ml), HCT-15 (colon cancer) (LC 50: 8.55x10^{-6} g/ml), SF-539 (CNS cancer) (LC 50: 2.18x10^{-5} g/ml), U251 (CNS cancer) (LC 50: 9.42x10^{-6} g/ml), LOX IMVI (melanoma) (LC 50: 7.09x10^{-6} g/ml), M14 (melanoma) (LC 50: 1.10x10^{-5} g/ml), SK-MEL-5 (melanoma) (LC 50: 8.42x10^{-6} g/ml), A498 (renal cancer) (LC 50: 1.81x10^{-5} g/ml), ACHN (renal cancer) (LC 50: 1.09x10^{-5} g/ml), DU-145 (prostate cancer) (LC 50: 5.08x10^{-5} g/ml) (Appendix, pages 169, 170).

A negative sensitivity response to the LC 50 parameter was obtained for the other quassinoids (samaderine A (i), 5β,6-dihydrosamaderine A (**ii**)) tested in this work.

3,4β-Dihydrosamaderine C (**vii**) was the most active of the quassinoids tested in this work, and was active in killing the cancer cells associated with non-small cell lung cancer, colon cancer, CNS cancer, melanoma, renal cancer, prostate cancer and breast cancer (Appendix, pages 169, 170).

However, these results were not of significant interest since the concentrations at which these compounds were active were not suitably low. They are therefore not being investigated further by the National Cancer Institute.

5.5. References

1. Capuron, J., *Adansonia*, Tome 1-fasc., 1961, **1**, 83-92.
2. Nooteboom, H.P., Generic delimitation in Simaroubaceae tribus Simaroubeae and a conspectus of the genus *Quassia* L., *Blumea*, 1963, **11**, 510-528.
3. Petitjean, A. and Petitjean, M., Û plantes utiles de Madagascar, Centre d'Information Technique *et* Economique (CIDST), Antananarivo, 1992, page number not supplied.
4. Rasoanaivo, P., Petitjean, A., Ratsimamanga-Urverg, S. and Rakoto-Ratsimamanga, A., Medicinal plants used to treat malaria in Madagascar, *J. Ethnopharmacol.*, 1992, **37**, 117-127.
5. Randrianarivelojosia, M., personal communication.
6. Coombes, P.H., Extractives from the Meliaceae and Simaroubaceae of Madagascar, PhD Thesis, University of Natal, South Africa, 2001, 116-144.
7. Merrien, A. and Polonsky, J., The natural occurrence of melianodiol and its diacetate in *Samadera madagascariensis* (Simaroubaceae): model experiments on melianodiol directed toward Simarolide, *Chem. Comm.*, 1971, 261-263.
8. Simão, S.M., Barreiros, E.L., da Silva, M.F.G.F. and Gottlieb, O.R., Chemogeographical evolution of quassinoids in Simaroubaceae, *Phytochemistry*, 1991, **30**, 853-865.
9. Gibbons, S., Craven, L., Dunlop, C., Gray, A.I., Hartley, T.G. and Waterman, P.G., The secondary metabolites of aff. *Samadera* SAC-2825: an Australian Simaroubaceae with unusual chemistry, *Phytochemistry*, 1997, **44**, 1109-1114.
10. Latif, Z., Craven, L., Hartley, T.G., Kemp, B.R., Potter, J., Rice, M.J., Waigh, R.D. and Waterman, P.G., An insecticidal quassinoid from the new Australian species *Quassia* sp. aff. *bidwillii, Biochemical Systematics and Ecology*, 2000, **28**, 183-184.
11. Zylber, J. and Polonsky, J., Constituents of *Samadera indica* bark. Structure of samaderine B and of samaderine C, *Bull. Soc. Chim. France*, 1964, 2016-2019.
12. Zylber, J., Etude des constituants de l'ecoye de *Samadera indica*; structures des samaderines, B, C et D, PhD Thesis, Universite de Paris, Orsay, 1967. In Polonsky, J., Quassinoid Bitter Principles, *Progress in the Chemistry of Organic Natural Products*, 1973, **30**, 101-150.
13. Polonsky, J., Zylber, J. and Wijesekera, R.O.B., Constituents of the bark of *Samadera indica, Bull. Soc. Chim. France*, 1962, 1715-1721.
14. Wani, M.C., Taylor, H.L., Wall, M.E., McPhail, T. and Onan, K.D., Plant antitumour agents: Isolation and structure of samaderine A (x-ray analysis) and a new antileukaemic quassinoid samaderine E from *Samadera indica, Chem. Comm.*, 1977, 295-296.
15. Onan, K.D. and McPhail, T., Crystal and molecular structure of samaderine A, a C_{18} quassinoid from *Samadera indica, J. Chem. Res. (S)*, 1978, 14; *(M)*, 0401-0431.
16. Wintersteiner, O., Krakower, G. and Moore, M., Lupenone and 18α-oleanan-19α-ol-3-one, *J. Org. Chem.*, 1965, **30**, 2847-2849.
17. Iyer, V.S. and Rangaswami, S., Indacanthinone, a crystalline chemical component from the wood of *Samadera indica*, *Curr. Sci.*, 1972, **41**, 140-141.

18. Winckler, F.L., *Rep. Pharm.*, 1835, **4**, 85. In Valenta, Z., Papadopolous, S. and Podešva, C., Quassin and neoquassin, *Tetrahedron*, 1961, **15**, 100-110.
19. Valenta, Z., Papadopolous, S. and Podešva, C., Quassin and neoquassin, *Tetrahedron*, 1961, **15**, 100-110.
20. Valenta, Z., Gray, A.H., Orr, D.E., Papadopolous, S. and Podešva, C., The stereochemistry of quassin, *Tetrahedron*, 1962, **18**, 1433-1441.
21. Casinovi, C.G., Ceccherelli, P. and Grandolini, G., 18-Hydroxyquassine, a new amaroid from *Quassia amara*, *Ann. 1st. Super. Sanita.*, 1966, **2**, 414-416.
22. Kupchan, S.M. and Streelman, D.R., Quassimarin, a new antileukemic quassinoid from *Quassia amara*, *J. Org. Chem.*, 1976, **41**, 3481-3482.
23. Grandolini, G., Casinovi, C.G., Barbetti, P. and Fardella, G., A new neoquassin derivative from *Quassia amara*, *Phytochemistry*, 1987, **26**, 3085-3087.
24. Barbetti, P., Grandolini, G., Fardella, G. and Chiappini, I., Quassinoids from *Quassia amara*, *Phytochemistry*, 1993, **32**, 1007-1013.
25. Lavie, D. and Kaye, I.A., Isolation of β-sitostenone from *Quassia amara*, *J. Chem. Soc.*, 1963, 5001-5002.
26. Barbetti, P., Grandolini, G., Fardella, G. and Chiappini, I., Indole alkaloids from *Quassia amara*, *Planta. Med.*, 1987, **53**, 289-290.
27. Barbetti, P., Grandolini, G., Fardella, G., Chiappini, I. and Mastalia, A., New canthin-6-one alkaloids from *Quassia amara*, *Planta. Med.*, 1990, **56**, 216-217.
28. Njar, V.C.O., Alao, T.O., Okogun, J.I. and Holland, H.L., 2-Methoxycanthin-6-one: a new alkaloid from the stem wood of *Quassia amara*, *Planta. Med.*, 1993, **59**, 259-261.
29. Carter, C.A.G., Tinto, W.F., Reynolds, W.F. and McLean, S., Quassinoids from *Quassia multiflora*: structural assignments by 2D NMR spectroscopy, *J. Nat. Prod.*, 1993, **56**, 130-133.
30. Miller, S.L., Tinto, W.F., McLean, S., Reynolds, W.F., Yu, M. and Carter, C.A.G., Isolation and characterisation of a new glabretal triterpene from *Quassia multiflora*, *J. Nat. Prod.*,1995, **58**, 1640-1642.
31. Tinto, W.F., McLean, S., Reynolds, W.F. and Carter, C.A.G., Quassiol A, a novel squalene triterpene from *Quassia multiflora*, *Tetrahedron Letters*, 1993, **34**, 1705-1708.
32. Miller, S.L., Tinto, W.F., McLean, S., Reynolds, W.F., Yu, M. and Carter, C.A.G., Quassiols B-D, new squalene triterpenes from *Quassia multiflora*, *Tetrahedron*, 1995, **51**, 11959-11966.
33. Tresca, J-P., Alais, L. and Polonsky, J., Bitter constituents of *Quassia africana*. simalikalactones A, B, C, and D, and simalikahemiacetal A, *C.R. Seances Acad. Sci., Ser. C*, 1971, **273**, 601-604.
34. Murae, T., Tsuyuki, T., Ikeda, T., Nishihama, T., Masuda, T. and Takahashi, T., Bitter principles of *Picrasma ailanthoides* Planchon. Nigakilactones A, B, C, D, E and F, *Tetrahedron*, 1971, **27**, 1545-1555.
35. Hikino, H., Ohta, T. and Takemoto, T., Stereostructure of picrasin B, simaroubolide of *Picrasma quassioides*, *Chem. Pharm. Bull.*, 1970, **18**, 219-220.
36. Lumonadio, L. and Vanhaelen, M., Indole alkaloids and quassin from *Quassia Africana*, *J. Nat. Prod.*, 1986, **49**, 940.

37. Ayafor, J.F., Tchuendem, M.K., Mbazoa, C.M., Ngadjui, B.T. and Tillequin, F., ^{13}C NMR and other spectral data of 4-methylthiocanthin-6-one from *Quassia africana*, *Bull. Chem. Soc. Ethiop.*, 1993, **7**, 121-124.

38. Koike, K. and Ohmoto, T., Indaquassins A and B, two novel quassinoids from the bark of *Quassia indica* (0.6kg), *Phytochemistry*, 1993, **34**, 505-509.

39. Aono, H., Koike, K., Kaneko, J. and Ohmoto, T., Alkaloids and quassinoids from *Ailanthus malabarica*, *Phytochemistry*, 1994, **37**, 579-584.

40. Kitagawa, I., Mahmud, T., Yokota, K., Nakagawa, S., Mayumi, T., Kobayashi, M. and Shibuya, H., Indonesian Medicinal Plants XVII. Characterization of quassinoids from the stems of *Quassia indica*, *Chem. Pharm. Bull.*, 1996, **44**, 2009-2014.

41. White, T.C., "Tree of Flora of Malaya", Longman, New York, 1983, **2**, 352.

42. Mulholland, D.A., Monkhe, T.V., Taylor, D.A.H. and Rajab, M.S., Triterpenoids from *Turraea holstii*, *Phytochemistry*, 1999, **52**, 123-126.

43. Kitagawa, I., Mahmud, T., Simanjuntak, P., Hori, K., Uji, T. and Shibuya, H., Indonesian medicinal plants. VIII. Chemical structures of three new triterpenoids, bruceajavanin A, dihydrobruceajavanin A, and bruceajavanin B, and a new alkaloidal glycoside, bruceacanthinoside, from the stems of *Brucea javanica* (Simaroubaceae), *Chem. Pharm. Bull.*, 1994, **42**, 1416-1421.

44. Ang, H.H., Hitotsuyanagi, Y. and Takeya, K., Eurycolactones A-C, novel quassinoids from *Eurycoma longifolia*, *Tetrahedron Letters*, 2000, **41**, 6849-6853.

45. Koike, K. and Ohmoto, T., Quassinoids from *Picrasma Javanica*, *Phytochemistry*, 1994, **35**, 459-463.

46. Jacobs, H. and Lewis, D.E., Cedronin and 7-*epi*-cedronin: x-ray crystal structure analysis, *J. Nat. Prod.*, 1987, **50**, 700-705.

47. Polonsky, J., Varon, Z. and Rabanal, R.M., 21,20-Anhydromelianone and melianone from *Simarouba amara* (Simaroubaceae); carbon-13 NMR spectral analysis of Δ^7-tirucallol-type triterpenes, *Isr. J. Chem.*, 1977, **16**, 16-19.

48. Puripattanavong, J., Weber, S., Brecht, V. and Frahm, A.W., Phytochemical investigation of *Aglaia andamanica*, *Planta Med.*, 2000, **66**, 740-745.

49. Zeng, L., Gu, Z.-M., Chang, C.-J., Wood, K.V. and McLaughlin, J.L., Meliavolkenin, a new bioactive triterpenoid from *Melia volkensii* (Meliaceae), *Bioorg. Med. Chem.*, 1995, **3**, 383-390.

50. Omobuwajo, O.R., Martin, M.-T., Perromat, G., Sévenet, T., Païs, M. and Awang, K., Apotirucallane triterpenes from *Aglaia argentea*, *J. Nat. Prod.*, 1996, **59**, 614-617.

51. Connolly, J.D., Labbe, C., Rycroft, D.S. and Taylor, D.A.H., Tetranortriterpenoids and related compounds. Part 22. New apotirucallol derivatives and tetranortriterpenoids from the wood and seeds of *Chisocheton paniculatus* (Meliaceae), *J. Chem. Soc. Perkin Trans. I*, 1979, 2959-2964.

52. http://www.scs.uiuc.edu/~mainzv/Basics/integ.htm (23/11/2004)

53. Coombes, P.H., Extractives from the Meliaceae and Simaroubaceae of Madagascar, PhD Thesis, University of Natal, South Africa, 2001, 164-221.

Chapter 6

Extractives from *Toddaliopsis bremekampii* leaves & branches

Photograph by Dr. Neil Crouch

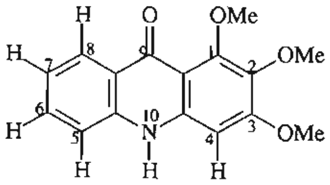
CHAPTER 6
Extractives from *Toddaliopsis bremekampii* leaves and branches

6.1. Introduction

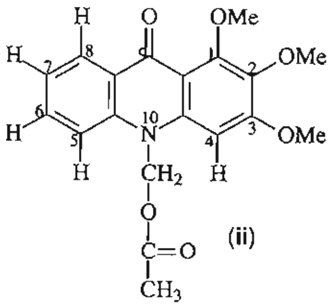
Toddaliopsis Engl. (Rutaceae, chapter 2, page 15) comprises three species,[1] of which two, *Toddaliopsis sansibarensis* (Engl.) Engl and *Toddaliopsis bremekampii* I. Verd. are found in Africa.[2,3] *T. bremekampii* I. Verd., whose common name is the wild-mandarin,[4] grows as a small evergreen tree to a height of about 4.6 m in dry woodland and scrub forest.[5] This species is distributed in northern KwaZulu-Natal, southern Mozambique, the Limpopo Province and south-eastern Zimbabwe.[4] *T. bremekampii* branches are cream to reddish brown in colour.[4] The leaves have a lemon scent when crushed. The round reddish brown fruits ripen from February to March and are eaten by birds.[4,5] *T. bremekampii* wood was previously used for building wagons.[5] The leaves and branches were investigated in this work. No work has been reported on the genus *Toddaliopsis*, and this work is the first report of a chemical investigation of *T. bremekampii.*

6.2. Results and Discussion

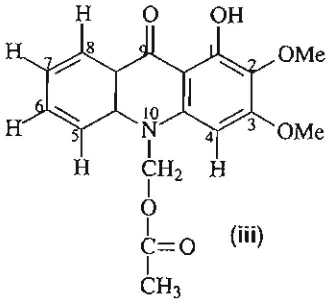
The leaves and branches of *T. bremekampii* collected from the Tembe Elephant Park Game Reserve in Mpumalanga Province, South Africa were investigated in this work. The dichloromethane extract of the leaves yielded four acridone alkaloids, 1,2,3-trimethoxyacridone (**i**), 1,2,3-trimethoxy-10-acetoxymethylacridone (**ii**), 1-hydroxy-2,3-dimethoxy-10-acetoxymethylacridone (**iii**) and 1,2,3-trimethoxy-10-methoxymethylacridone (**iv**).



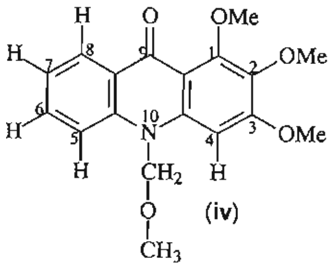
(i)



(ii)



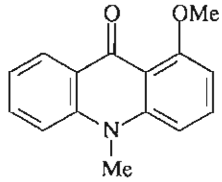
(iii)



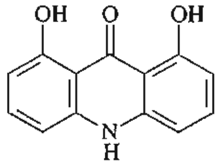
(iv)

The other extracts did not contain any compounds of interest. All four of these alkaloids have not been reported previously.

Compounds (**ii** - **iv**) are the only acridone alkaloids with substituted N-methyl groups to be reported. All but two acridone alkaloids have been previously reported from the Rutaceae family. Only 1-methoxy-10-methylacridone (**1**) and 1,8-dihydroxyacridone (**2**) have been reported outside the Rutaceae family from *Samadera bidwilli* (Simaroubaceae).[6] These alkaloids (**i** - **iv**) showed anti-inflammatory properties (discussed later in this chapter), and will be further screened for anti-malarial and other activities by the newly established South African Drug Discovery Platform.

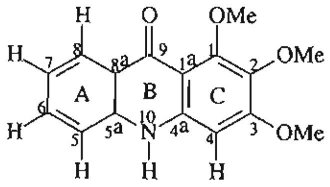


1-Methoxy-10-methylacridone (**1**)



1,8-Dihydroxyacridone (**2**)

6.3.1. Structural elucidation of Compound i: 1,2,3-Trimethoxyacridone (i)



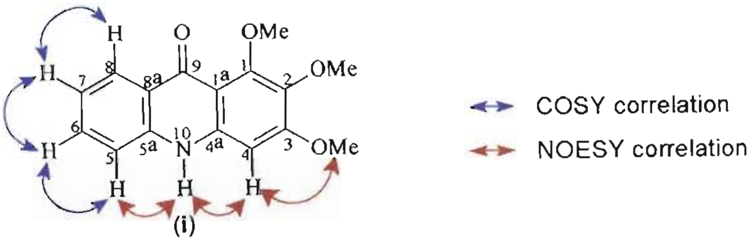
(**i**)

The positive CI mass spectrum **[242]** of compound (**i**) showed a $[M+H]^+$ peak at *m/z* 286.1081 corresponding to the molecular formula $C_{16}H_{15}NO_4$ for the compound. The IR spectrum **[240]** of compound (**i**) showed an N-H stretching band at 3275 cm^{-1} and a C=O stretching band at 1633 cm^{-1}.

Inspection of the ^{1}H NMR spectrum **[243]** of compound (**i**) showed the presence of five aromatic proton resonances (δ_H 8.41, 7.53 (2H), 7.17, 6.75), three methoxy group proton resonances (δ_H 4.00, 3.84, 3.74) and a NH group proton resonance (δ_H 10.39).

Inspection of the ^{13}C NMR **[244]** and ADEPT **[245]** spectra of compound (**i**) showed the presence of sixteen carbon resonances, four fully substituted carbon resonances (δ_C 140.39, 140.10, 121.88, 110.31), three oxygenated fully substituted carbon resonances (δ_C 158.30, 153.55, 137.81), a carbonyl carbon resonance (δ_C 176.99), five methine carbon resonances (δ_C 132.73, 126.56, 121.38, 116.65, 94.27) and three oxygenated methyl carbon resonances (δ_C 61.97, 61.54, 55.84).

The COSY spectrum **[249]** showed correlations between the four aromatic proton resonances H-5 (δ_H 7.53), H-6 (δ_H 7.53), H-7 (δ_H 7.17) and H-8 (δ_H 8.41), which indicated the presence of an *ortho*-disubstituted aromatic ring A in compound (**i**).

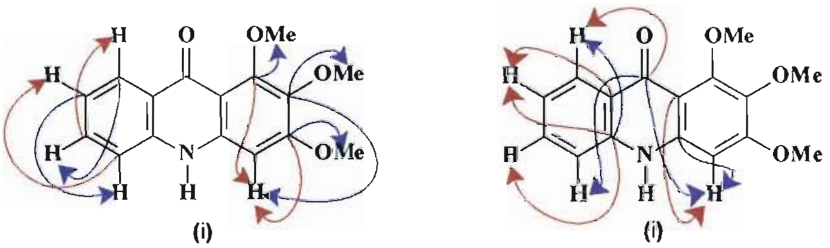


(i)

COSY and NOESY NMR correlations

The HMBC **[247-248]** correlations between the C-9 carbonyl carbon resonance at δ_C 176.99 and the H-5 and H-8 proton resonances confirmed that compound (i) is an acridone alkaloid. The H-8 proton resonance at δ_H 8.41 is characteristic of acridone alkaloids as a result of the deshielding effects of the NH and C=O groups. The methoxy groups were attached to ring C.

The H-6, H-7 and H-8 proton resonances showed HMBC **[247-248]** correlations to the fully substituted carbon resonance at δ_C 140.10, which was assigned to C-5a. The H-7 proton resonance showed a HMBC **[247-248]** correlation to the fully substituted carbon resonance at δ_C 121.88, which was assigned to C-8a.



(i) (i)

HMBC NMR correlations (C→H)

When three oxygenated carbons are adjacent to each other, the middle carbon resonance is doubly shielded and resonates at > 130 ppm. The other two carbon resonances are relatively deshielded and occurs around 159 – 151 ppm. Therefore the resonance at δ_C 137.81 was assigned to C-2.

The NH group proton resonance (δ_H 10.39) showed correlations in the NOESY NMR spectrum **[250]** to the H-5 proton resonance of ring A and to the proton resonance (δ_H 6.75) of ring C, which indicated that this ring C proton was attached at C-4. The H-4 proton resonance showed a correlation in the NOESY NMR spectrum **[250]** to the methoxy group proton resonance at δ_H 3.74, which lead to the assignment of this methoxy group to C-3. The oxygenated fully substituted carbon resonance at δ_C 158.30 was therefore assigned to C-3 in the HMBC NMR spectra **[247-248]**. The H-4 proton resonance showed HMBC **[247-248]** correlations (4 *J* coupling across the zig-zag bond) to the C-9 carbonyl carbon resonance (δ_C 176.99), the three oxygenated fully substituted carbon resonances at δ_C 137.81 (C-2), 153.55 (C-1) and 158.30 (C-3), and the two fully substituted carbon resonances at δ_C 110.31 and 140.39, which were assigned to C-1a and C-4a respectively.

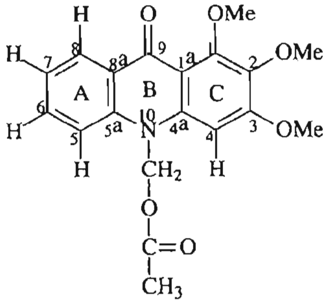
Compound (**i**) is the novel 1,2,3-trimethoxyacridone (**i**). The NMR data for 1,2,3-trimethoxyacridone (**i**) is shown in table 6.1.

Table 6.1. : NMR data for 1,2,3-trimethoxyacridone (i)

C	$\delta^{13}C$ / ppm ($CDCl_3$)	$\delta^{1}H$ / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	153.55 (C)	-	1-OCH_3, 4		
2	137.81 (C)	-	2-OCH_3, 4		
3	158.30 (C)	-	3-OCH_3, 4		
4	94.27 (CH)	6.75 (s)			3-OCH_3, NH
5	116.65 (CH)	7.53 *	7	6	6, NH
6	132.73 (CH)	7.53 *	8	5, 7	5, 7
7	121.38 (CH)	7.17 (m)	5	6, 8	6, 8
8	126.56 (CH)	8.41 (d, 8.06)	6	7	7
9	176.99 (C)	-	4, 5, 8		
10 NH	-	10.39 (bs)			4, 5
4a	140.39 (C)	-	4		
1a	110.31 (C)	-	4		
8a	121.88 (C)	-	7		
5a	140.10 (C)	-	6, 7, 8		
1-OCH_3	61.97 (CH_3)	4.00 (s)			
2-OCH_3	61.54 (CH_3)	3.84 (s)			
3-OCH_3	55.84 (CH_3)	3.74 (s)			4

* The H-5 and H-6 resonances are superimposed.

6.3.2. Structural elucidation of Compound ii: 1,2,3-Trimethoxy-10-acetoxymethylacridone (ii)



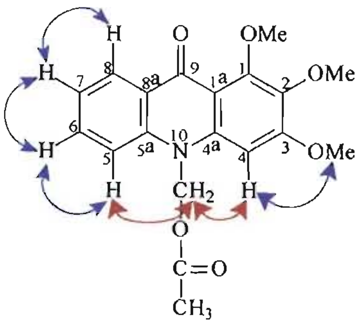
(ii)

The positive CI mass spectrum **[254]** of compound (**ii**) showed a $[M+H]^+$ peak at *m/z* 358.1291 corresponding to the molecular formula $C_{19}H_{19}NO_6$ for the compound. The IR spectrum **[252]** of compound (**ii**) showed C=O stretching bands at 1733 and 1644 cm^{-1} for the acetoxy and pyridone respectively,[7] and no stretching bands were observed for the N-H group.

Inspection of the ^1H NMR spectrum **[255]** of compound (**ii**) showed the presence of five aromatic proton resonances (δ_H 8.43, 7.65, 7.50, 7.30, 6.88), three methoxy group proton resonances (δ_H 4.008, 4.006, 3.99), a two proton methylene group proton resonance (δ_H 6.29) and an acetoxy methyl group proton resonance (δ_H 2.20).

Inspection of the ^{13}C NMR **[256]** and ADEPT **[257]** spectra showed the presence of nineteen carbon resonances, comprising two carbonyl carbon resonances (δ_C 176.82, 170.56), four fully substituted carbon resonances (δ_C 141.30, 140.85, 123.99, 112.14), three oxygenated fully substituted carbon resonances (δ_C 158.16, 154.67, 138.80), five methine carbon resonances (δ_C 133.27, 127.58, 122.62, 114.34, 93.22), one methylene carbon resonance (δ_C 71.48), one methyl group carbon resonance (δ_C 21.01) and three oxygenated methyl carbon resonances (δ_C 61.87, 61.43, 56.20). The NMR correlations in compound **(ii)** were similar to those of compound **(i)**, and suggested that the structure of compound **(ii)** was similar to compound **(i)**.

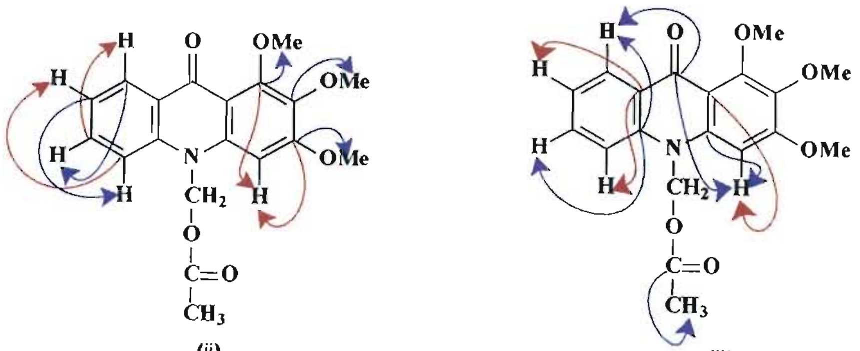
The aromatic ring proton resonances at δ_H 7.50 (d, 8.61 Hz), 7.65 (dd, 6.96, 8.61 Hz), 7.30 (dd, 6.96, 8.06 Hz) and 8.43 (d, 8.06 Hz) showed correlations to each other in the COSY NMR spectrum **[261]**, which indicated the presence of an *ortho*-disubstituted aromatic ring A in compound **(ii)**, thereby leaving the three methoxy groups on ring C, as observed for compound **(i)**.



(ii)

COSY and NOESY NMR correlations

The H-5 (δ_H 7.50) and H-7 (δ_H 7.30) proton resonances of ring A showed HMBC **[259-260]** correlations to the fully substituted carbon resonance at δ_C 123.99, which was assigned to C-8a. The H-6 (δ_H 7.65) and H-8 (δ_H 8.43) proton resonances of ring A showed HMBC **[259-260]** correlations to the fully substituted carbon resonance at δ_C 141.30, which was assigned to C-5a.



(ii)

(ii)

HMBC NMR correlations (C→H)

The proton resonance at δ_H 6.88 showed a correlation in the NOESY NMR spectrum **[262-263]** to the methoxy group proton resonance at δ_H 4.006, and also to the methylene group two proton resonance at δ_H 6.29, which, in turn, showed a correlation in the NOESY NMR spectrum **[262-263]** to the H-5 resonance (δ_H 7.50). This indicated that the proton (δ_H 6.88) was attached at C-4 and the methylene group was attached to the nitrogen atom. The acetoxy group was placed on the methylene group as a result of the methylene group carbon resonance occurring at δ_C 71.48. This is expected for methylene group carbons that are attached to both a nitrogen atom and an oxygen atom.[8] The H-4 proton resonance showed HMBC **[259-260]** correlations to the C-9 carbonyl carbon resonance (δ_C 176.82), the two oxygenated fully substituted carbon resonances at δ_C 138.80 (C-2) and 158.16 (C-3), and the two fully substituted carbon resonances at δ_C 112.14 and 140.85, which were assigned to C-1a and C-4a respectively. A characteristic feature of acridone alkaloids that can be seen here is the deshielded C-4a and C-5a carbon resonances which occur at ~ δ_H 140.

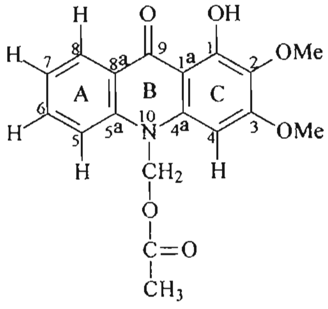
Compound (**ii**) is the novel 1,2,3-trimethoxy-10-acetoxymethylacridone (ii). The NMR data for 1,2,3-trimethoxy-10-acetoxymethylacridone (**ii**) is shown in table 6.2.

Table 6.2. : NMR data for 1,2,3-trimethoxy-10-acetoxymethylacridone (ii)

C	$\delta^{13}C$ / ppm ($CDCl_3$)	δ^1H / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	154.67 (C)	-	1-OCH_3		
2	138.80 (C)	-	2-OCH_3, 4		
3	158.16 (C)	-	3-OCH_3, 4		
4	93.22 (CH)	6.88 (s)		3-OCH_3	3-OCH_3, N-CH_2
5	114.34 (CH)	7.50 (d, 8.61)	7	6	6, N-CH_2
6	133.27 (CH)	7.65 (dd, 6.96, 8.61)	8	5, 7	5, 7
7	122.62 (CH)	7.30 (dd, 6.96, 8.06)	5	6, 8	6, 8
8	127.58 (CH)	8.43 (d, 8.06)	6	7	7
9	176.82 (C)	-	4, 8		
4a	140.85 (C)	-	4		
1a	112.14 (C)	-	4		
8a	123.99 (C)	-	5, 7		
5a	141.30 (C)	-	6, 8		
N-CH_2	71.48 (CH_2)	6.29 (bs)			4, 5
1-OCH_3	61.87 (CH_3)	4.008 (s)			
2-OCH_3	61.43 (CH_3)	3.99 (s)			
3-OCH_3	56.20 (CH_3)	4.006 (s)		4	4
$O\underline{C}OCH_3$	170.56 (C)	-	$OCOC\underline{H}_3$		
$OCO\underline{C}H_3$	21.01 (CH_3)	2.20 (s)			

6.3.3. Structural elucidation of Compound iii:

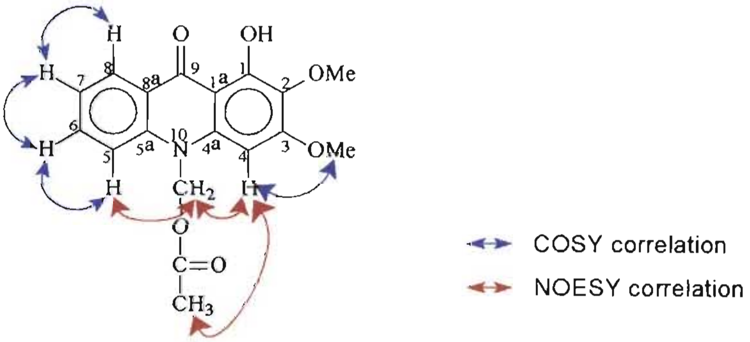
1-Hydroxy-2,3-dimethoxy-10-acetoxymethylacridone (iii)



(iii)

The positive CI mass spectrum **[267]** of compound (**iii**) showed a $[M+H]^+$ peak at *m/z* 344.1131 corresponding to the molecular formula $C_{18}H_{17}NO_6$ for the compound, and a difference relative to compound (**ii**), of a CH_2 group. The IR spectrum **[265]** of compound (**iii**) showed an O-H stretching band at 3455 cm^{-1} and C=O stretching bands at 1747 and 1643 cm^{-1} for the acetoxy and pyridone carbonyl groups respectively.

Inspection of the ^1H **[268]** and ^{13}C NMR **[269]** spectra of compound (**iii**) showed this compound to be very similar to compound (ii). However, the ^1H NMR spectrum **[268]** of compound (iii) showed one less methoxy group proton resonance, and the C-9 carbonyl carbon resonance, the fully substituted C-1a carbon resonance, the oxygenated fully substituted C-2 carbon resonance and the oxygenated fully substituted C-1 carbon resonance had shifted from δ_C 176.82, 112.14, 138.80 and 154.67 in the ^{13}C NMR spectrum **[256]** of compound (ii) to δ_C 181.58, 105.54, 130.98 and 156.13 respectively in the ^{13}C NMR spectrum **[269]** of compound (iii).

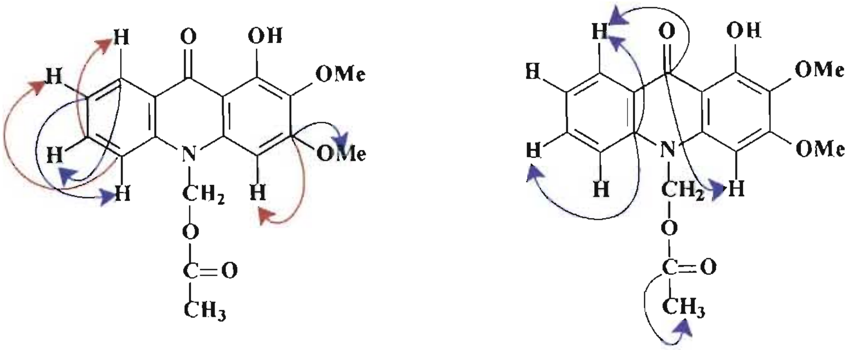


(iii)

COSY and NOESY NMR correlations

The proton resonance at δ_H 6.59 showed correlations in the NOESY NMR spectrum **[274]** to a methoxy group proton resonance (δ_H 3.99), the methylene group proton resonance (δ_H 6.33), and the acetoxy methyl group proton resonance (δ_H 2.19), which indicated that this proton was attached to C-4.

The two methoxy group proton resonances showed correlations to each other in the NOESY NMR spectrum **[274]**. These observations indicated that C-1 no longer had a methoxy group but instead a hydroxy group attached to it, and the two methoxy groups were attached to C-2 (δ_C 130.98) and C-3 (δ_C 159.56).



(iv) (iv)

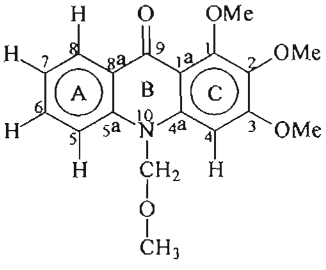
HMBC NMR correlations (C→H)

Compound (**iii**) is the novel 1-hydroxy-2,3-dimethoxy-10-acetoxymethylacridone (**iii**). The NMR data for 1-hydroxy-2,3-dimethoxy-10-acetoxymethylacridone (**iii**) is shown in table 6.3.

Table 6.3. : NMR data for 1-hydroxy-2,3-dimethoxy-10-acetoxymethylacridone (iii)

C	$\delta^{13}C$ / ppm ($CDCl_3$)	$\delta^{1}H$ / ppm ($CDCl_3$)	HMBC (C→H)	COSY	NOESY
1	156.13 (C)	-			
2	130.98 (C)	-	2-OCH_3, 4		
3	159.56 (C)	-	3-OCH_3, 4		
4	87.45 (CH)	6.59 (s)			3-OCH_3, N-CH_2
5	114.64 (CH)	7.58 (d, 8.61)	7	6	6, N-CH_2
6	134.40 (CH)	7.73 (dd, 7.14, 8.61)	8	5, 7	5, 7
7	122.73 (CH)	7.34 (dd, 7.14, 8.06)	5	6, 8	6, 8
8	126.74 (CH)	8.41 (d, 8.06)	6	6, 7	7
9	181.58 (C)	-	4, 8		
4a	139.70 (C)	-			
1a	105.54 (C)	-			
8a	121.02 (C)	-	4		
5a	141.66 (C)	-	6, 8		
N-CH_2	70.28 (CH_2)	6.33 (bs)			4, 5
2-OCH_3	60.81 (CH_3)	3.90 (s)			
3-OCH_3	56.21 (CH_3)	3.99 (s)			4
$O\underline{C}OCH_3$	170.43 (C)	-	$OCOC\underline{H}_3$		
$OCO\underline{C}H_3$	20.94 (CH_3)	2.19 (s)			4

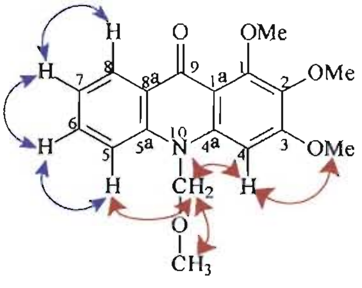
6.3.4. Structural elucidation of Compound iv: 1,2,3-Trimethoxy-10-methoxymethylacridone (iv)



(iv)

The positive CI mass spectrum **[278]** of compound (**iv**) showed a $[M+H]^+$ peak at *m/z* 330.1337 corresponding to the molecular formula $C_{18}H_{19}NO_5$ for the compound. The IR spectrum **[276]** of compound (**iv**) showed a C=O stretching band at 1643 cm^{-1}, and no O-H or N-H bands were observed.

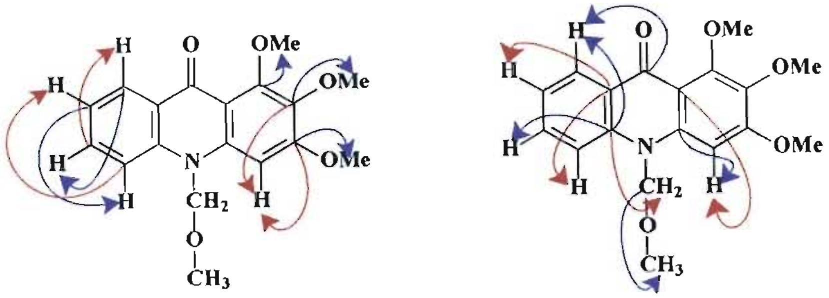
The NMR spectra of compound (**iv**) showed that it was not too pure since the compound started to decompose in $CDCl_3$, and the impurity peaks have been crossed out. The ^1H **[279]** and ^{13}C NMR **[280]** spectra of compound (**iv**) indicated that this compound was very similar to compound (**ii**). However, the ^1H NMR spectrum **[279]** of compound (**iv**) showed the absence of the acetoxy group proton resonance and an extra methoxy group proton resonance (δ_H 3.58), which occurred further upfield than the other three methoxy group proton resonances at δ_H 3.90, 4.00 and 4.01. The methylene group proton resonance had also shifted from δ_H 6.29 in the ^1H NMR spectrum **[255]** of compound (**ii**) to δ_H 5.52 in the ^1H NMR spectrum **[279]** of compound (**iv**). The methylene group carbon resonance had shifted from δ_C 71.48 in the ^{13}C NMR spectrum **[256]** of compound (**ii**) to δ_C 79.59 in the ^{13}C NMR spectrum **[280]** of compound (**iv**).



(iv)

COSY and NOESY NMR correlations

The corresponding proton resonance (δ_H 5.52) showed correlations in the NOESY NMR spectrum **[284]** to the H-4 (δ_H 6.80), the H-5 (δ_H 7.49) and the upfield methoxy group (δ_H 3.58) proton resonances, which suggested that the methoxy group was attached to the methylene group.



(iv) (iv)

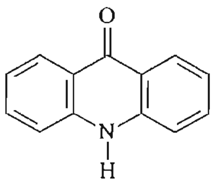
HMBC NMR correlations (C→H)

Compound (**iv**) is the novel 1,2,3-trimethoxy-10-methoxymethylacridone (**iv**). The NMR data for 1,2,3-trimethoxy-10-methoxymethylacridone (**iv**) is shown in table 6.4.

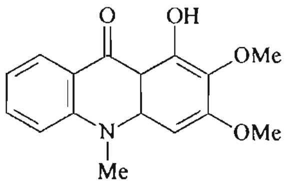
Table 6.4. : NMR data for 1,2,3-trimethoxy-10-methoxymethylacridone (iv)

C	δ^{13}C / ppm (CDCl$_3$)	δ^{1}H / ppm (CDCl$_3$)	HMBC (C→H)	COSY	NOESY
1	154.39 (C)	-	1-OCH$_3$		
2	138.60 (C)	-	2-OCH$_3$, 4		
3	157.96 (C)	-	3-OCH$_3$, 4		
4	93.54 (CH)	6.80 (s)			3-OCH$_3$, N-CH$_2$
5	114.42 (CH)	7.49 (d, 8.61)	7	6	6, N-CH$_2$
6	133.04 (CH)	7.62 (dd, 7.14, 8.61)	8	5, 7	5, 7
7	122.11 (CH)	7.26 (dd, 7.14, 8.06)	5	6, 8	6, 8
8	127.52 (CH)	8.43 (d, 8.06)	6	7	7
9	176.90 (C)	-	8		
4a	141.85 (C)	-	4		
1a	112.18 (C)	-	4		
8a	123.82 (C)	-	5, 7		
5a	141.62 (C)	-	6, 8, N-CH$_2$		
N-CH$_2$	79.59 (CH$_2$)	5.52 (s)	N-CH$_2$-OC$\underline{\text{H}}_3$		4, 5, N-CH$_2$-OC$\underline{\text{H}}_3$
N-CH$_2$-O$\underline{\text{C}}$H$_3$	55.92 (CH$_3$)	3.58 (s)			N-CH$_2$
1-OCH$_3$	61.85 (CH$_3$)	3.90 (s)			
2-OCH$_3$	61.47 (CH$_3$)	4.01 (s)			
3-OCH$_3$	55.95 (CH$_3$)	4.00 (s)			4

Only acridones with the N-H (*e.g.* acridone (**a**)) and N-methyl (*e.g.* arborinin (**b**)) have been previously reported.[9] This work reports acridones which possess new nitrogen substituents.



(a)



(b)

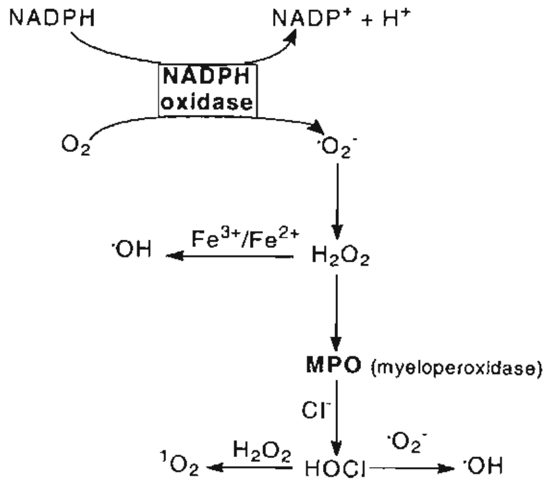
6.4. Biological activity of the alkaloids

This part of my research was carried out by myself in the medicinal chemistry laboratory at the University of Utrecht under the supervision of Dr. A.J.J. van den Berg. The alkaloids isolated in this work were tested in the chemiluminescence and DPPH (α,α-diphenyl-β-picrylhydrazyl) assays for their anti-inflammatory and anti-oxidant activities respectively, screens that were available in the laboratory.

6.4.1. The Chemiluminescence bioassay

General Introduction

Reactive oxygen species (ROS) produced by activated polymorphonuclear leukocytes (PMNs), play an important role in a host's defence against invading microorganisms.[10,11] During acute infection or other inflammatory stresses, PMNs are released from the bone marrow and move to the site of inflammation where they serve as the host's defense against infectious microorganisms.[10,11] PMNs are filled with proteolytic and other cytotoxic enzymes and are able to phagocytose and convert molecular oxygen into highly ROS.[10,11] Therefore, the ingested microorganisms may be killed inside the phagosome by the combined action of the enzyme activity and the ROS production.[11] Upon activation, the PMNs undergo an oxidative burst, which involves the intake of a large amount of oxygen which is converted into ROS.[10,11,12] The oxidative burst depends on the activity of the enzyme NADPH oxidase, which accepts an electron from NADPH and donates it to the molecular oxygen, thereby reducing the oxygen to the superoxide anion ($^{\bullet}O_2^-$).[12,13] Most of the $^{\bullet}O_2^-$ is converted into other ROS such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$) and hypohalites, which have bacteriocidal and cytotoxic activities.[10,11,12]



Reactive oxygen species production[11]

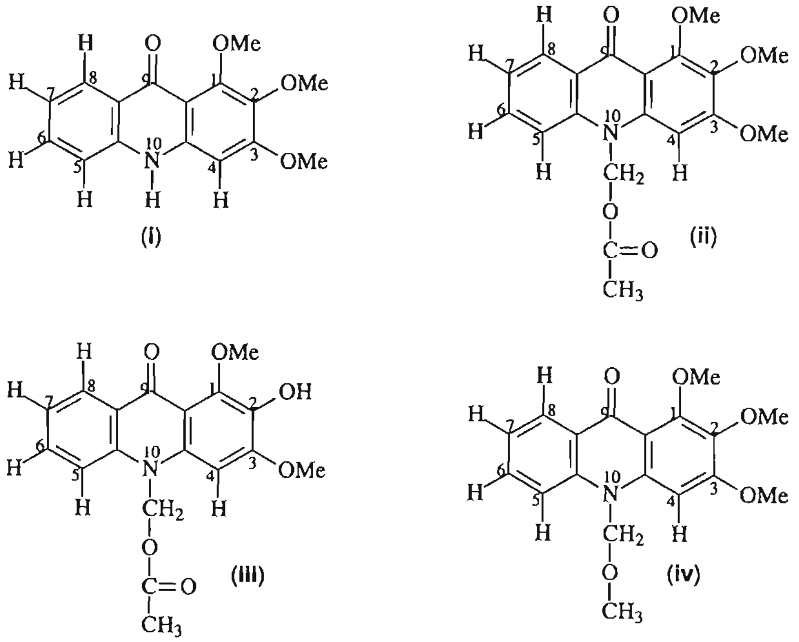
Although ROS formation by activated neutrophils may be a physiological response which is beneficial to the host, these ROS can be detrimental in many inflammatory states by causing excessive tissue damage.[10,11] Therefore compounds that can interfere with ROS production may be useful in preventing tissue damage.

In an experimental environment, ROS production by human neutrophils can be detected using light-emission enhancers such as luminol and lucigenin. In this work, the alkaloid's ability to inhibit ROS production by PMNs was measured by means of luminol-enhanced chemiluminescence. The luminol undergoes oxidation to generate an endoperoxide, which generates light by photon emission when it decomposes. Luminol largely detects HOCl and is therefore dependent on the MPO/ H_2O_2 system. However, luminol can detect intra- and extracellularly produced ROS.[13,14]

Results

Measuring ROS production

The experimental procedures are described in chapter 7, page 142. The PMNs were extracted from the blood of four different healthy donors. The effect of the alkaloids on the chemiluminescence for each sample is shown in table 6.5 below.



(i)

(ii)

(iii)

(iv)

Alkaloids tested in the assay

Table 6.5. : The IC 50 values, in μg/mL, of the alkaloids i – iv for each donor.

	Donor 1	Donor 2	Donor 3	Donor 4	Average	Activity Rating 1=most active 4=least active
Alkaloid (i)	101.41	126.45	56.83	5.68	**72.59**	**3**
Alkaloid (ii)	62.23	63.75	52.66	14.58	**48.31**	**2**
Alkaloid (iii)	4.33	4.95	4.53	3.02	**4.21**	**1 (4/4 cases)**
Alkaloid (iv)	58.48	124.19	112.2	21.66	**79.13**	**4**

The IC 50 values are the concentrations at which the alkaloids inhibit the chemiluminescence. Therefore the alkaloids with the lower IC 50 values are active at lower concentrations, implying that they are more active relative to one another. Each alkaloid performs differently in each of the donor's blood since the blood differs from one donor to the next. Therefore a large variation is observed for each alkaloid across the four donors. For example alkaloid (i) was very active when tested with the blood of donor 4, however, the same alkaloid was not so active when tested with the other donors' blood.

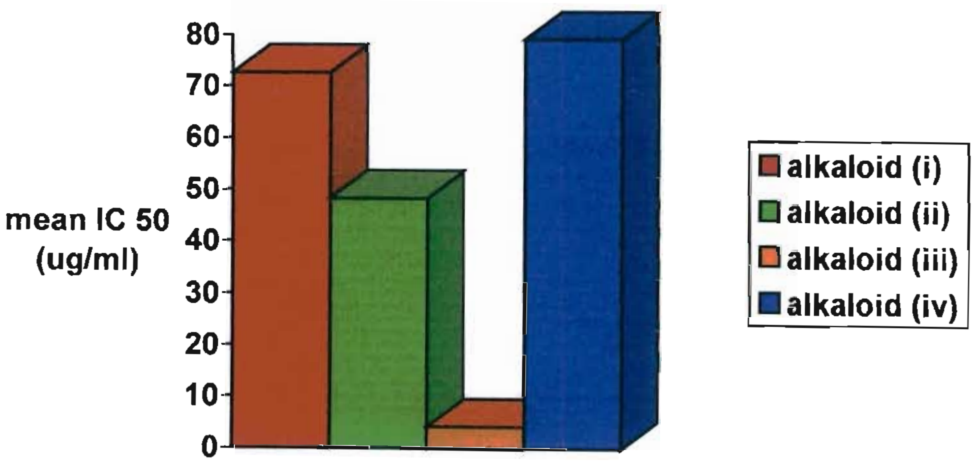


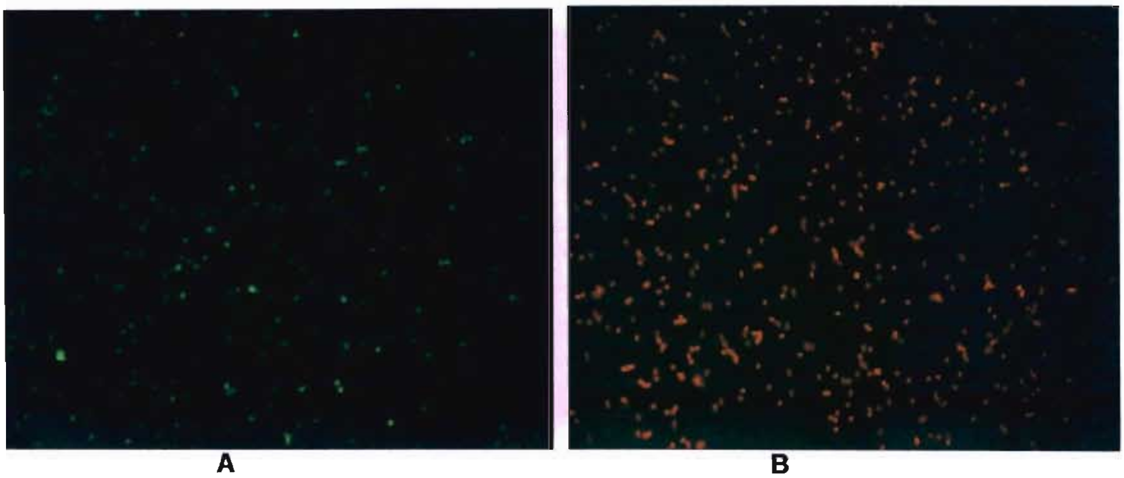
Figure 6.1.:The effects of the alkaloids on chemiluminescence. The bars represent the mean IC 50 values for each alkaloid.

Alkaloids (ii) and (iii) are similar in structure and only differ from each other by the nature of the oxygenated group at C-2. Alkaloid (iii) is the most active of all the alkaloids tested in this work. This suggests that acridone alkaloids with a hydroxy group adjacent to the carbonyl group show greater inhibitory activity on the luminol-enhanced chemiluminescence than acridone alkaloids with a methoxy group at that same position.

Alkaloids (i), (ii) and (iv) only differ from each other by the substituents attached to the nitrogen atom. These results show that alkaloids with the N-CH_2OCOCH_3 group are more active than alkaloids with the N-H or N-CH_2OCH_3 groups. However, again, with the small test sample definite conclusions cannot be drawn.

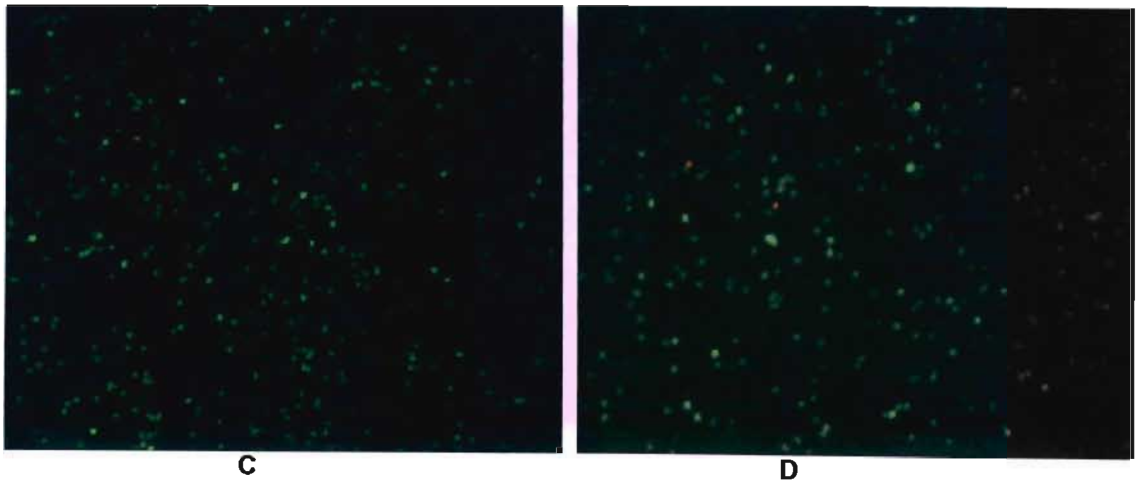
6.4.2. The toxicity test

These alkaloids were tested for their toxicity against PMNs and were found to be non-toxic at concentrations twice their IC 50 values, as illustrated in the photographs below. The experimental procedure is described in chapter 7, page 143.



A
PMNs + HBSS buffer

B
PMNs + Triton = 100% celldeath control



C
PMNs + alkaloid (i)

D
PMNs + alkaloid (ii)

Photographs by Ir. H. C. Quarles van Ufford

Photograph A depicts the live PMNs (green spots), while photograph B depicts the dead PMNs (red spots) to which the toxic triton was added. Photographs C and D depict the PMNs, to which the alkaloid was added.

6.4.3. The DPPH assay for anti-oxidant activity

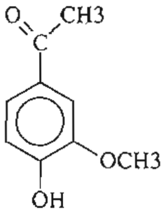
Anti-oxidant activity is determined by using a stable free radical.[15] The radical formed from α,α-diphenyl-β-picrylhydrazyl (DPPH) in this work was used to estimate the free radical scavenging activity of the alkaloids. The experimental procedures are described in chapter 7, page 143. The alkaloids tested in this work did not show anti-oxidant activity since they did not quench the free radicals produced by DPPH.

6.4.4. Discussion

A positive result in the chemiluminescence bioassay could be a result of the following:
a) the alkaloids could be toxic, thereby killing the PMNs and preventing ROS production.
b) the alkaloids could be $^{\bullet}O_2^{-}$ scavenging outside the neutrophils.
c) the alkaloids could be inhibiting the NADPH activity, thereby preventing the ROS production.
d) the alkaloids could be scavenging on the ROS within the PMNs.

Since the alkaloids were found to be non-toxic and not to show anti-oxidant activity, they should play a role in either the inhibiton of NADPH activity, or ROS production within the neutrophils, or both.

The alkaloids tested in this work have good activity. Apocynin (**c**), 4-hydroxy-3-methoxyacetophenone, is a potent inhibitor of ROS production and has been found to be extremely active at 10 μM.[16] Alkaloid (**iii**) shows activity at 12.2 μM, which is quite active.



(c)

Further testing on these alkaloids can determine their exact mode of action, and also test their action against inflammatory diseases such as arthritis, colitis, atherosclerosis etc. A better understanding of the mode of action of these alkaloids may contribute to their use as lead-compounds in the development of safe non-steroidal anti-inflammatory drugs. Further testing will be conducted on these compounds for other activities at the newly established South African Drug Discovery Platform.

6.5. References

1. http://mobot.mobot.org/cgi-bin/search_vast (http://www.mobot.org/) (23/11/2004)
2. http://www.museums.org.za/bio/plants/rutaceae/ (23/11/2004)
3. http://www.easternarc.org/eucamp/near-endemiceastusambara.html (23/11/2004)
4. Schmidt, E., Lötter, M., McCleland, W., Trees and shrubs of Mpumalanga and Kruger National Park, 2002, 236-237.
5. Palmer, E. and Pitman, N., Trees of southern Africa covering all known indigenous species in the Republic of South Africa, South-West Africa, Botswana, Lesotho and Swaziland, 1972, **2**, 993-994.
6. Gibbons, S., Craven, L., Dunlop, C., Gray, A.I., Hartley, T.G. and Waterman, P.G., The secondary metabolites of aff. *Samadera* SAC-2825: an Australian Simaroubaceae with unusual chemistry, *Phytochemistry*, 1997, **44**, 1109-1114.
7. Bandara, B.M.R., Gunatilaka, A.A.L., Wijeratne, E.M.K. and MacLeod, J.K., Acridone alkaloids and coumarins from *Pleiospermium alatum*, *Phytochemistry*, 1990, **29**, 297-301.
8. Stermitz, F.R. and Sharifi, I.A., Alkaoilds of *Zanthoxylum monophyllum* and *Z. Punctatum*, *Phytochemistry*, 1977, **16**, 2003-2006.
9. Bergenthal, D., Mester, I., Rozsa, Zs. and Reisch, J., ^{13}C-NMR-Spektren einiger acridon-alkaloide, *Phytochemistry*, 1979, **18**, 161-163.
10. Stites, D.P., Stobo, J.D. and Wells, J.V., Phagocytic cells: chemotaxis and effector functions of macrophages and granulocytes, basic and clinical immunology, Prentice-Hall International, Inc., sixth edition, London UK, 1987, **9**, 96-113.
11. DeLeo, F.R. and Quinn, M.T., Assembly of the phagocyte NADPH oxidase: molecular interaction of oxidase proteins, *J. Leukoc. Biol.* 1996, **60**, 677-691.
12. Hampton, M.B., Kettle, A.J. and Winterbourn, C.C., Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing, *Blood*, 1998, **92**, 3007-3017.
13. DeChatelet, L.R., Long, G.D., Shirley, P.S., Bass, D.A., Thomas, M.J., Henderson, F.W. and Cohen, M.S., Mechanism of the luminol-dependent chemiluminescence of human neutrophils, *J. Immunol.*, 1982, **129**, 1589-1593.
14. Vilim, V. and Wilhelm, J., What do we measure by a luminol-dependent chemiluminescence of phagocytes?, *Free Radic. Biol. Med.*, 1989, **6**, 623-629.
15. Blois, M.S., Antioxidant determinations by the use of a stable free radical, *Nature*, 1958, **181**, 1199-1200.
16. van den Worm, E., Investigations on apocynin, a potent NADPH oxidase inhibitor, PhD Thesis, Utrecht University, The Netherlands, 2001, 29-70.

Chapter 7

Experimental

CHAPTER 7
Experimental

Nuclear Magnetic Resonance Spectroscopy (NMR Spectroscopy)

Nuclear magnetic resonance spectroscopy was performed on a 400MHz Varian UNITY-INOVA NMR spectrometer. All spectra were recorded at room temperature in deuterated chloroform ($CDCl_3$). The chemical shifts were all recorded in ppm relative to TMS.

Infrared Spectroscopy (IR Spectroscopy)

The infra-red spectra were recorded using a Nicolet Impact 400D Fourier-Transform Infra-red (FT-IR) spectrometer. The samples were dissolved in dichloromethane and analysed on a NaCl window. The spectra were calibrated against an air background.

Ultraviolet Absorption Spectrometry/Spectroscopy (UV Spectroscopy)

The ultra-violet absorption spectra were obtained on a Varian DMS 300 UV-visible spectrometer. The solvent in which the spectra were recorded was dichloromethane.

Melting Points (Mp)

Melting points were determined on a Kofler micro-hot stage melting point apparatus and are uncorrected.

Optical Rotations

Optical rotations were measured at 20.0°C in chloroform using a Perkin Elmer model 341 polarimeter, serial number 8995. A quartz Microcell with a tube length of 100 mm and a volume of 1.0 ml was used.

Mass Spectrometry

High resolution mass spectra obtained for compounds (**i**), (**ii**), (**iv**) and (**vi**) (chapter 5) were recorded on a Kratos 9/50 HRMS instrument by Dr. Philip Boshoff at the Cape Technikon. High resolution mass spectra obtained for compounds (**iii**), (**v**), (**vii**) (chapter 5), and (**i**), (**ii**), (**iii**) and (**iv**) (chapter 6) were recorded on a VG 70-SE HRMS instrument with electron impact and chemical ionization by Mr. John Hill at Kent Mass Spectrometry. The low resolution mass spectra obtained for compounds (**viii**), (**ix**), (**x**), (**xi**), (**xii**), (**xiii**), (**xiv**), (**xv**), (**xvi**), (**xvii**) and (**xviii**) (chapter 5) were recorded on an Agilent MS 5975 instrument connected to GC 6890 at the University of Natal, Durban.

General Chromatography

The isolation process employed column and thin layer chromatographic techniques. In column chromatography, different sized columns were used ranging from 2-8 cm in diameter depending on the amount of sample available and the purification stage. Separation of crude extracts was generally carried out on a column using silica gel (Merck Art. 9385). All separations were carried out under gravity. Both the column and thin layer chromatographic techniques made use of varying ratios of dichloromethane and hexane or dichloromethane and ethyl acetate or dichloromethane and methanol. Thin layer chromatography was carried out on 0.2 mm silica-gel, aluminium-backed plates (Merck Art. 5554). The plates were developed using anisaldehyde : conc. H_2SO_4 : methanol [1:2:97] spray reagent. The plates were first analysed under UV light (254 nm and 366 nm) and then by heating.

Dry-packing

This procedure was employed for loading methanol extracts onto a column. Since these extracts only dissolved in methanol and no separation would occur if methanol was loaded onto the column, these extracts had to be dry-packed. This was done by dissolving the extract in methanol followed by mixing with silica gel until all the extract had been absorbed onto the silica gel. The powdery extract was then dried and loaded onto the column.

Preparative Thin Layer Chromatography (PTLC)

The aluminium-backed plates (Merck Art. 5554) and glass-backed plates (Merck Art. 5717) were lined with the sample 1.5 cm from the bottom of the plate. The samples were loaded onto the plates by running a capillary tube (containing the sample) from one end of the plate to the other end. The plates were then developed in a chromatography tank. The compounds of interest were detected under UV light. The bands were then cut and dissolved in methanol : dichloromethane (1:1) solution.

General Acetylation Procedure

Pyridine (1 ml) and acetic anhydride (1 ml) were added to the sample (15 mg) in a round bottom flask. The sample was left to stand at room temperature for 48 hours. Methanol (5 ml) was then added to the sample to react with the excess acetic anhydride, and toluene (4 x 10 ml) was added successively to remove the pyridine. After each addition, the solvent was evaporated off on the rotavapor. Thereafter, methanol (5 x 10 ml) was added to remove the remaining toluene. The sample was then spotted on a t.l.c plate to see whether the reaction had gone to completion or needed to be separated from the starting material.

General Plant Extraction Procedure

The species in this study were collected by Dr. Milijaona Randrianarivelojosia from Madagascar and Dr. Neil Crouch from South Africa. The plants were dried and ground, then successively extracted with hexane, dichloromethane, ethyl acetate and methanol using a soxhlet apparatus. Extraction with each solvent was carried out for approximately 48 hours. The solvents from the extracts obtained were evaporated using a BUCHI rotavapor.

Bioassay Techniques

The chemiluminescence and DPPH (α,α-diphenyl-β-picrylhydrazyl) assay techniques were employed in this work, in the medicinal chemistry laboratory at the University of Utrecht under the supervision of Dr. A.J.J. van den Berg.

The Chemiluminescence Assay

Polymorphonuclear leukocytes (PMNs) were isolated from venous blood of healthy donors (see isolation of PMNs below). In white 96-well, flat-bottomed microtitre plates, pure samples were serially diluted to final volumes of 50 μl. A PMN suspension (50 μl) (1 x 10^7 cells/ml) and luminol (50 μl) (120 μM) were added to each well. Serum treated zymosan (50 μl) was added to activate the PMNs. The chemiluminescence was monitored during a 30 minute period using a Titertek Luminoskan luminometer. Peak levels were used to calculate the activity of the test samples in comparison with the control. Controls were identically setup as experimental, with buffer replacing the test sample.

Isolation of PMNs

Phosphate buffered saline (PBS)/heparin (15 ml) was mixed with the buffy-coat (human blood) (15 ml). The mixture was underlayed with Percoll solution (12 ml) and then centrifuged for 20 minutes at 2400 rpm. The supernatant was removed leaving behind the pellets of PMNs and erythrocytes. The pellets were resuspended in ice-cold lysis buffer (50 ml) and incubated on ice until the solution turned black (5-15 minutes). After centrifugation (5 minutes, 1400 rpm), the supernatant was removed. The pellets were resuspended in ice-cold lysis buffer (3 ml), then made up to 50 ml with ice-cold lysis buffer, and incubated for 5 minutes. The mixture was centrifuged (5 minutes, 1400 rpm, 4 °C) and the supernatant was removed. The pellets were resuspended in HBSS-gel (Hanks Buffered Salt Solution mixed with gelatin) (2 ml), and made up to 10 ml with HBSS-gel. The PMNs concentration was measured by mixing them with Turk solution (dilution factor = 10) and viewing a drop of this mixture on a slide under a light microscope. The PMN cells were counted and the concentration was adjusted to 1 x 10^7 cells/ml with HBSS-gel.

The DPPH Assay

In white 96-well, flat-bottomed microtitre plates, pure samples were serially diluted to final volumes of 100 μl with ethanol (75%). DPPH (100 μl) was added to each well containing the pure sample. Controls were varying volumes of DPPH (0, 50, 100 μl) in ethanol (75%). DPPH$^\bullet$ (paramagnetic) can either accept an electron or proton and become stable (diamagnetic).[1] DPPH$^\bullet$ in ethanol solution is purple in colour and when reduced it goes pale yellow to colourless.[1]

Toxicity Test

The PMNs were labelled with the stain 5-carboxyfluorescein (CFDA), resuspended to a concentration of 1 x 10^7 cells/ml, and incubated with equal volumes of pure sample at 37 °C for 15 minutes. The dead cells were stained with propidium iodide (PI). The percentage of dead cells were measured using a fluorescence microscope. The PMNs with the toxic 0.02% v/v triton X100 served as the positive control for 100% cell death.

Plant Experimental

Samadera madagascariensis

Dried leaves (633.98 g) of *Samadera madagascariensis* Jussieu (voucher no.: 007MJ/Mdul-TAN) from Foulpointe, in the rainy eastern part of Madagascar, were collected and identified by Dr. M. Randrianarivelojosia of the University of Antananarivo, Madagascar. The hexane extract (38.65 g) was subjected to column chromatography on a 3 cm column (crude) using silica gel (Merck 9385) as the stationary phase and a solvent step gradient of dichloromethane : ethyl acetate as the mobile phase. The following compounds were isolated: protosamaderine A (**viii**) (27.1 mg) (fractions 239-250 : 35% ethyl acetate in dichloromethane), chisocheton compound A (**x**) (10.1 mg) (fractions 151-183 : 10% ethyl acetate in dichloromethane), protosamaderine B (**xi**) (18.2 mg) (fractions 206-213 : 30% ethyl acetate in dichloromethane), protosamaderine C (**xii**) (22.1 mg) (fractions 151-183 : 10% ethyl acetate in dichloromethane).

The dichloromethane extract (41.26 g) was loaded onto a crude column in the same way as the hexane extract. 1,2-Dihydrobruceajavanin A (**ix**) (50.8 mg) eluted in fractions 2-3 (30% ethyl acetate in dichloromethane).

The ethyl acetate extract (31.56 g) was loaded onto a crude column in the same way as the hexane extract. Samaderine A (**i**) (32.3 mg) eluted in fractions 39-40 (20% ethyl acetate in dichloromethane) and 2-chlorosamaderine A (**iii**) (10.2 mg) eluted in fractions 24-64 (20% ethyl acetate in dichloromethane).

The methanol extract (56.23 g) had to be dry-packed before being loaded onto a crude column. The following compounds were isolated: 5β,6-dihydrosamaderine A (**ii**) (12.2 mg) (fractions 131-168 : 10%

ethyl acetate in dichloromethane), samaderine DN (**iv**) (18.5 mg) (fractions 139-152 : 10% ethyl acetate in dichloromethane), samaderine B (**v**) (27.3 mg) (fraction 145 : 10% ethyl acetate in dichloromethane), cedronin (**vi**) (8.1 mg) (fractions 217-247 : 30% ethyl acetate in dichloromethane), 3,4β-dihydrosamaderine C (**vii**) (13.2 mg) (fractions 370-392 : 35% ethyl acetate in dichloromethane), protosamaderine D (**xiii**) (14.4 mg) (fractions 270-280 : 40% ethyl acetate in dichloromethane), protosamaderine E (**xiv**) (5.8 mg) (fractions 169-216 : 30% ethyl acetate in dichloromethane), protosamaderine F (**xv**) (8.0 mg) (fractions 169-216 : 30% ethyl acetate in dichloromethane), protosamaderine G (**xvi**) (8.2 mg) (fractions 217-247 : 30% ethyl acetate in dichloromethane), protosamaderine H (**xvii**) (8.1 mg) (fractions 281-342 : 40% ethyl acetate in dichloromethane), protosamaderine I (**xviii**) (11.0 mg) (fractions 270-280 : 40% ethyl acetate in dichloromethane). All these compounds were further purified by preparative thin layer chromatography using the same solvent systems.

Physical Data of Compounds

Compound (i):

Name: Samaderine A (**i**)

Physical appearance: yellow crystalline material

Yield: 32.3 mg

Mp: 250-252 °C (Lit.[2]: 253-258 °C)

Optical rotation: $[\alpha]_{D}^{20^\circ}$ ($CHCl_3$): - 21.88 ° [c = 0.064] (Lit.[2]: - 31.3 °[c = 0.259 (pyridine)])

IR: ν_{max}^{NaCl} : 3487 (O-H), 2919 (C-H stretch), 2853 (C-H stretch), 1793 (C=O), 1717 (C=O), 1661 (C=O), 1631, cm^{-1}.

HRMS at *m/z*: 330.1106 [$C_{18}H_{18}O_6$] requires 330.1106

EIMS at *m/z*: 330.1106, 315.0870, 312.1018, 302.1177, 271.0971, 243.1006, 215.0719, 203.0699, 187.0748, 175.0749, 149.0607, 125.0597, 97.1015, 85.1020, 57.0702.

Compound (ii):

Name: 5β,6-Dihydrosamaderine A (**ii**)

Physical appearance: white solid

Yield: 12.2 mg

Optical rotation: $[\alpha]_{D}^{20^\circ}$ ($CHCl_3$): + 75.00 ° [c = 0.032]

IR: ν_{max}^{NaCl} : 3489 (O-H), 2920 (C-H stretch), 2851 (C-H stretch), 1785 (C=O), 1701 (C=O), 1623 (C=O) cm^{-1}.

HRMS at *m/z*: 332.1262 [$C_{18}H_{20}O_6$] requires 332.1262
EIMS at *m/z*: 332.1262, 317.1023, 315.1223, 288.1359, 260.0949, 245.1165, 217.0875, 189.0867, 165.0568, 150.0682, 135.0733, 123.0809, 110.0735, 69.0338.

Compound (iii):
Name: 2-Chlorosamaderine A **(iii)**

Physical appearance: yellow solid
Yield: 10.2 mg

Optical rotation: $[\alpha]_D^{20°}$ ($CHCl_3$): - 12.50 ° [c = 0.016]

IR: ν_{max}^{NaCl} : 3488 (O-H), 2929 (C-H stretch), 2863 (C-H stretch), 1795 (C=O), 1733 (C=O), 1672 (C=O), 1625, 1466, 1383, 1256, 1173 cm^{-1}.

HRMS at *m/z*: 365.0794 [$C_{18}H_{17}ClO_6$] requires 365.0792
CIMS at *m/z*: 367, 365, 347, 331, 273, 239, 201, 167, 149, 113, 83, 71, 57.

Compound (iv):
Name: Samaderine DN **(iv)**

Physical appearance: yellow solid
Yield: 18.5 mg

Optical rotation: $[\alpha]_D^{20°}$ ($CHCl_3$): + 13.64 ° [c = 0.066]

IR: ν_{max}^{NaCl} : 3457 (O-H), 2925 (C-H stretch), 2862 (C-H stretch), 1788 (C=O), 1730 (C=O), 1655 (C=O), 1621, 1460, 1381, 1265 cm^{-1}.

HRMS at *m/z*: 390.1313 [$C_{20}H_{22}O_8$] requires 390.1313
EIMS at *m/z*: 390.1313, 375.1056, 372.1207, 331.1187, 313.1064, 255.1021, 239.1070, 227.1060, 211.1107, 187.0759, 125.0596, 91.0541, 57.0704.

Compound (v):

Name: Samaderine B (**v**)

Physical appearance: white crystalline material

Yield: 27.3 mg

Mp: 229-231 °C (Lit.[3]: 235-240 °C)

Optical rotation: $[\alpha]_{D}^{20^\circ}$ ($CHCl_3$): + 38.46 ° [c = 0.026] (Lit.[3]: + 67.5 °[c = not given (pyridine)])

IR: ν_{max}^{NaCl} : 3455 (O-H), 2927 (C-H stretch), 2855 (C-H stretch), 1788 (C=O), 1715 (C=O), 1672 (C=O), 1261, 1176, 1121, 1055, 1006 cm^{-1}.

HRMS at *m/z*: 363.1445 [$C_{19}H_{22}O_7$+H] requires 363.1444

CIMS at *m/z*: 363, 345, 317, 271, 235, 197, 127, 95, 71, 57.

Compound (vi):

Name: Cedronin (**vi**)

Physical appearance: yellow crystalline material

Yield: 8.1 mg

Mp: 275-277 °C (Lit.[4]: 275-280 °C)

Optical rotation: $[\alpha]_{D}^{20^\circ}$ ($CHCl_3$): - 13.64 ° [c = 0.022] (Lit.[4]: - 12.6 °[c = 0.714 (pyridine)])

IR: ν_{max}^{NaCl} : 3468 (O-H), 2917 (C-H stretch), 2854 (C-H stretch), 1733 (C=O), 1458 cm^{-1}.

HRMS at *m/z*: 364.1527 [$C_{19}H_{24}O_7$] requires 364.1527

EIMS at *m/z*: 364.1527, 349.1284, 346.1412, 335.1468, 331.1167, 328.1298, 317.1388, 313.1080, 299.1286, 233.0826, 201.0910, 173.0962, 149.0244, 125.0594, 109.0652, 95.0495, 69.0339.

Compound (vii):

Name: 3,4β-Dihydrosamaderine C (**vii**)

Physical appearance: yellow solid

Yield: 13.2 mg

Optical rotation: $[\alpha]_{D}^{20^\circ}$ ($CHCl_3$): - 18.18 ° [c = 0.022]

IR: ν_{max}^{NaCl} : 3428 (O-H), 2928 (C-H stretch), 2849 (C-H stretch), 1736 (C=O), 1460, 1263, 1168 cm^{-1}.

HRMS at *m/z*: 367.1758 [$C_{19}H_{26}O_7$ + H] requires 367.1757
CIMS at *m/z*: 367, 349, 331, 319, 275, 257, 239, 223, 205, 179, 149, 127, 111, 85, 71, 57.

Compound (viii):
Name: Protosamaderine A (**viii**)
(21*S*,23*R*-epoxy-7α-acetoxy-21*S*,24*S*,25-trihydroxyapotirucalla-14-en-3-one)
(7-acetyl-21-desmethyl-1,2-dihydroholstinone B)

Physical appearance: white solid
Yield: 27.1 mg

Optical rotation: $[\alpha]_D^{20°}$ ($CHCl_3$): - 7.14 ° [c = 0.056]

IR: ν_{max}^{NaCl} : 3436 (O-H), 2926 (C-H stretch), 2859 (C-H stretch), 1717 (C=O), 1461, 1378, 1250 cm^{-1}.

LRMS at *m/z*: 546 [$C_{32}H_{50}O_7$], 492, 475, 432, 368, 269, 223, 179, 91, 67.

Compound (ix):
Name: 1,2-Dihydrobruceajavanin A (**ix**)
(21*S*,23*R*, 24*S*,25-diepoxy-7,21*S*-diacetoxyapotirucalla-14-en-3-one)

Physical appearance: white crystalline material
Yield: 50.8 mg
Mp: 202-204 °C (Lit.^{5}: 206-207 °C)

Optical rotation: $[\alpha]_D^{20°}$ ($CHCl_3$): - 2.74 ° [c = 1.422] (Lit.^{5}: - 3.3 °[c = 1.0 ($CHCl_3$)])

IR: ν_{max}^{NaCl} : 2932 (C-H stretch), 1737 (C=O), 1453, 1377, 1238 cm^{-1}.

LRMS at *m/z*: 510 [$C_{34}H_{50}O_7$ - CH_3CO_2H], 450, 432, 397, 366, 301, 281, 227, 145, 105, 81.

Compound (x):
Name: Chisocheton compound A (**x**)
(21*S*,23*R*, 24*S*,25-diepoxy-21*S*-acetoxy-7-hydroxyapotirucalla-14-en-3-one)

Physical appearance: yellow crystalline material
Yield: 10.1 mg
Mp: 207-209 °C (Lit.[6]: 209-211 °C)

Optical rotation: $[\alpha]_D^{20°}$ ($CHCl_3$): + 13.95 ° [c = 0.086] (Lit.[6]: + 12.8 °[concentration not given])

IR: ν_{max}^{NaCl}: 3458 (O-H), 2926 (C-H stretch), 2866 (C-H stretch), 1741 (C=O), 1705 (C=O), 1463, 1379, 1240 cm^{-1}.

LRMS at *m/z*: 528 [$C_{32}H_{48}O_6$], 510, 468, 378, 297, 227, 173, 145, 119, 95, 69.

Compound (xi):
Name: Protosamaderine B (**xi**)
(21*S*,23*R*-epoxy-21*S*,24*S*-diacetoxy-7,25-dihydroxyapotirucalla-14-en-3-one)
(21-desmethyl-21,24-diacetyl-1,2-dihydroholstinone B)

Physical appearance: white solid
Yield: 18.2 mg

Optical rotation: $[\alpha]_D^{20°}$ ($CHCl_3$): + 12.35 ° [c = 0.324]

IR: ν_{max}^{NaCl}: 3490 (O-H), 2944 (C-H stretch), 2876 (C-H stretch), 1738 (C=O), 1460, 1380, 1244, 1039, cm^{-1}.

LRMS at *m/z*: 528 [$C_{34}H_{52}O_8 - CH_3CO_2H$], 468, 429, 379, 355, 328, 301, 281, 185, 145, 119, 91, 69.

Compound (xii):
Name: Protosamaderine C (**xii**)
(21*S*,23*R*-epoxy-25-hydroxy-7,21*S*,24*S*-triacetoxyapotirucalla-14-en-3-one)
(21-desmethyl-1,2-dihydro-7,21,24-triacetylholstinone B)

Physical appearance: yellow solid
Yield: 22.1 mg
Mp: 116-118 °C

Optical rotation: $[\alpha]_D^{20°}$ ($CHCl_3$): + 2.96 ° [c = 0.304]

IR: ν_{max}^{NaCl}: 3480 (O-H), 2944 (C-H stretch), 1736 (C=O), 1458, 1377, 1241, 1033 cm^{-1}.

LRMS at *m/z*: 510 [$C_{36}H_{54}O_9$ - 2 x CH_3CO_2H], 492, 450, 397, 366, 314, 281, 227, 145, 119, 91, 69.

Compound (xiii):

Name: Protosamaderine D (**xiii**)
(21*S*,23*R*-epoxy-25-acetoxy-7,24*S*-dihydroxy-21*S*-methoxyapotirucalla-14-en-3-one)
(25-acetyl-1,2-dihydroholstinone B)

Physical appearance: white solid
Yield: 14.4 mg

Optical rotation: $[\alpha]_D^{20°}$ ($CHCl_3$): - 14.55 ° [c = 0.110]

IR: ν_{max}^{NaCl}: 3425 (O-H), 2931 (C-H stretch), 2859 (C-H stretch), 1707 (C=O), 1458, 1381, 1252, 1054 cm^{-1}.

LRMS at *m/z*: 542 [$C_{33}H_{52}O_7 - H_2O$], 524, 470, 395, 297, 259, 183, 159, 131, 107, 69.

Compound (xiv):

Name: Protosamaderine E (**xiv**)
(21*S*,23*R*-epoxy-7,24*S*-diacetoxy-25-hydroxy-21*S*-methoxyapotirucalla-14-en-3-one)
(7,24-diacetyl-1,2-dihydroholstinone B)

Physical appearance: white solid
Yield: 5.8 mg

Optical rotation: $[\alpha]_D^{20°}$ ($CHCl_3$): - 22.58 ° [c = 0.062]

IR: ν_{max}^{NaCl}: 3456 (O-H), 2923 (C-H stretch), 2851 (C-H stretch), 1724 (C=O), 1463, 1380, 1247, 1036.

LRMS at *m/z*: 542 [$C_{35}H_{54}O_8$ – CH_3CO_2H], 510, 450, 379, 346, 316, 281, 133, 96, 73.

Compound (xv):

Name: Protosamaderine F (**xv**)
(21*S*,23*R*-epoxy-7-acetoxy-24*S*,25-dihydroxy-21*S*-methoxyapotirucalla-14-en-3-one)
(7-acetyl-1,2-dihydroholstinone B)

Physical appearance: white solid
Yield: 8.0 mg

Optical rotation: $[\alpha]_D^{20°}$ ($CHCl_3$): - 15.18 ° [c = 0.112]

IR: ν_{max}^{NaCl}: 3450 (O-H), 2934 (C-H stretch), 1718 (C=O), 1591, 1463, 1380, 1252, 1102, 1036 cm^{-1}.

LRMS at *m/z*: 542 [$C_{33}H_{52}O_7 - H_2O$], 489, 465, 385, 344, 308, 281, 256, 226, 183, 153, 126, 95, 74.

Compound (xvi):
Name: Protosamaderine G (**xvi**)
(21*R*,23*R*-epoxy-7-acetoxy-24*S*,25-dihydroxy-21*R*-methoxyapotirucalla-14-en-3-one)
(7-acetyl-1,2-dihydroholstinone C)

Physical appearance: yellow solid
Yield: 8.2 mg

Optical rotation: $[\alpha]_D^{20°}$ ($CHCl_3$): - 33.72 ° [c = 0.086]

IR: ν_{max}^{NaCl}: 3391 (O-H), 2926 (C-H stretch), 2876 (C-H stretch), 1724 (C=O), 1468, 1391, 1253, 1043 cm^{-1}.

LRMS at *m/z*: 542 [$C_{33}H_{52}O_7 - H_2O$], 510, 452, 397, 328, 310, 281, 227, 171, 119, 93, 75.

Compound (xvii):
Name: Protosamaderine H (**xvii**)
(21*R*,23*R*-epoxy-7,24*S*,25-trihydroxy-21*R*-methoxyapotirucalla-14-en-3-one)
(1,2-dihydroholstinone C)

Physical appearance: white solid
Yield: 8.1 mg

Optical rotation: $[\alpha]_D^{20°}$ ($CHCl_3$): - 42.22 ° [c = 0.090]

IR: ν_{max}^{NaCl}: 3458 (O-H), 2935 (C-H stretch), 2862 (C-H stretch), 1702 (C=O), 1461, 1384, 1267, 1117, 1038 cm^{-1}.

LRMS at *m/z*: 518 [$C_{31}H_{50}O_5$], 489, 429, 400, 378, 326, 267, 223, 187, 165, 125, 104, 77, 60.

Optical rotation: $[\alpha]_D^{20^\circ}$ ($CHCl_3$): - 15.18 ° [c = 0.112]

IR: ν_{max}^{NaCl} : 3450 (O-H), 2934 (C-H stretch), 1718 (C=O), 1591, 1463, 1380, 1252, 1102, 1036 cm^{-1}.

LRMS at *m/z*: 542 [$C_{33}H_{52}O_7 - H_2O$], 489, 465, 385, 344, 308, 281, 256, 226, 183, 153, 126, 95, 74.

Compound (xvi):
Name: Protosamaderine G (**xvi**)
(21*R*,23*R*-epoxy-7-acetoxy-24*S*,25-dihydroxy-21*R*-methoxyapotirucalla-14-en-3-one)
(7-acetyl-1,2-dihydroholstinone C)

Physical appearance: yellow solid
Yield: 8.2 mg

Optical rotation: $[\alpha]_D^{20^\circ}$ ($CHCl_3$): - 33.72 ° [c = 0.086]

IR: ν_{max}^{NaCl} : 3391 (O-H), 2926 (C-H stretch), 2876 (C-H stretch), 1724 (C=O), 1468, 1391, 1253, 1043 cm^{-1}.

LRMS at *m/z*: 542 [$C_{33}H_{52}O_7 - H_2O$], 510, 452, 397, 328, 310, 281, 227, 171, 119, 93, 75.

Compound (xvii):
Name: Protosamaderine H (**xvii**)
(21*R*,23*R*-epoxy-7,24*S*,25-trihydroxy-21*R*-methoxyapotirucalla-14-en-3-one)
(1,2-dihydroholstinone C)

Physical appearance: white solid
Yield: 8.1 mg

Optical rotation: $[\alpha]_D^{20^\circ}$ ($CHCl_3$): - 42.22 ° [c = 0.090]

IR: ν_{max}^{NaCl} : 3458 (O-H), 2935 (C-H stretch), 2862 (C-H stretch), 1702 (C=O), 1461, 1384, 1267, 1117, 1038 cm^{-1}.

LRMS at *m/z*: 518 [$C_{31}H_{50}O_5$], 489, 429, 400, 378, 326, 267, 223, 187, 165, 125, 104, 77, 60.

Compound (xviii):

Name: Protosamaderine I (**xviii**)
(21*R*,23*R*-epoxy-24*S*-acetoxy-7,25-dihydroxy-21*R*-methoxyapotirucalla-14-en-3-one)
(24-acetyl-1,2-dihydroholstinone C)

Physical appearance: white solid
Yield: 11.0 mg

Optical rotation: $[\alpha]_D^{20^\circ}$ ($CHCl_3$): - 25.00 ° [c = 0.060]

IR: ν_{max}^{NaCl}: 3396 (O-H), 2926 (C-H stretch), 2868 (C-H stretch), 1738 (C=O), 1713 (C=O), 1594, 1468, 1383, 1250, 1120, 1048 cm^{-1}.

LRMS at *m/z*: 542 [$C_{33}H_{52}O_7 - H_2O$], 528, 515, 451, 424, 390, 354, 324, 253, 176, 151, 121, 84, 60.

Toddaliopsis bremekampii

Leaves (43.61 g) and branches (206.12 g) of *Toddaliopsis bremekampii* (voucher no.: N. Crouch 943, NH) from the Tembe Elephant Park Game Reserve in Mpumalanga Province, South Africa, were collected and identified by Dr. N. Crouch of the National Botanical Institute, Durban, South Africa. The dichloromethane extract (12.34 g) of the leaves was loaded onto a glass-backed preparative thin layer chromatography plate and 10% to 20% ethyl acetate in dichloromethane was used as the mobile phase. The following compounds were isolated: 1,2,3-trimethoxyacridone (**i**) (18.2 mg), 1,2,3-trimethoxy-10-acetoxymethylacridone (**ii**) (20.2 mg), 1-hydroxy-2,3-dimethoxy-10-acetoxymethylacridone (**iii**) (22.4 mg) and 1,2,3-trimethoxy-10-methoxymethylacridone (**iv**) (10.3 mg). All these compounds were further purified by preparative thin layer chromatography onto aluminium-backed plates using the same solvent systems. All other extracts did not contain any compounds of interest.

Compound (i):

Name: 1,2,3-trimethoxyacridone (**i**)

Physical appearance: yellow solid
Yield: 18.2 mg

UV: $\lambda_{max}^{CH_2Cl_2}$: 263 nm (log ε 4.34), 297 nm (log ε 3.57), 367 nm (log ε 3.68), 382 nm (log ε 3.69).

IR: ν_{max}^{NaCl}: 3275 (N-H), 2928 (C-H), 2843 (C-H), 1633 (C=O), 1600, 1478, 1312, 1252, 1141, 1102 cm^{-1}.

HRMS at *m/z*: 286.1081 [$C_{16}H_{15}NO_4$ + H] requires 286.1079
CIMS at *m/z*: 286, 133, 57.

Compound (ii):
Name: 1,2,3-trimethoxy-10-acetoxymethylacridone (**ii**)

Physical appearance: yellow solid
Yield: 20.2 mg

UV: $\lambda_{max}^{CH_2Cl_2}$: 263 nm (log ε 4.21), 307 nm (log ε 3.50), 375 nm (log ε 3.53).

IR: ν_{max}^{NaCl} : 2933 (C-H), 2858 (C-H), 1733 (C=O), 1644 (C=O), 1607, 1487, 1291, 1266, 1196, 1135 cm^{-1}.

HRMS at *m/z*: 358.1291 [$C_{19}H_{19}NO_6$ + H] requires 358.1291
CIMS at *m/z*: 358, 286, 197, 149, 61.

Compound (iii):
Name: 1-hydroxy-2,3-dimethoxy-10-acetoxymethylacridone (**iii**)

Physical appearance: yellow solid
Yield: 22.4 mg

UV: $\lambda_{max}^{CH_2Cl_2}$: 223 nm (log ε 3.48), 263 nm (log ε 3.64), 272 nm (log ε 3.64), 320 nm (log ε 3.29), 390 nm (log ε 2.94).

IR: ν_{max}^{NaCl} : 3455 (OH), 2926 (C-H), 2860 (C-H), 1747 (C=O), 1643 (C=O), 1610, 1472, 1202 cm^{-1}.

HRMS at *m/z*: 344.1131 [$C_{18}H_{17}NO_6$ + H] requires 344.1134
EIMS at *m/z*: 344, 286, 198, 149, 61.

Compound (iv):
Name: 1,2,3-trimethoxy-10-methoxymethylacridone (**iv**)

Physical appearance: yellow solid
Yield: 10.3 mg

UV : $\lambda_{max}^{CH_2Cl_2}$: 264 nm (log ε 3.22), 379 nm (log ε 2.47).

Chapter 8

Conclusion

CHAPTER 8
Conclusion

The findings contained in this work have proved to be interesting and beneficial, as novel and biologically active compounds have been isolated.

Investigation of *Samadera madagascariensis* leaves has yielded a variety of C_{18} and C_{19} quassinoids, and a series of related protolimonoids. Thirteen of the eighteen compounds isolated from this plant were not reported previously. This work provided further evidence that *S. madagascariensis* is indeed chemically synonymous with *Samadera indica* and *Quassia indica*.

Four of the seven quassinoids isolated in this work were not reported previously. The novel 5β,6-dihydrosamaderine A (**ii**) (C_{18} type) differs from the known samaderine A (**i**) only by the absence of the $\Delta^{5,6}$–double bond, however, this is the only report of a C_{18} quassinoid without the $\Delta^{5,6}$–double bond. The 2-chloro substituent in 2-chlorosamaderine A (**iii**) (C_{18} type) makes this compound novel. The novel 3,4β-dihydrosamaderine C (**vii**) (C_{19} type) differs from the known samaderine C only by the absence of the $\Delta^{3,4}$–double bond. The C-1 ester substituent in samaderine DN (**iv**) (C_{18} type) makes this compound different. The isolation of the three novel C_{18} quassinoids in this work, increase the number of C_{18} quassinoids reported by 60 %. 2-Chlorosamaderine A (**iii**), cedronin (**vi**) and 3,4β-dihydrosamaderine C (**vii**) displayed anticarcinogenic properties.

Nine of the eleven protolimonoids isolated in this work were not reported previously. These protolimonoids all have the same tetracyclic skeleton, and differ from each other by the oxygenated substituent at C-7, C-21 and C-24. 1,2-Dihydrobruceajavanin A (**ix**) and chisocheton compound A (**x**) are the only two protolimonoids with the 24,25-epoxide ring isolated in this work. All other protolimonoids isolated in this work possess the 24,25 diol. All these protolimonoids (**viii**) – (**xviii**) have the *S* configuration at C-24. It is proposed that the 24*S*,25-diols from *S. madagascariensis* leaves isolated in this work have been formed *via* a SN_1 mechanism, thereby resulting in retention of the *S* configuration at C-24.

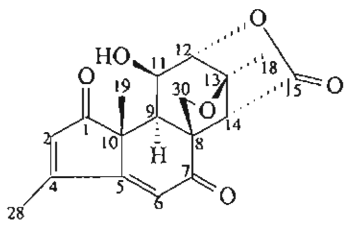
Investigation of the branches of *Toddaliopsis bremekampii* yielded four novel acridone alkaloids. This work is of particular interest since this is the first species of the genus to have been examined. This is the first report of trimethoxyacridone alkaloids without a methylated nitrogen. This is also the first report of isolated acridones that possess substituted N-methyl groups. These non-toxic acridone alkaloids showed anti-inflammatory properties by either inhibiting the NADPH activity or by scavenging on the reactive oxygen species within the human neutrophils. The presence of hydroxy groups in the acridone alkaloids increased their activity on the luminol-enhanced chemiluminescence. The alkaloids with acetoxy groups showed greater activity compared to the others.

The isolation of the acridone alkaloids from *Toddaliopsis bremekampii* in this work is not surprising since the Rutaceae is a plant family well known for containing alkaloids.

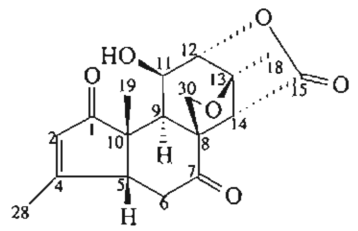
Research in Natural Product Chemistry is of vital importance since it involves the discovery of novel and biologically active compounds that may be lead compounds for the synthesis of better and safer drugs.

Appendix

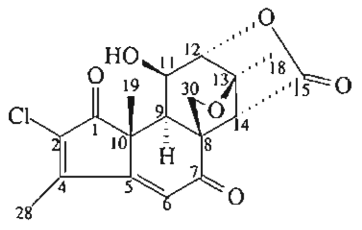
- Structures of compounds isolated in this work
- Mean graphs
- Dose response curves



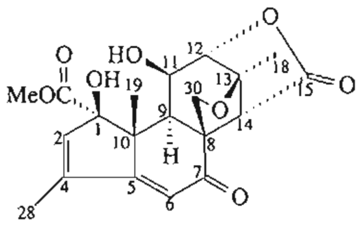
(i)
Samaderine A



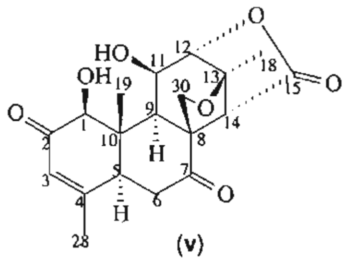
(ii)
5β,6-Dihydrosamaderine A



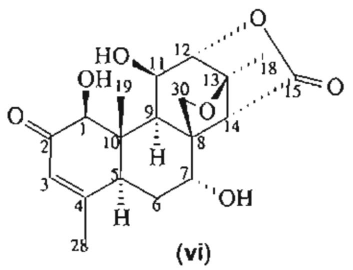
(iii)
2-Chlorosamaderine A



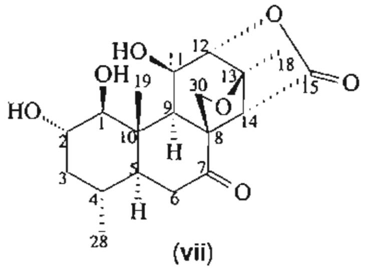
(iv)
Samaderine DN



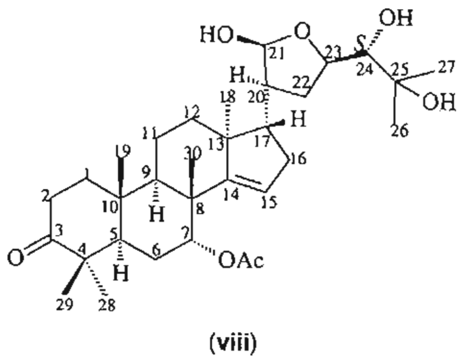
(v)
Samaderine B



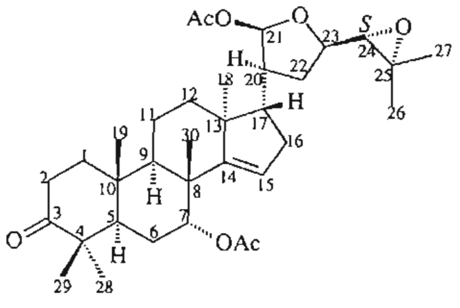
(vi)
Cedronin



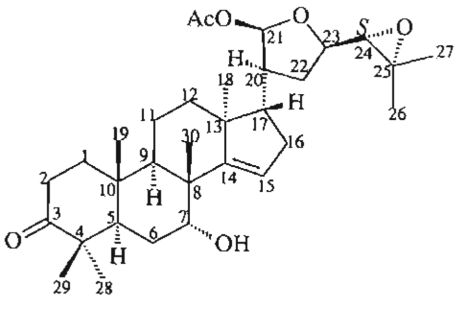
(vii)
3,4β-Dihydrosamaderine C



(viii)
Protosamaderine A

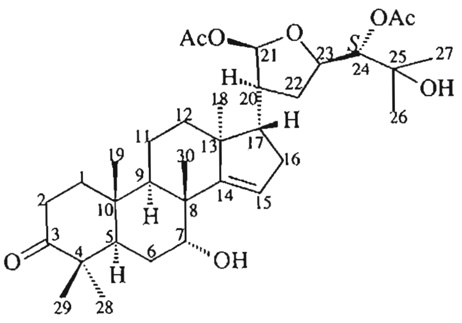


(ix)
1,2-Dihydrobruceajavanin A

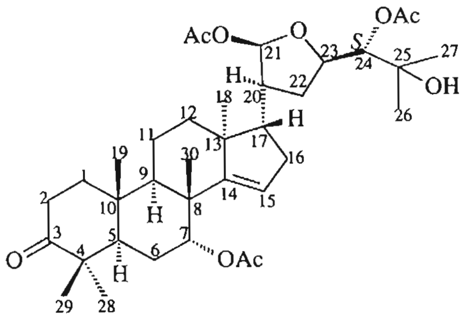


(x)
Chisocheton compound A

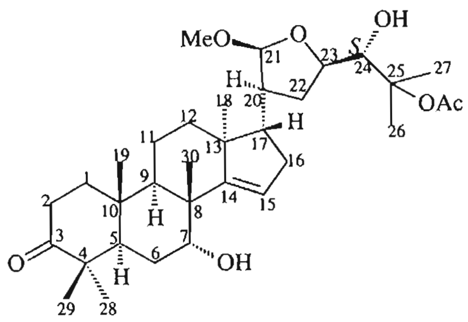
Compounds isolated from *Samadera madagascariensis*



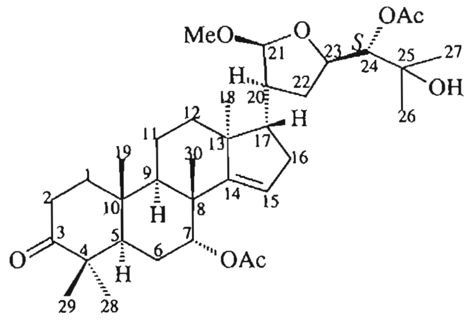
(xi)
Protosamaderine B



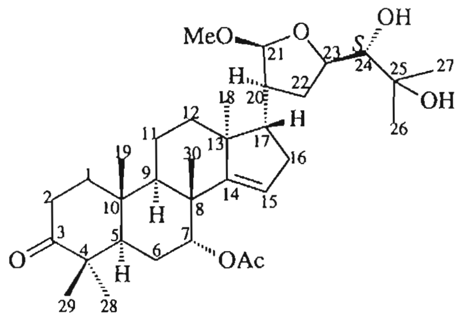
(xii)
Protosamaderine C



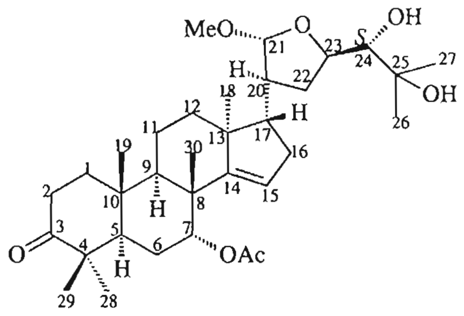
(xiii)
Protosamaderine D



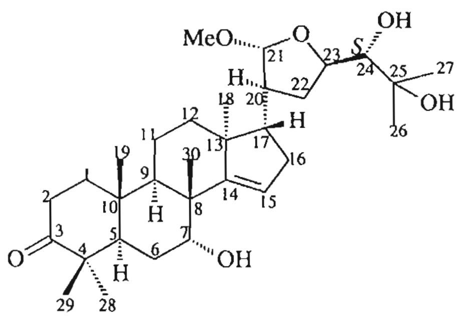
(xiv)
Protosamaderine E



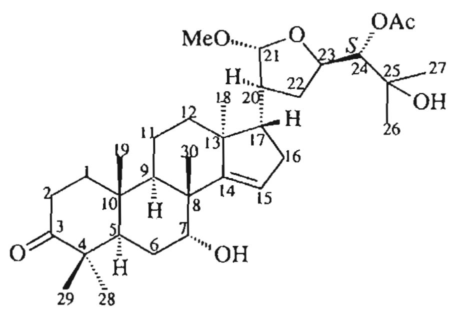
(xv)
Protosamaderine F



(xvi)
Protosamaderine G

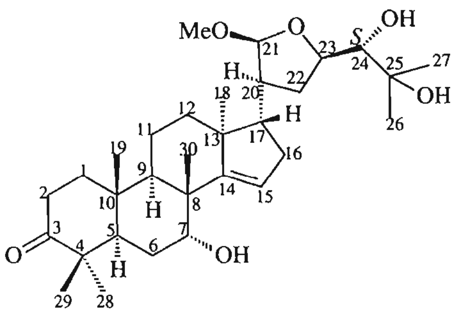


(xvii)
Protosamaderine H

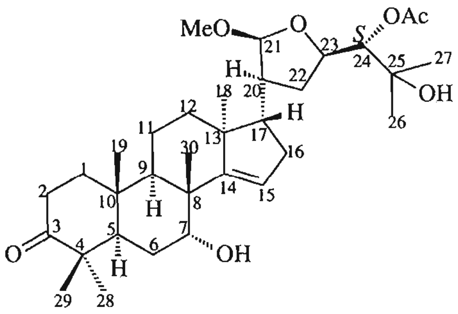


(xviii)
Protosamaderine I

Compounds isolated from *Samadera madagascariensis*

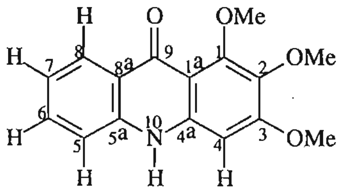


(xviia)



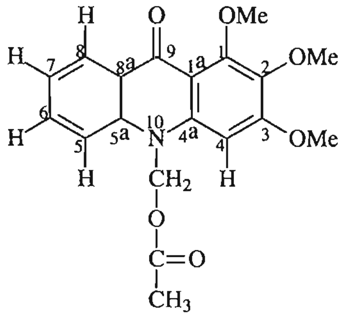
(xviiia)

Compounds isolated from *Samadera madagascariensis*



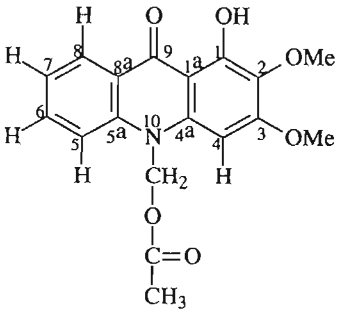
(i)

1,2,3-Trimethoxyacridone



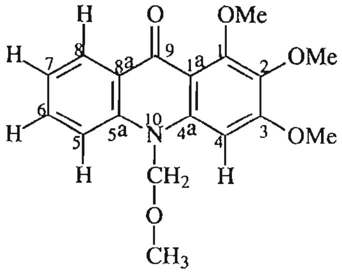
(ii)

1,2,3-Trimethoxy-10-acetoxymethylacridone



(iii)

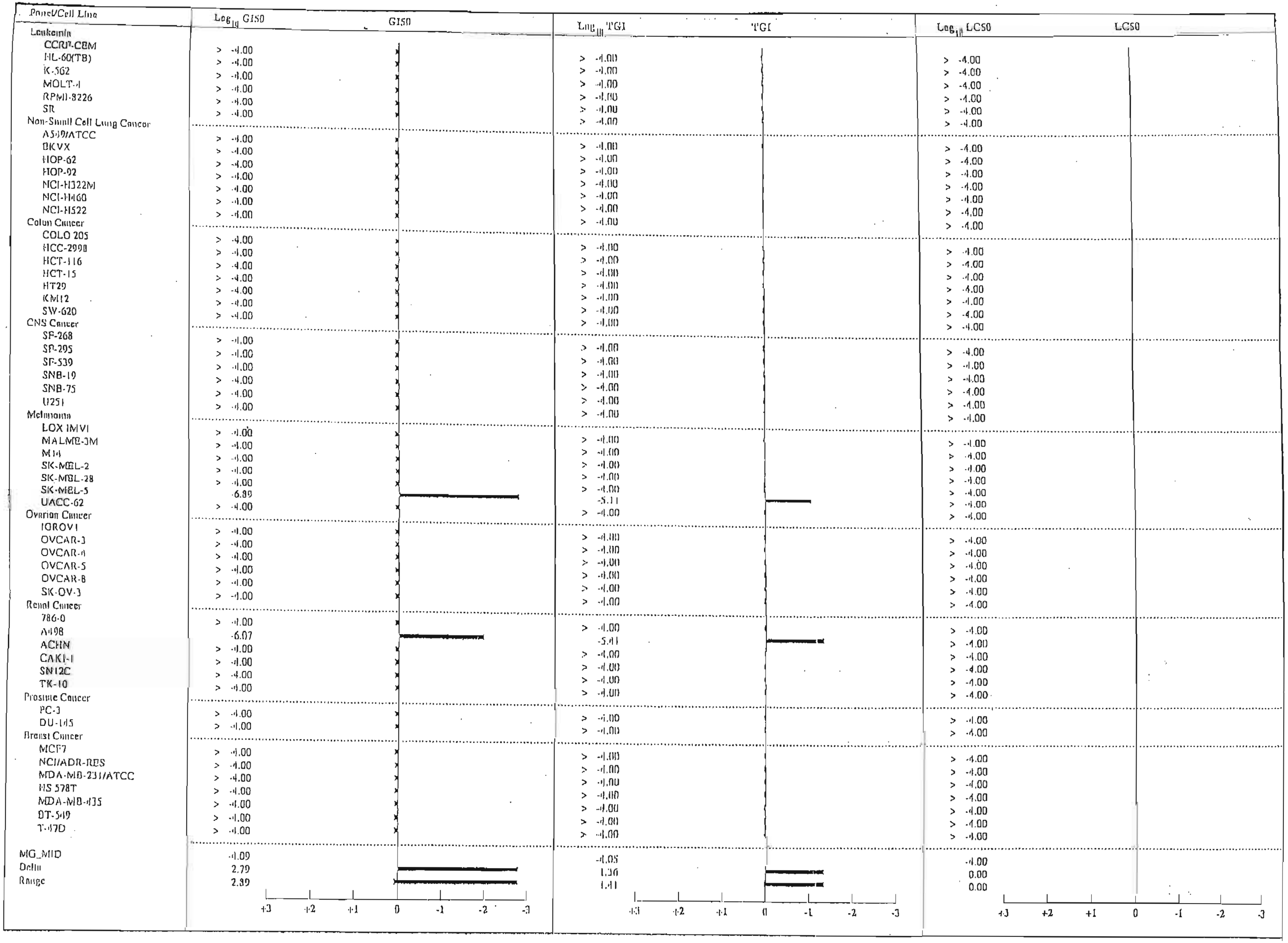
1-Hydroxy-2,3-dimethoxy-10-acetoxymethylacridone



(iv)

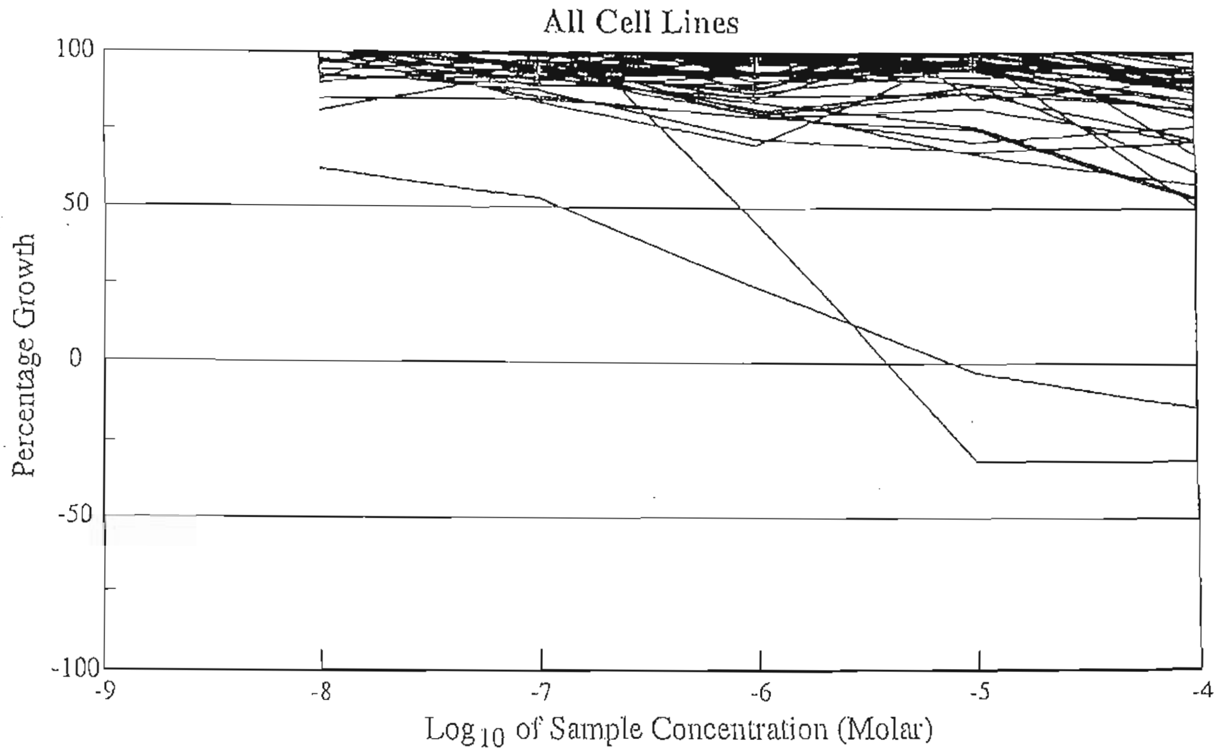
1,2,3-Trimethoxy-10-methoxymethylacridone

Compounds isolated from *Toddaliopsis bremekampii*

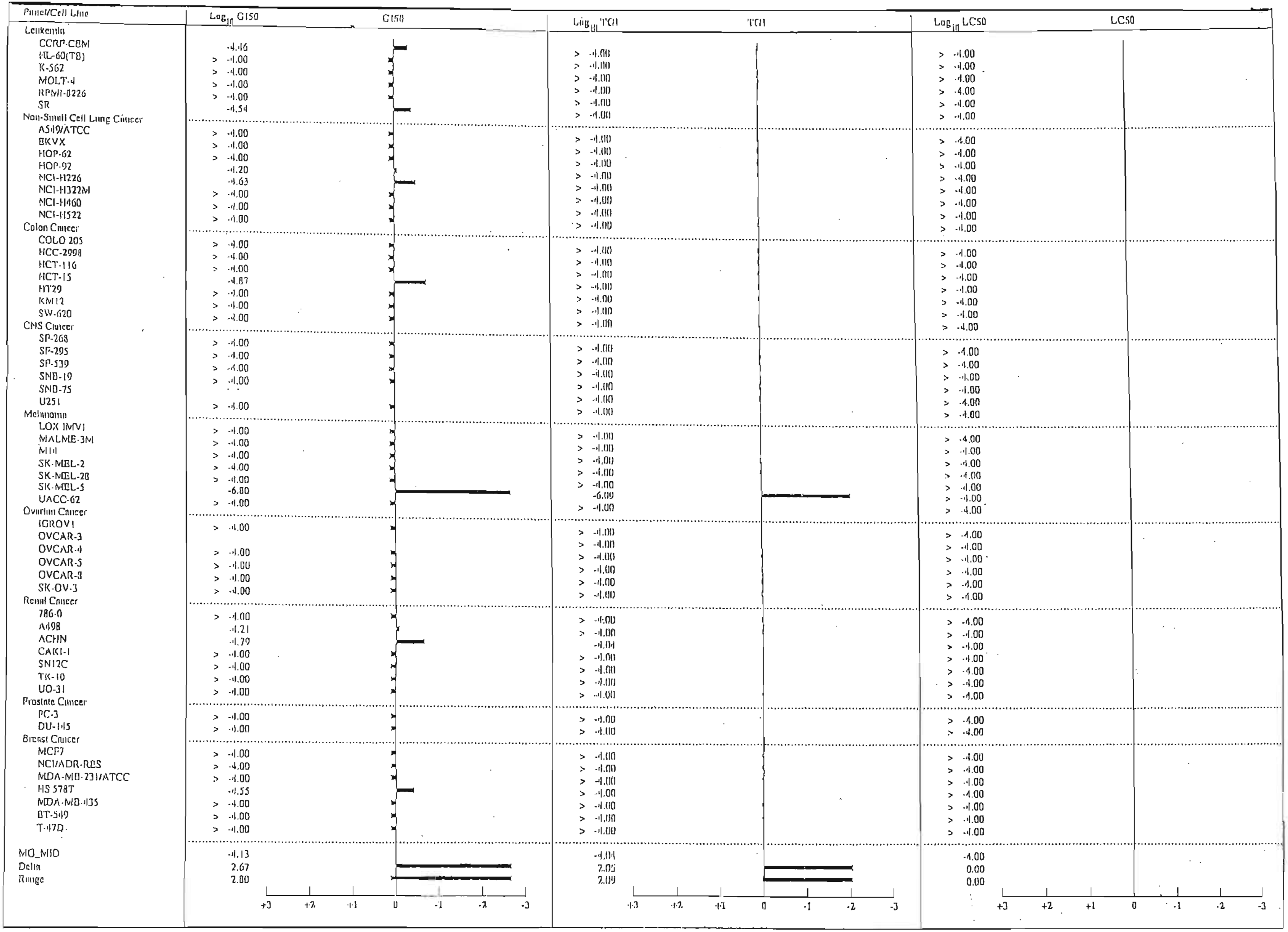


Panel/Cell Line	Log_{10} GI50	Log_{10} TGI	Log_{10} LC50
Leukemia			
CCRF-CEM	> -4.00	> -4.00	> -4.00
HL-60(TB)	> -4.00	> -4.00	> -4.00
K-562	> -4.00	> -4.00	> -4.00
MOLT-4	> -4.00	> -4.00	> -4.00
RPMI-8226	> -4.00	> -4.00	> -4.00
SR	> -4.00	> -4.00	> -4.00
Non-Small Cell Lung Cancer			
A549/ATCC	> -4.00	> -4.00	> -4.00
EKVX	> -4.00	> -4.00	> -4.00
HOP-62	> -4.00	> -4.00	> -4.00
HOP-92	> -4.00	> -4.00	> -4.00
NCI-H322M	> -4.00	> -4.00	> -4.00
NCI-H460	> -4.00	> -4.00	> -4.00
NCI-H522	> -4.00	> -4.00	> -4.00
Colon Cancer			
COLO 205	> -4.00	> -4.00	> -4.00
HCC-2998	> -4.00	> -4.00	> -4.00
HCT-116	> -4.00	> -4.00	> -4.00
HCT-15	> -4.00	> -4.00	> -4.00
HT29	> -4.00	> -4.00	> -4.00
KM12	> -4.00	> -4.00	> -4.00
SW-620	> -4.00	> -4.00	> -4.00
CNS Cancer			
SF-268	> -4.00	> -4.00	> -4.00
SF-295	> -4.00	> -4.00	> -4.00
SF-539	> -4.00	> -4.00	> -4.00
SNB-19	> -4.00	> -4.00	> -4.00
SNB-75	> -4.00	> -4.00	> -4.00
U251	> -4.00	> -4.00	> -4.00
Melanoma			
LOX IMVI	> -4.00	> -4.00	> -4.00
MALME-3M	> -4.00	> -4.00	> -4.00
M14	> -4.00	> -4.00	> -4.00
SK-MEL-2	> -4.00	> -4.00	> -4.00
SK-MEL-28	> -4.00	> -4.00	> -4.00
SK-MEL-5	-6.89	-5.11	> -4.00
UACC-62	> -4.00	> -4.00	> -4.00
Ovarian Cancer			
IGROV1	> -4.00	> -4.00	> -4.00
OVCAR-3	> -4.00	> -4.00	> -4.00
OVCAR-4	> -4.00	> -4.00	> -4.00
OVCAR-5	> -4.00	> -4.00	> -4.00
OVCAR-8	> -4.00	> -4.00	> -4.00
SK-OV-3	> -4.00	> -4.00	> -4.00
Renal Cancer			
786-0	> -4.00	> -4.00	> -4.00
A498	-6.07	-5.41	> -4.00
ACHN	> -4.00	> -4.00	> -4.00
CAKI-1	> -4.00	> -4.00	> -4.00
SN12C	> -4.00	> -4.00	> -4.00
TK-10	> -4.00	> -4.00	> -4.00
Prostate Cancer			
PC-3	> -4.00	> -4.00	> -4.00
DU-145	> -4.00	> -4.00	> -4.00
Breast Cancer			
MCF7	> -4.00	> -4.00	> -4.00
NCI/ADR-RES	> -4.00	> -4.00	> -4.00
MDA-MB-231/ATCC	> -4.00	> -4.00	> -4.00
HS 578T	> -4.00	> -4.00	> -4.00
MDA-MB-435	> -4.00	> -4.00	> -4.00
BT-549	> -4.00	> -4.00	> -4.00
T-47D	> -4.00	> -4.00	> -4.00
MG_MID	-4.09	-4.05	-4.00
Delta	2.79	1.36	0.00
Range	2.89	1.41	0.00

Mean Graphs for Samaderine A (i)

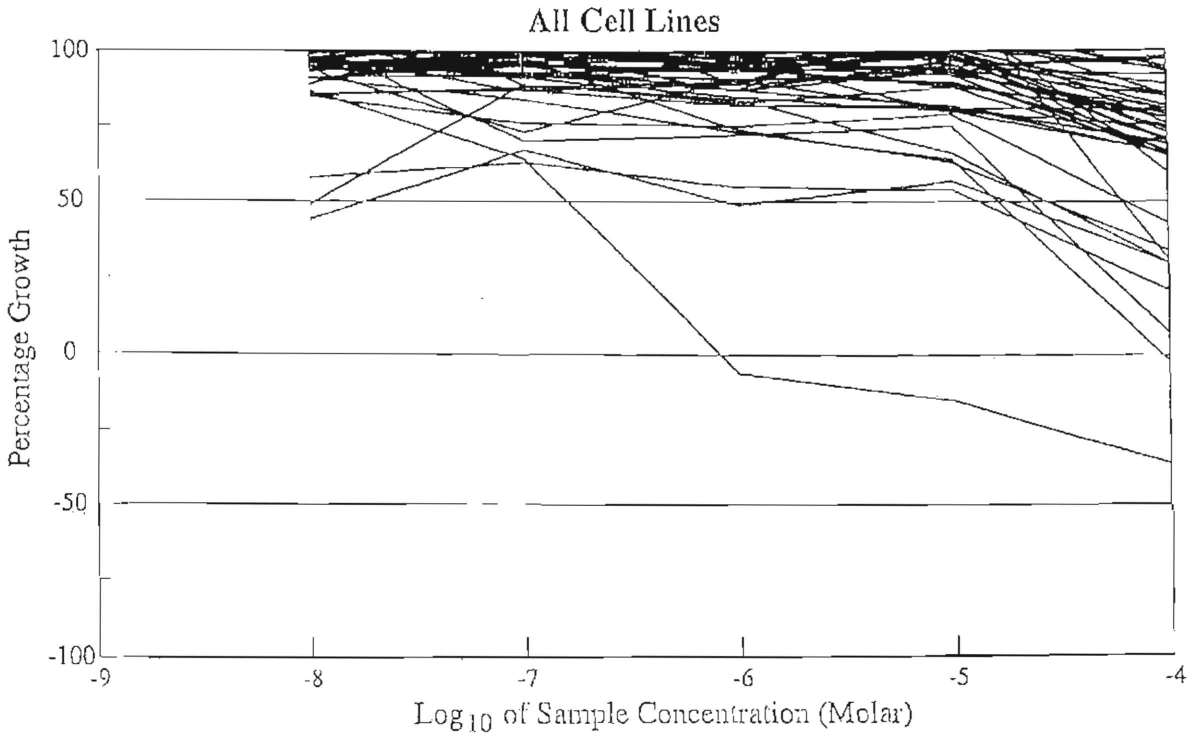


Dose Response Curves for Samaderine A (i)

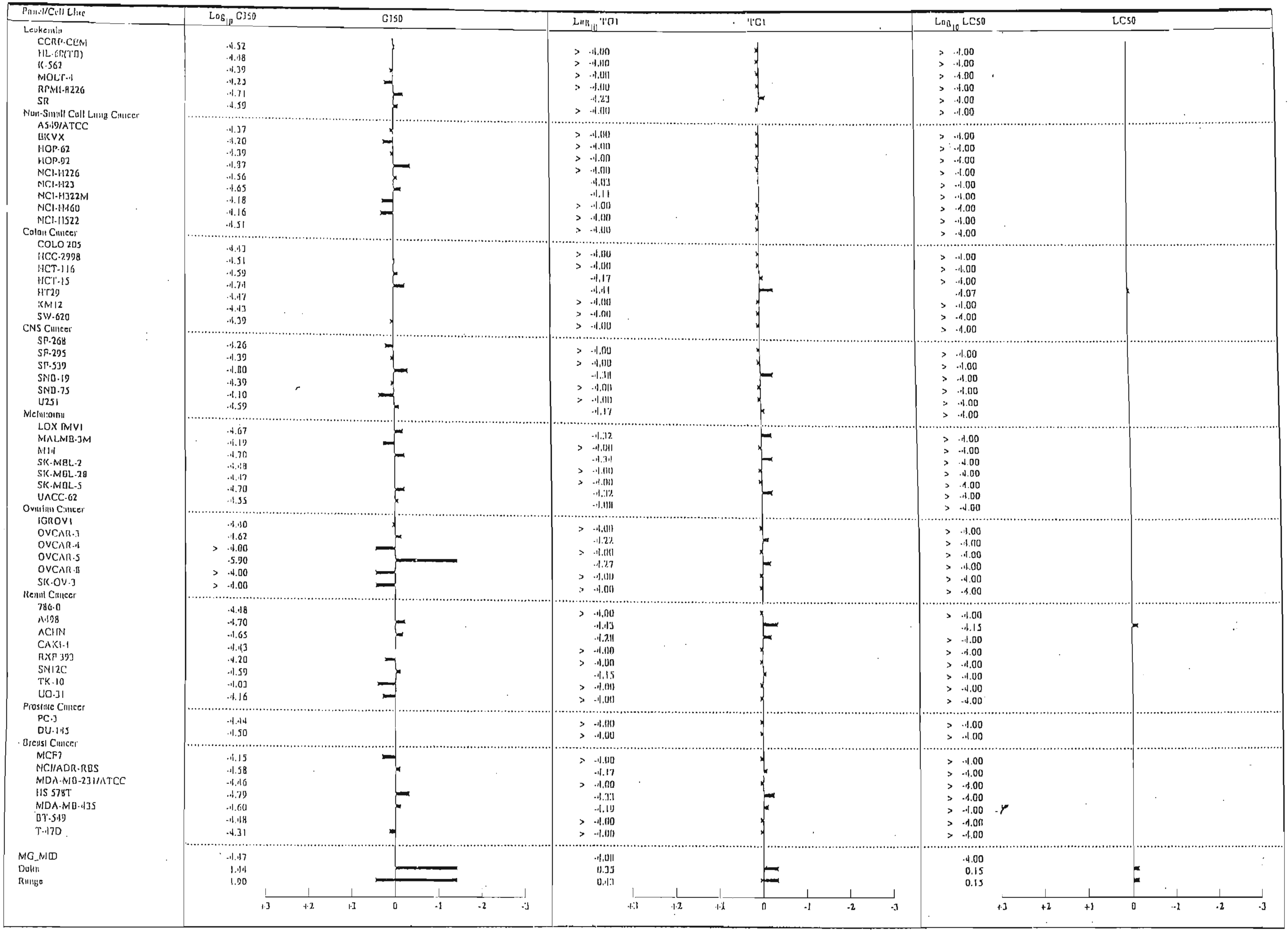


Panel/Cell Line	Log_{10} GI50	Log_{10} TGI	Log_{10} LC50
Leukemia			
CCRF-CEM	-4.46	> -4.00	> -4.00
HL-60(TB)	> -4.00	> -4.00	> -4.00
K-562	> -4.00	> -4.00	> -4.00
MOLT-4	> -4.00	> -4.00	> -4.00
RPMI-8226	> -4.00	> -4.00	> -4.00
SR	-4.54	> -4.00	> -4.00
Non-Small Cell Lung Cancer			
A549/ATCC	> -4.00	> -4.00	> -4.00
EKVX	> -4.00	> -4.00	> -4.00
HOP-62	> -4.00	> -4.00	> -4.00
HOP-92	-4.20	> -4.00	> -4.00
NCI-H226	-4.63	> -4.00	> -4.00
NCI-H322M	> -4.00	> -4.00	> -4.00
NCI-H460	> -4.00	> -4.00	> -4.00
NCI-H522	> -4.00	> -4.00	> -4.00
Colon Cancer			
COLO 205	> -4.00	> -4.00	> -4.00
HCC-2998	> -4.00	> -4.00	> -4.00
HCT-116	> -4.00	> -4.00	> -4.00
HCT-15	-4.87	> -4.00	> -4.00
HT29	> -4.00	> -4.00	> -4.00
KM12	> -4.00	> -4.00	> -4.00
SW-620	> -4.00	> -4.00	> -4.00
CNS Cancer			
SF-268	> -4.00	> -4.00	> -4.00
SF-295	> -4.00	> -4.00	> -4.00
SF-539	> -4.00	> -4.00	> -4.00
SNB-19	> -4.00	> -4.00	> -4.00
SNB-75		> -4.00	> -4.00
U251	> -4.00	> -4.00	> -4.00
Melanoma			
LOX IMVI	> -4.00	> -4.00	> -4.00
MALME-3M	> -4.00	> -4.00	> -4.00
M14	> -4.00	> -4.00	> -4.00
SK-MEL-2	> -4.00	> -4.00	> -4.00
SK-MEL-28	> -4.00	> -4.00	> -4.00
SK-MEL-5	-6.80	-6.09	> -4.00
UACC-62	> -4.00	> -4.00	> -4.00
Ovarian Cancer			
IGROV1	> -4.00	> -4.00	> -4.00
OVCAR-3		> -4.00	> -4.00
OVCAR-4	> -4.00	> -4.00	> -4.00
OVCAR-5	> -4.00	> -4.00	> -4.00
OVCAR-8	> -4.00	> -4.00	> -4.00
SK-OV-3	> -4.00	> -4.00	> -4.00
Renal Cancer			
786-0	> -4.00	> -4.00	> -4.00
A498	-4.21	> -4.00	> -4.00
ACHN	-4.79	-4.04	> -4.00
CAKI-1	> -4.00	> -4.00	> -4.00
SN12C	> -4.00	> -4.00	> -4.00
TK-10	> -4.00	> -4.00	> -4.00
UO-31	> -4.00	> -4.00	> -4.00
Prostate Cancer			
PC-3	> -4.00	> -4.00	> -4.00
DU-145	> -4.00	> -4.00	> -4.00
Breast Cancer			
MCF7	> -4.00	> -4.00	> -4.00
NCI/ADR-RES	> -4.00	> -4.00	> -4.00
MDA-MB-231/ATCC	> -4.00	> -4.00	> -4.00
HS 578T	-4.55	> -4.00	> -4.00
MDA-MB-435	> -4.00	> -4.00	> -4.00
BT-549	> -4.00	> -4.00	> -4.00
T-47D	> -4.00	> -4.00	> -4.00
MG_MID	-4.13	-4.04	-4.00
Delta	2.67	2.05	0.00
Range	2.80	2.09	0.00

Mean Graphs for 5β,6-Dihydrosamaderine A (ii)

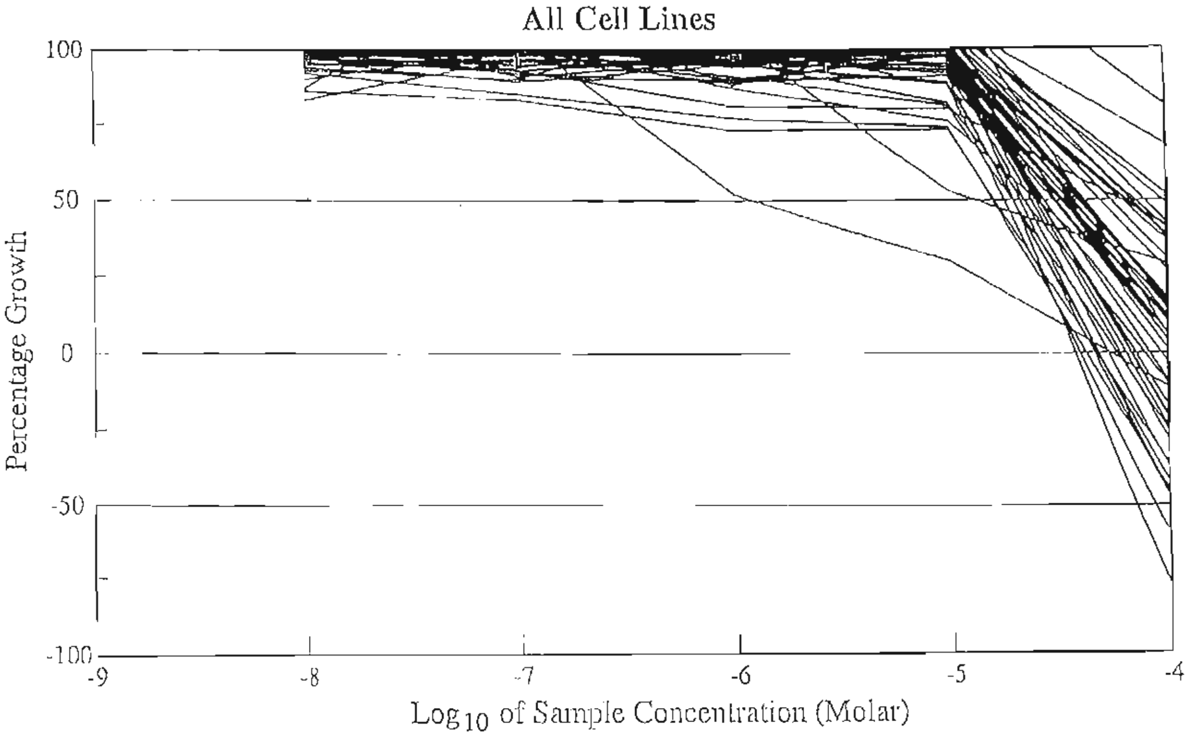


Dose Response Curves for 5β,6-Dihydrosamaderine A (ii)

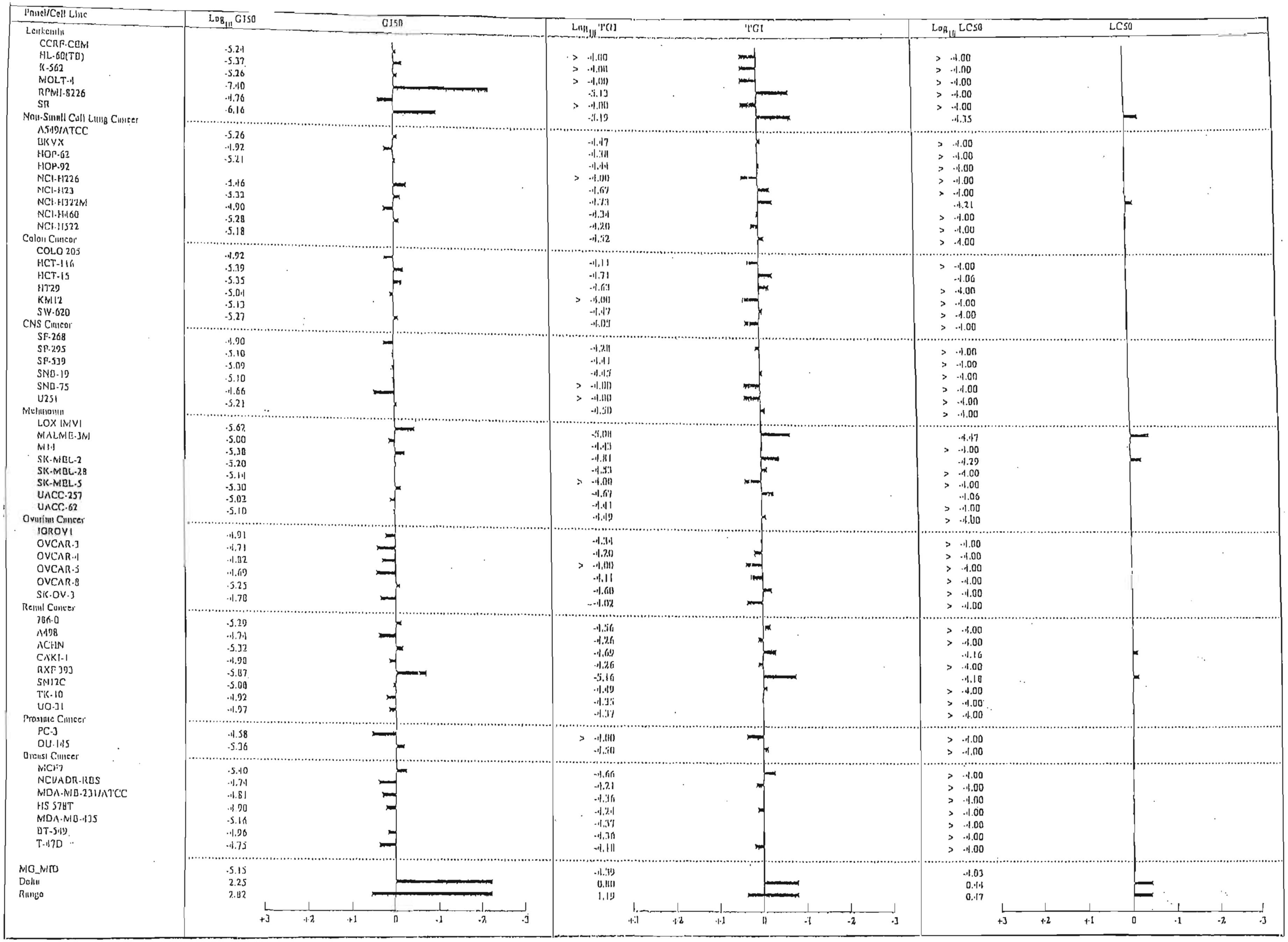


Panel/Cell Line	Log$_{10}$ GI50	Log$_{10}$ TGI	Log$_{10}$ LC50
Leukemia			
CCRF-CEM	-4.52	> -4.00	> -4.00
HL-60(TB)	-4.48	> -4.00	> -4.00
K-562	-4.39	> -4.00	> -4.00
MOLT-4	-4.25	> -4.00	> -4.00
RPMI-8226	-4.71	-4.23	> -4.00
SR	-4.59	> -4.00	> -4.00
Non-Small Cell Lung Cancer			
A549/ATCC	-4.37	> -4.00	> -4.00
EKVX	-4.20	> -4.00	> -4.00
HOP-62	-4.39	> -4.00	> -4.00
HOP-92	-4.87	> -4.00	> -4.00
NCI-H226	-4.56	-4.03	> -4.00
NCI-H23	-4.65	-4.11	> -4.00
NCI-H322M	-4.18	> -4.00	> -4.00
NCI-H460	-4.16	> -4.00	> -4.00
NCI-H522	-4.51	> -4.00	> -4.00
Colon Cancer			
COLO 205	-4.43	> -4.00	> -4.00
HCC-2998	-4.51	> -4.00	> -4.00
HCT-116	-4.59	-4.17	> -4.00
HCT-15	-4.74	-4.41	-4.07
HT29	-4.47	> -4.00	> -4.00
KM12	-4.43	> -4.00	> -4.00
SW-620	-4.39	> -4.00	> -4.00
CNS Cancer			
SF-268	-4.26	> -4.00	> -4.00
SF-295	-4.39	> -4.00	> -4.00
SF-539	-4.80	-4.38	> -4.00
SNB-19	-4.39	> -4.00	> -4.00
SNB-75	-4.10	> -4.00	> -4.00
U251	-4.59	-4.17	> -4.00
Melanoma			
LOX IMVI	-4.67	-4.32	> -4.00
MALME-3M	-4.19	> -4.00	> -4.00
M14	-4.70	-4.34	> -4.00
SK-MEL-2	-4.48	> -4.00	> -4.00
SK-MEL-28	-4.47	> -4.00	> -4.00
SK-MEL-5	-4.70	-4.32	> -4.00
UACC-62	-4.55	-4.08	> -4.00
Ovarian Cancer			
IGROV1	-4.40	> -4.00	> -4.00
OVCAR-3	-4.62	-4.22	> -4.00
OVCAR-4	> -4.00	> -4.00	> -4.00
OVCAR-5	-5.90	-4.27	> -4.00
OVCAR-8	> -4.00	> -4.00	> -4.00
SK-OV-3	> -4.00	> -4.00	> -4.00
Renal Cancer			
786-0	-4.48	> -4.00	> -4.00
A498	-4.70	-4.43	-4.15
ACHN	-4.65	-4.28	> -4.00
CAKI-1	-4.43	> -4.00	> -4.00
RXF 393	-4.20	> -4.00	> -4.00
SN12C	-4.59	-4.15	> -4.00
TK-10	-4.03	> -4.00	> -4.00
UO-31	-4.16	> -4.00	> -4.00
Prostate Cancer			
PC-3	-4.44	> -4.00	> -4.00
DU-145	-4.50	> -4.00	> -4.00
Breast Cancer			
MCF7	-4.15	> -4.00	> -4.00
NCI/ADR-RES	-4.58	-4.17	> -4.00
MDA-MB-231/ATCC	-4.46	> -4.00	> -4.00
HS 578T	-4.79	-4.33	> -4.00
MDA-MB-435	-4.60	-4.19	> -4.00
BT-549	-4.48	> -4.00	> -4.00
T-47D	-4.31	> -4.00	> -4.00
MG_MID	-4.47	-4.08	-4.00
Delta	1.44	0.35	0.15
Range	1.90	0.43	0.15

Mean Graphs for 2-Chlorosamaderine A (iii)

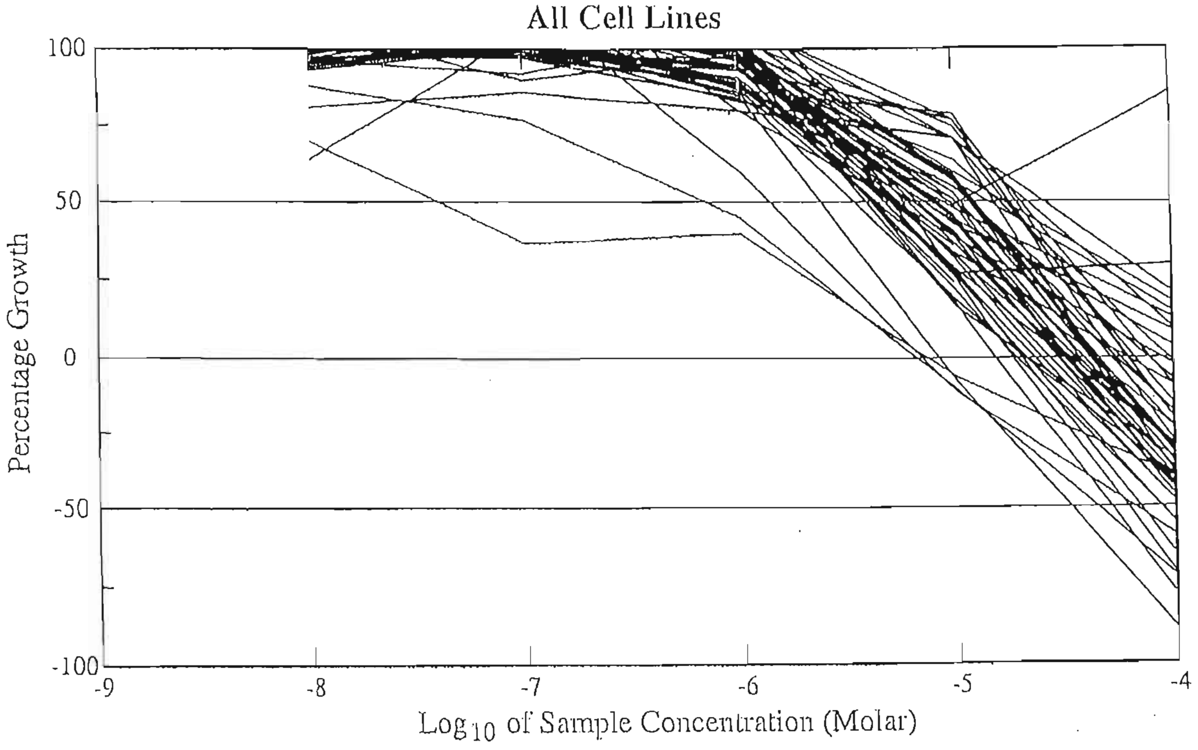


Dose Response Curves for 2-Chlorosamaderine A (iii)

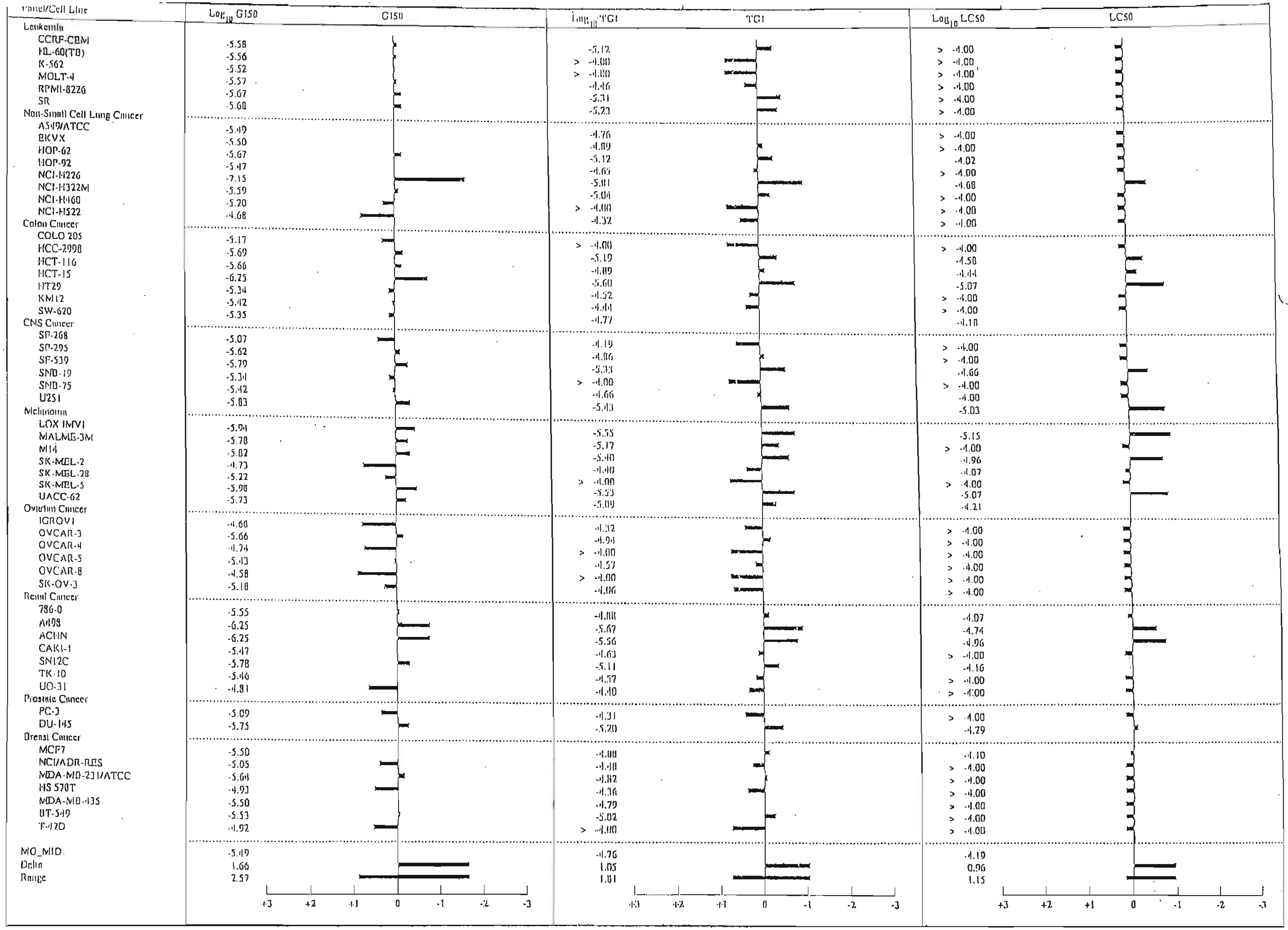


Panel/Cell Line	Log_{10} GI50	Log_{10} TGI	Log_{10} LC50
Leukemia			
CCRF-CEM	-5.24	> -4.00	> -4.00
HL-60(TB)	-5.37	> -4.00	> -4.00
K-562	-5.26	> -4.00	> -4.00
MOLT-4	-7.40	-5.13	> -4.00
RPMI-8226	-4.76	> -4.00	> -4.00
SR	-6.16	-5.19	-4.35
Non-Small Cell Lung Cancer			
A549/ATCC	-5.26	-4.47	> -4.00
EKVX	-4.92	-4.38	> -4.00
HOP-62	-5.21	-4.44	> -4.00
HOP-92		> -4.00	> -4.00
NCI-H226	-5.46	-4.67	> -4.00
NCI-H23	-5.33	-4.73	-4.21
NCI-H322M	-4.90	-4.34	> -4.00
NCI-H460	-5.28	-4.20	> -4.00
NCI-H522	-5.18	-4.52	> -4.00
Colon Cancer			
COLO 205	-4.92	-4.11	> -4.00
HCT-116	-5.39	-4.71	-4.06
HCT-15	-5.35	-4.63	> -4.00
HT29	-5.04	> -4.00	> -4.00
KM12	-5.13	-4.47	> -4.00
SW-620	-5.27	-4.09	> -4.00
CNS Cancer			
SF-268	-4.90	-4.28	> -4.00
SF-295	-5.10	-4.41	> -4.00
SF-539	-5.09	-4.43	> -4.00
SNB-19	-5.10	> -4.00	> -4.00
SNB-75	-4.66	> -4.00	> -4.00
U251	-5.21	-4.50	> -4.00
Melanoma			
LOX IMVI	-5.62	-5.08	-4.47
MALME-3M	-5.00	-4.43	> -4.00
M14	-5.38	-4.81	-4.29
SK-MEL-2	-5.20	-4.53	> -4.00
SK-MEL-28	-5.14	> -4.00	> -4.00
SK-MEL-5	-5.30	-4.67	-4.06
UACC-257	-5.02	-4.41	> -4.00
UACC-62	-5.10	-4.49	> -4.00
Ovarian Cancer			
IGROV1	-4.91	-4.34	> -4.00
OVCAR-3	-4.71	-4.20	> -4.00
OVCAR-4	-4.82	> -4.00	> -4.00
OVCAR-5	-4.69	-4.11	> -4.00
OVCAR-8	-5.25	-4.60	> -4.00
SK-OV-3	-4.78	-4.02	> -4.00
Renal Cancer			
786-0	-5.29	-4.56	> -4.00
A498	-4.74	-4.26	> -4.00
ACHN	-5.32	-4.69	-4.16
CAKI-1	-4.90	-4.26	> -4.00
RXF 393	-5.87	-5.16	-4.18
SN12C	-5.08	-4.49	> -4.00
TK-10	-4.92	-4.35	> -4.00
UO-31	-4.97	-4.37	> -4.00
Prostate Cancer			
PC-3	-4.58	> -4.00	> -4.00
DU-145	-5.36	-4.50	> -4.00
Breast Cancer			
MCF7	-5.40	-4.66	> -4.00
NCI/ADR-RES	-4.74	-4.21	> -4.00
MDA-MB-231/ATCC	-4.81	-4.36	> -4.00
HS 578T	-4.90	-4.24	> -4.00
MDA-MB-435	-5.16	-4.37	> -4.00
BT-549	-4.96	-4.30	> -4.00
T-47D	-4.75	-4.10	> -4.00
MG_MID	-5.15	-4.39	-4.03
Delta	2.25	0.80	0.44
Range	2.82	1.19	0.47

Mean Graphs for Cedronin (vi)

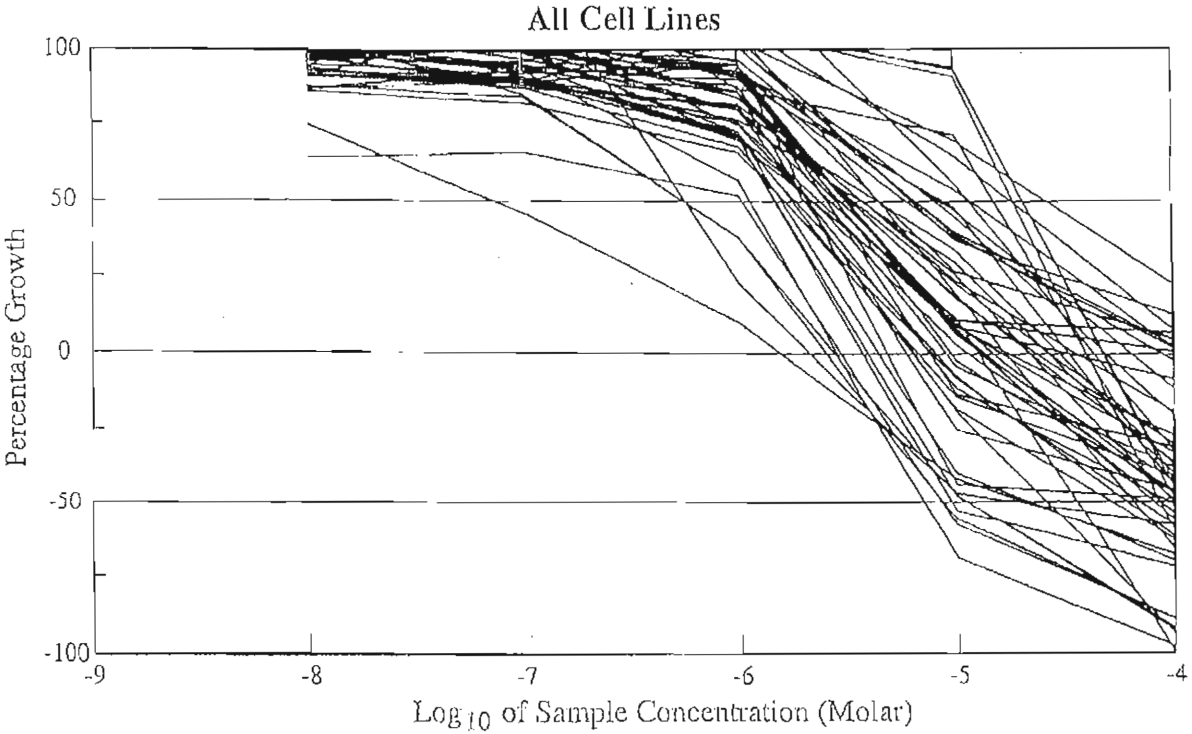


Dose Response Curves for Cedronin (vi)



Panel/Cell Line	Log_{10} GI50	Log_{10} TGI	Log_{10} LC50
Leukemia			
CCRF-CEM	-5.58	-5.12	> -4.00
HL-60(TB)	-5.56	> -4.00	> -4.00
K-562	-5.52	> -4.00	> -4.00
MOLT-4	-5.57	-4.46	> -4.00
RPMI-8226	-5.67	-5.31	> -4.00
SR	-5.60	-5.23	> -4.00
Non-Small Cell Lung Cancer			
A549/ATCC	-5.49	-4.76	> -4.00
EKVX	-5.50	-4.89	> -4.00
HOP-62	-5.67	-5.12	-4.02
HOP-92	-5.47	-4.65	> -4.00
NCI-H226	-7.15	-5.81	-4.68
NCI-H322M	-5.59	-5.04	> -4.00
NCI-H460	-5.20	> -4.00	> -4.00
NCI-H522	-4.68	-4.32	> -4.00
Colon Cancer			
COLO 205	-5.17	> -4.00	> -4.00
HCC-2998	-5.69	-5.19	-4.58
HCT-116	-5.66	-4.89	-4.44
HCT-15	-6.25	-5.60	-5.07
HT29	-5.34	-4.52	> -4.00
KM12	-5.42	-4.44	> -4.00
SW-620	-5.35	-4.77	-4.18
CNS Cancer			
SF-268	-5.07	-4.19	> -4.00
SF-295	-5.62	-4.86	> -4.00
SF-539	-5.79	-5.33	-4.66
SNB-19	-5.34	> -4.00	> -4.00
SNB-75	-5.42	-4.66	-4.00
U251	-5.83	-5.43	-5.03
Melanoma			
LOX IMVI	-5.94	-5.55	-5.15
MALME-3M	-5.78	-5.17	> -4.00
M14	-5.82	-5.40	-4.96
SK-MEL-2	-4.73	-4.40	-4.07
SK-MEL-28	-5.22	> -4.00	> -4.00
SK-MEL-5	-5.98	-5.53	-5.07
UACC-62	-5.73	-5.09	-4.21
Ovarian Cancer			
IGROV1	-4.68	-4.32	> -4.00
OVCAR-3	-5.66	-4.94	> -4.00
OVCAR-4	-4.74	> -4.00	> -4.00
OVCAR-5	-5.43	-4.57	> -4.00
OVCAR-8	-4.58	> -4.00	> -4.00
SK-OV-3	-5.18	-4.06	> -4.00
Renal Cancer			
786-0	-5.55	-4.88	-4.07
A498	-6.25	-5.67	-4.74
ACHN	-6.25	-5.56	-4.96
CAKI-1	-5.47	-4.63	> -4.00
SN12C	-5.78	-5.11	-4.16
TK-10	-5.46	-4.57	> -4.00
UO-31	-4.91	-4.40	> -4.00
Prostate Cancer			
PC-3	-5.09	-4.31	> -4.00
DU-145	-5.75	-5.20	-4.29
Breast Cancer			
MCF7	-5.50	-4.88	-4.10
NCI/ADR-RES	-5.05	-4.48	> -4.00
MDA-MB-231/ATCC	-5.64	-4.82	> -4.00
HS 578T	-4.93	-4.36	> -4.00
MDA-MB-435	-5.50	-4.79	> -4.00
BT-549	-5.53	-5.02	> -4.00
T-47D	-4.92	> -4.00	> -4.00
MG_MID	-5.49	-4.76	-4.19
Delta	1.66	1.05	0.96
Range	2.57	1.81	1.15

Mean Graphs for 3,4β-Dihydrosamaderine C (vii)



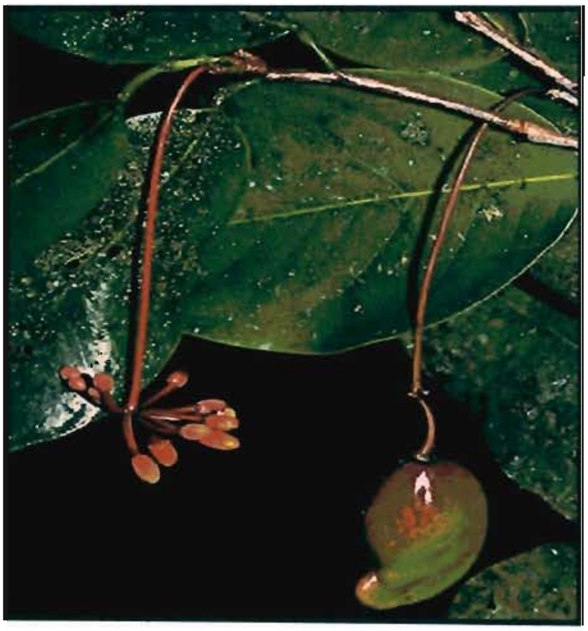
Dose Response Curves for 3,4β-Dihydrosamaderine C (vii)

PART II
Spectra

CONTENTS

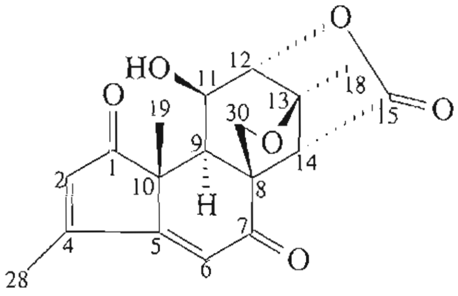
PAGE NO.

Spectra of Compounds from *Samadera madagascariensis* leaves

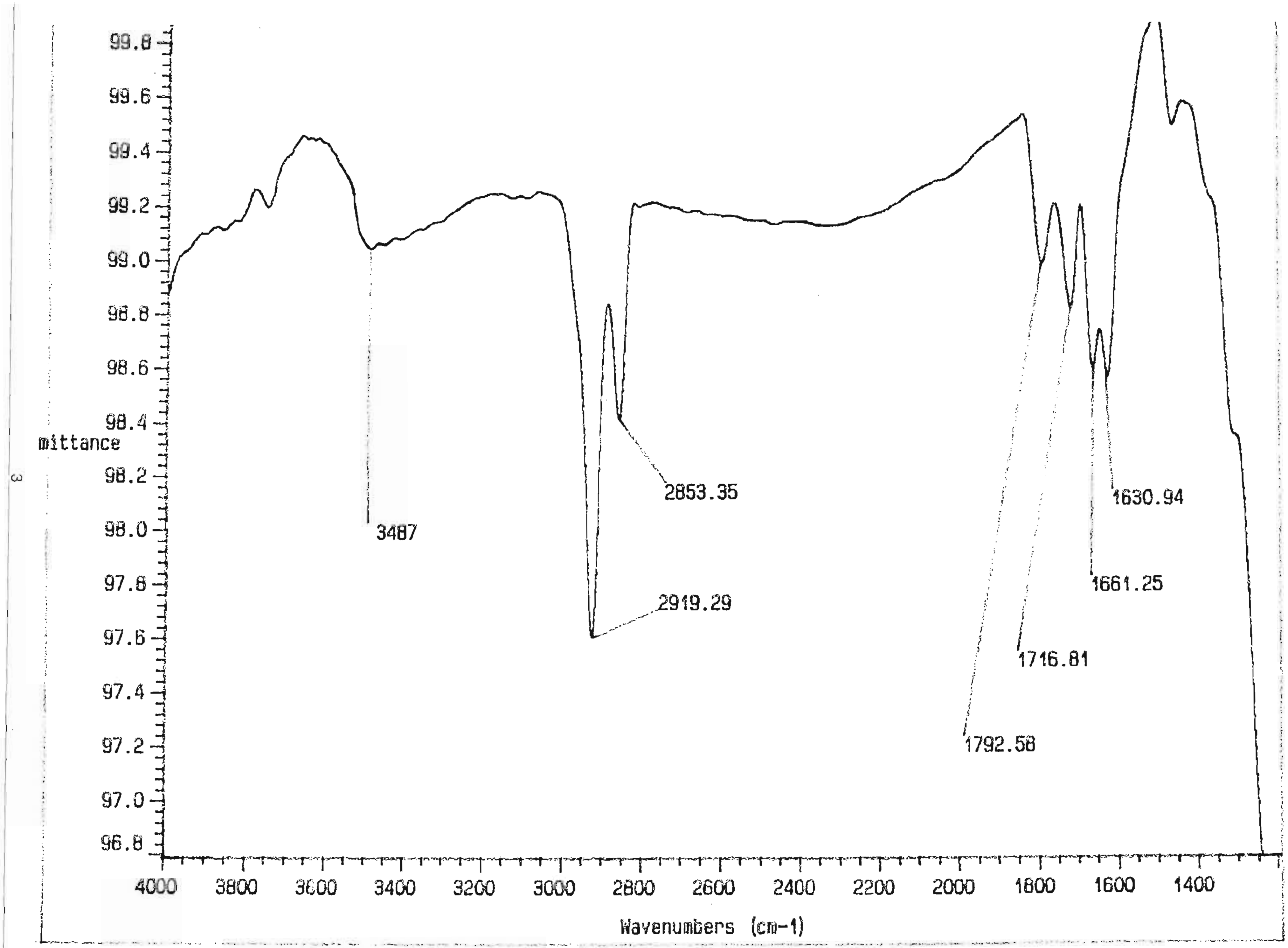


Photograph by Dr. Harison Rabarison

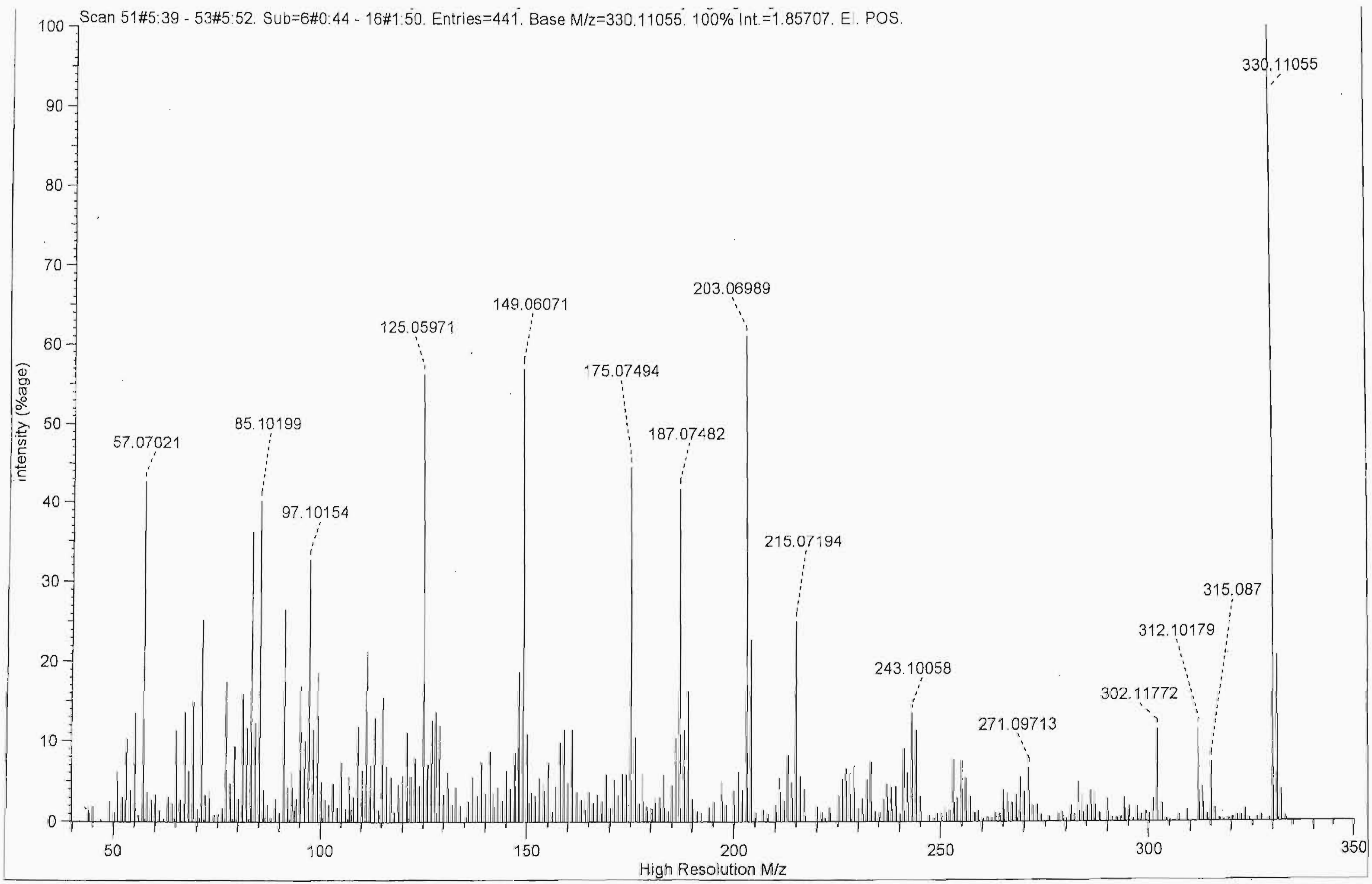
Samaderine A (i)



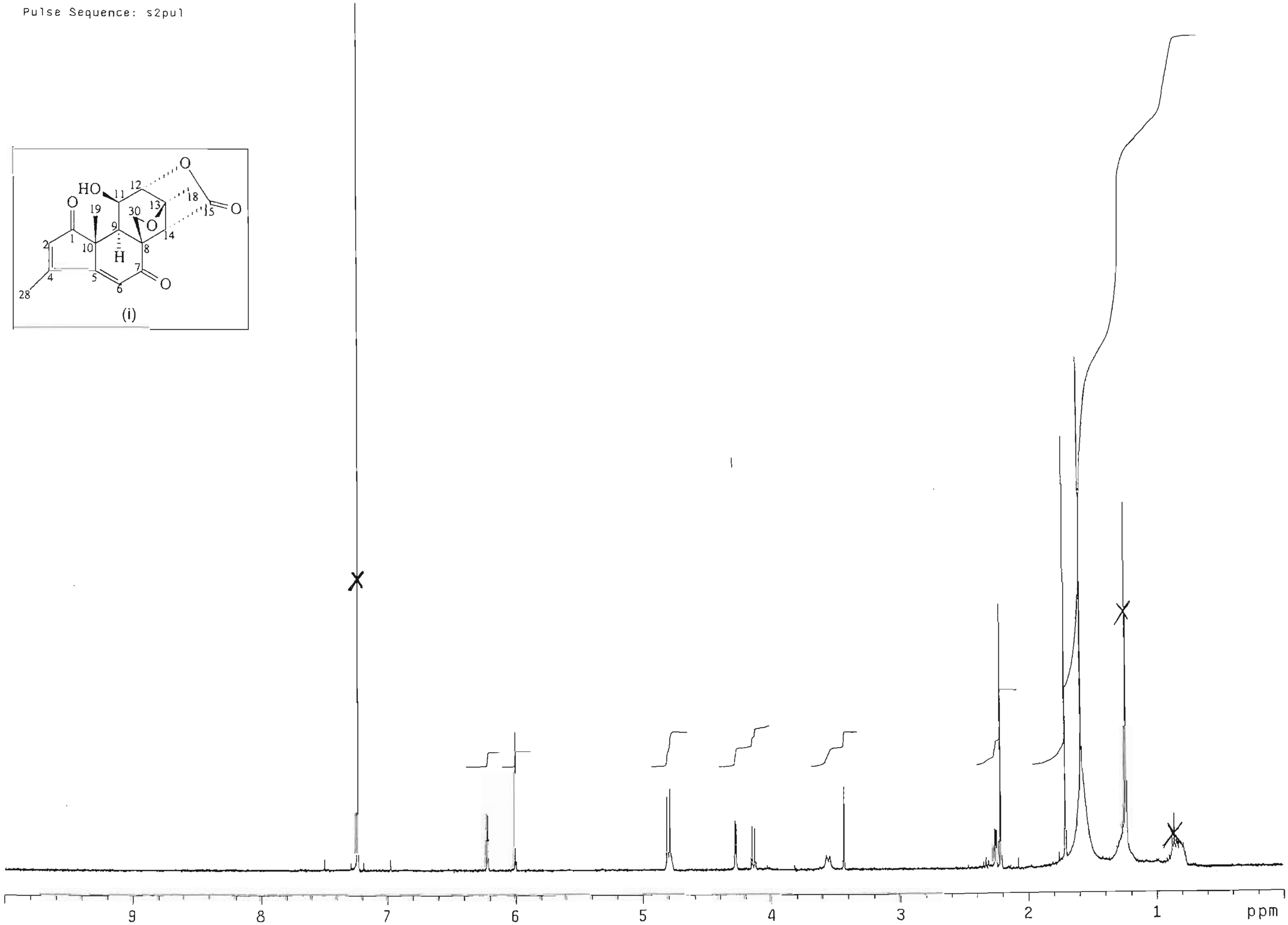
(i)



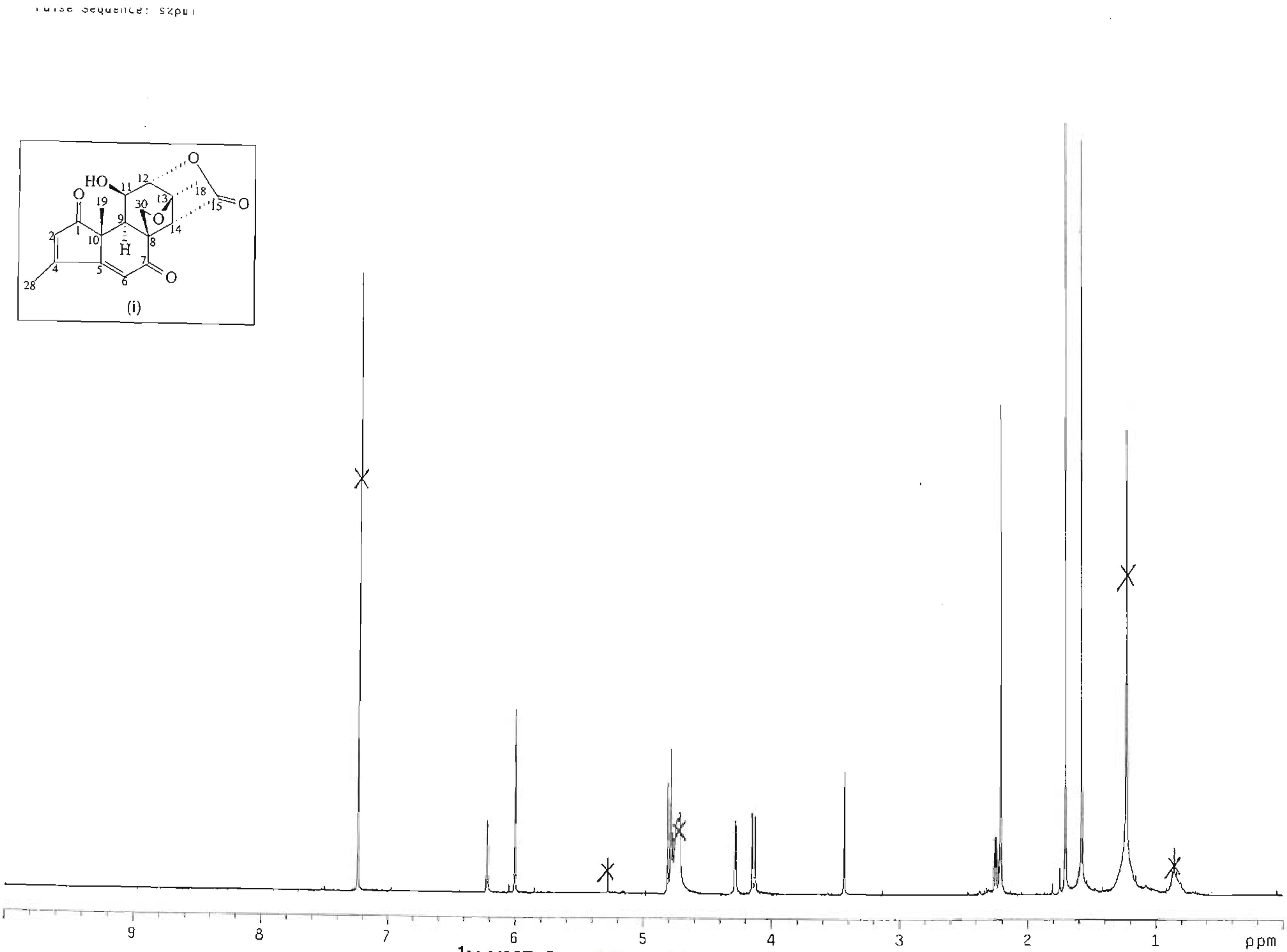
IR Spectrum of Samaderine A (i)



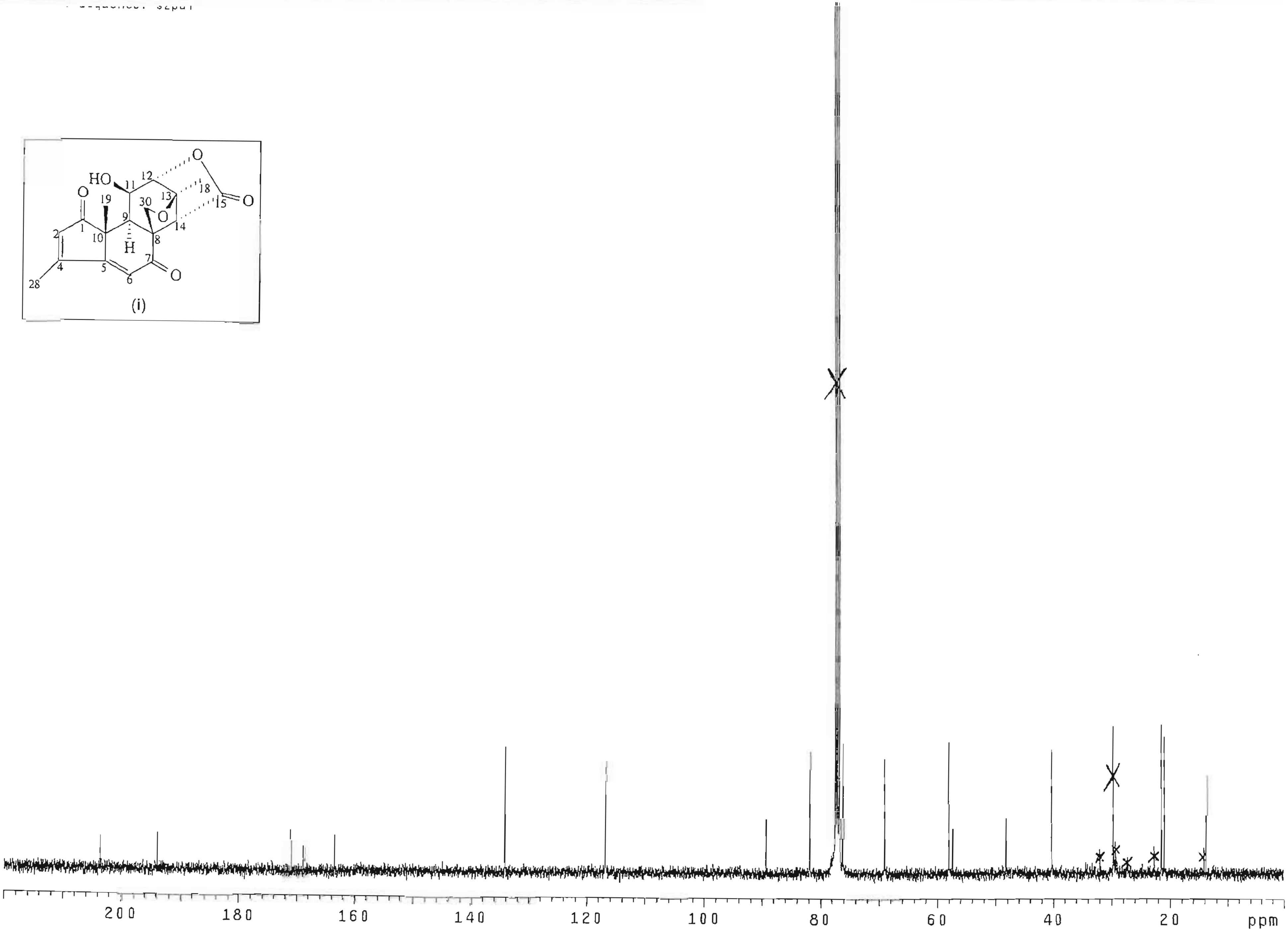
Mass Spectrum of Samaderine A (i)



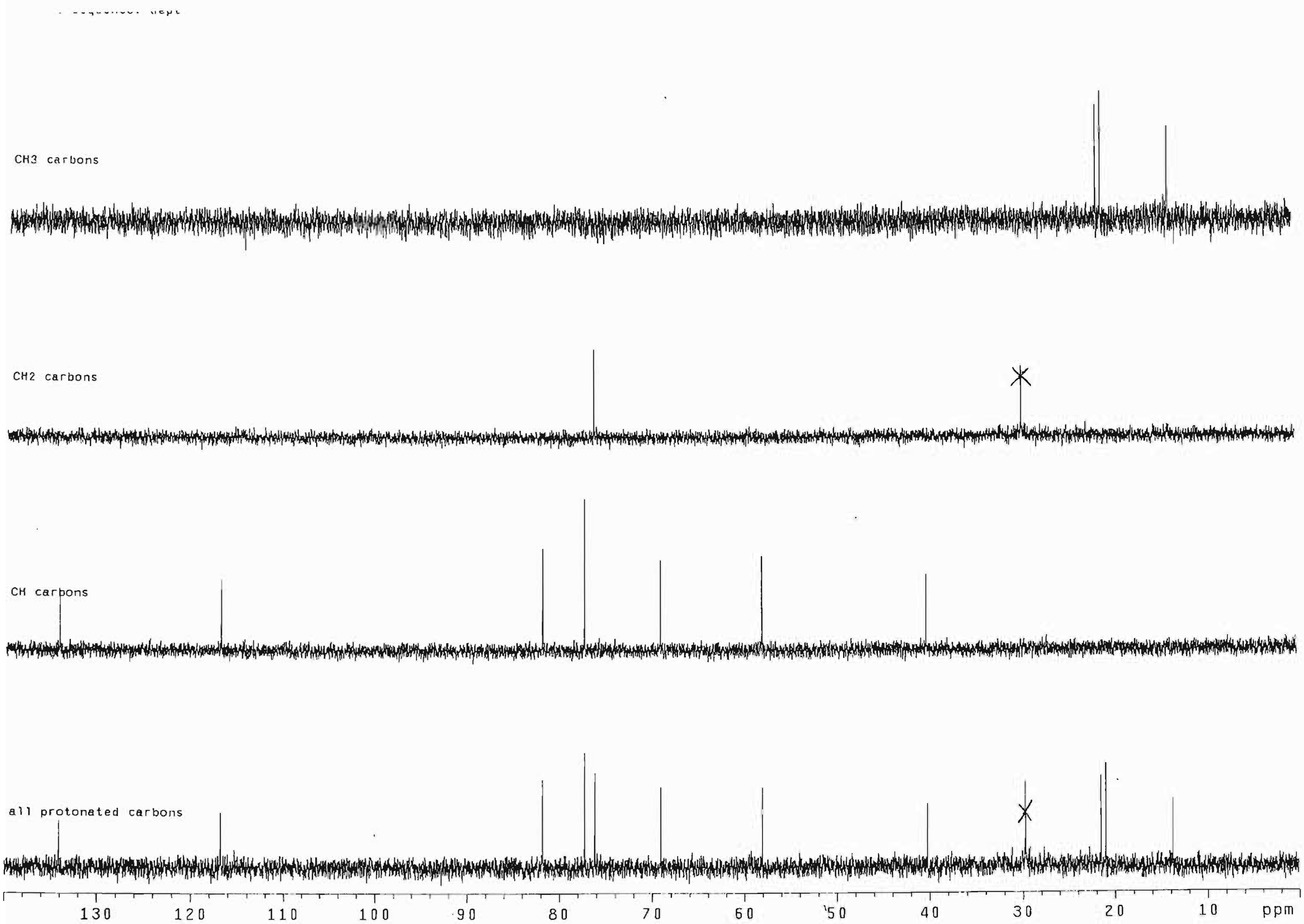
¹H NMR Spectrum of Samaderine A (i)



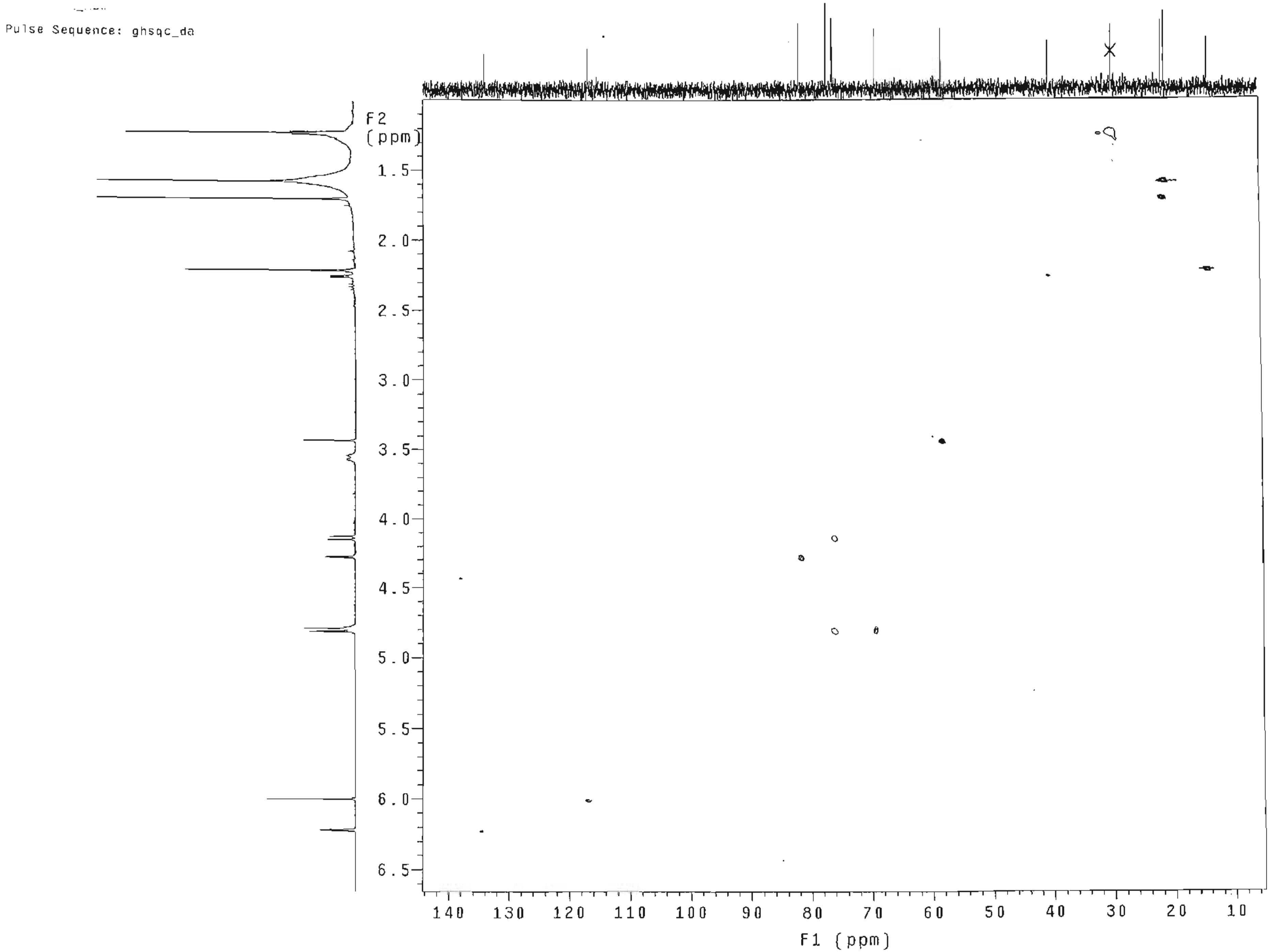
^{1}H NMR Spectrum of Samaderine A (i) (D$_2$O added)



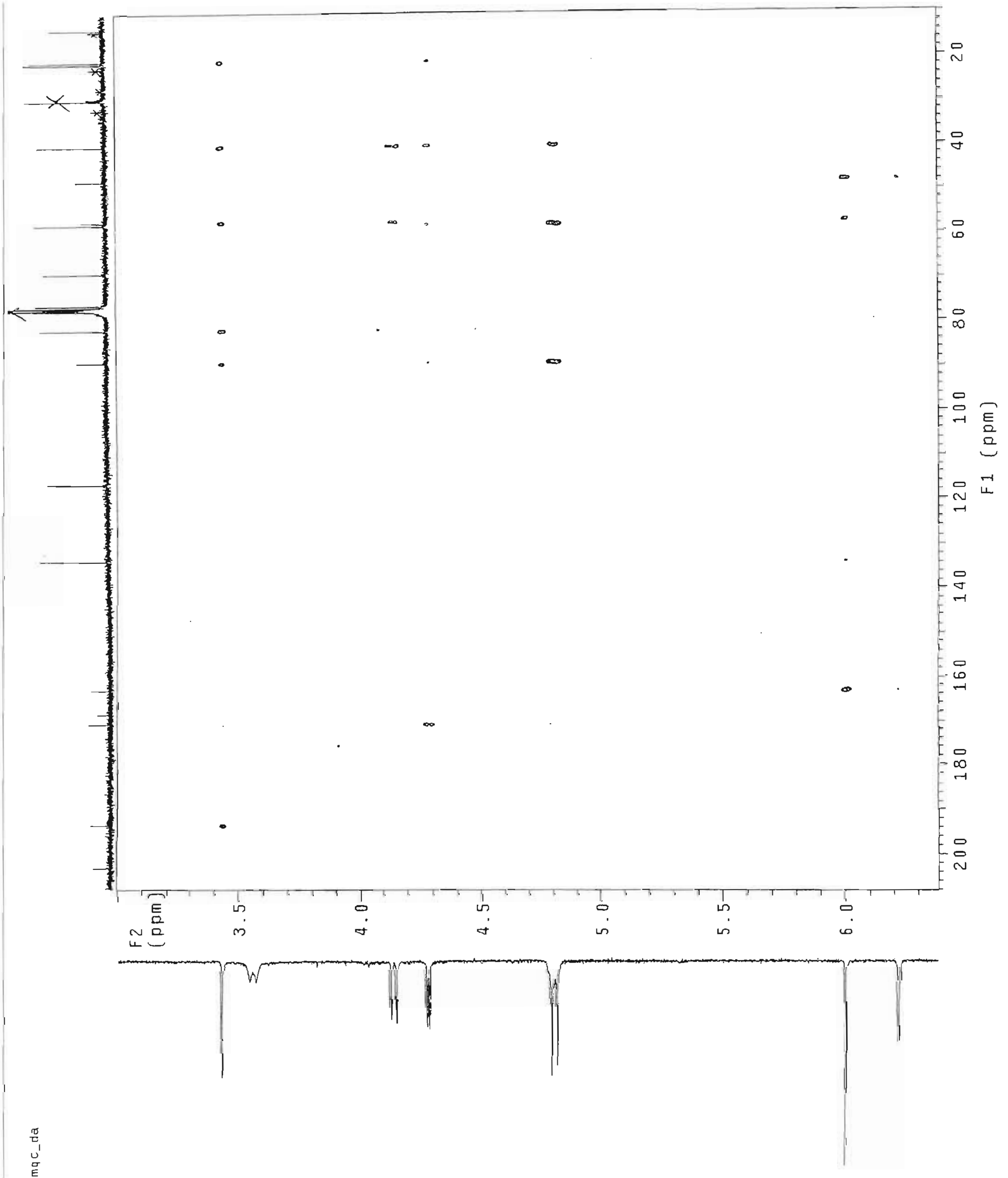
^{13}C NMR Spectrum of Samaderine A (i)



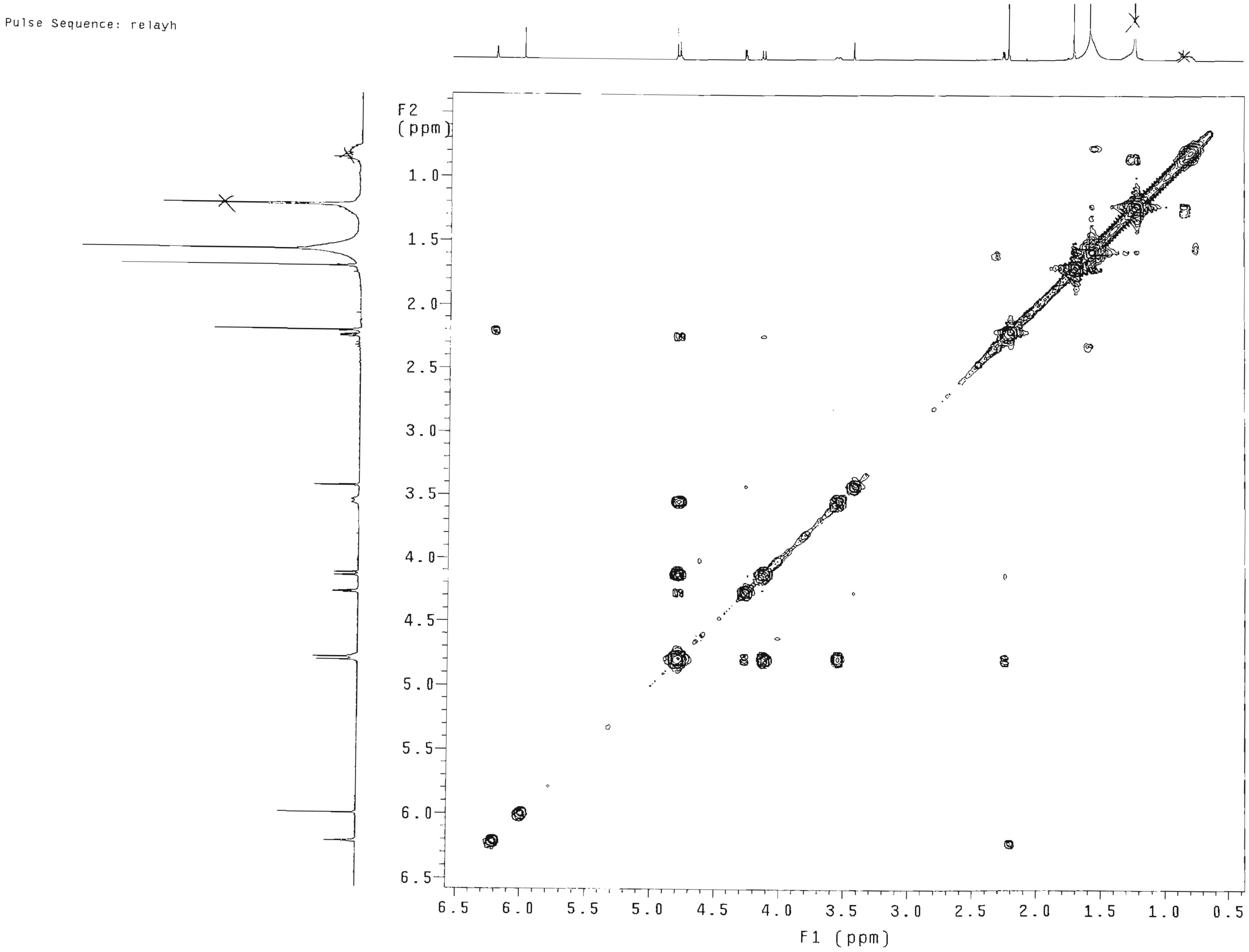
ADEPT NMR Spectrum of Samaderine A (i)



HSQC NMR Spectrum of Samaderine A (i)



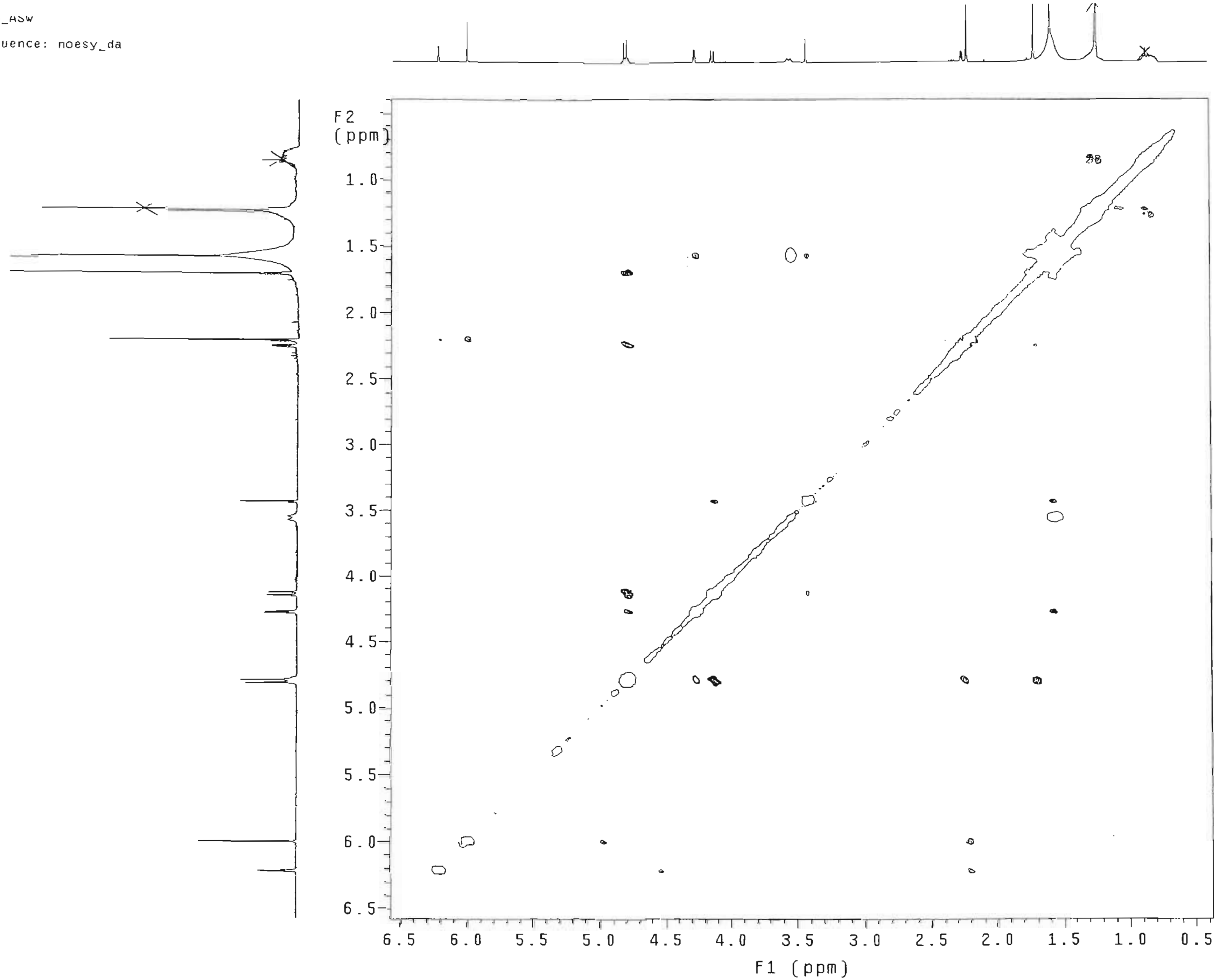
HMBC NMR Spectrum of Samaderine A (i)



COSY NMR Spectrum of Samaderine A (i)

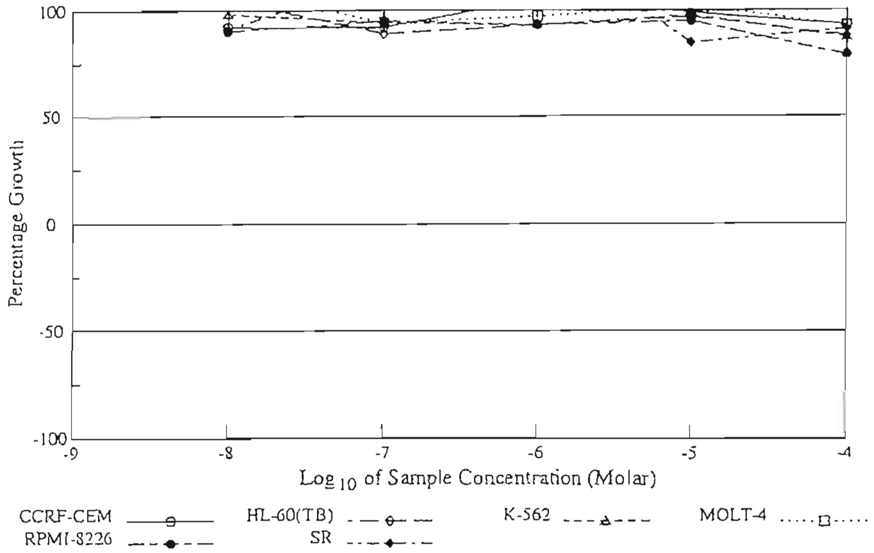
probe=5mm_ASW

Pulse Sequence: noesy_da

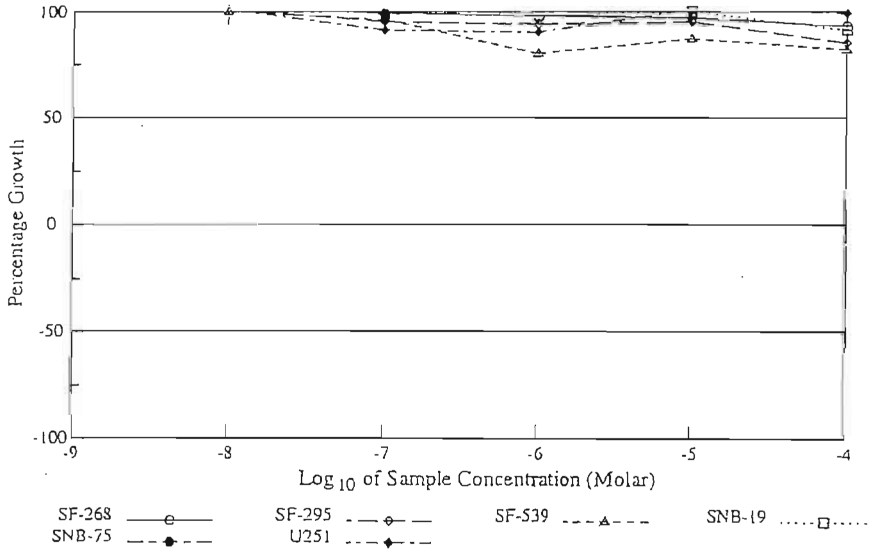


NOESY NMR Spectrum of Samaderine A (i)

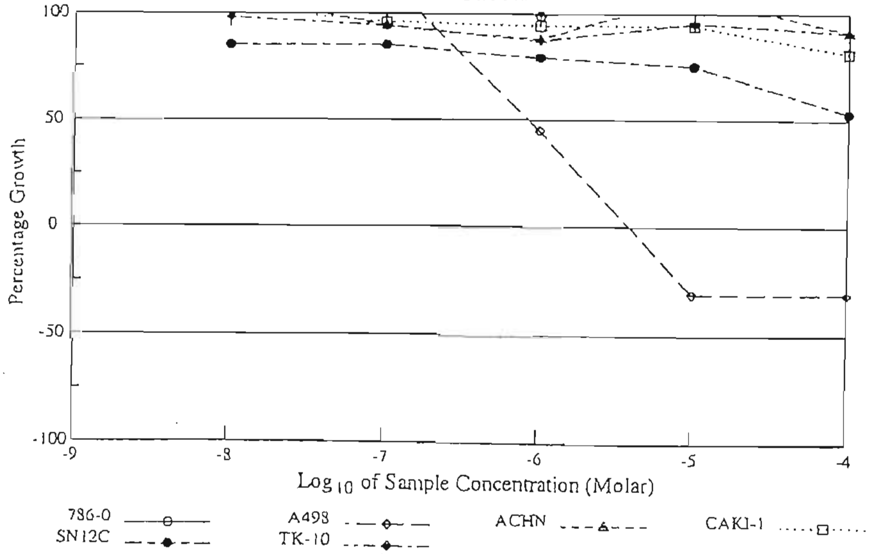
Leukemia



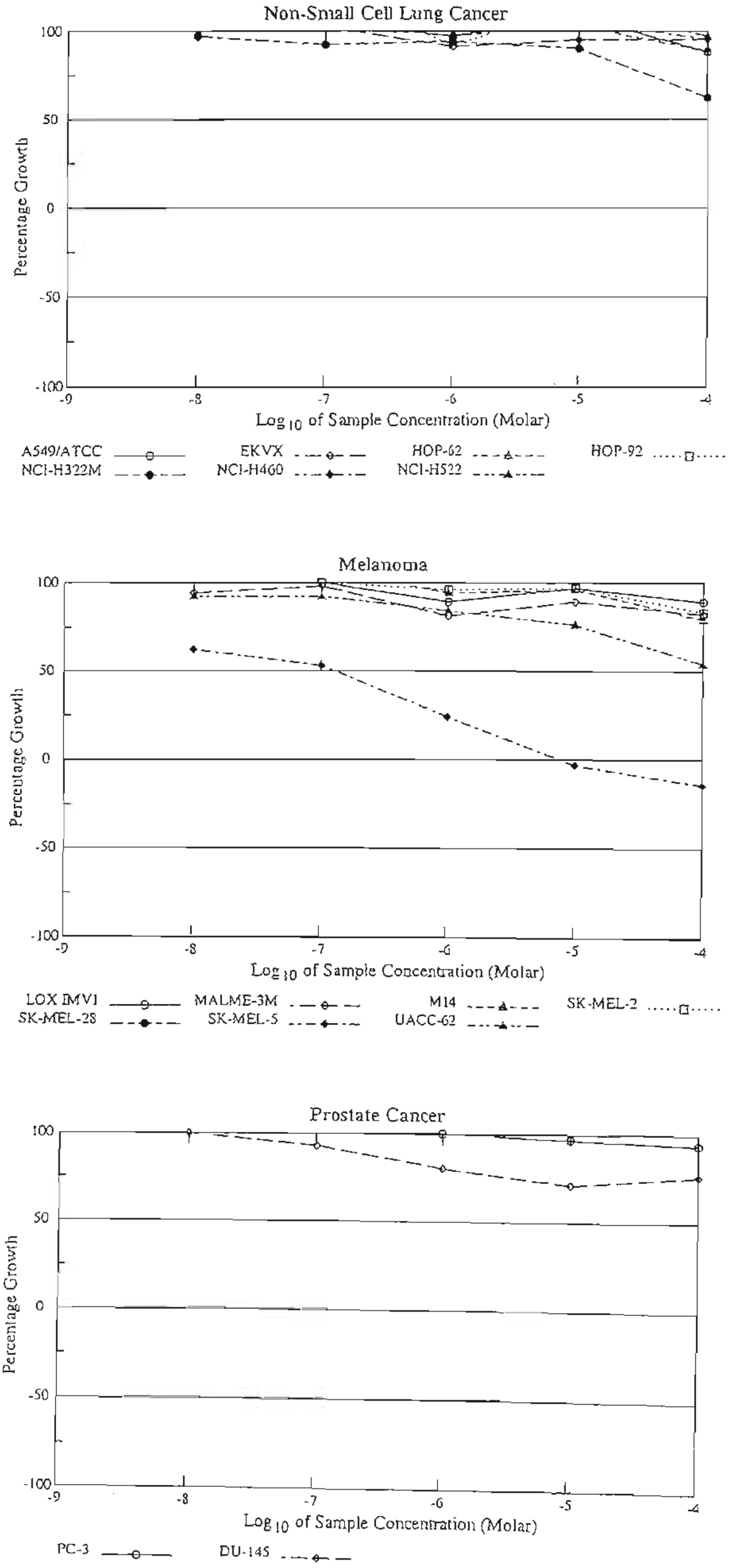
CNS Cancer



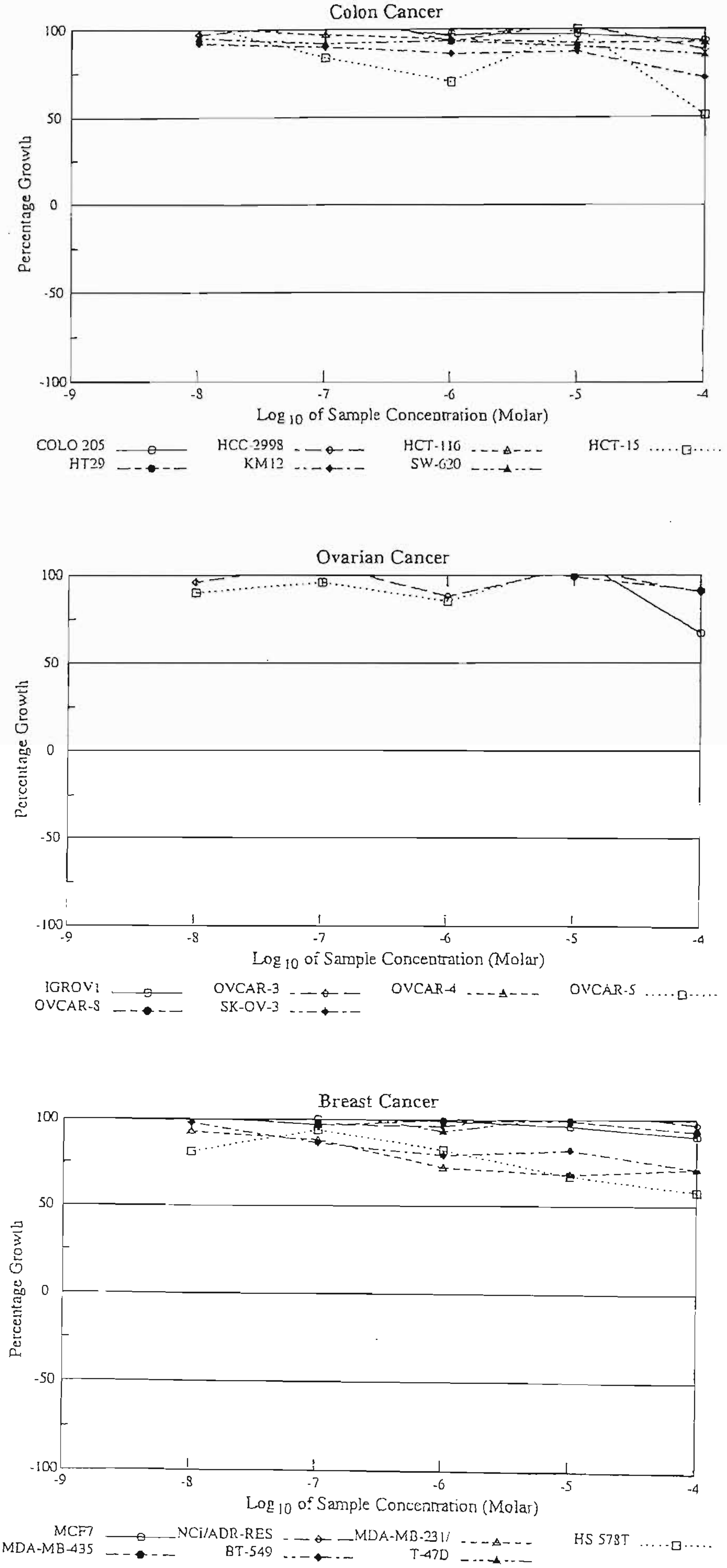
Renal Cancer



Dose Response Curves for Samaderine A (i)

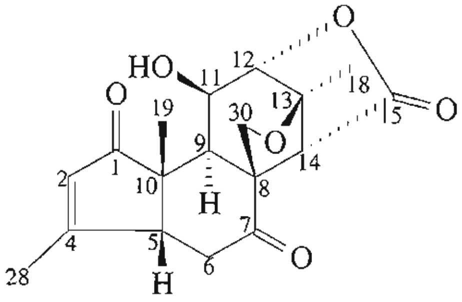


Dose Response Curves for Samaderine A (i)

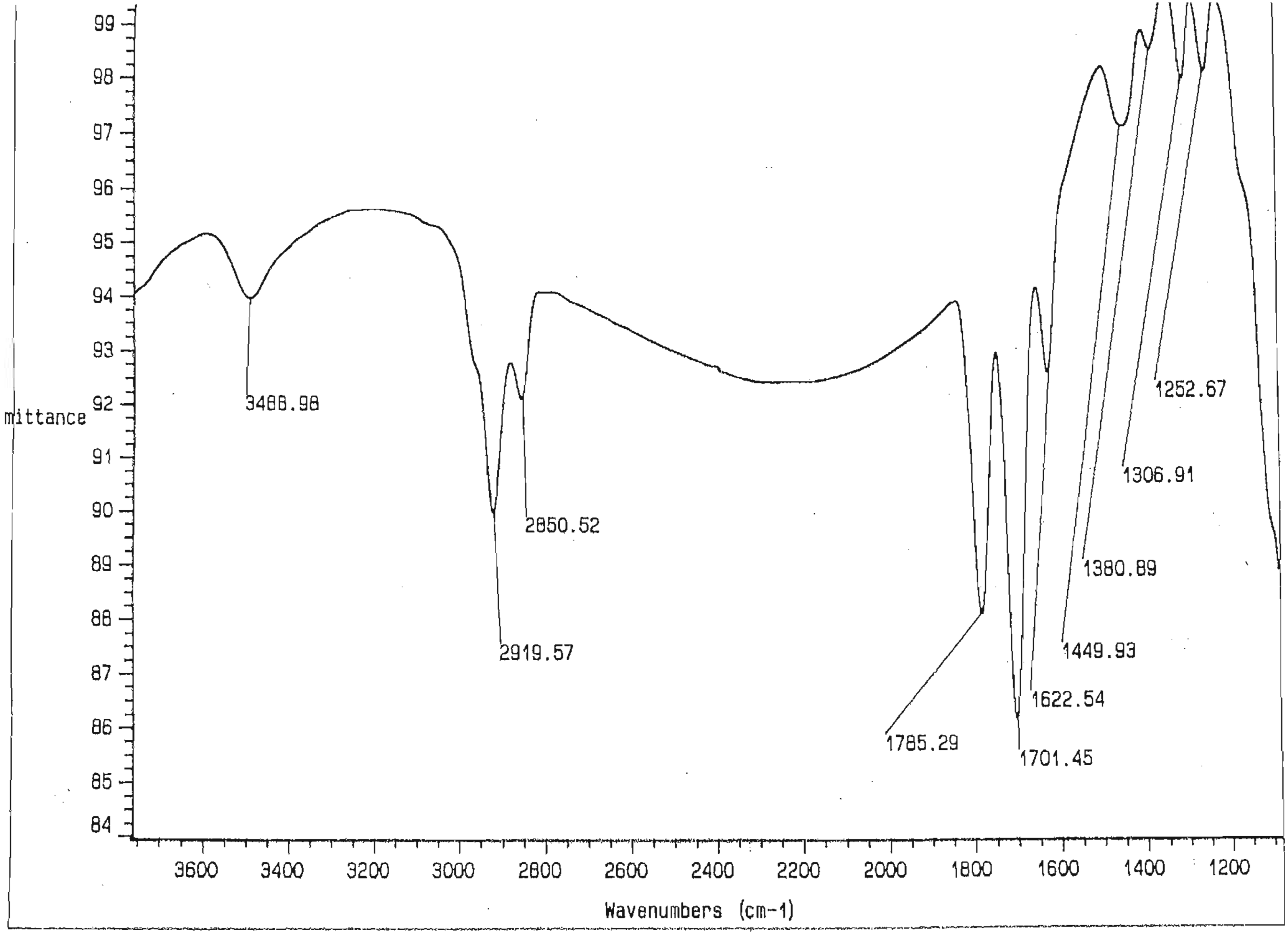


Dose Response Curves for Samaderine A (i)

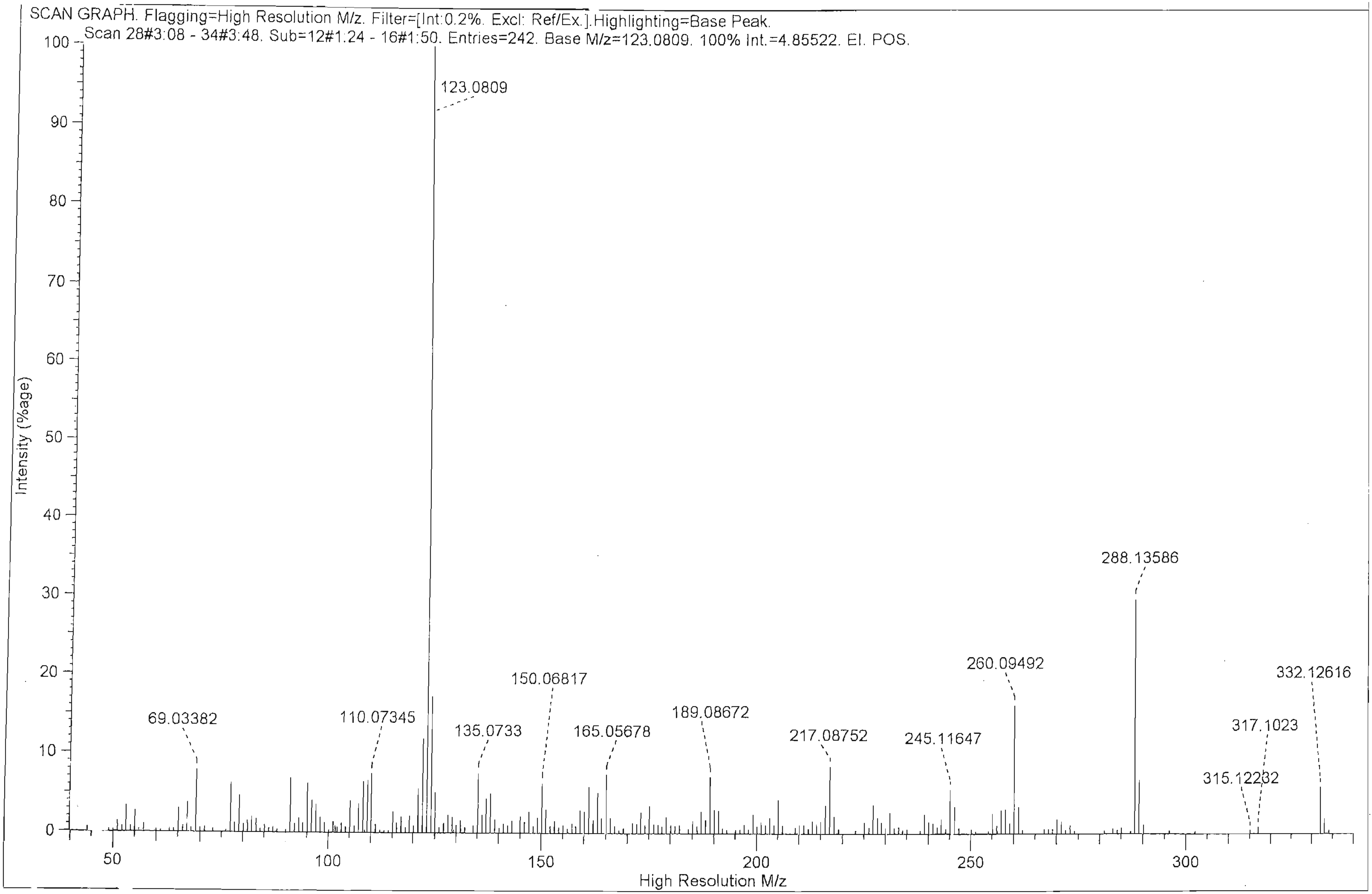
5β,6-Dihydrosamaderine A (ii)



(ii)

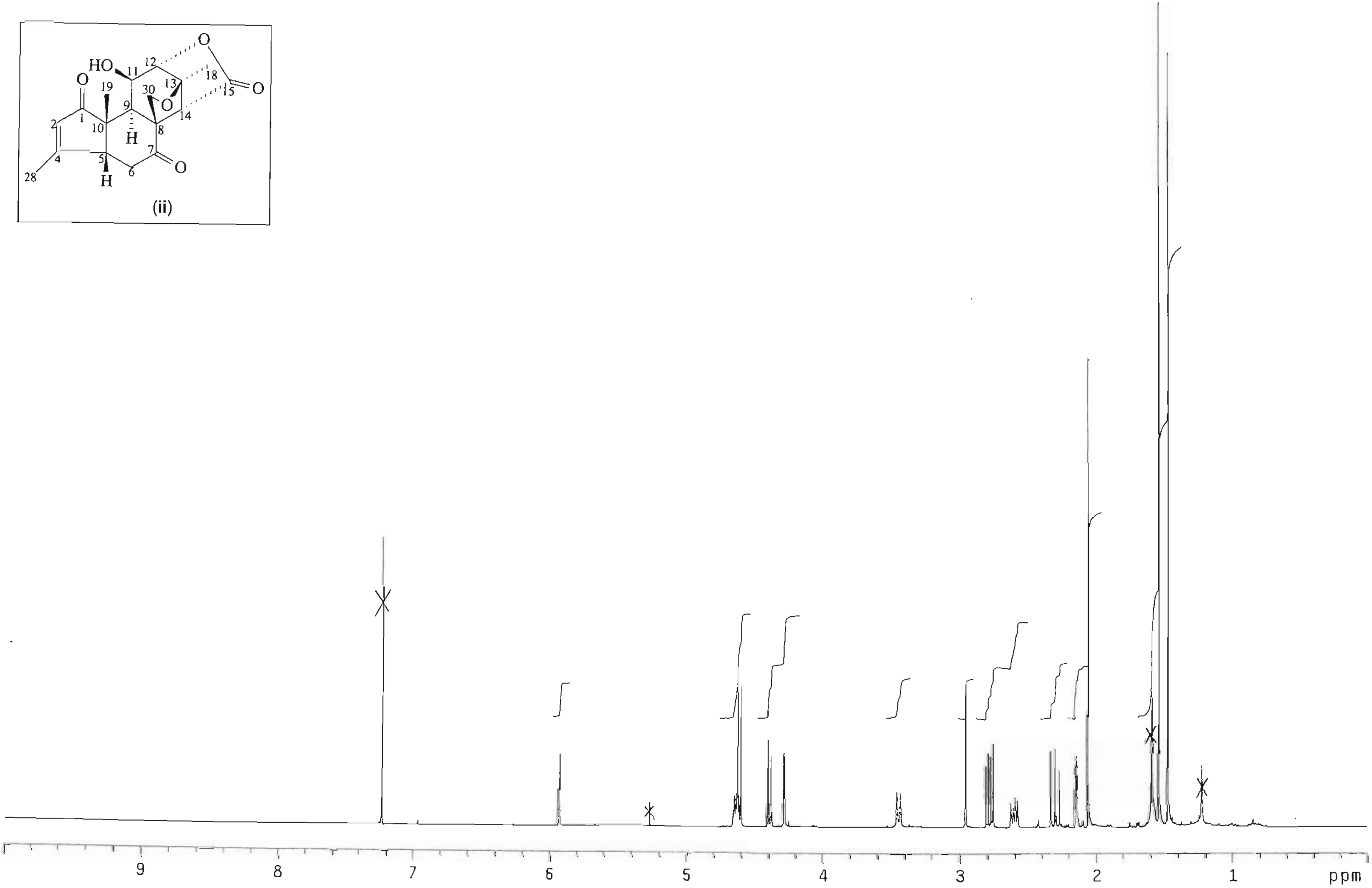


IR Spectrum of 5β,6-Dihydrosamaderine A (ii)



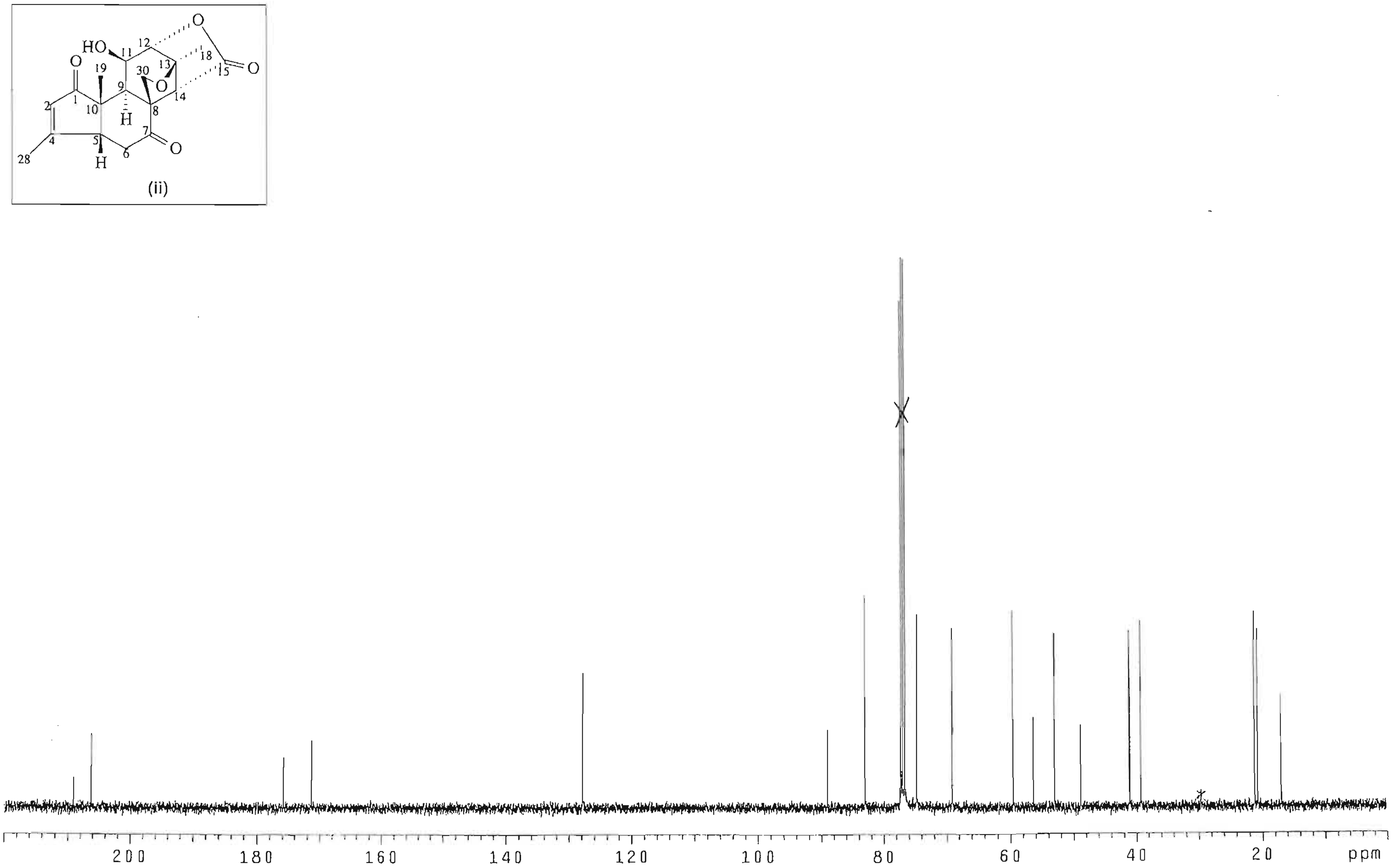
Mass Spectrum of 5β,6-Dihydrosamaderine A (ii)

Pulse Sequence: s2pul

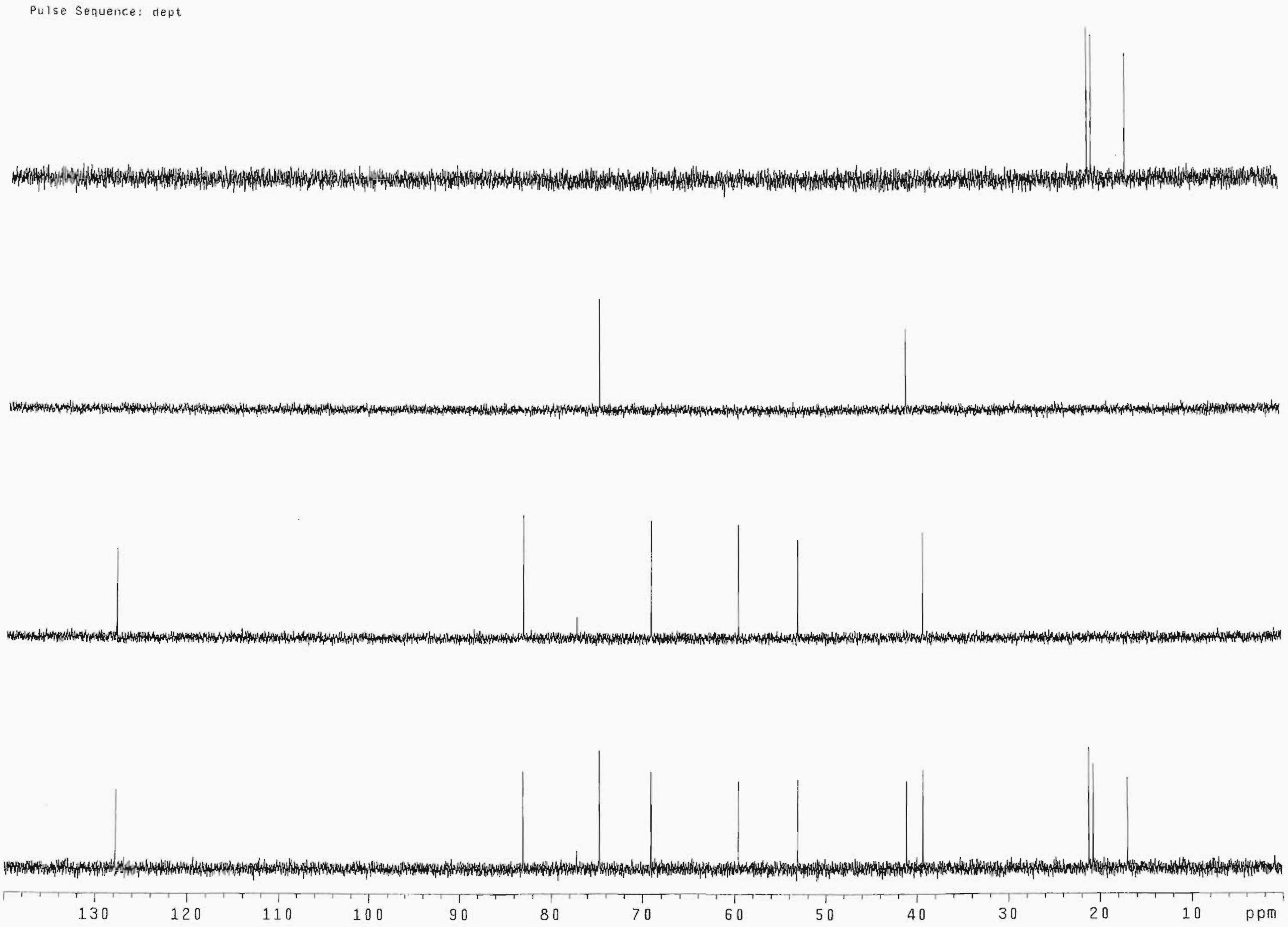


¹H NMR Spectrum of 5β,6-Dihydrosamaderine A (ii)

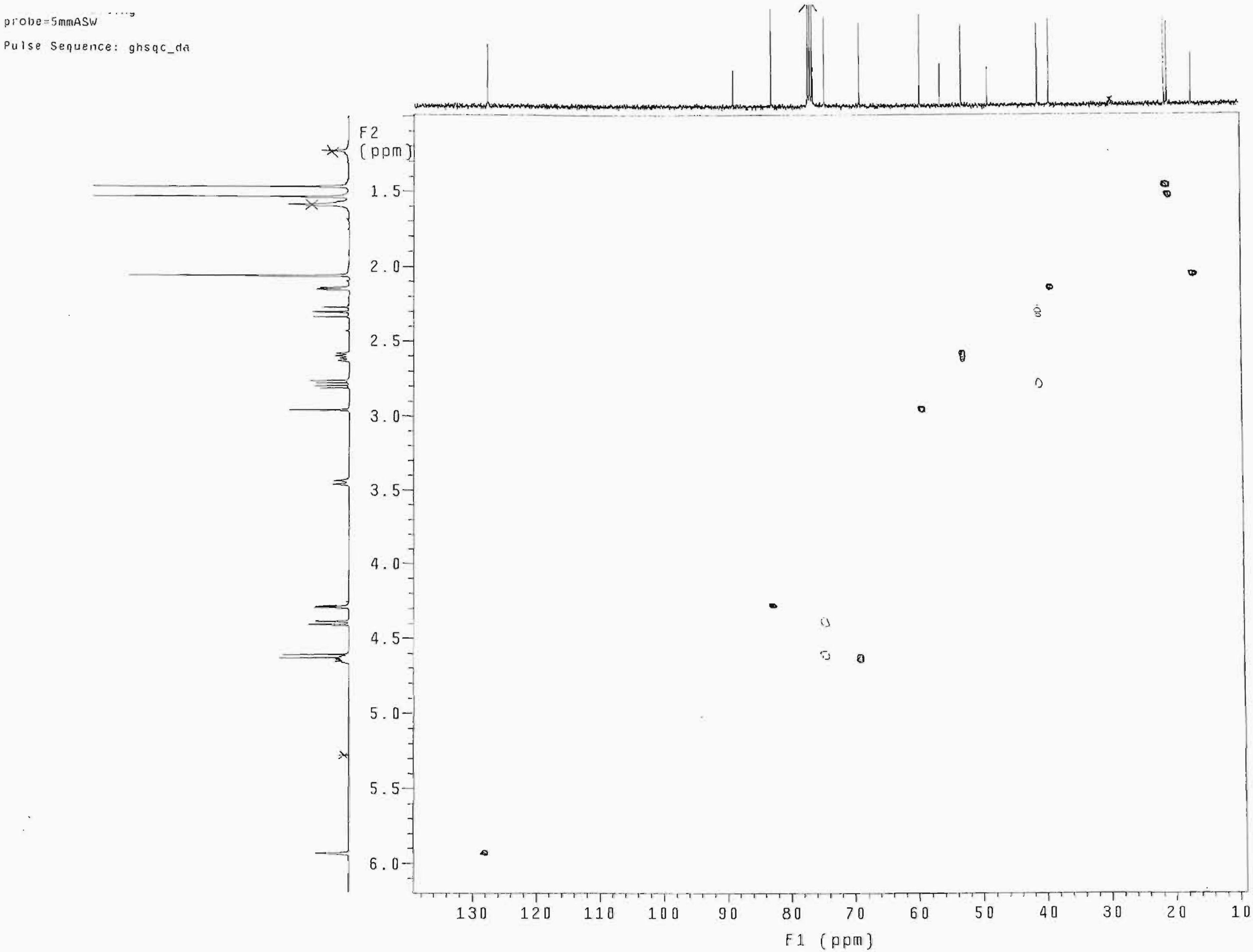
Pulse Sequence: s2pul



^{13}C NMR Spectrum of 5β,6-Dihydrosamaderine A (ii)



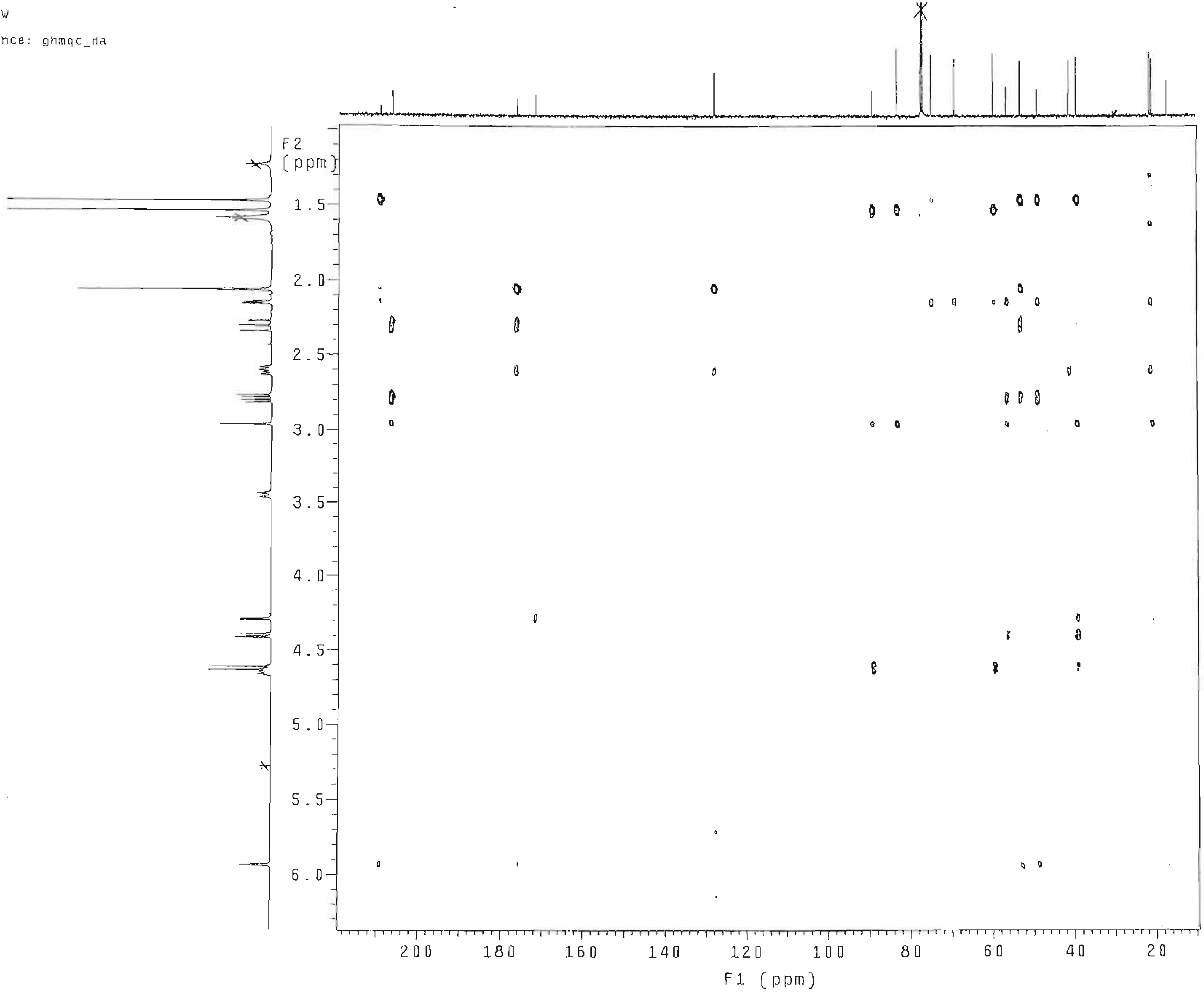
ADEPT NMR Spectrum of 5β,6-Dihydrosamaderine A (ii)



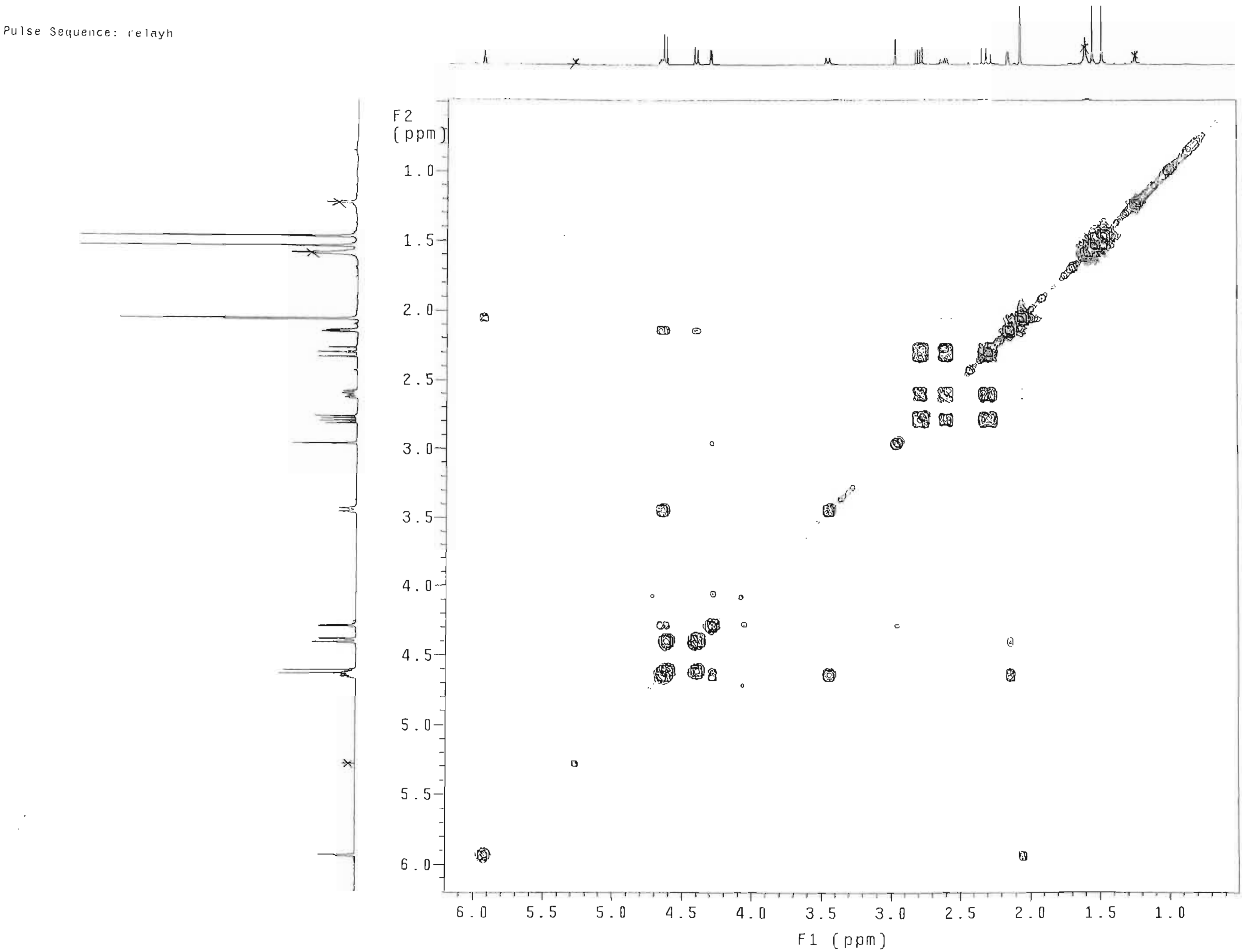
HSQC NMR Spectrum of 5β,6-Dihydrosamaderine A (ii)

probe=5mmASW

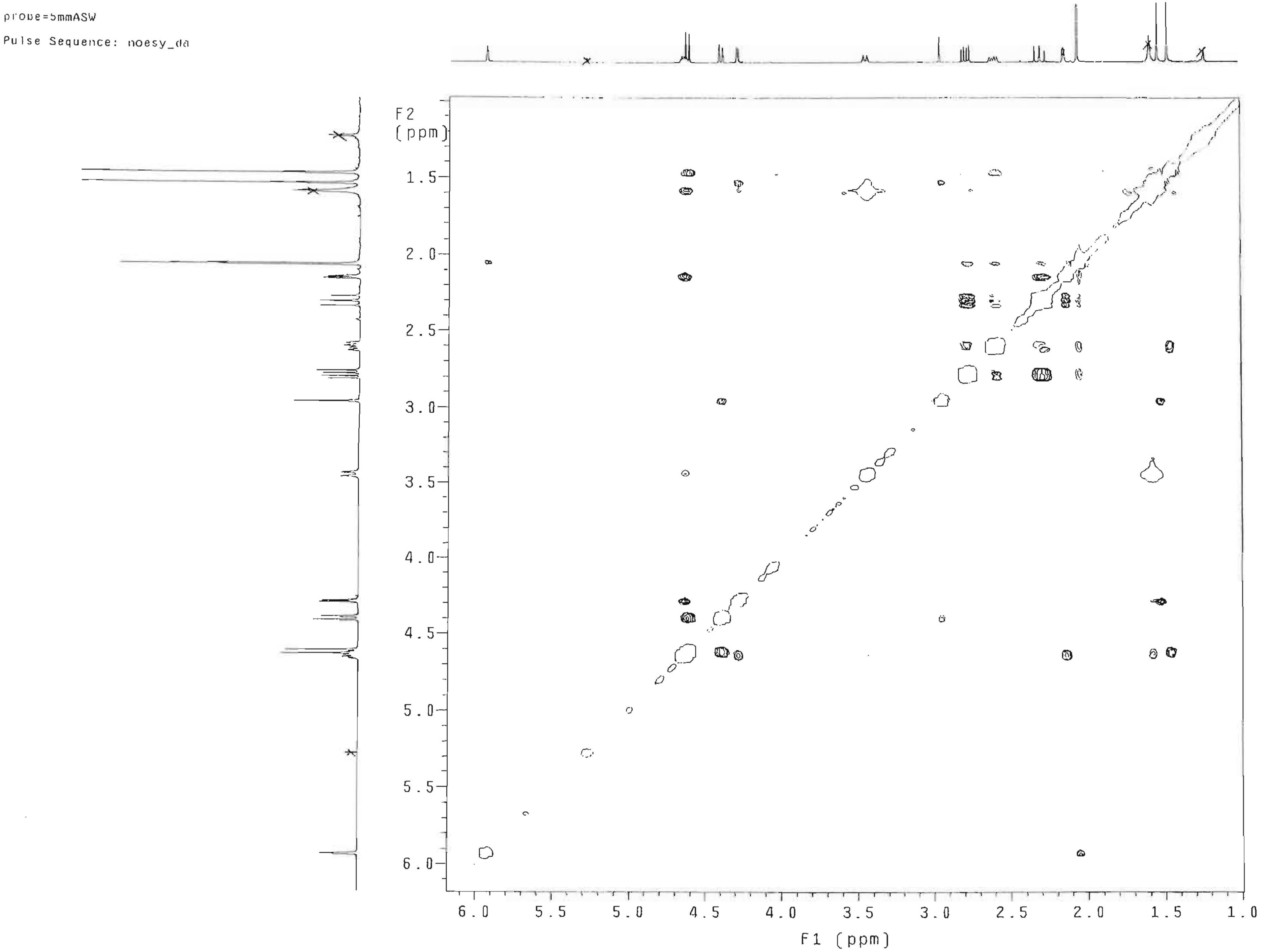
Pulse Sequence: ghmqc_da



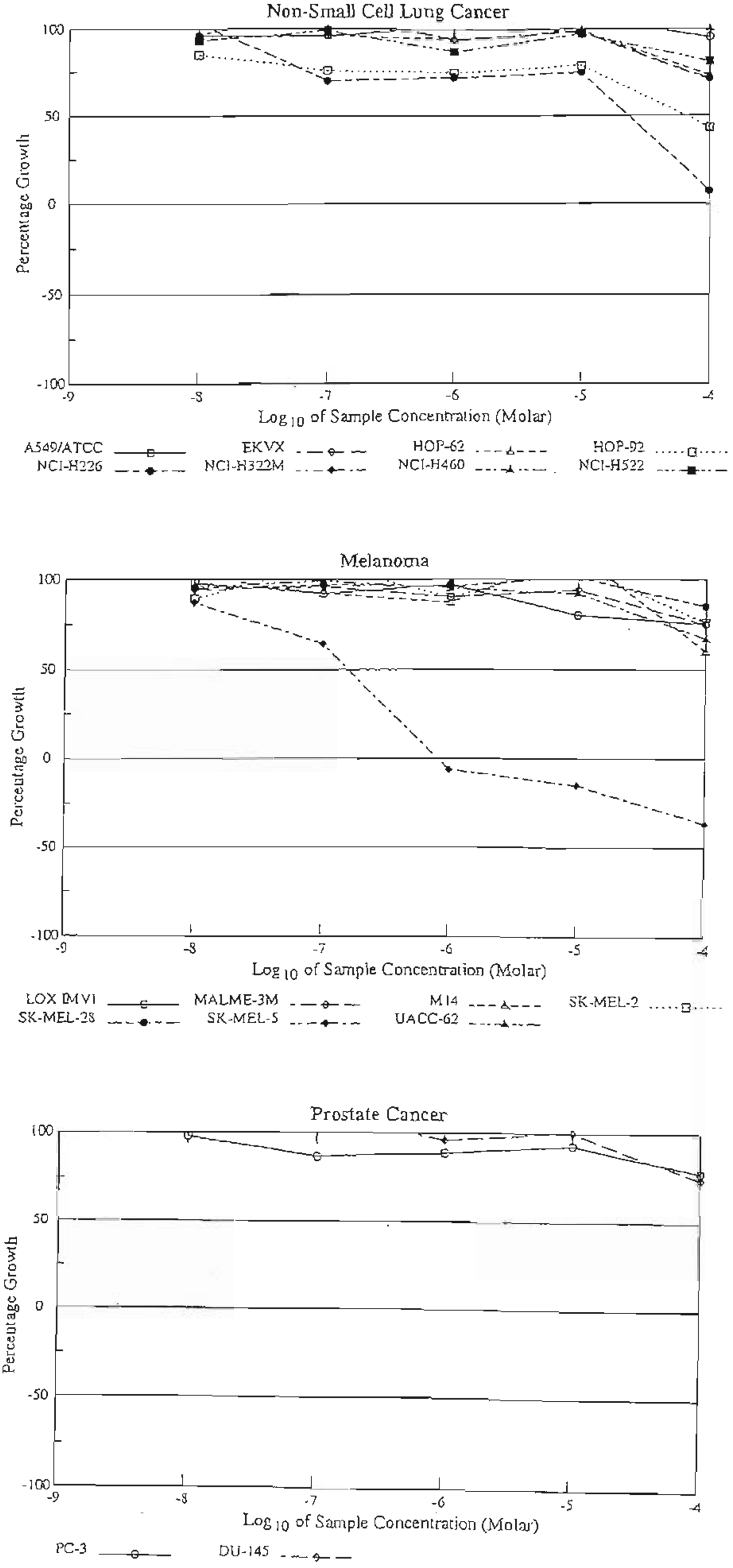
HMBC NMR Spectrum of 5β,6-Dihydrosamaderine A (ii)



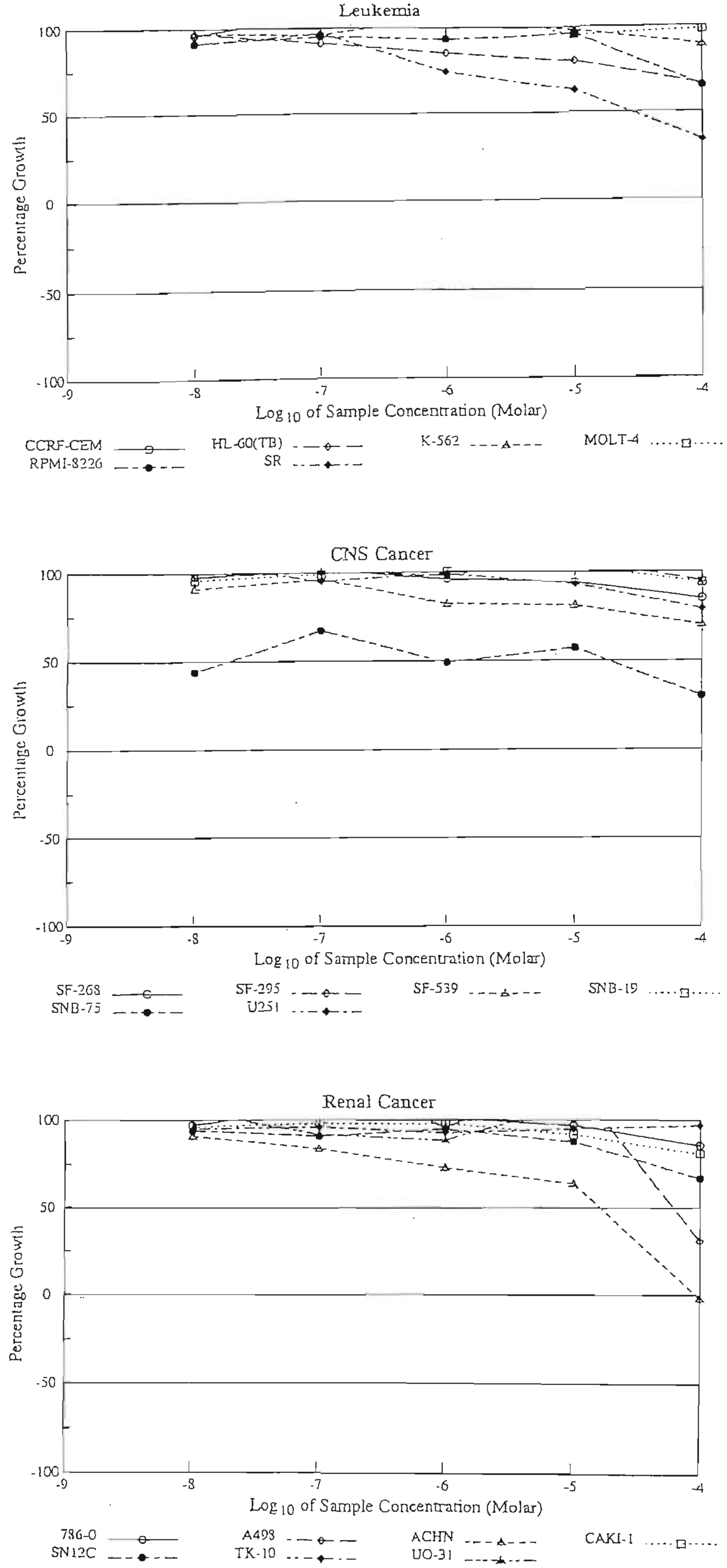
COSY NMR Spectrum of 5β,6-Dihydrosamaderine A (ii)



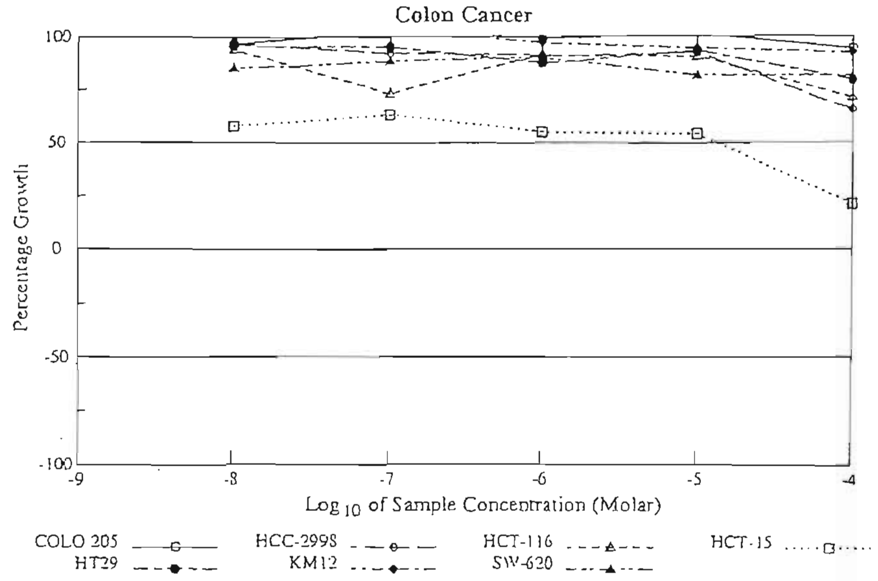
NOESY NMR Spectrum of 5β,6-Dihydrosamaderine A (ii)

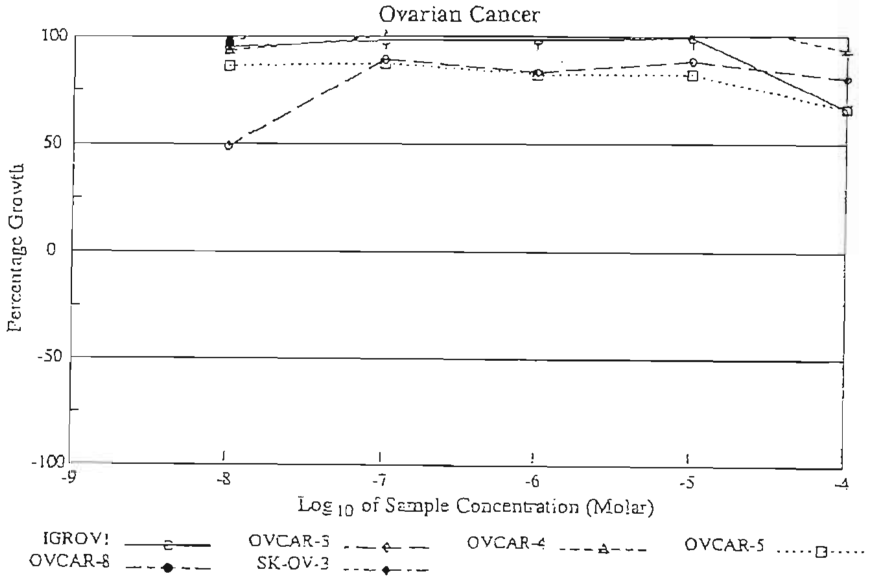


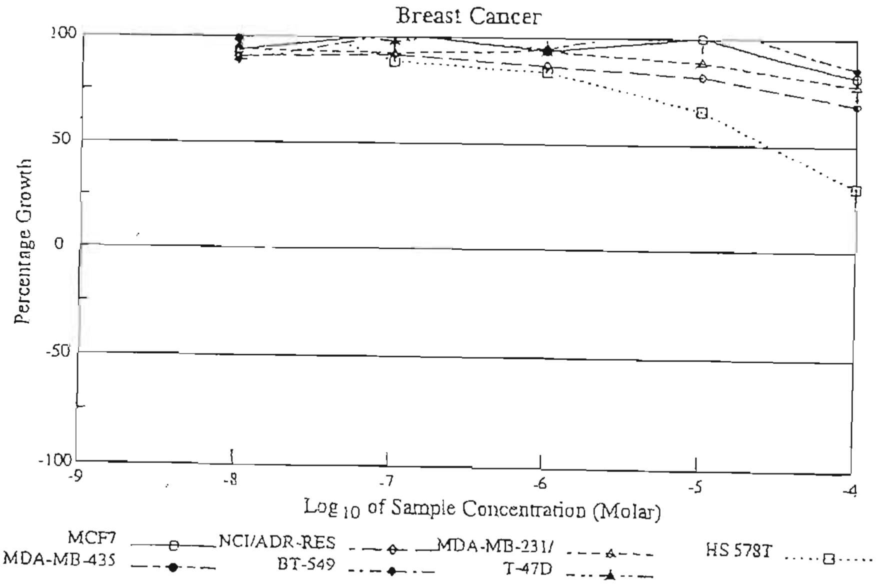
Dose Response Curves for 5β,6-Dihydrosamaderine A (ii)



Dose Response Curves for 5β,6-Dihydrosamaderine A (ii)

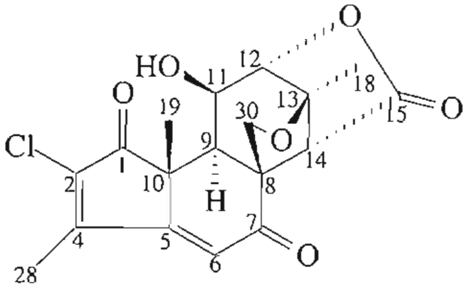




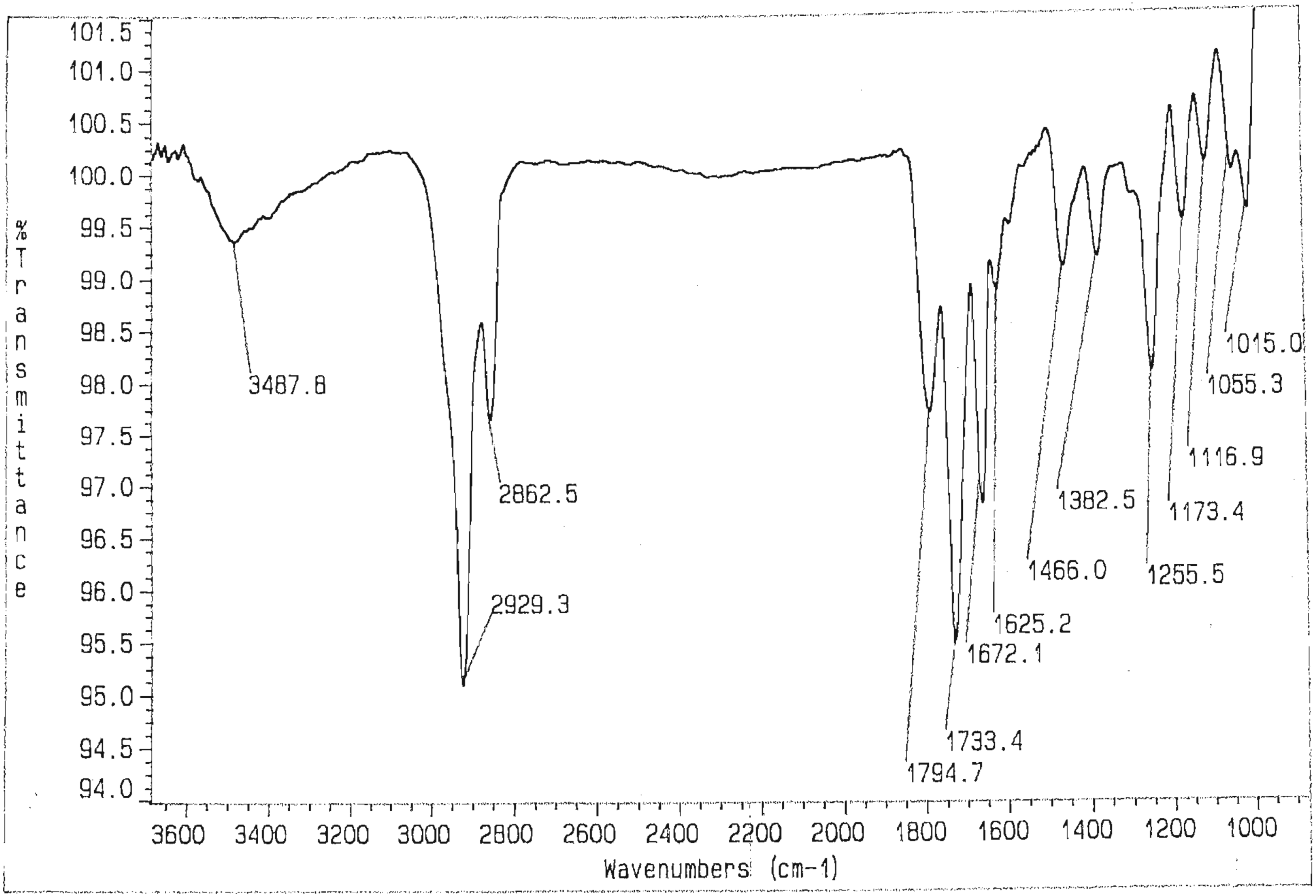


Dose Response Curves for 5β,6-Dihydrosamaderine A (ii)

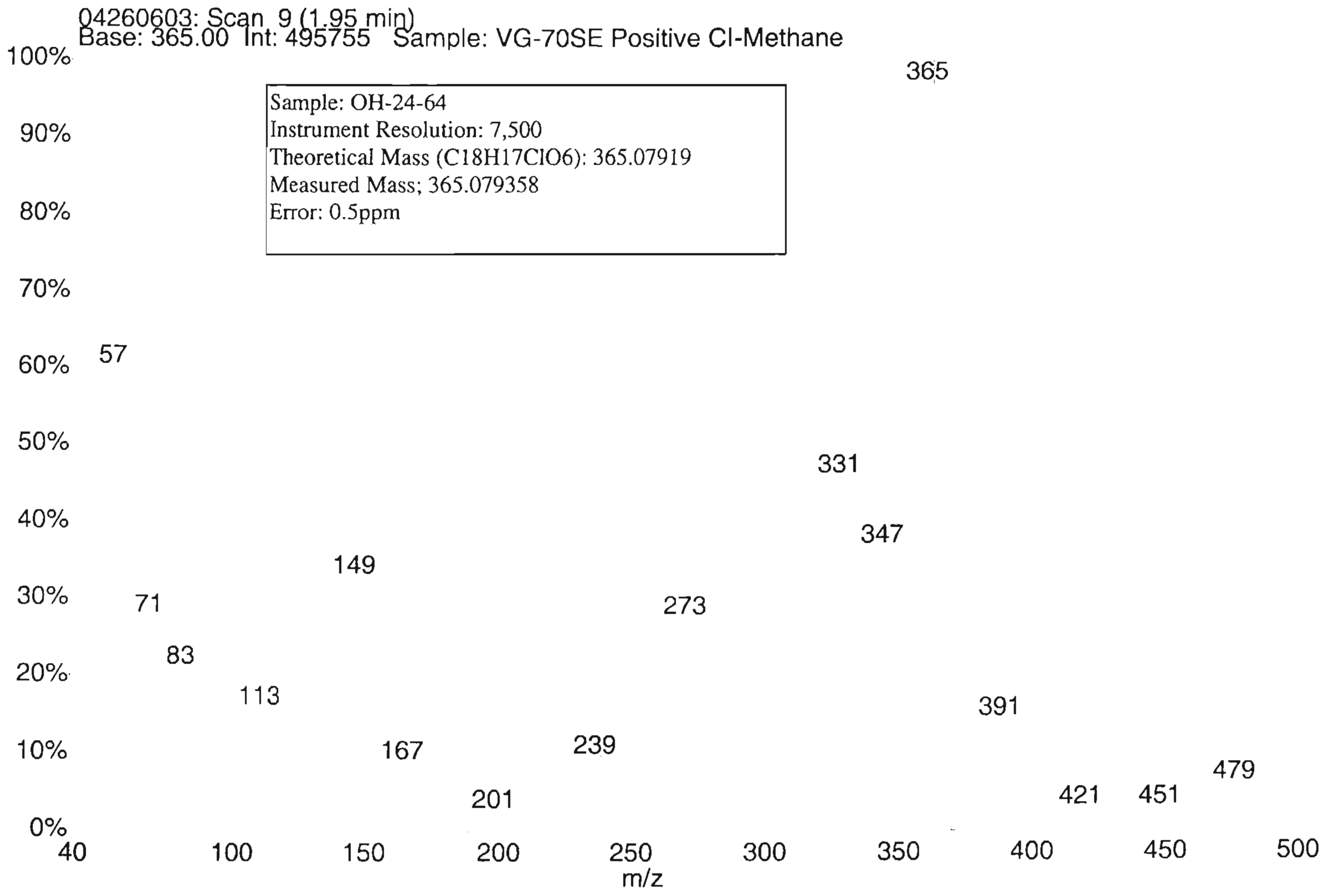
2-Chlorosamaderine A (iii)



(iii)

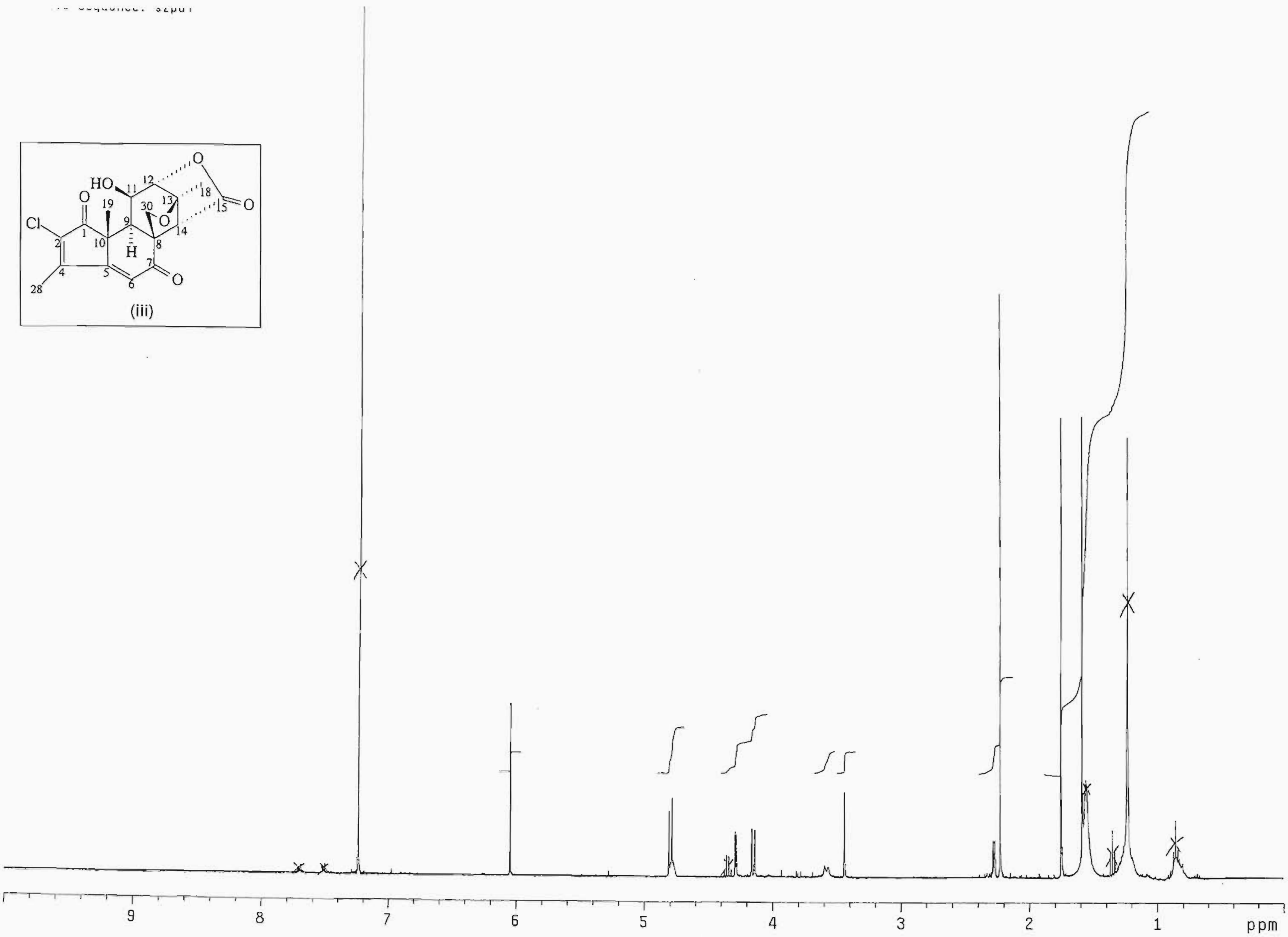


IR Spectrum of 2-Chlorosamaderine A (iii)

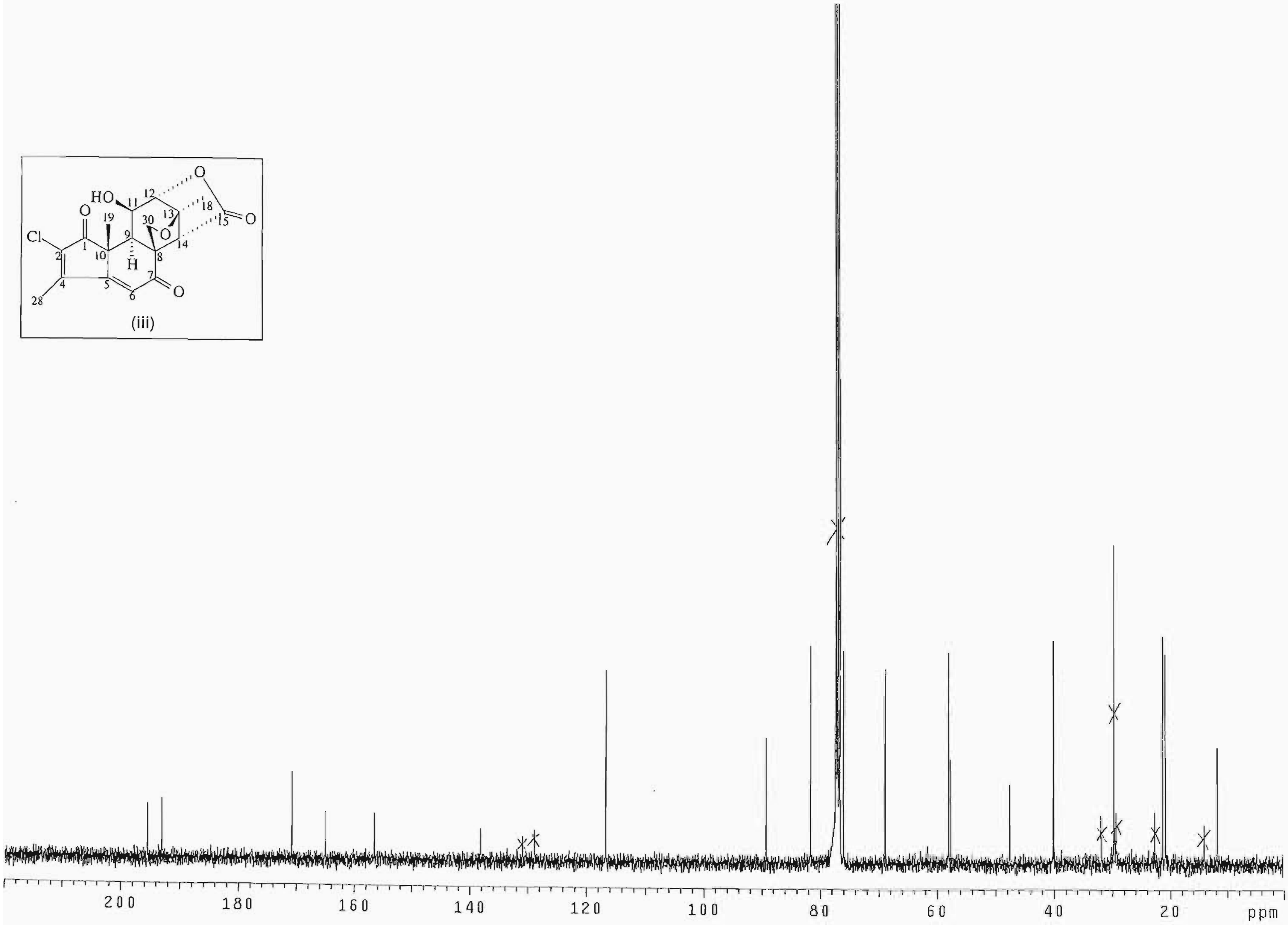


Sample: OH-24-64
Instrument Resolution: 7,500
Theoretical Mass ($C_{18}H_{17}ClO_6$): 365.07919
Measured Mass; 365.079358
Error: 0.5ppm

Mass Spectrum of 2-Chlorosamaderine A (iii)



^{1}H NMR Spectrum of 2-Chlorosamaderine A (iii)

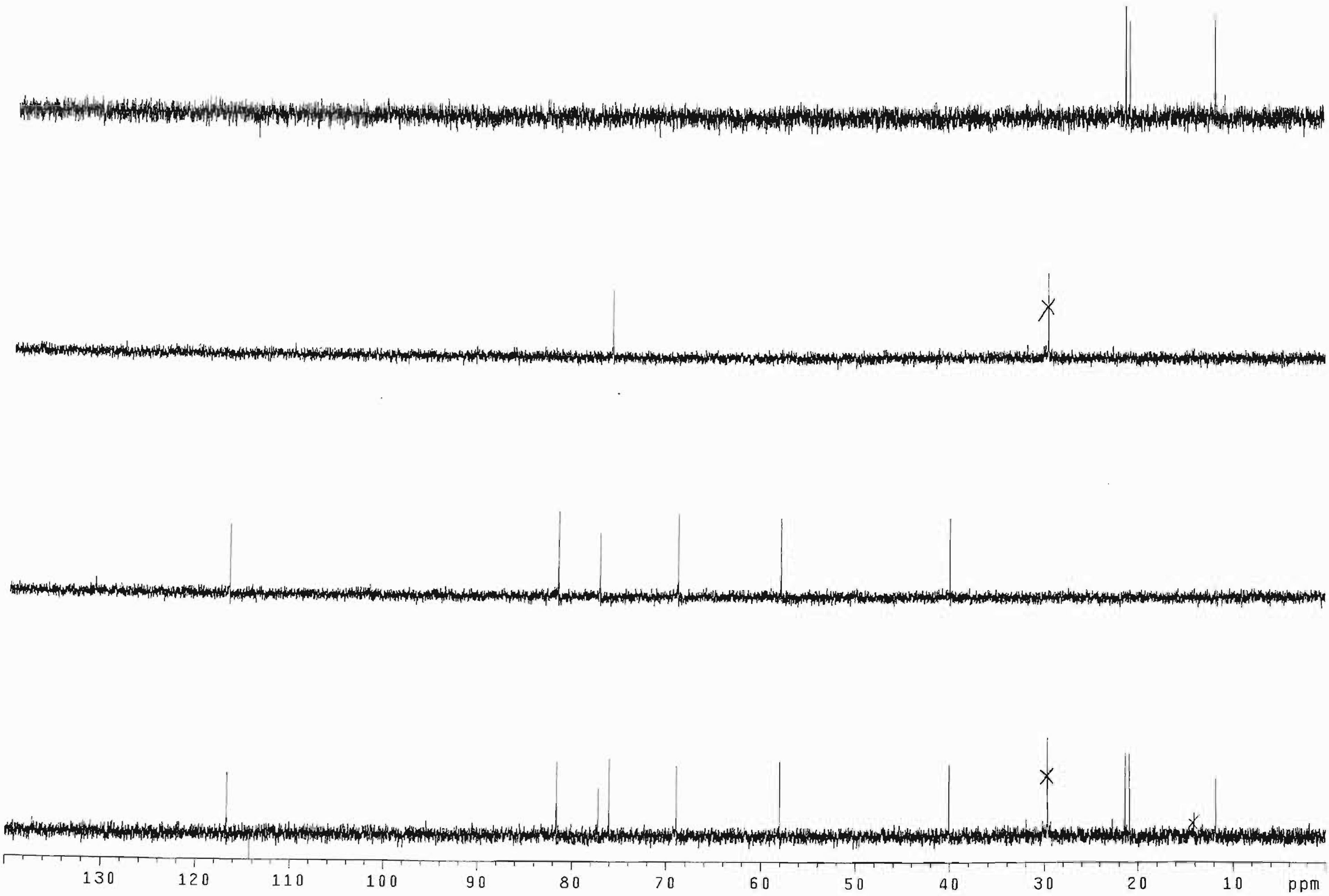


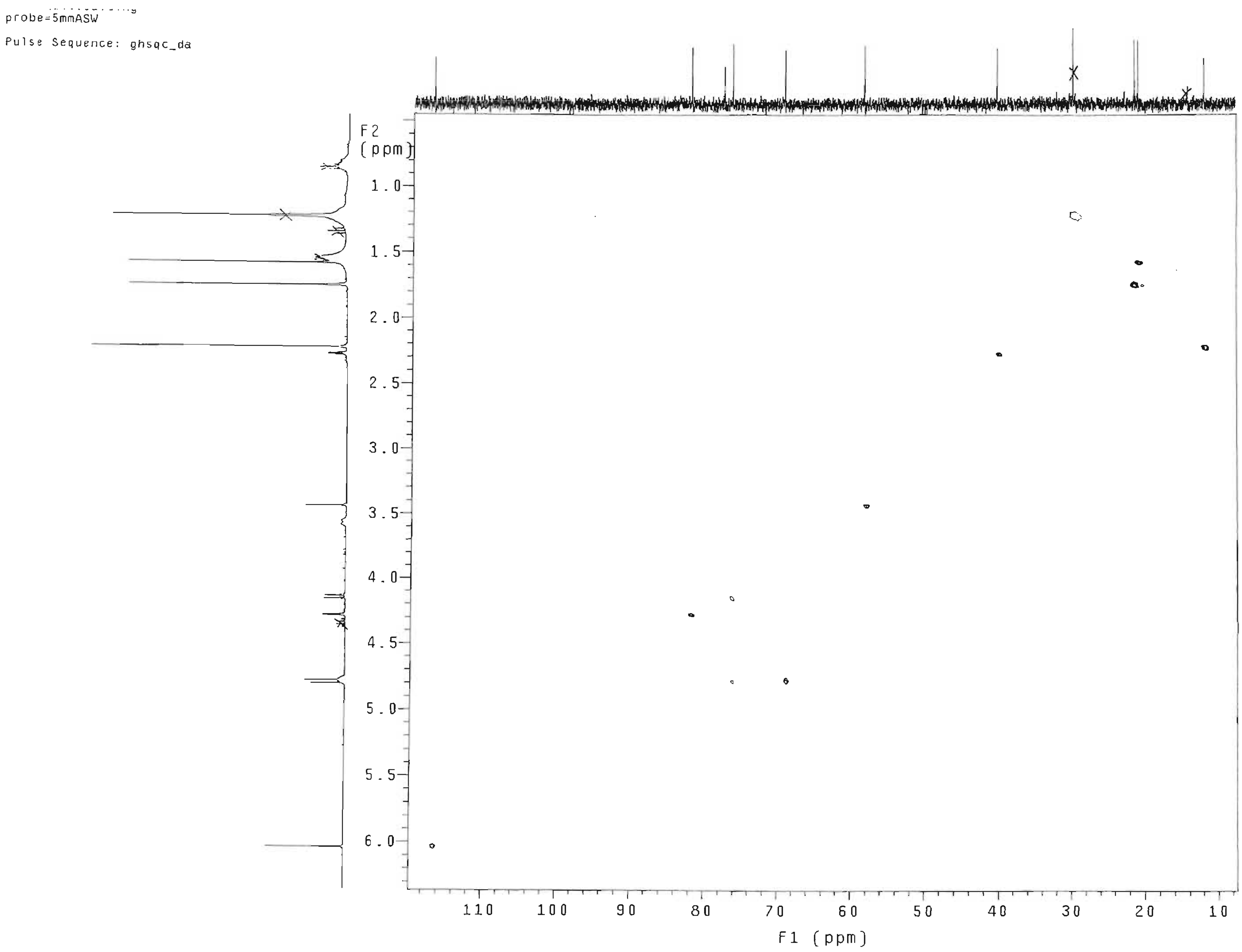
^{13}C NMR Spectrum of 2-Chlorosamaderine A (iii)

✓

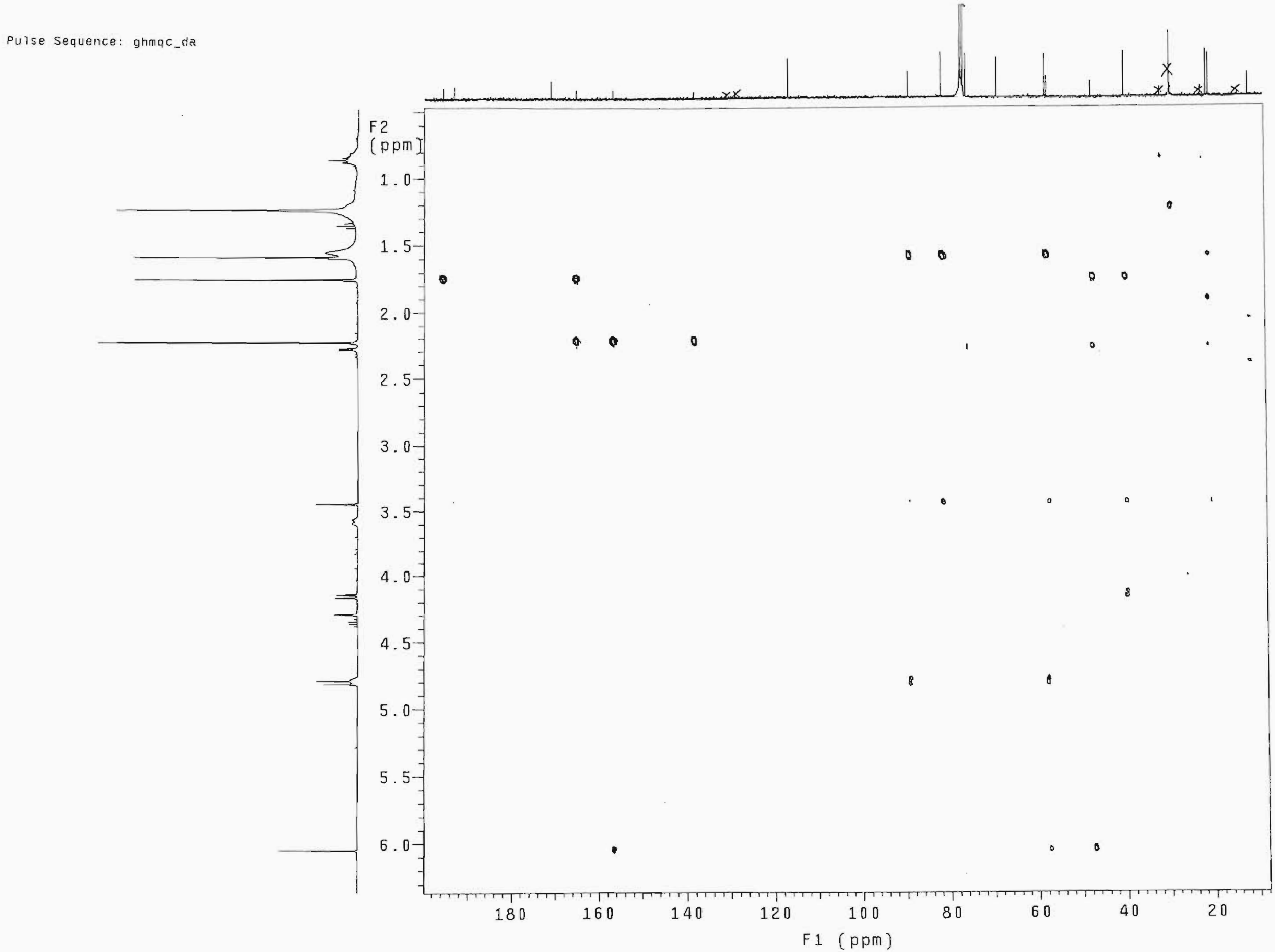
probe=5mmASW

Pulse Sequence: dept

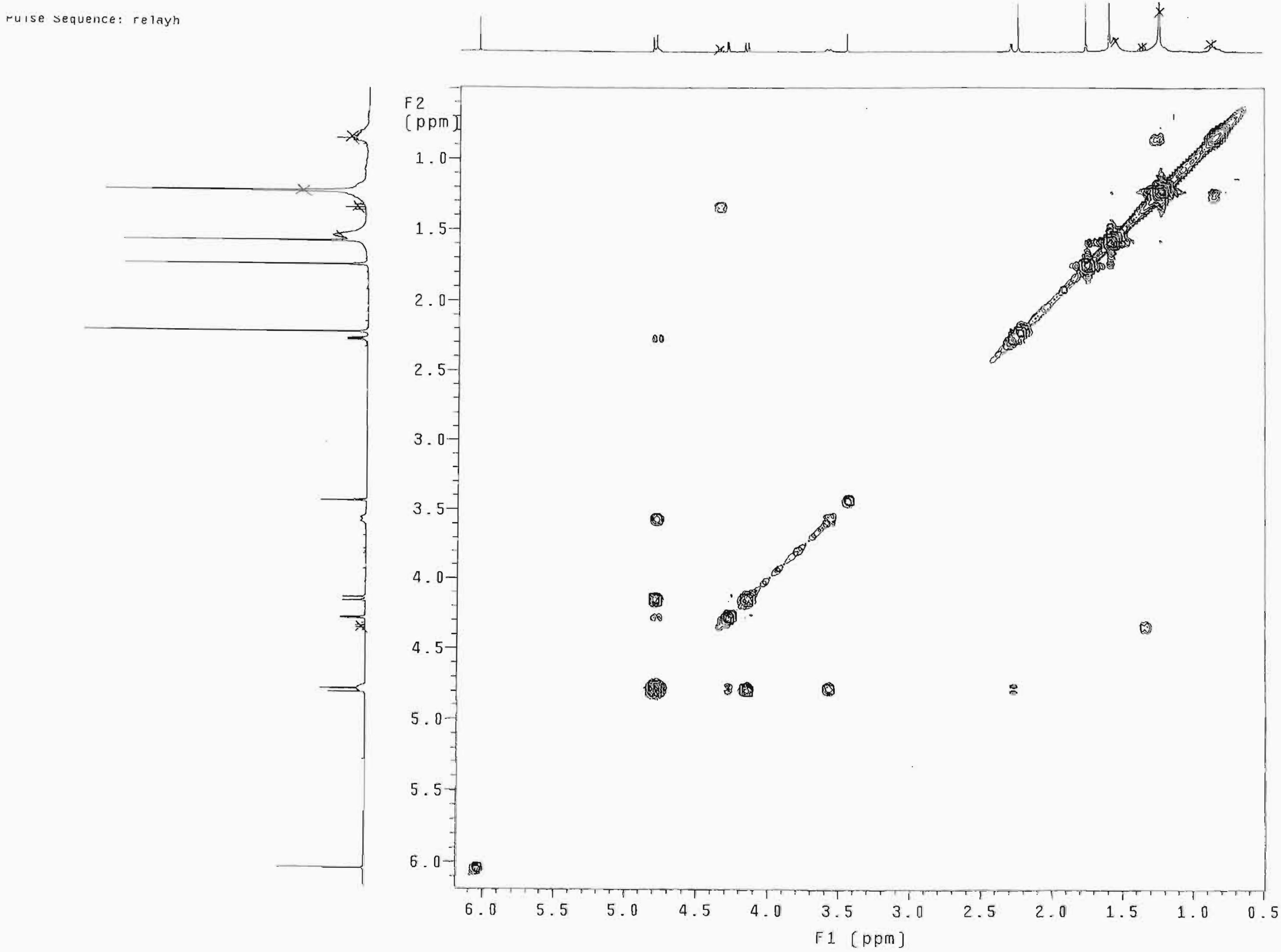




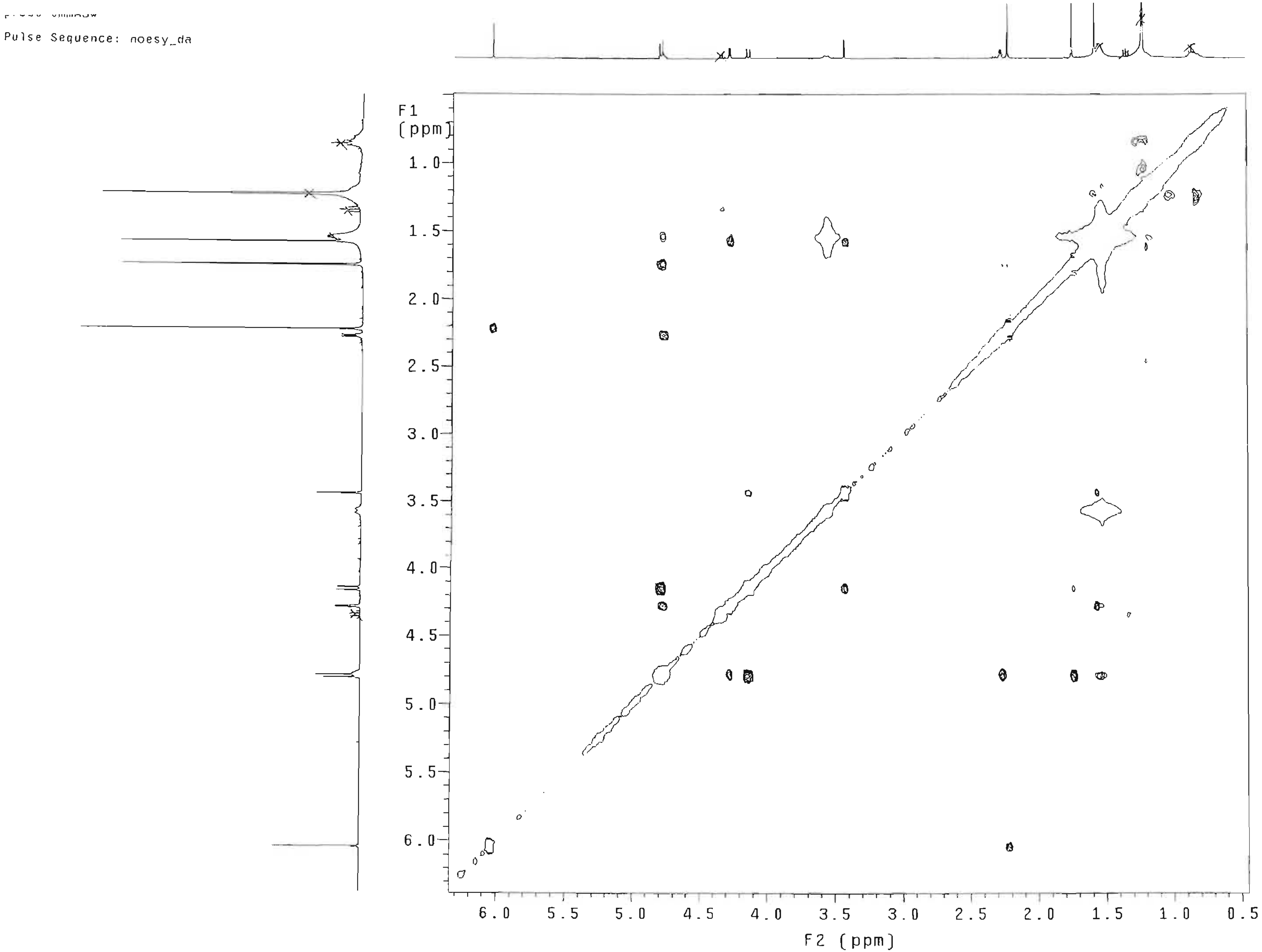
HSQC NMR Spectrum of 2-Chlorosamaderine A (iii)



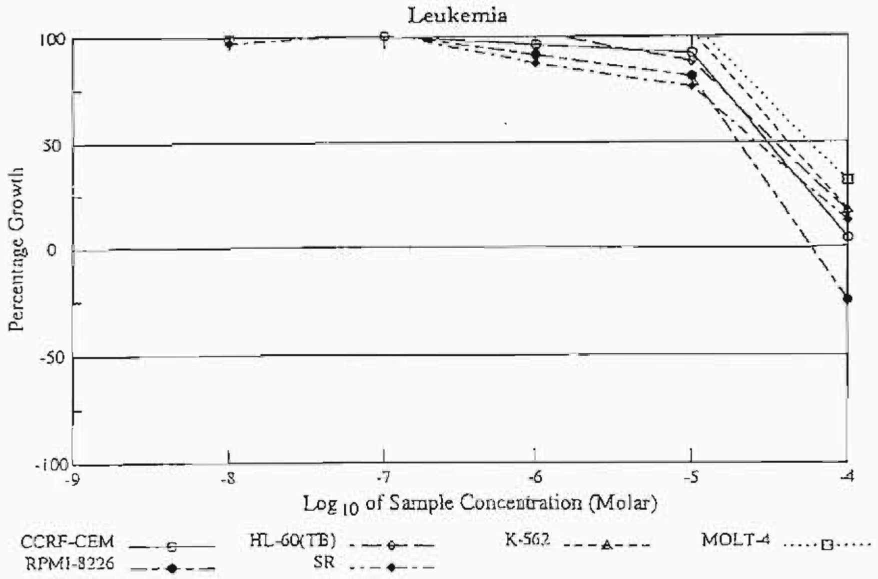
HMBC NMR Spectrum of 2-Chlorosamaderine A (iii)

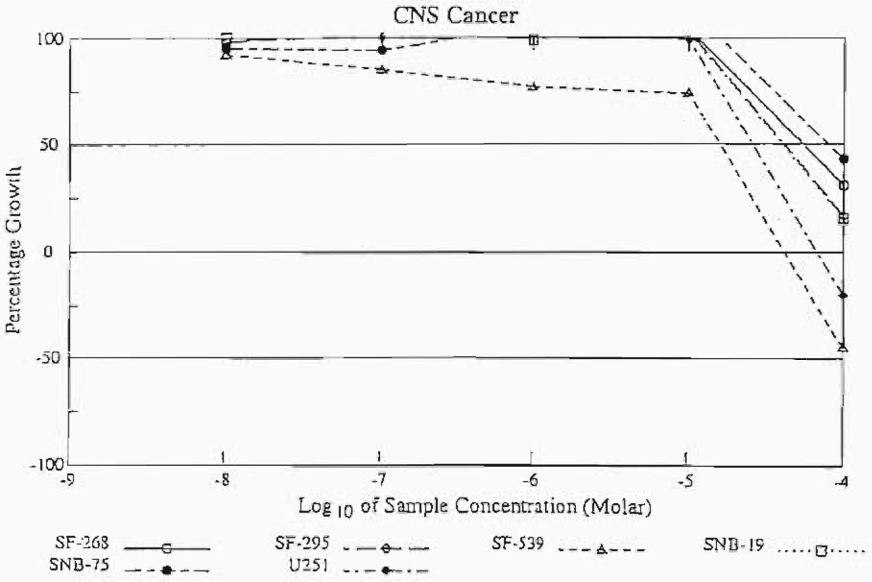


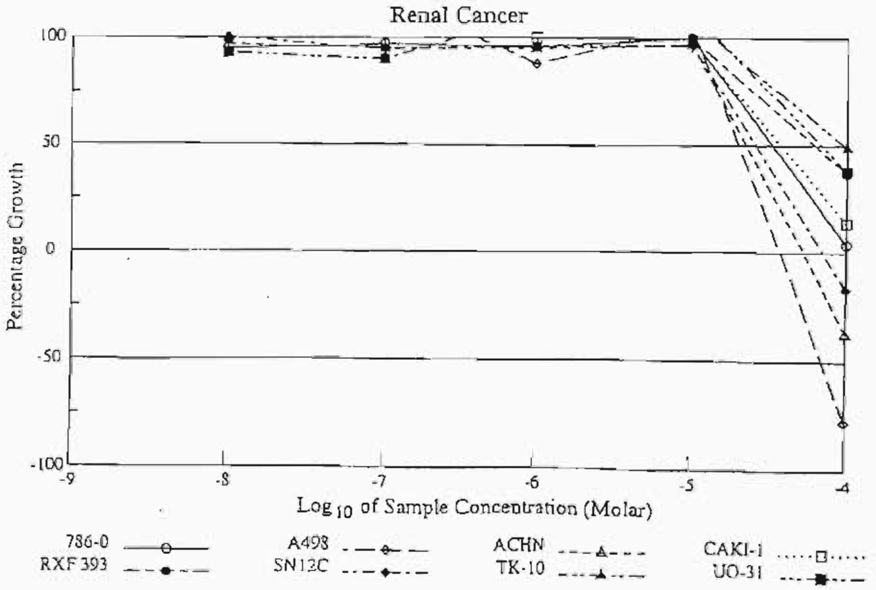
COSY NMR Spectrum of 2-Chlorosamaderine A (iii)



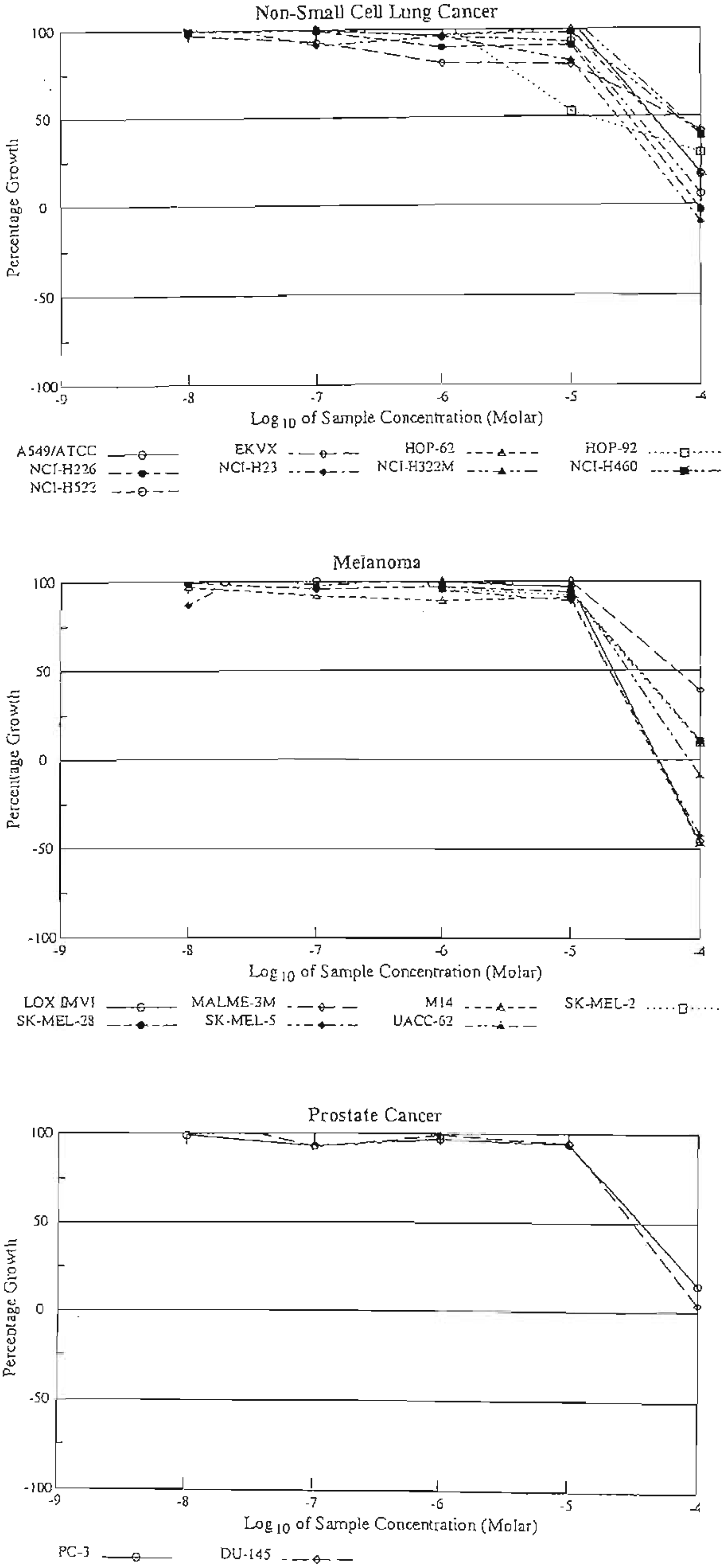
NOESY NMR Spectrum of 2-Chlorosamaderine A (iii)


Leukemia

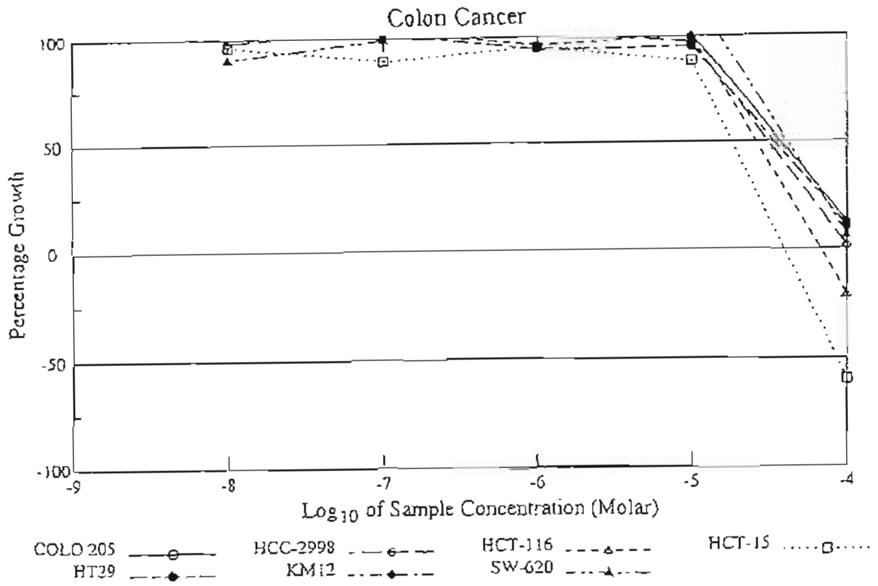

CNS Cancer

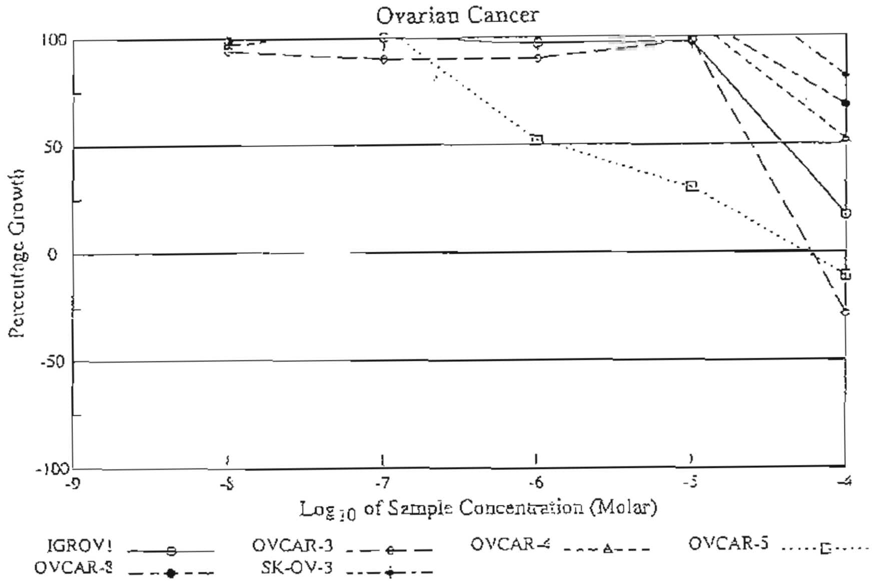

Renal Cancer

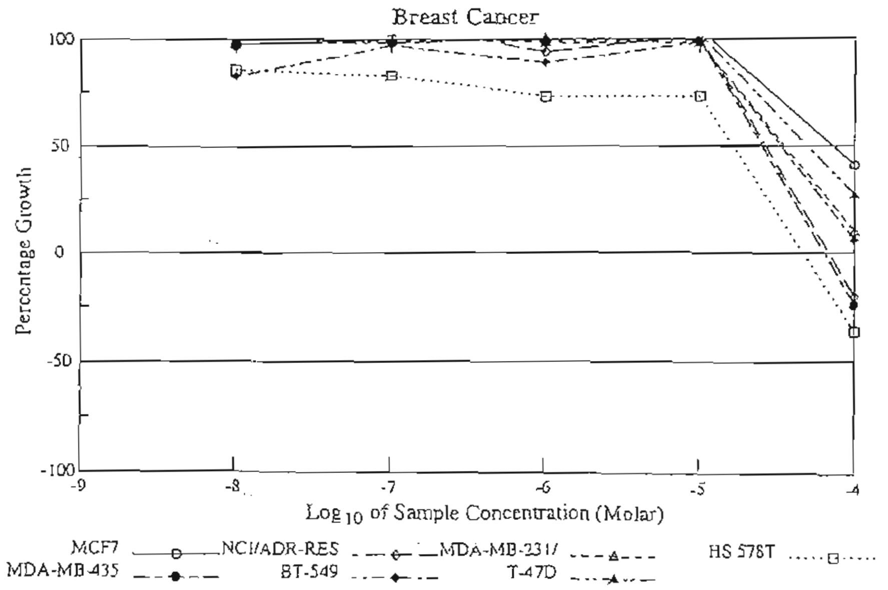
Dose Response Curves for2-Chlorosamaderine A (iii)



Dose Response Curves for2-Chlorosamaderine A (iii)

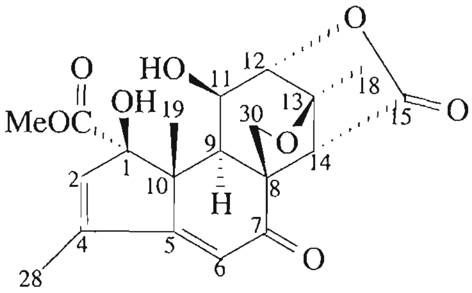




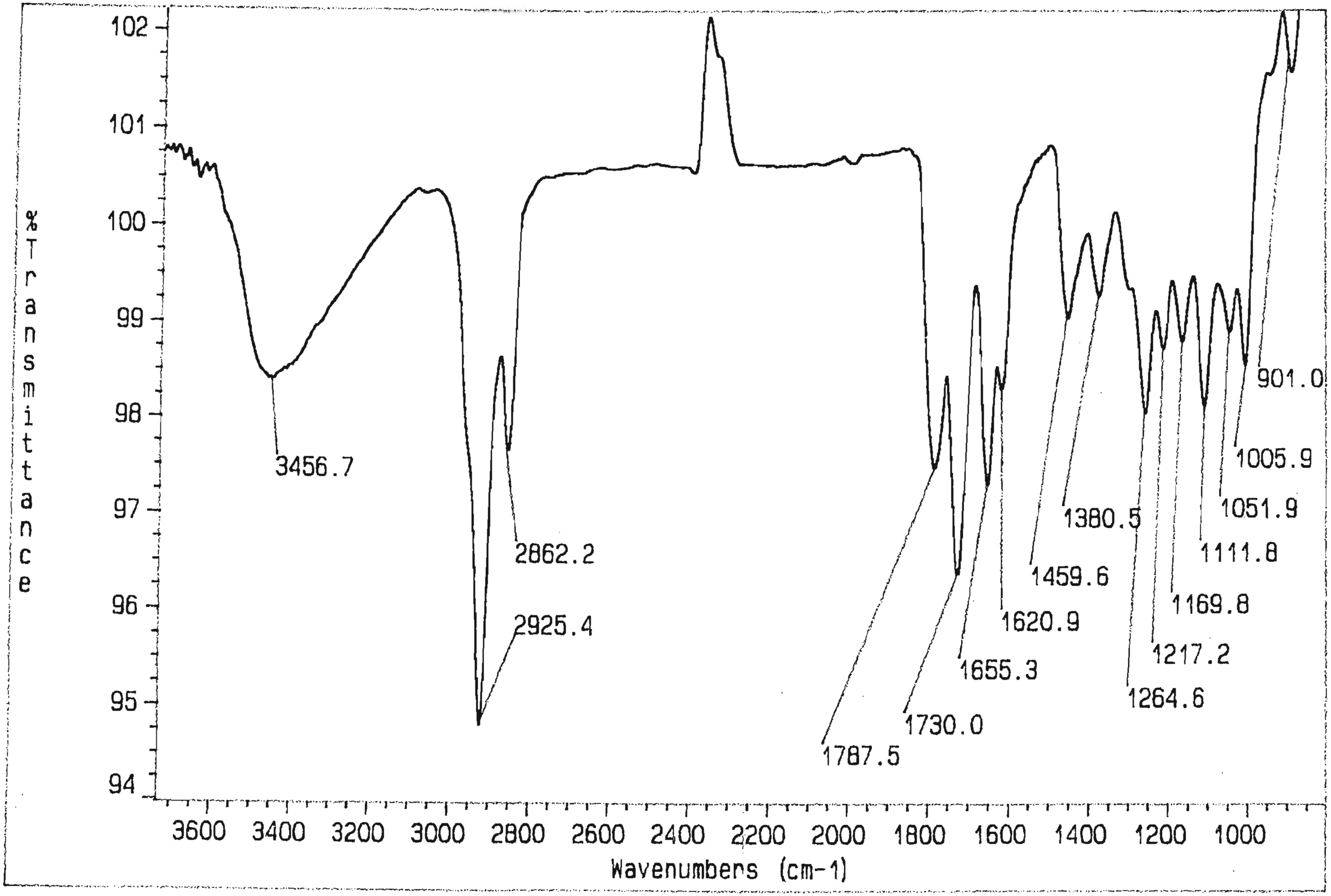


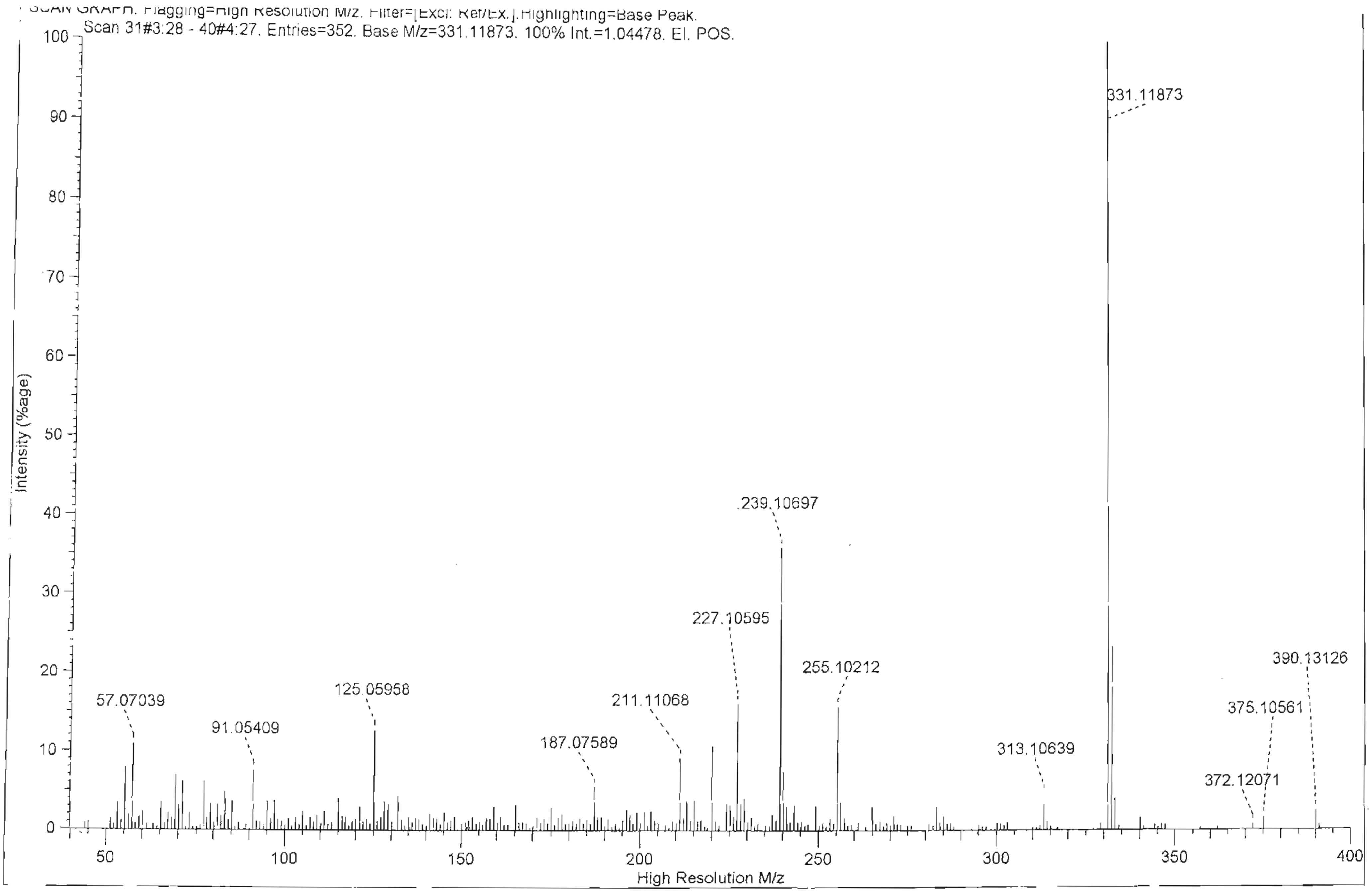
Dose Response Curves for2-Chlorosamaderine A (iii)

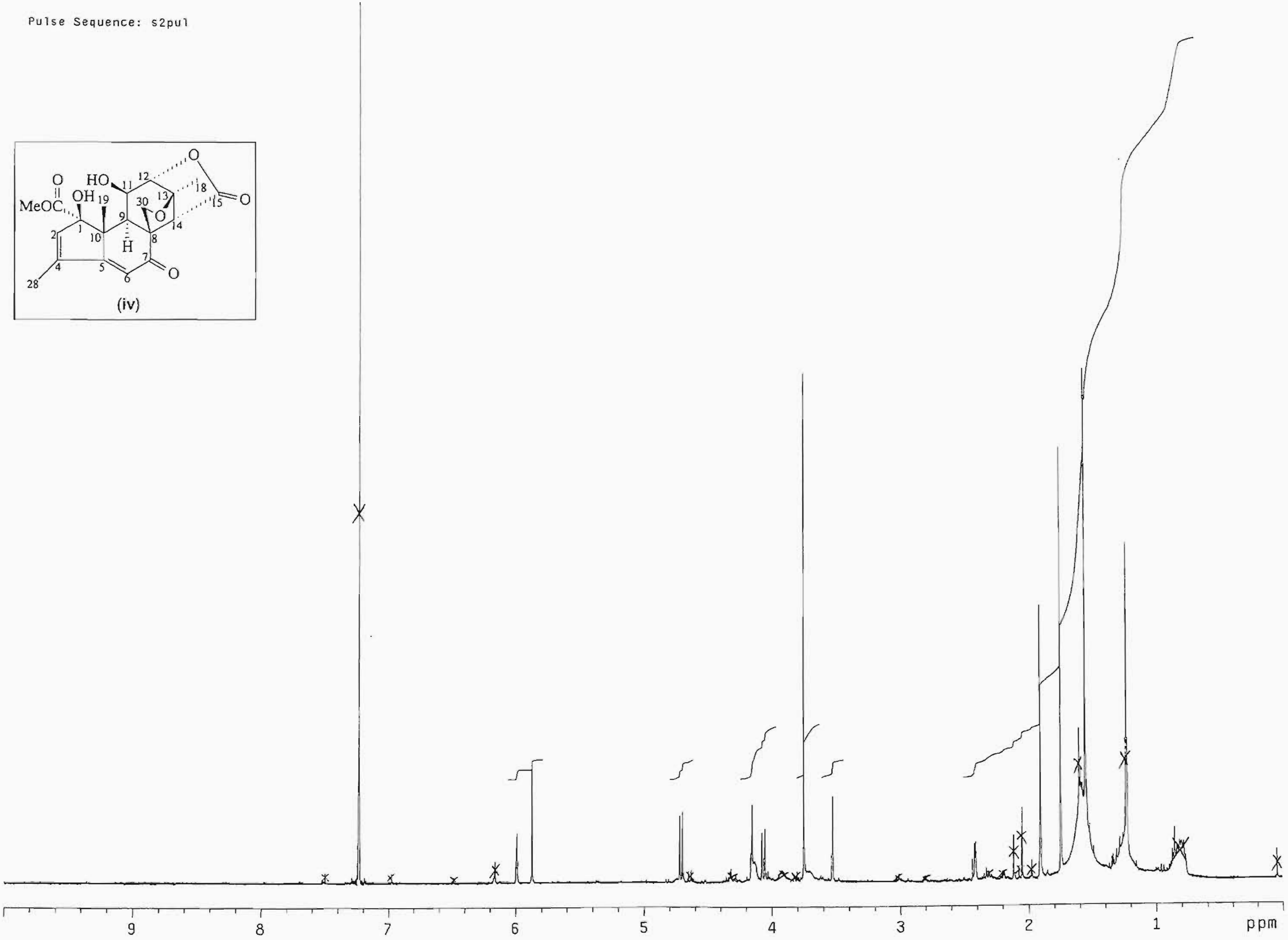
Samaderine DN (iv)



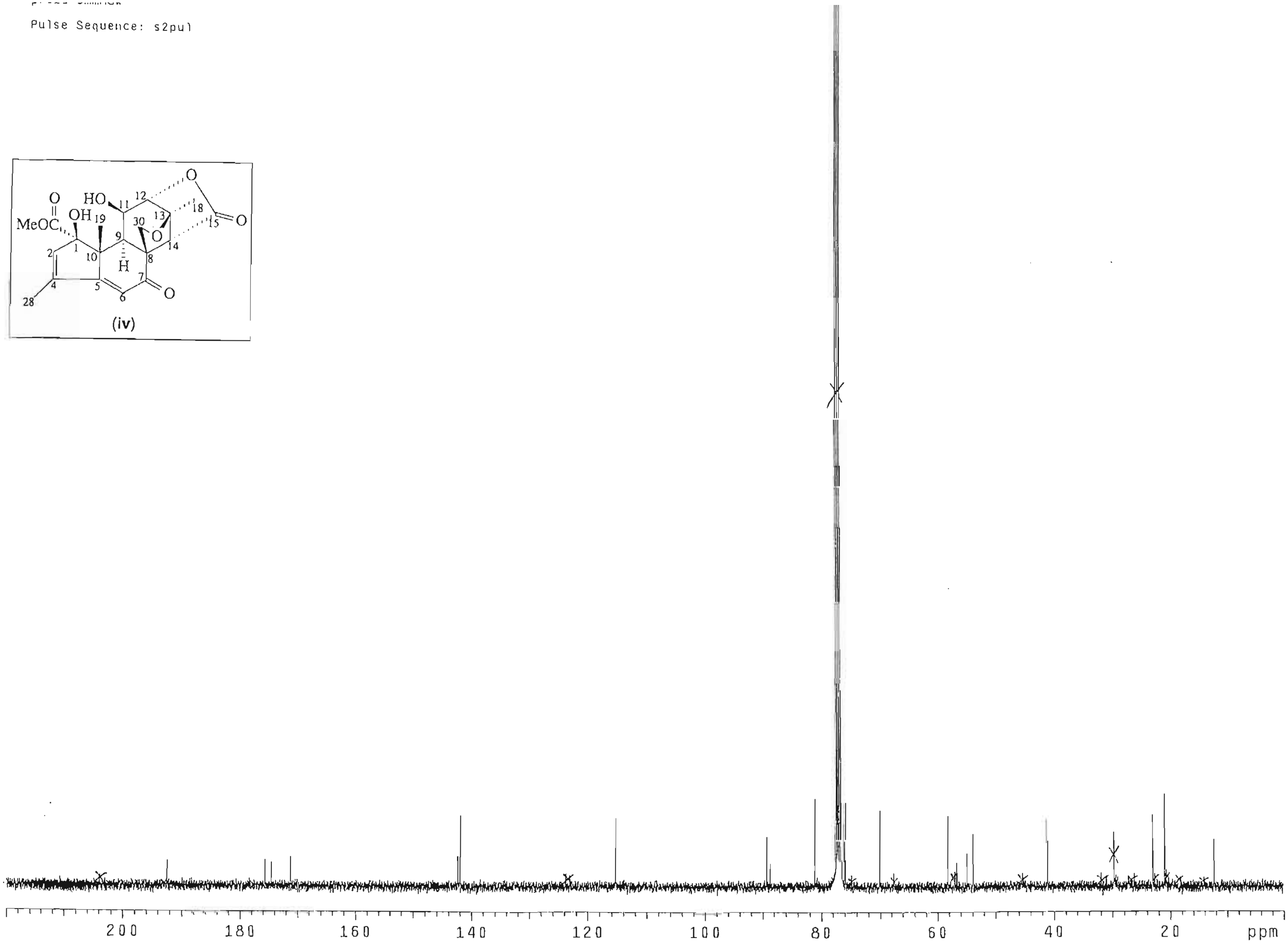
(iv)


%Transmittance
102
101
100
99
98
97
96
95
94
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
Wavenumbers (cm-1)
3456.7
2862.2
2925.4
1787.5
1730.0
1655.3
1620.9
1459.6
1380.5
1264.6
1217.2
1169.8
1111.8
1051.9
1005.9
901.0


SCAN GRAPH. Flagging=High Resolution M/z. Filter=[Excl: Ref/Ex.].Highlighting=Base Peak.
Scan 31#3:28 - 40#4:27. Entries=352. Base M/z=331.11873. 100% Int.=1.04478. EI. POS.
Intensity (%age)
100
90
80
70
60
50
40
30
20
10
0
High Resolution M/z
50
100
150
200
250
300
350
400
331.11873
239.10697
227.10595
255.10212
390.13126
375.10561
372.12071
313.10639
211.11068
187.07589
125.05958
91.05409
57.07039



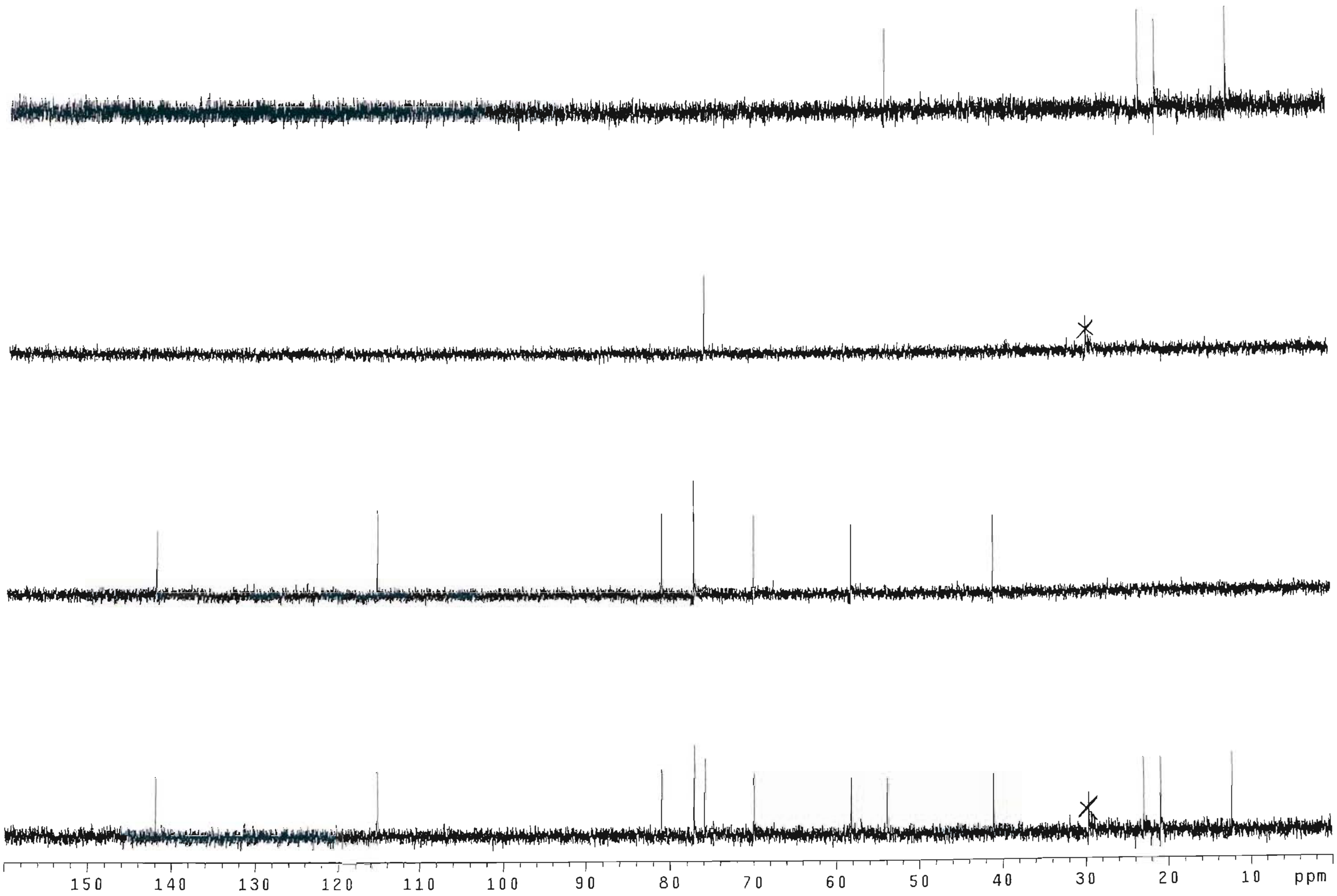
^{1}H NMR Spectrum of Samaderine DN (iv)



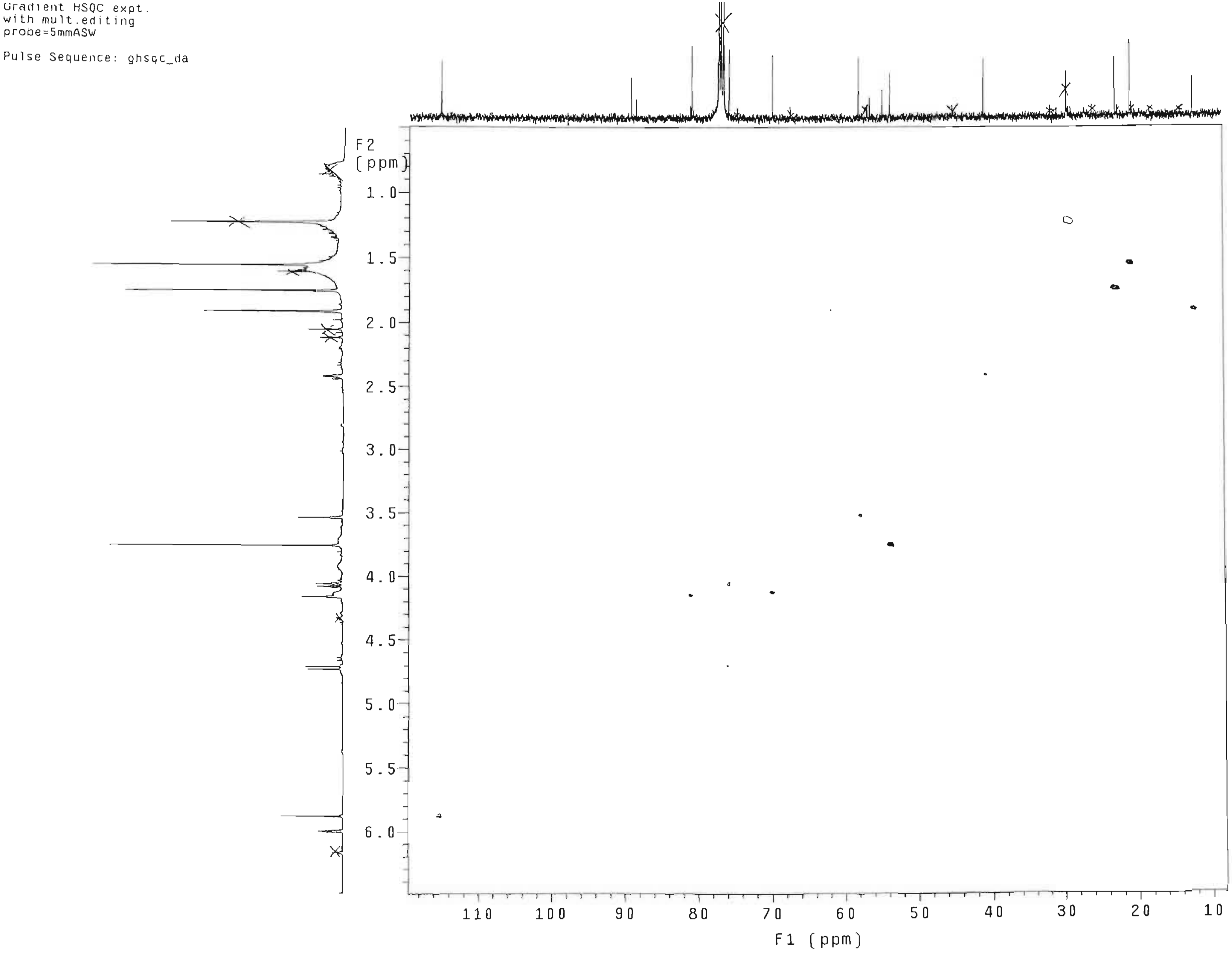
^{13}C NMR Spectrum of Samaderine DN (iv)

probe=5mmASW

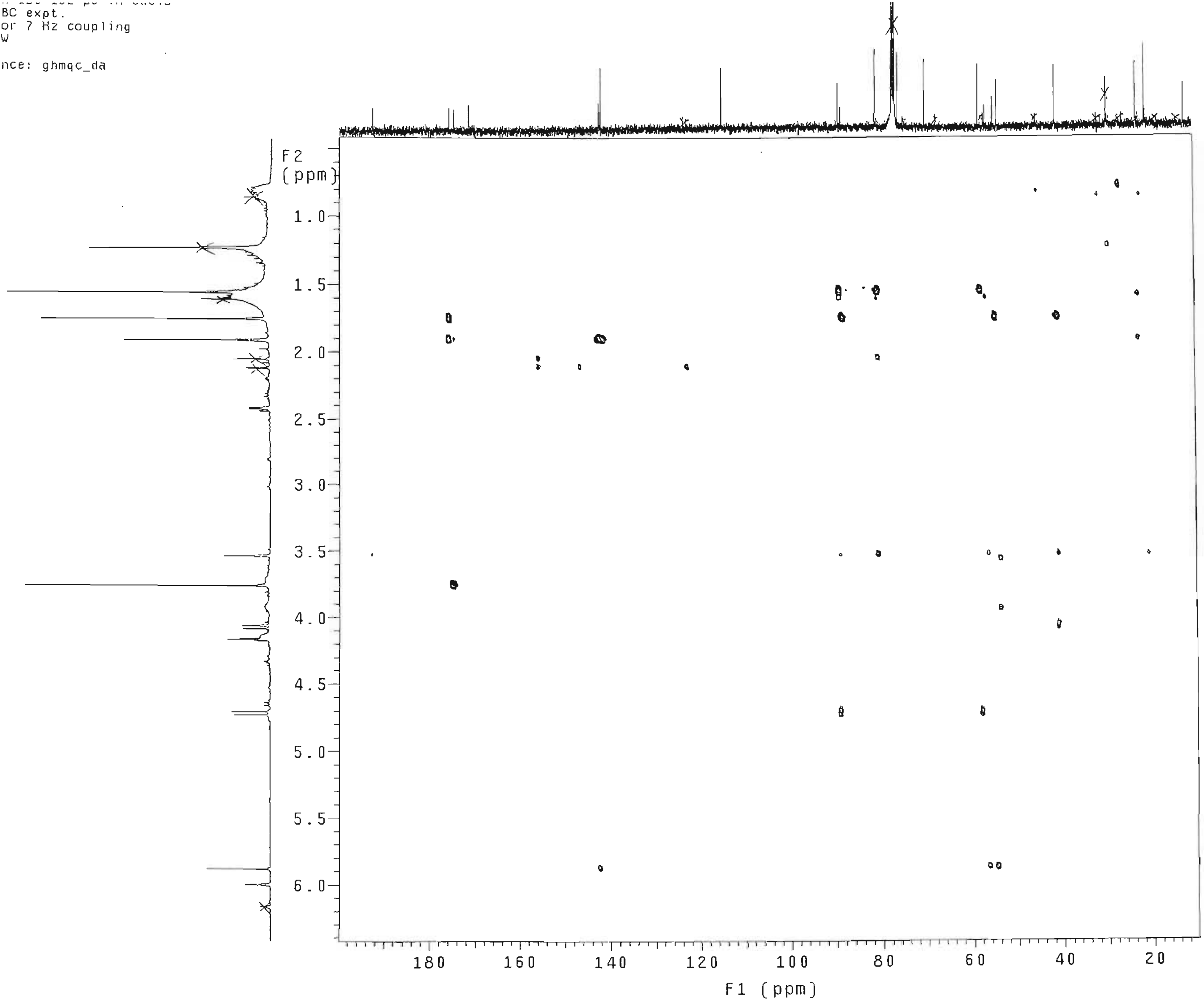
Pulse Sequence: dept



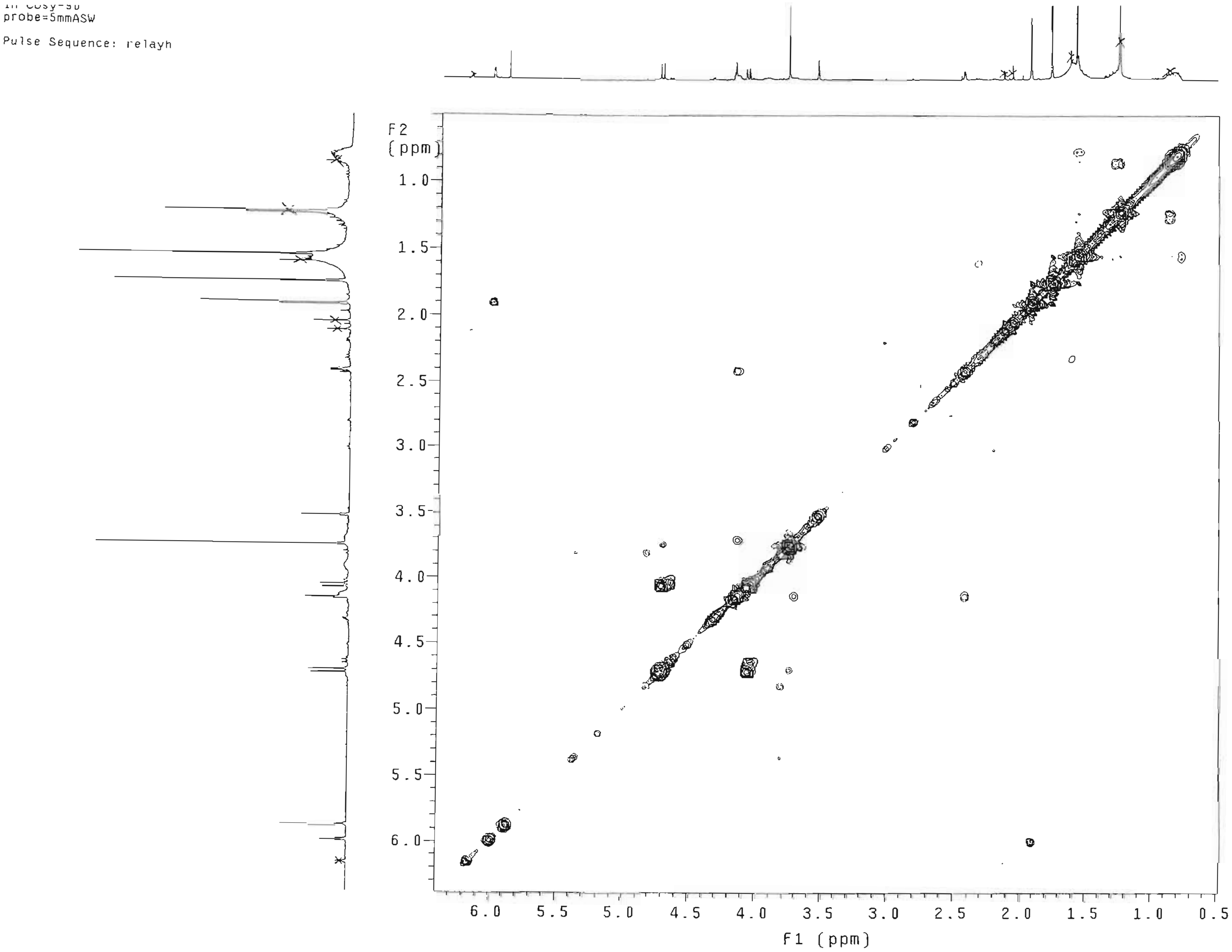
ADEPT NMR Spectrum of Samaderine DN (iv)



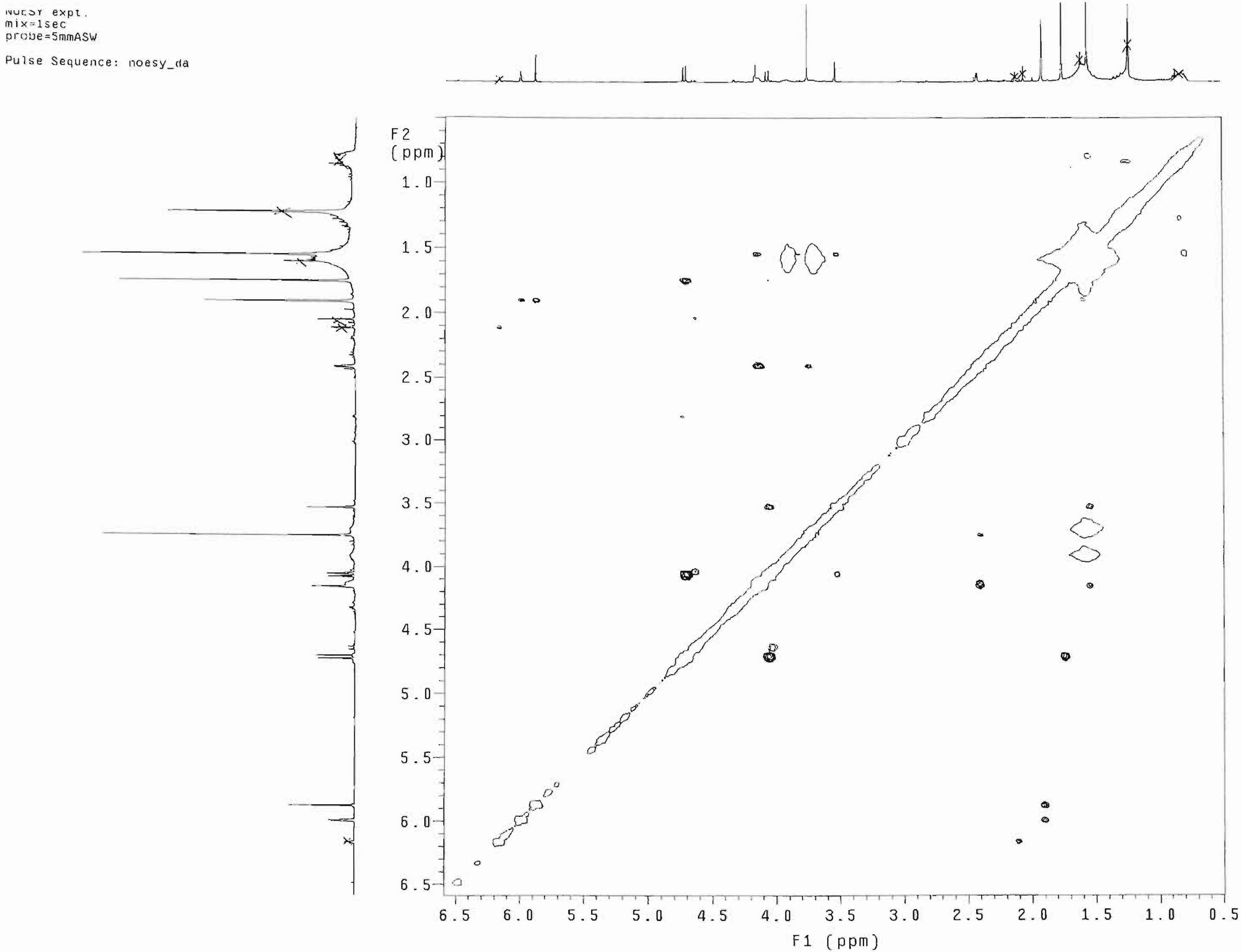
HSQC NMR Spectrum of Samaderine DN (iv)



HMBC NMR Spectrum of Samaderine DN (iv)

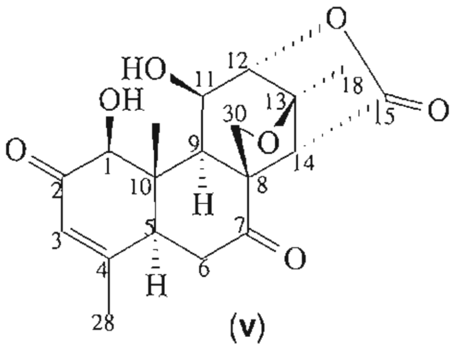


COSY NMR Spectrum of Samaderine DN (iv)

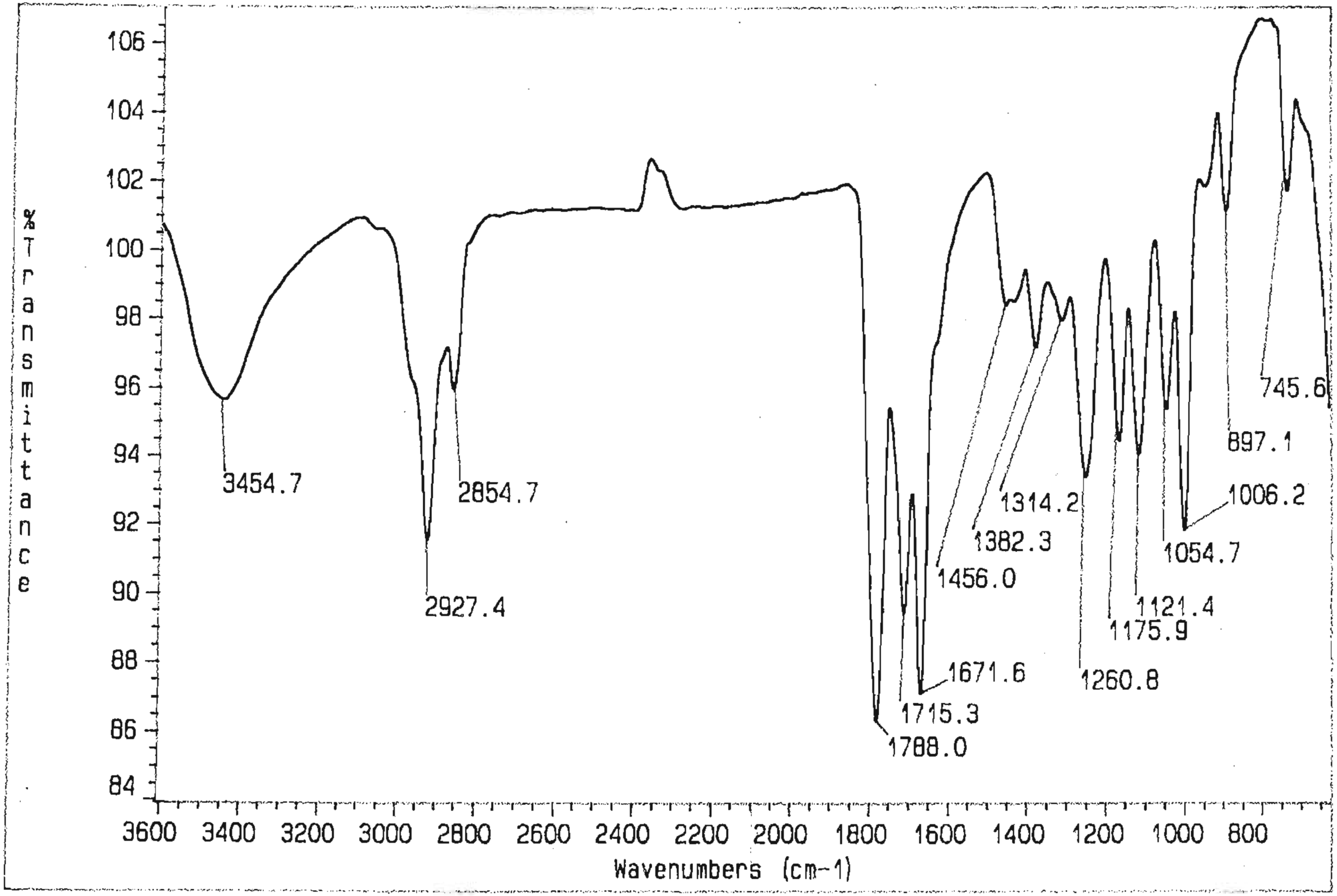


NOESY NMR Spectrum of Samaderine DN (iv)

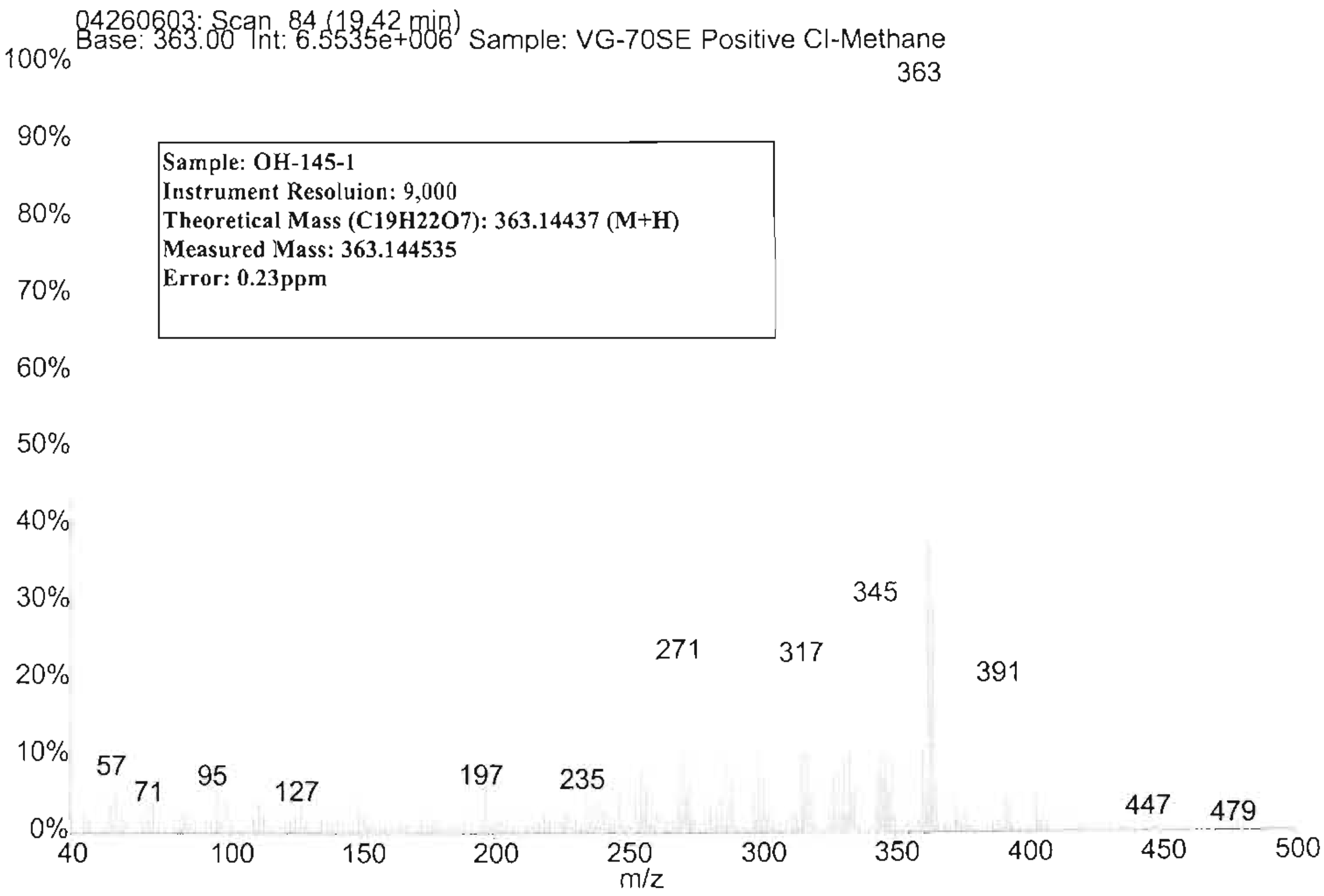
Samaderine B (v)



(v)

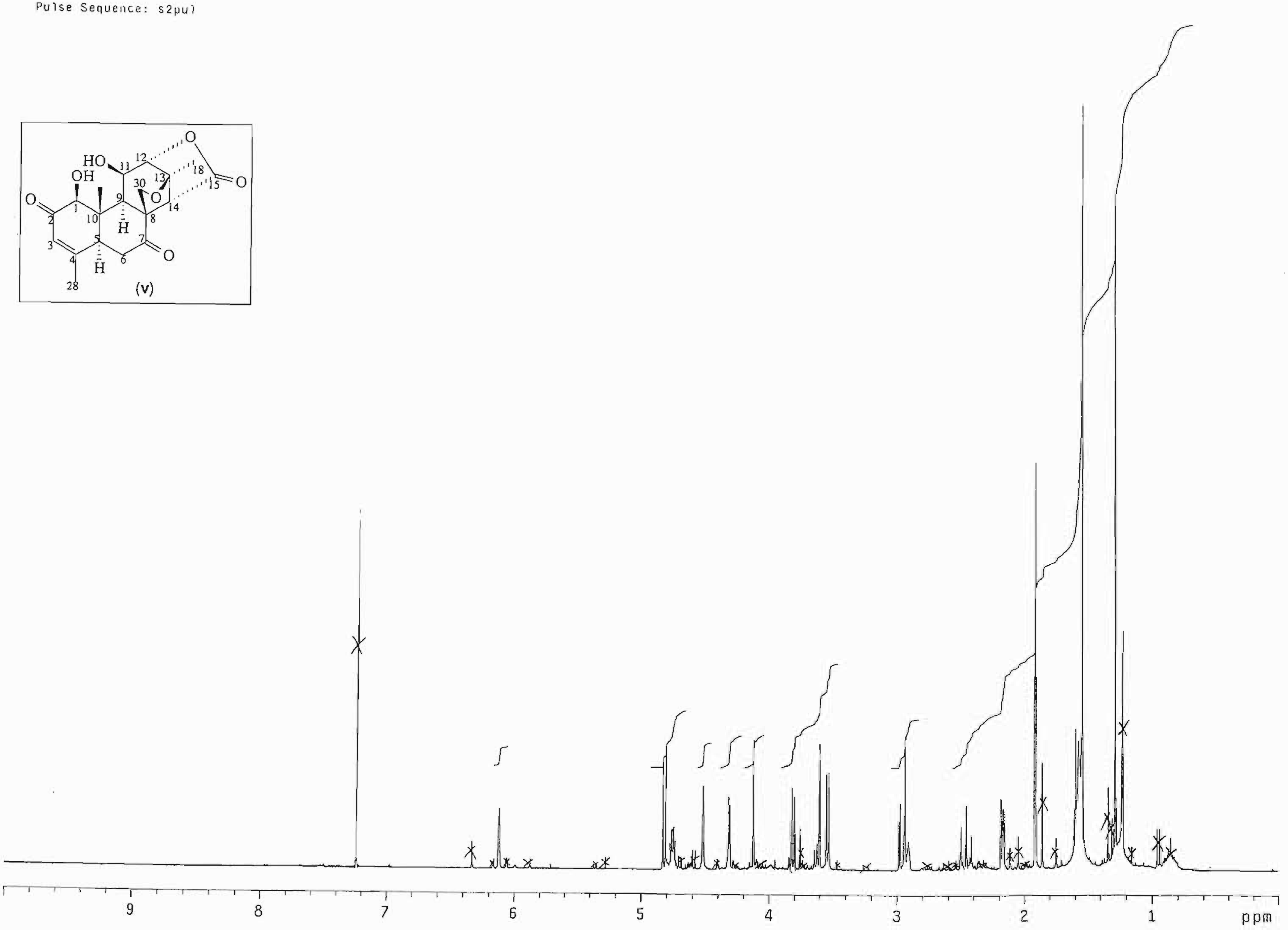


IR Spectrum of Samaderine B (v)

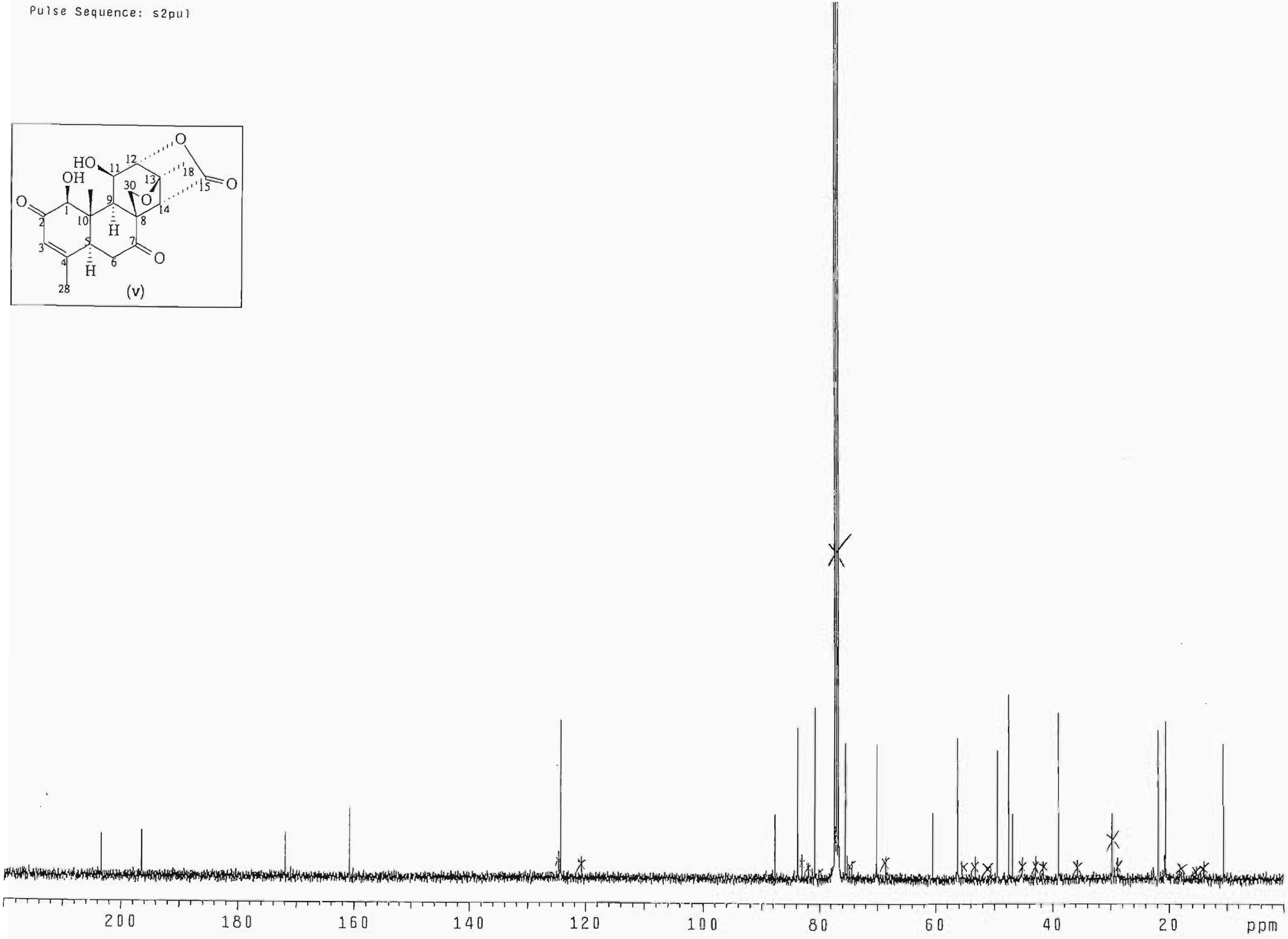


Sample: OH-145-1
Instrument Resoluion: 9,000
Theoretical Mass ($C_{19}H_{22}O_7$): 363.14437 (M+H)
Measured Mass: 363.144535
Error: 0.23ppm

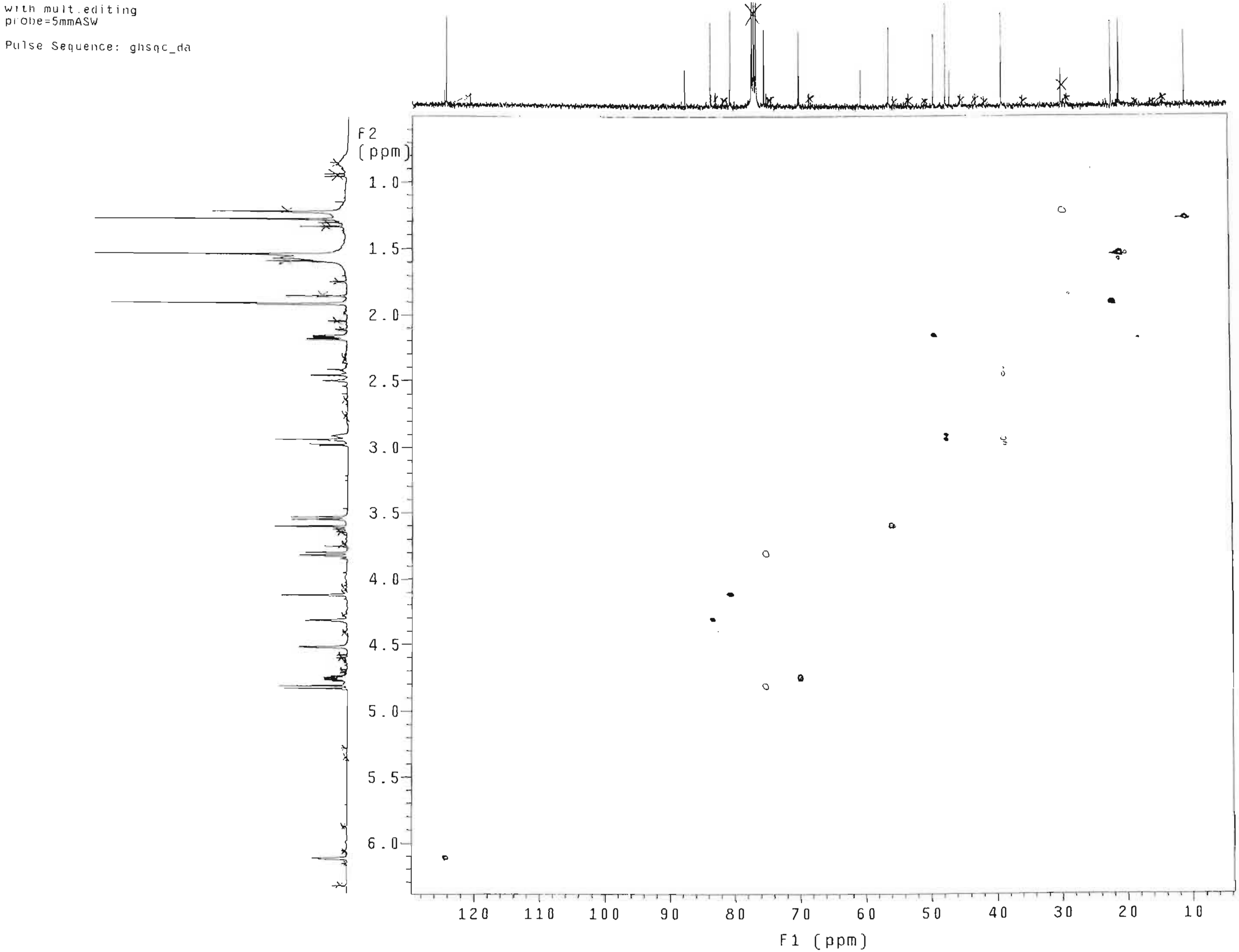
Mass Spectrum of Samaderine B (v)



^{1}H NMR Spectrum of Samaderine B (v)



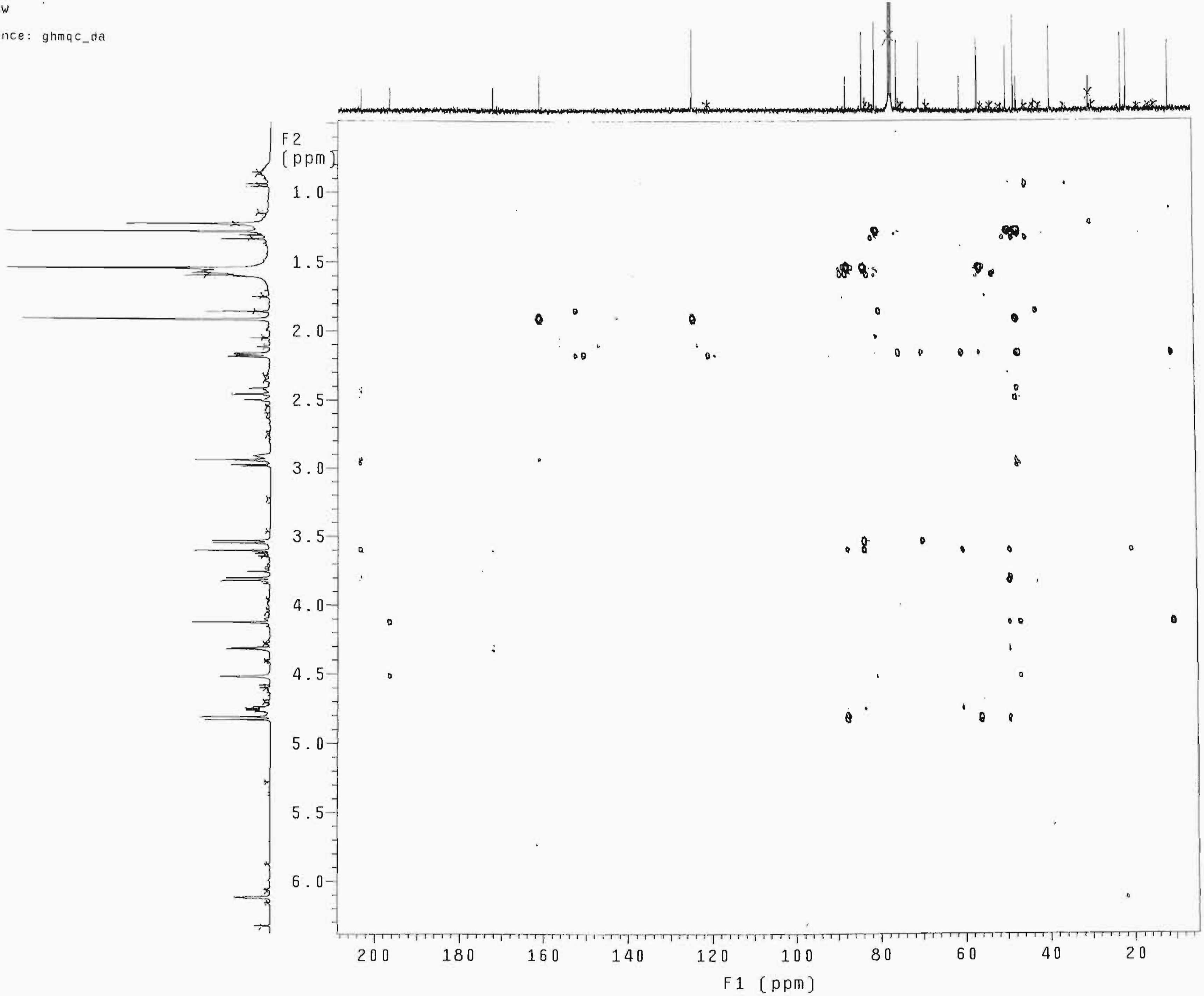
^{13}C NMR Spectrum of Samaderine B (v)



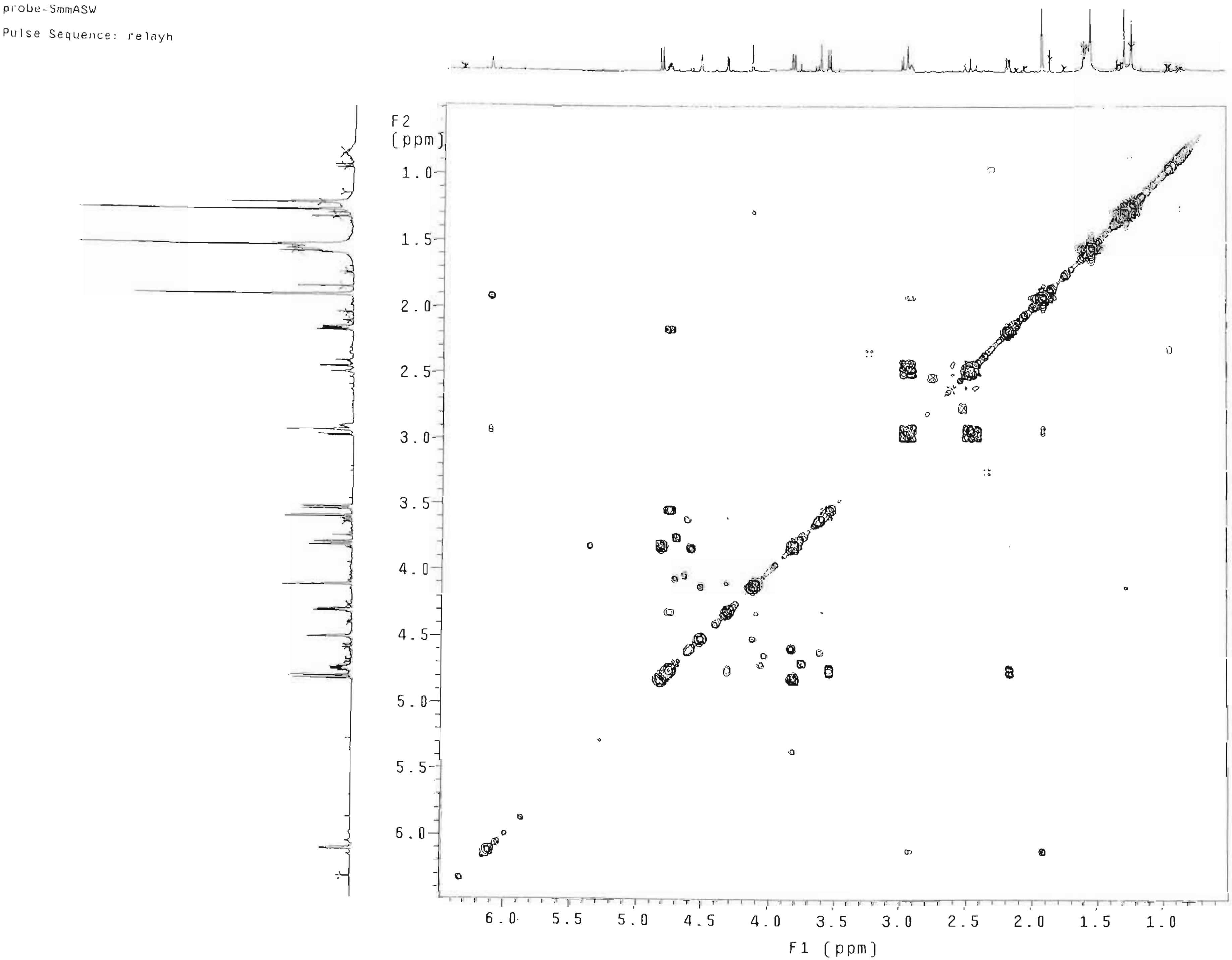
HSQC NMR Spectrum of Samaderine B (v)

probe=5mmASW

Pulse Sequence: ghmqc_da



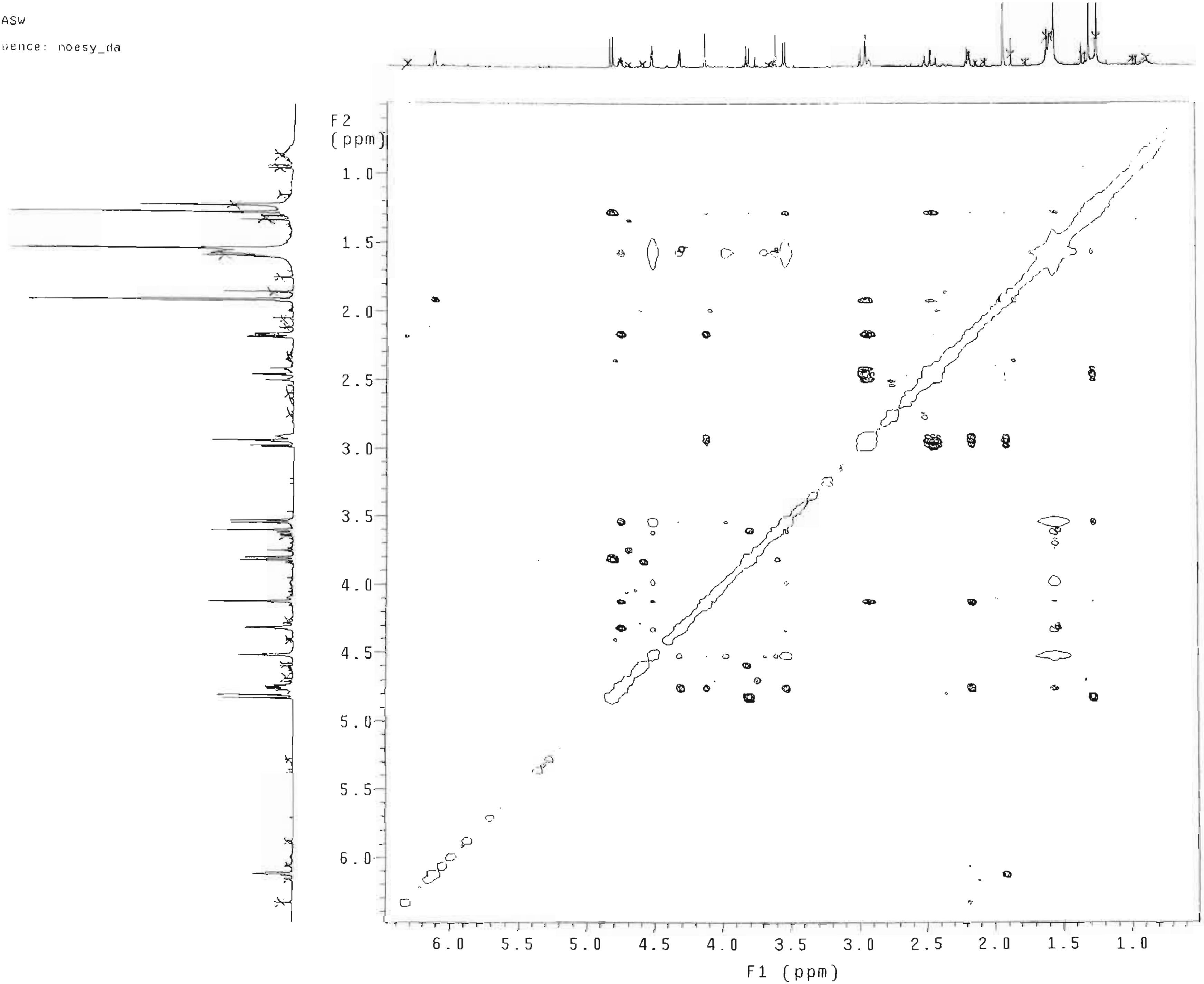
HMBC NMR Spectrum of Samaderine B (v)



COSY NMR Spectrum of Samaderine B (v)

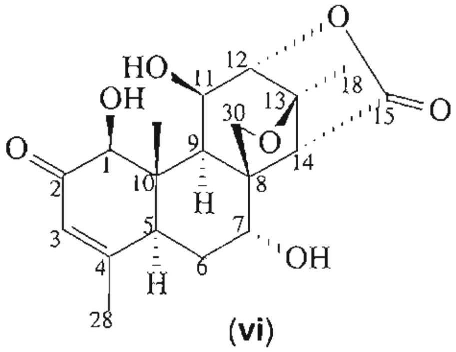
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da

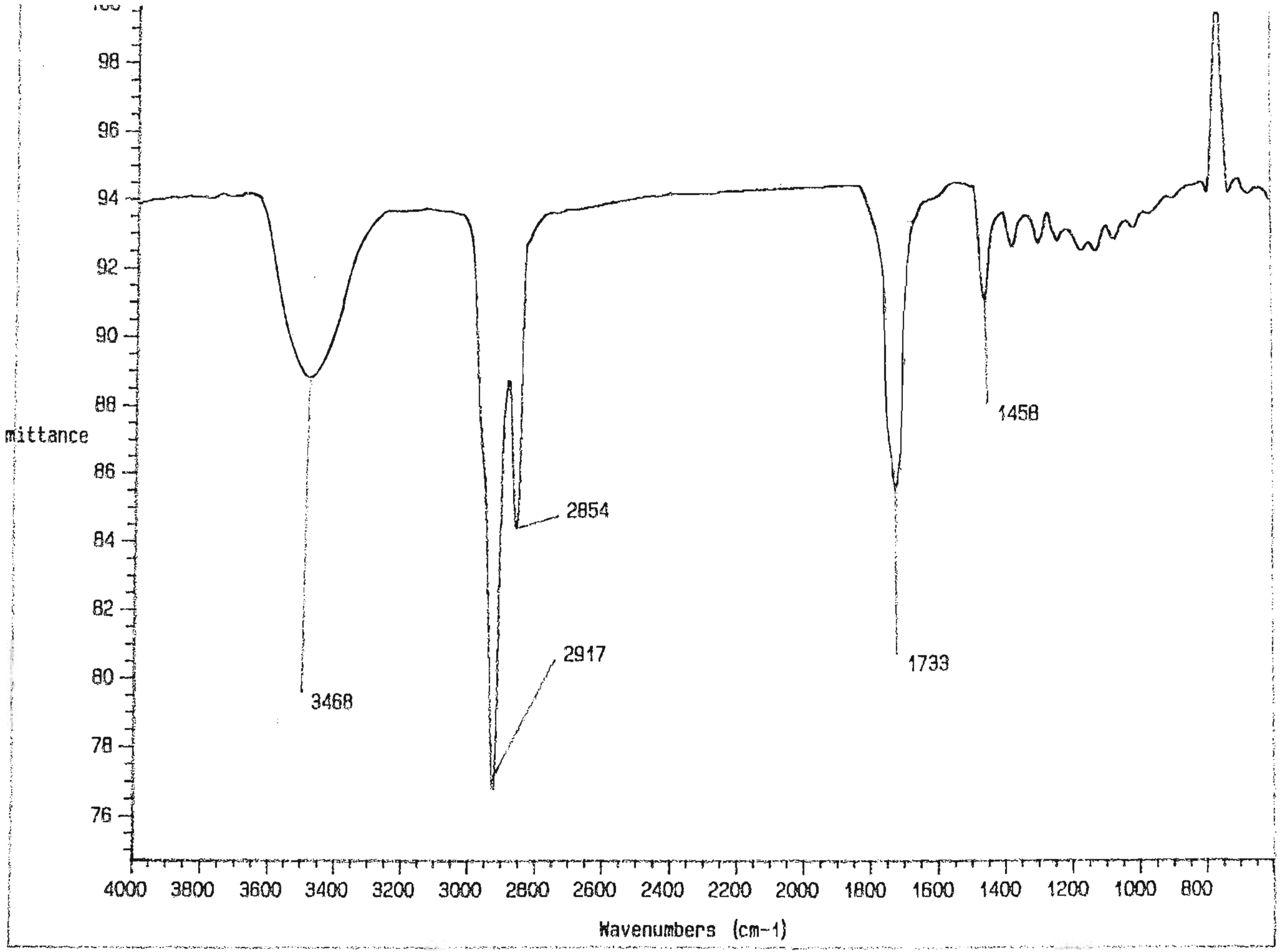


NOESY NMR Spectrum of Samaderine B (v)

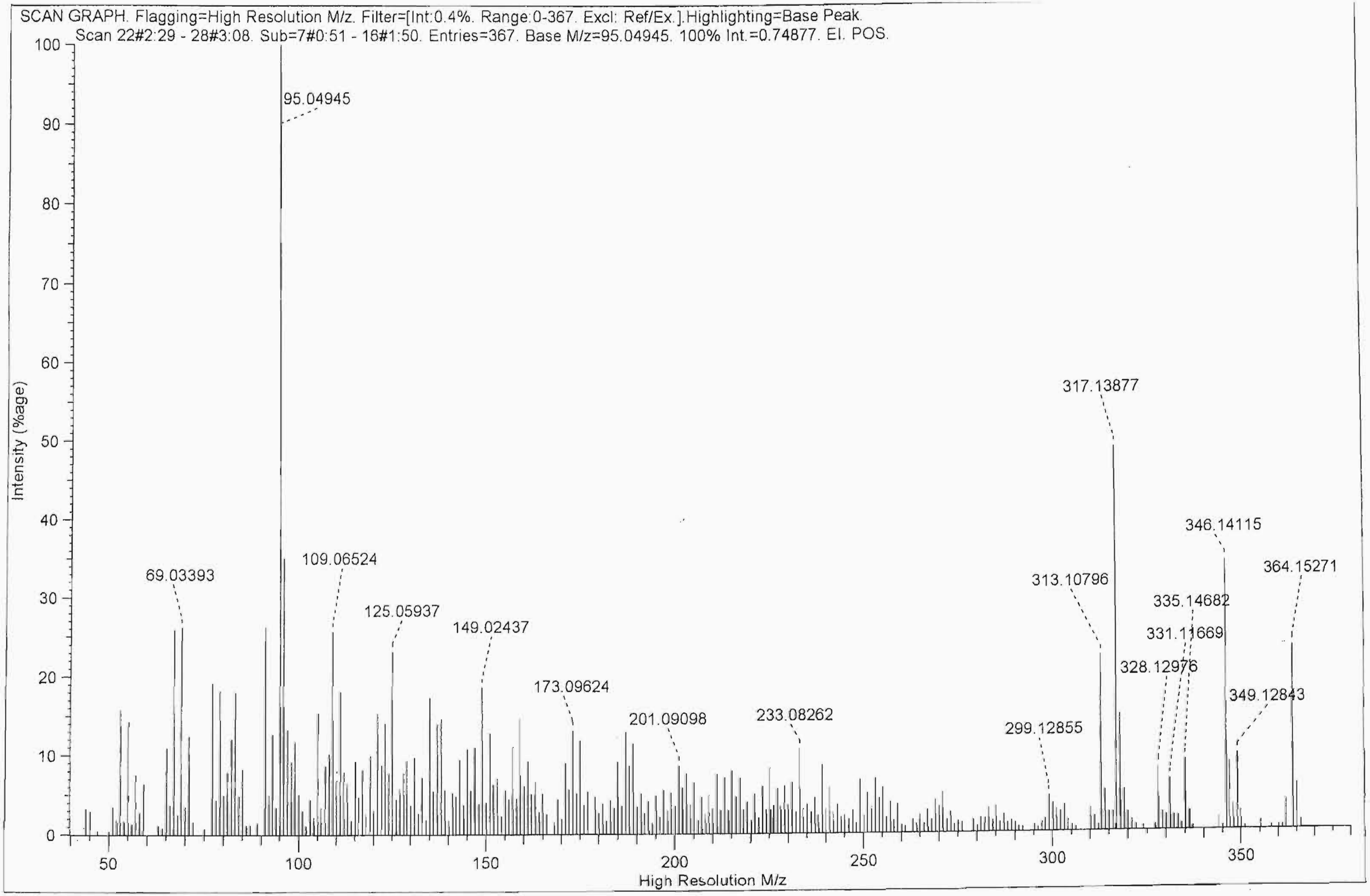
Cedronin (vi)



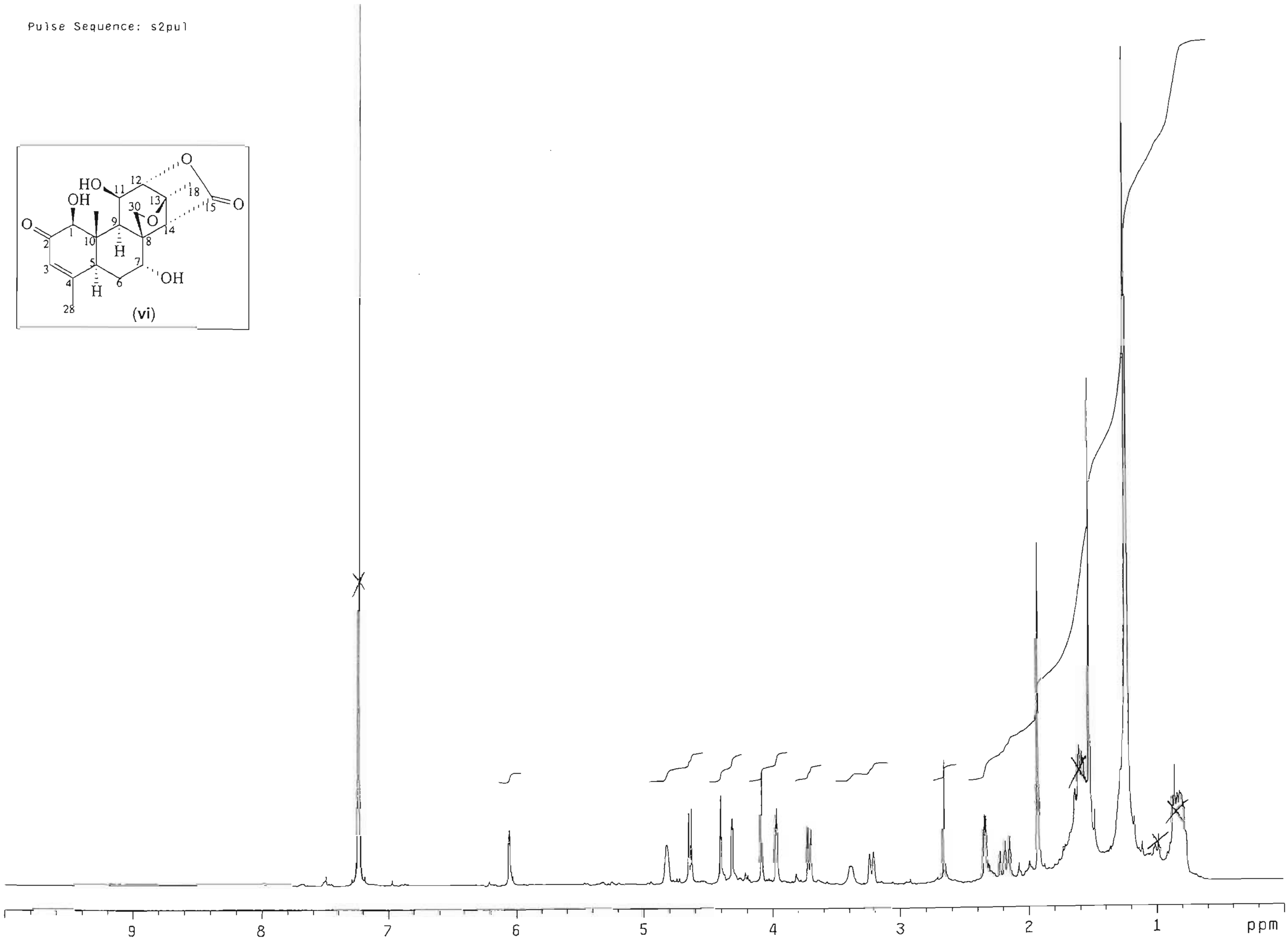
(vi)



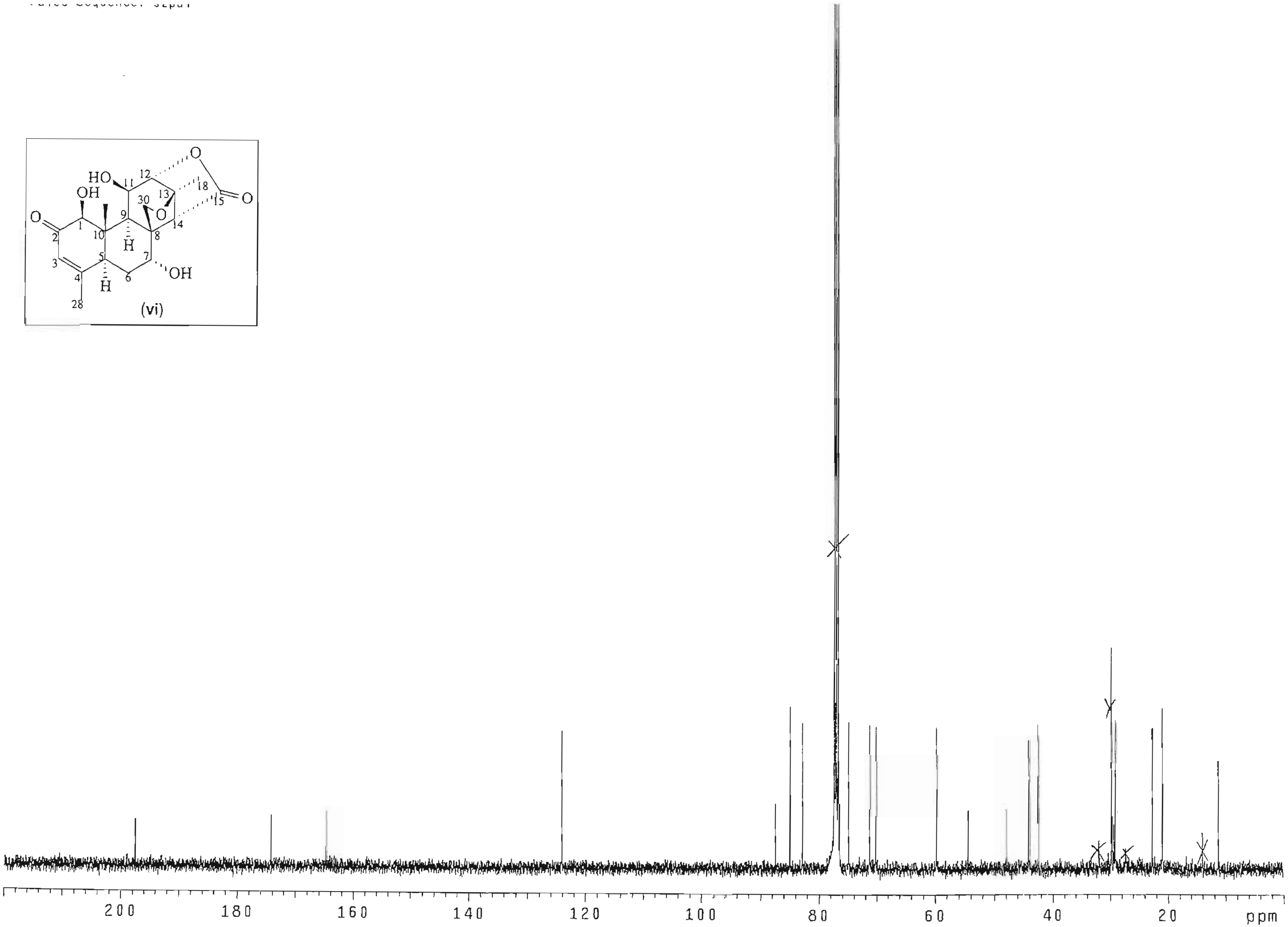
IR Spectrum of Cedronin (vi)



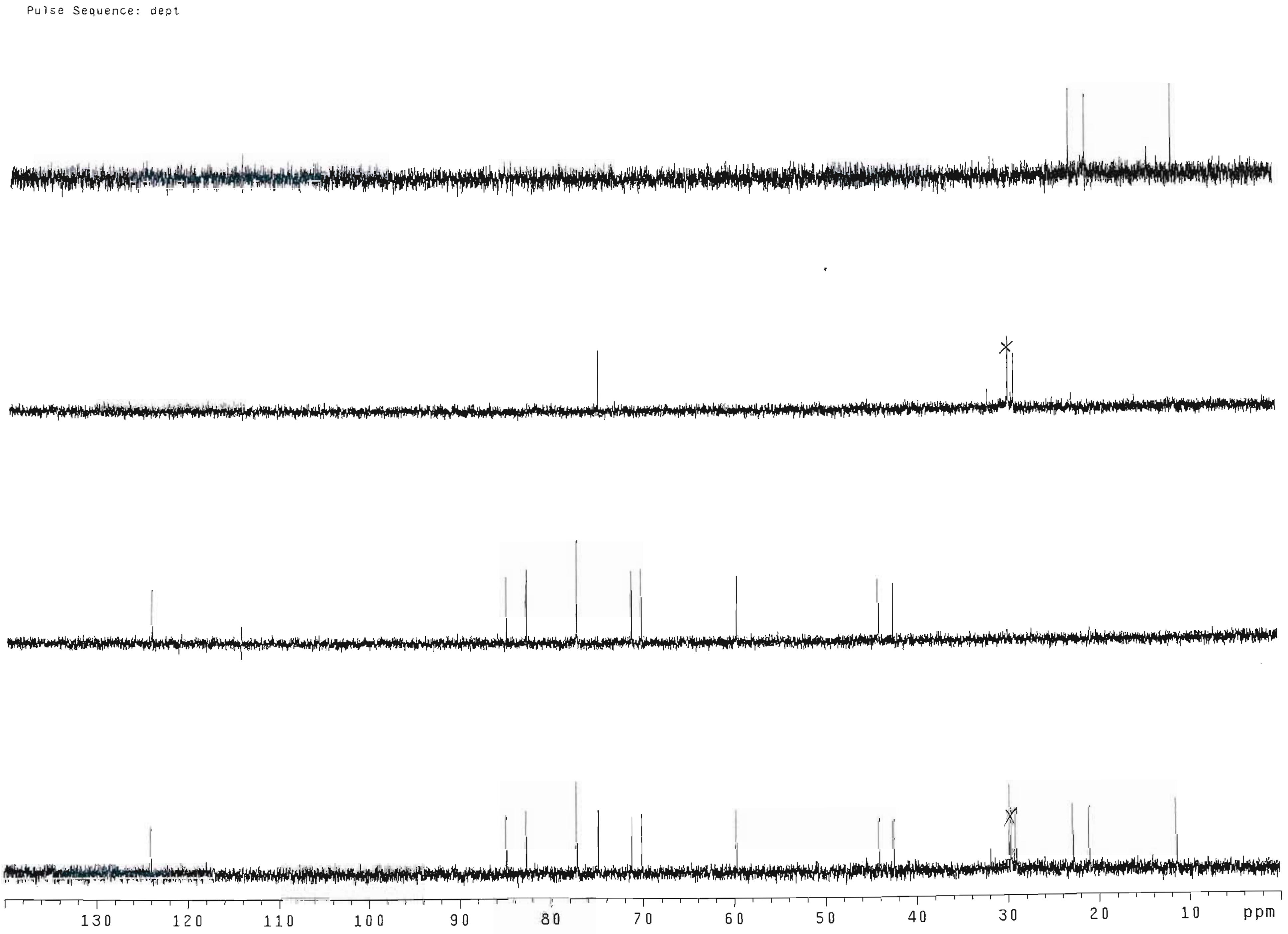
Mass Spectrum of Cedronin (vi)



¹H NMR Spectrum of Cedronin (vi)



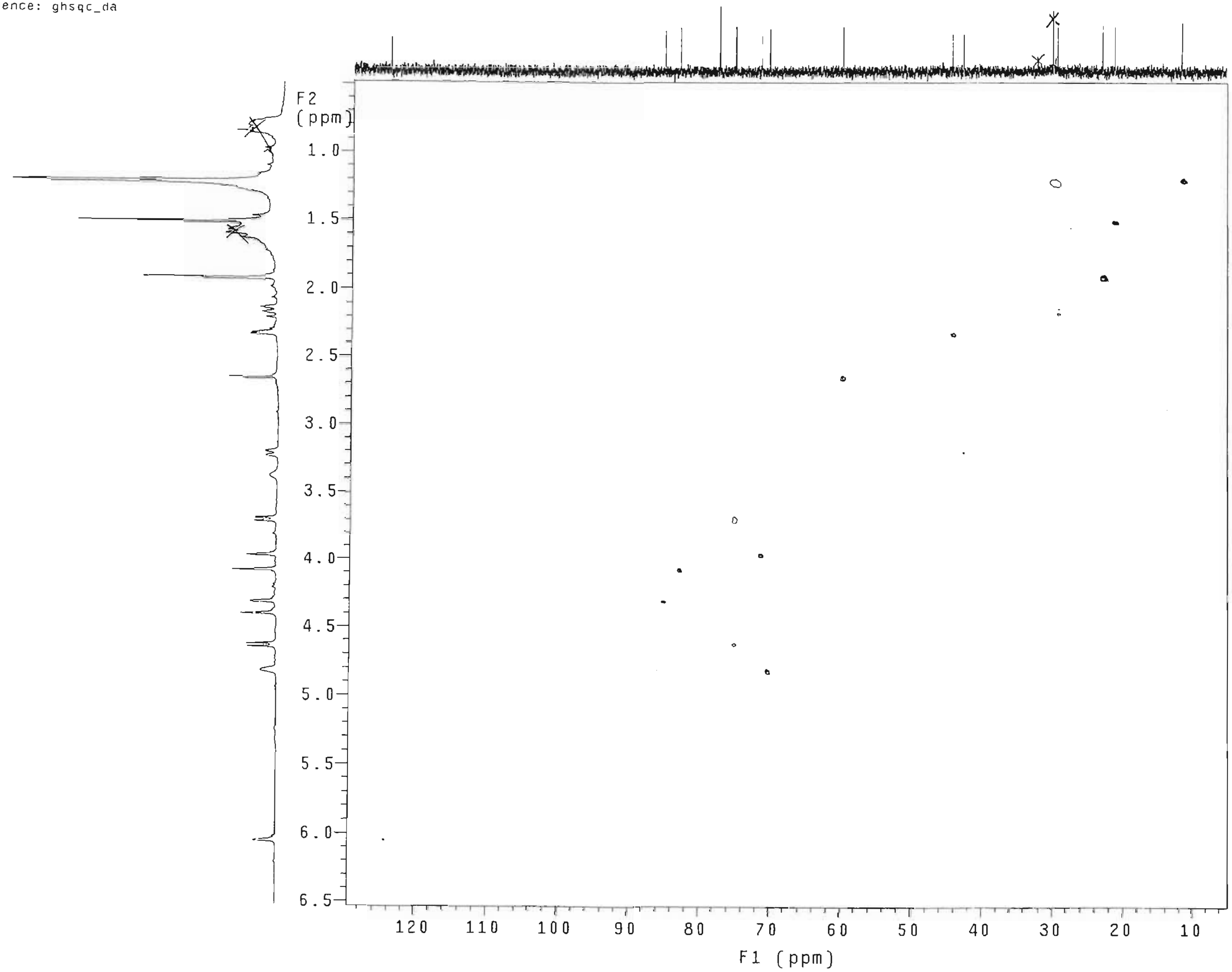
^{13}C NMR Spectrum of Cedronin (vi)



ADEPT NMR Spectrum of Cedropin (vi)

probe=5mmASW

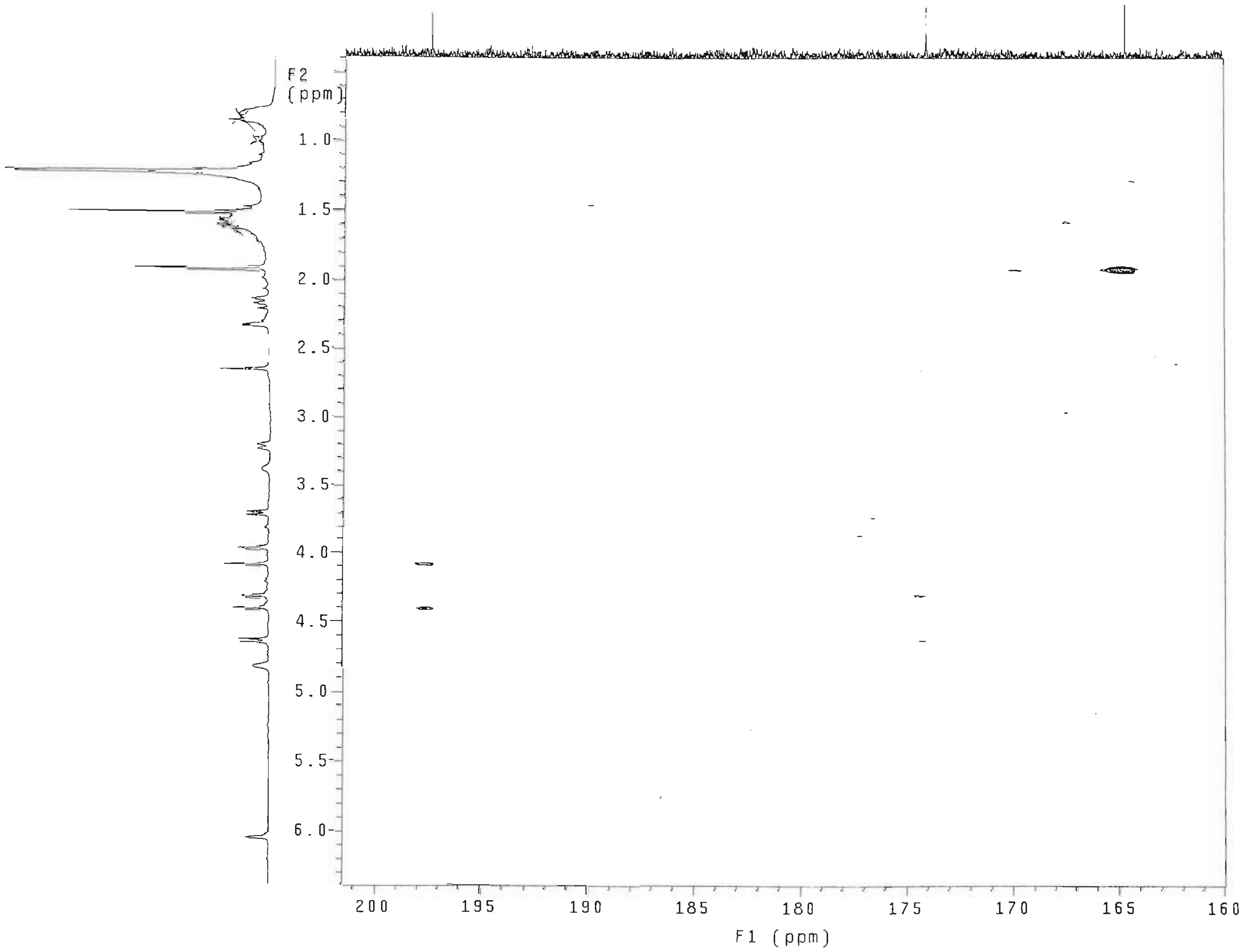
Pulse Sequence: ghsqc_da



HSQC NMR Spectrum of Cedronin (vi)

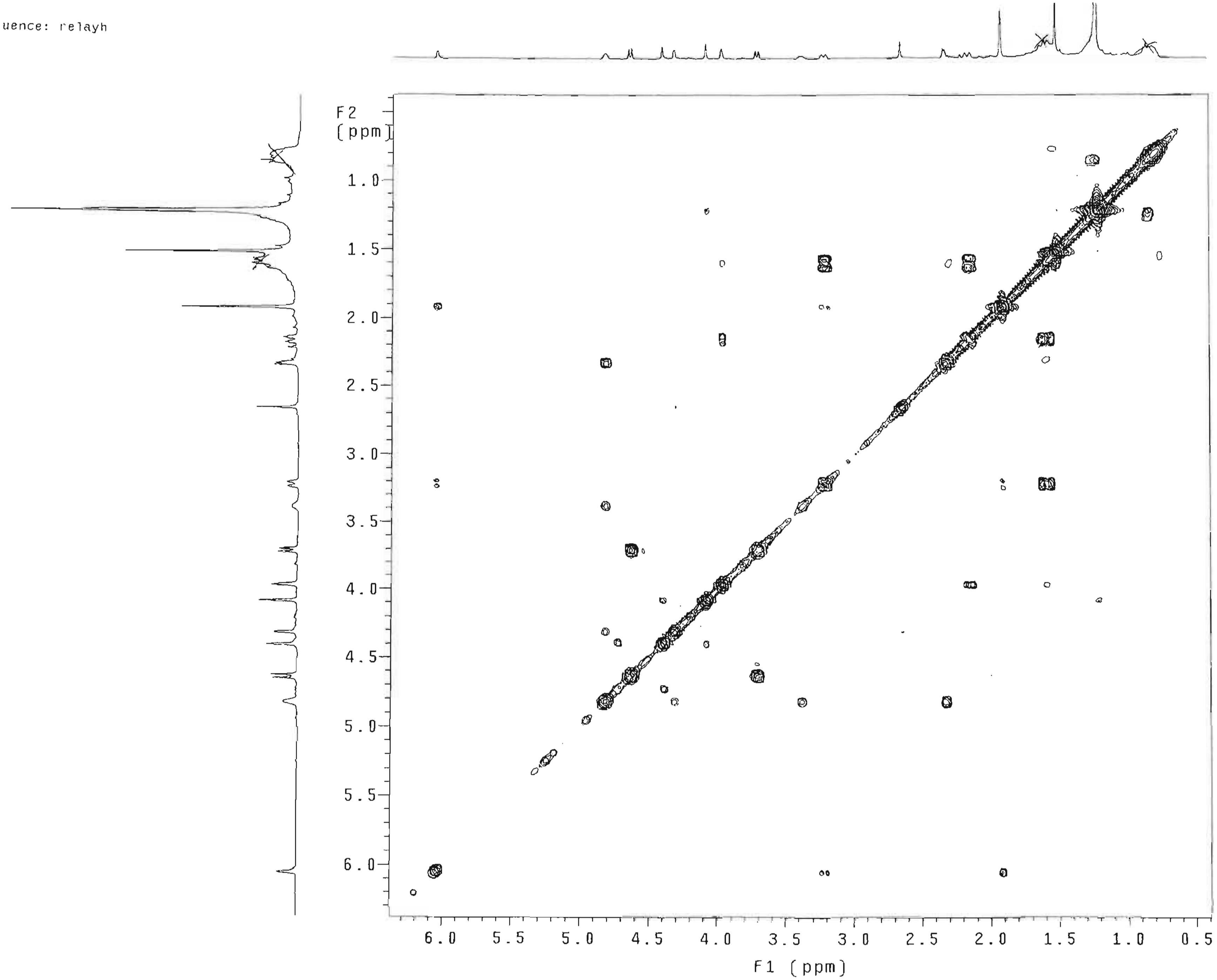
probe=5mmASW

Pulse Sequence: ghmqc_da

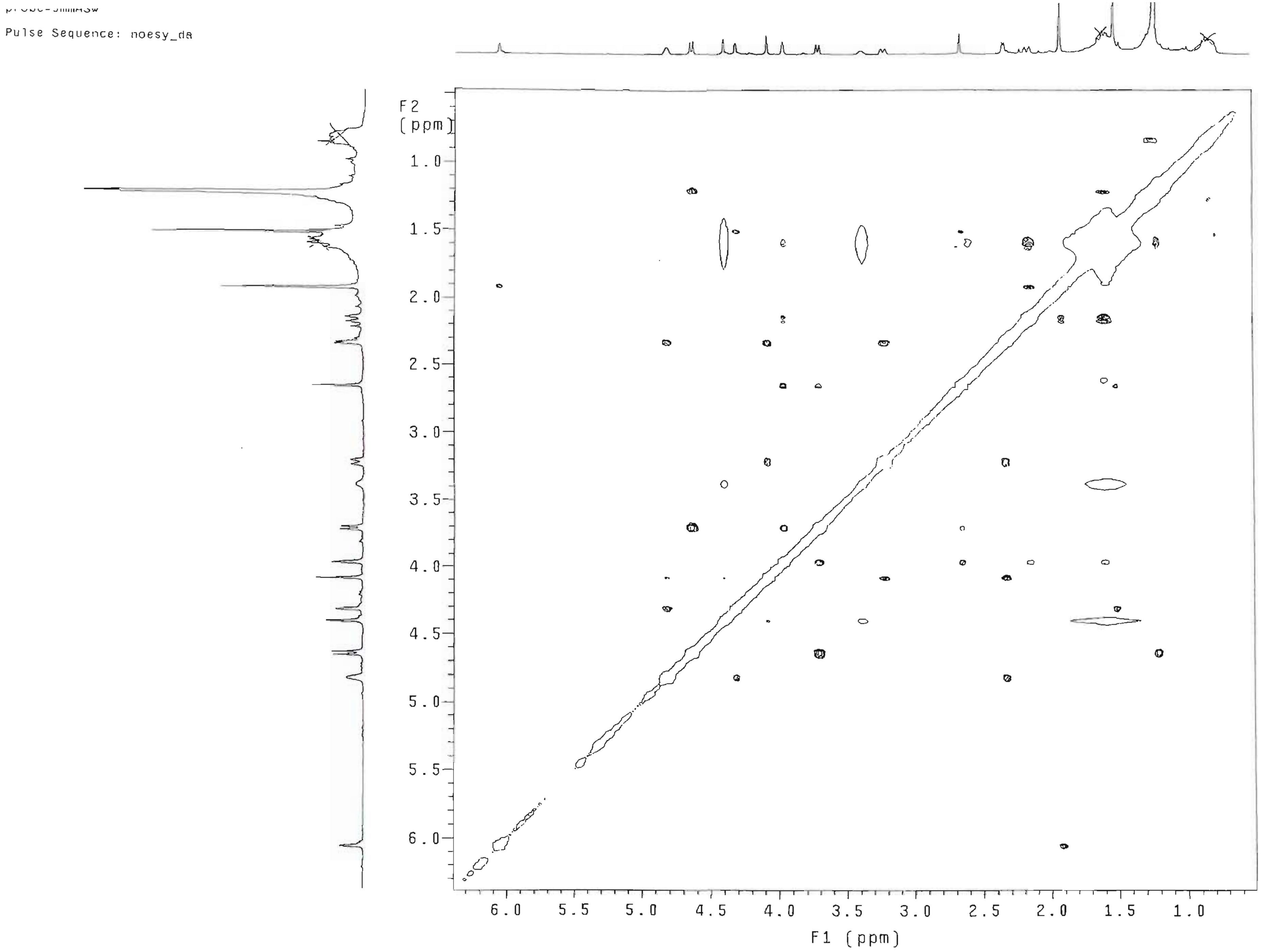


HMBC NMR Spectrum of Cedronin (vi)

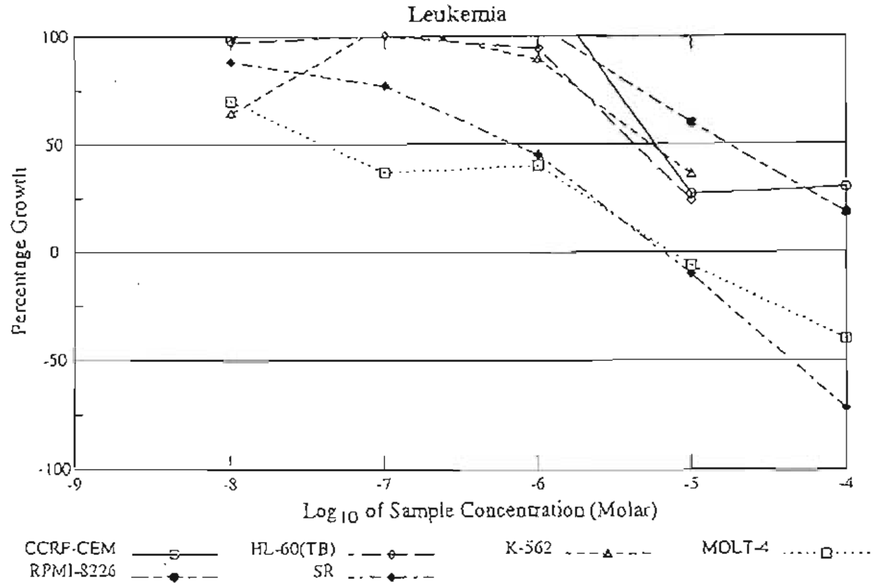
Pulse Sequence: relayh

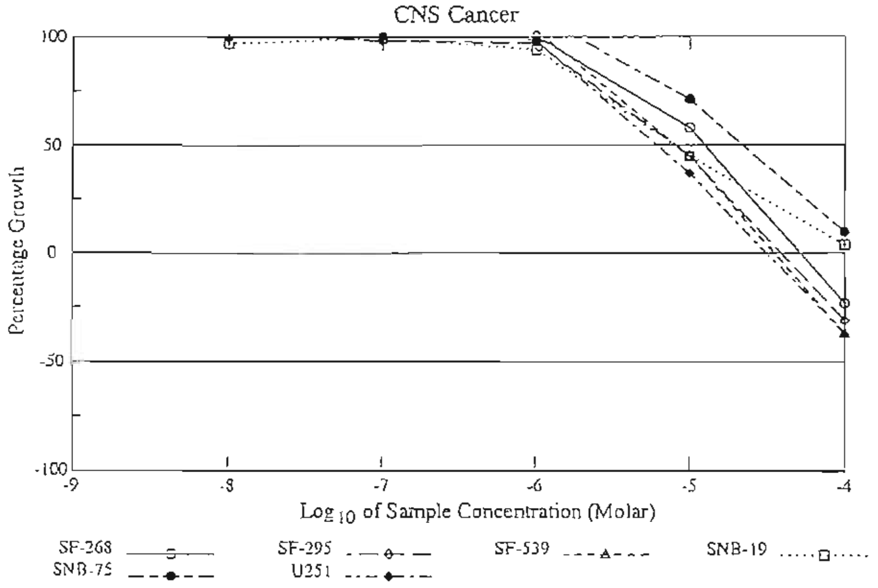


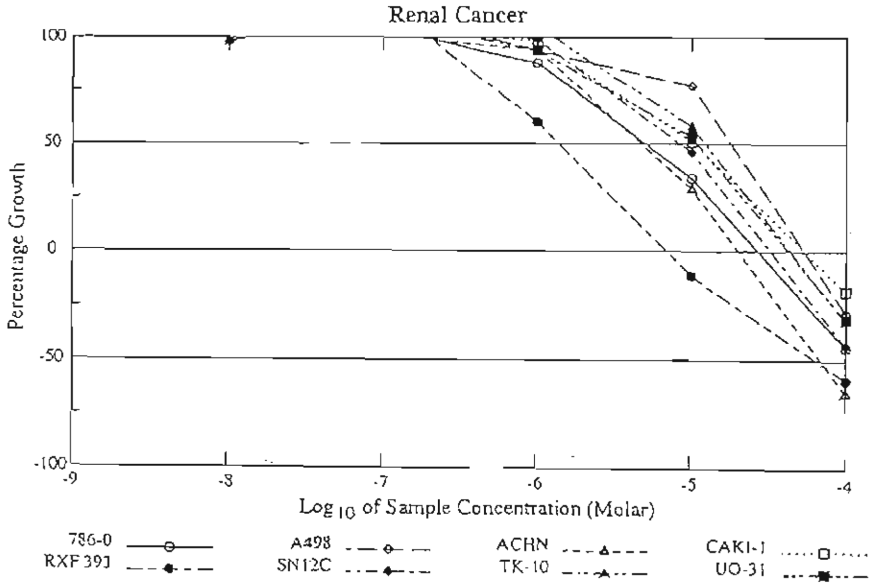
COSY NMR Spectrum of Cedronin (vi)



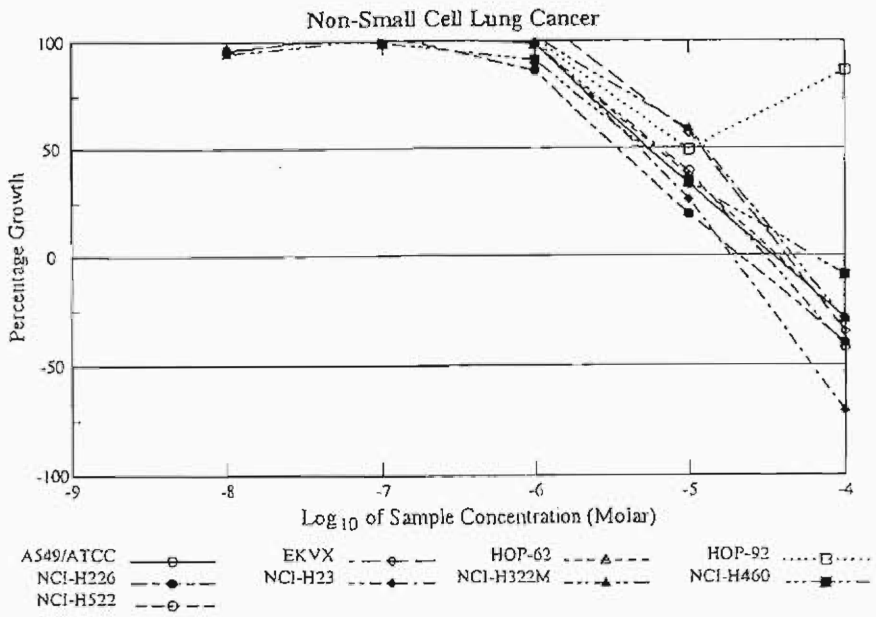
NOESY NMR Spectrum of Cedronin (vi)

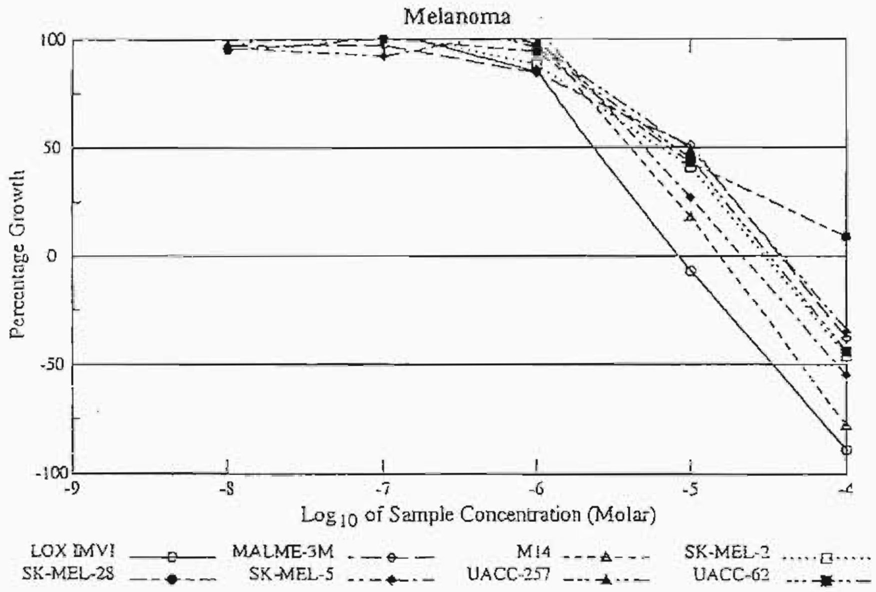


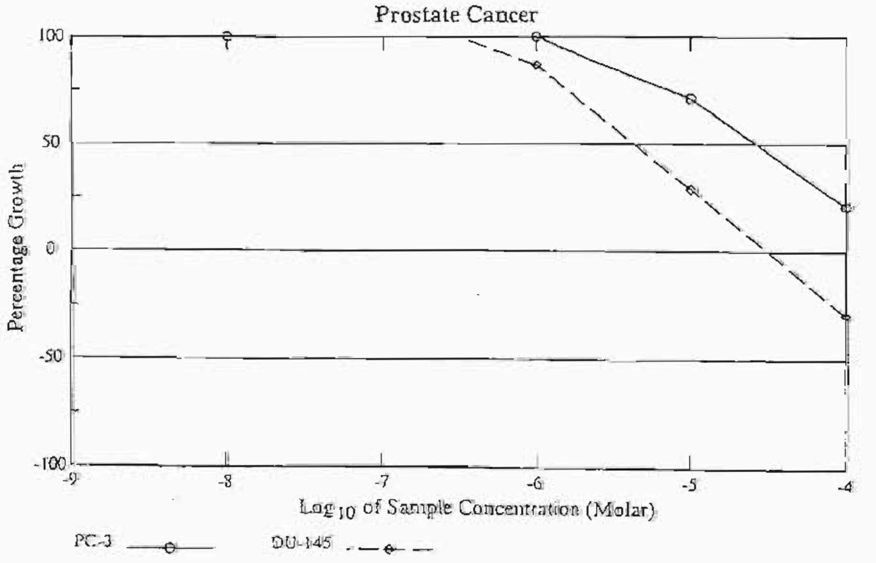




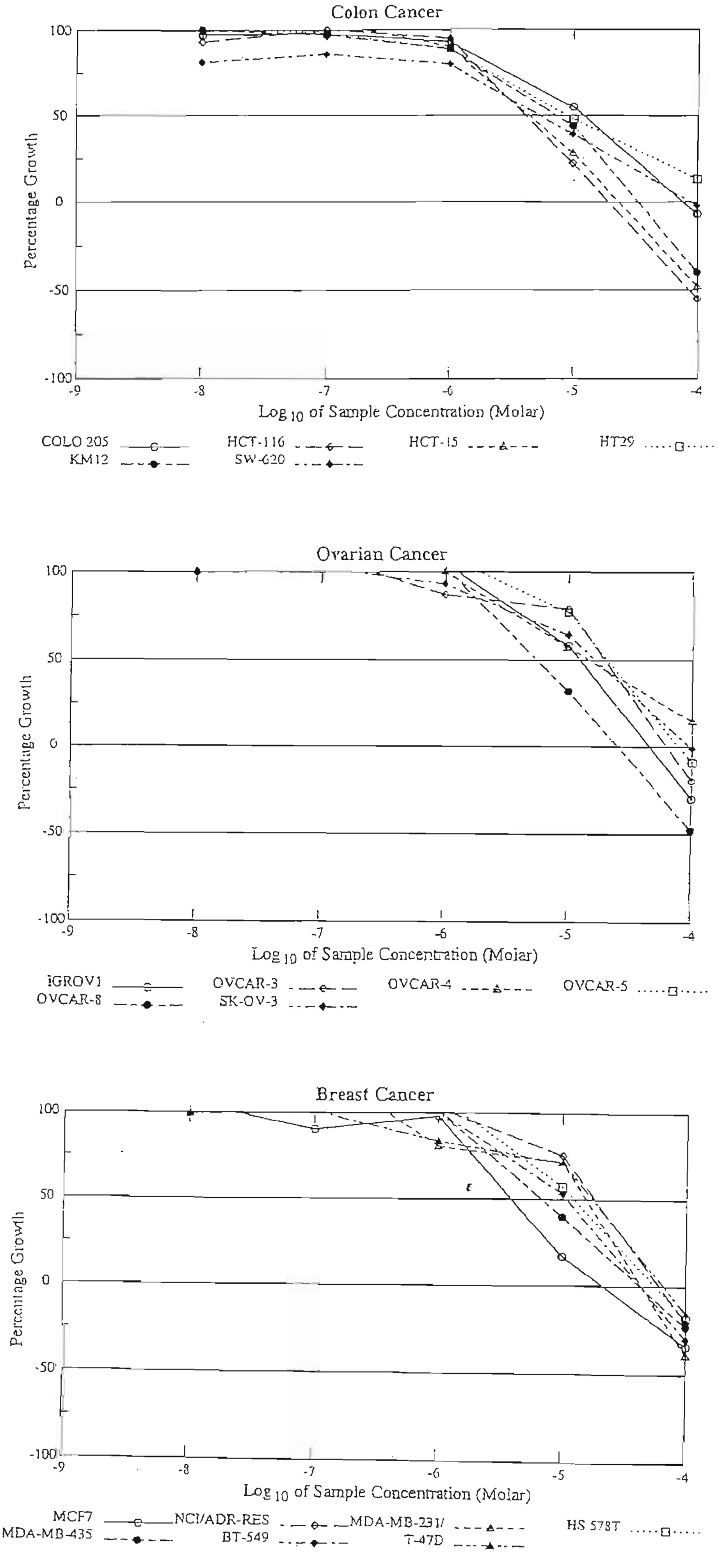
Dose Response Curves for Cedronin (vi)





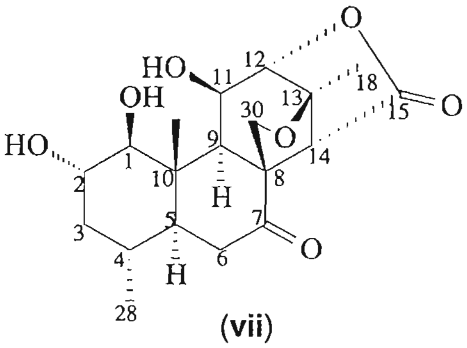


Dose Response Curves for Cedronin (vi)

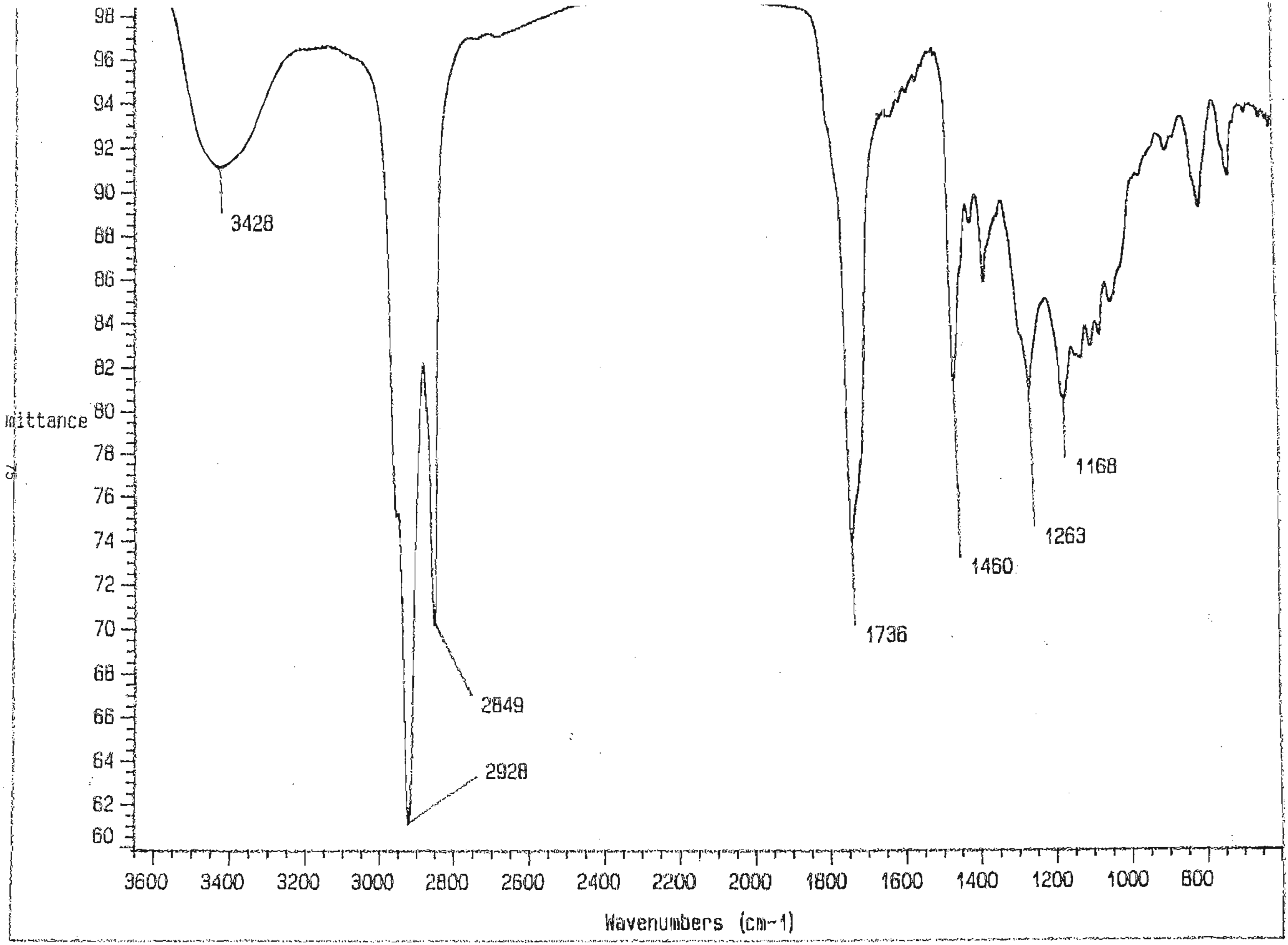


Dose Response Curves for Cedronin (vi)

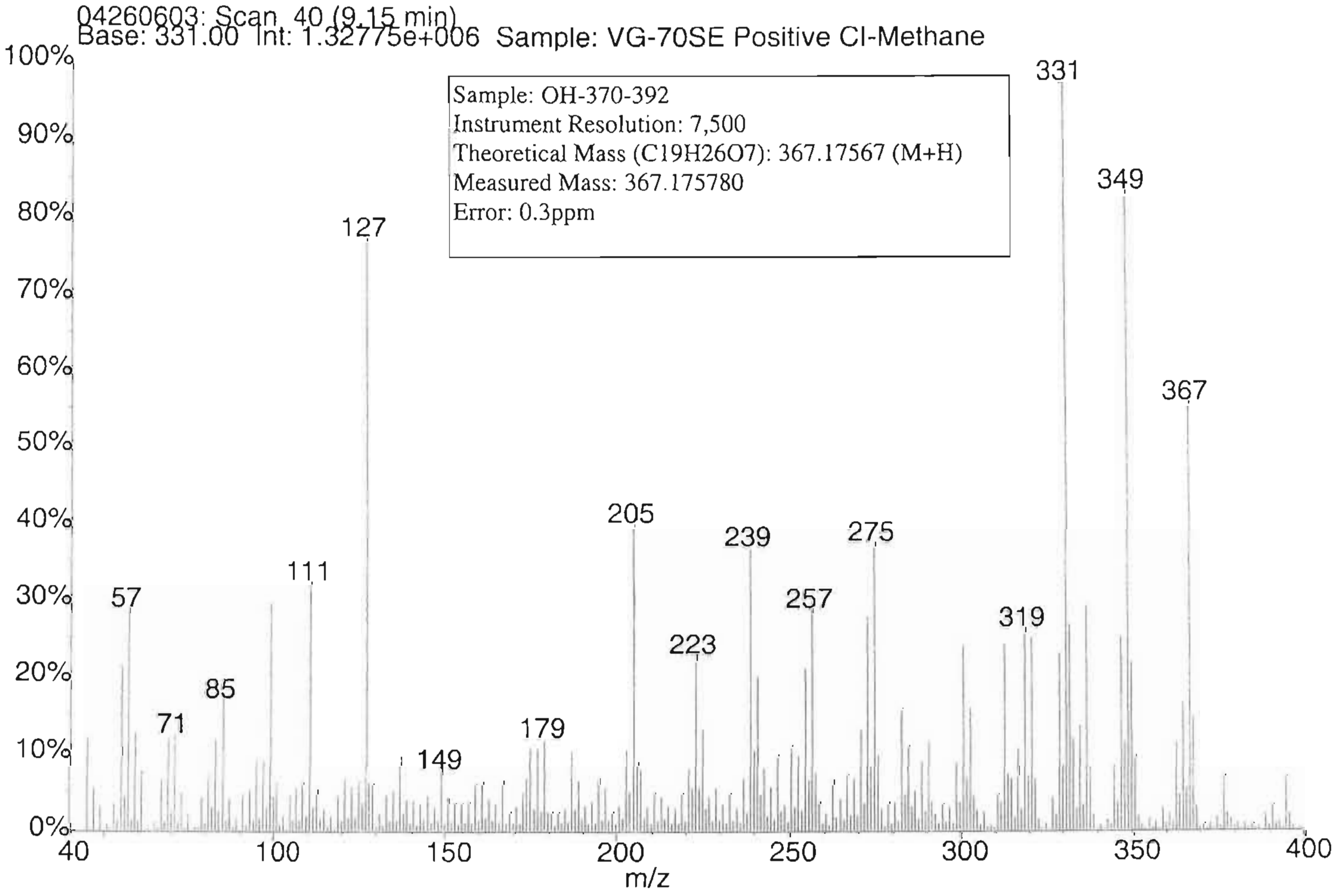
3,4β-Dihydrosamaderine C (vii)



(vii)

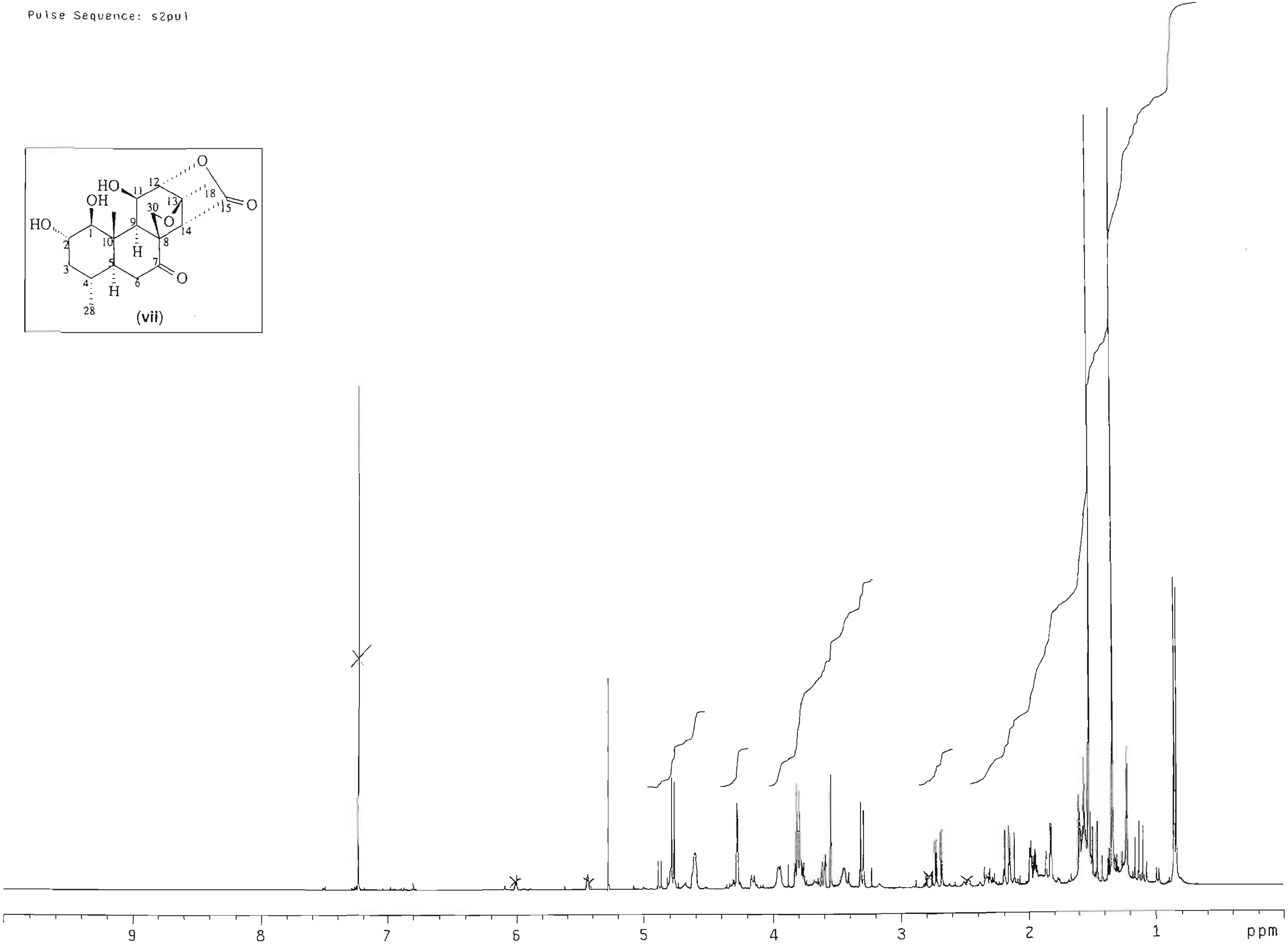


IR Spectrum of 3,4β-Dihydrosamaderine C (vii)

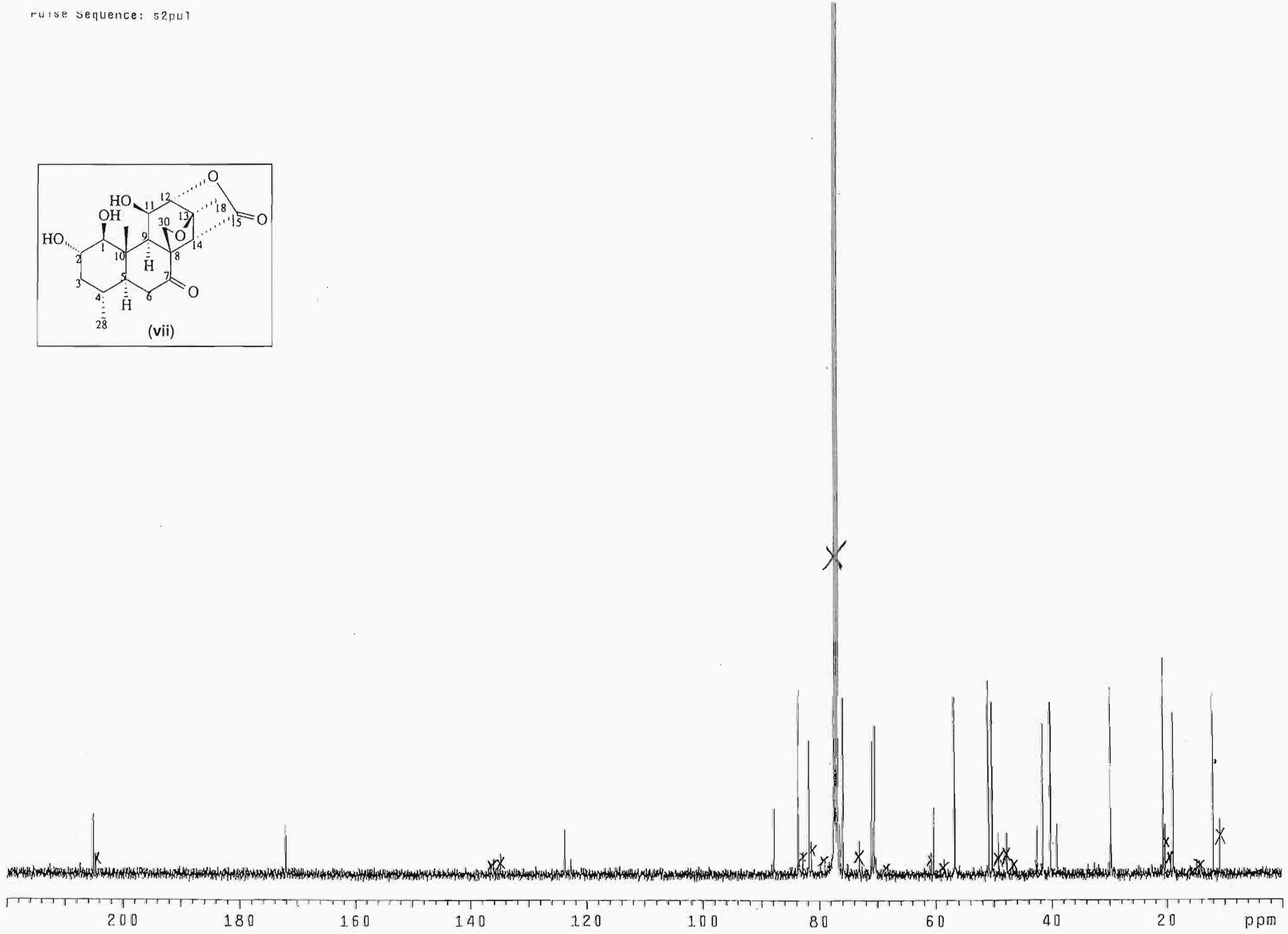


Sample: OH-370-392
Instrument Resolution: 7,500
Theoretical Mass ($C_{19}H_{26}O_7$): 367.17567 (M+H)
Measured Mass: 367.175780
Error: 0.3ppm

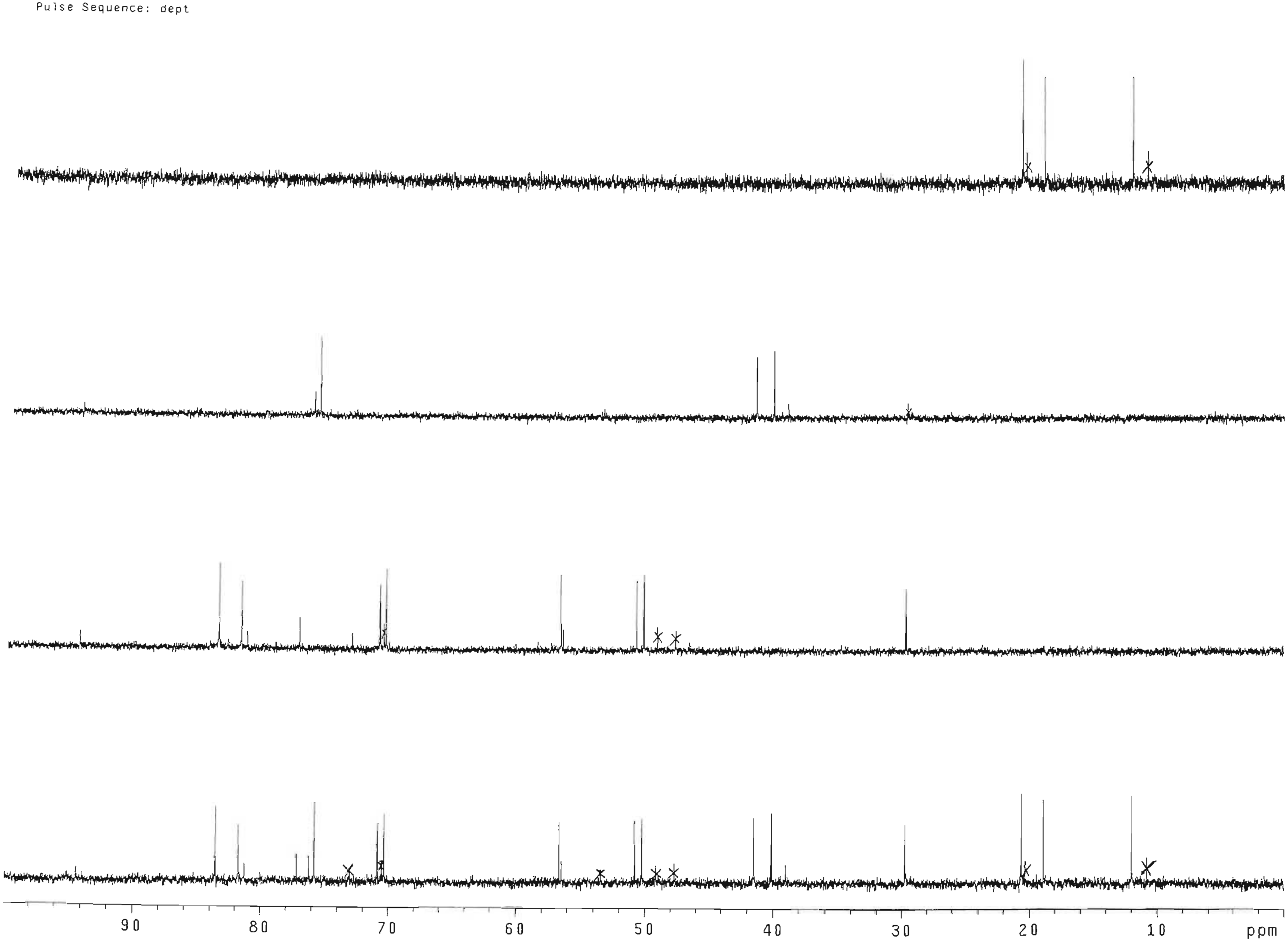
Mass Spectrum of 3,4β-Dihydrosamaderine C (vii)



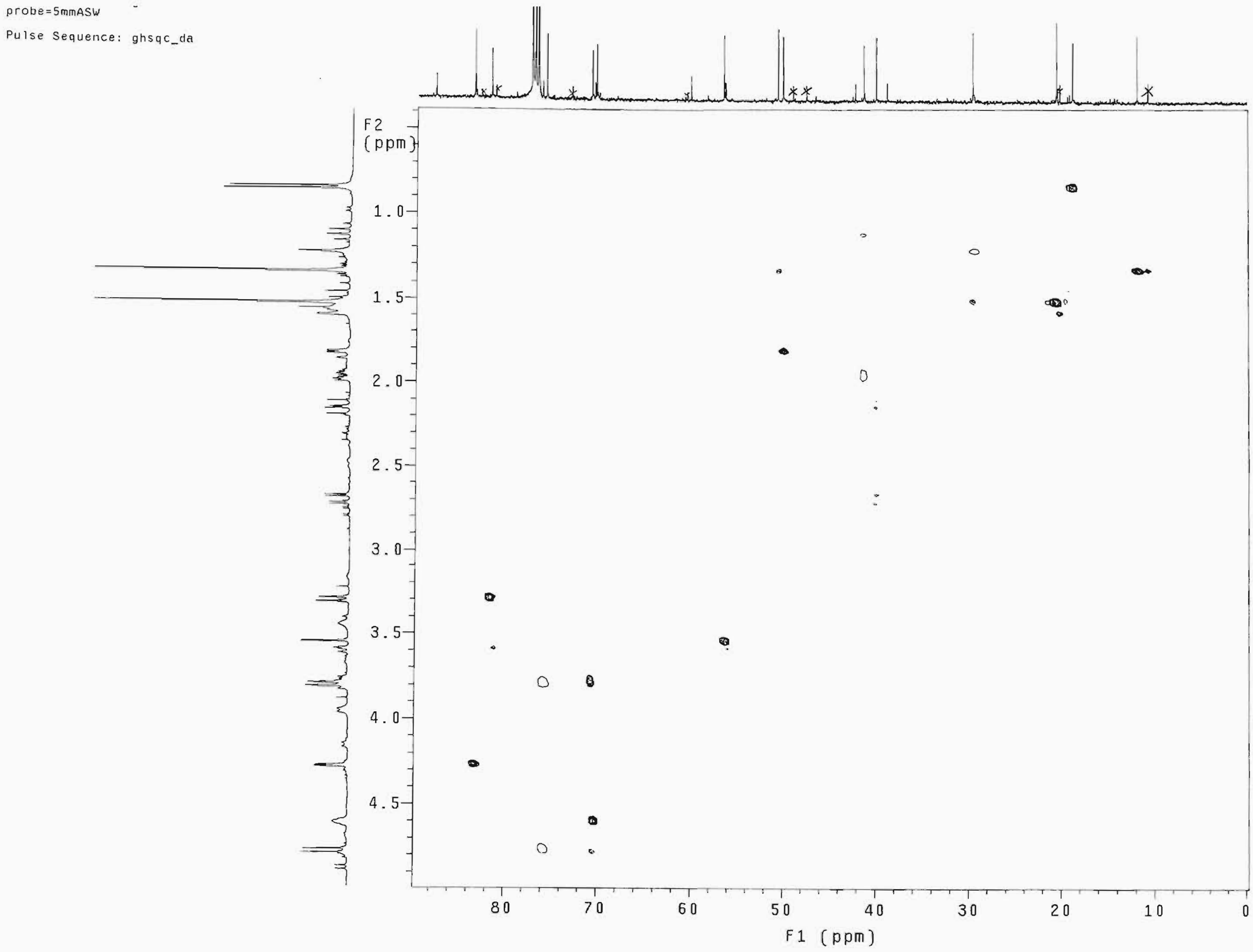
^{1}H NMR Spectrum of 3,4β-Dihydrosamaderine C (vii)



^{13}C NMR Spectrum of 3,4β-Dihydrosamaderine C (vii)

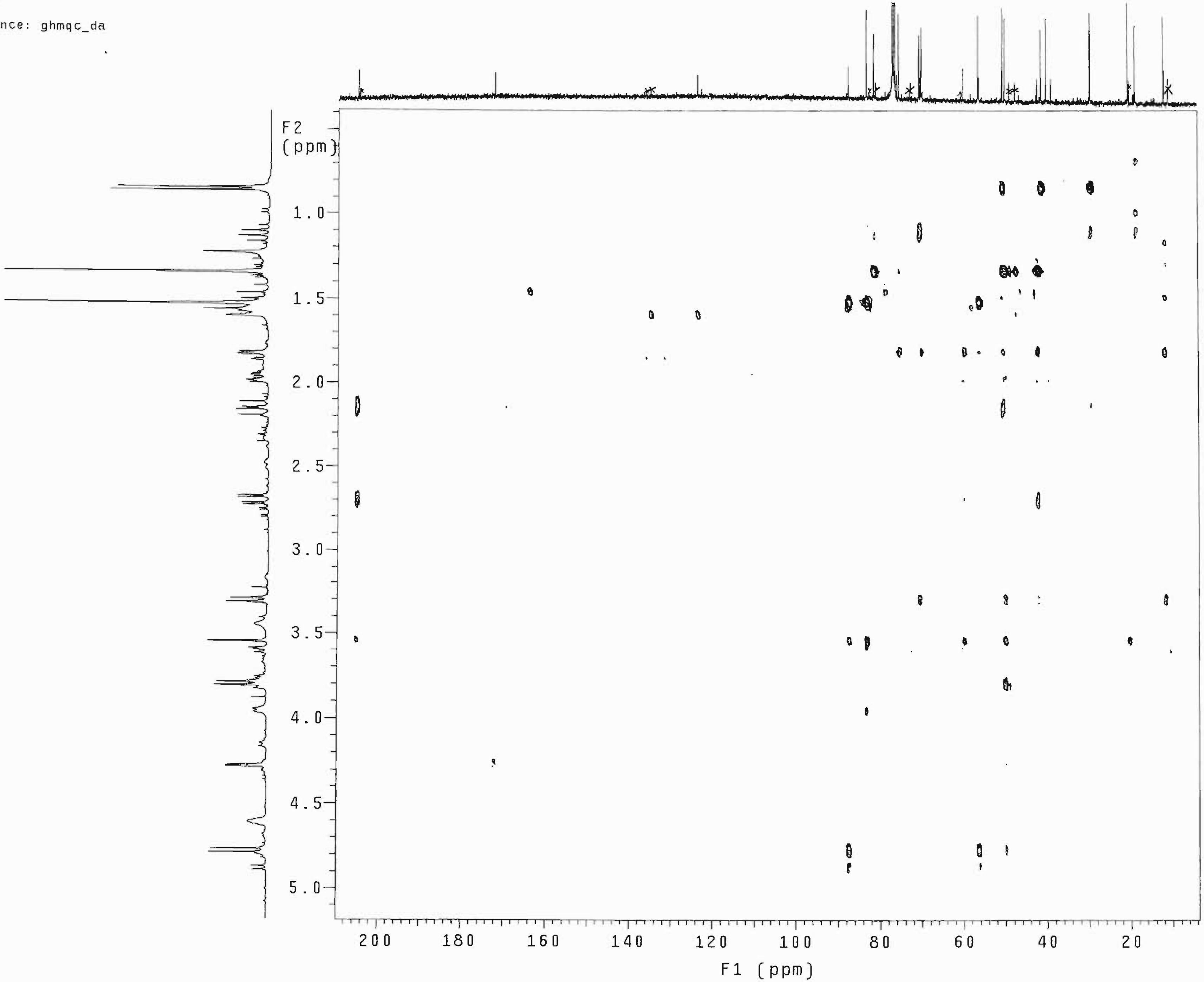


ADEPT NMR Spectrum of 3,4β-Dihydrosamaderine C (vii)

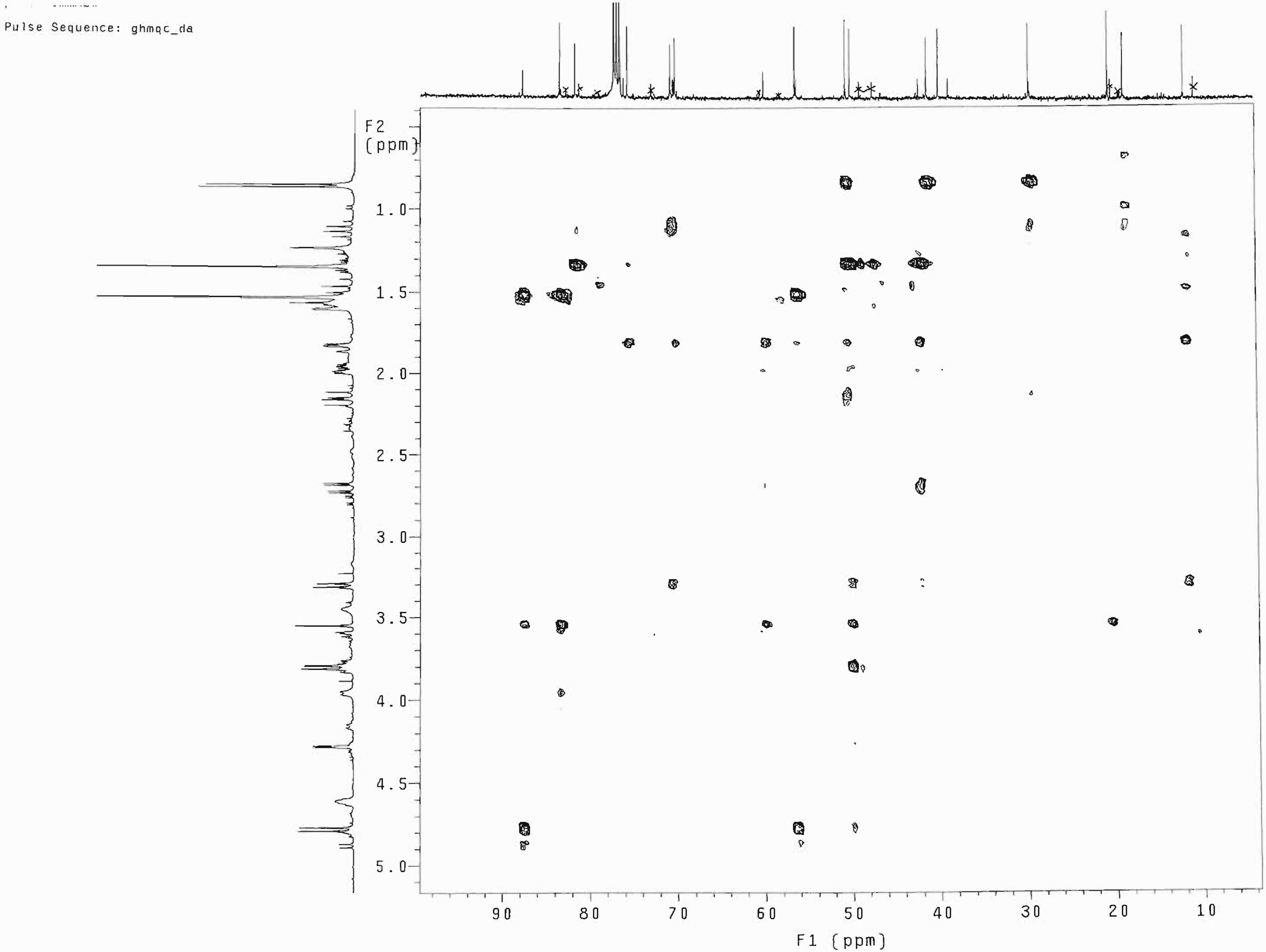


HSQC NMR Spectrum of 3,4β-Dihydrosamaderine C (vii)

Pulse Sequence: ghmqc_da



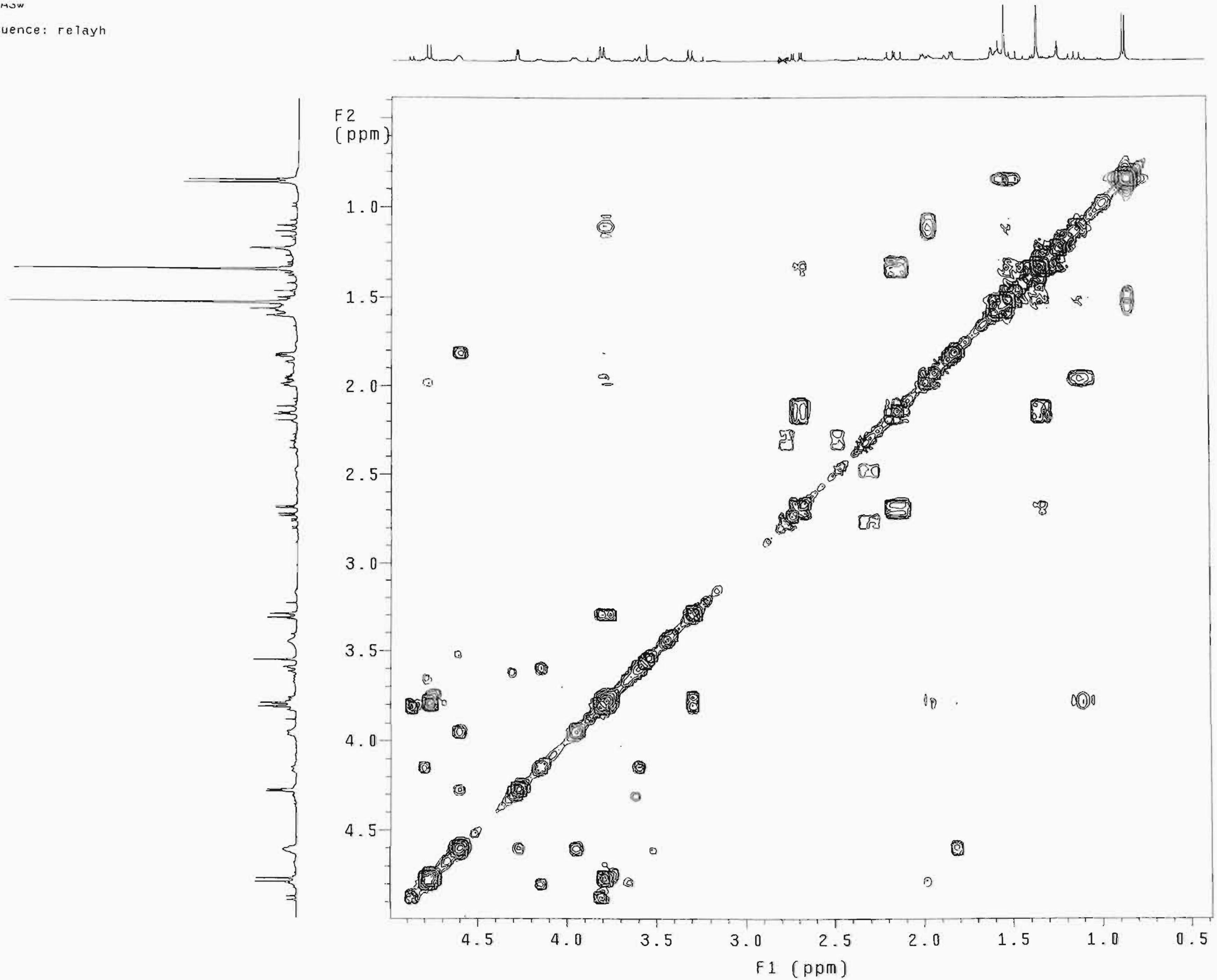
HMBC NMR Spectrum of 3,4β-Dihydrocemodorine C (vii)



HMBC NMR Spectrum of 3,4β-Dihydroasamedorine C (vii)

probe=5mmASW

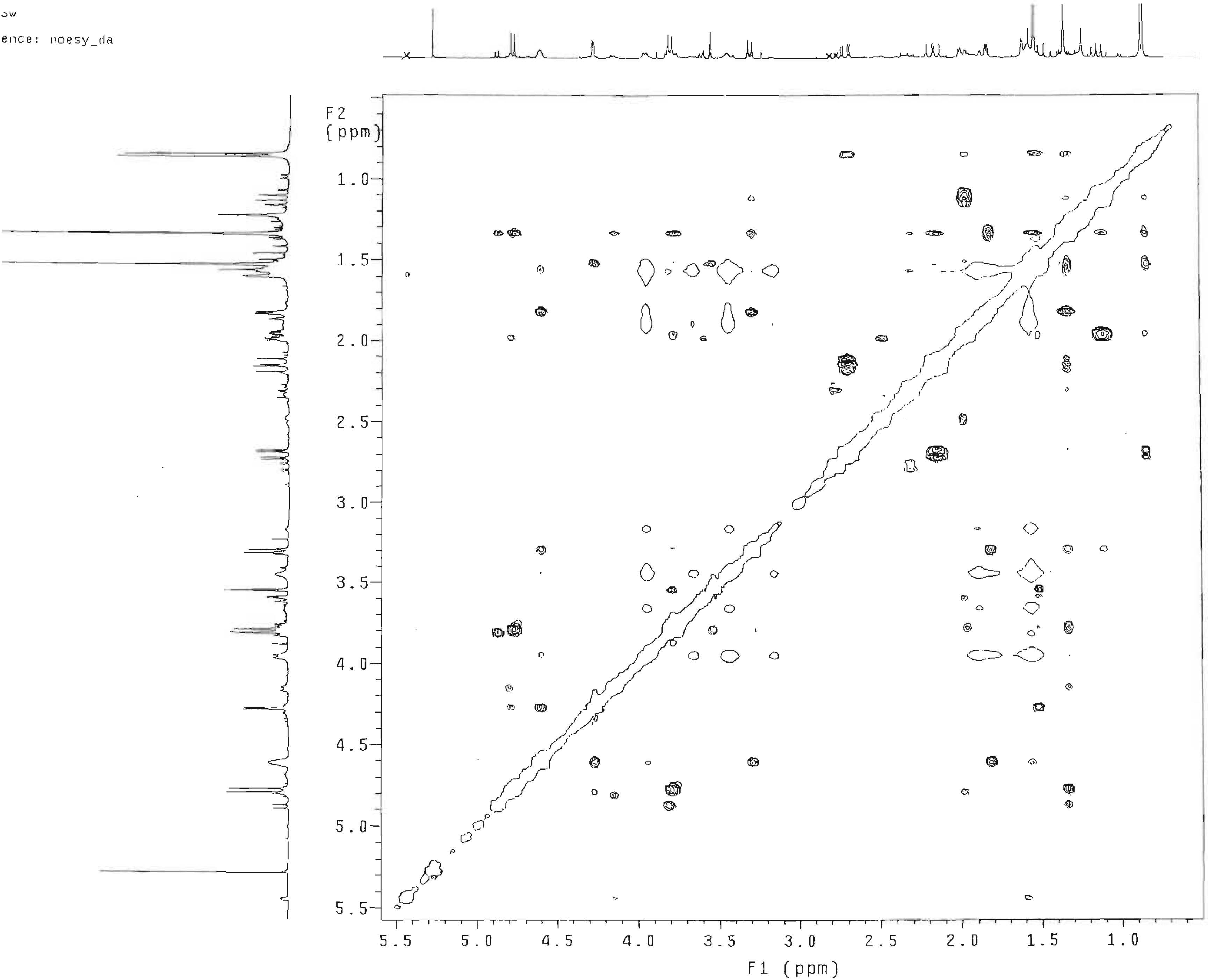
Pulse Sequence: relayh



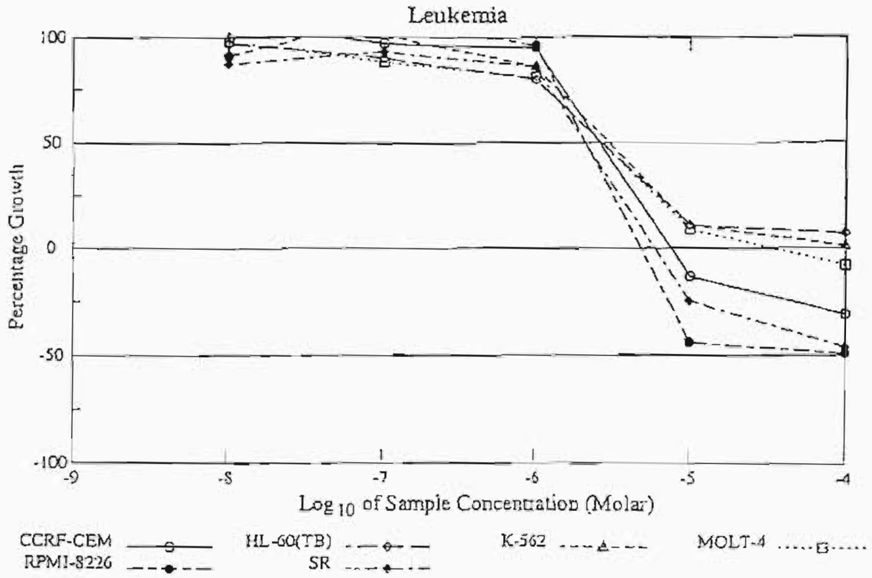
COSY NMR Spectrum of 3,4β-Dihydrosamaderine C (vii)

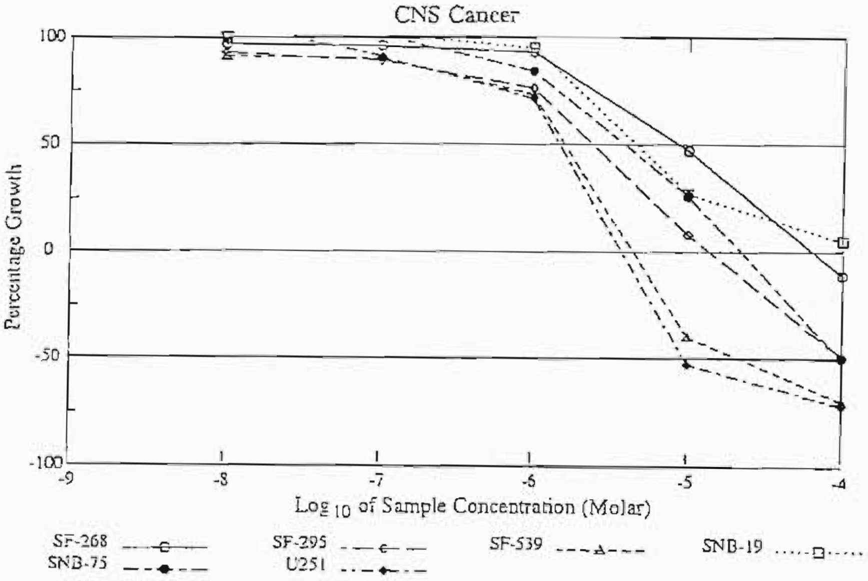
probe=5mmASW

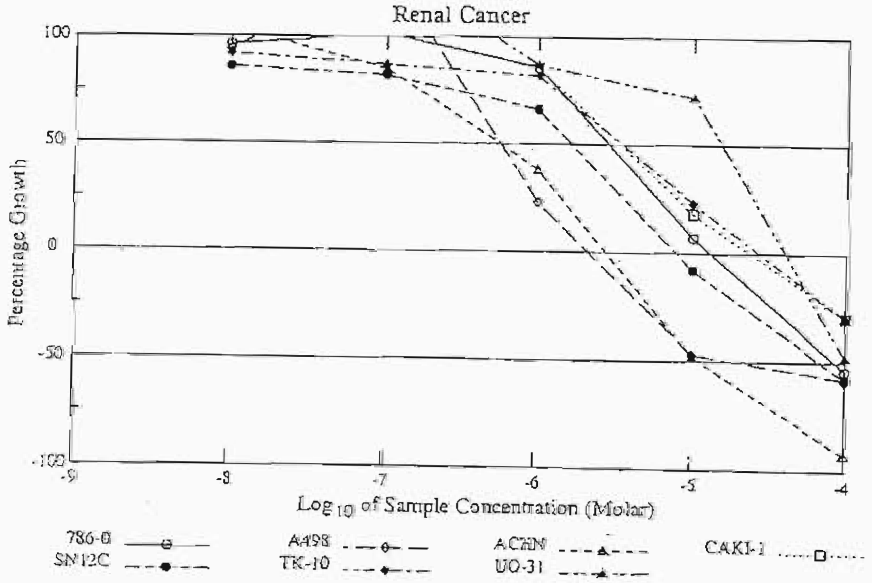
Pulse Sequence: noesy_da



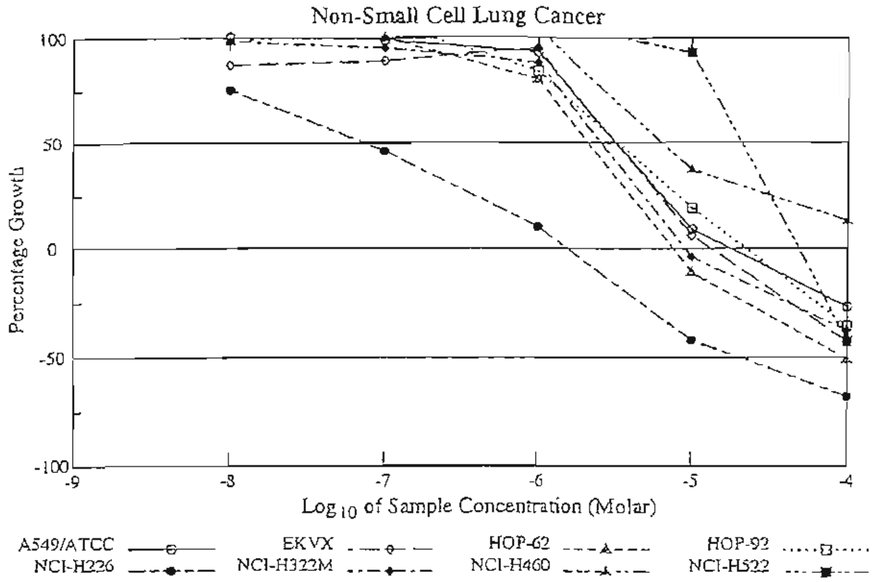
NOESY NMR Spectrum of 3,4β-Dihydrosamaderine C (vii)

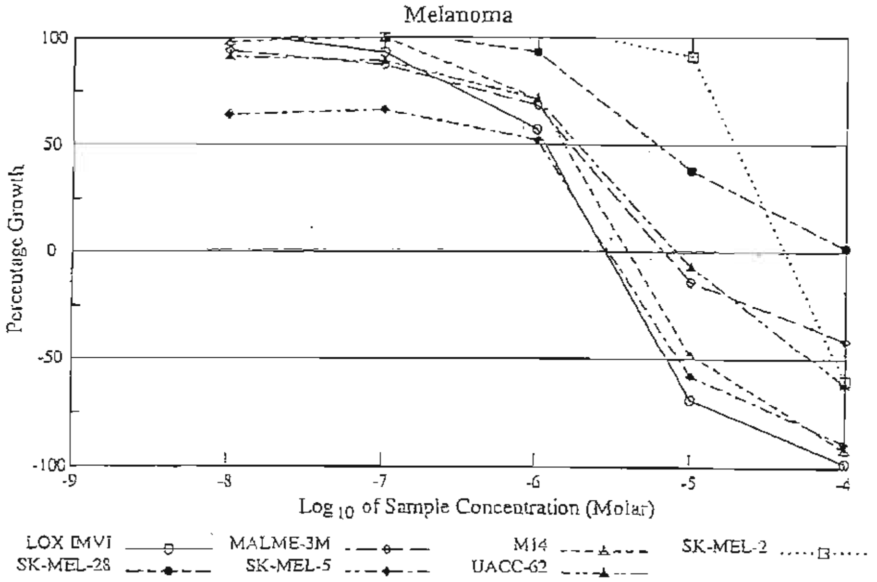


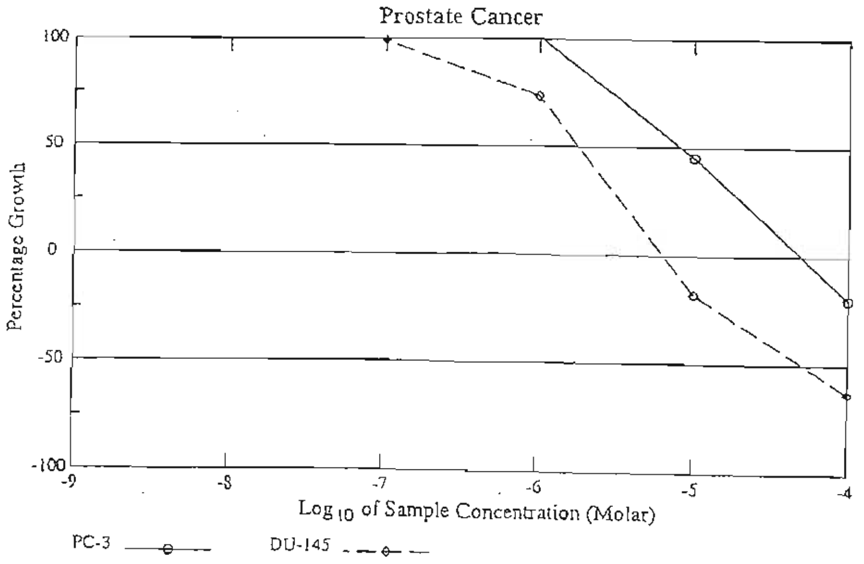




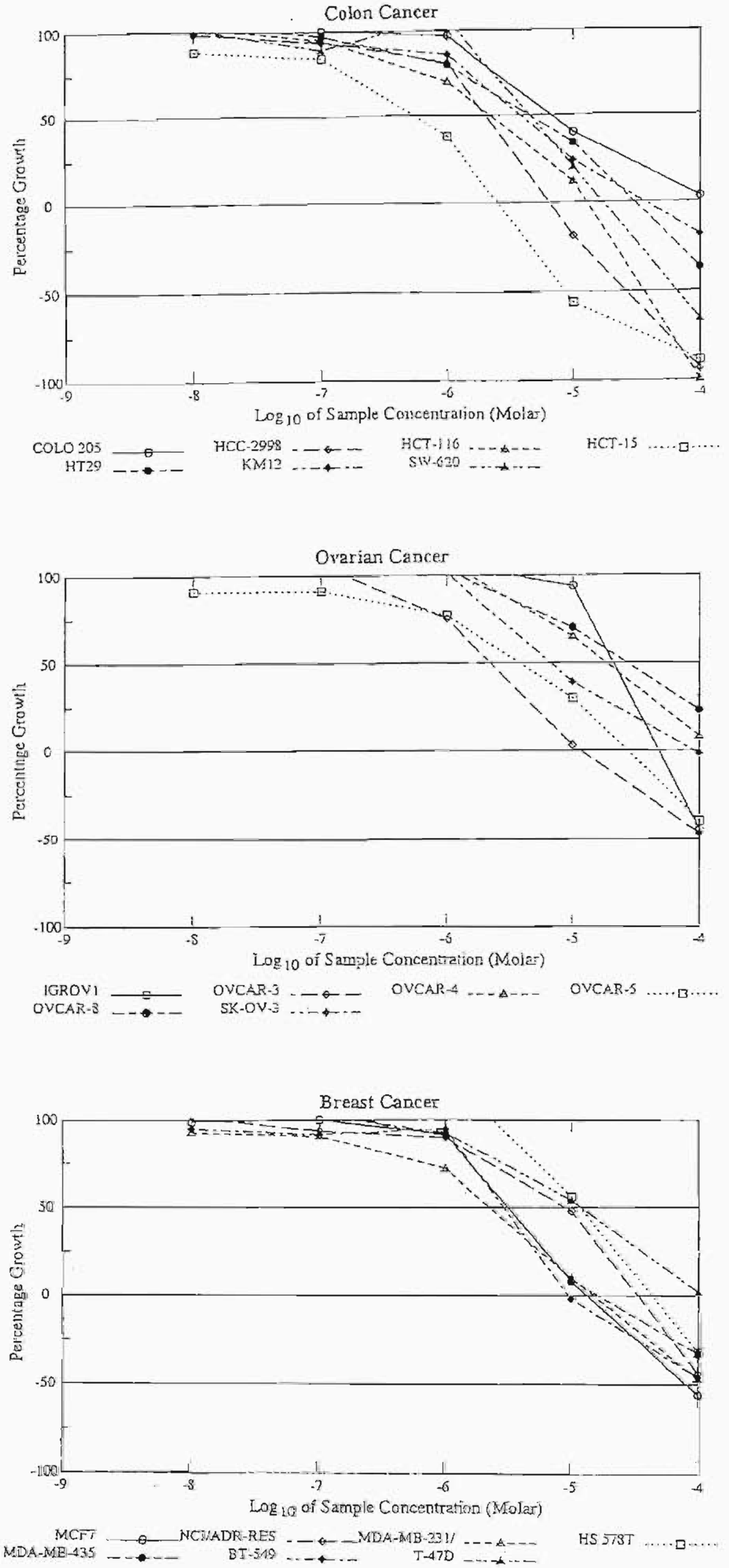
Dose Response Curves for 3,4β-Dihydrosamaderine C (vii)





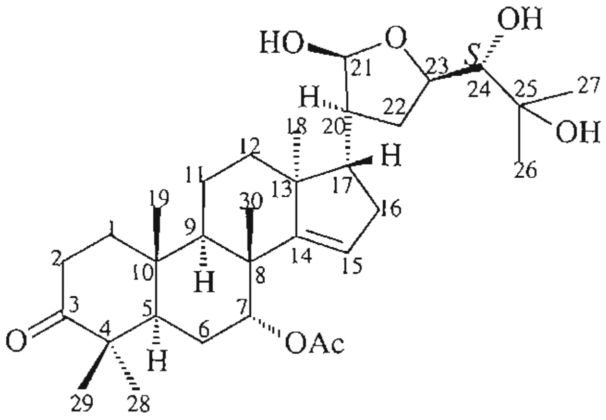


Dose Response Curves for 3,4β-Dihydrosamaderine C (vii)

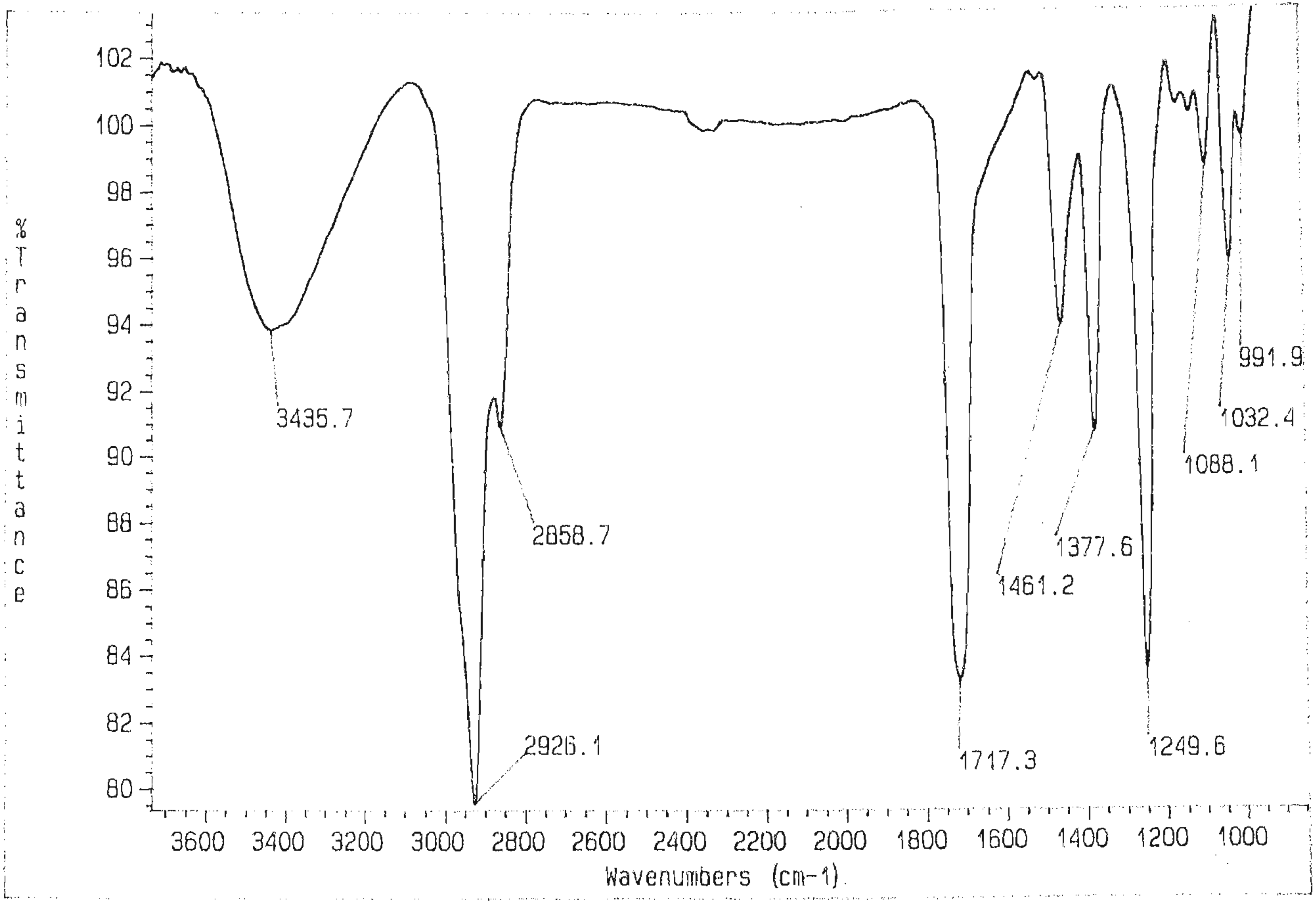


Dose Response Curves for 3,4β-Dihydrosamaderine C (vii)

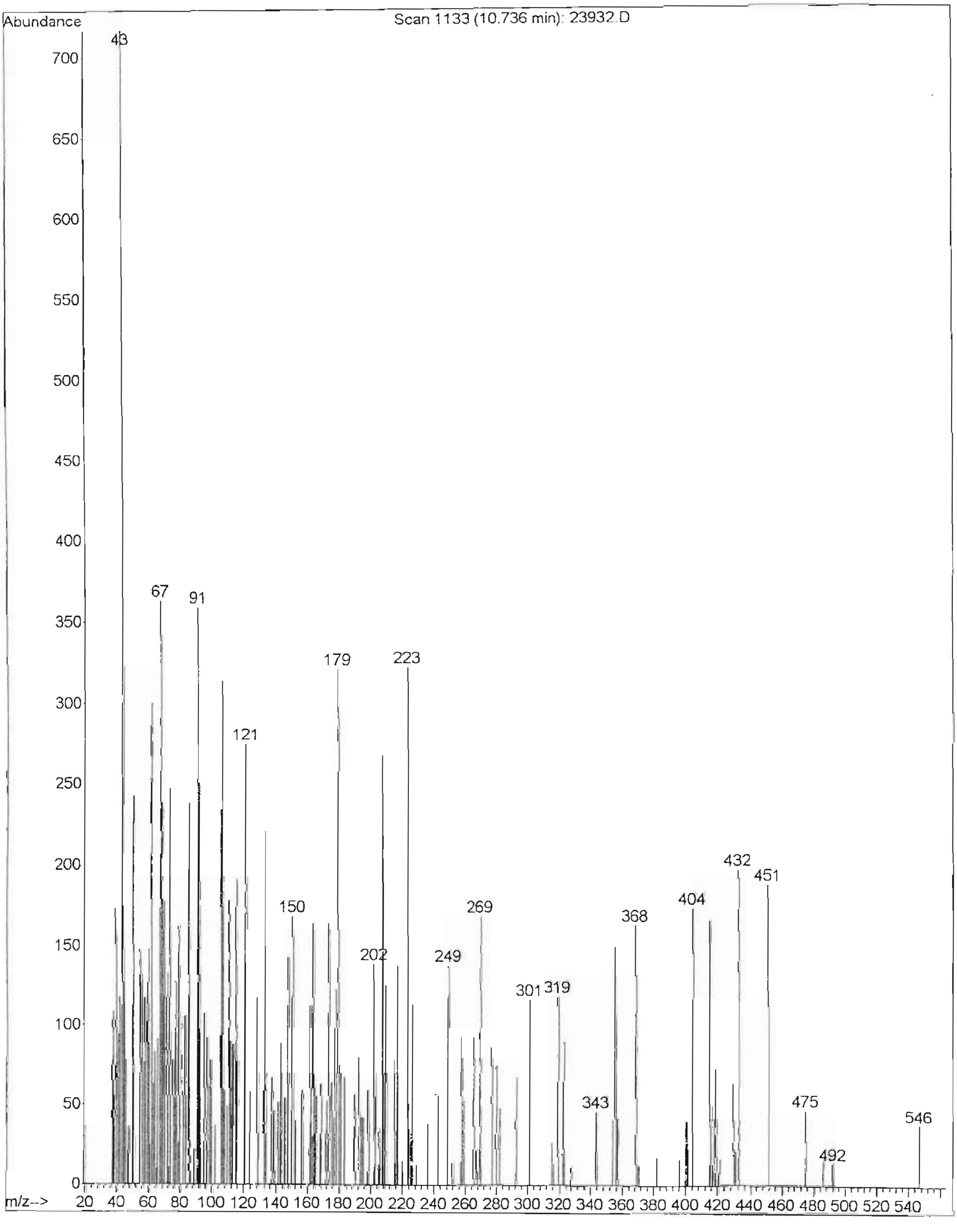
Protosamaderine A (viii)



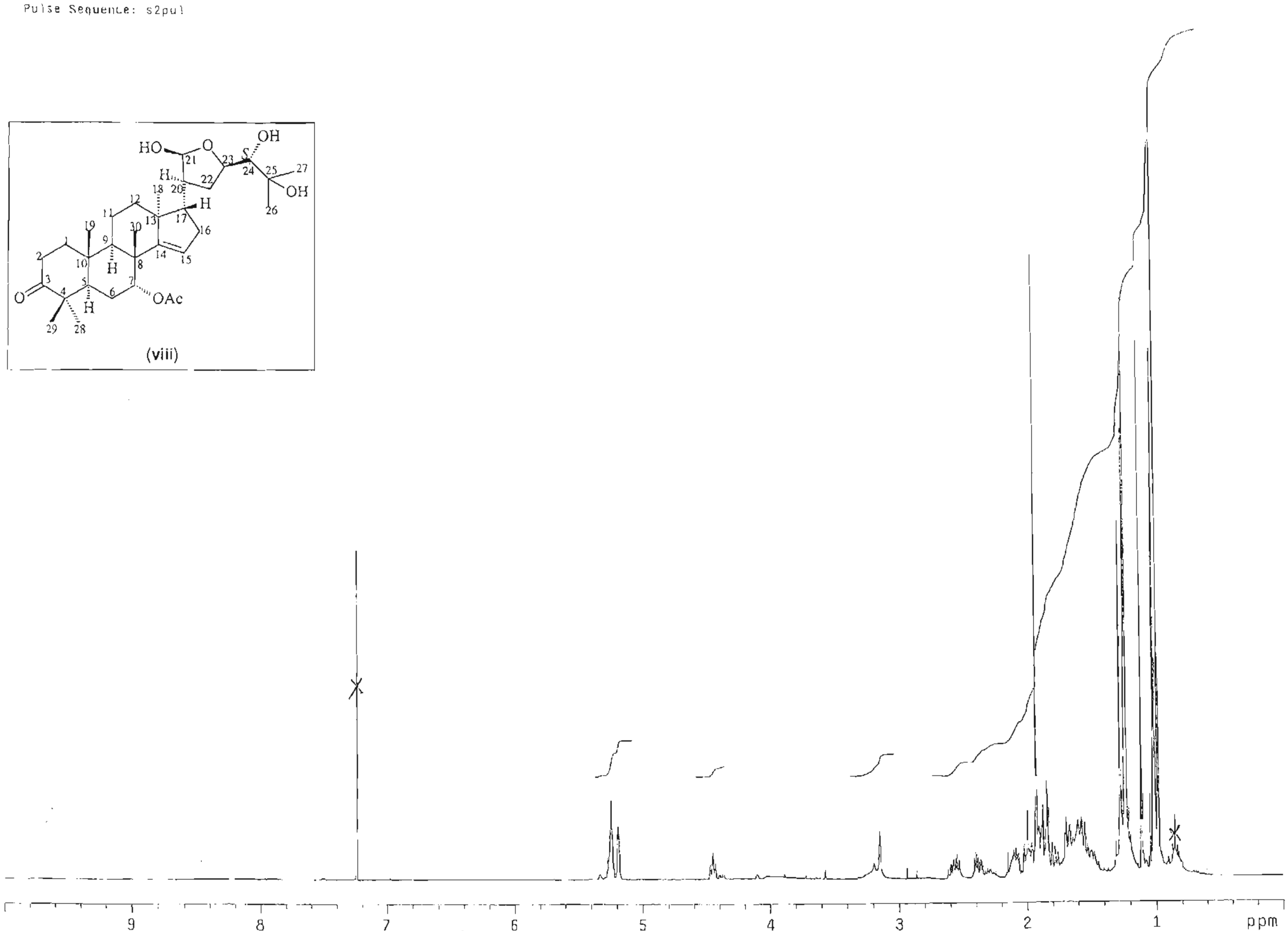
(viii)



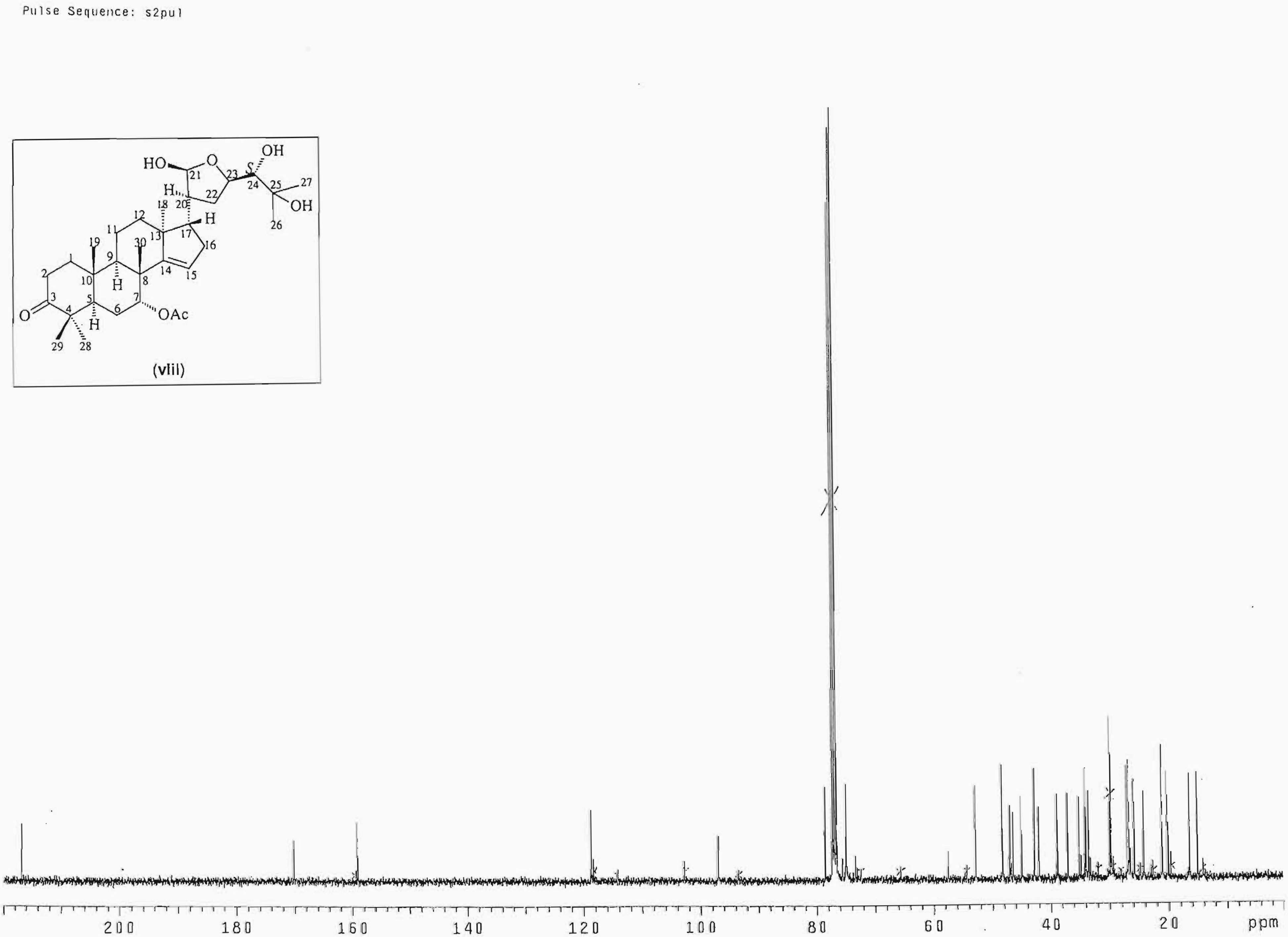
IR Spectrum of Protosamaderine A (viii)



Mass Spectrum of Protosamaderine A (viii)



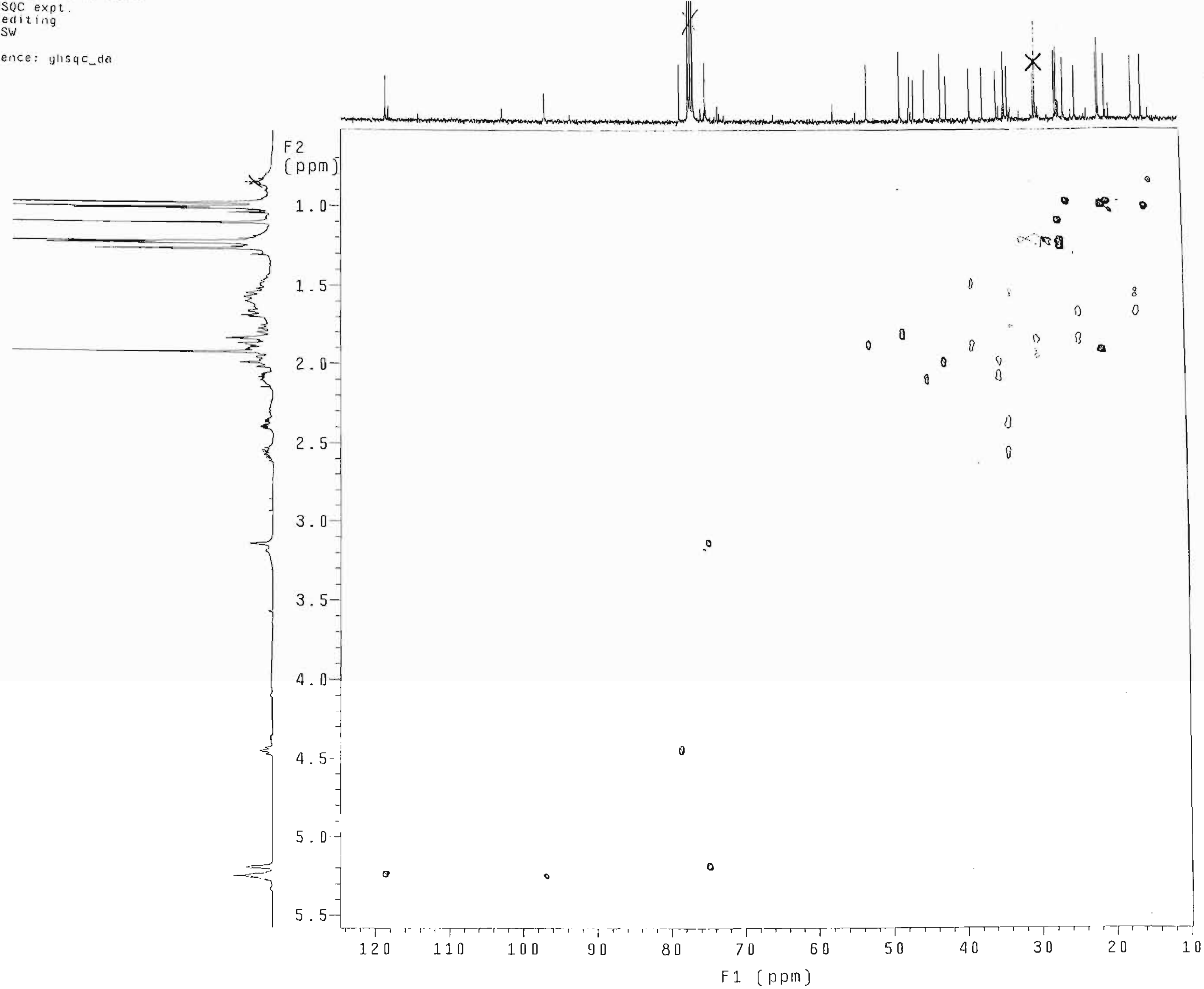
^{1}H NMR Spectrum of Protosamaderine A (viii)



^{13}C NMR Spectrum of Protosamaderine A (viii)

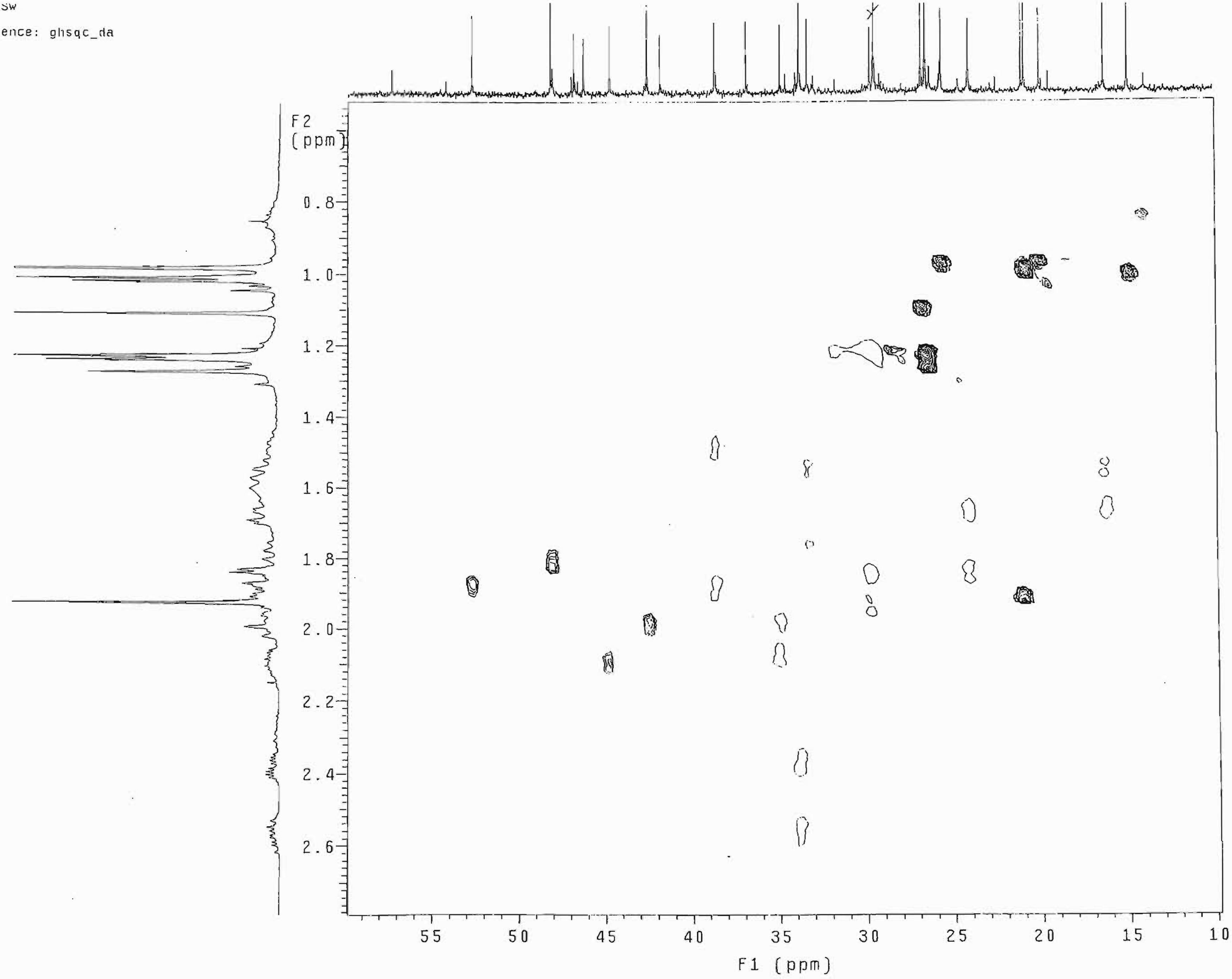
HQsm32.Sm-H-239-3-2 in cdcl3
Gradient HSQC expt.
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da

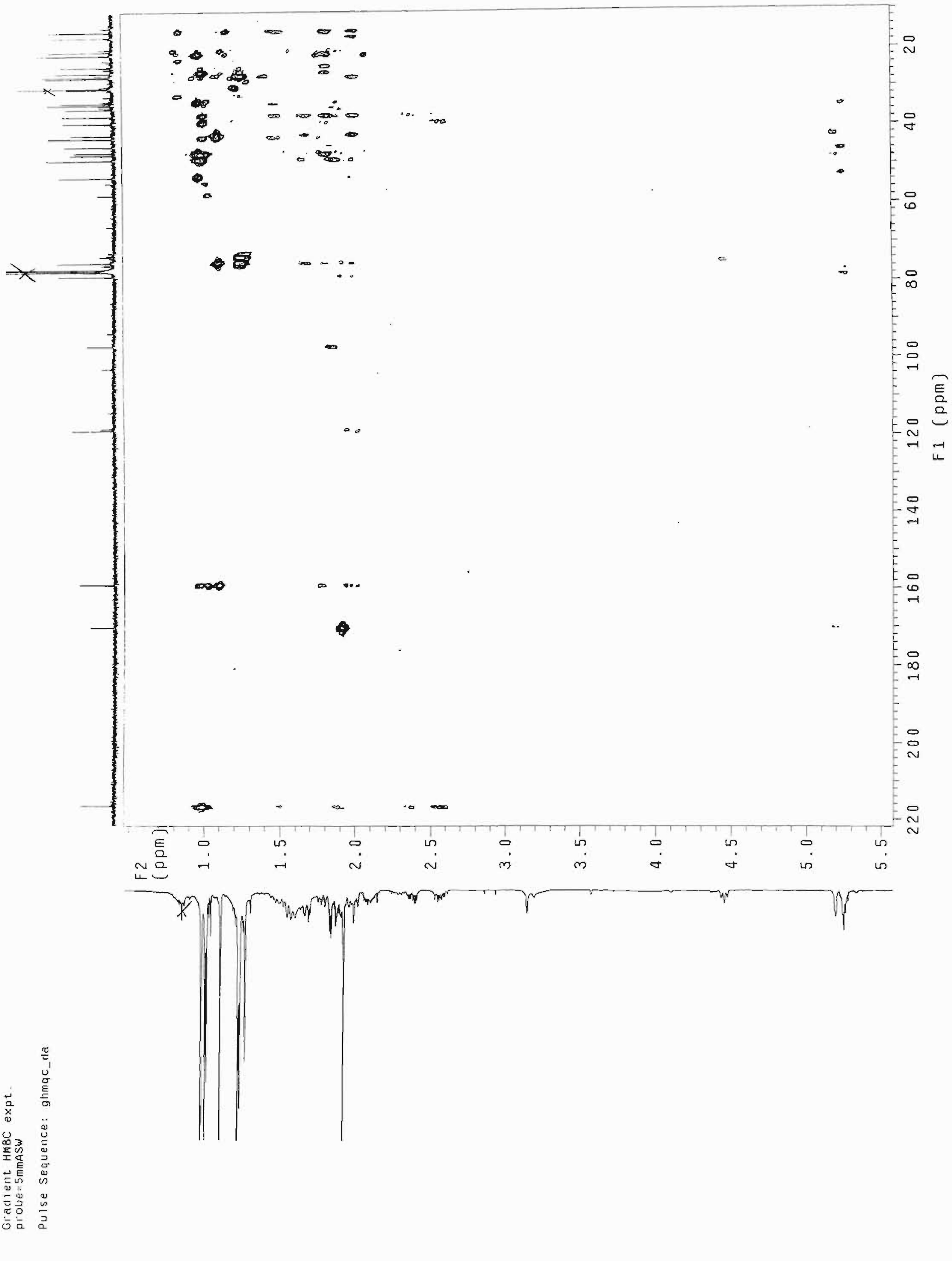


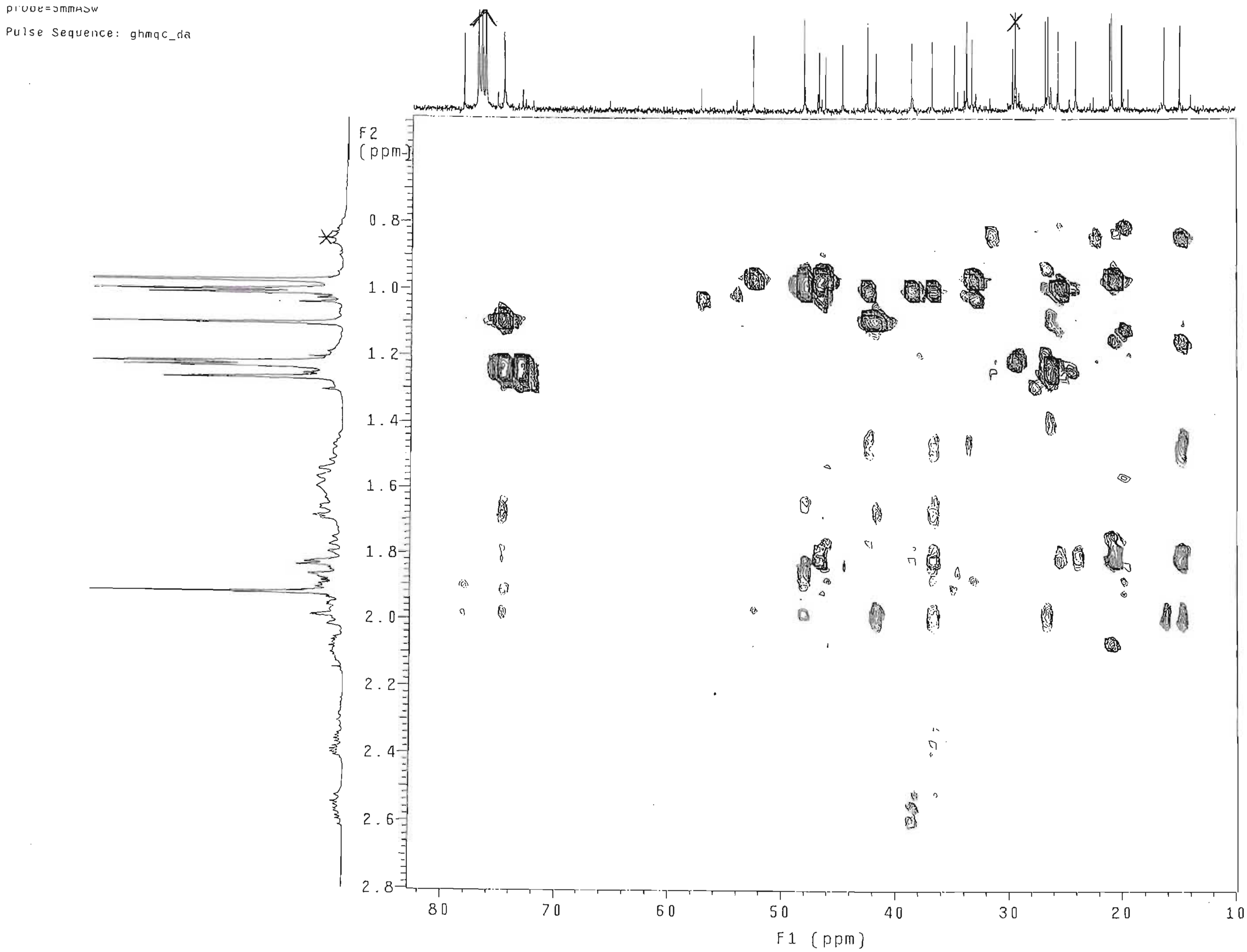
probe=5mmASW

Pulse Sequence: ghsqc_da

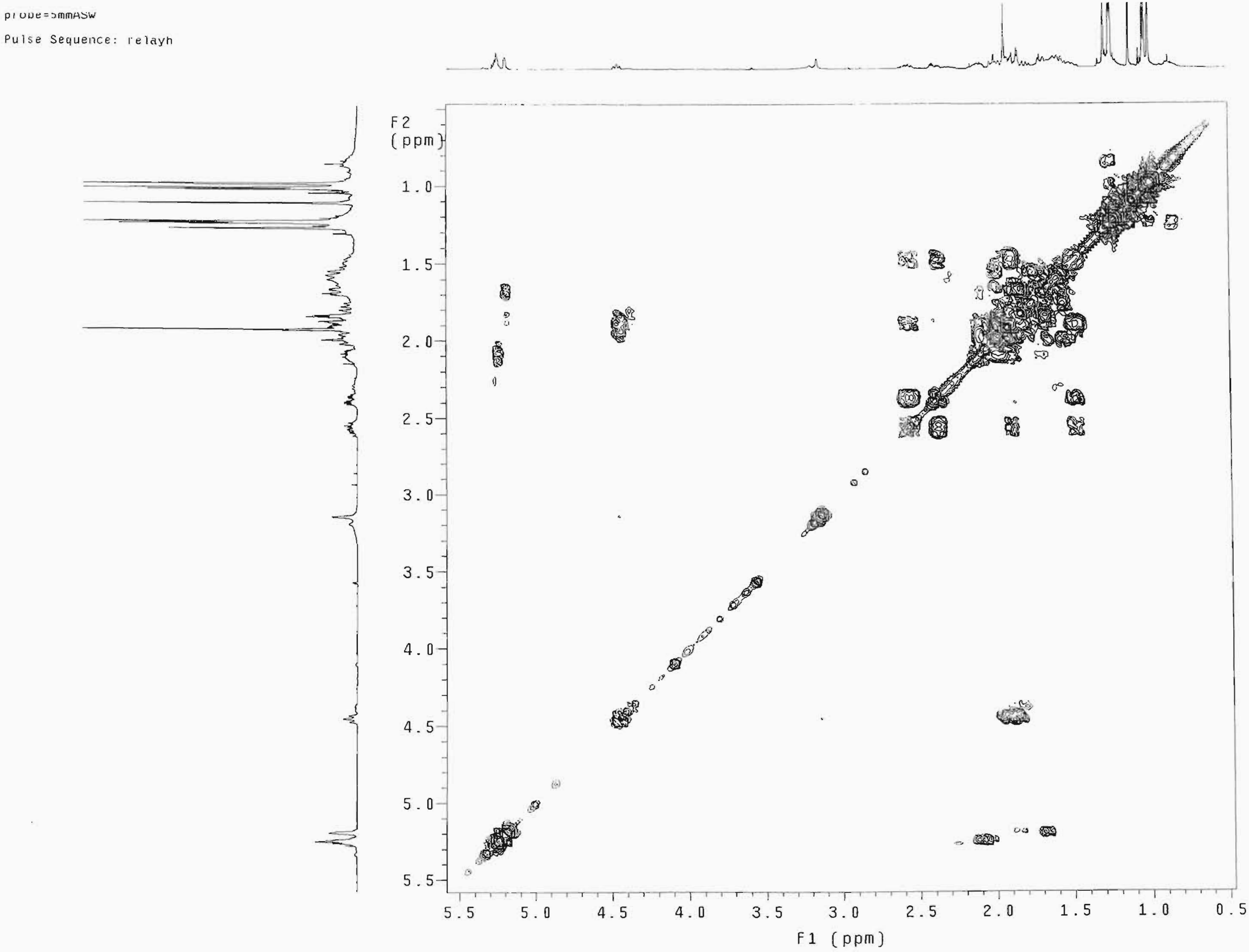


HSQC NMR Spectrum of Protoesmaderine A (viii)


Gradient HMBC expt.
probe=5mmASW
Pulse Sequence: ghmqc_da
F2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
F1 (ppm)
20
40
60
80
100
120
140
160
180
200
220



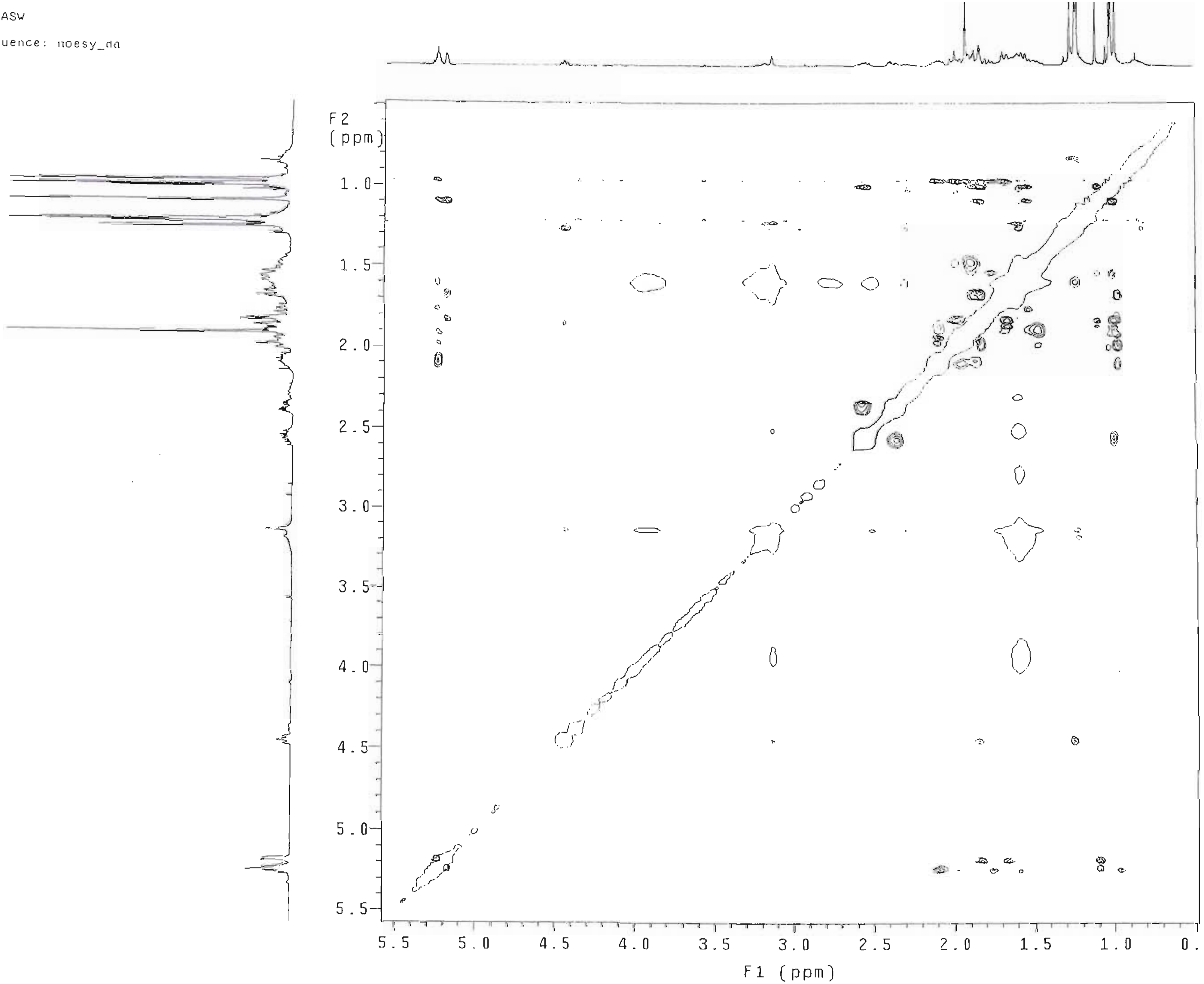
HMBC NMR Spectrum of Protosamaderine A (viii)



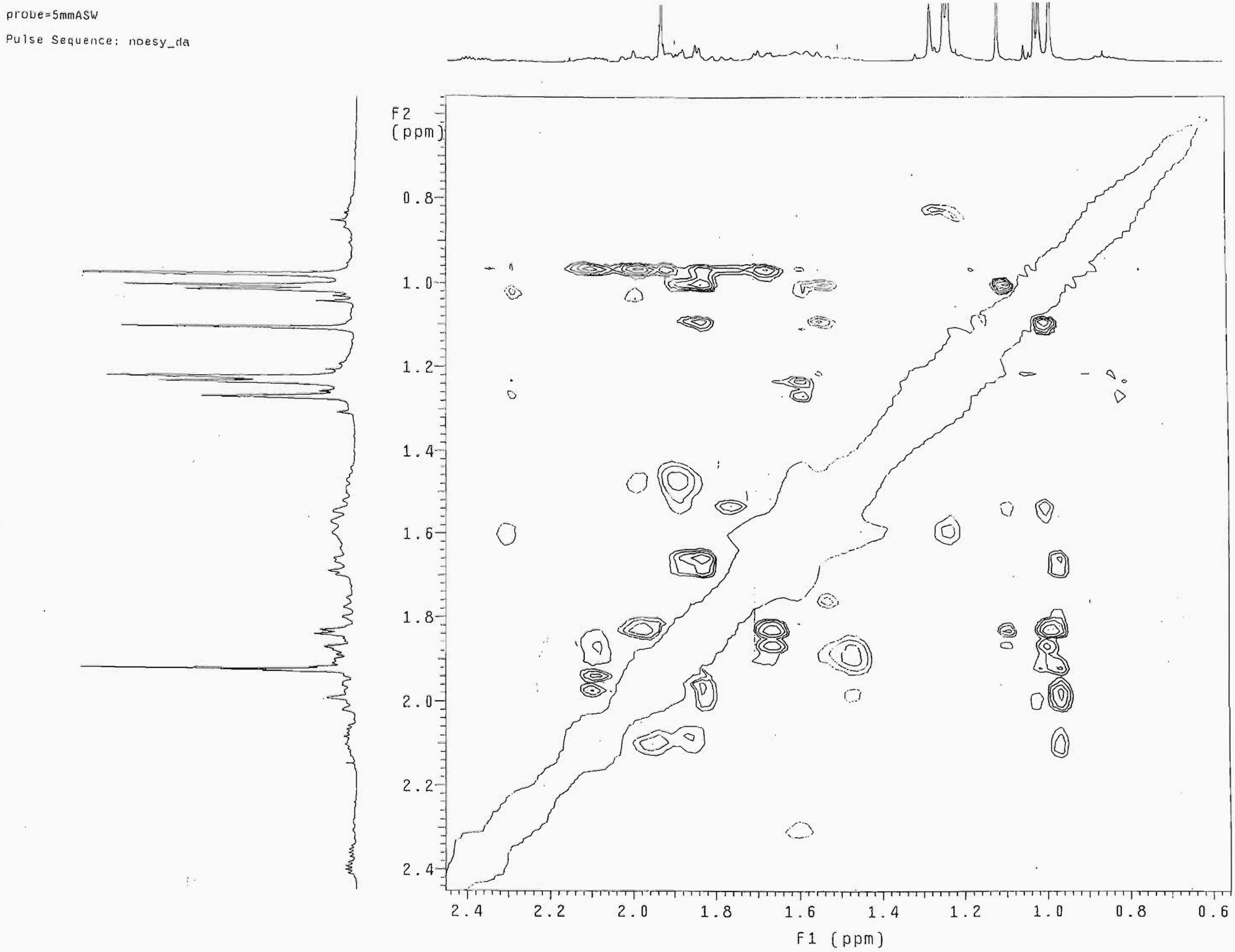
COSY NMR Spectrum of Protoasmaderine A (viii)

probe=5mmASW

Pulse Sequence: noesy_da

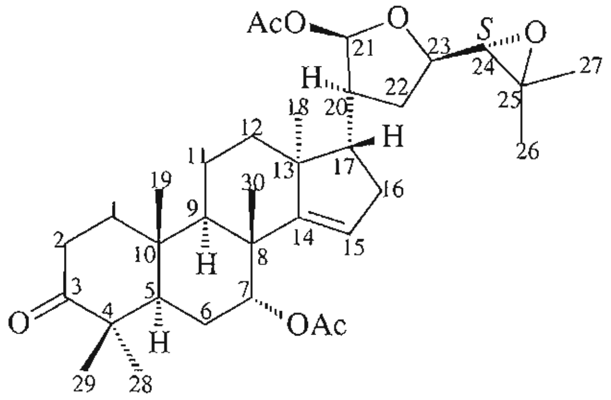


NOESY NMR Spectrum of [illegible] A (viii)

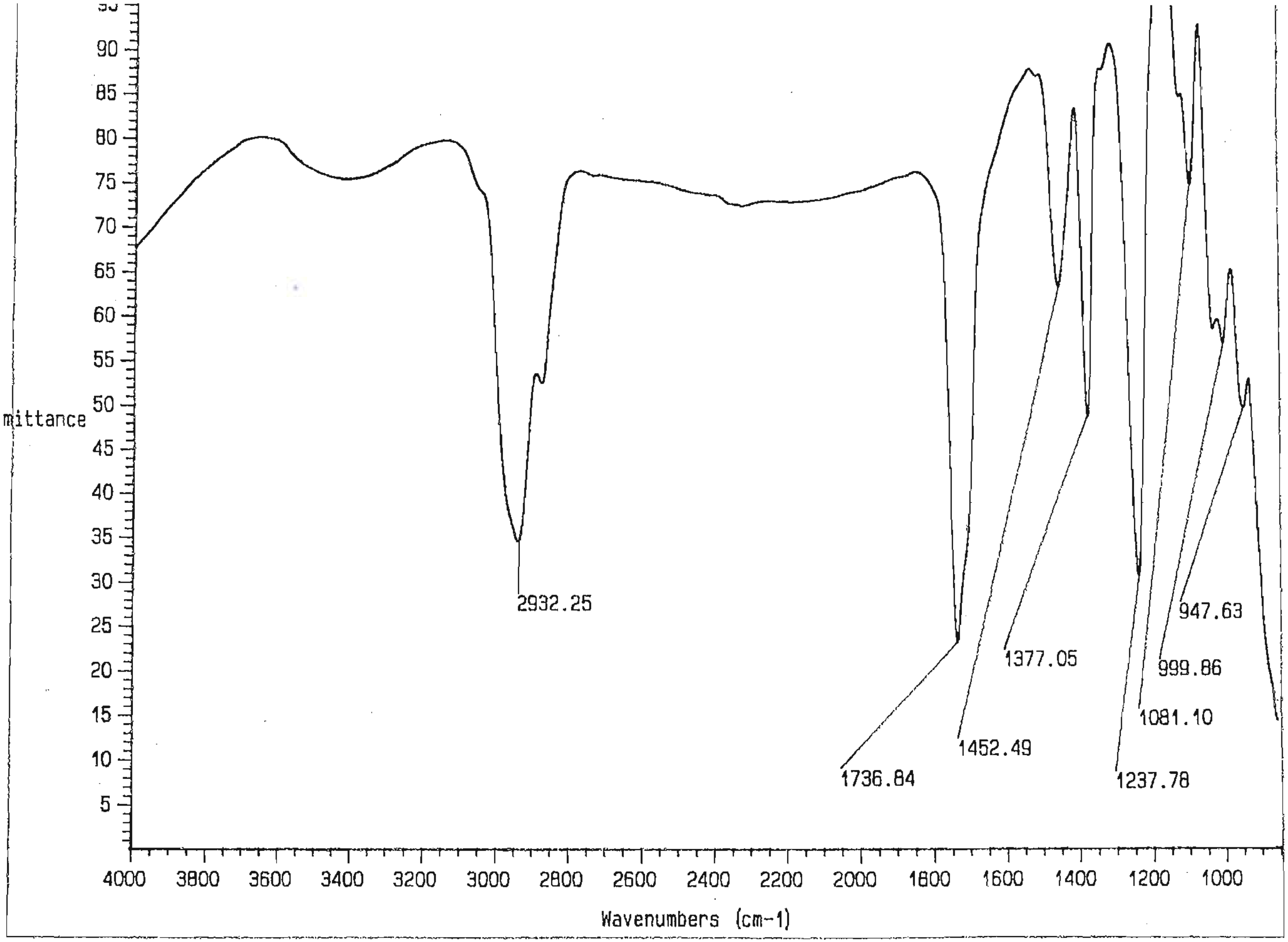


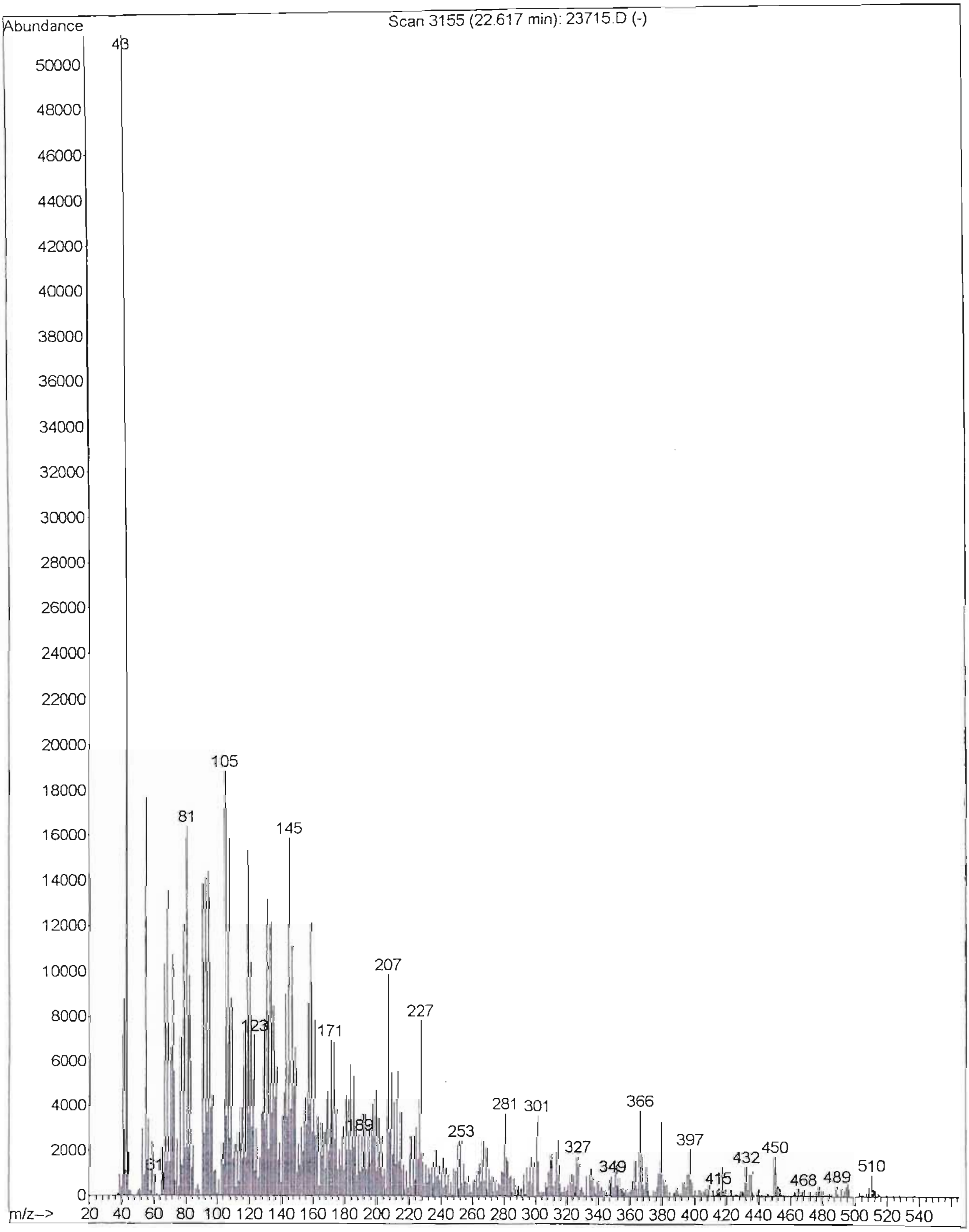
NOESY NMR Spectrum of Protosamaderine A (viii)

1,2-Dihydrobruceajavanin A (ix)

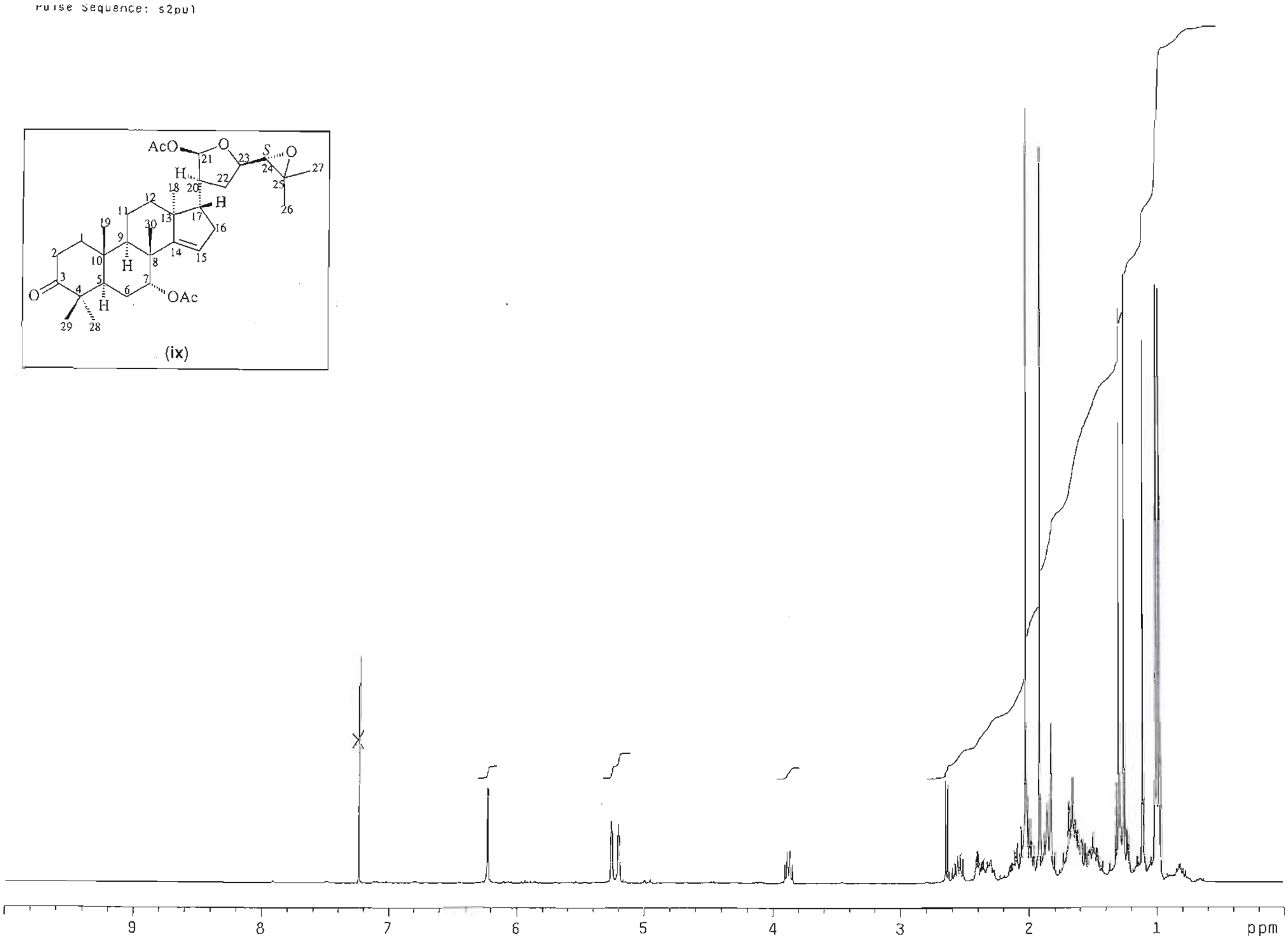


(ix)

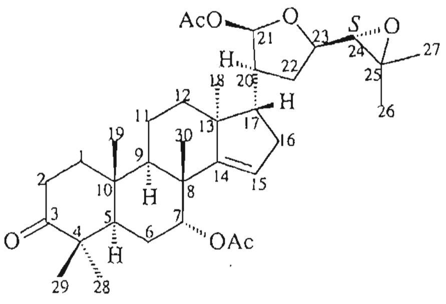

mittance
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
2932.25
1736.84
1452.49
1377.05
1237.78
1081.10
999.86
947.63
4000
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
Wavenumbers (cm-1)



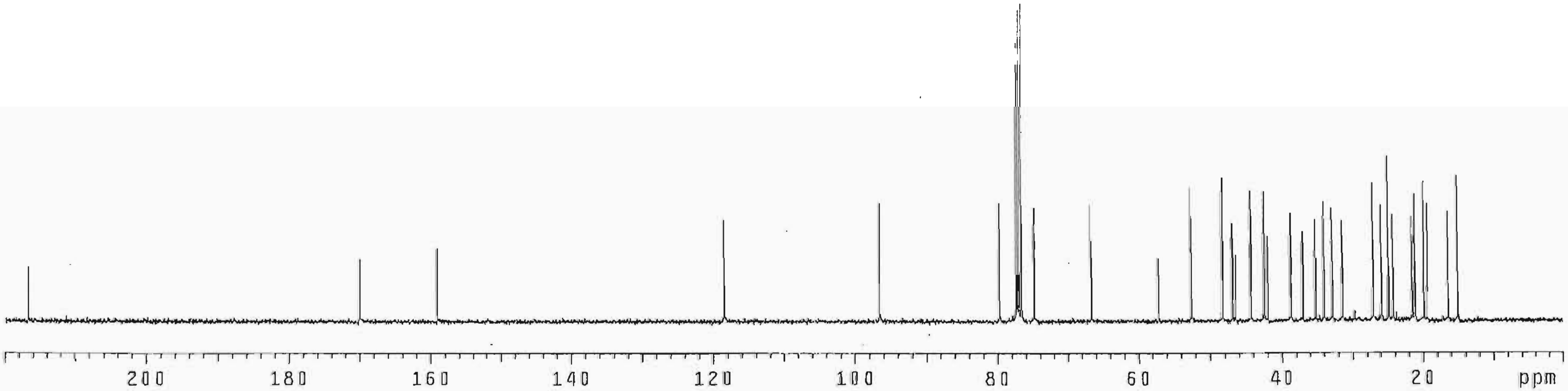
Mass Spectrum of 1,2-Dihydrobruceajavanin A (ix)



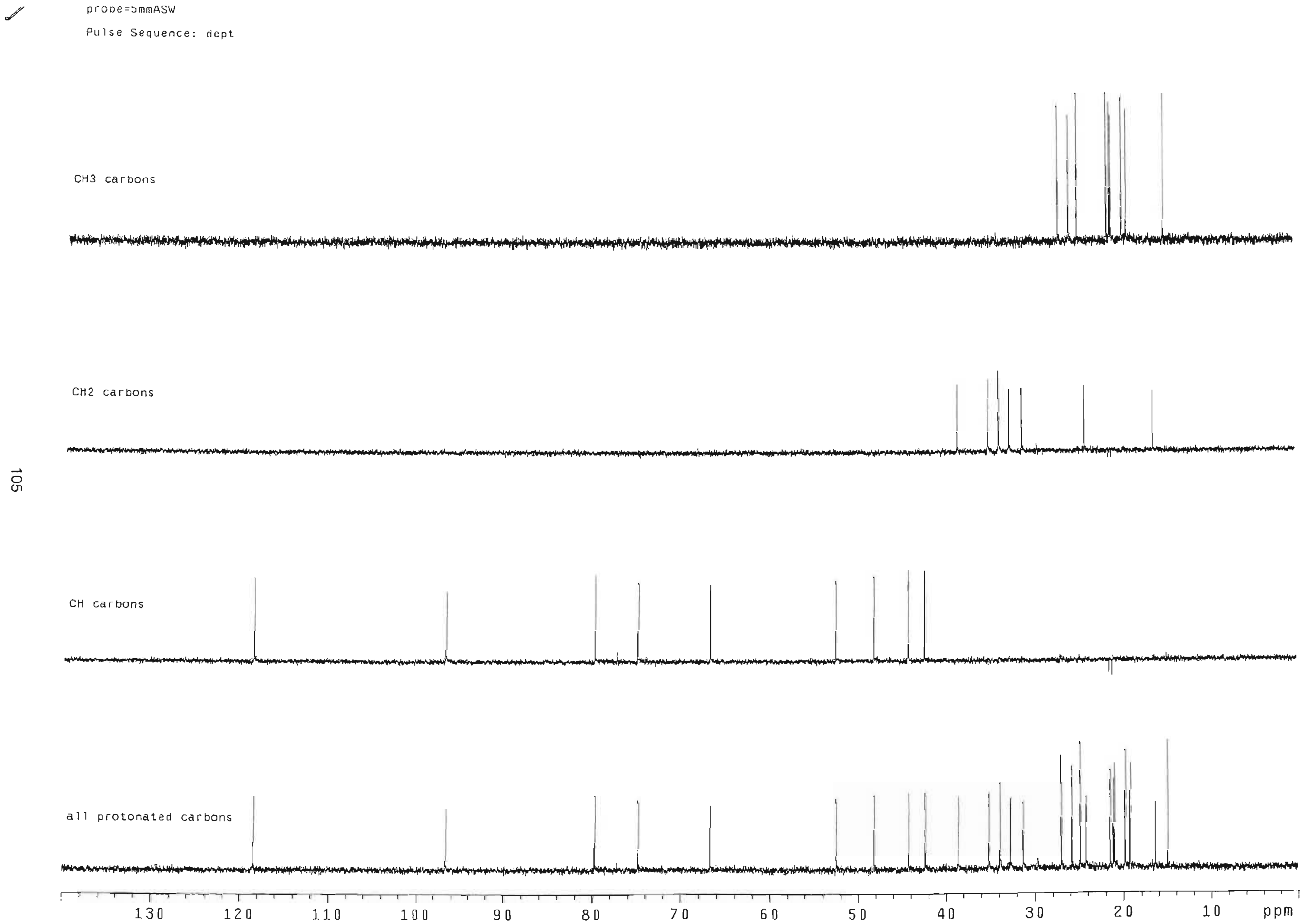
^{1}H NMR Spectrum of 1,2-Dihydrobruceajavanin A (ix)

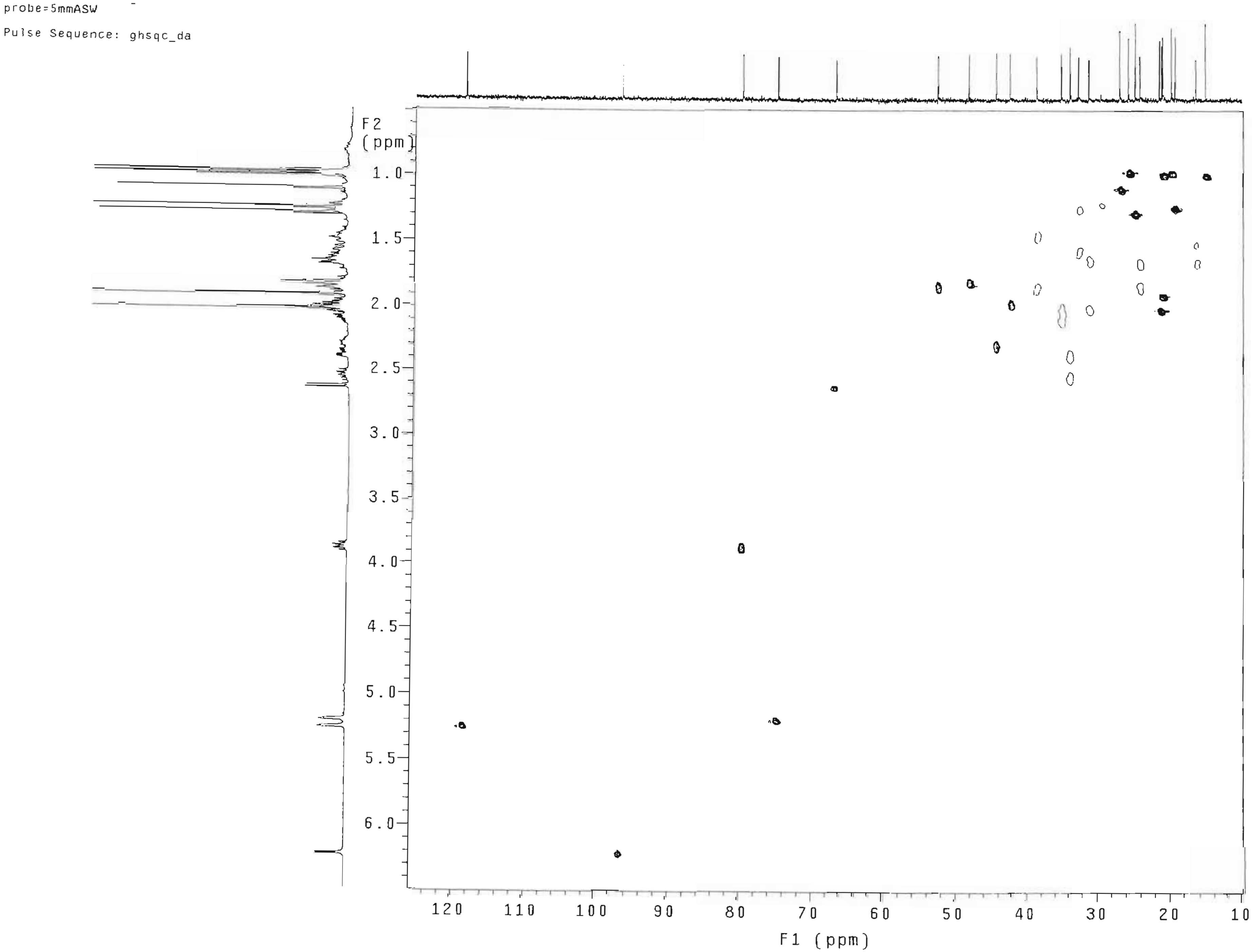


(ix)



^{13}C NMR Spectrum of 1,2-Dihydrobruceajavanin A (ix)

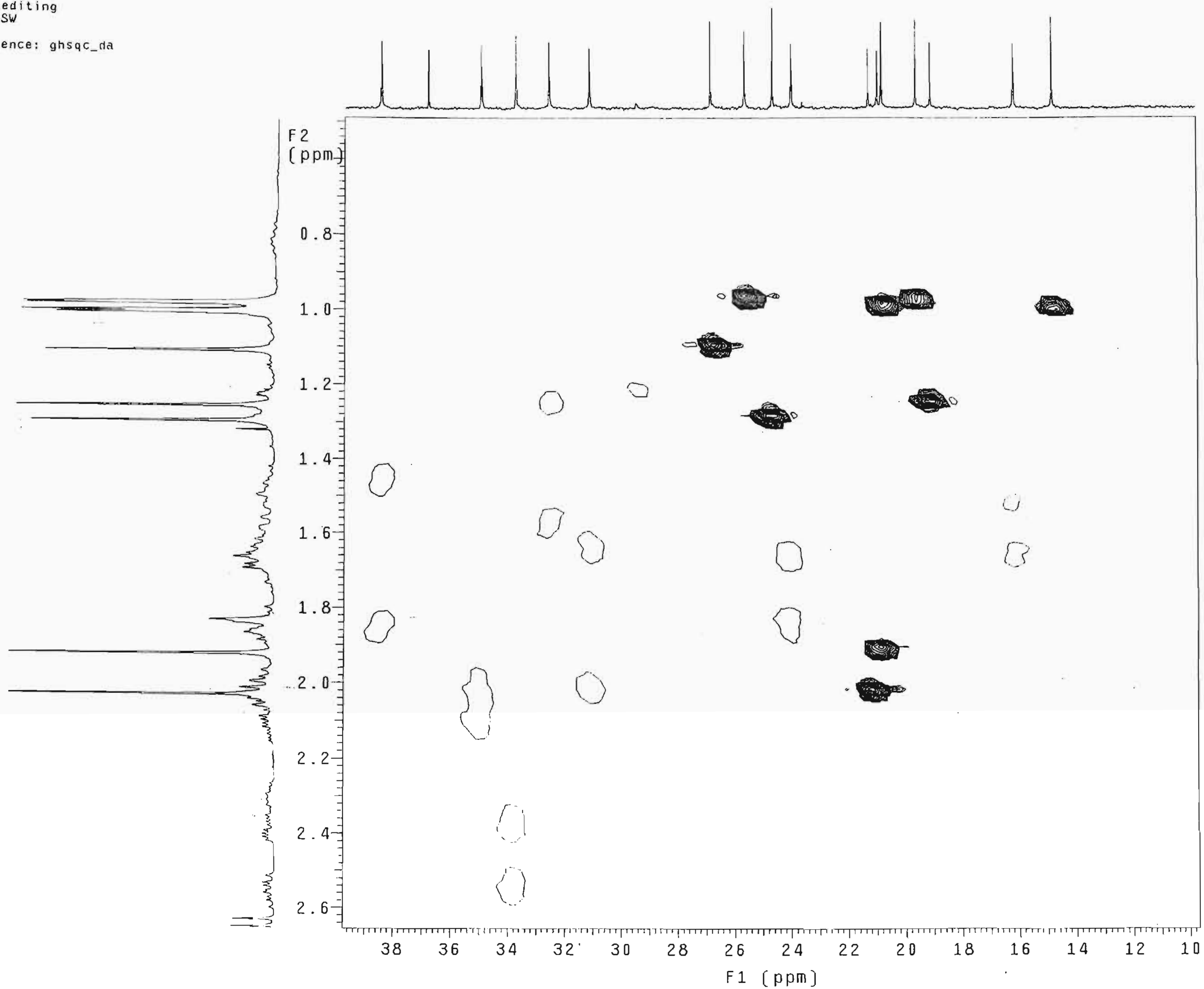

probe=5mmASW
Pulse Sequence: dept
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm



HSQC NMR Spectrum of 1,2-Dihydrobruceajavanin A (ix)

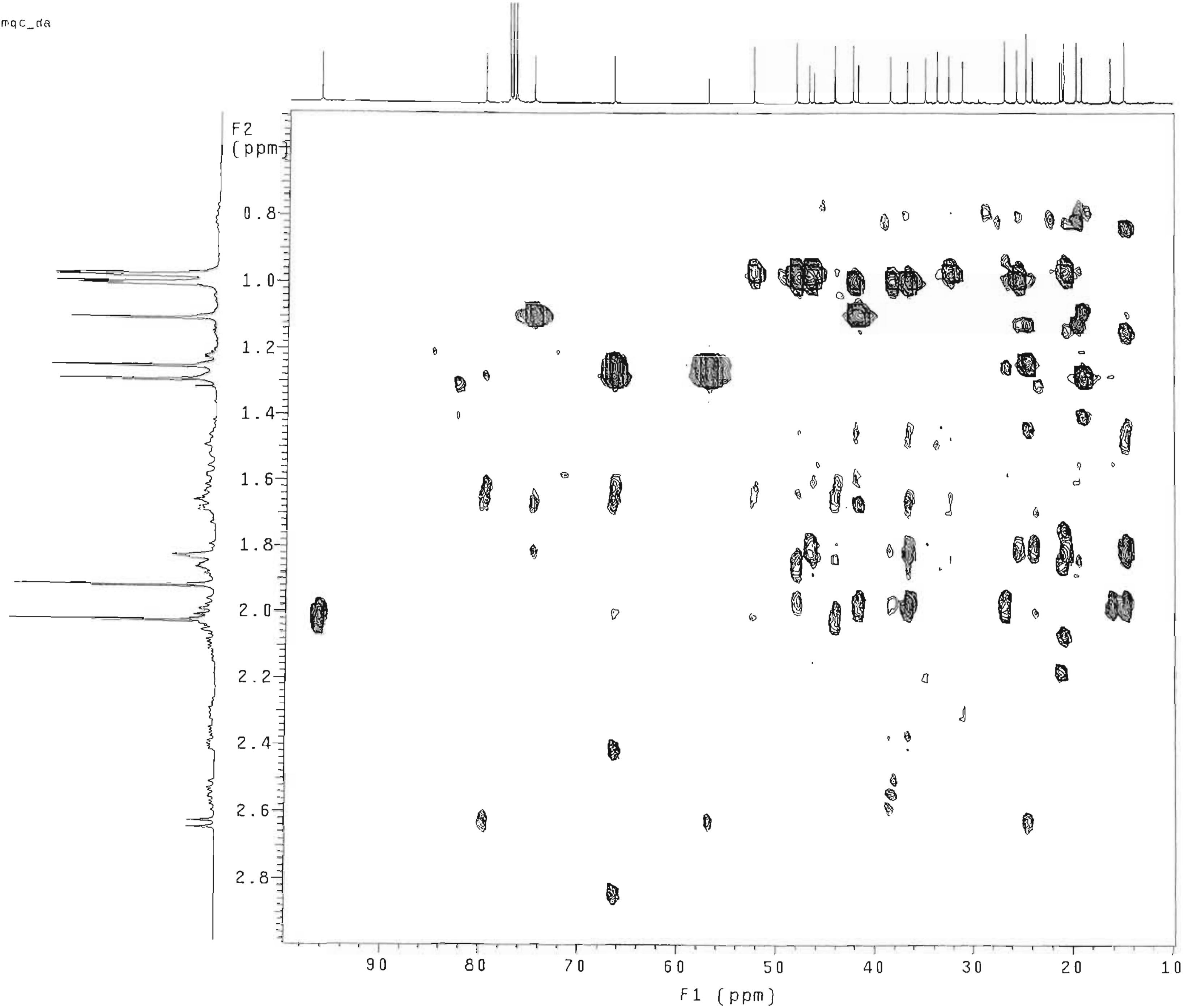
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da

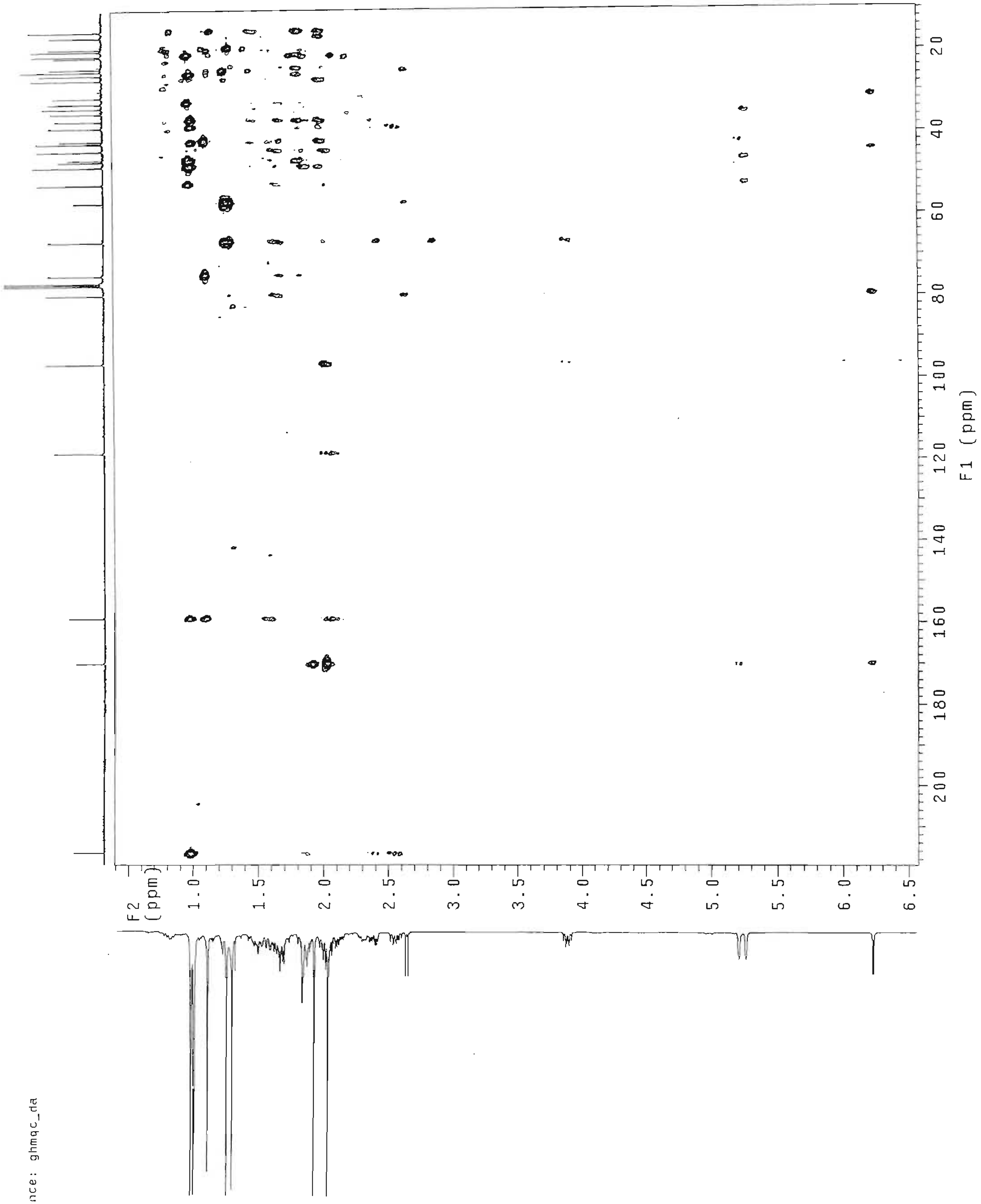


HSQC NMR Spectrum of 1,2-Dihydrobruceajavanin A (ix)

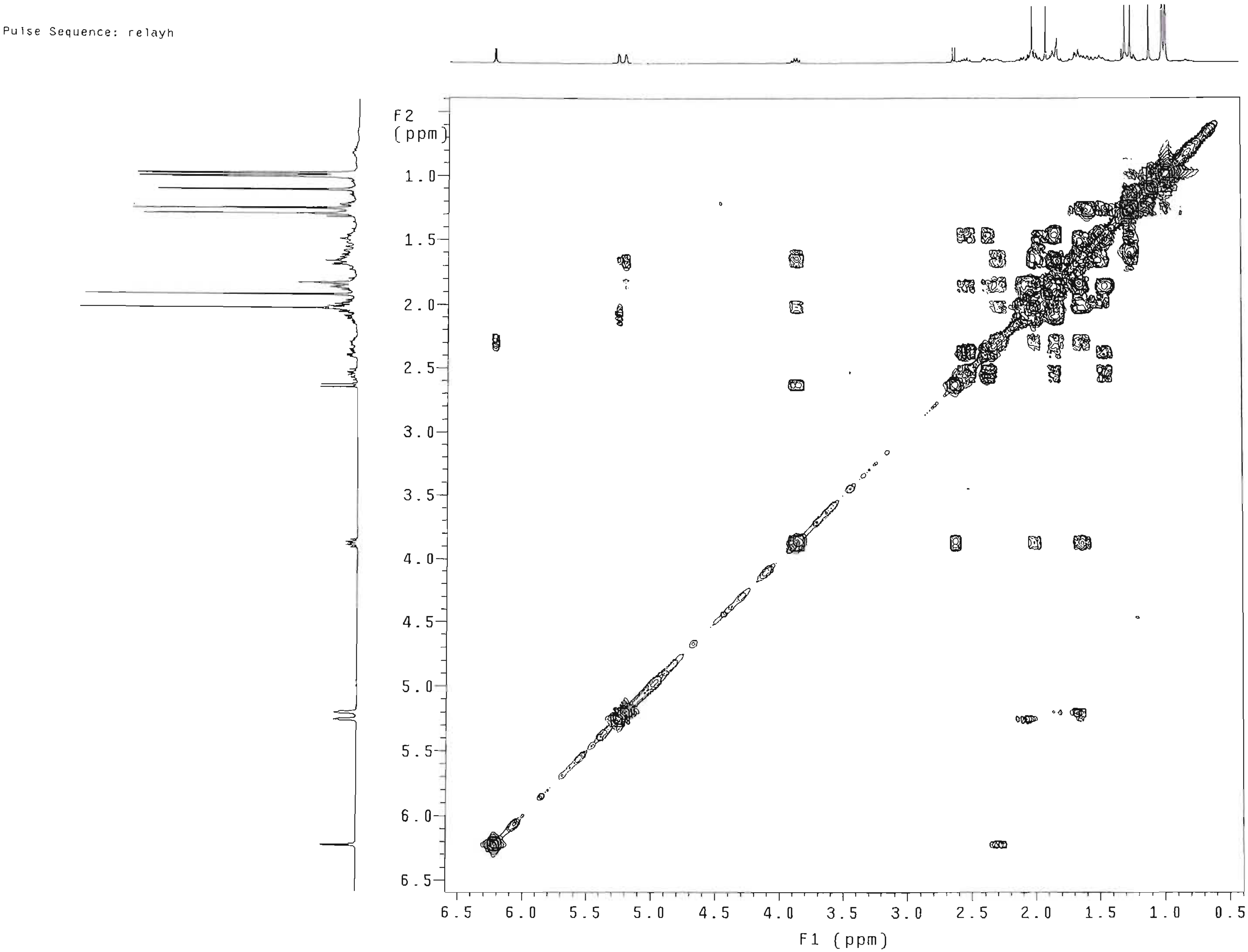
Pulse Sequence: ghmqc_da



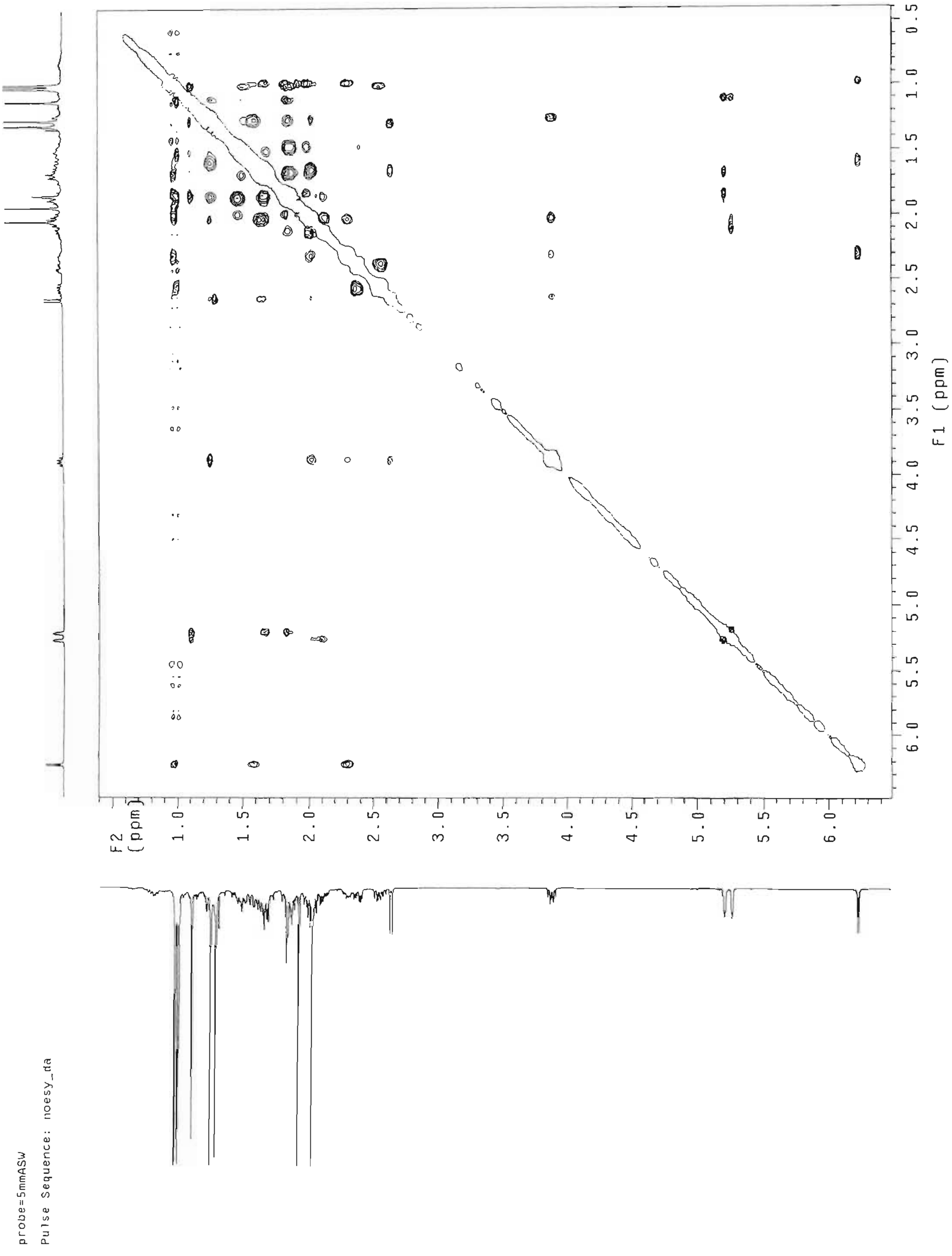
HMBC NMR Spectrum of 1,2-Dihydro[illegible]



HMBC NMR Spectrum of 1,2-Dih

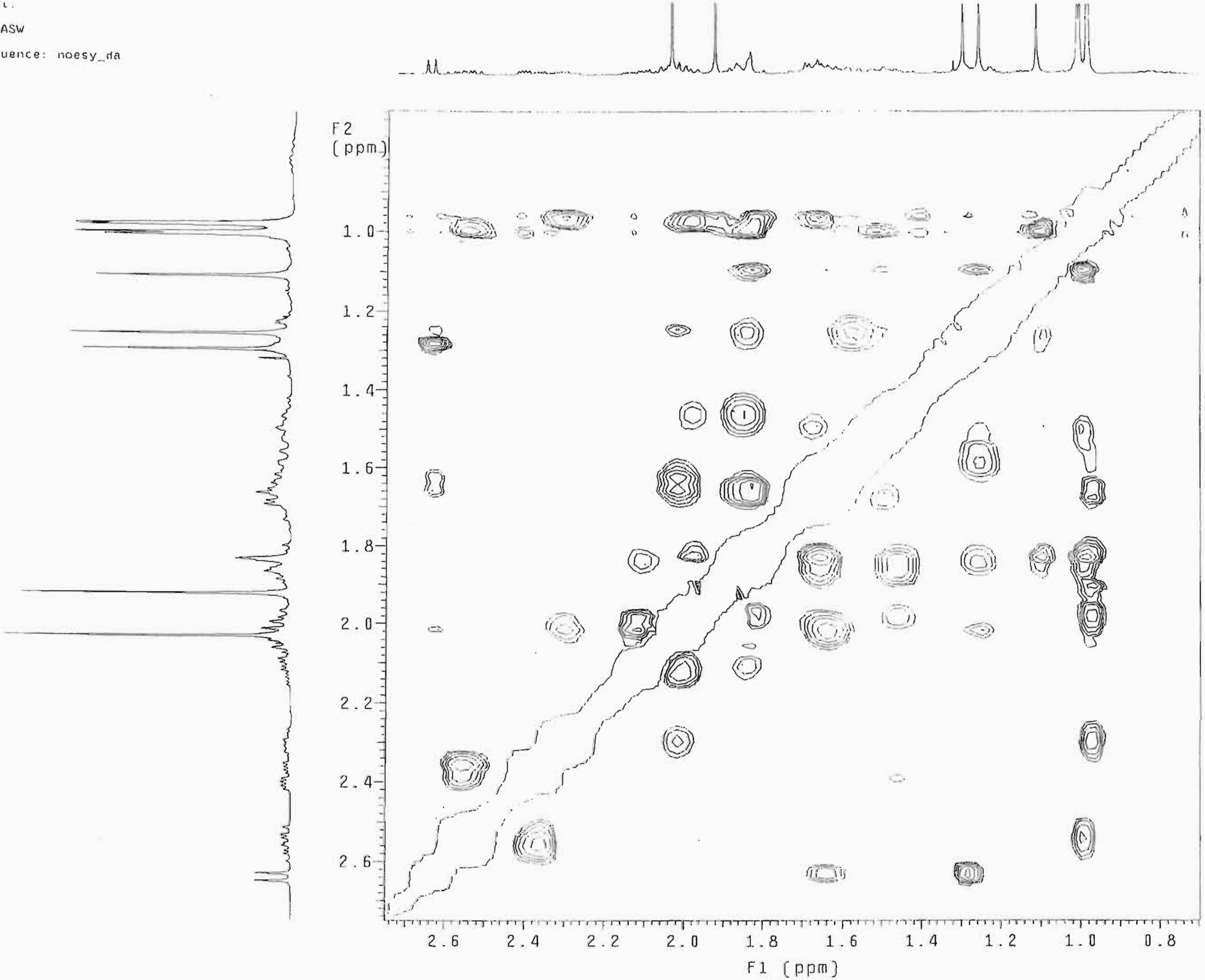


COSY NMR Spectrum of 1,2-Dihydrobruceajavanin A (ix)

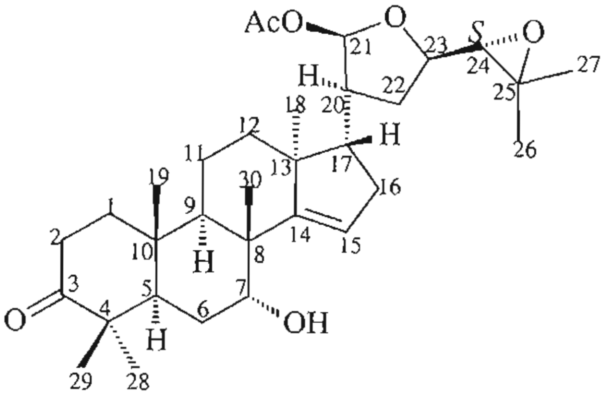

probe=5mmASW
Pulse Sequence: noesy_da
F2 (ppm)
F1 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
0.5

NOESY expt.
mix=1sec
probe=5mmASW

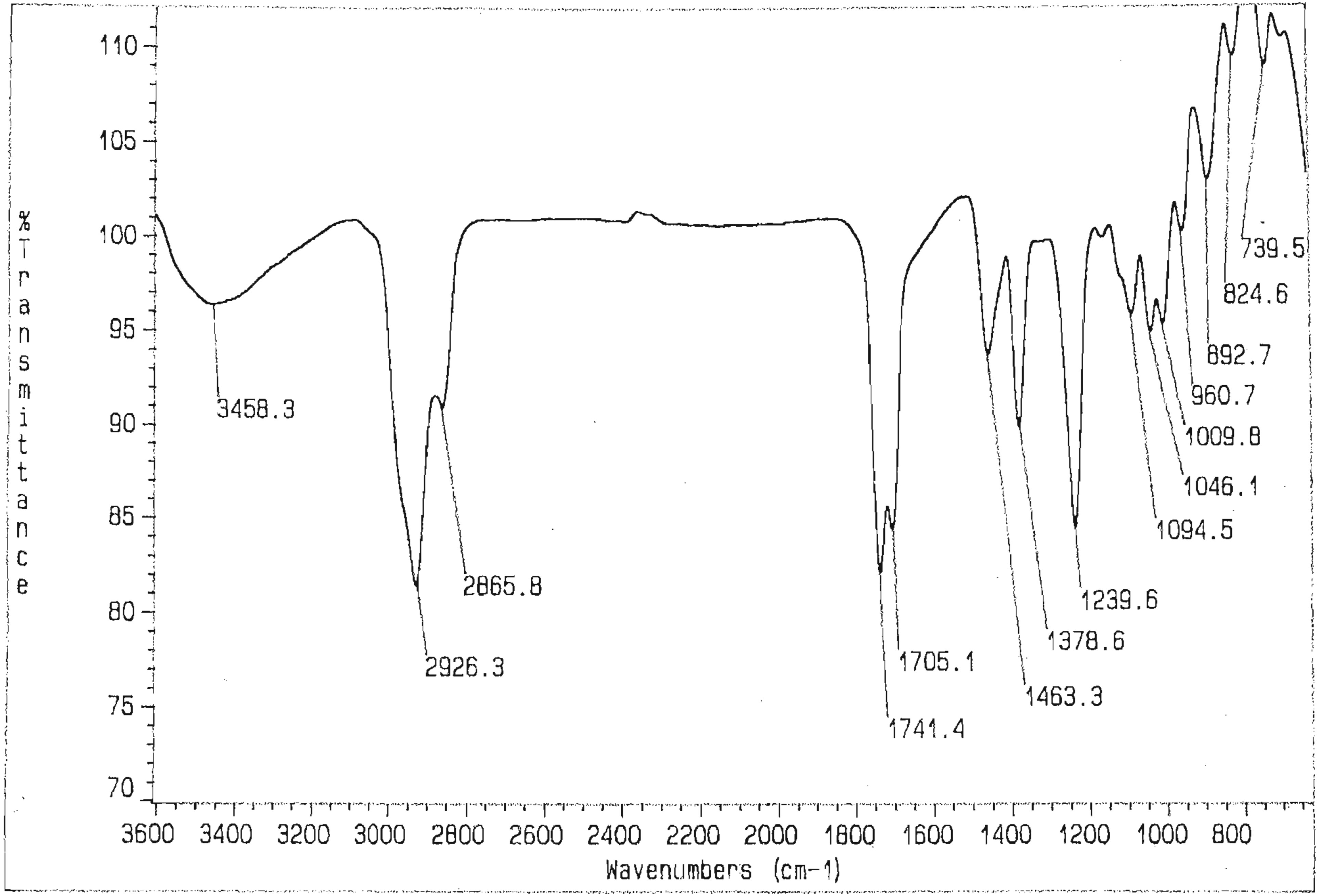
Pulse Sequence: noesy_da



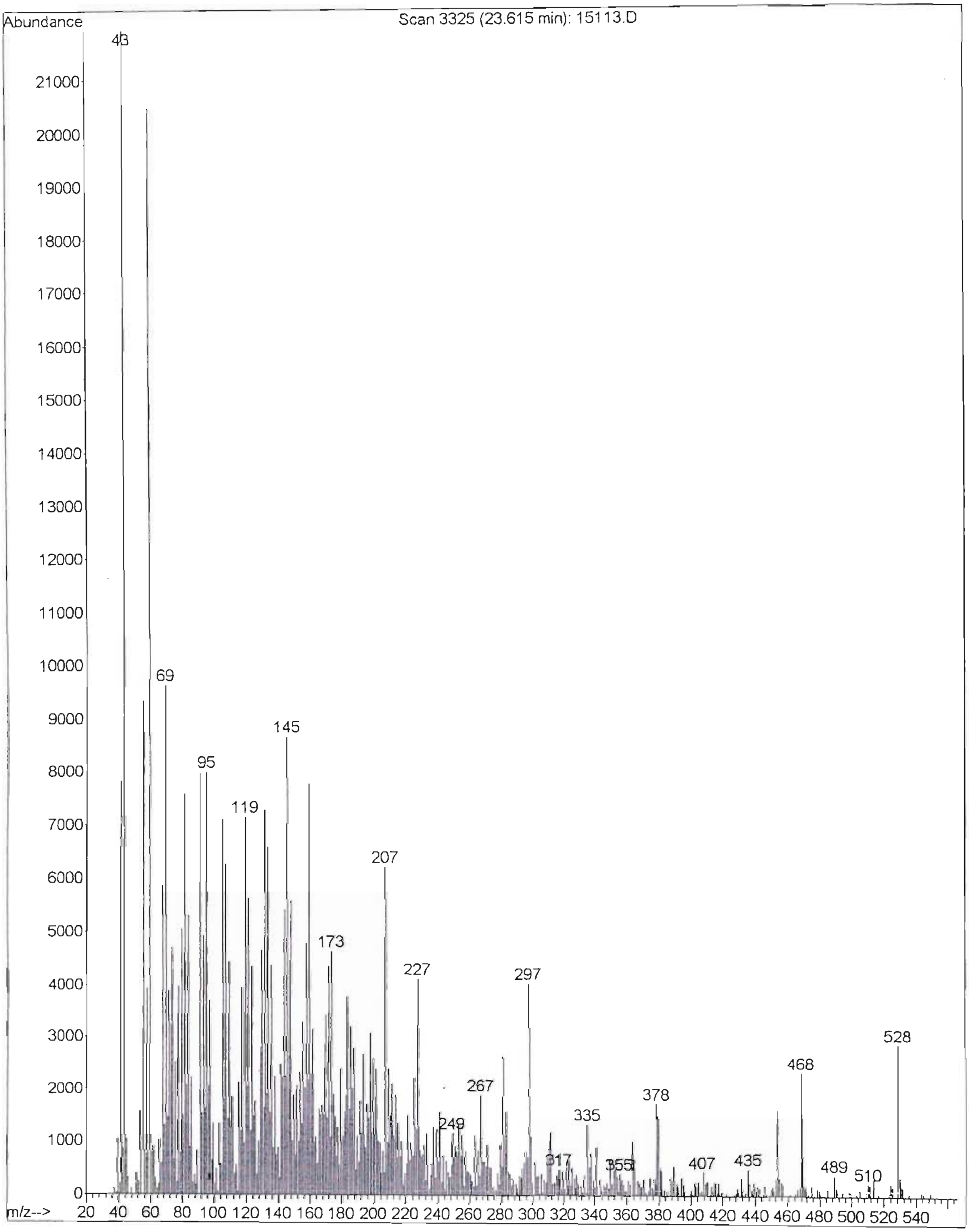
Chisocheton compound A (**x**)



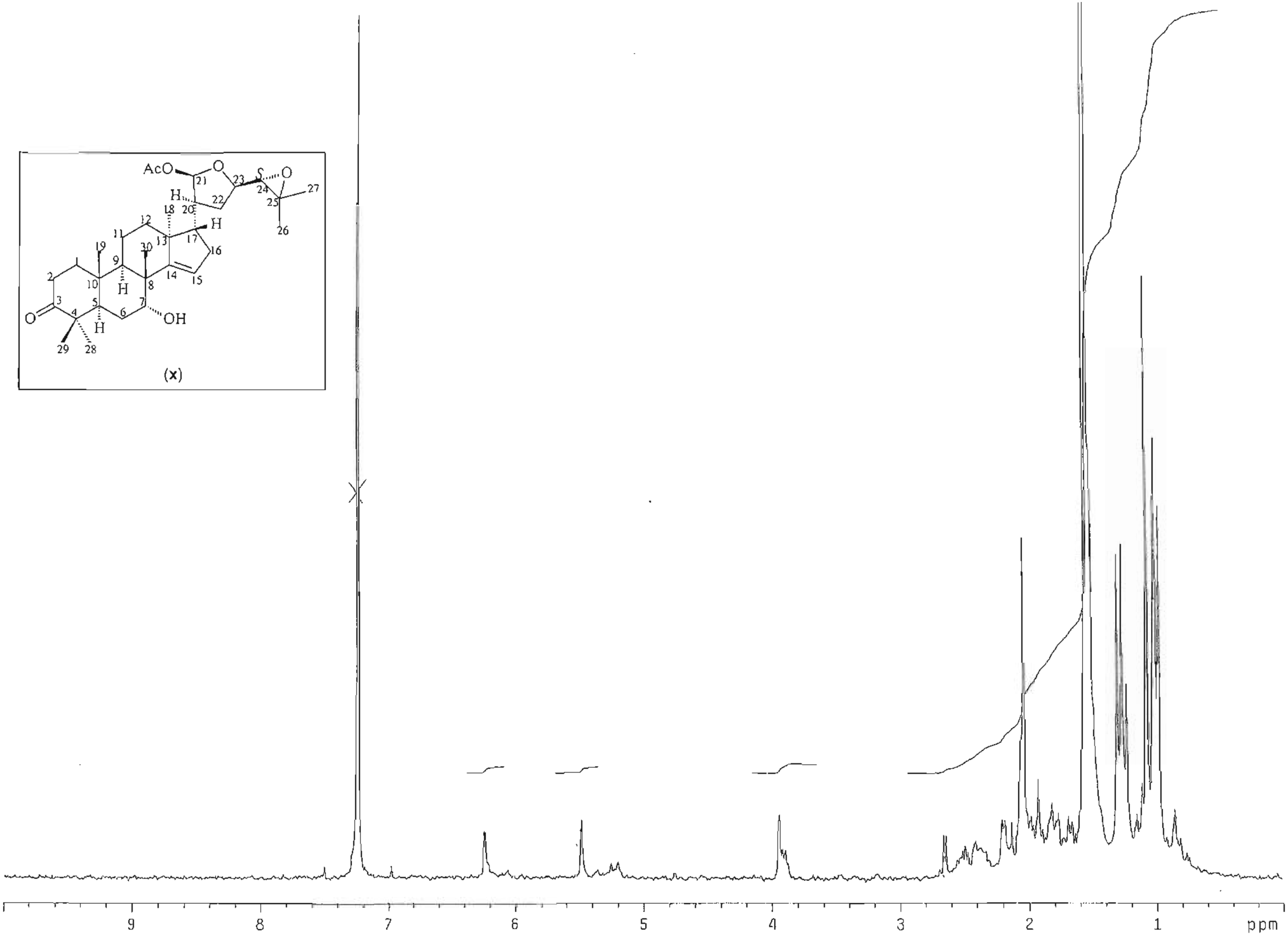
(**x**)



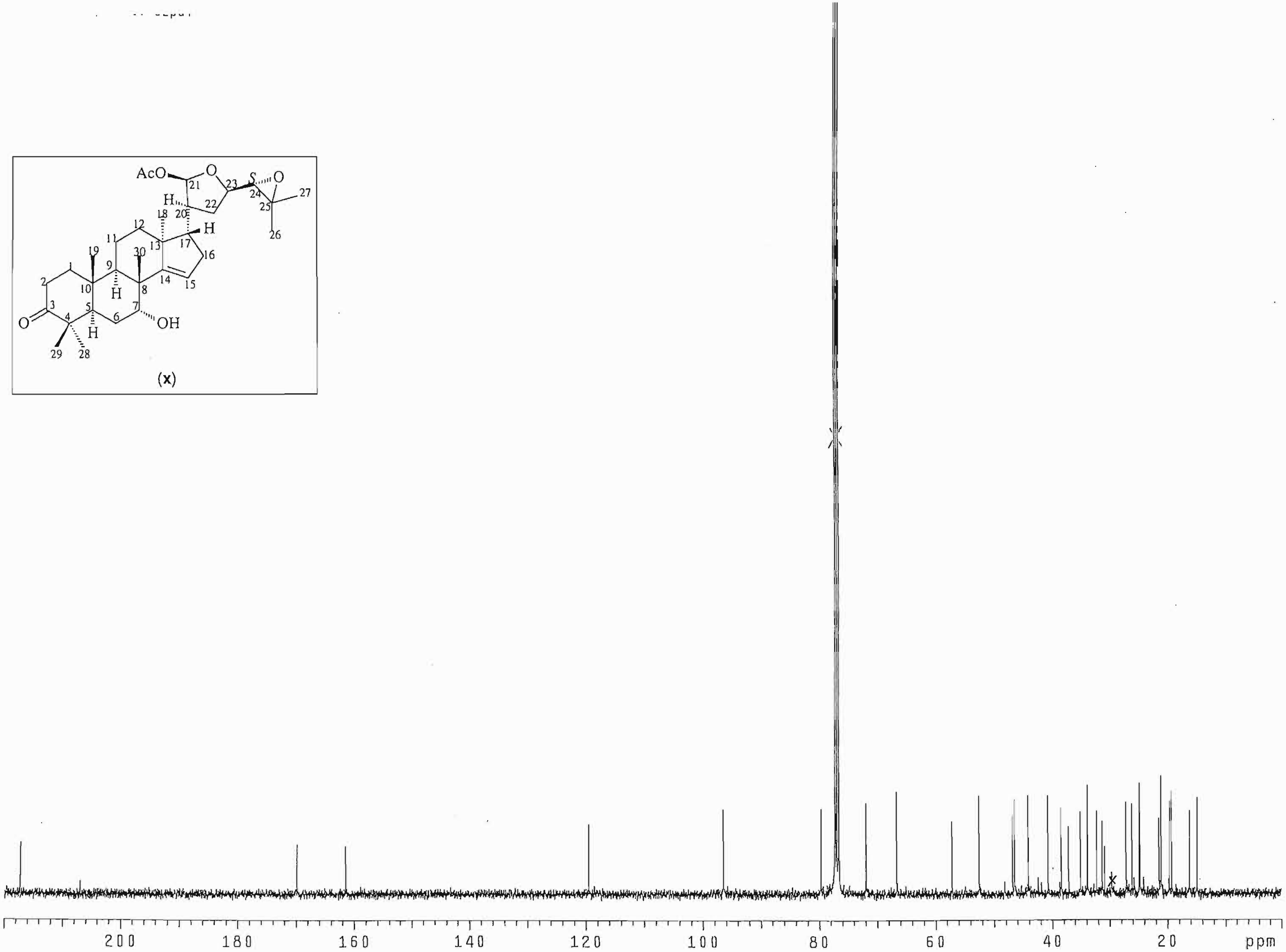
IR Spectrum of Chisocheton compound A (x)



Mass Spectrum of Chisocheton compound A (x)

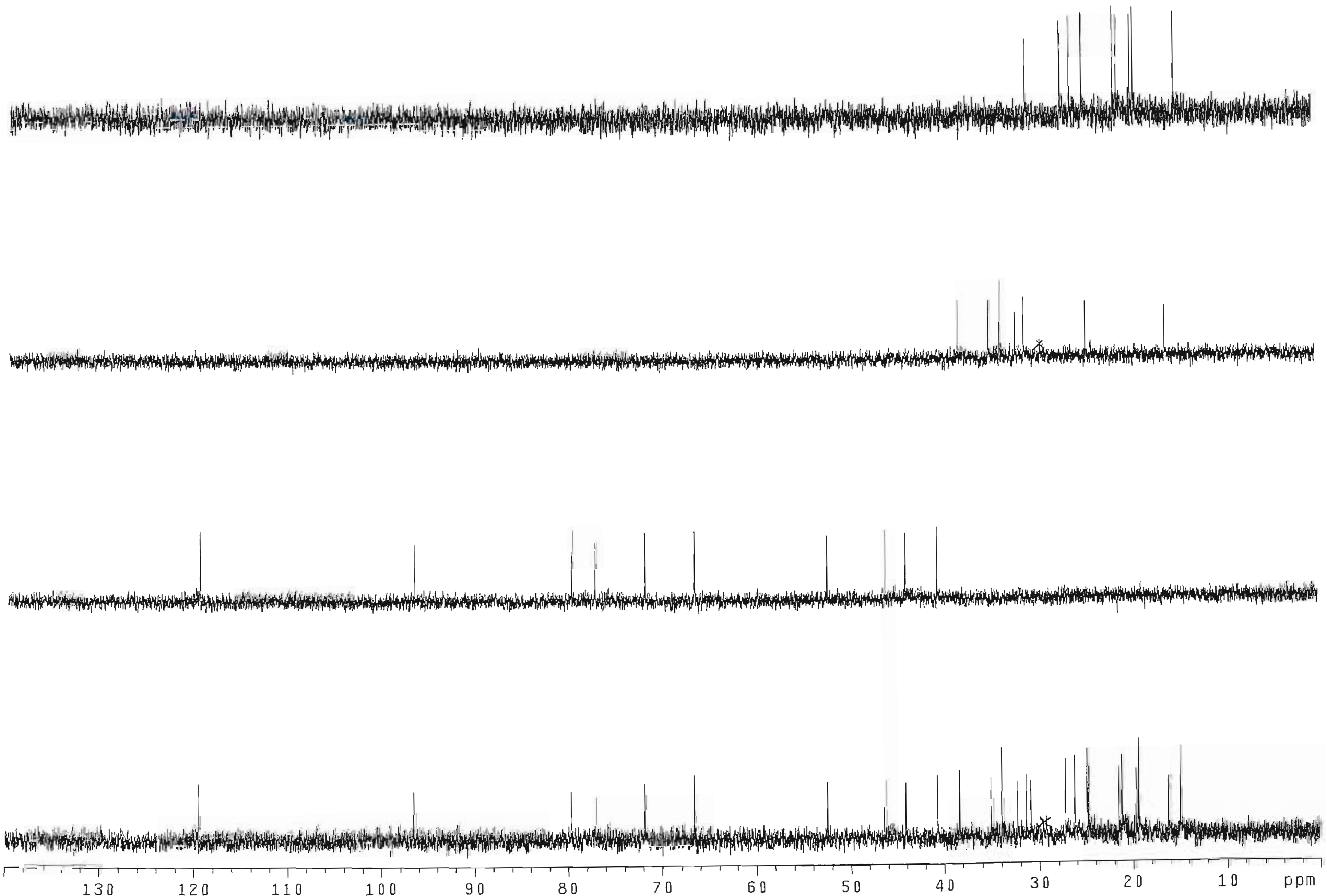


^{1}H NMR Spectrum of Chisocheto



^{13}C NMR Spectrum of Chisocheton compound A (x)

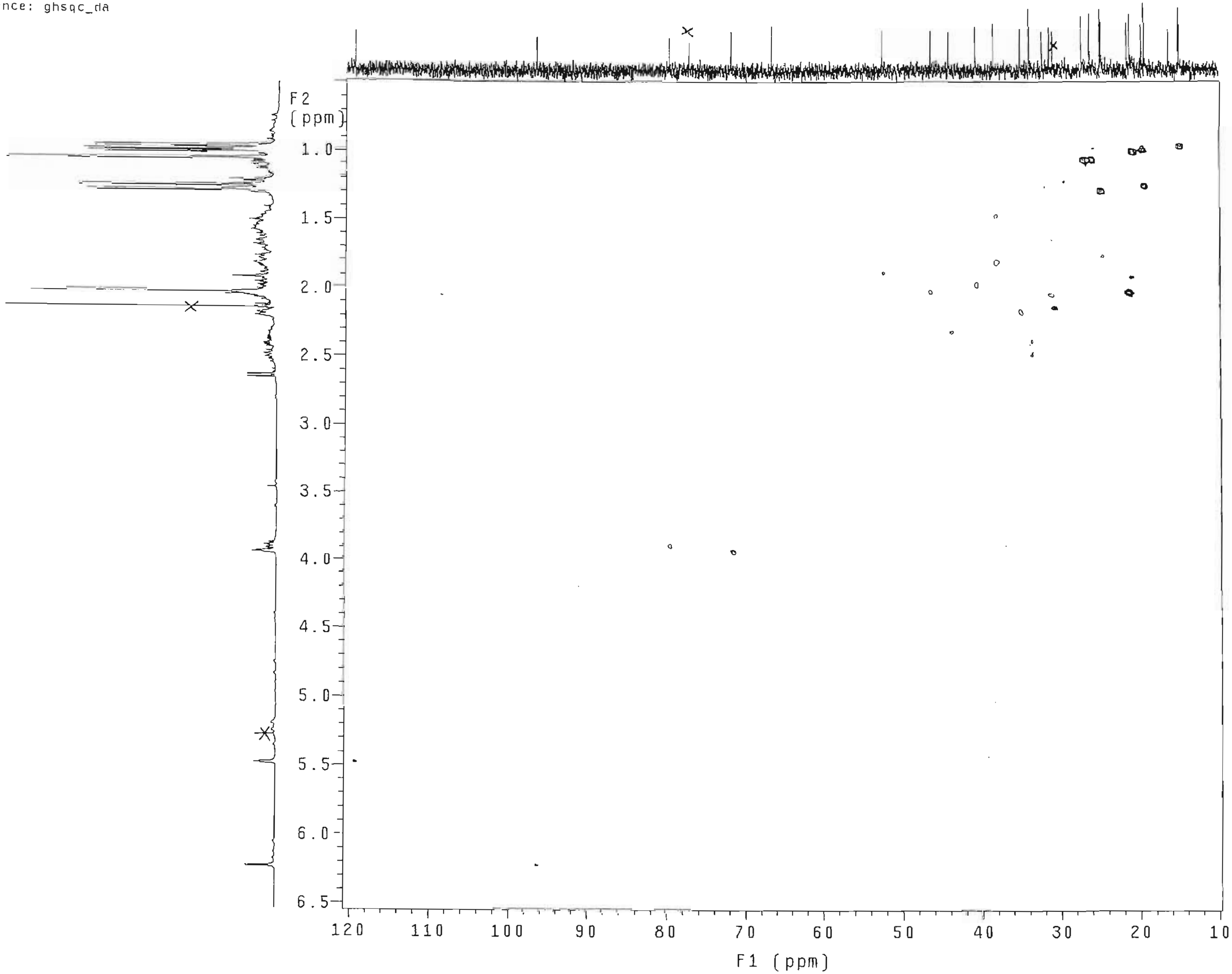
Pulse Sequence: dept



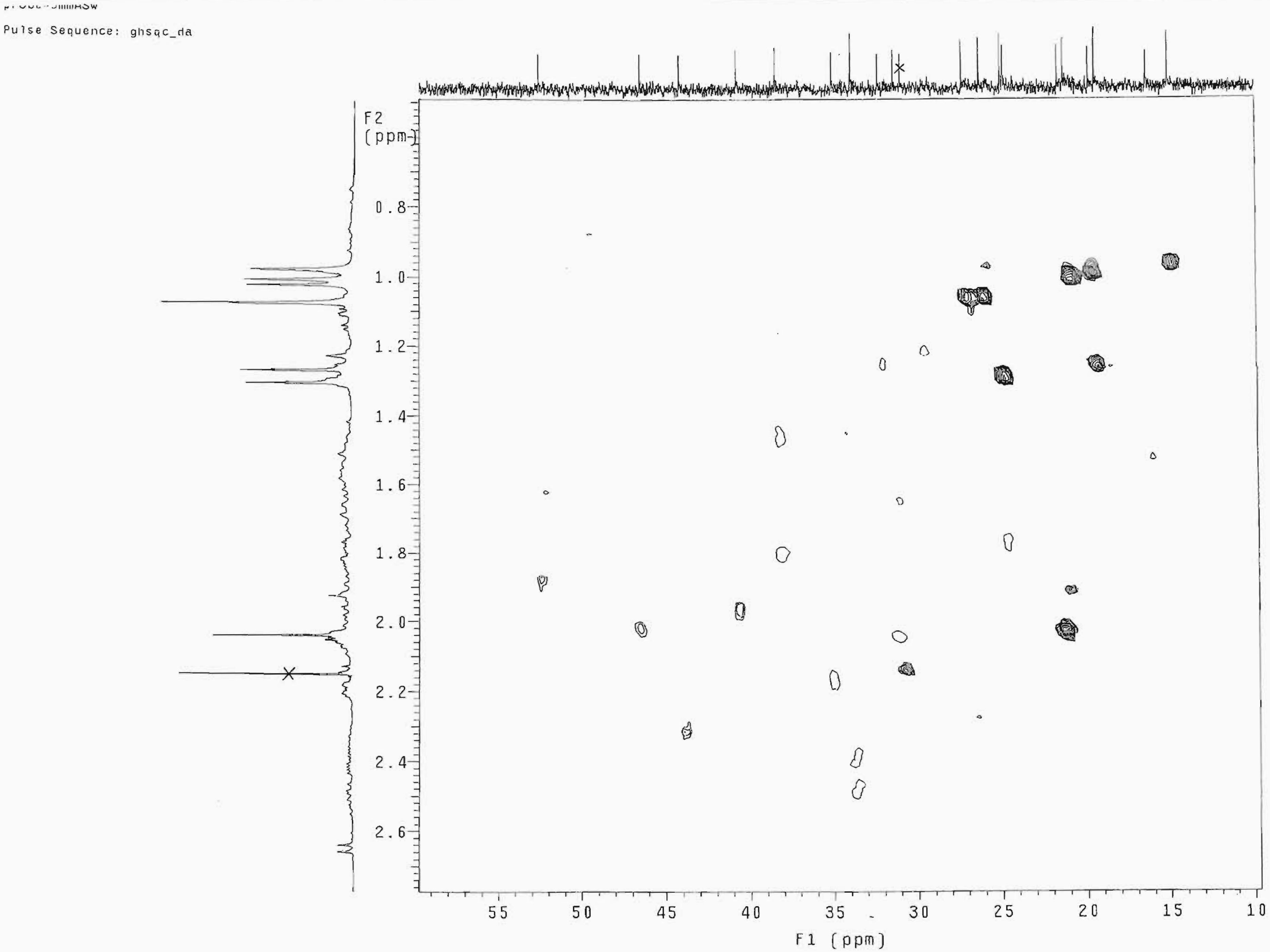
ADEPT NMR Spectrum of Chisocheton compound A (x)

probe=5mmASW

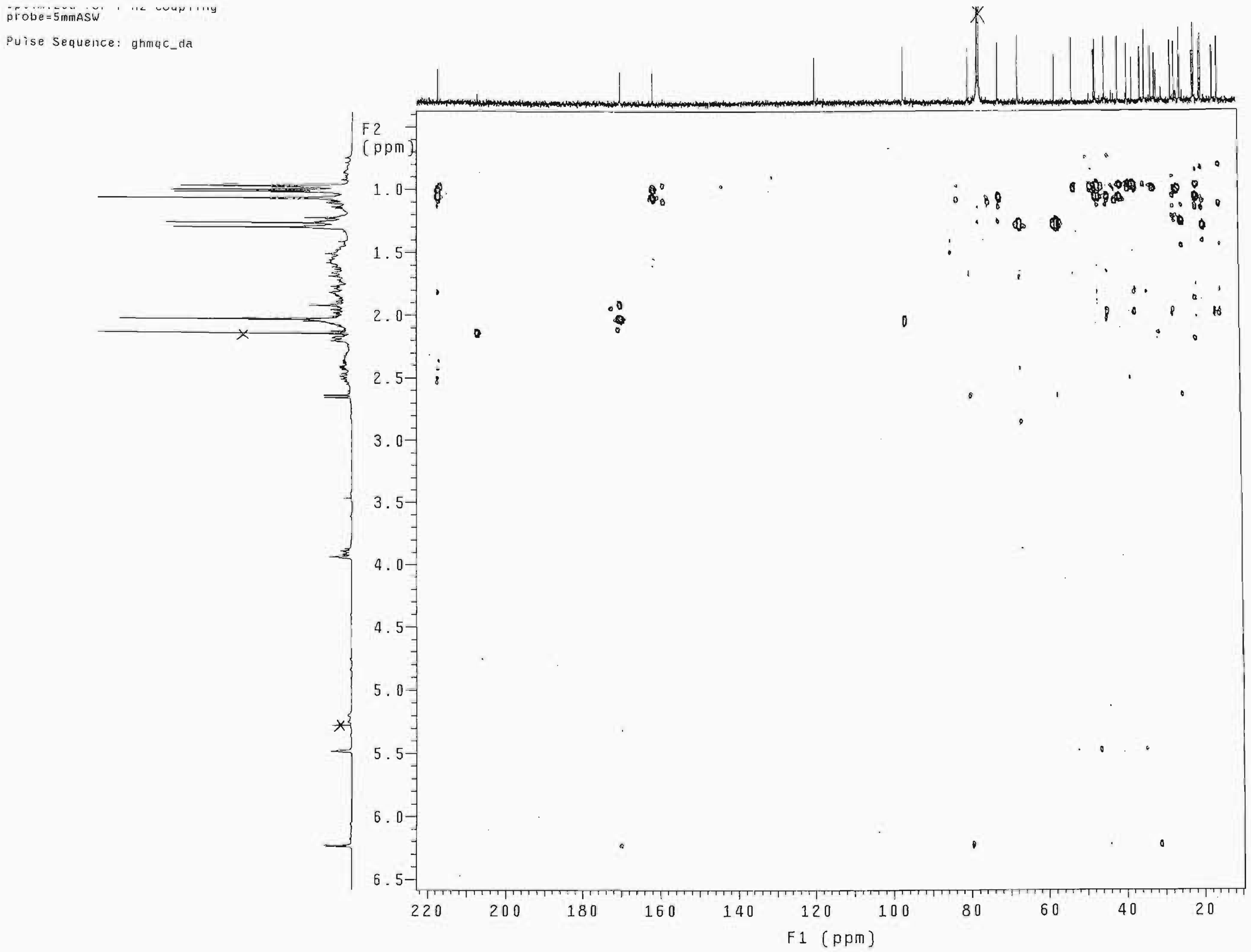
Pulse Sequence: ghsqc_da



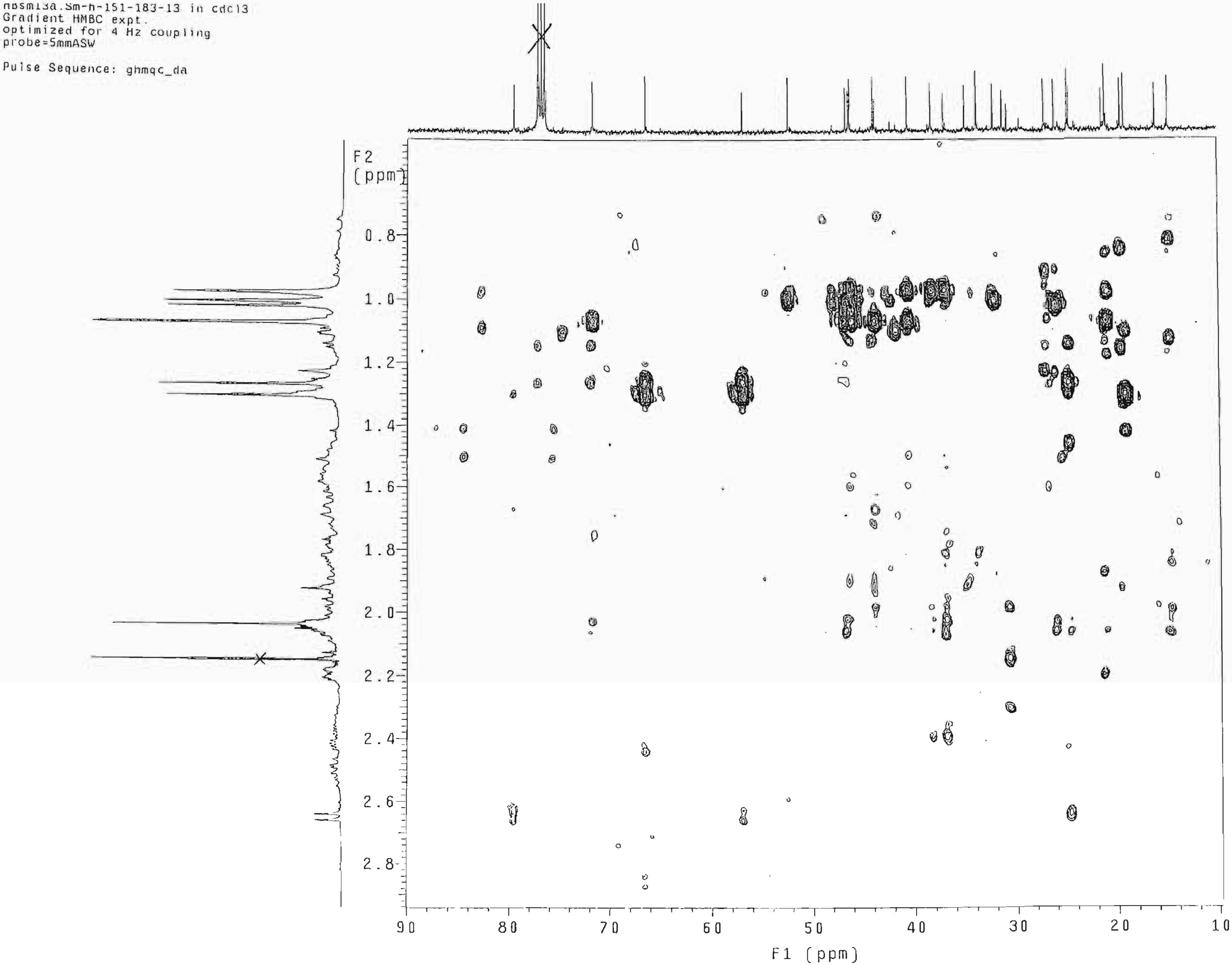
HSQC NMR Spectrum of Chisocheton compound A (x)



HSQC NMR Spectrum of Chisocheton compound A (x)

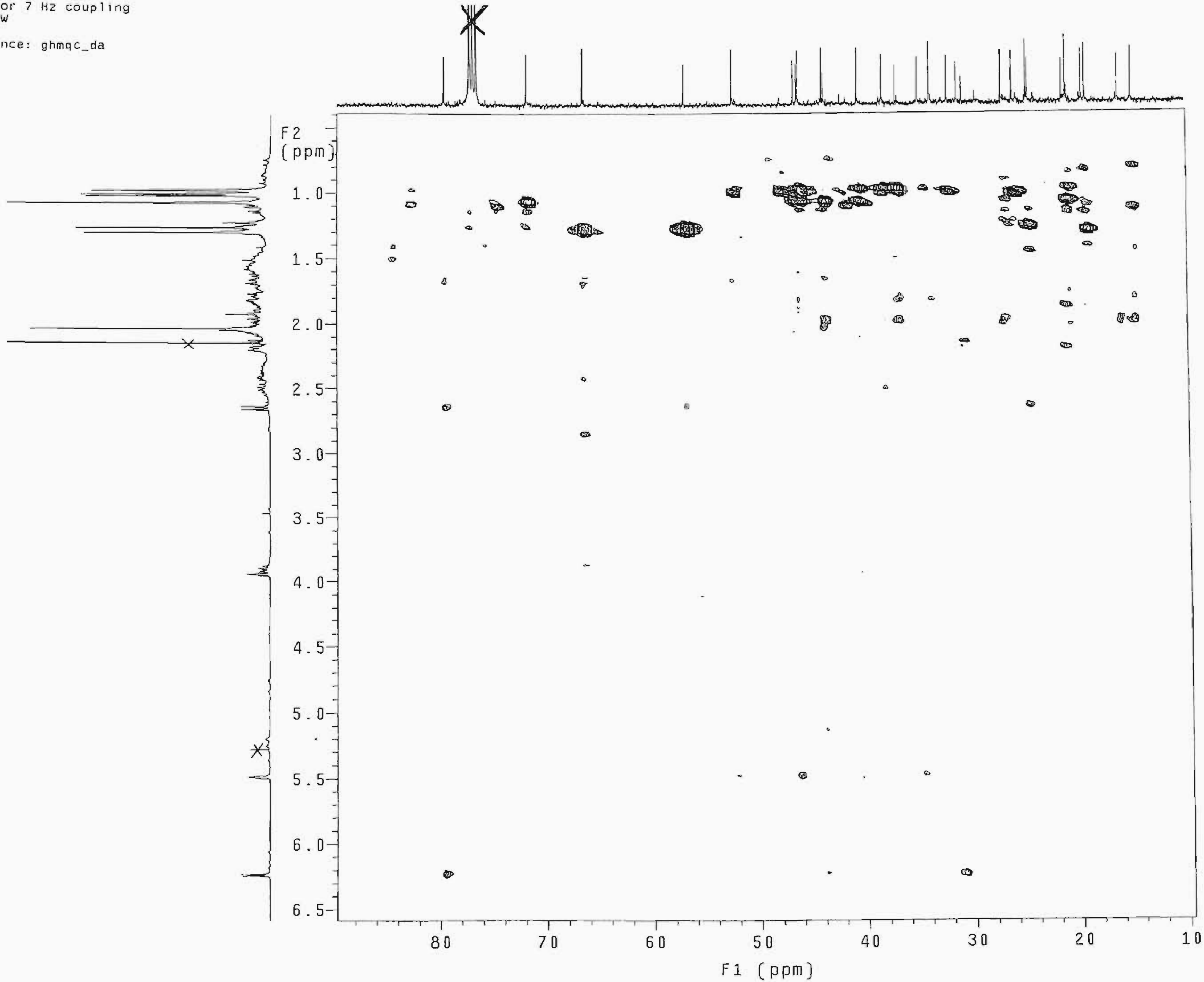


HMBC NMR Spectrum of Chisocheton compound A (x)

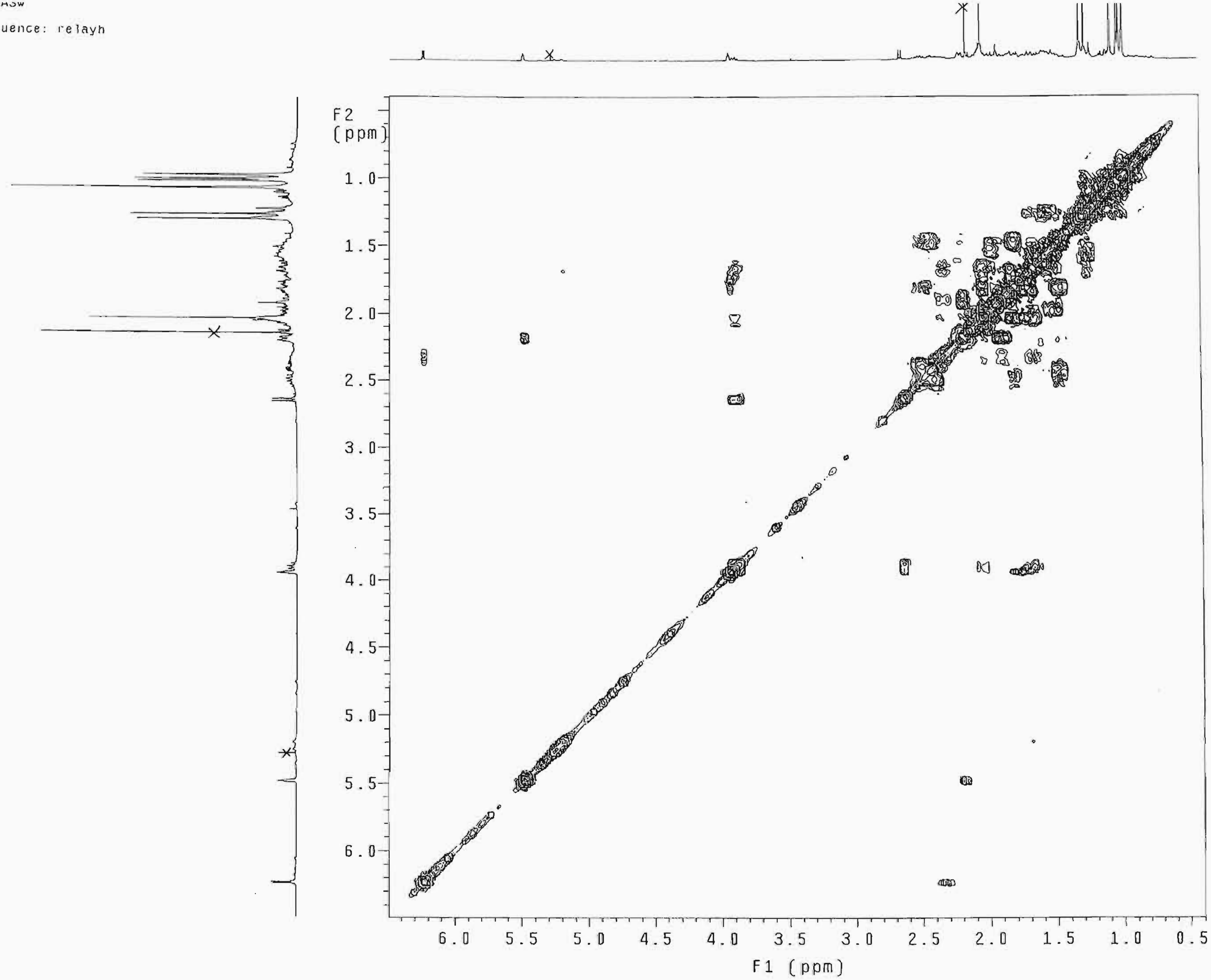

HBsm13a.Sm-h-151-183-13 in cdcl3
Gradient HMBC expt.
optimized for 4 Hz coupling
probe=5mmASW
Pulse Sequence: ghmqc_da
F2 (ppm)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
90
80
70
60
50
40
30
20
10
F1 (ppm)

optimized for 7 Hz coupling
probe=5mmASW

Pulse Sequence: ghmqc_da



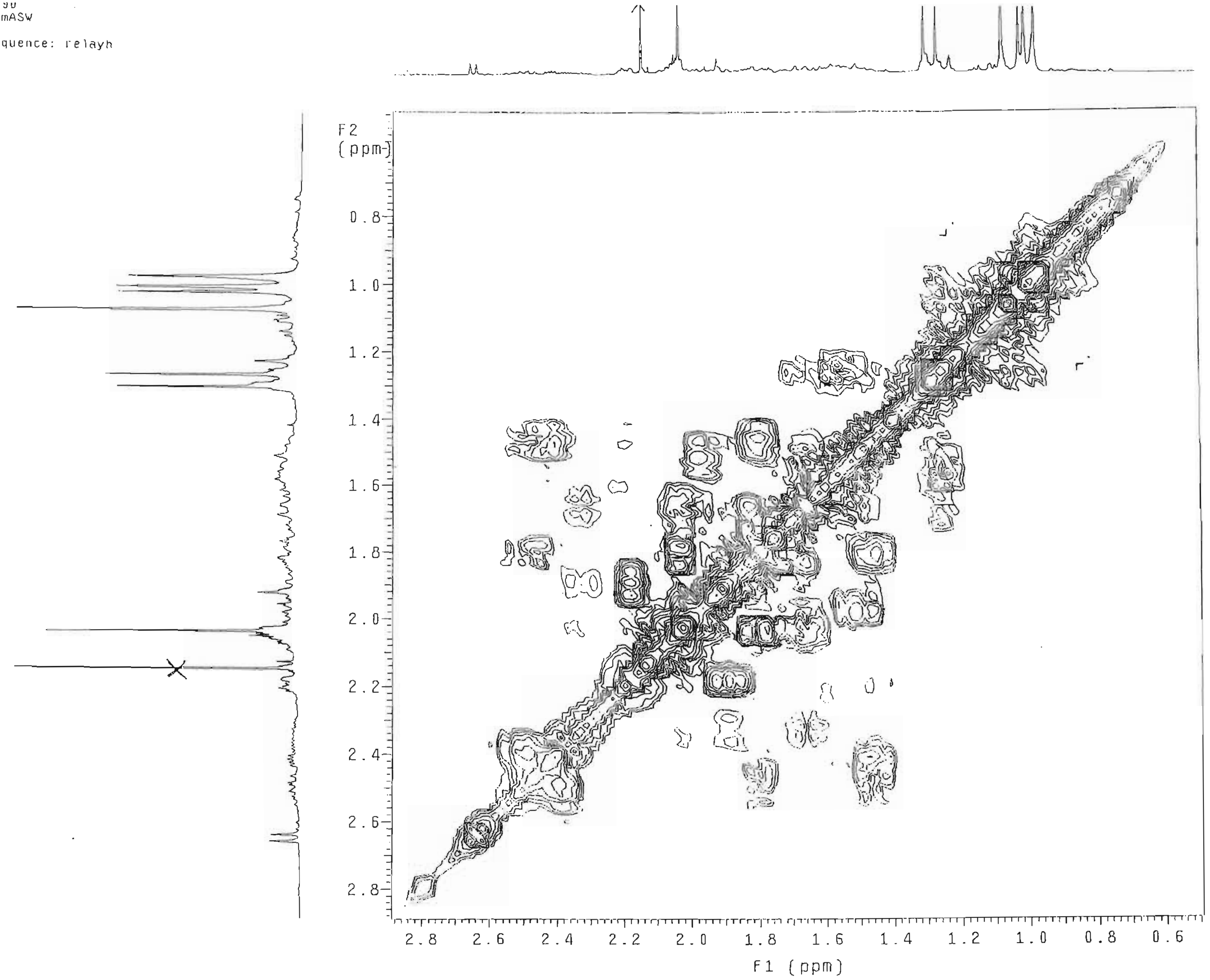
HMBC NMR Spectrum of Chisocheton compound A (x)



COSY NMR Spectrum of Chisocheton compound A (x)

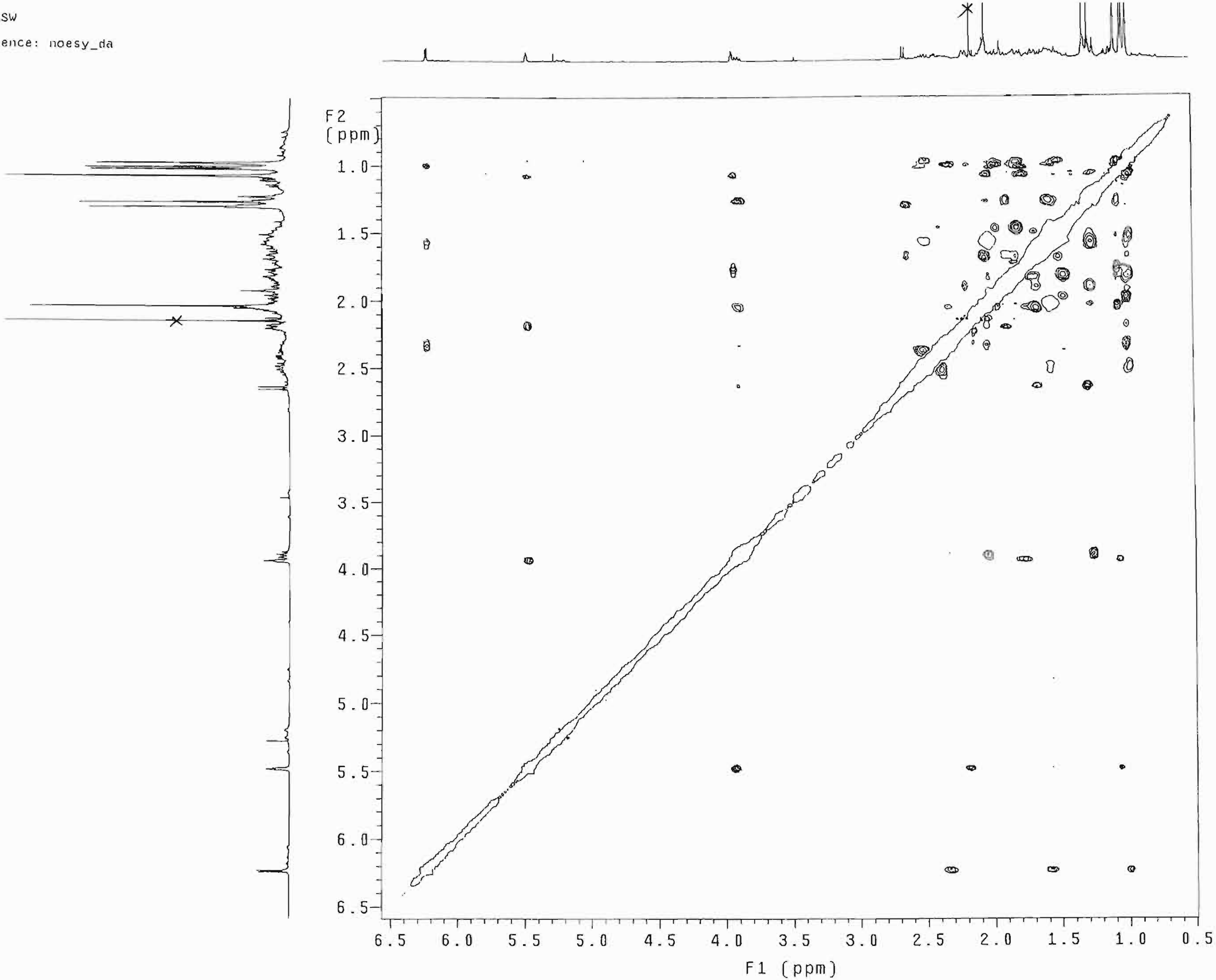
probe=5mmASW

Pulse Sequence: relayh

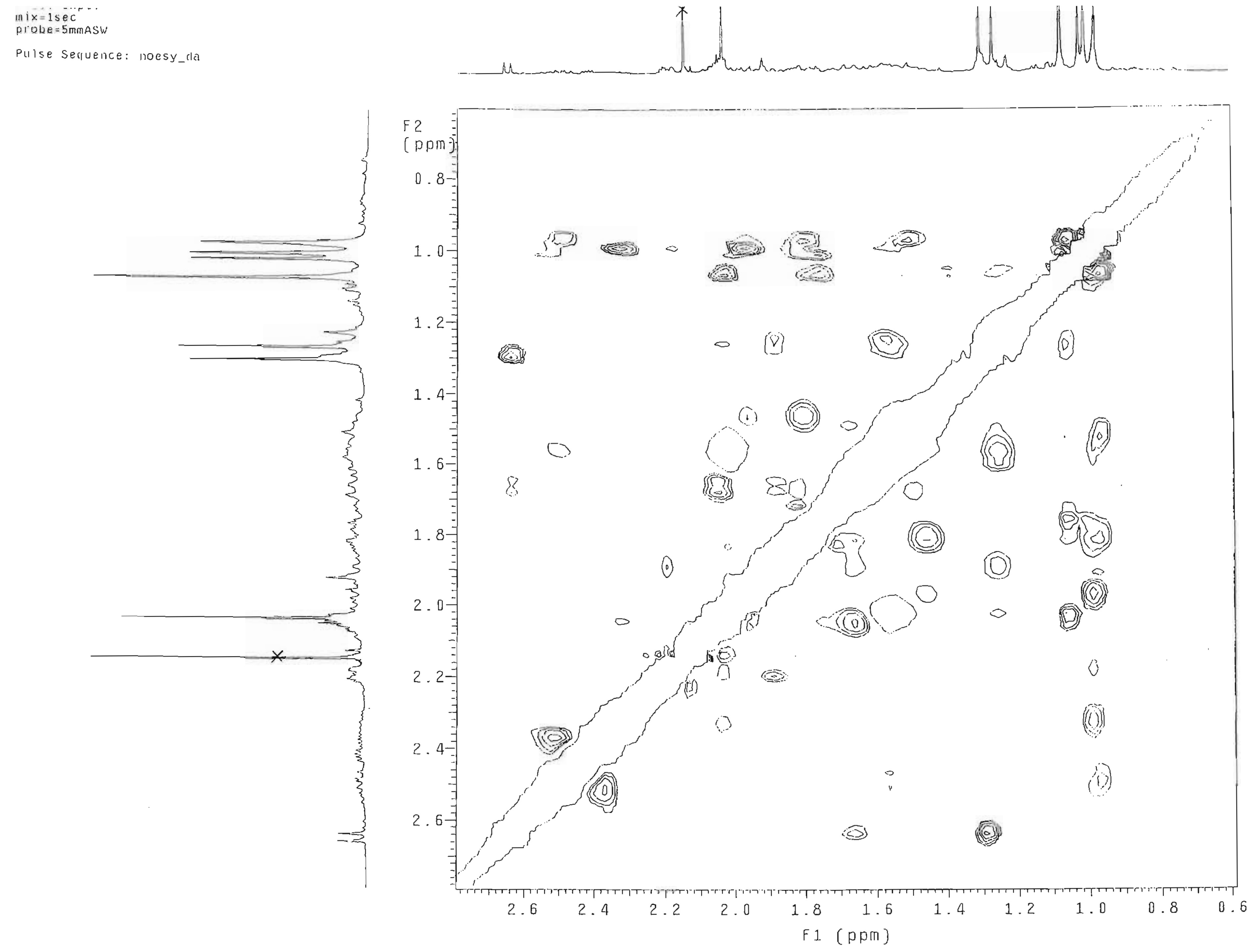


mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da

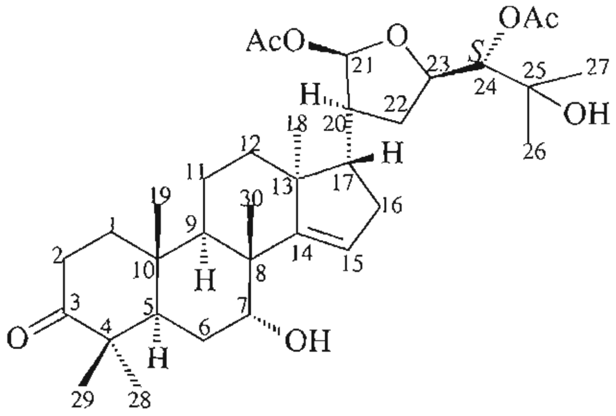


NOESY NMR Spectrum of Chisocheton compound A (x)

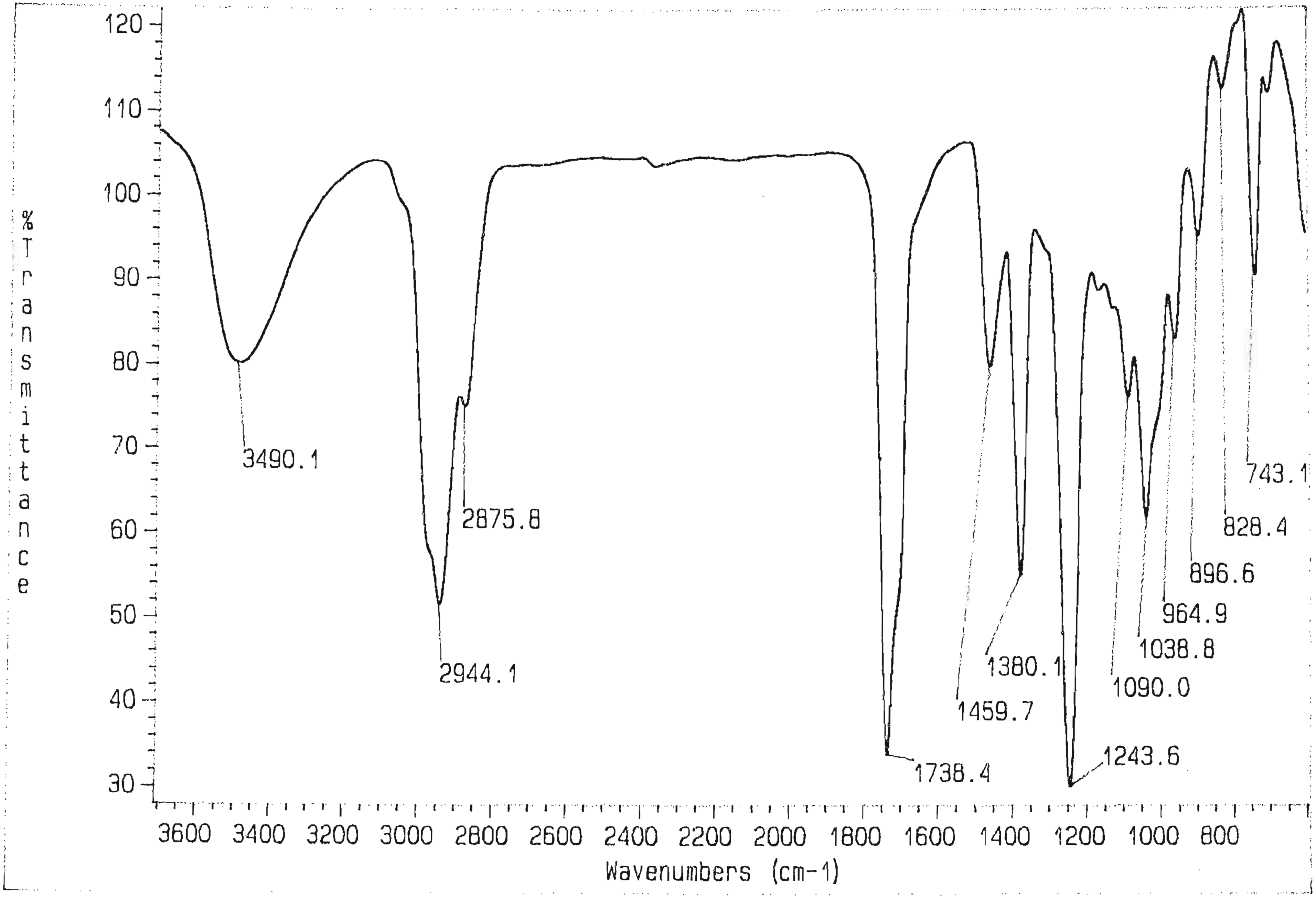


NOESY NMR Spectrum of Chisocheton compound A (x)

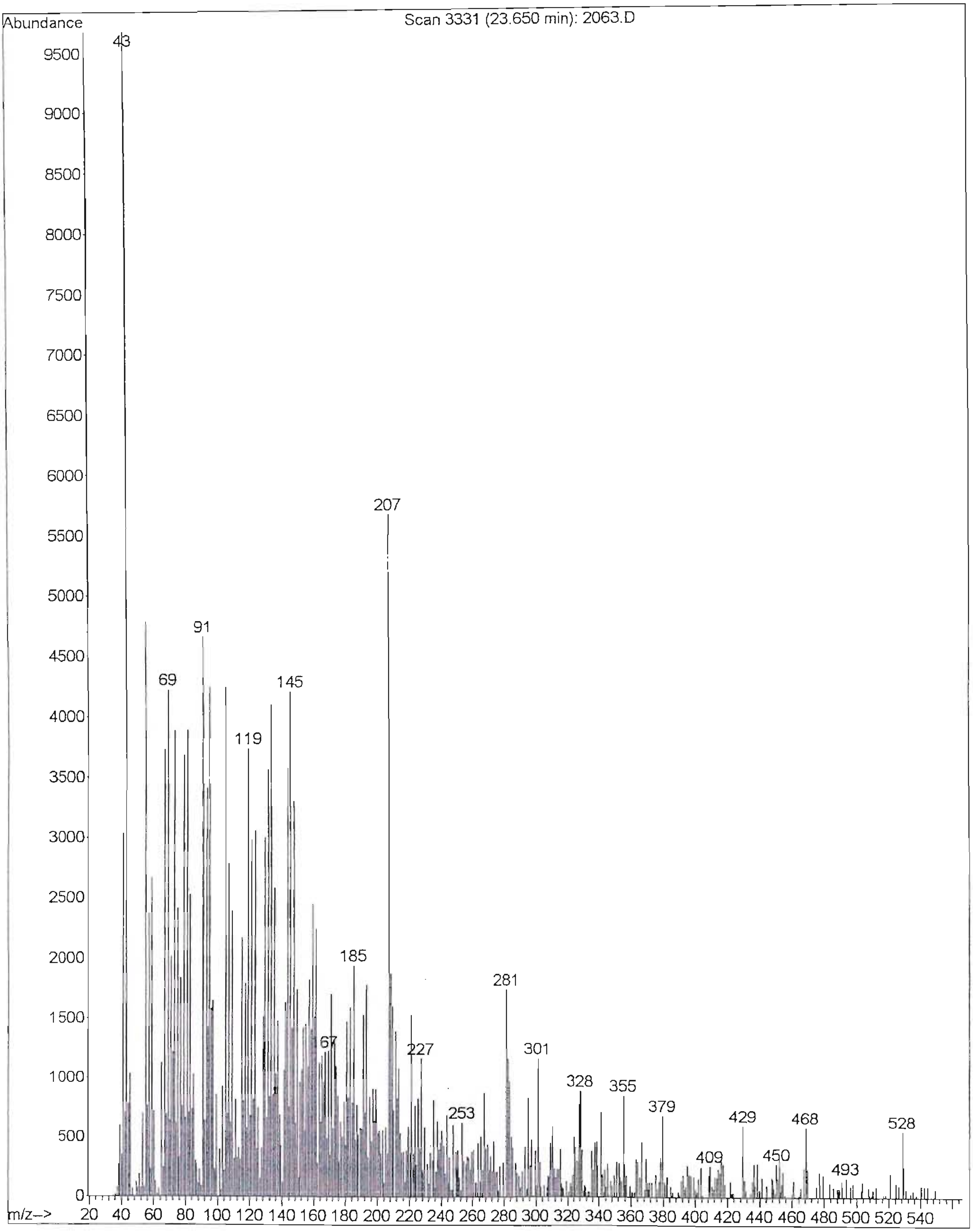
Protosamaderine B (xi)



(xi)

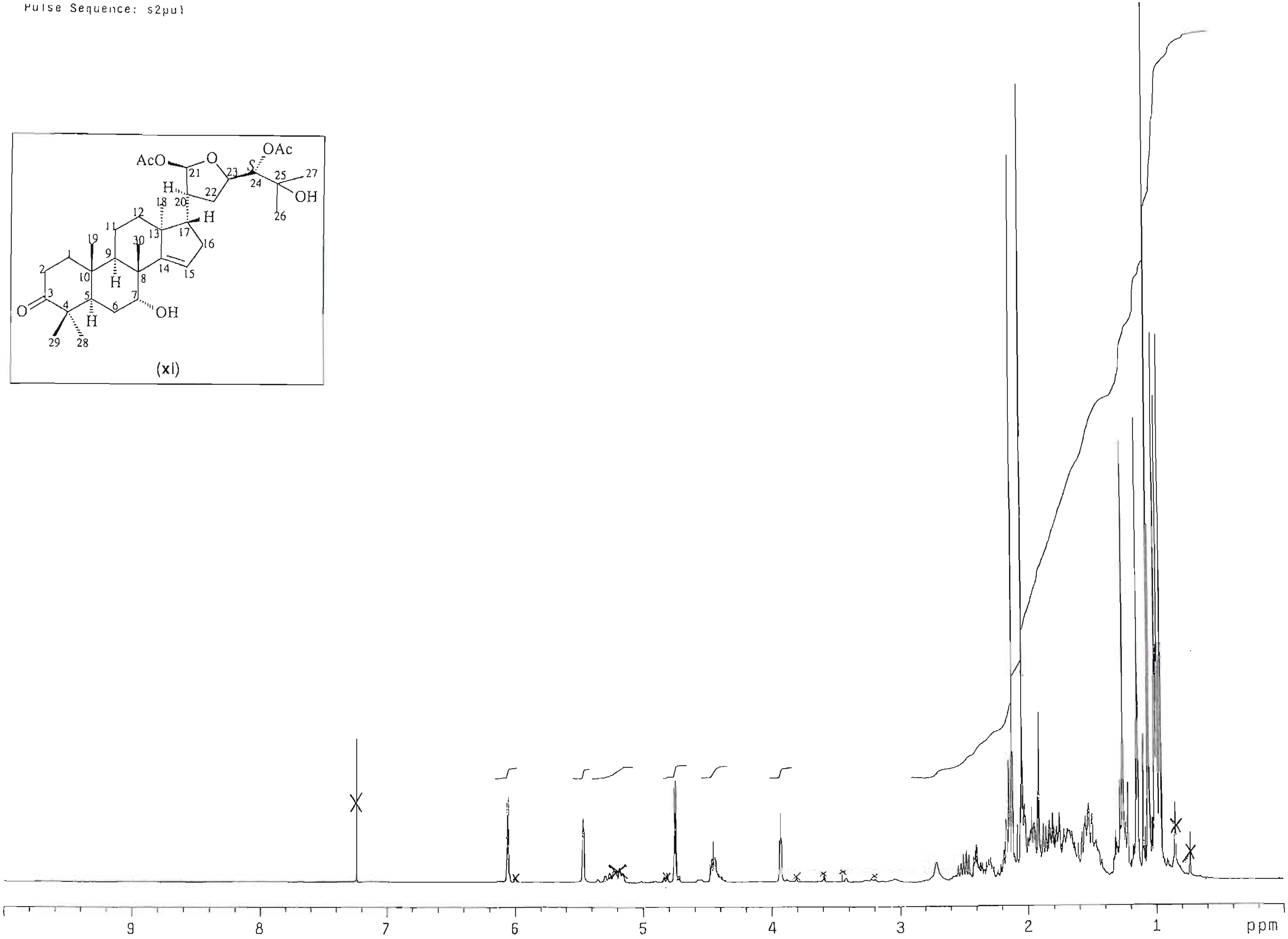


IR Spectrum of Protosamaderine B (xi)



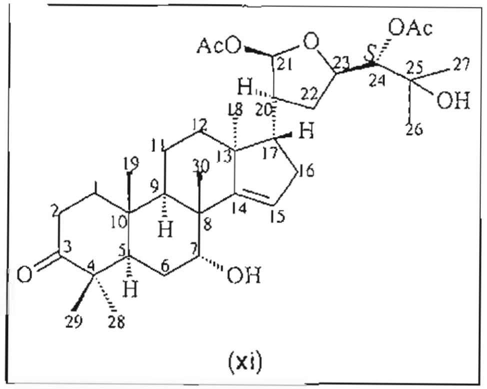
Mass Spectrum of Protosamaderine B (xi)

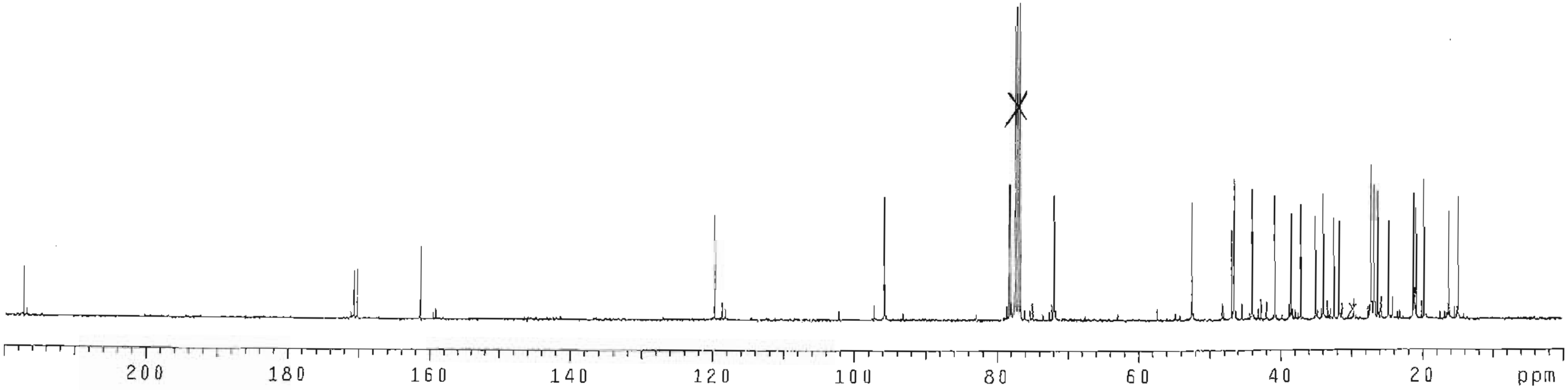
Pulse Sequence: s2pul



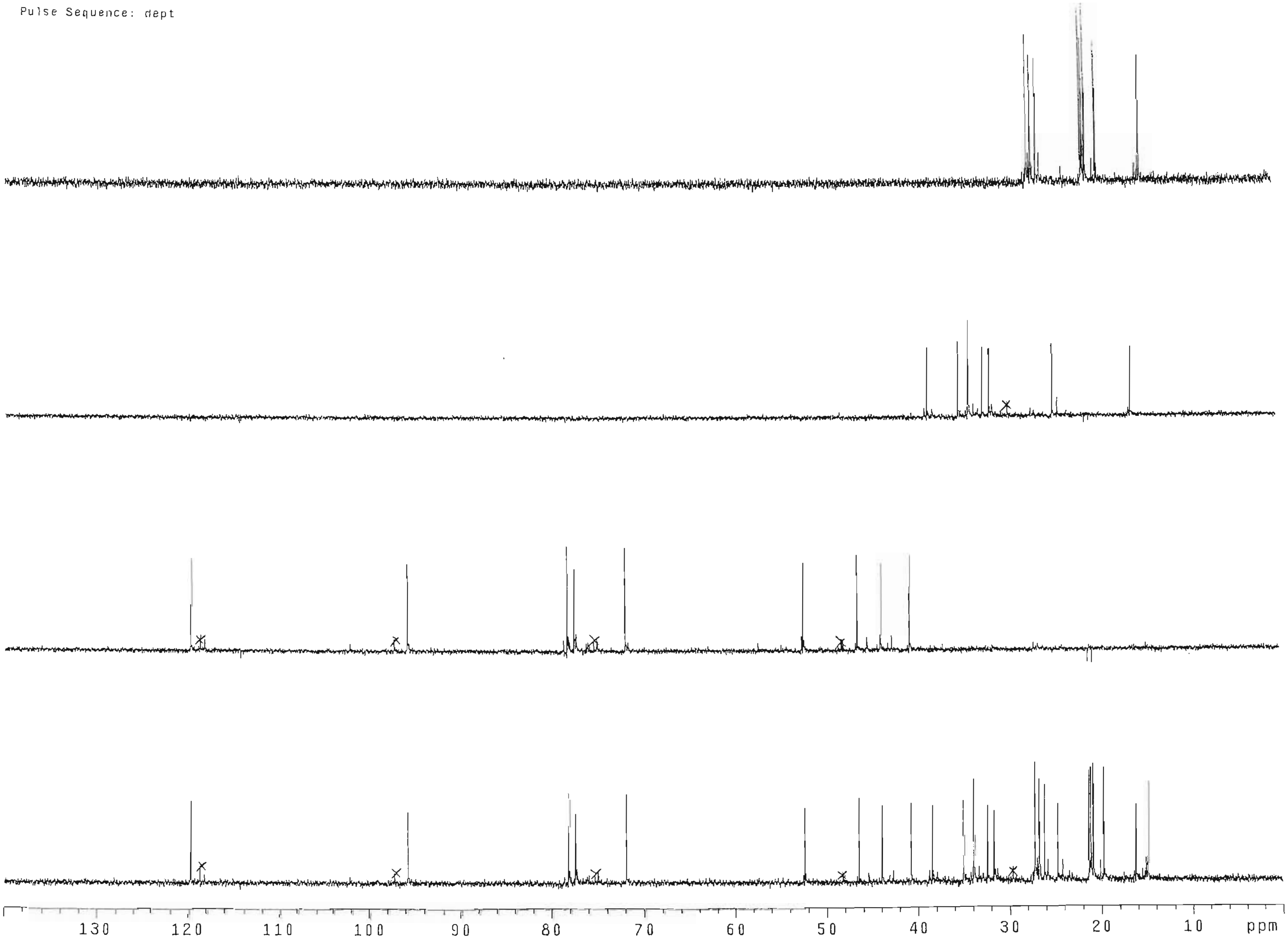
^{1}H NMR Spectrum of Protosamaderine B (xi)

Pulse Sequence: s2pul





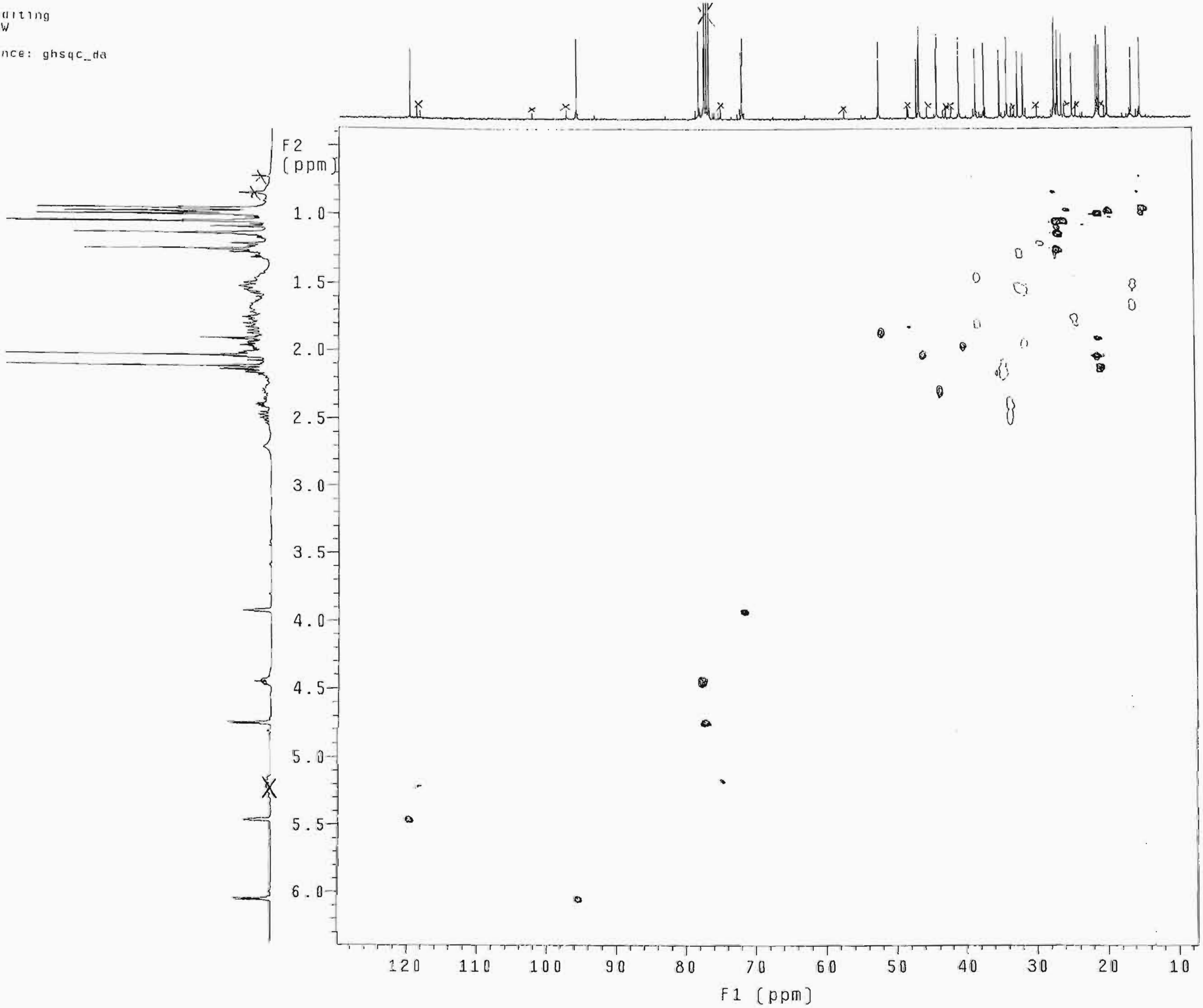
^{13}C NMR Spectrum of [illegible] B (xi)



ADEPT NMR Spectrum of Protoesmodarine B (xi)

with mult.editing
probe=5mmASW

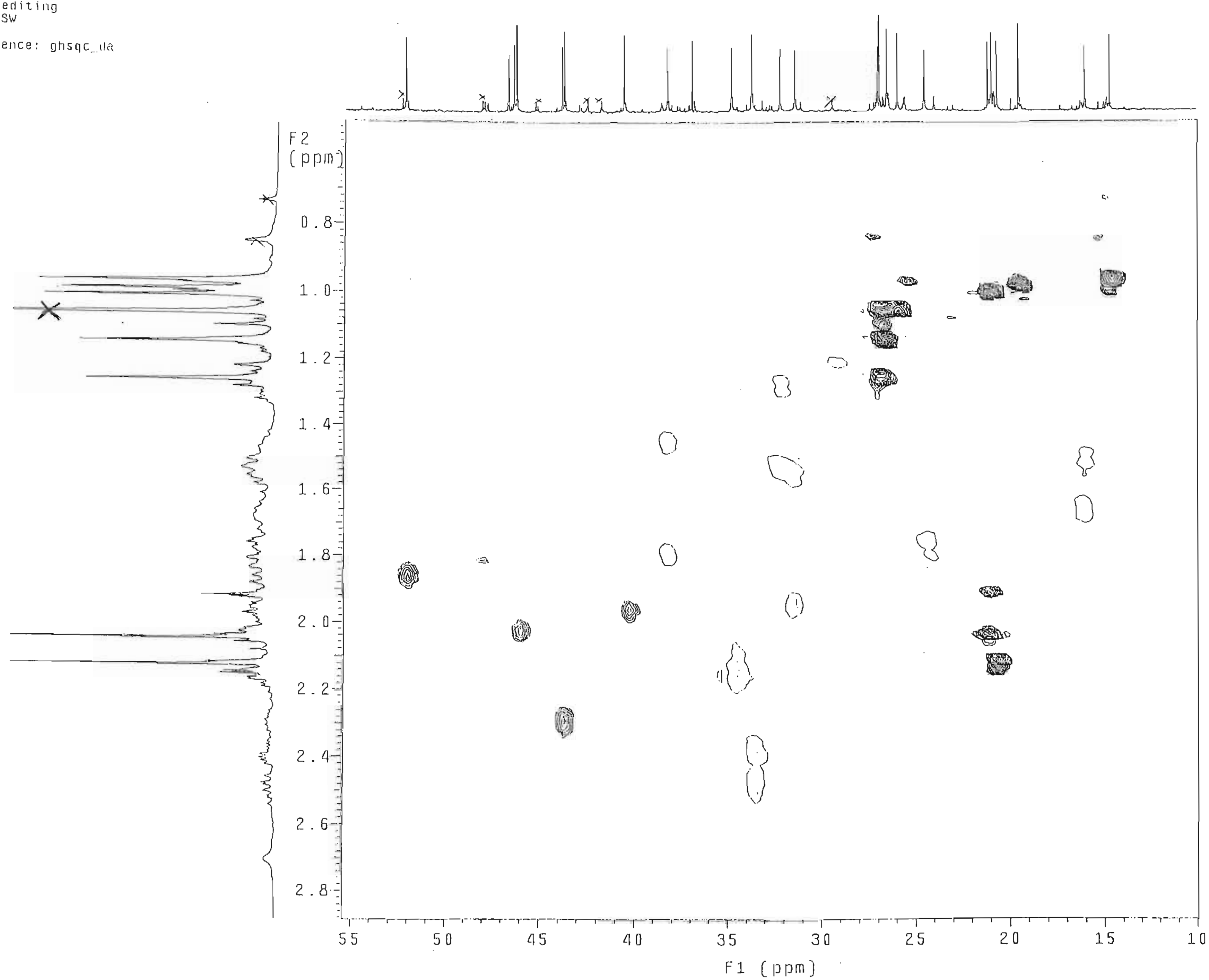
Pulse Sequence: ghsqc_da



HSQC NMR Spectrum of Protosamaderine B (xi)

Gradient HSQC expt.
with mult.editing
probe=5mmASW

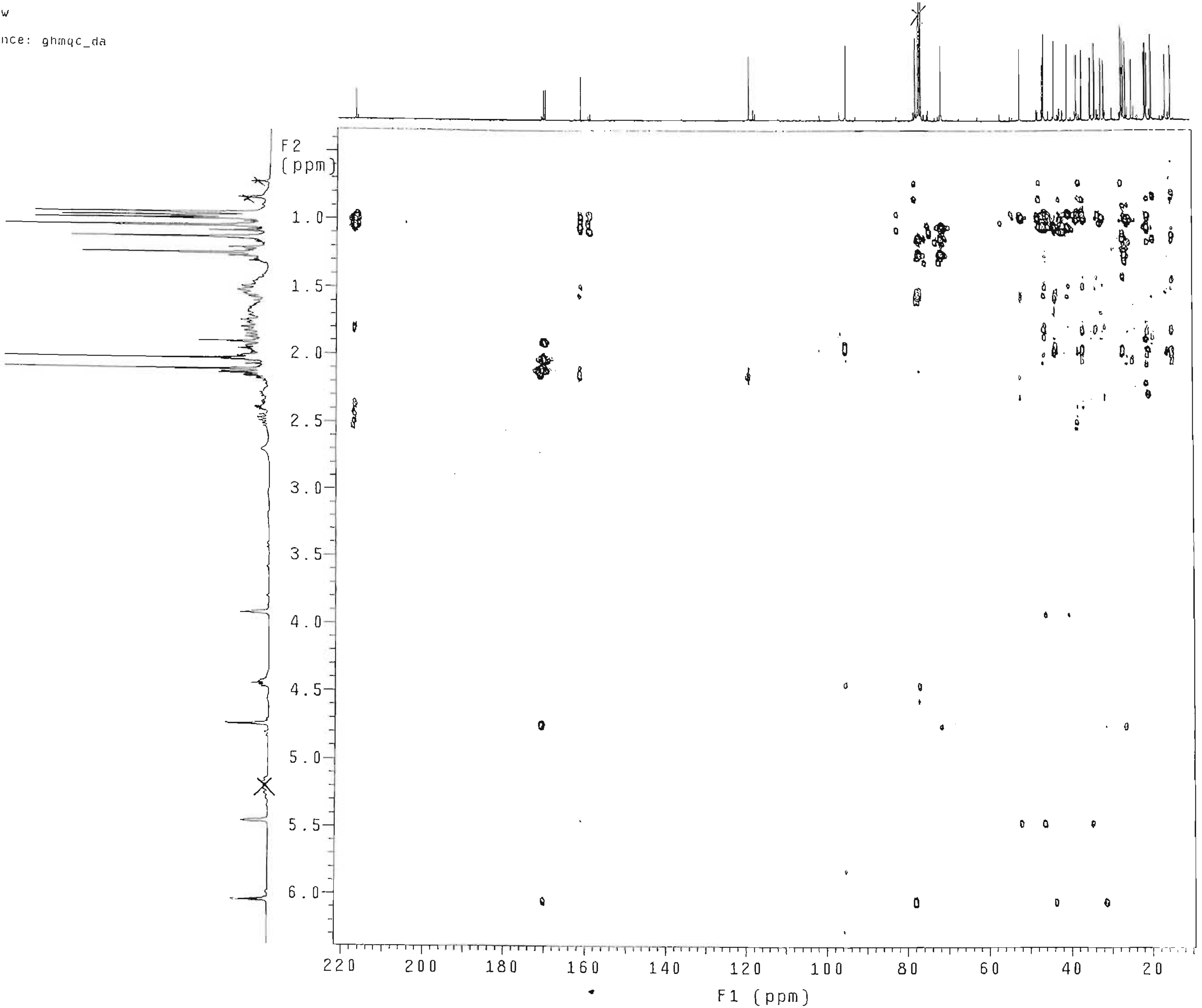
Pulse Sequence: ghsqc_ua



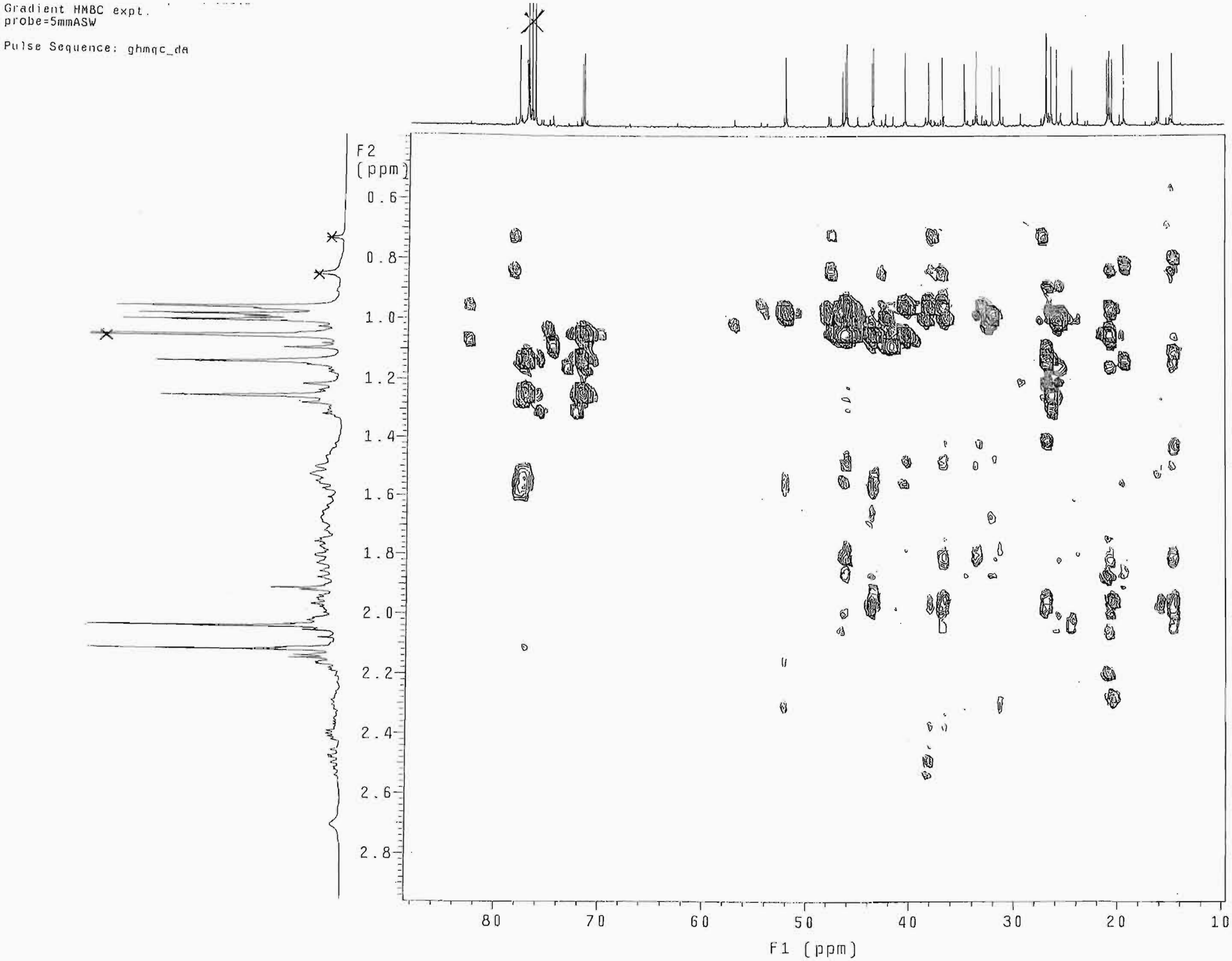
HSQC NMR Spectrum of Protosamadorine B (xi)

probe=5mmASW

Pulse Sequence: ghmqc_da

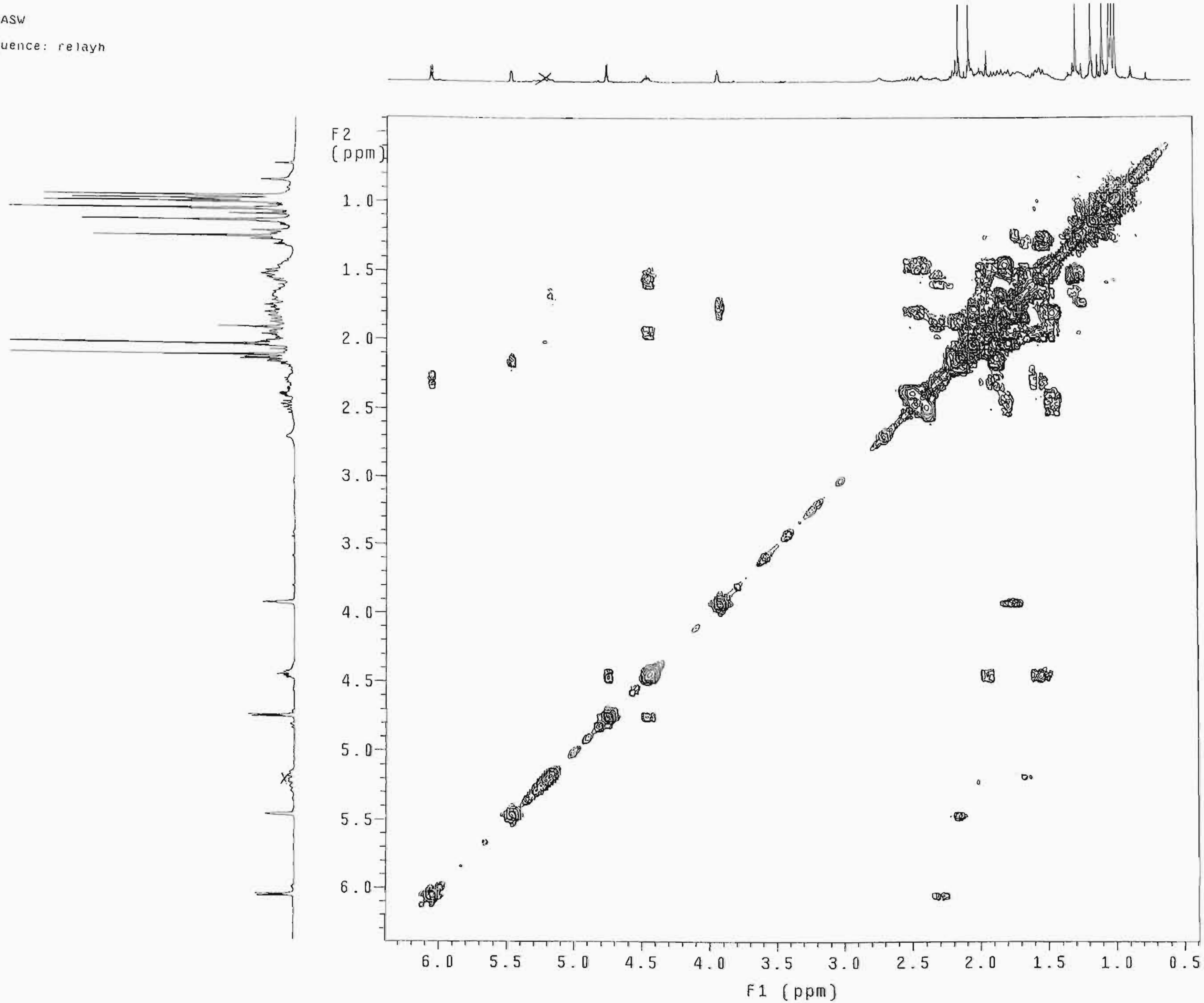


HMBC NMR Spectrum of Protoasmodarine B (xi)


Gradient HMBC expt.
probe=5mmASW
Pulse Sequence: ghmqc_da
F2 (ppm)
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
80
70
60
50
40
30
20
10
F1 (ppm)

probe=5mmASW

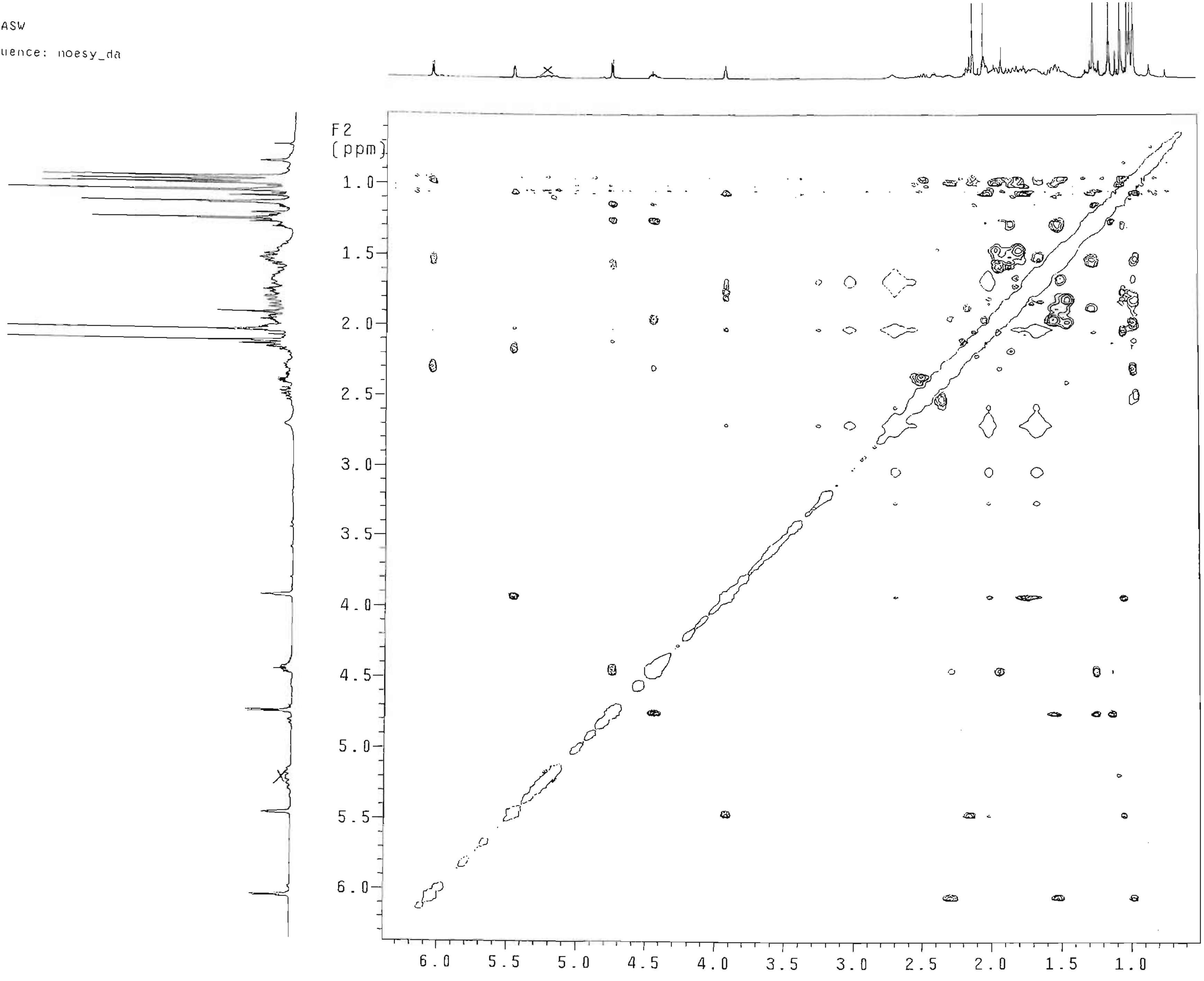
Pulse Sequence: relayh



COSY NMR Spectrum of [illegible]

mix=1sec
probe=5mmASW

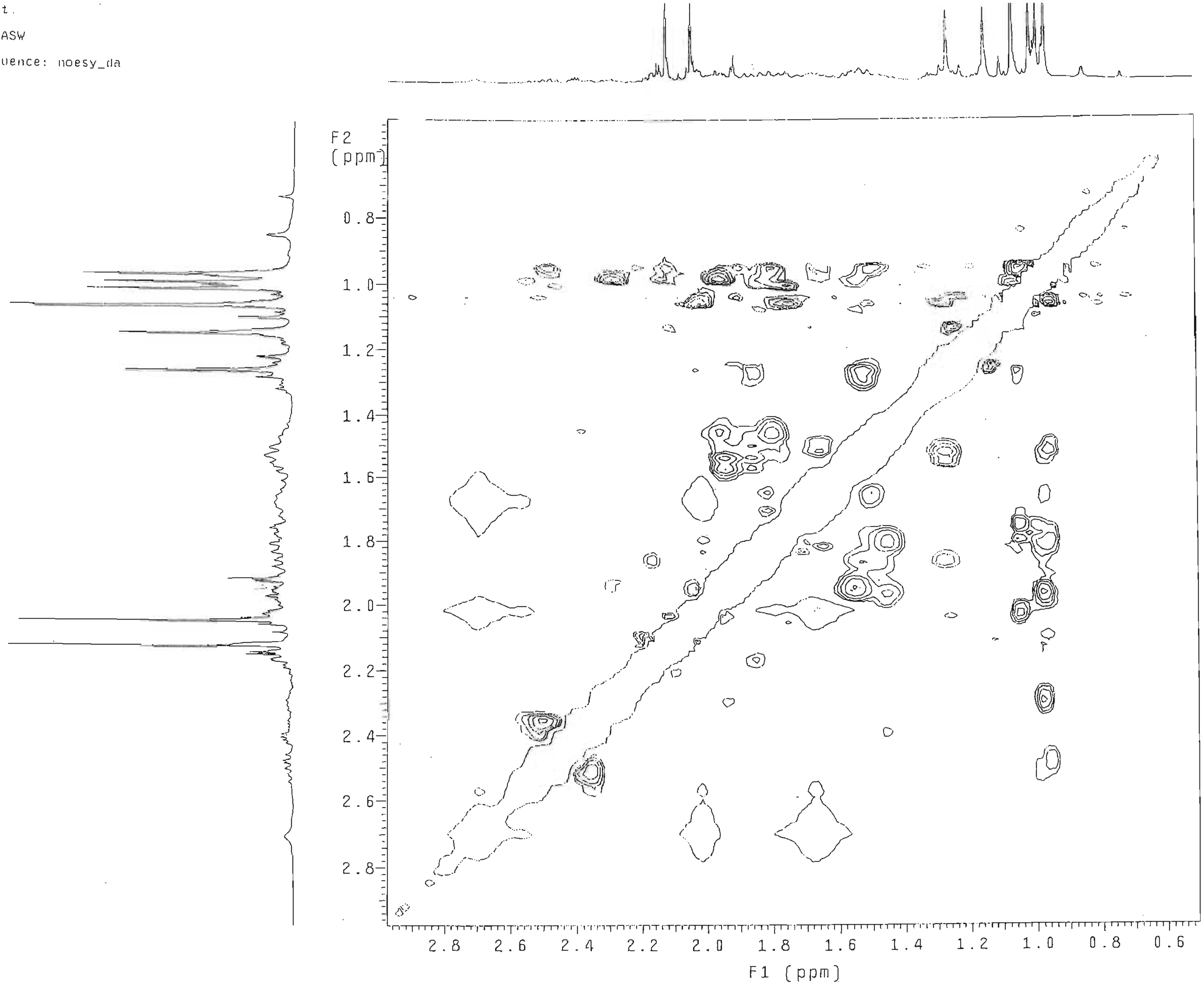
Pulse Sequence: noesy_da



NOESY NMR Spectrum of

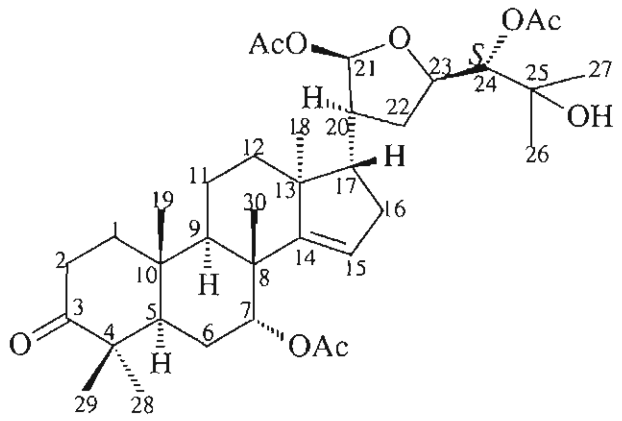
NOESY expt.
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da

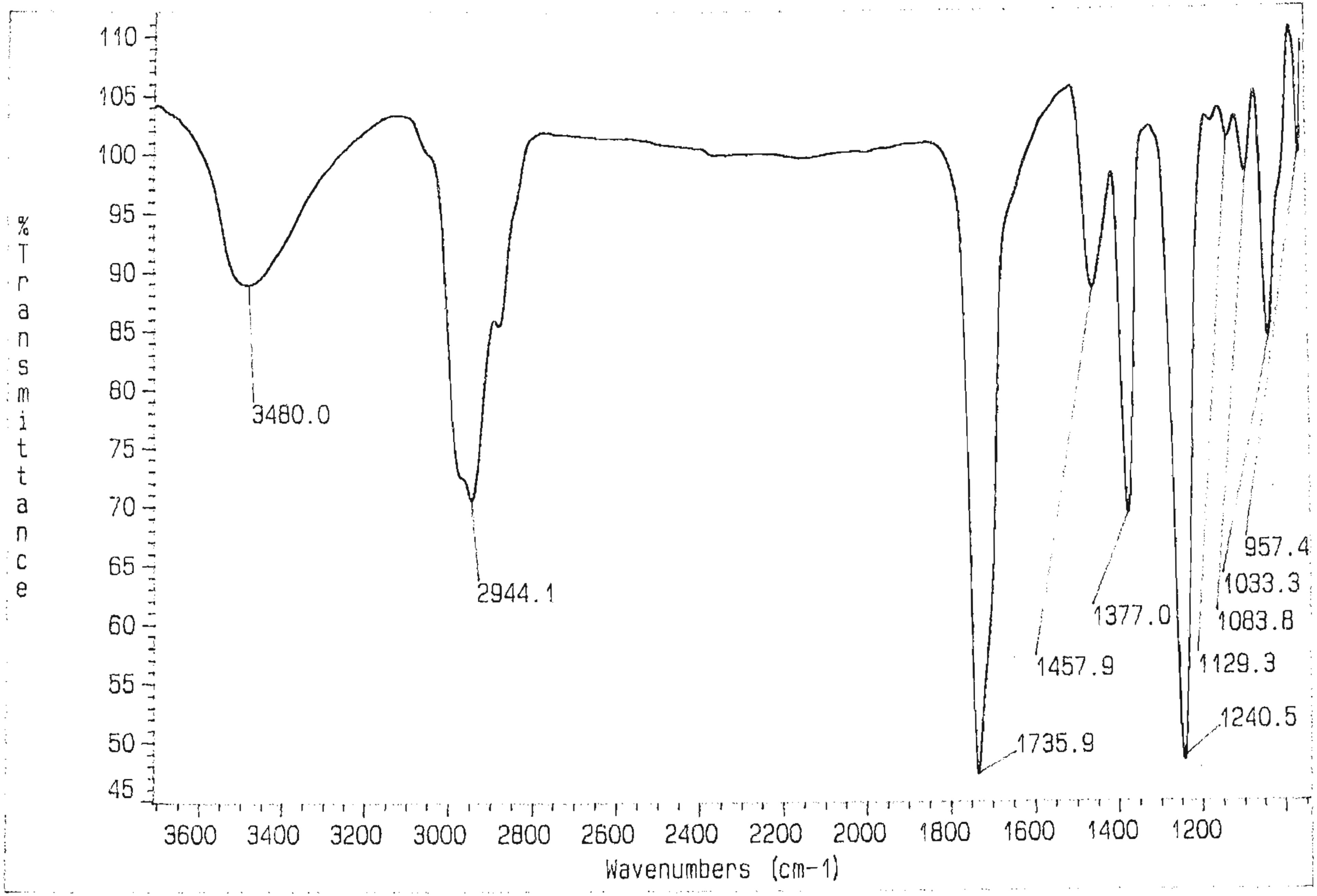


NOESY NMR Spectrum of Protosamaderine B (xi)

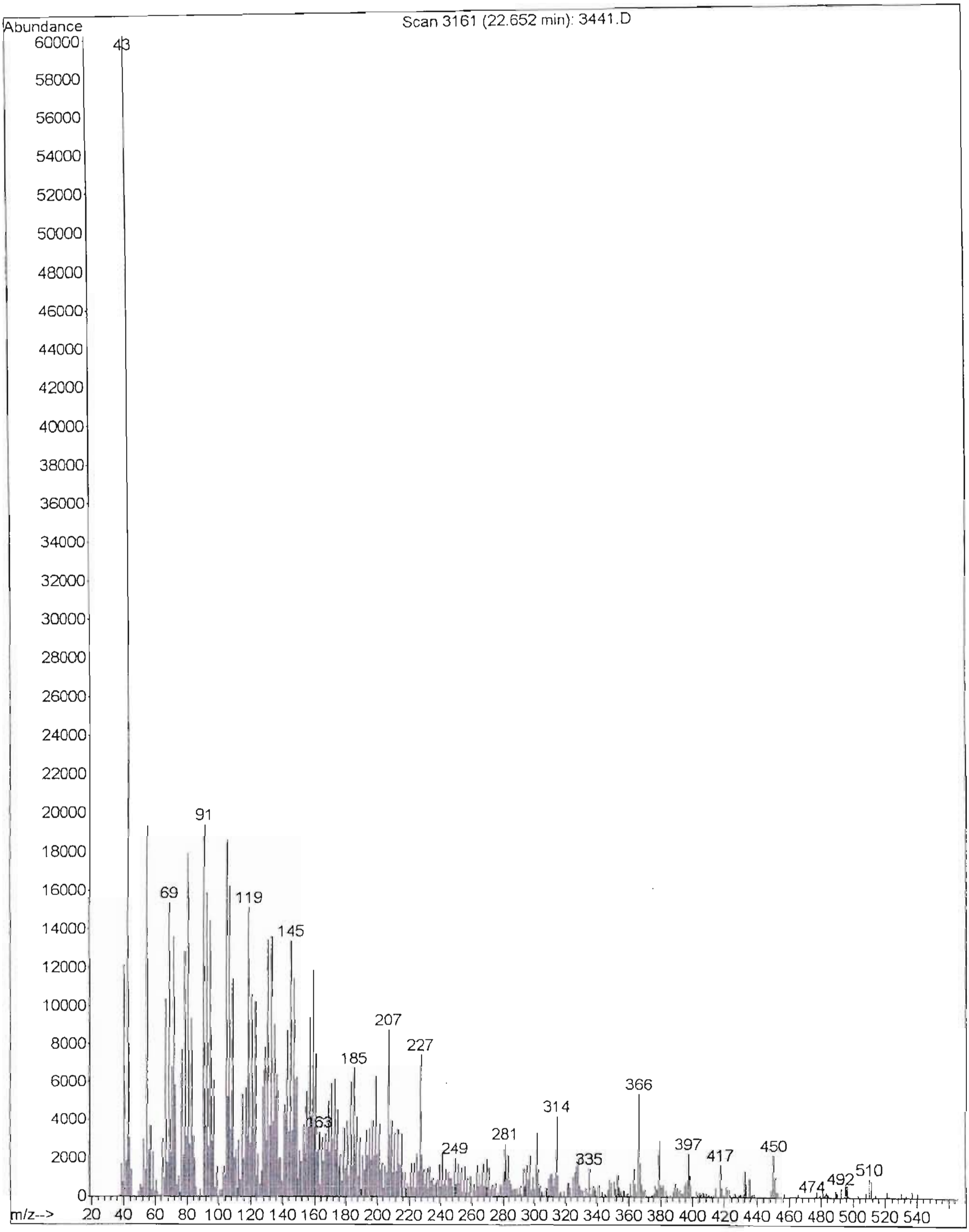
Protosamaderine C (xii)



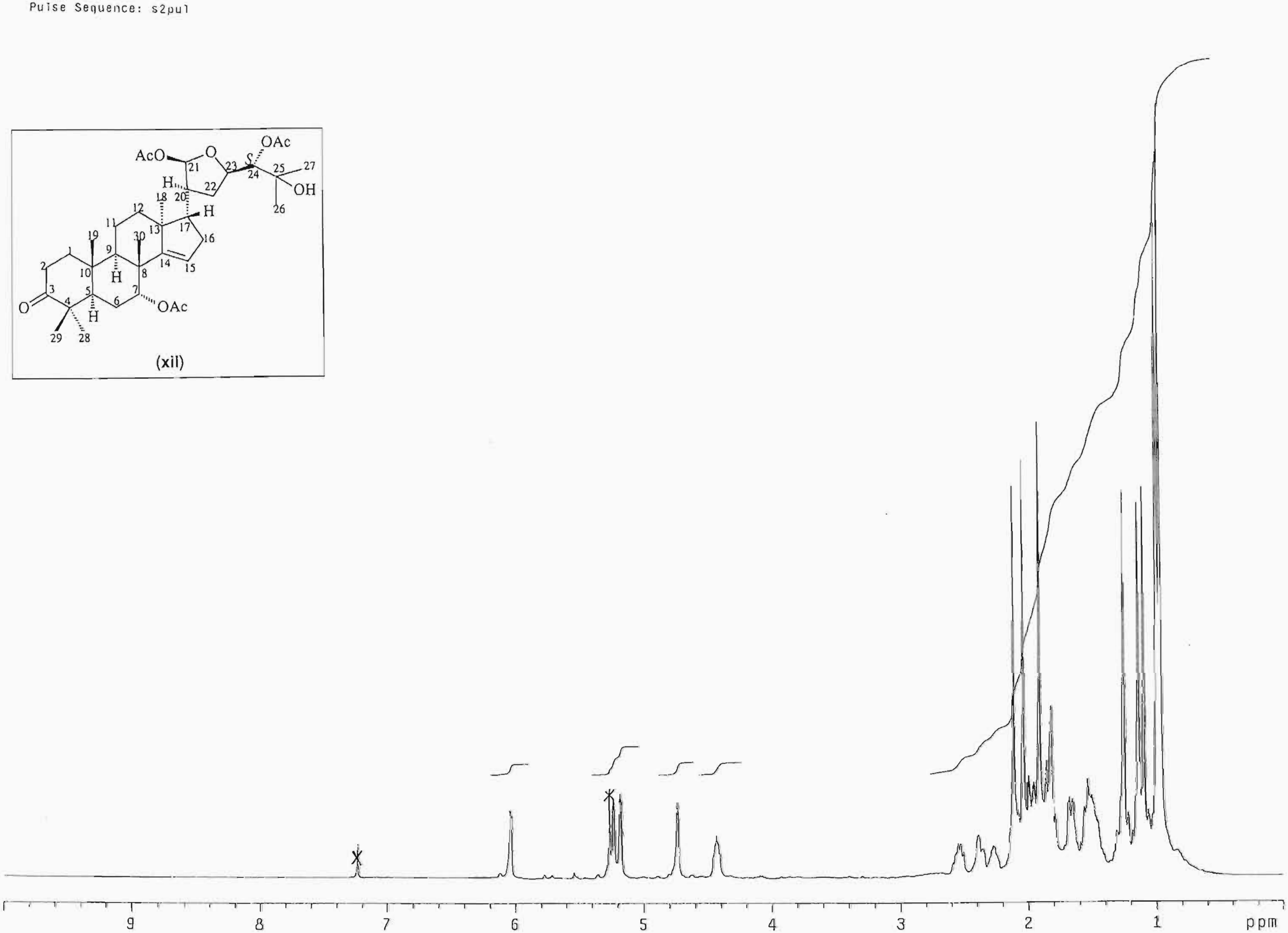
(xii)



IR Spectrum of Protosamaderine C (xii)

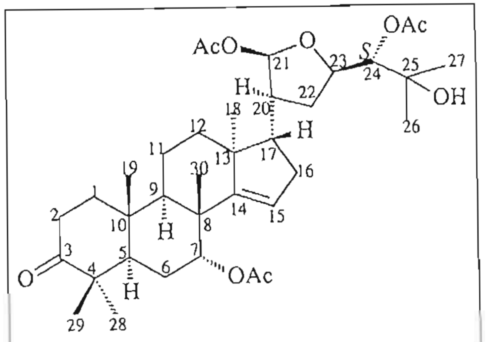


Mass Spectrum of Protosamaderine C (xii)

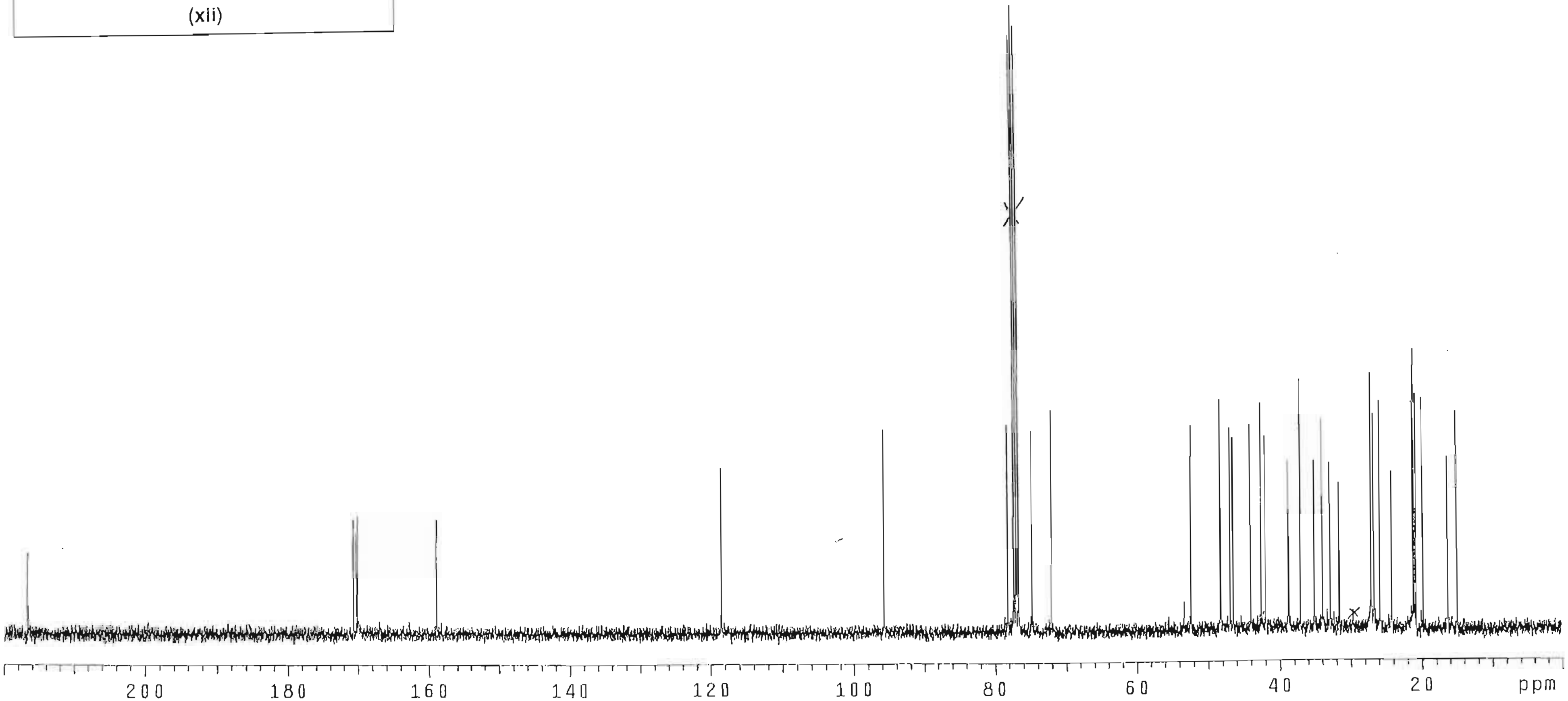


^{1}H NMR Spectrum of Protosamaderine C (xii)

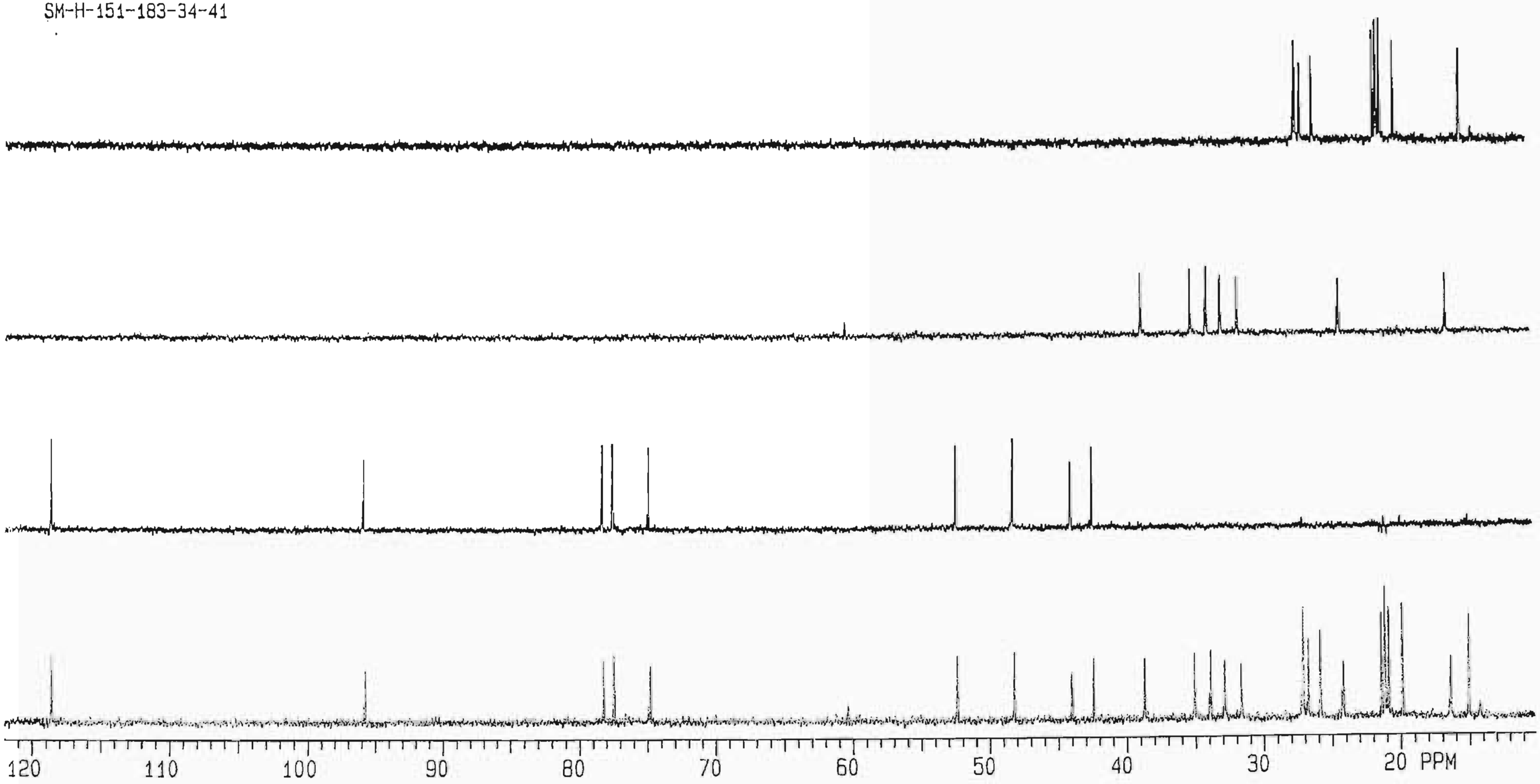
Pulse Sequence: s2pul



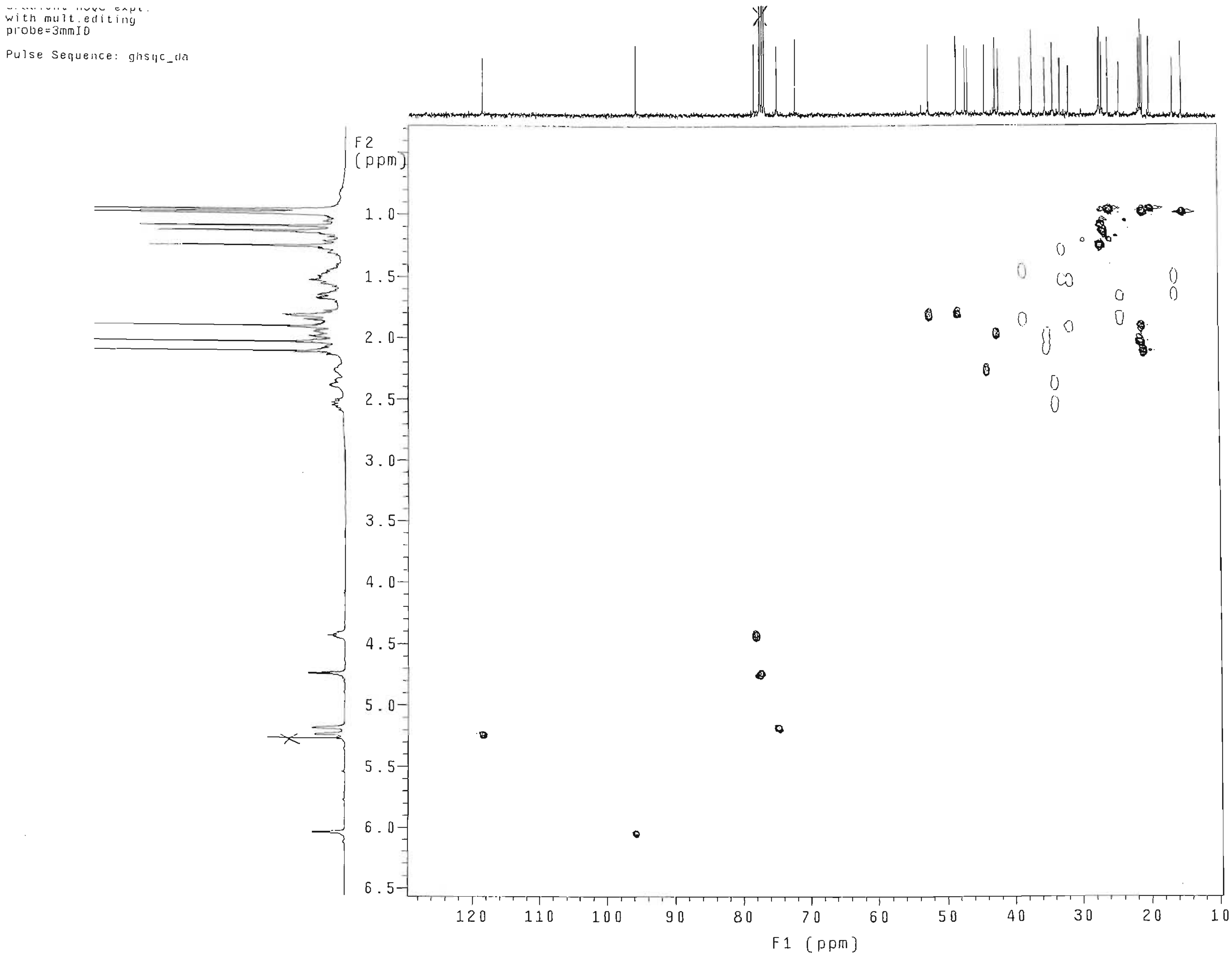
(xii)



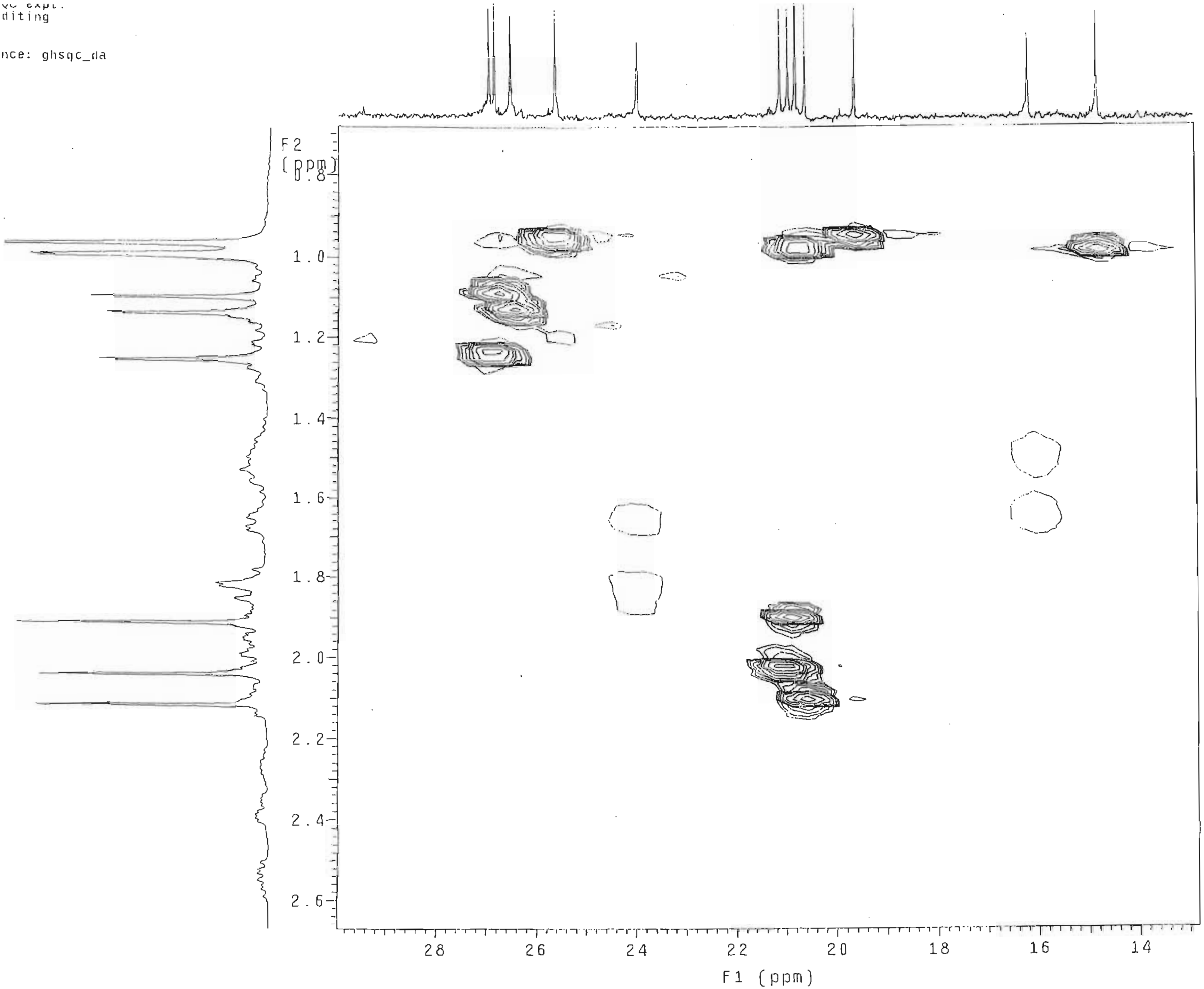
^{13}C NMR Spectrum of Protosamaderine C (xii)



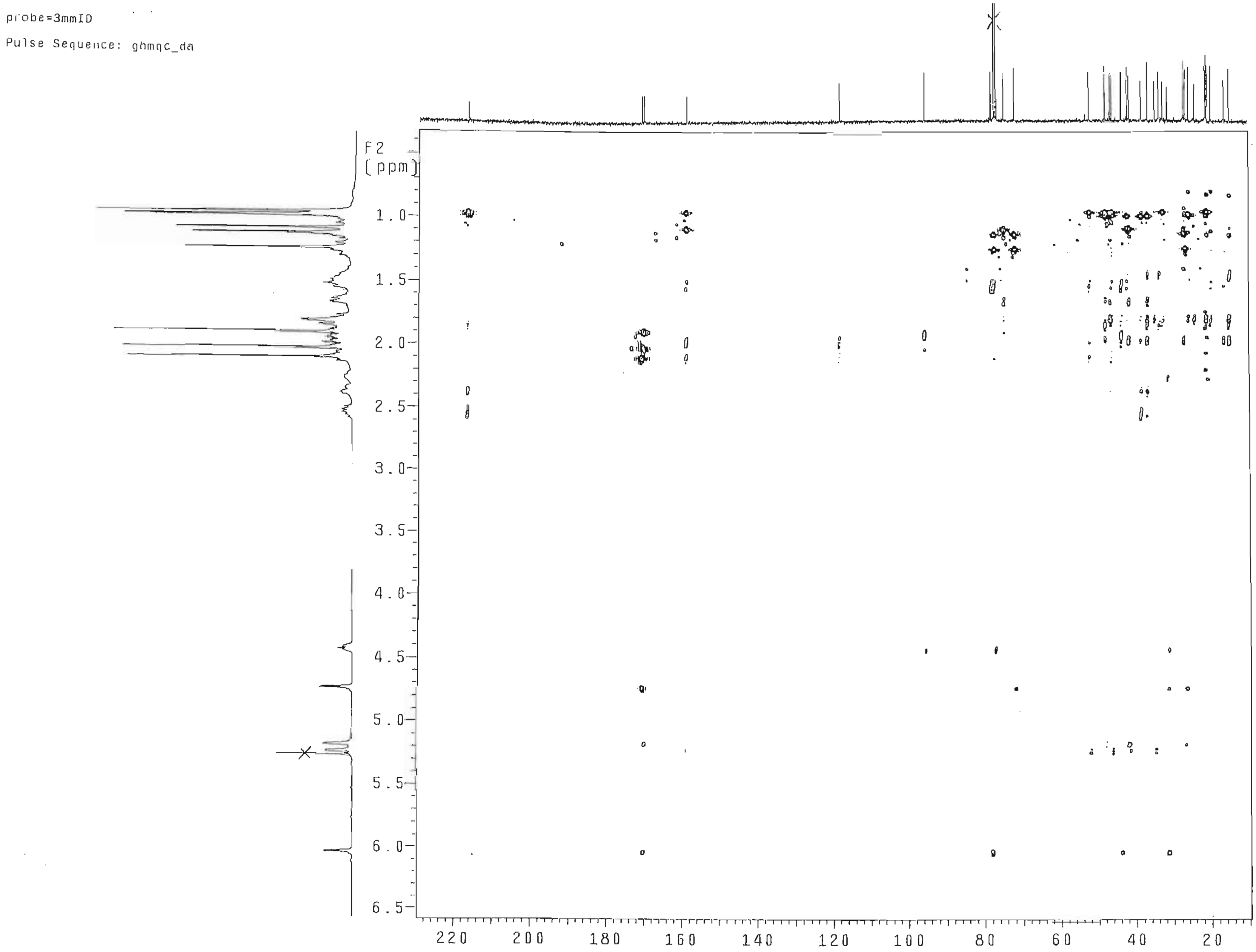
ADEPT NMR Spectrum of Protoesmaderine C (xii)



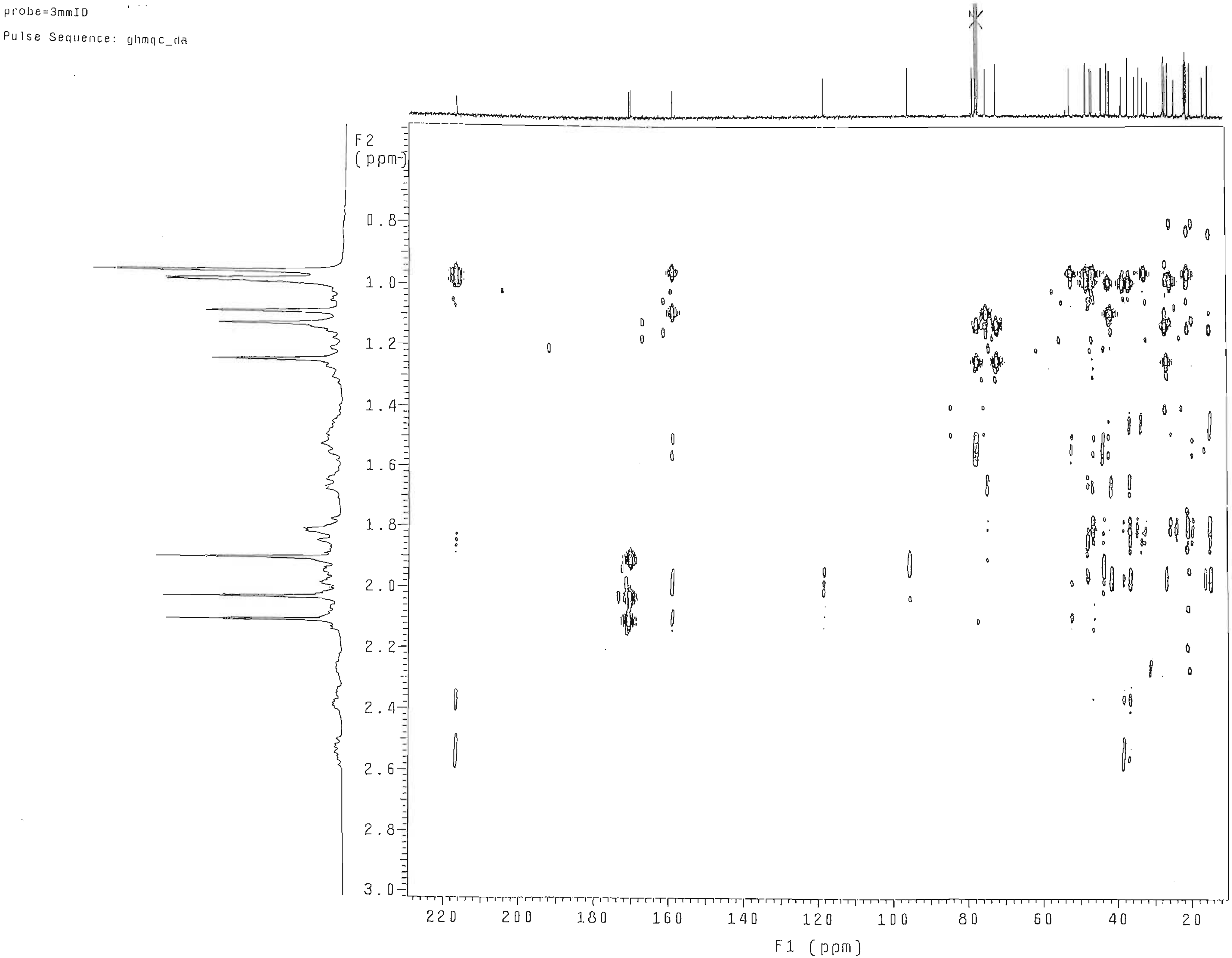
HSQC NMR Spectrum of Protosamaderine C (xii)



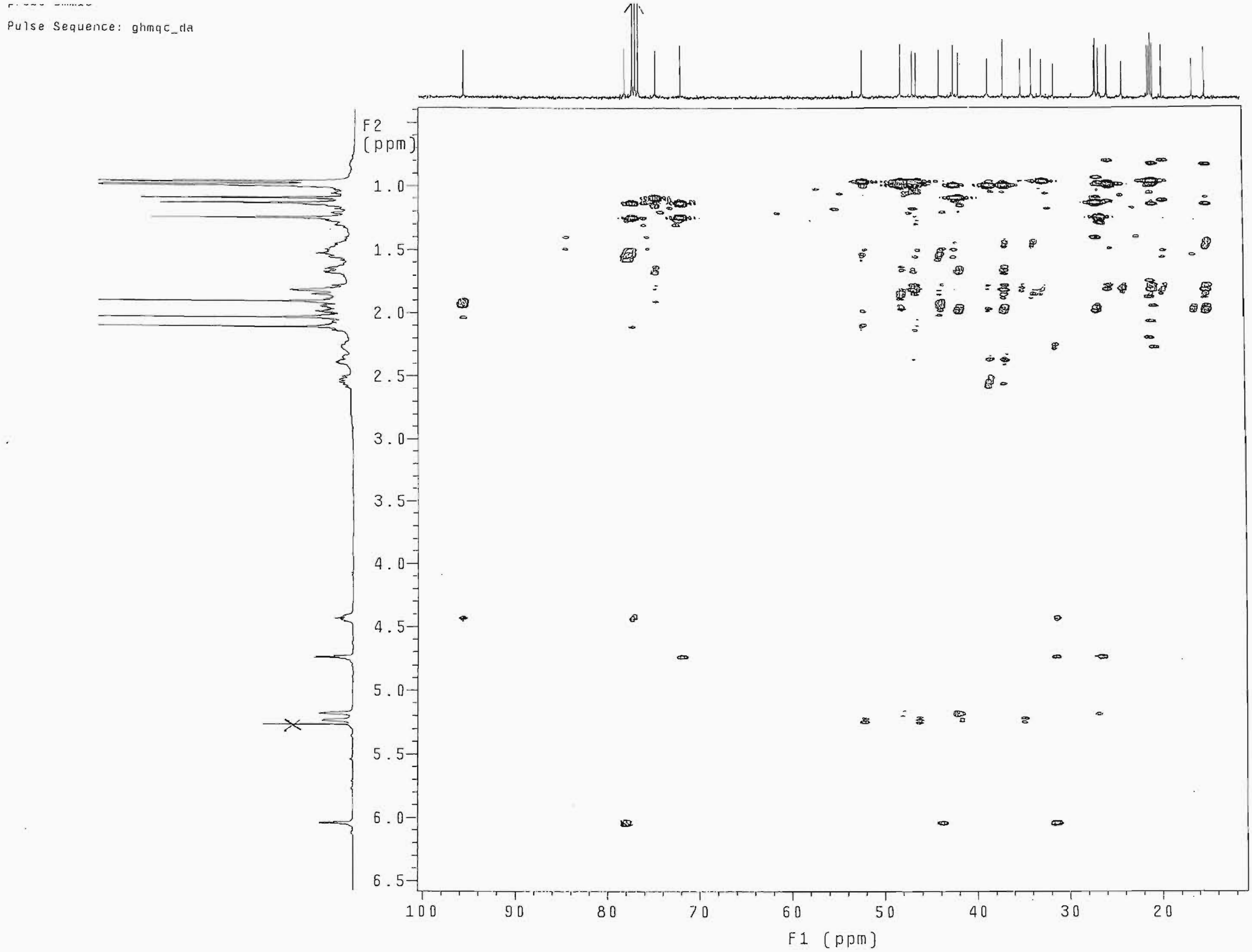
HSQC NMR Spectrum of Protoasmodarine C (vii)



HMBC NMR Spectrum of Protoseemaderine C (xii)

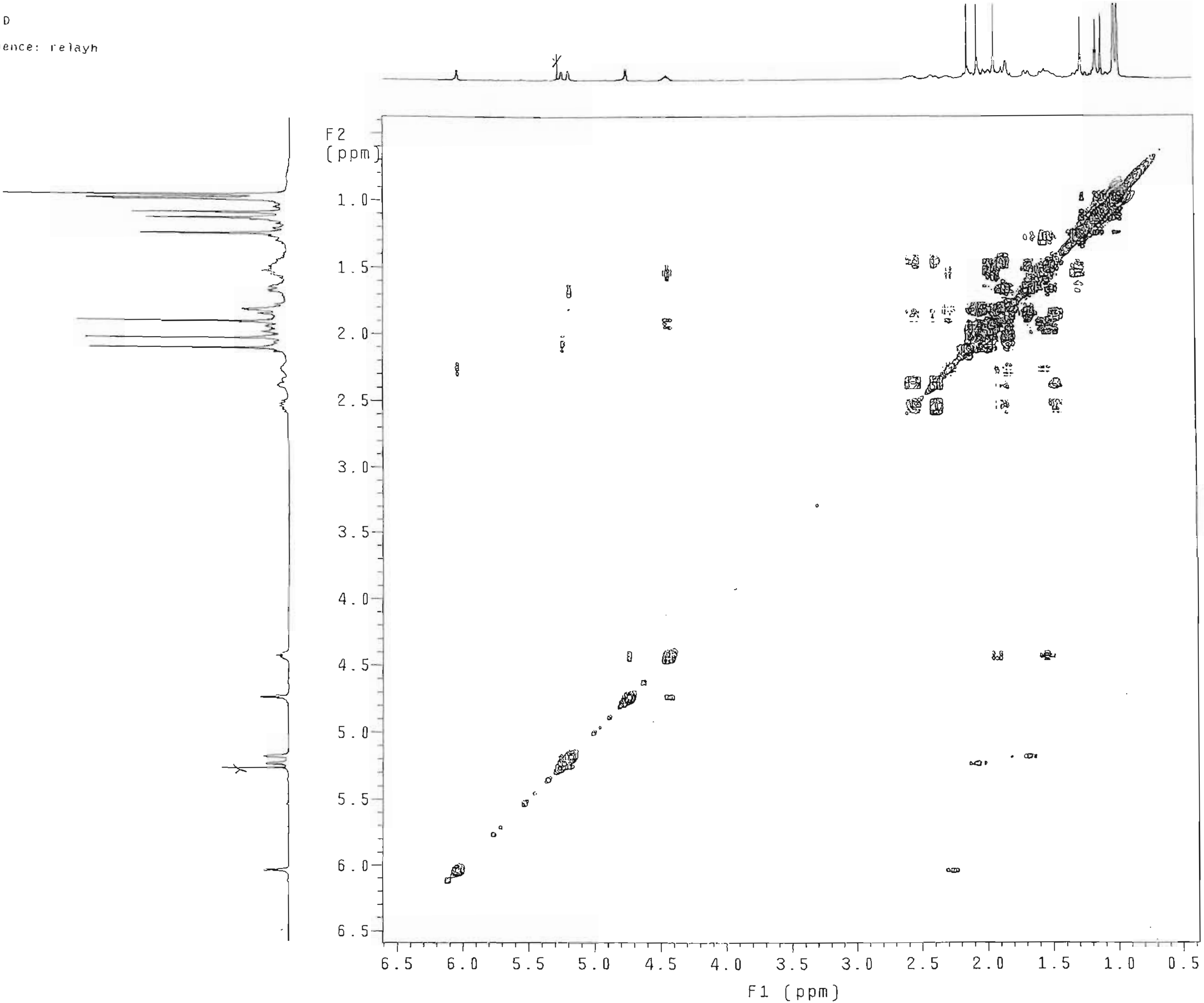


HMBC NMR Spectrum of Protosamaderine C (xii)



HMBC NMR Spectrum of Protosamaderine C (xii) probe=3mmID

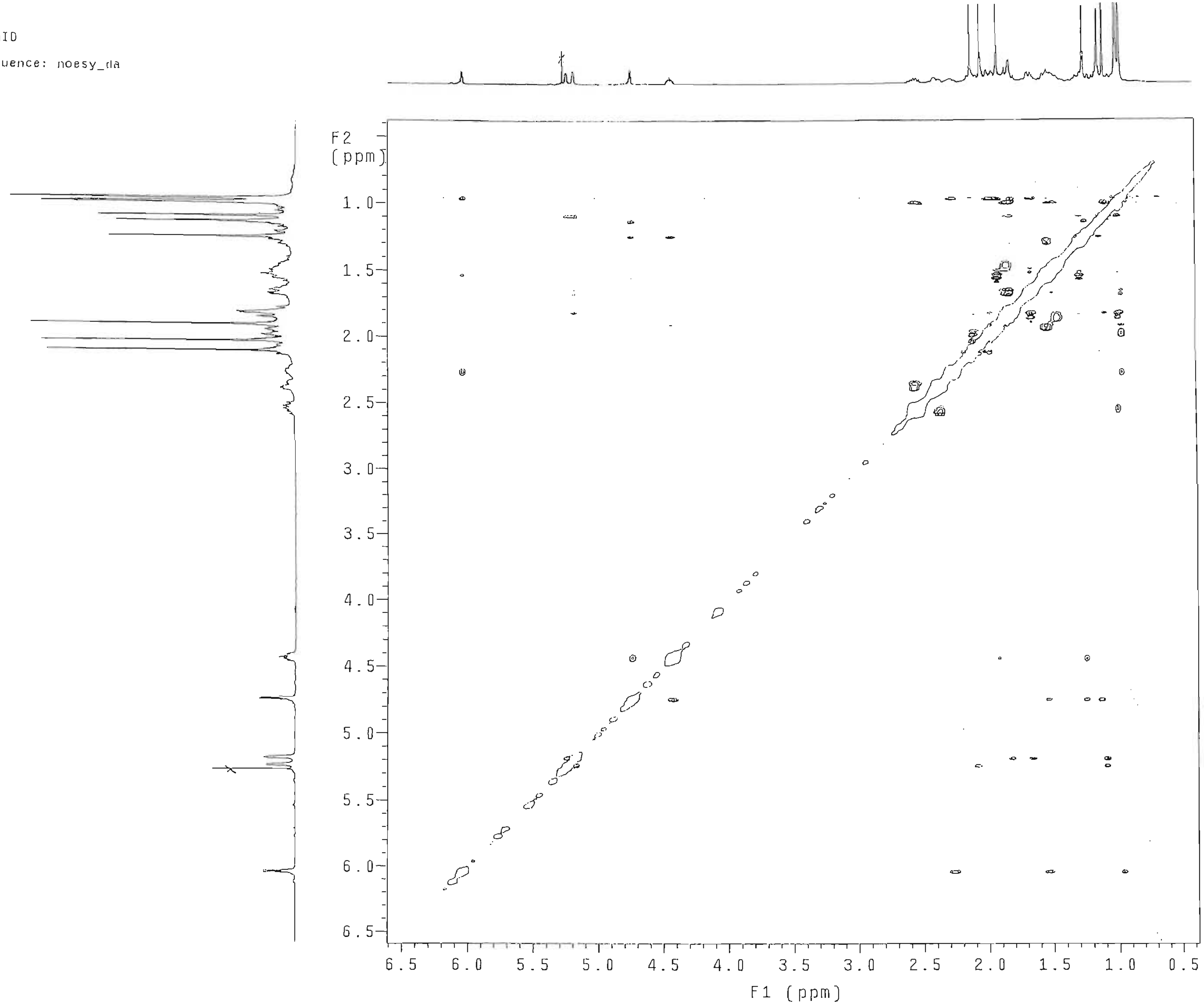
Pulse Sequence: relayh



COSY NMR Spectrum of [illegible]

mix=1sec
probe=3mmID

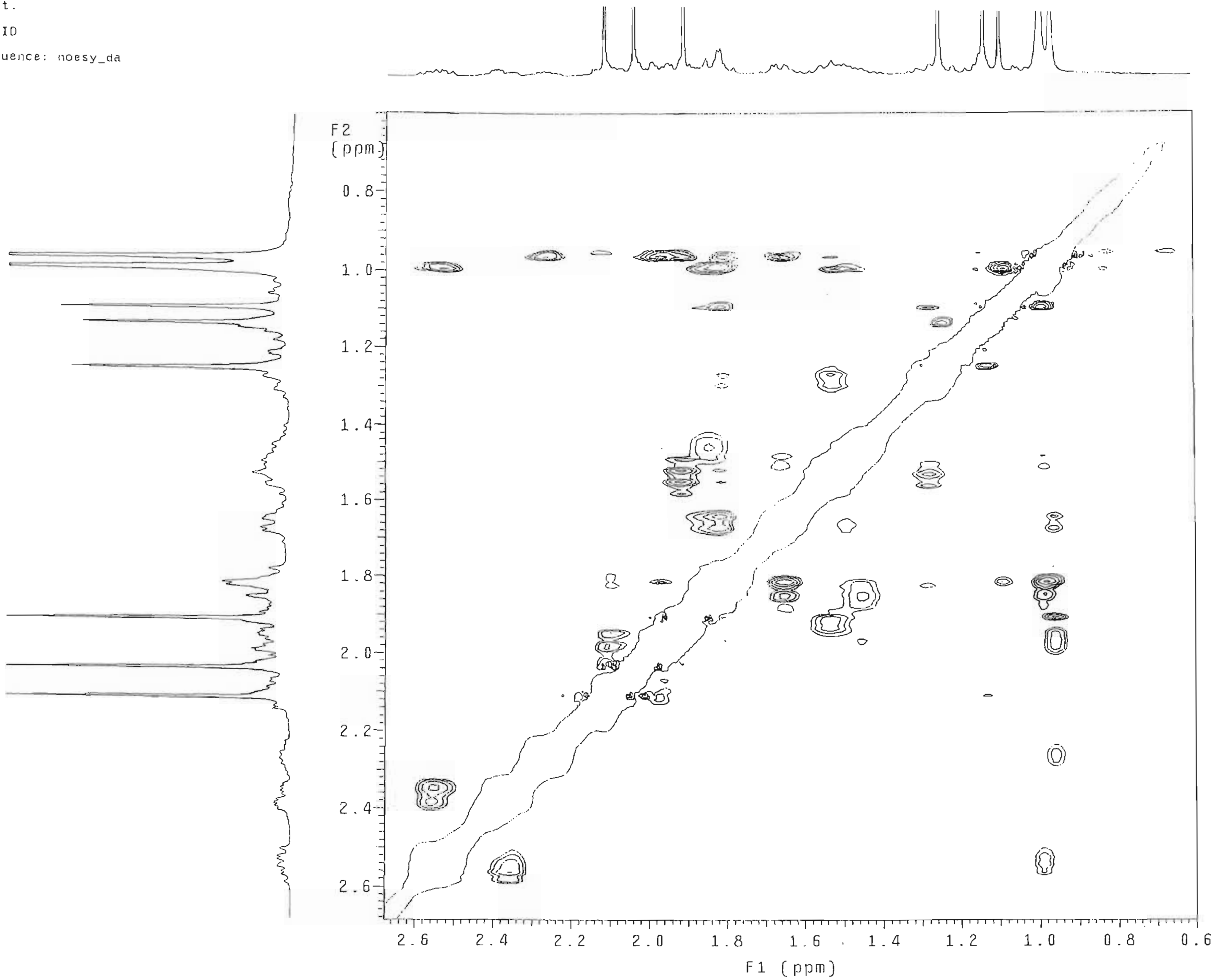
Pulse Sequence: noesy_da



NOESY NMR Spectrum of [illegible]

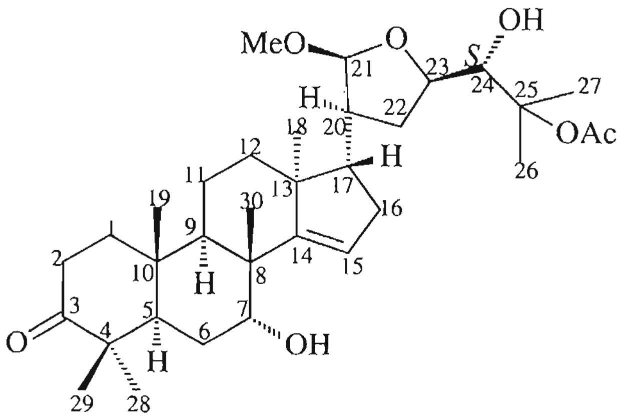
NOESY expt.
mix=1sec
probe=3mmID

Pulse Sequence: noesy_da

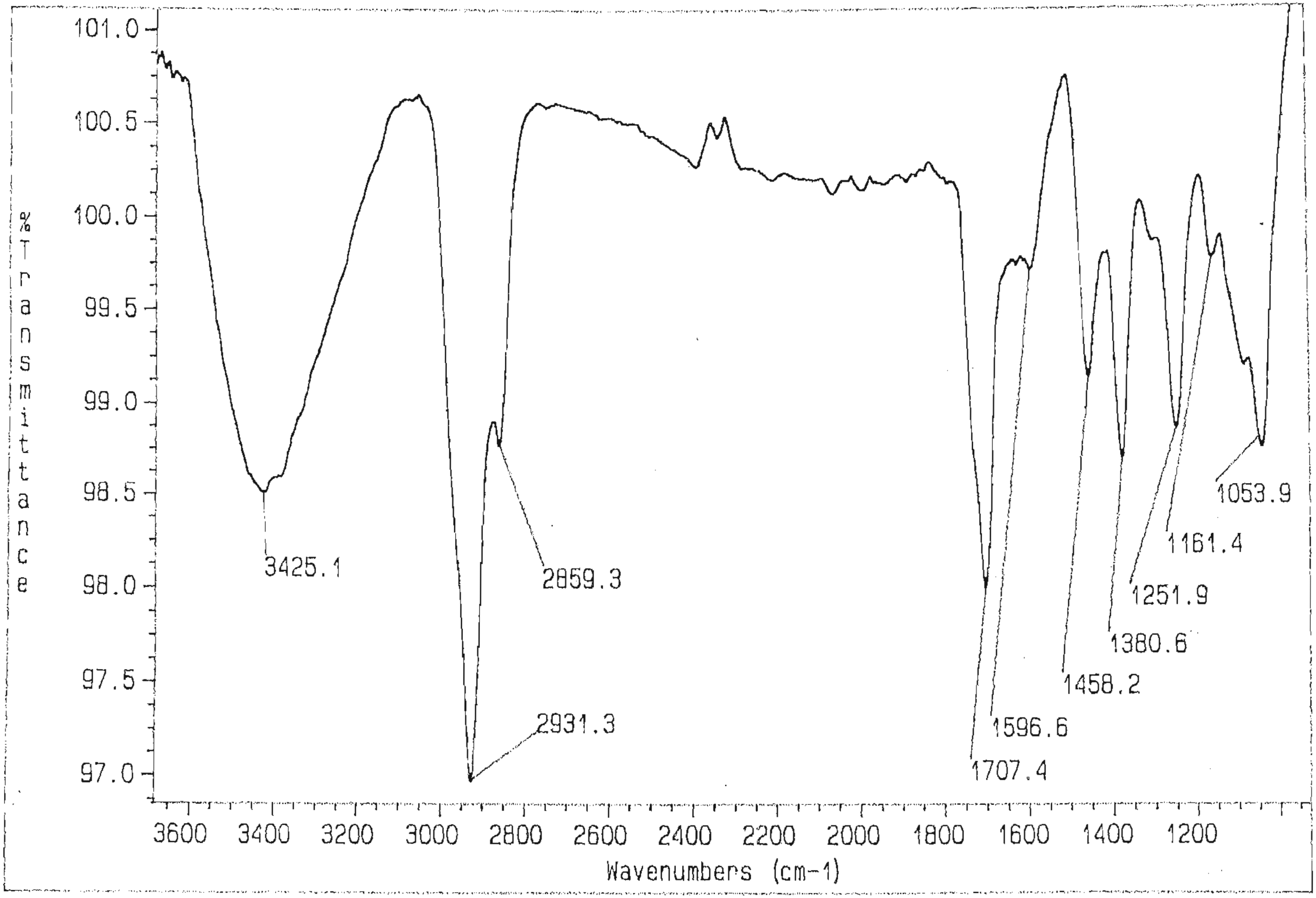


NOESY NMR Spectrum of Protosamaderine C (vii)

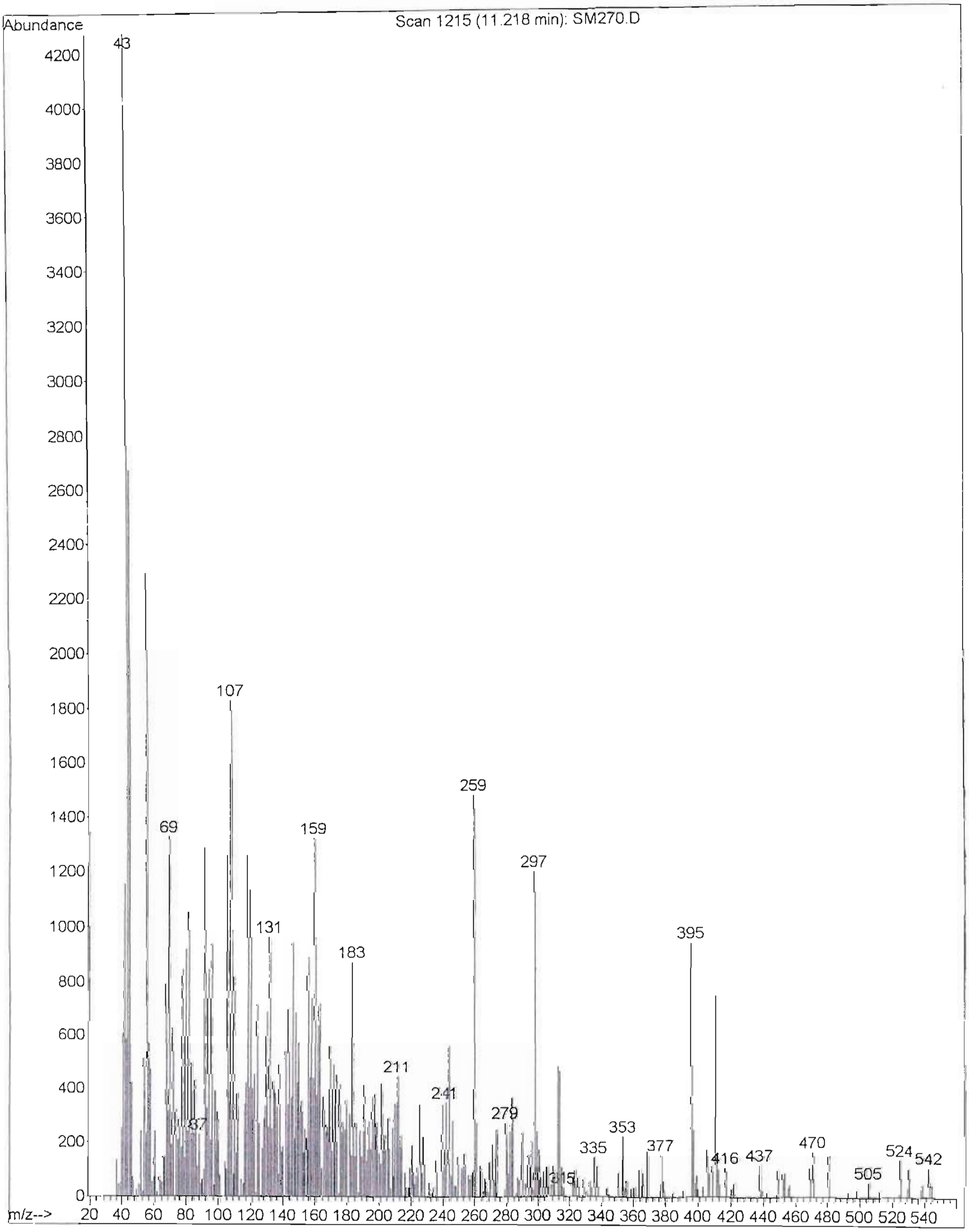
Protosamaderine D (xiii)



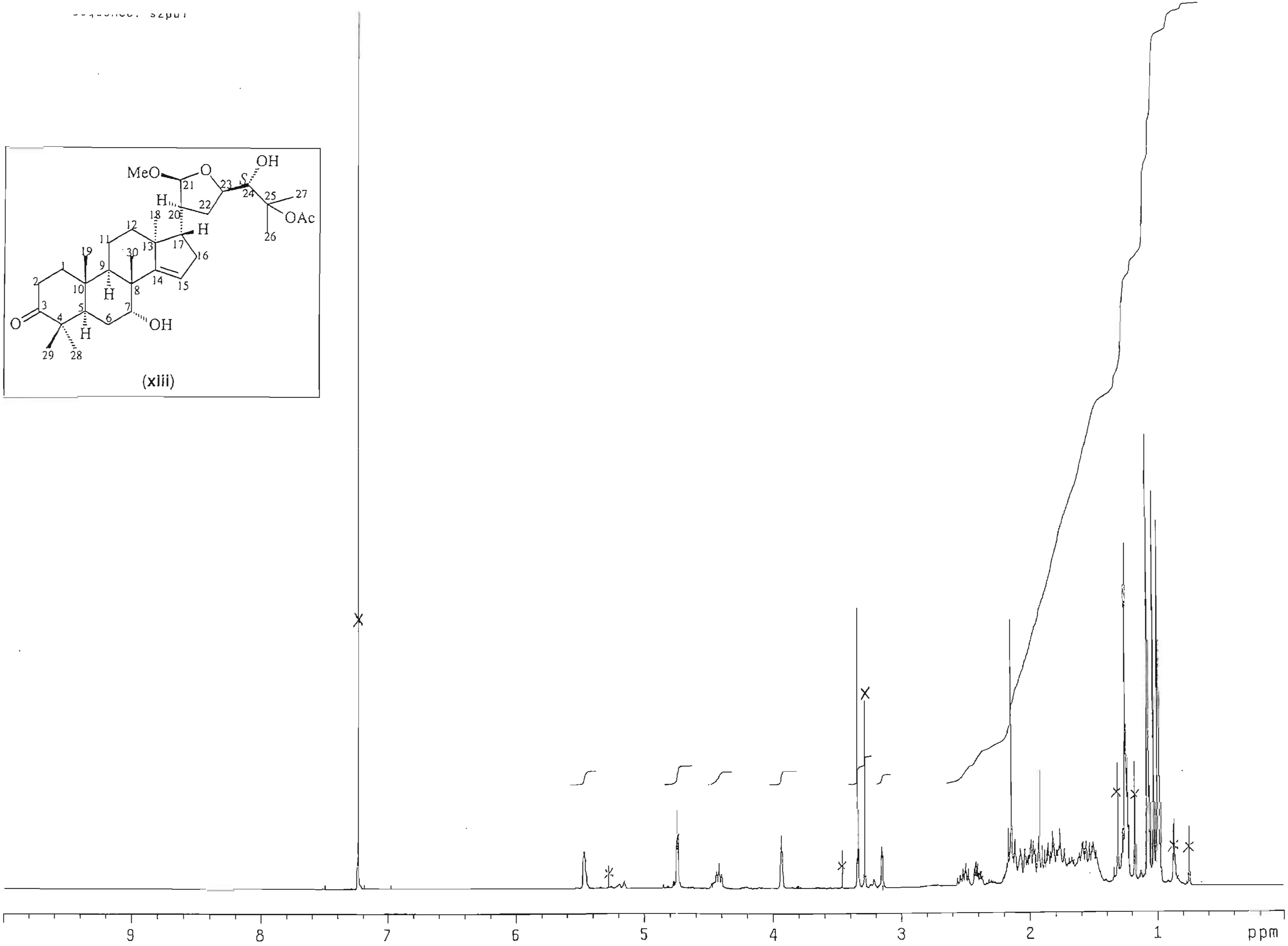
(xiii)



IR Spectrum of Protosamaderine D (xiii)

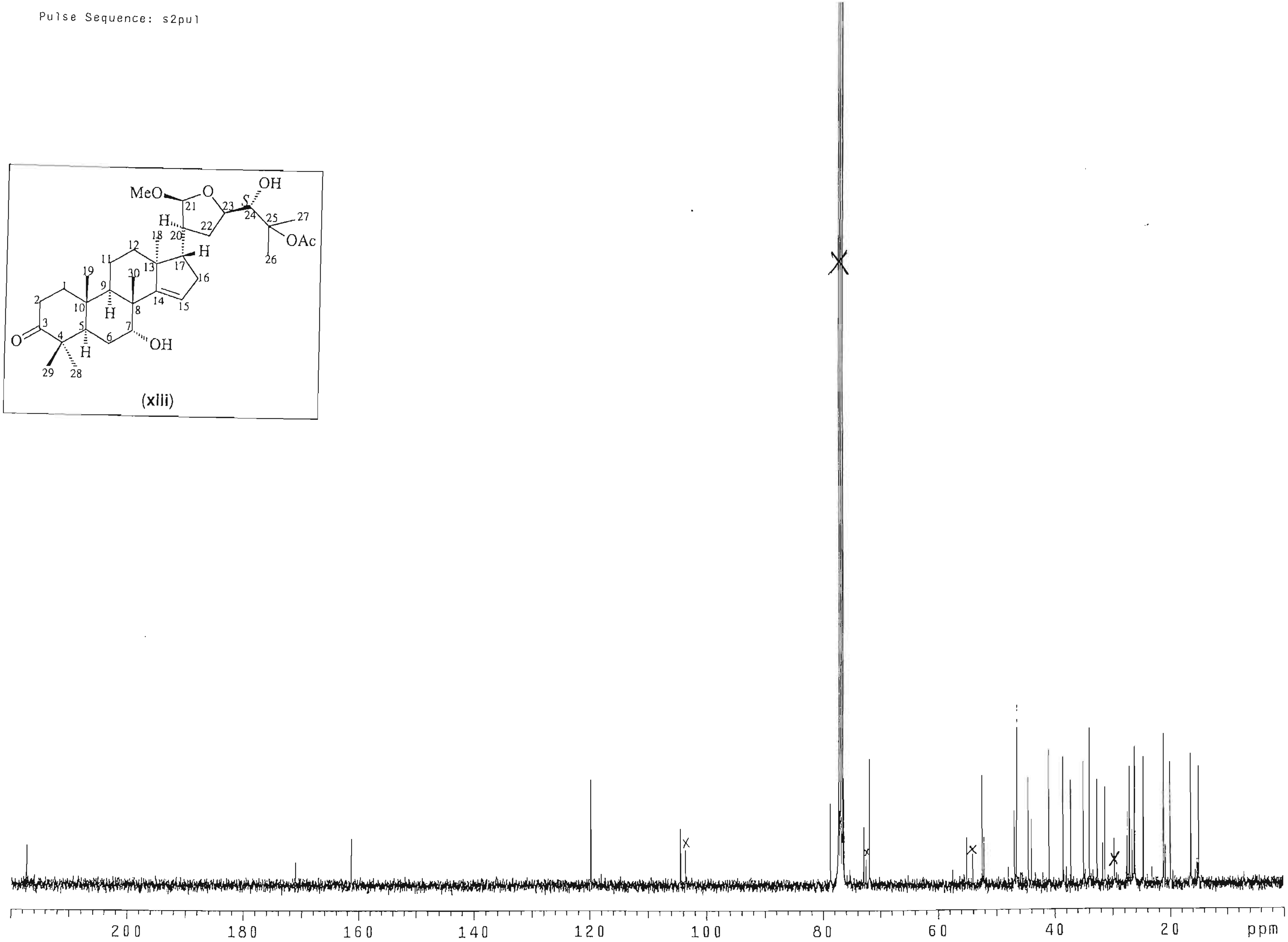


Mass Spectrum of Protosamaderine D (xiii)



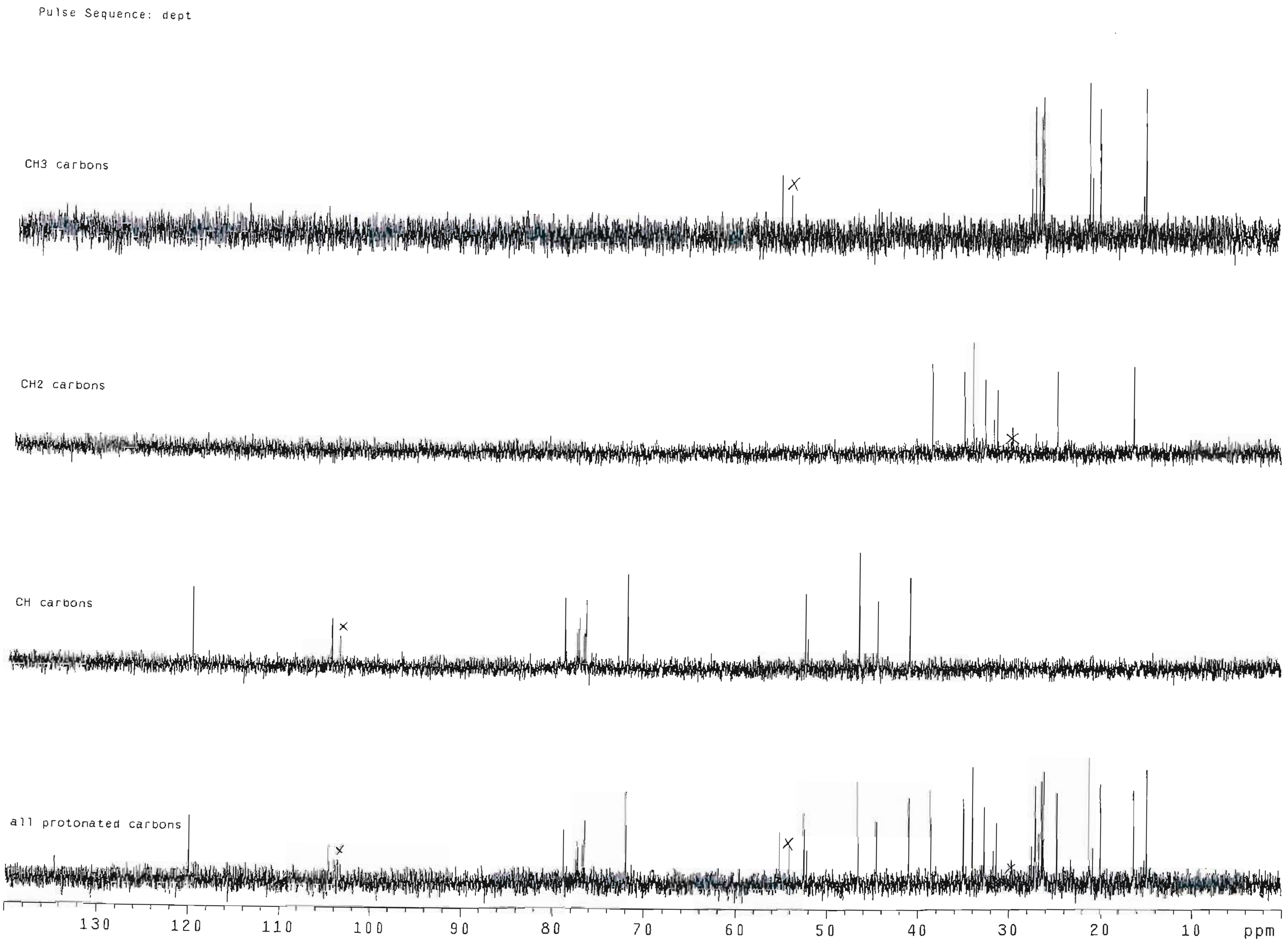
^1H NMR Spectrum of Protosamaderine D (xiii)

Pulse Sequence: s2pul



(xlii)

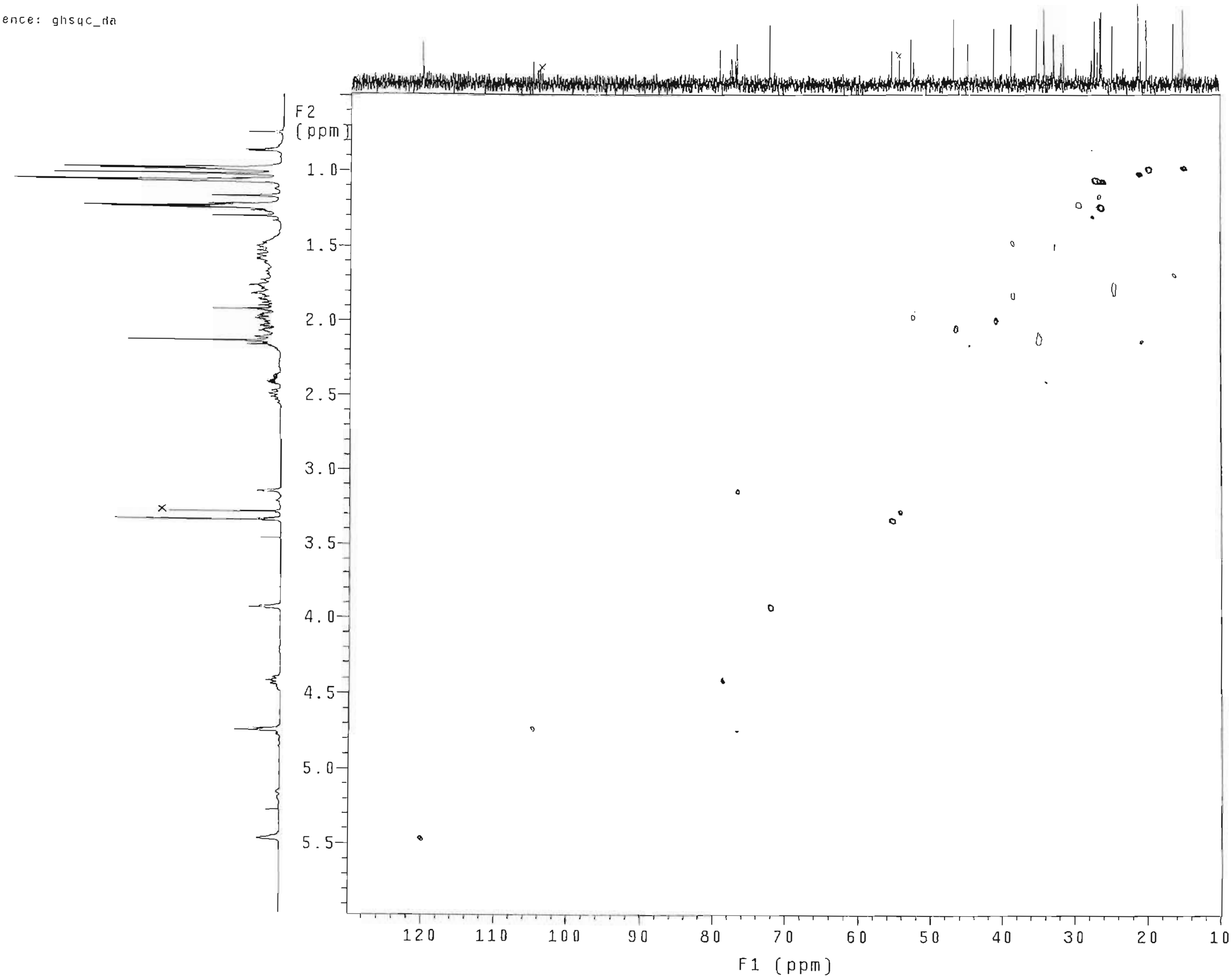
^{13}C NMR Spectrum of Protosamaderine D (xlii)



ADEPT NMR Spectrum of Protosamaderine D (xiii)

probe=5mmASW

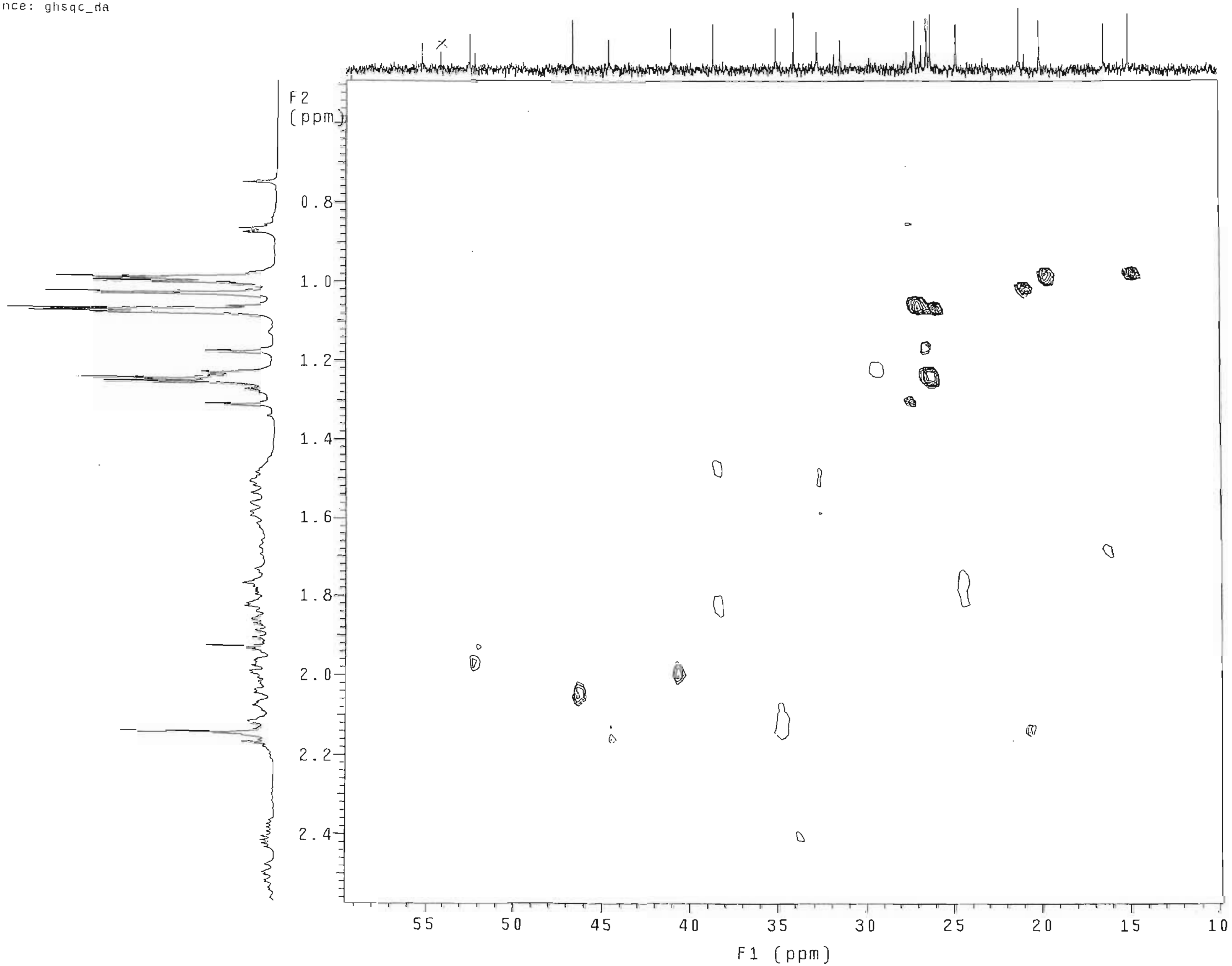
Pulse Sequence: ghsqc_da



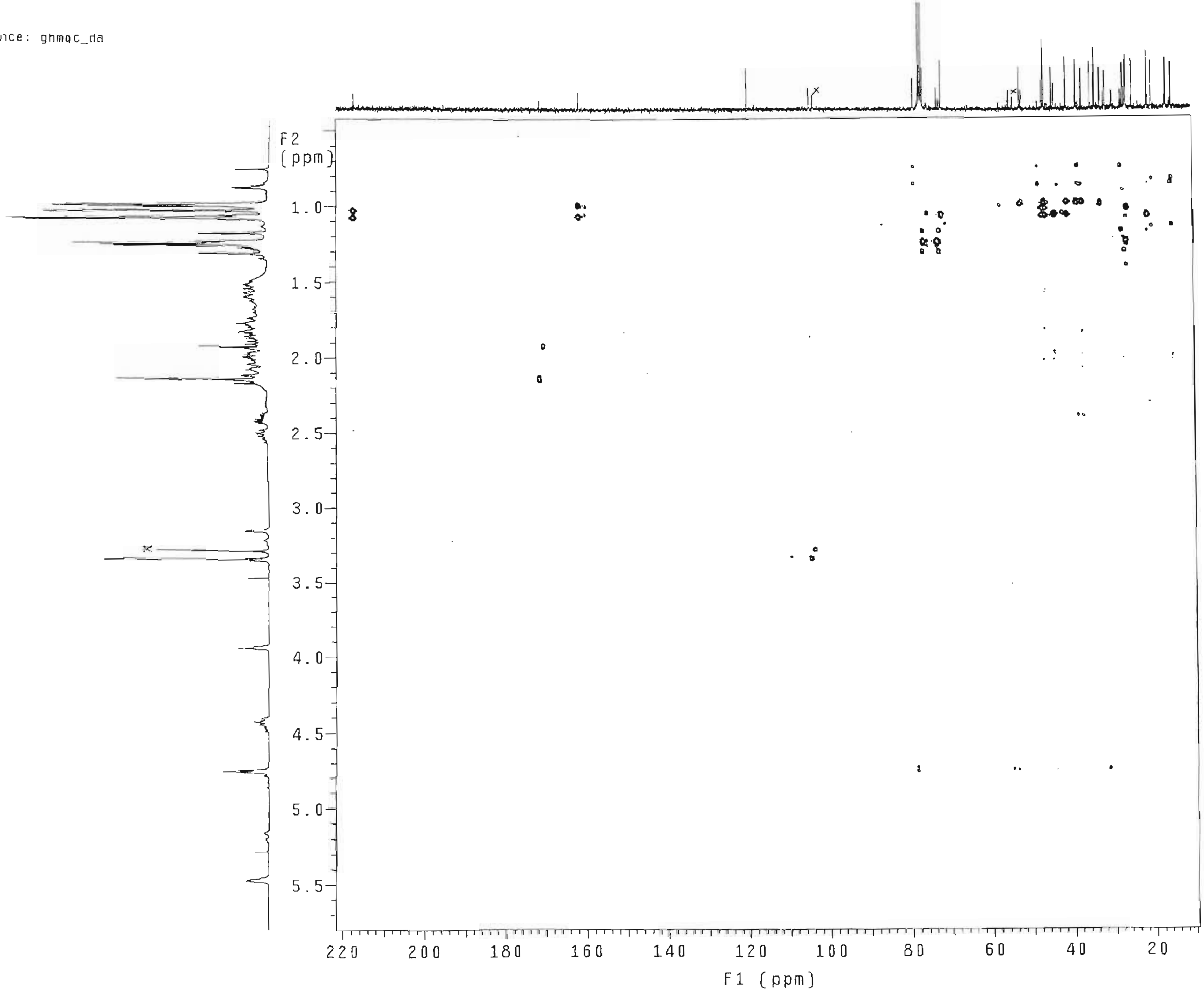
HSQC NMR Spectrum of Protoesamoderine D (xiii)

probe=5mmASW

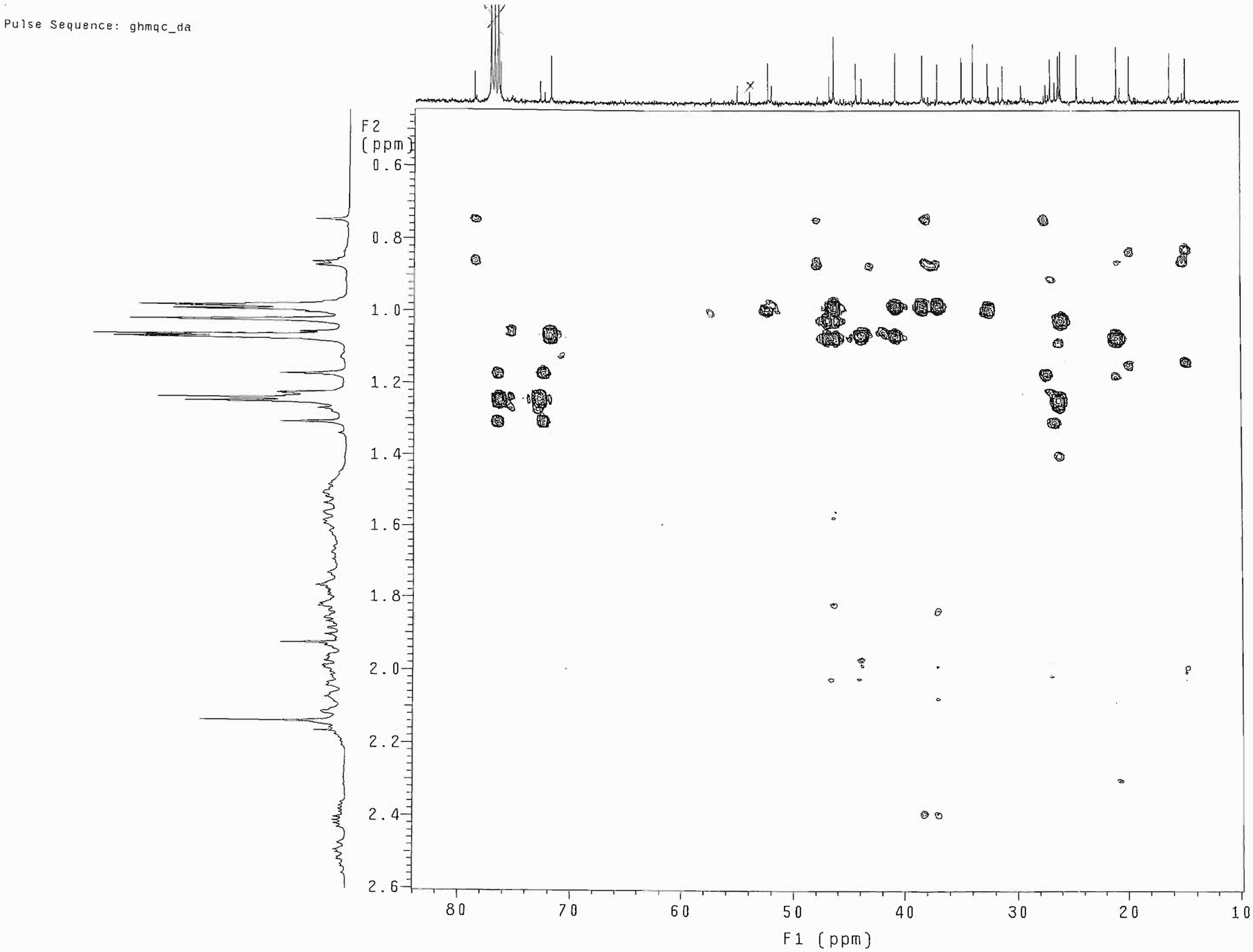
Pulse Sequence: ghsqc_da



HSQC NMR Spectrum of Protoasmoderine D (xiii)

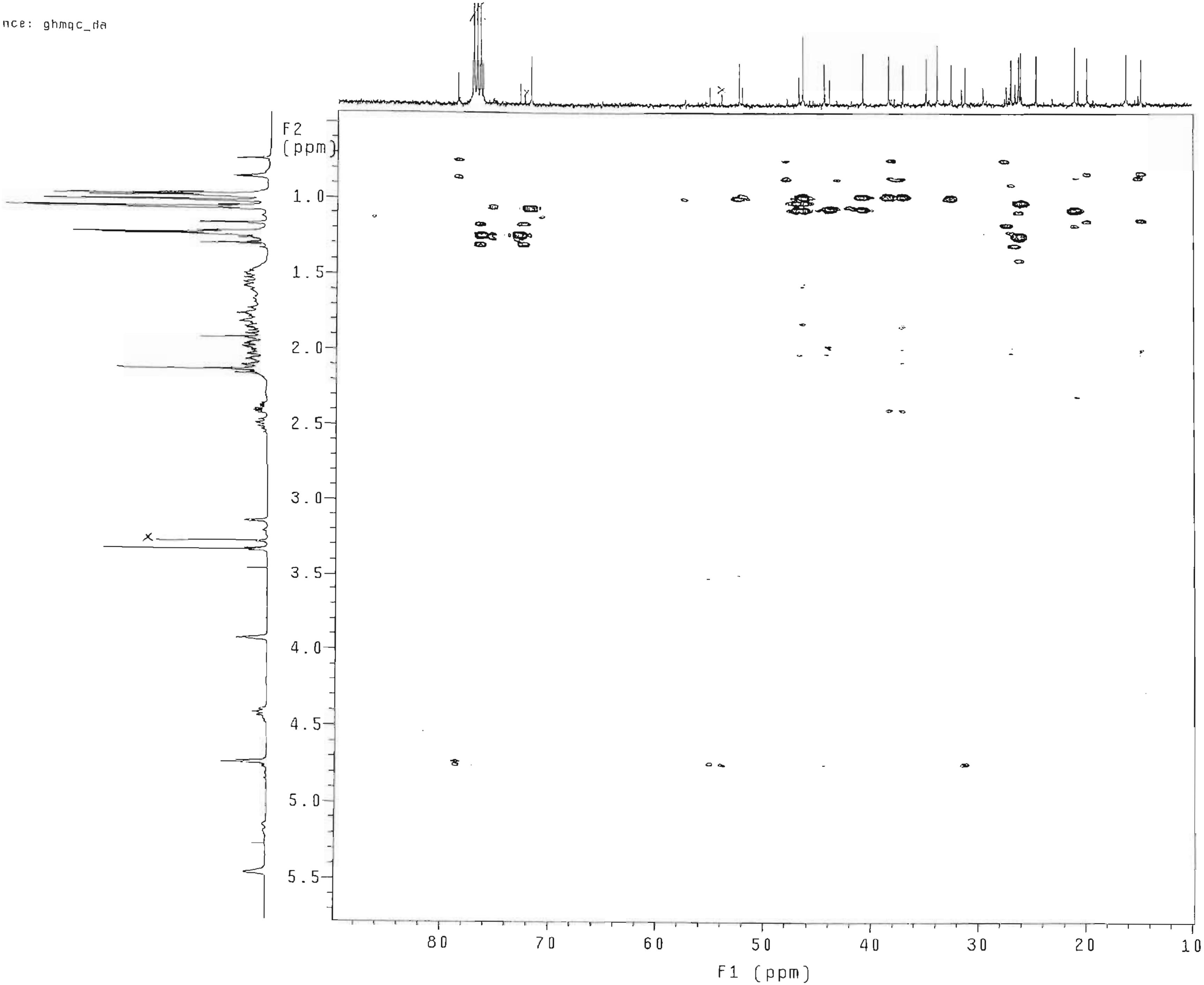


HMBC NMR Spectrum of Protoesamaderine D (viii)



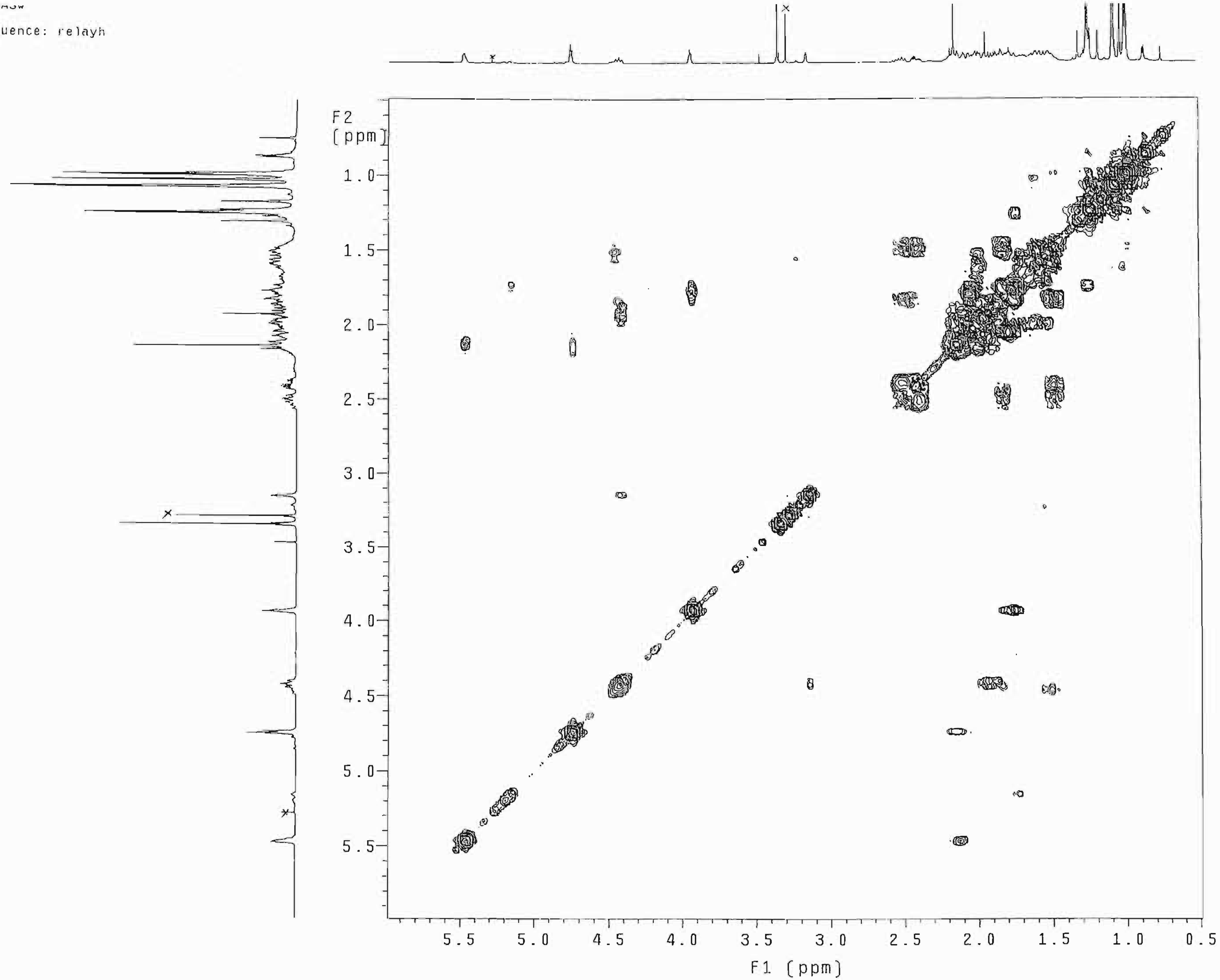
HMBC NMR Spectrum of Protosamaderine D (xiii)

Pulse Sequence: ghmqc_da



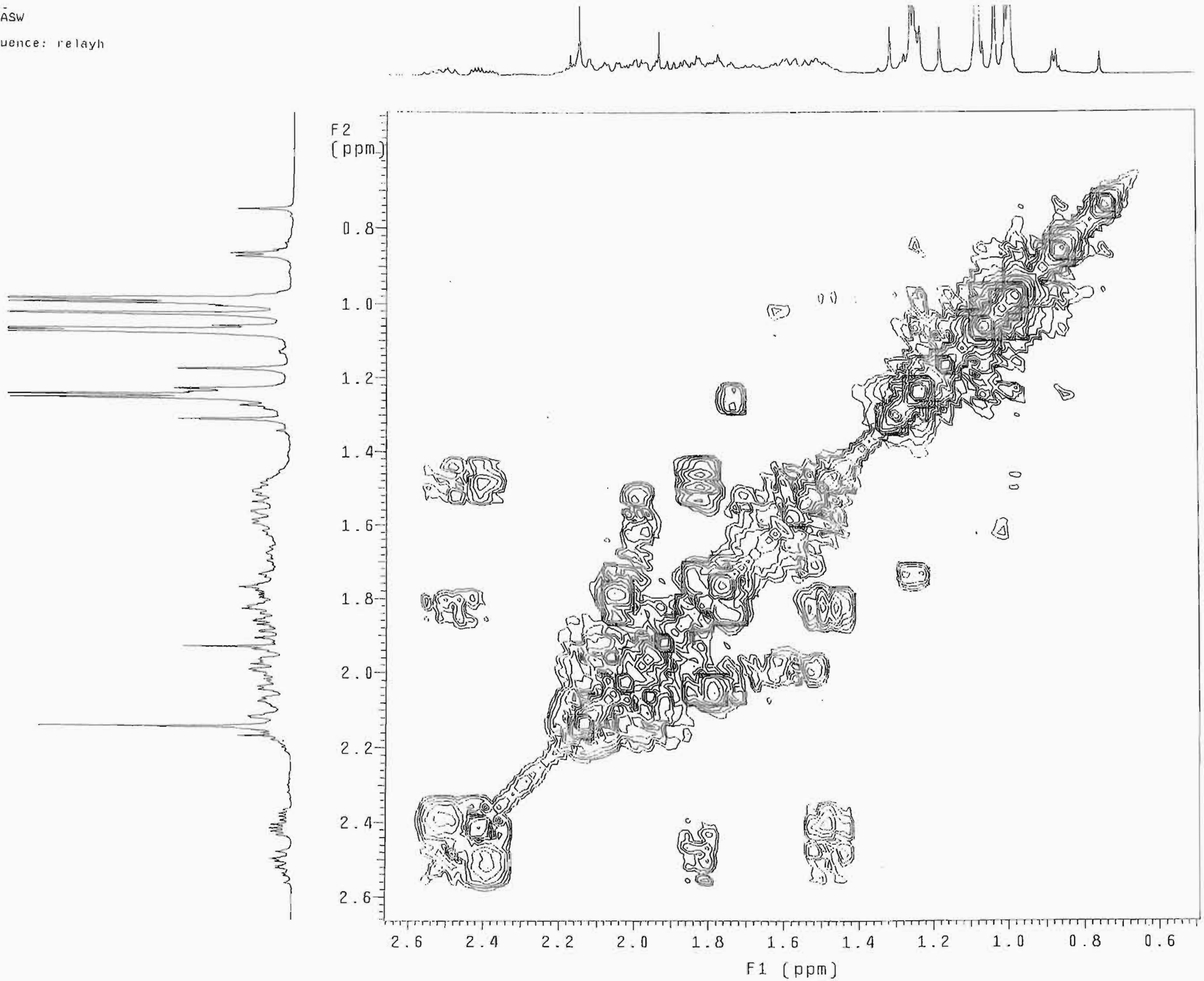
HMBC NMR Spectrum of Protosamaderine D (xiii)

Pulse Sequence: relayh



COSY NMR Spectrum of Protoasmaderine D (xiii)

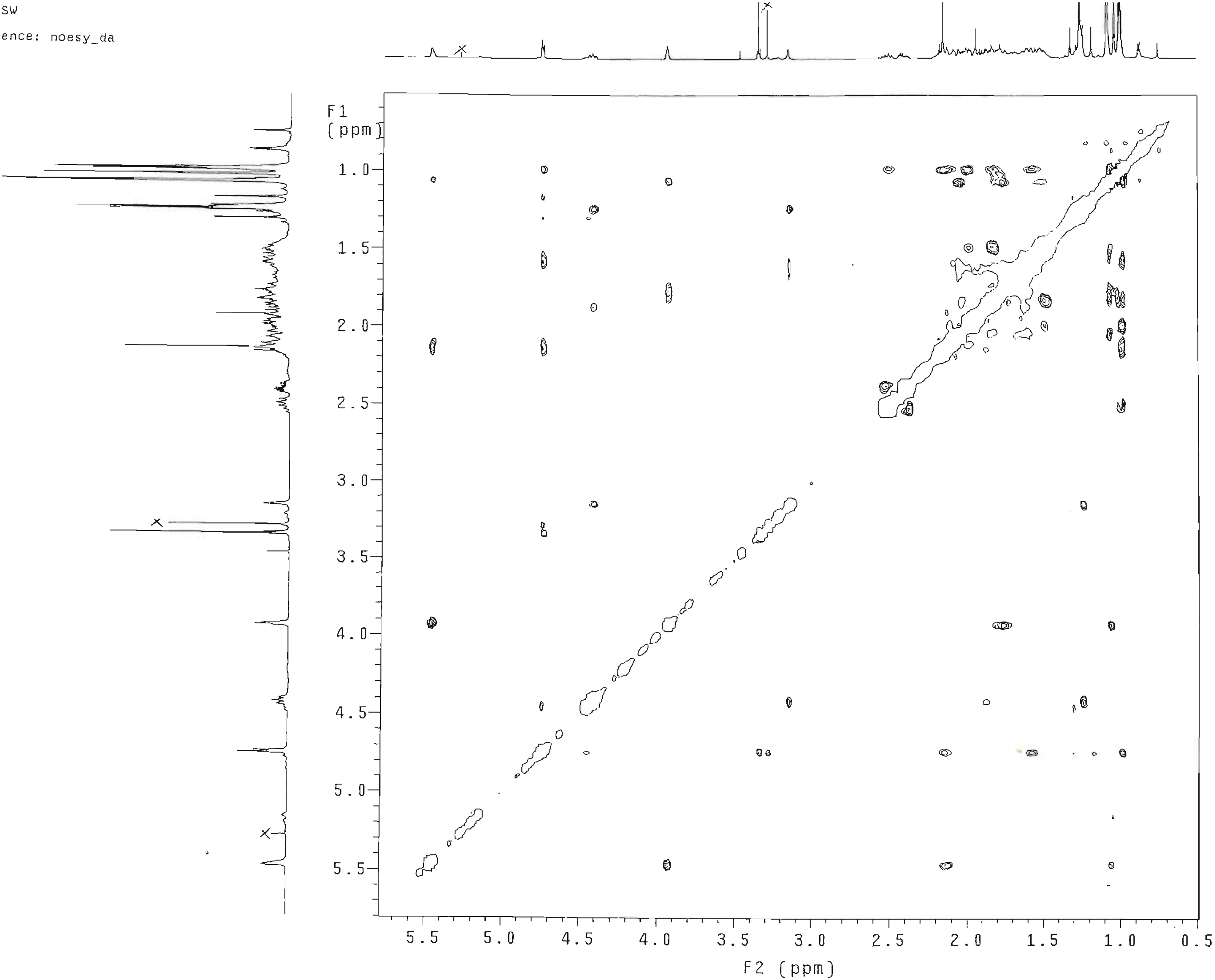
probe=5mmASW
Pulse Sequence: relayh



COSY NMR Spectrum of [illegible] D (viii)

probe=5mmASW

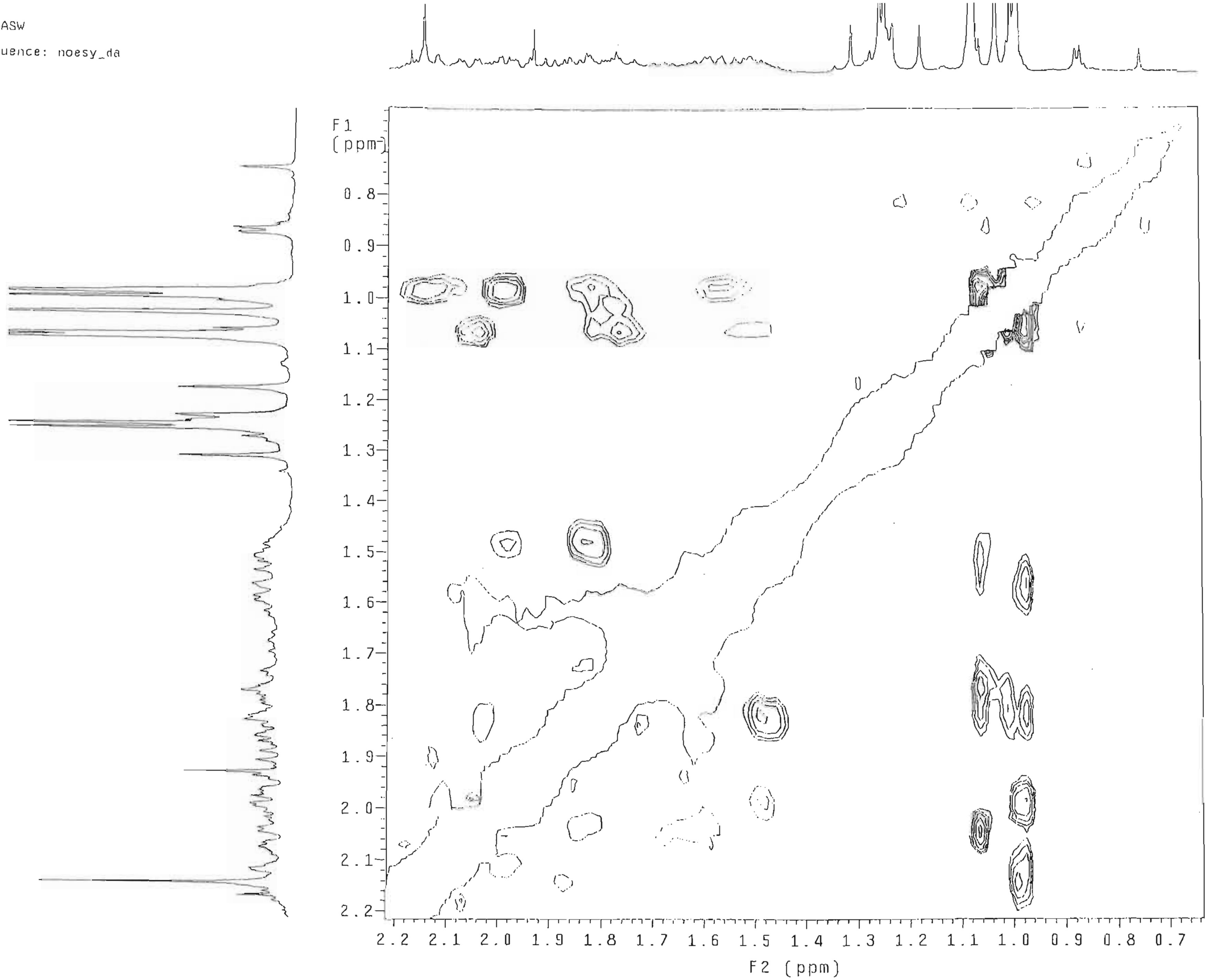
Pulse Sequence: noesy_da



NOESY NMR Spectrum of Protoescamoderine D (xiii)

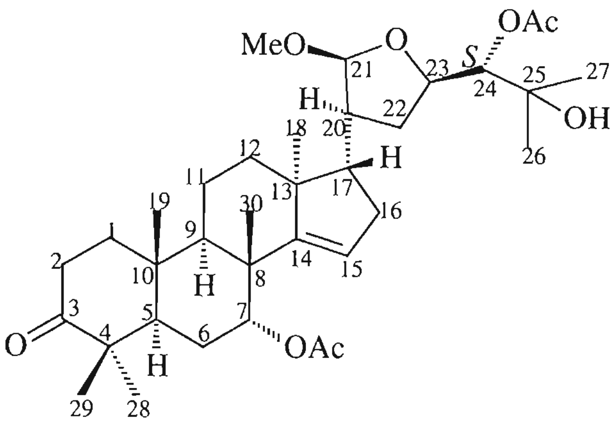
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da

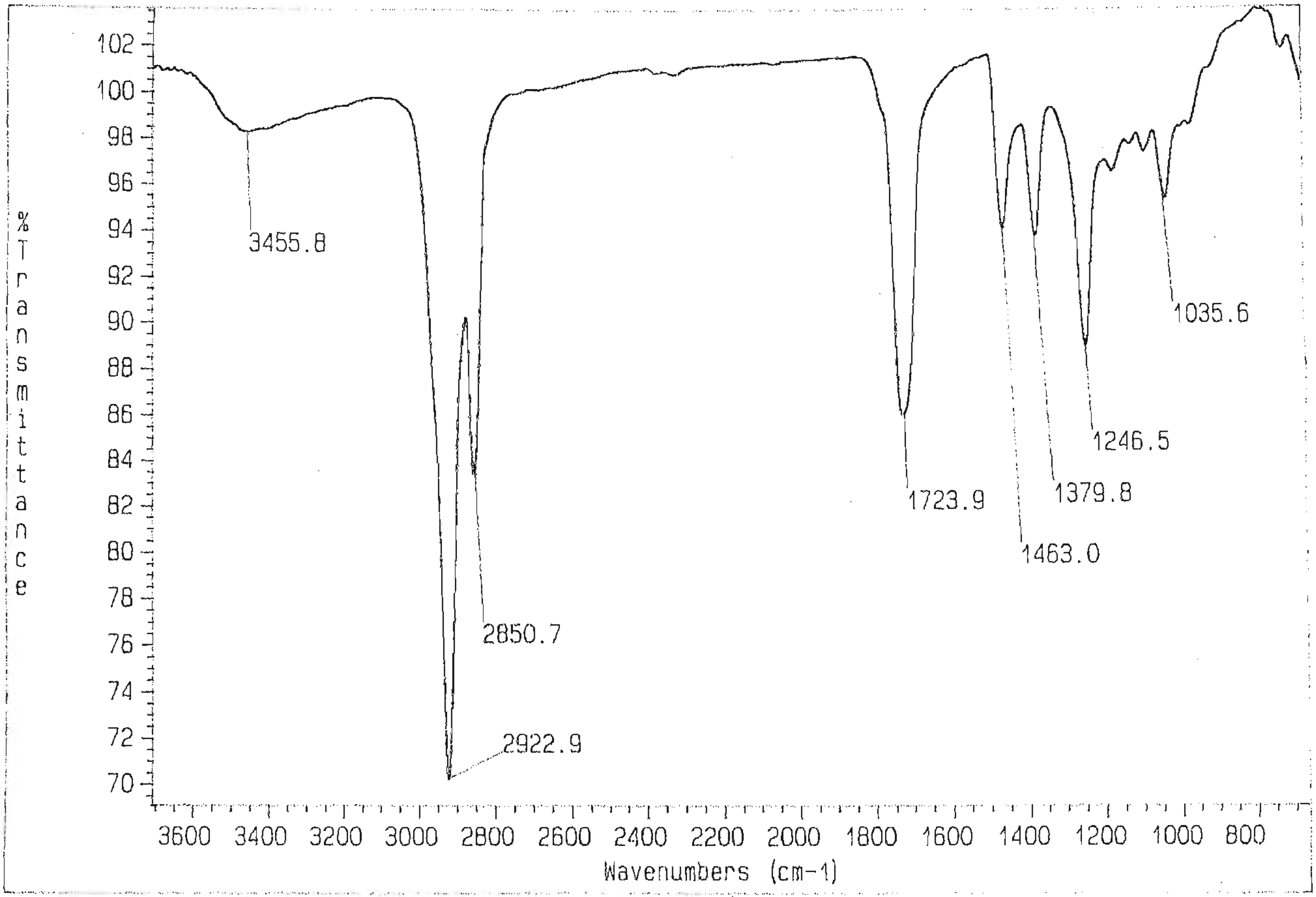


NOESY NMR Spectrum of Protosamaderine D (xiii)

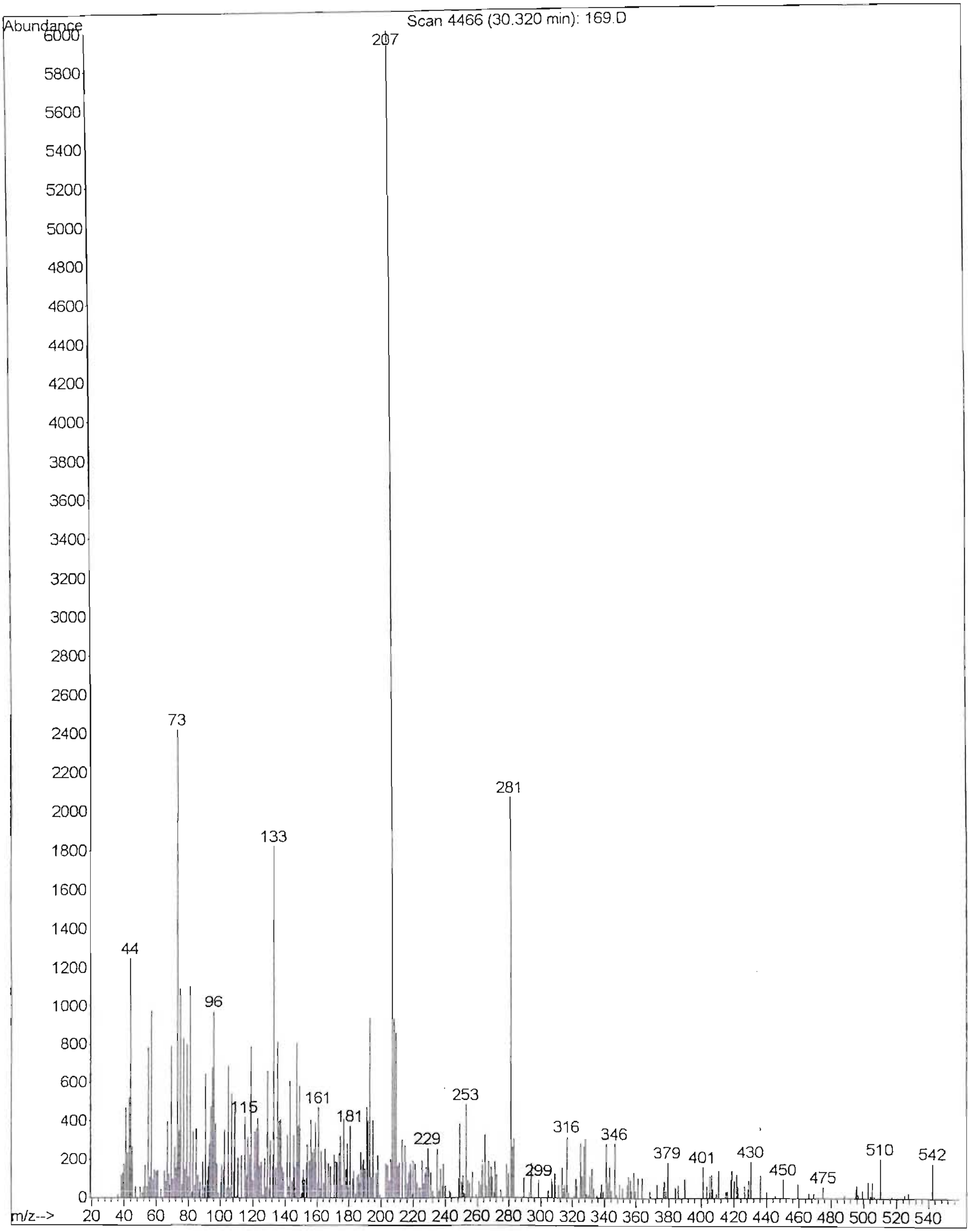
Protosamaderine E (xiv)



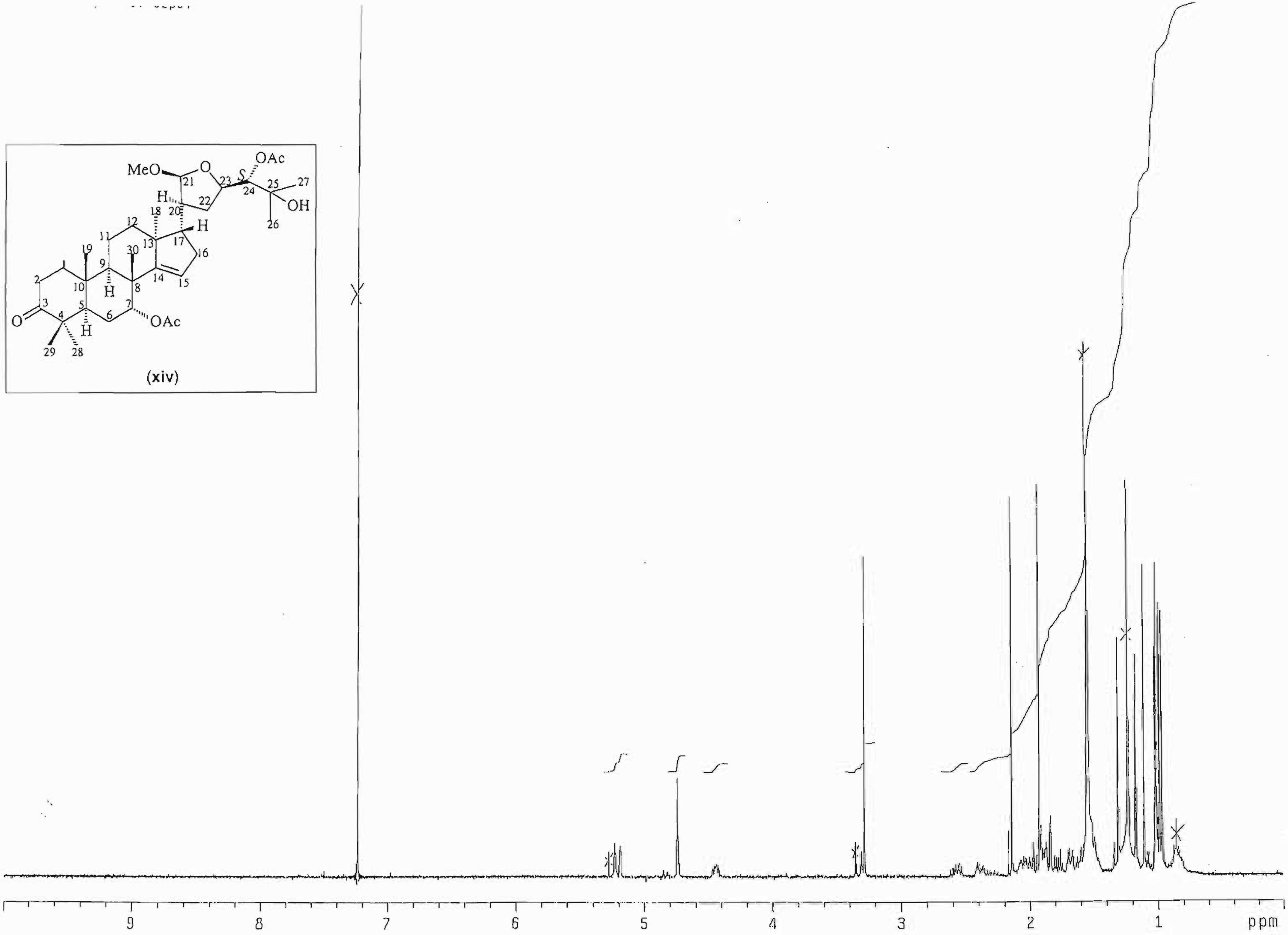
(xiv)



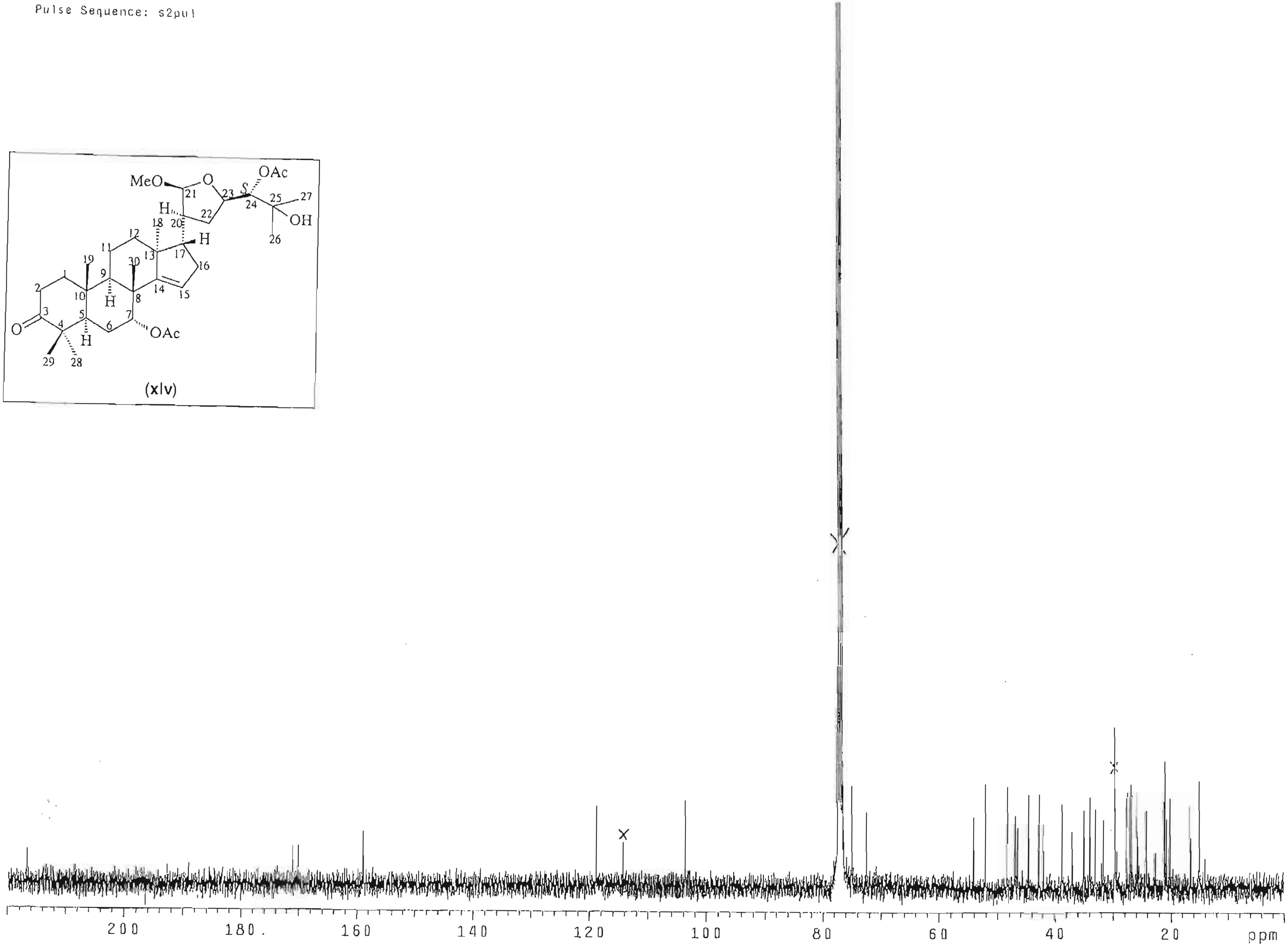
IR Spectrum of Protosamaderine E (xiv)



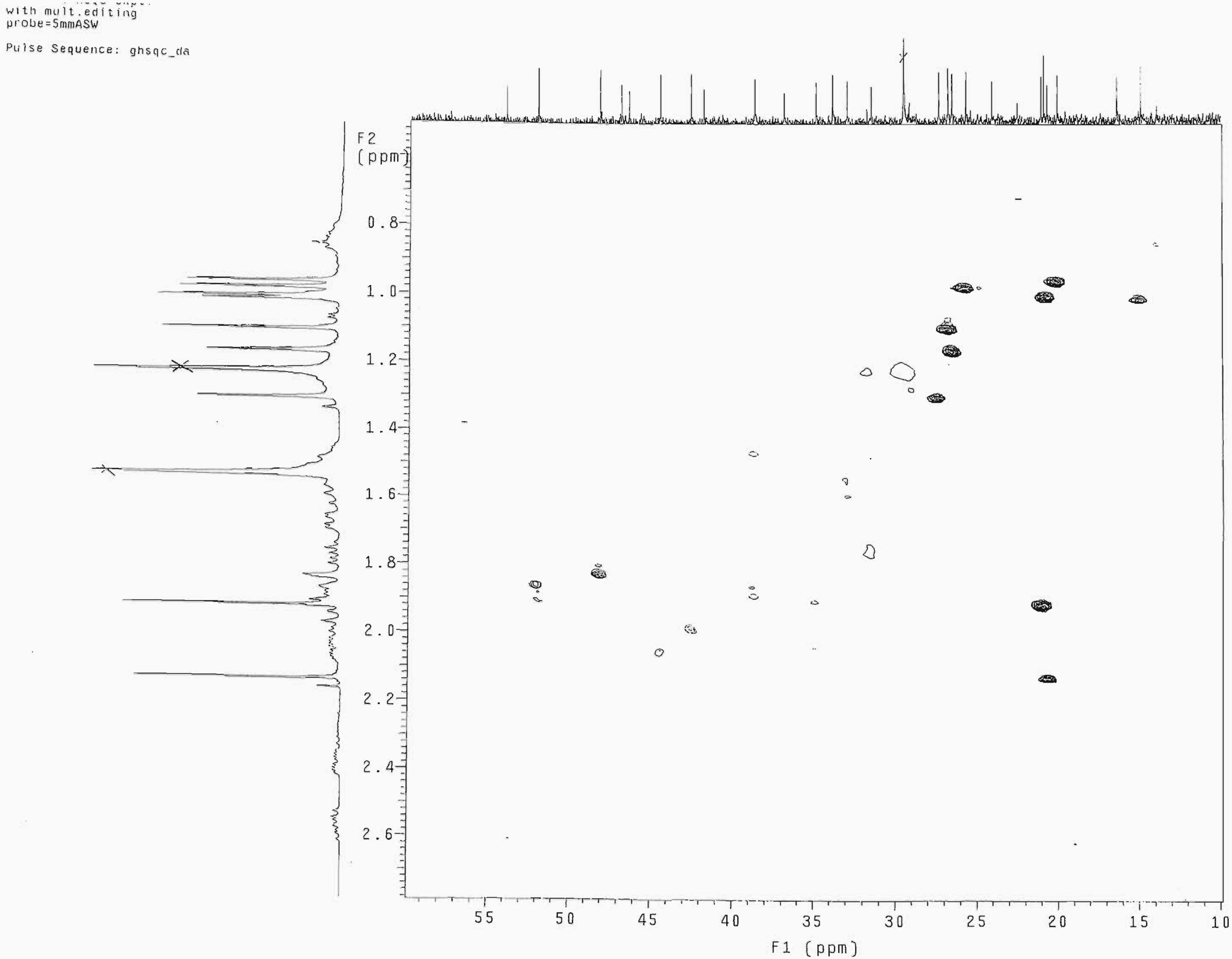
Mass Spectrum of Protosamaderine E (xiv)



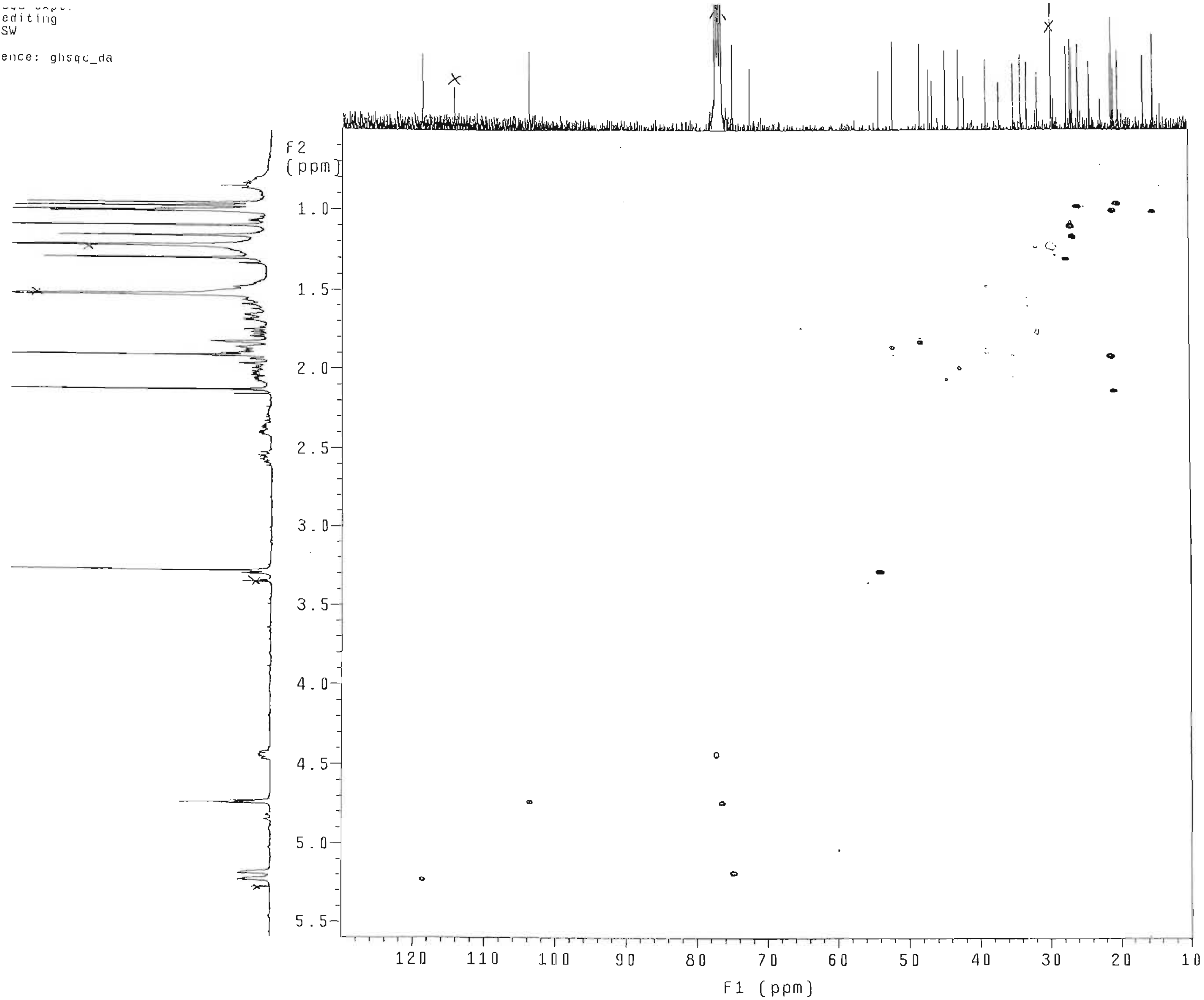
¹H NMR Spectrum of Protoseamedarine E (xiv)



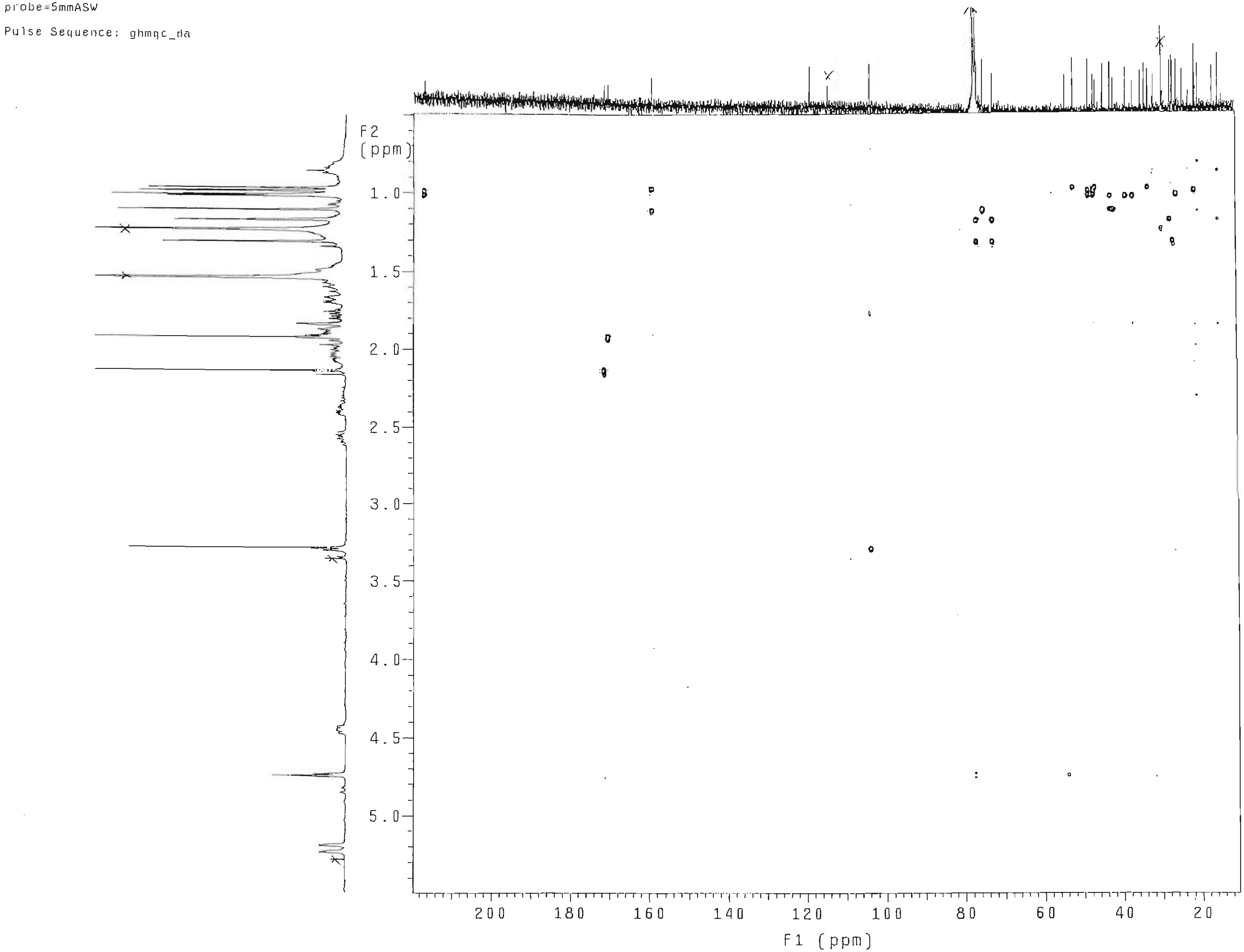
^{13}C NMR Spectrum of Protosamaderine E (xlv)



HSQC NMR Spectrum of Protosamaderine E (xiv)



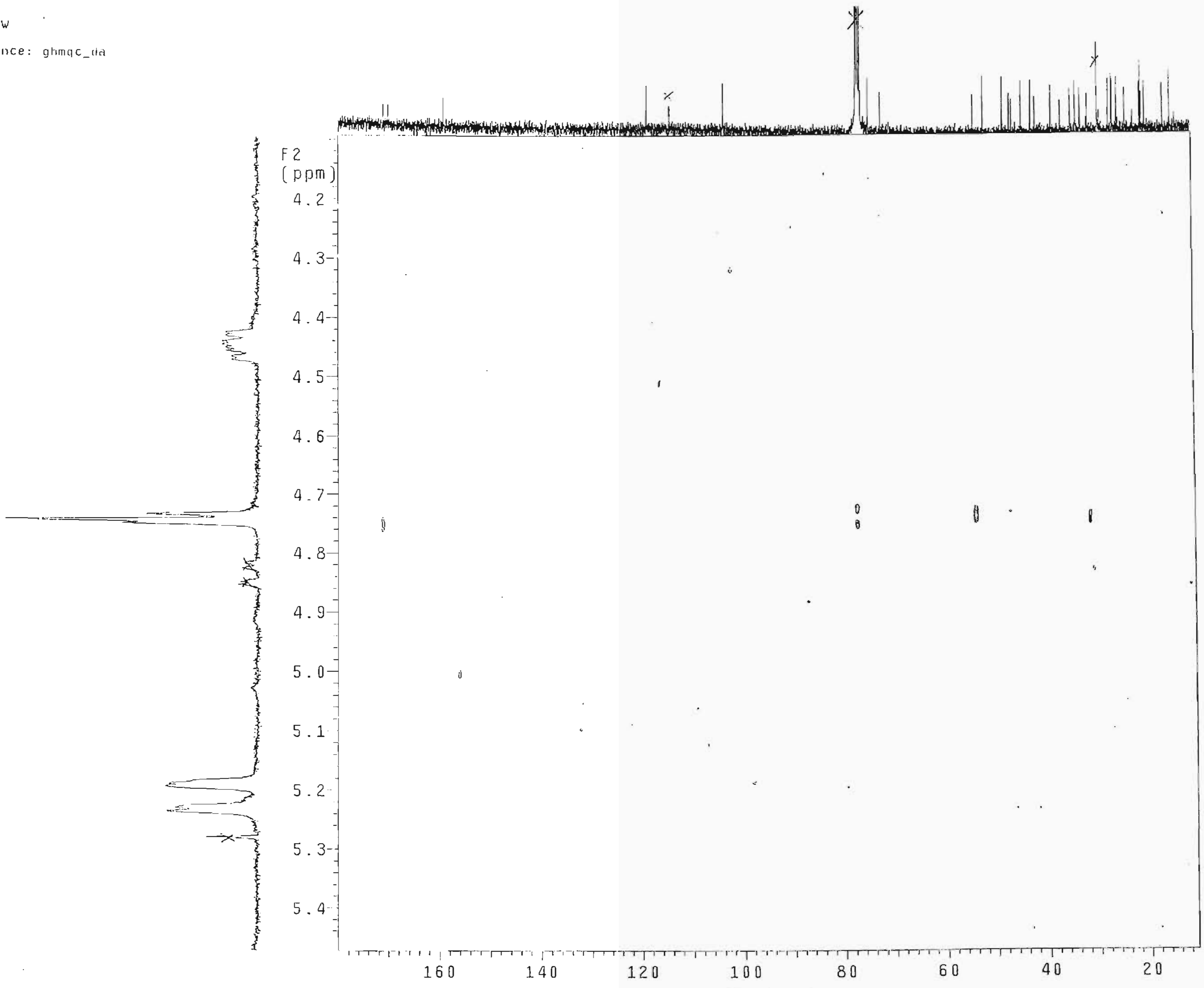
HSQC NMR Spectrum of Protoesamaderine E (xiv)



HMBC NMR Spectrum of [illegible]

probe=5mmASW

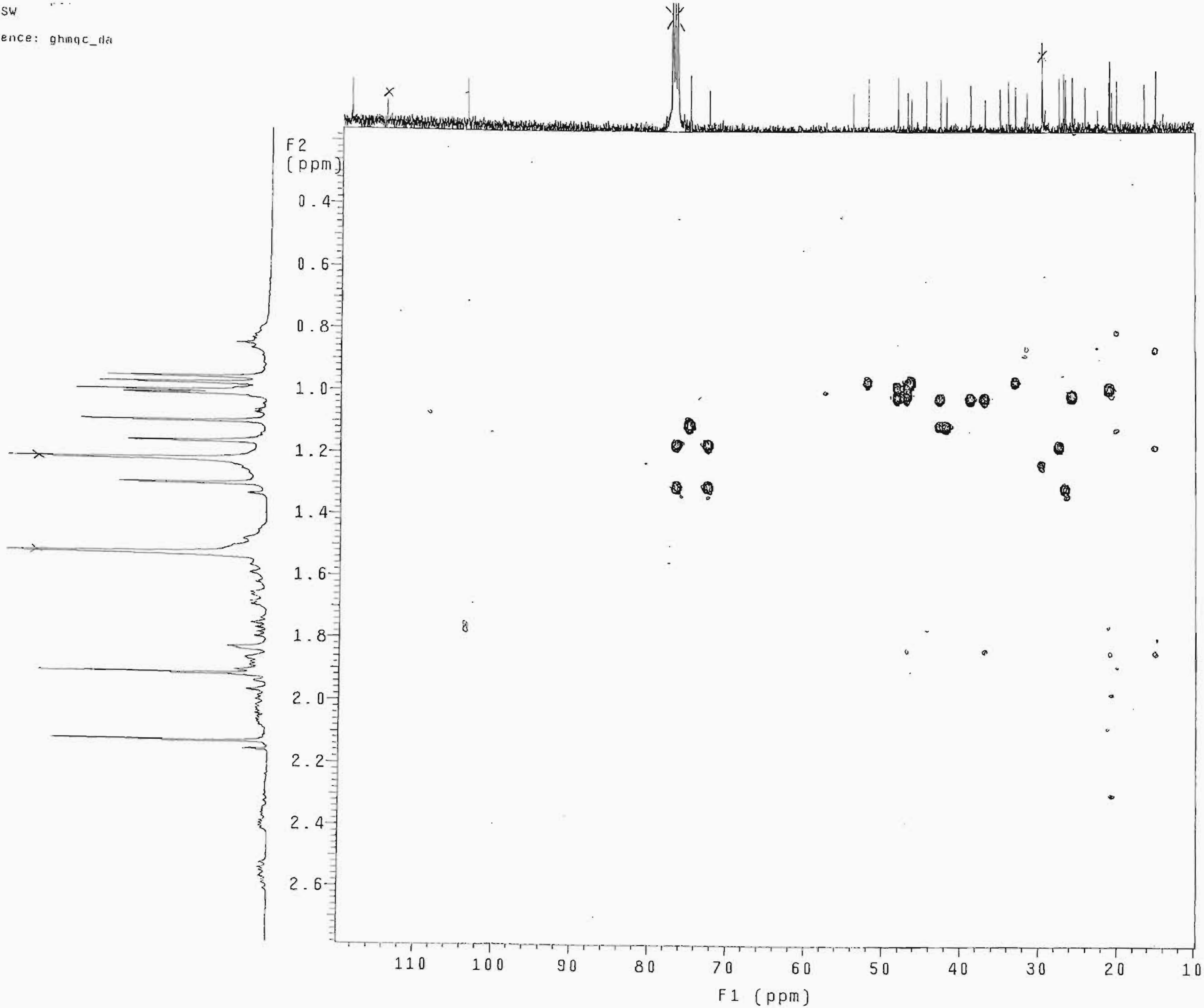
Pulse Sequence: ghmqc_da



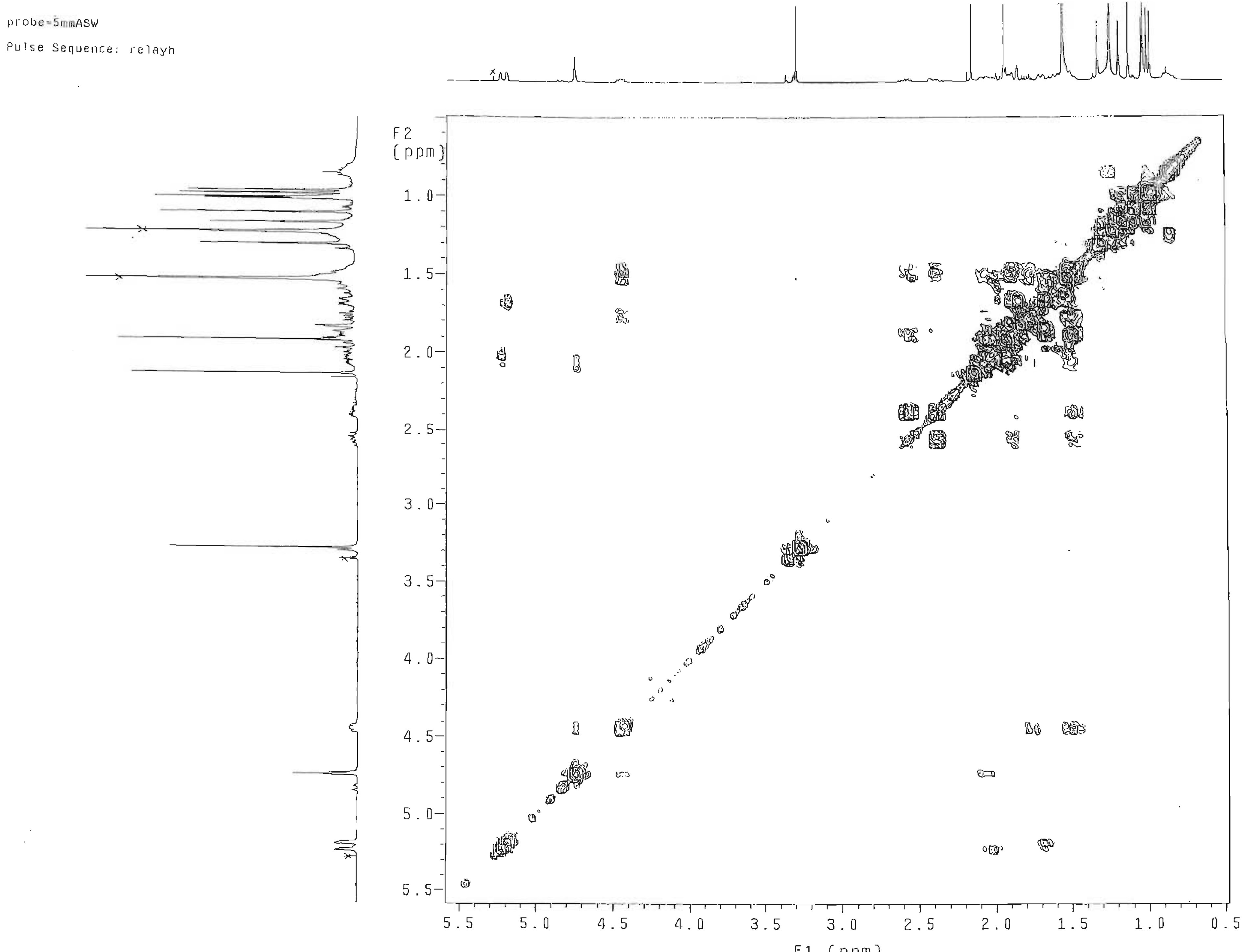
HMBC NMR Spectrum of

probe=5mmASW

Pulse Sequence: ghmqc_da



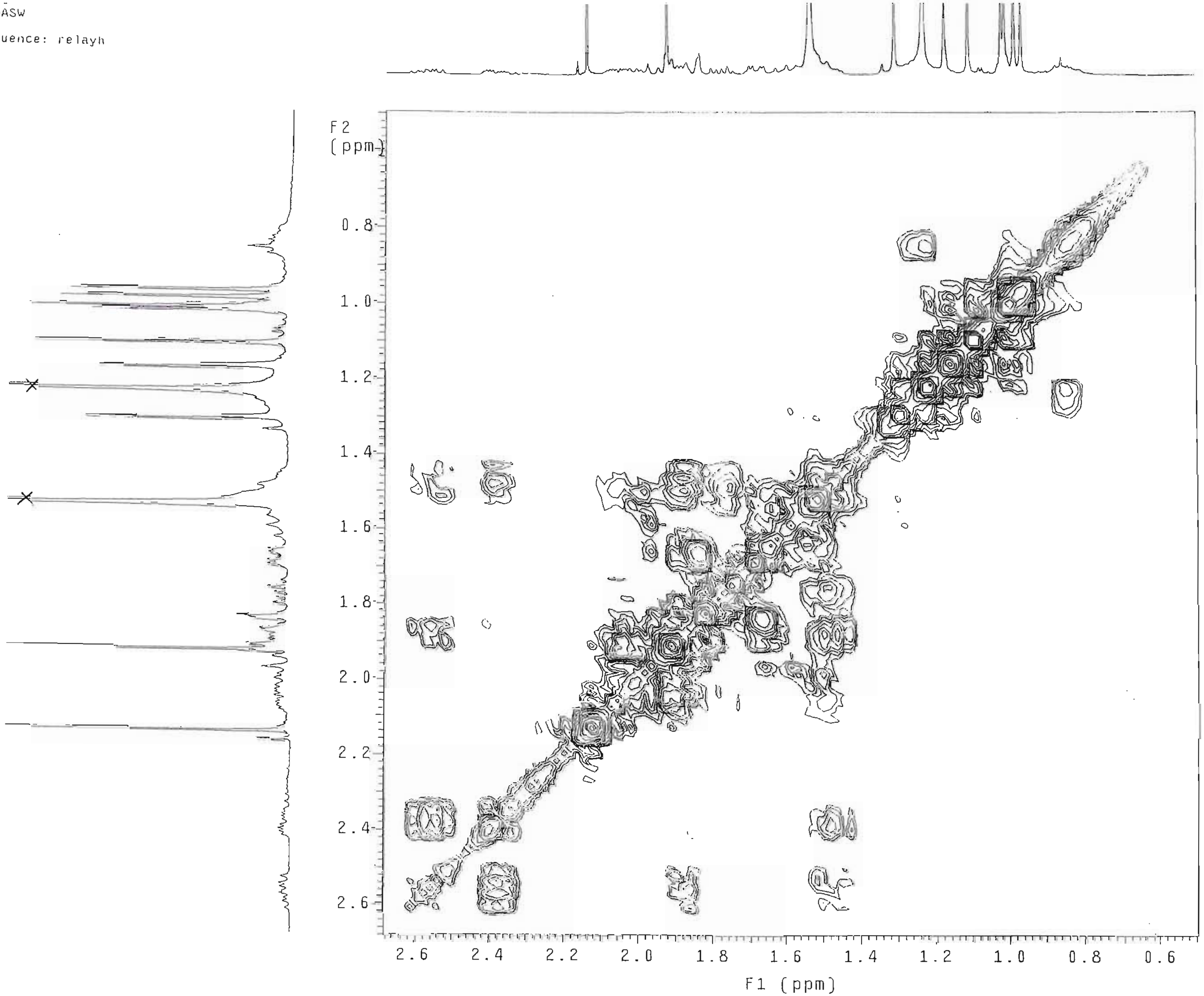
HMBC NMR Spectrum of Protoasamoderine E (xiv)



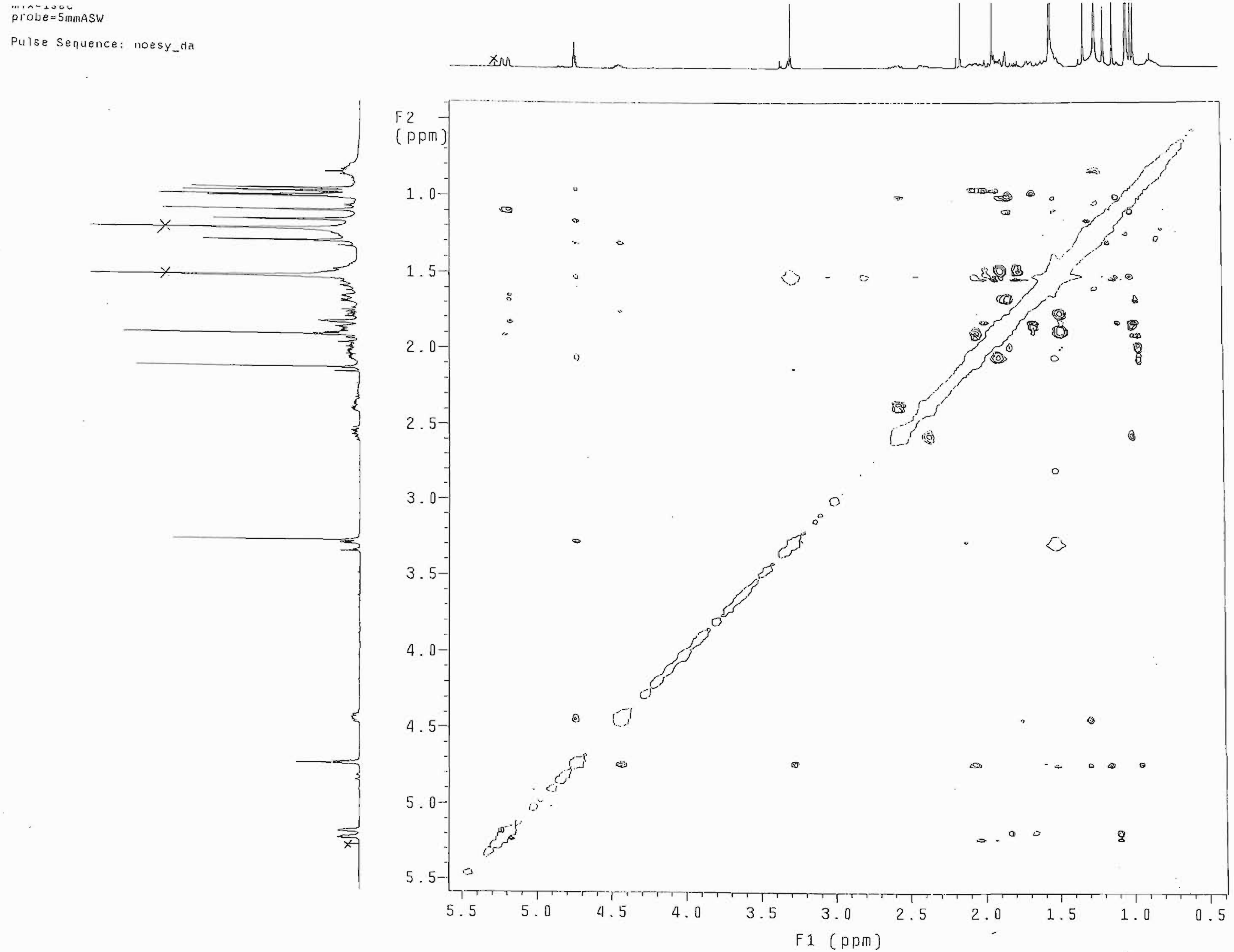
COSY NMR Spectrum of

probe=5mmASW

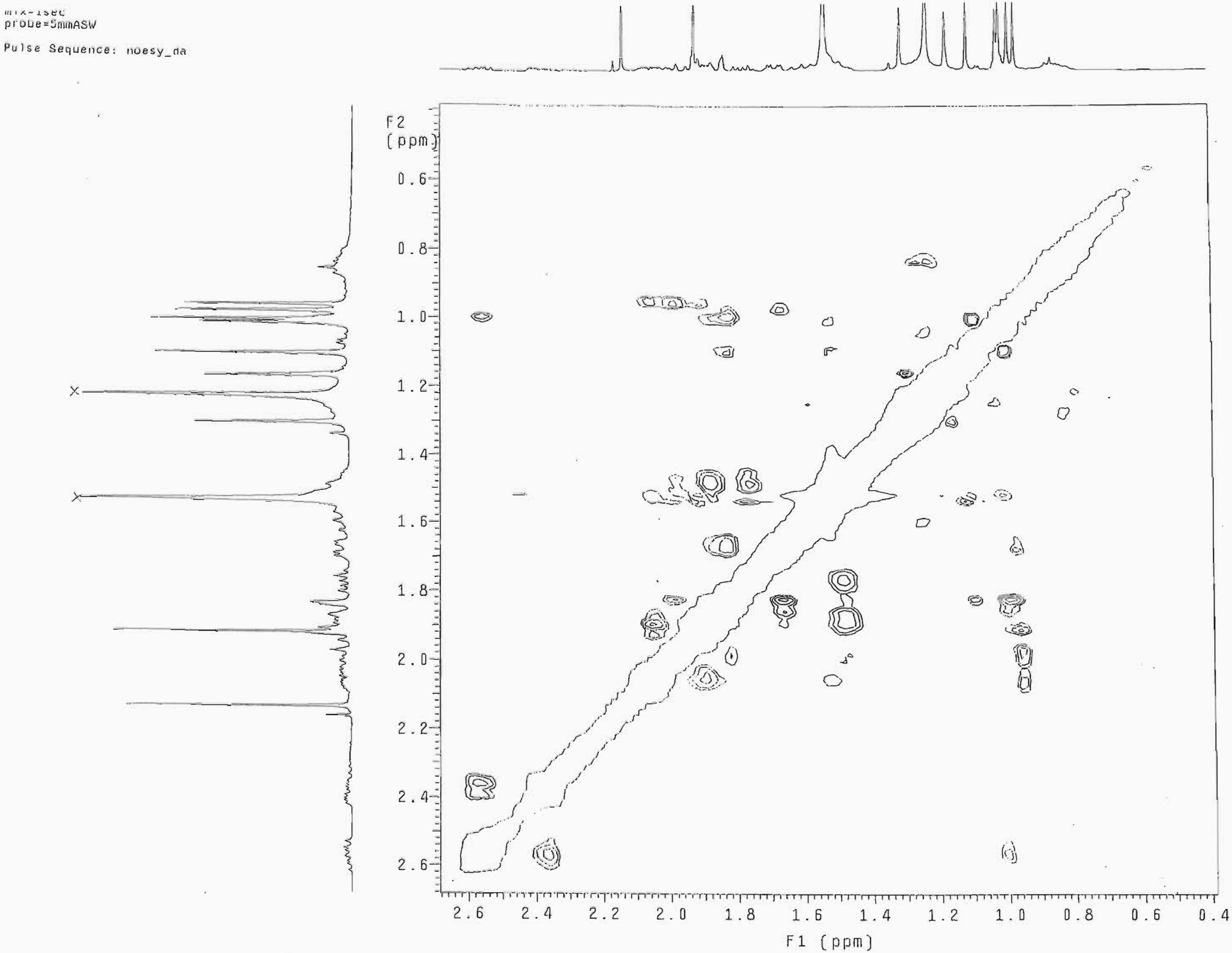
Pulse Sequence: relayh



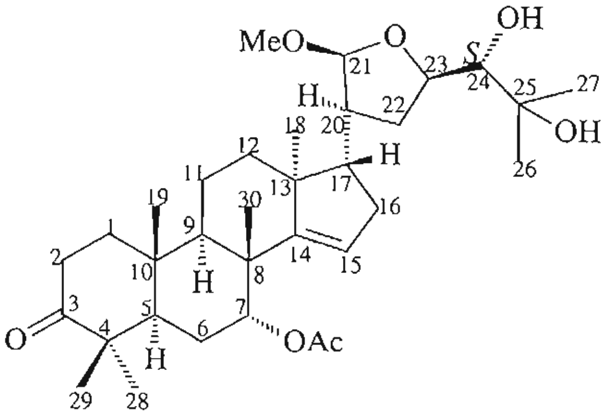
COSY NMR Spectrum of Protoasmaderine F (xiv)



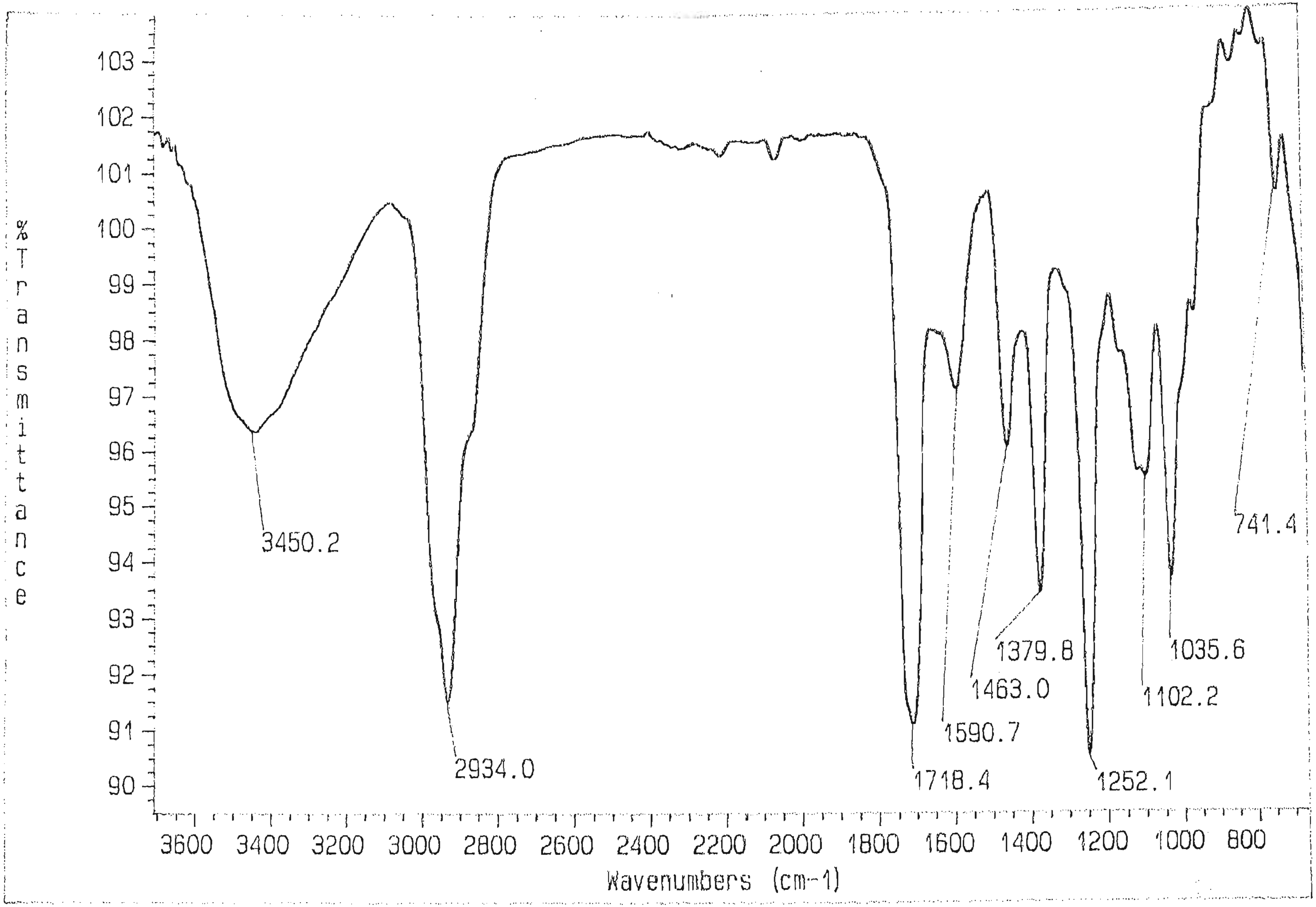
NOESY NMR Spectrum of [illegible]


mix=1sec
probe=5mmASW
Pulse Sequence: noesy_da
F2 (ppm)
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
F1 (ppm)

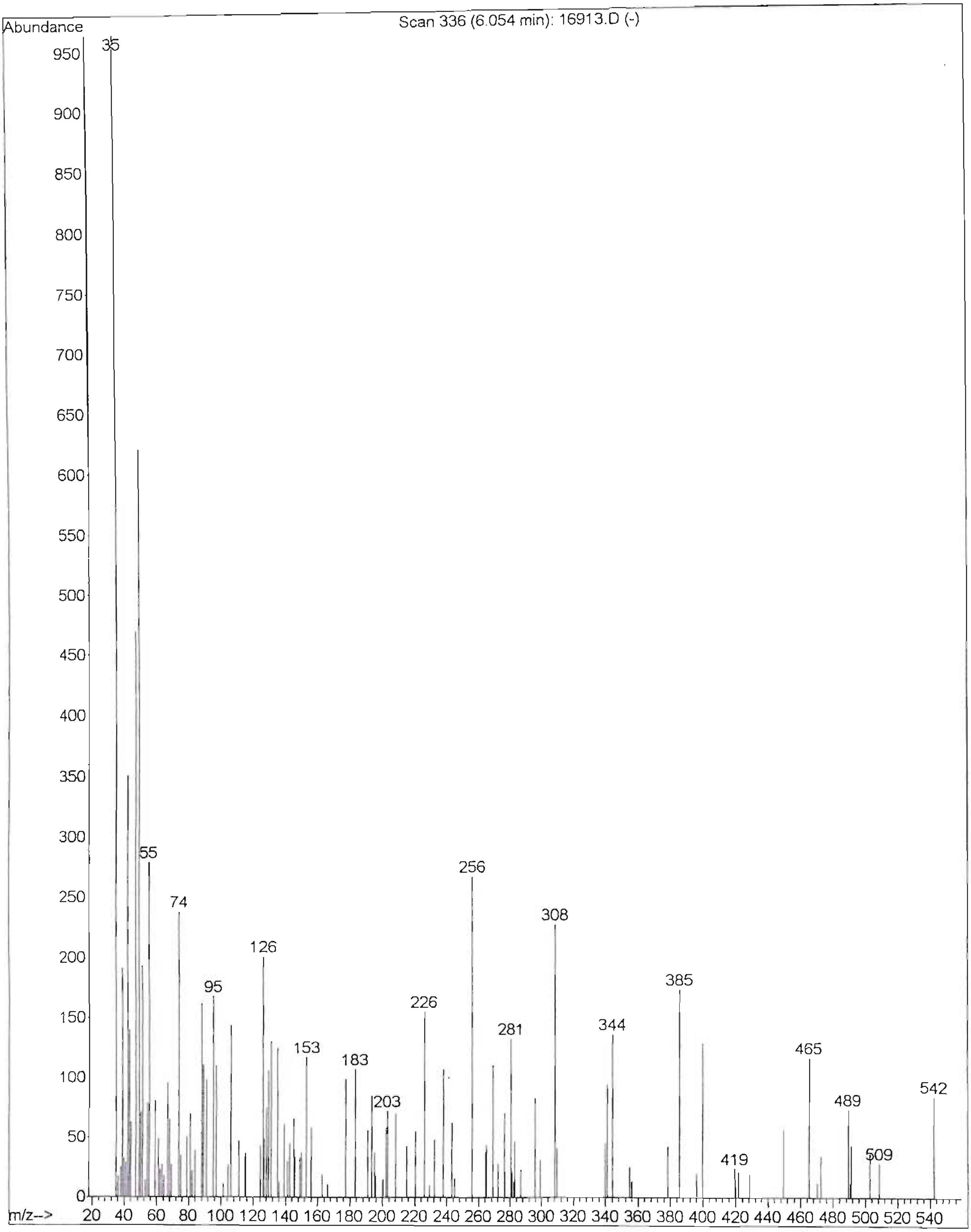
Protosamaderine F (xv)



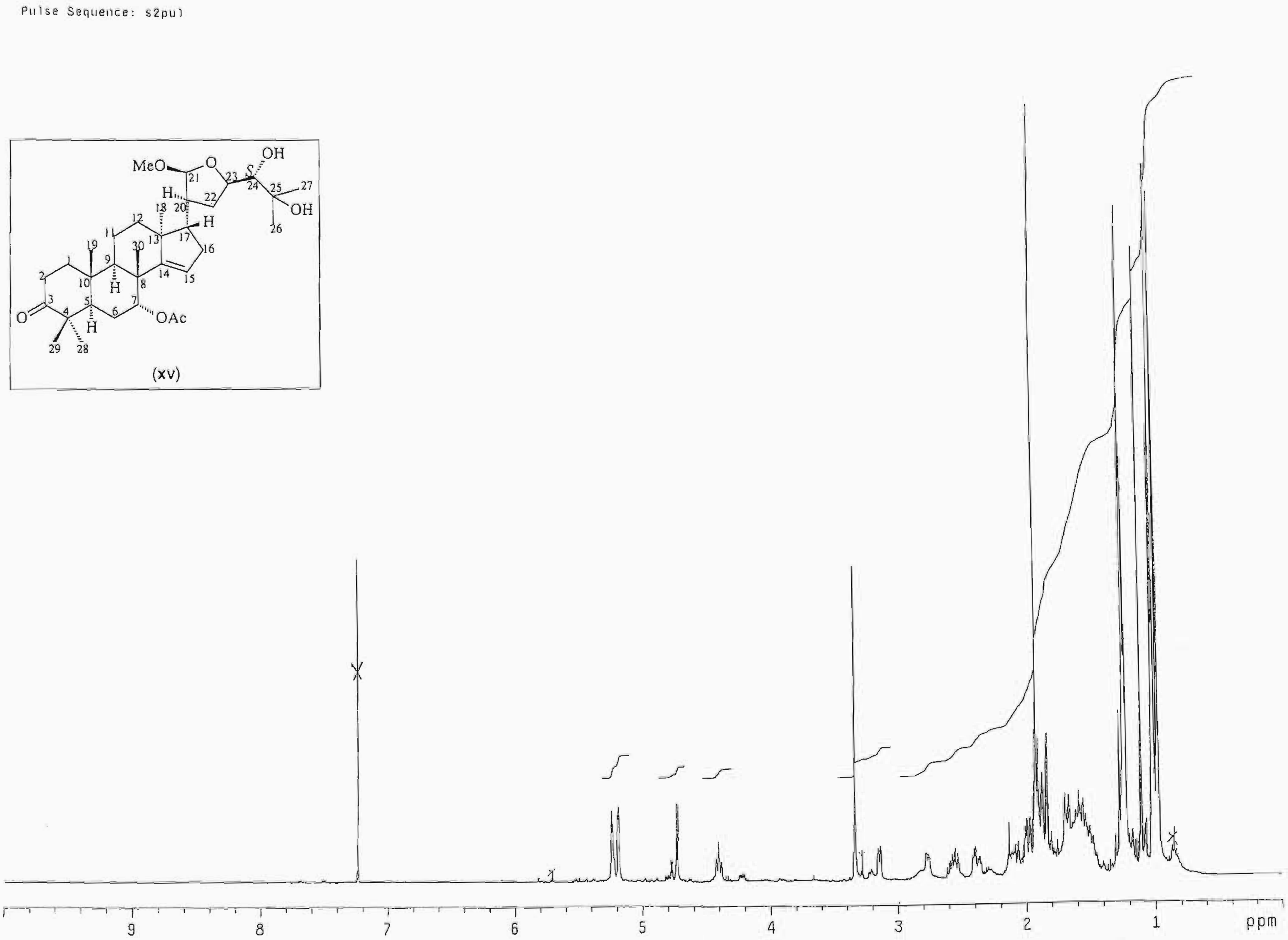
(xv)



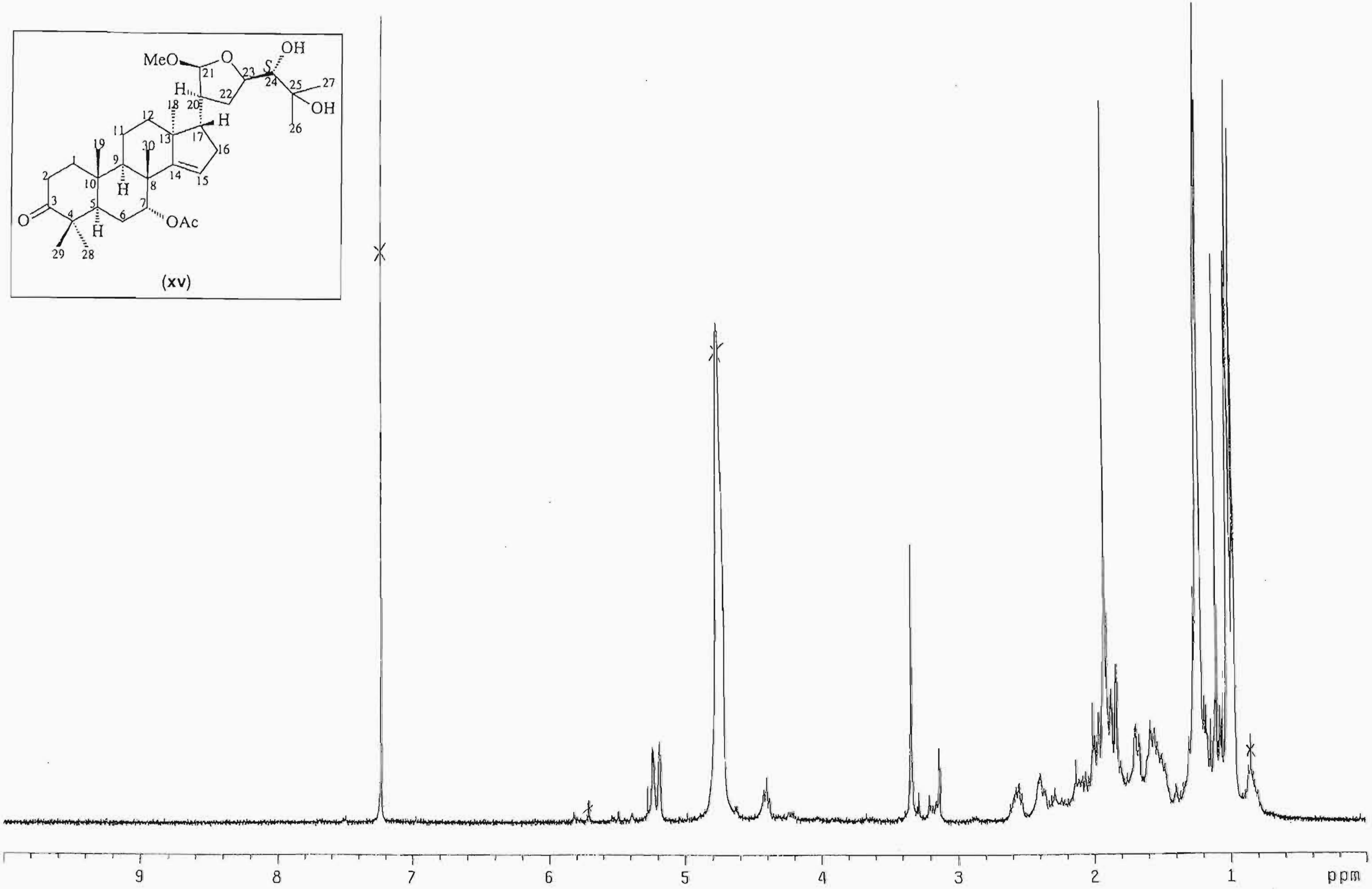
IR Spectrum of Protosamaderine F (xv)



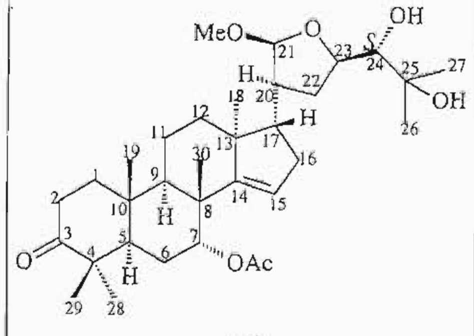
Mass Spectrum of Protosamaderine F (xv)

Pulse Sequence: s2pul
MeO
O
OH
OH
H
H
O
OAc
H
H
21
22
23
24
25
26
27
S
20
18
17
16
15
14
13
12
11
19
30
9
10
8
7
6
5
4
3
2
1
29
28
(xv)
9
8
7
6
5
4
3
2
1
ppm

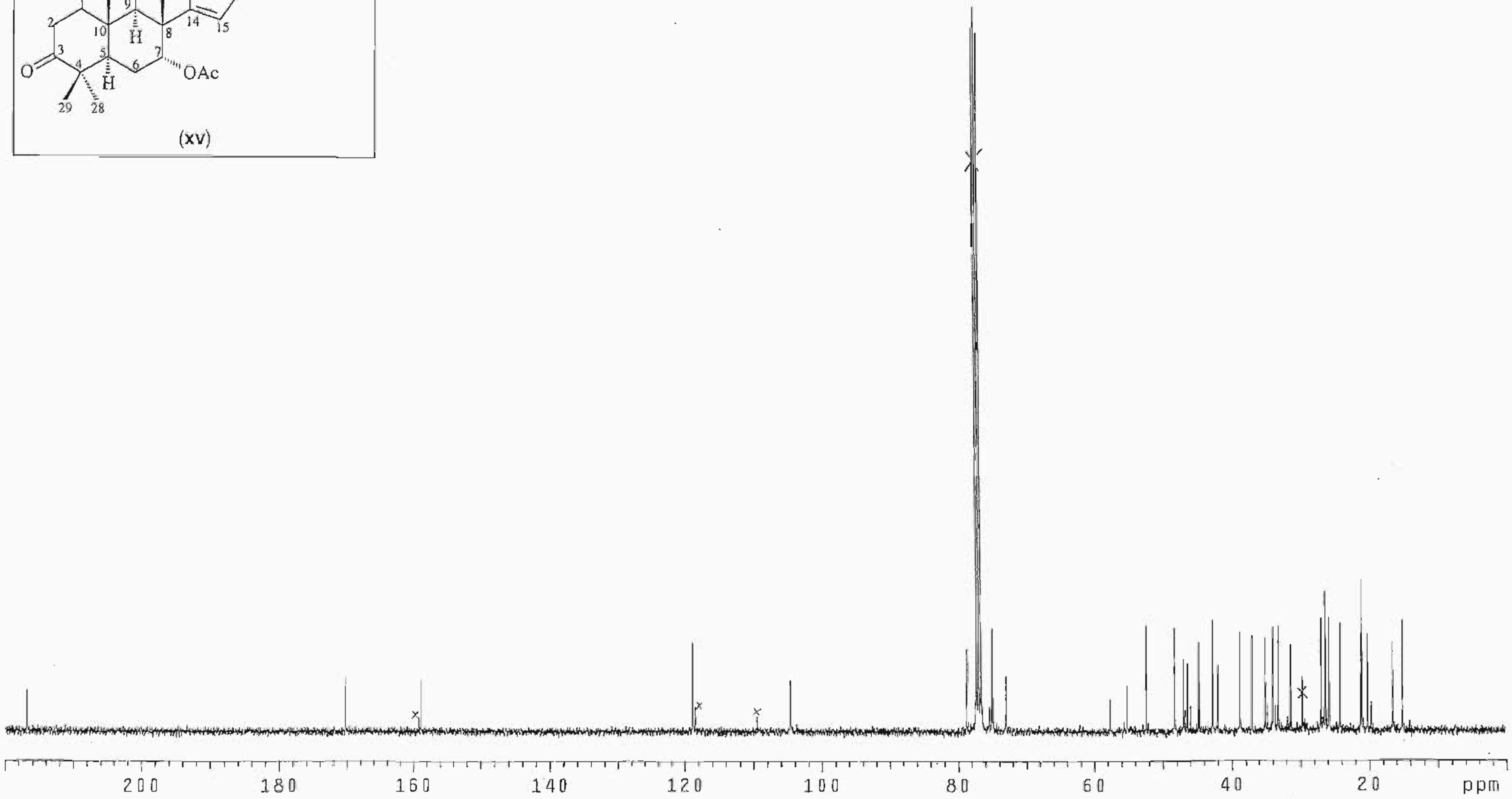
Pulse Sequence: s2pul



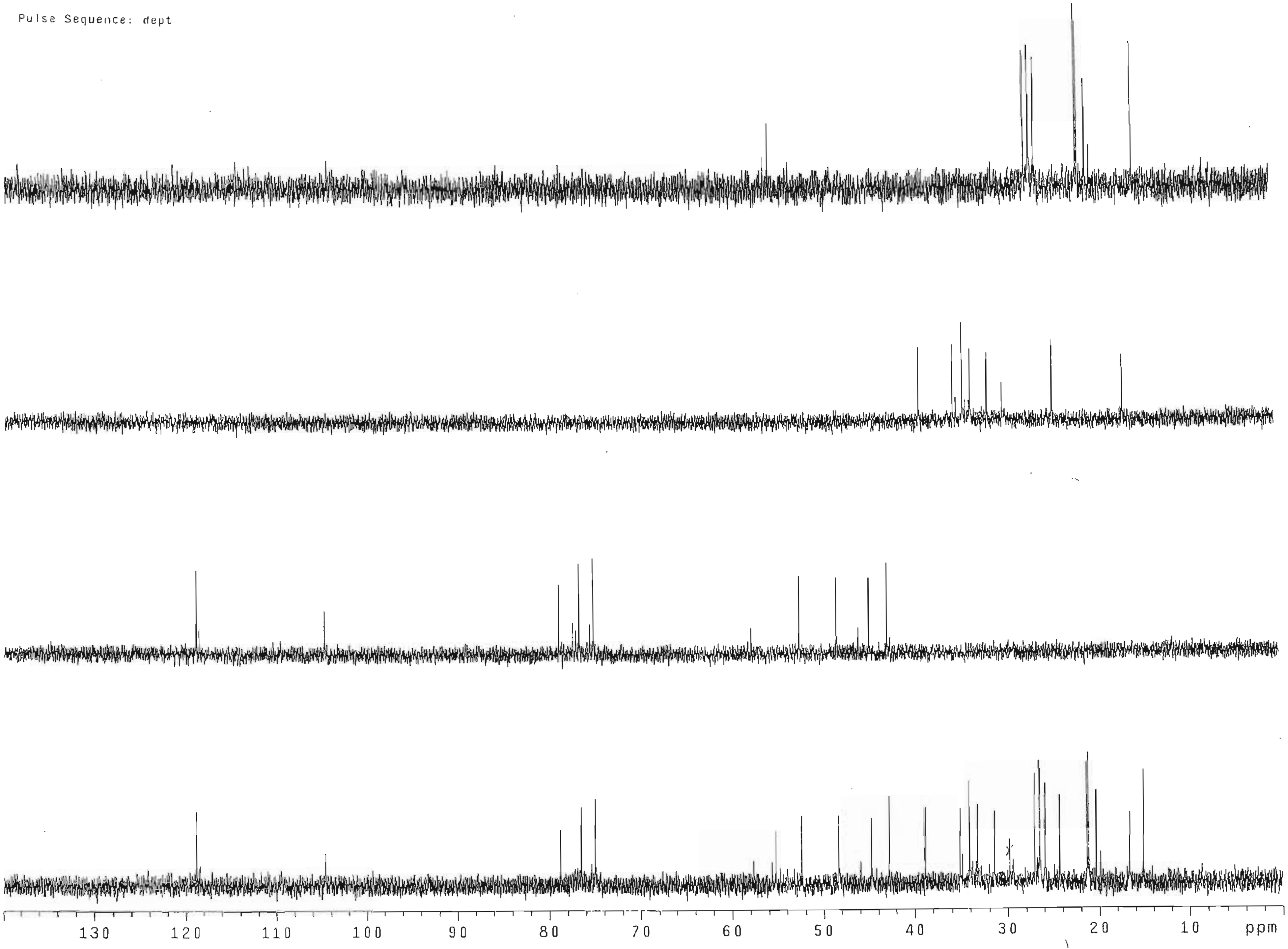
Pulse Sequence: s2pul



(xv)



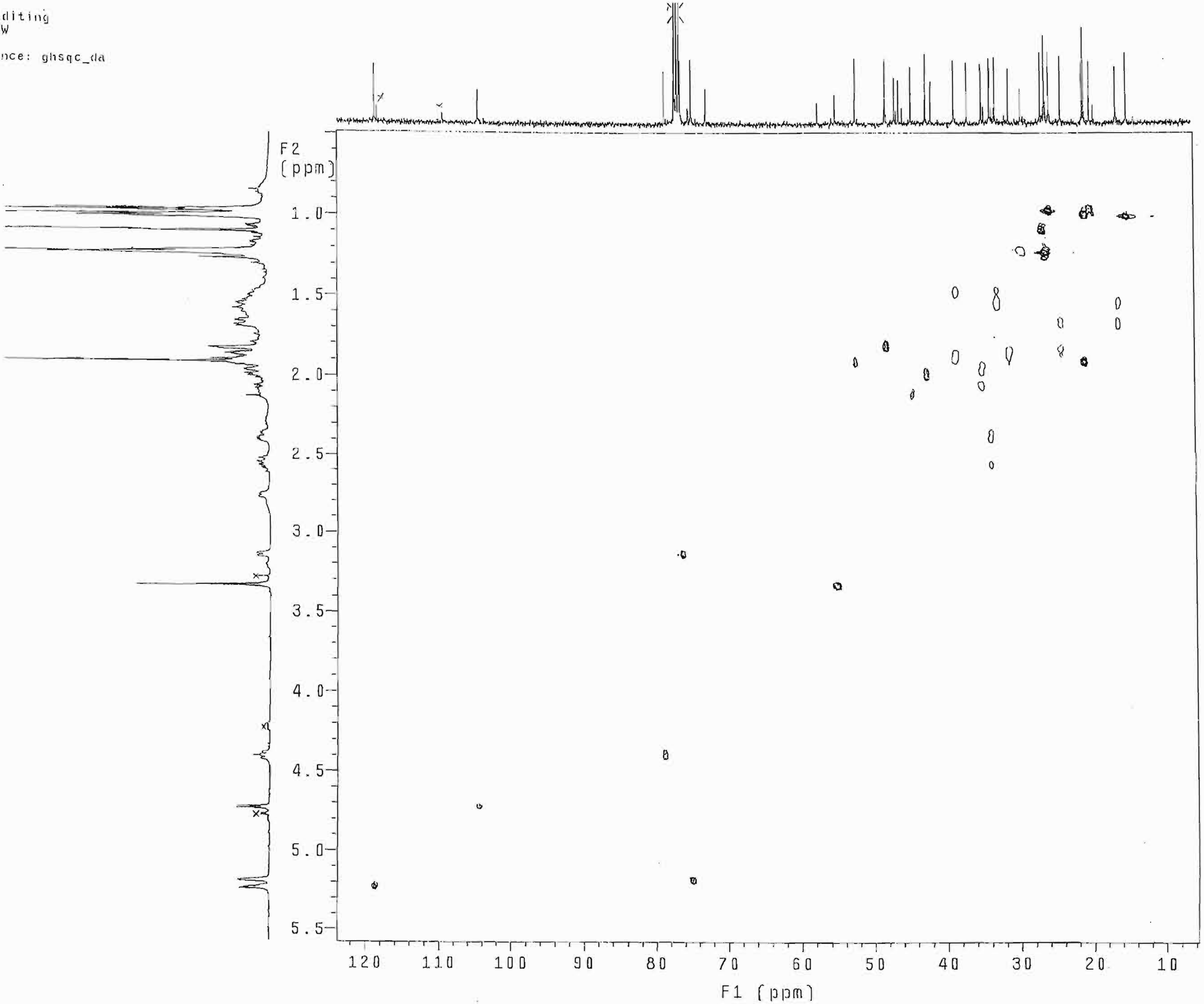
^{13}C NMR Spectrum of Protoceramoderins F (xv)



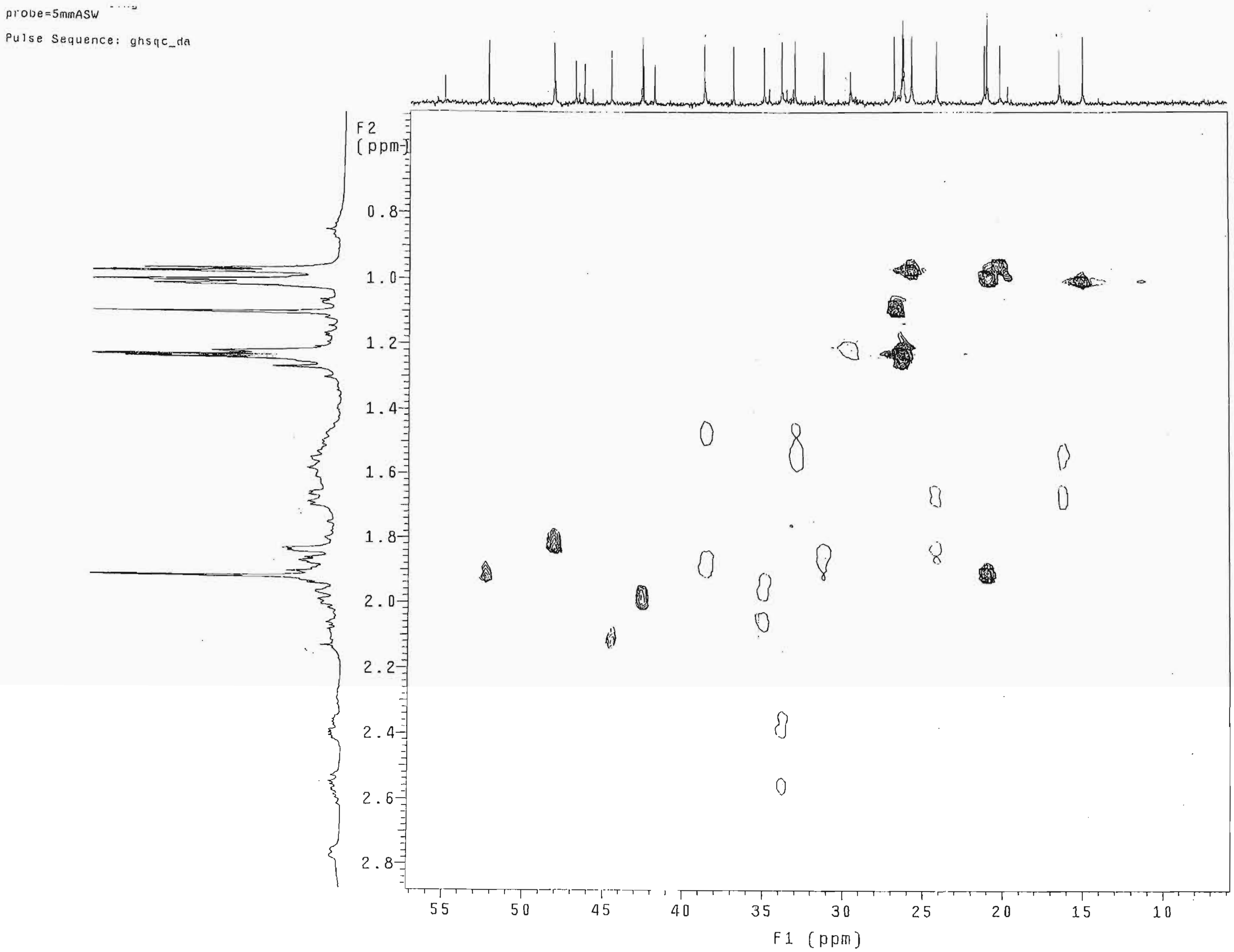
ADEPT NMR Spectrum of Protosamaderine F (xv)

with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da



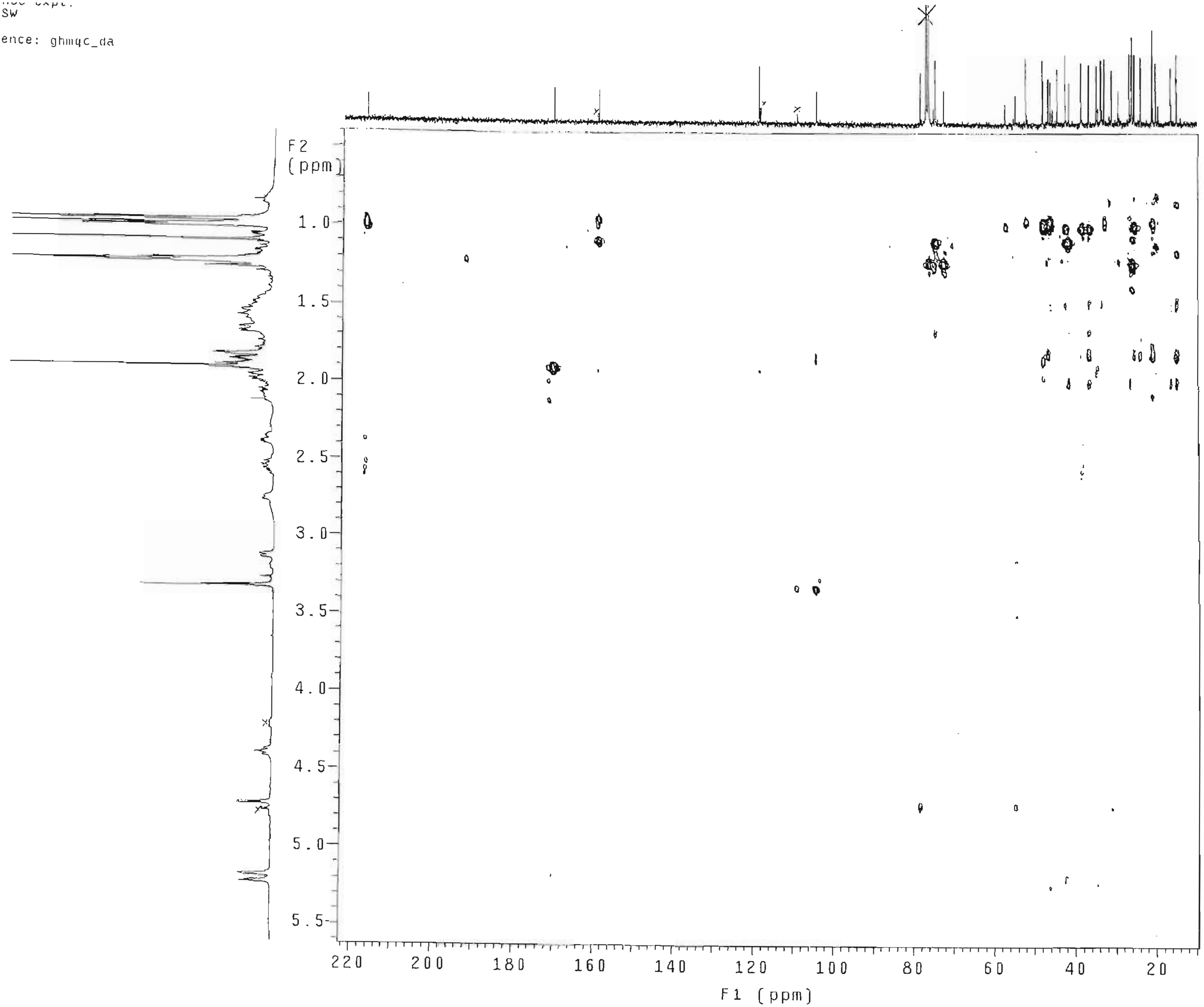
HSQC NMR Spectrum of Protoasmaderine F (xx)



HSQC NMR Spectrum of Protosamaderine E (xv)

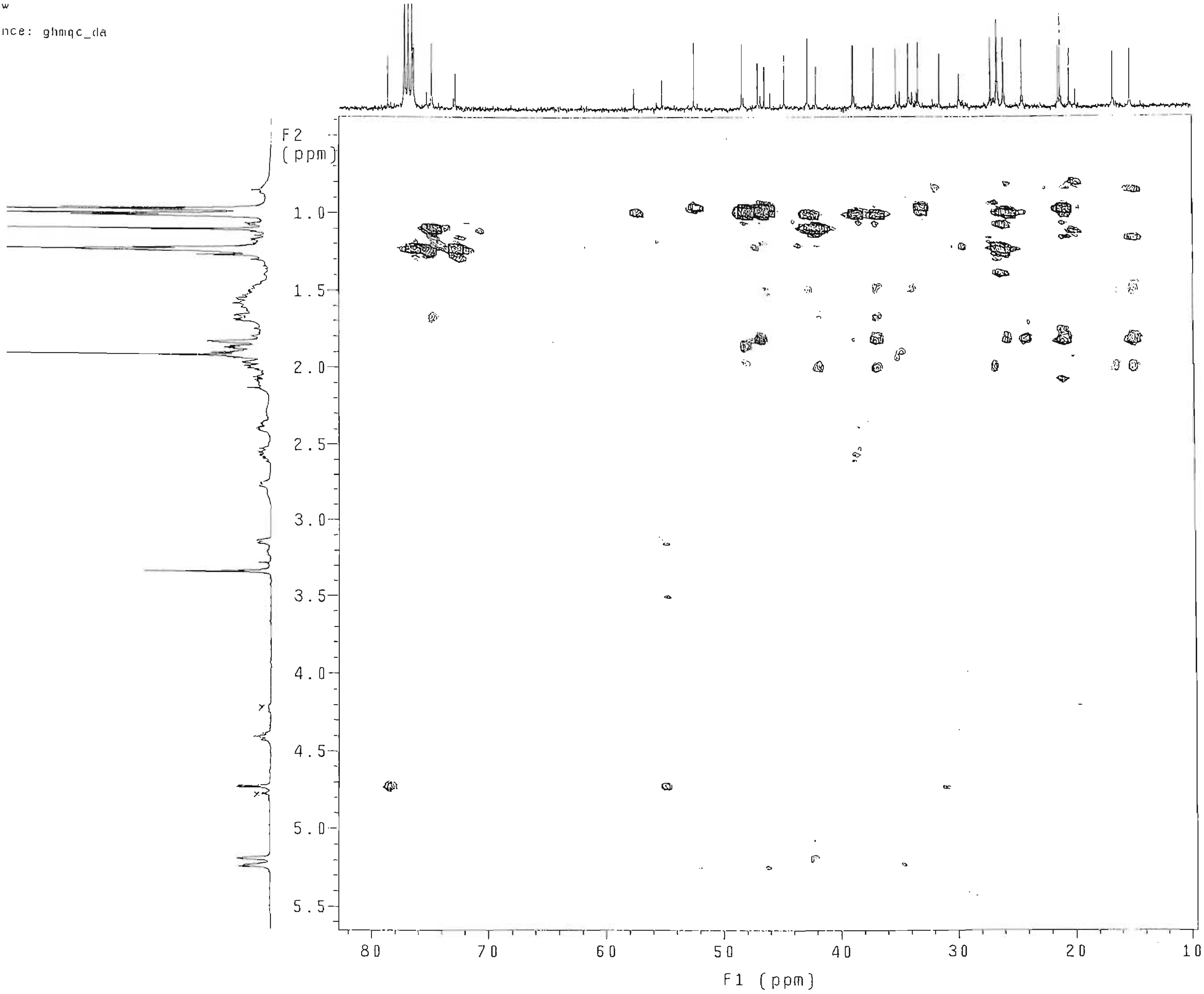
probe=5mmASW

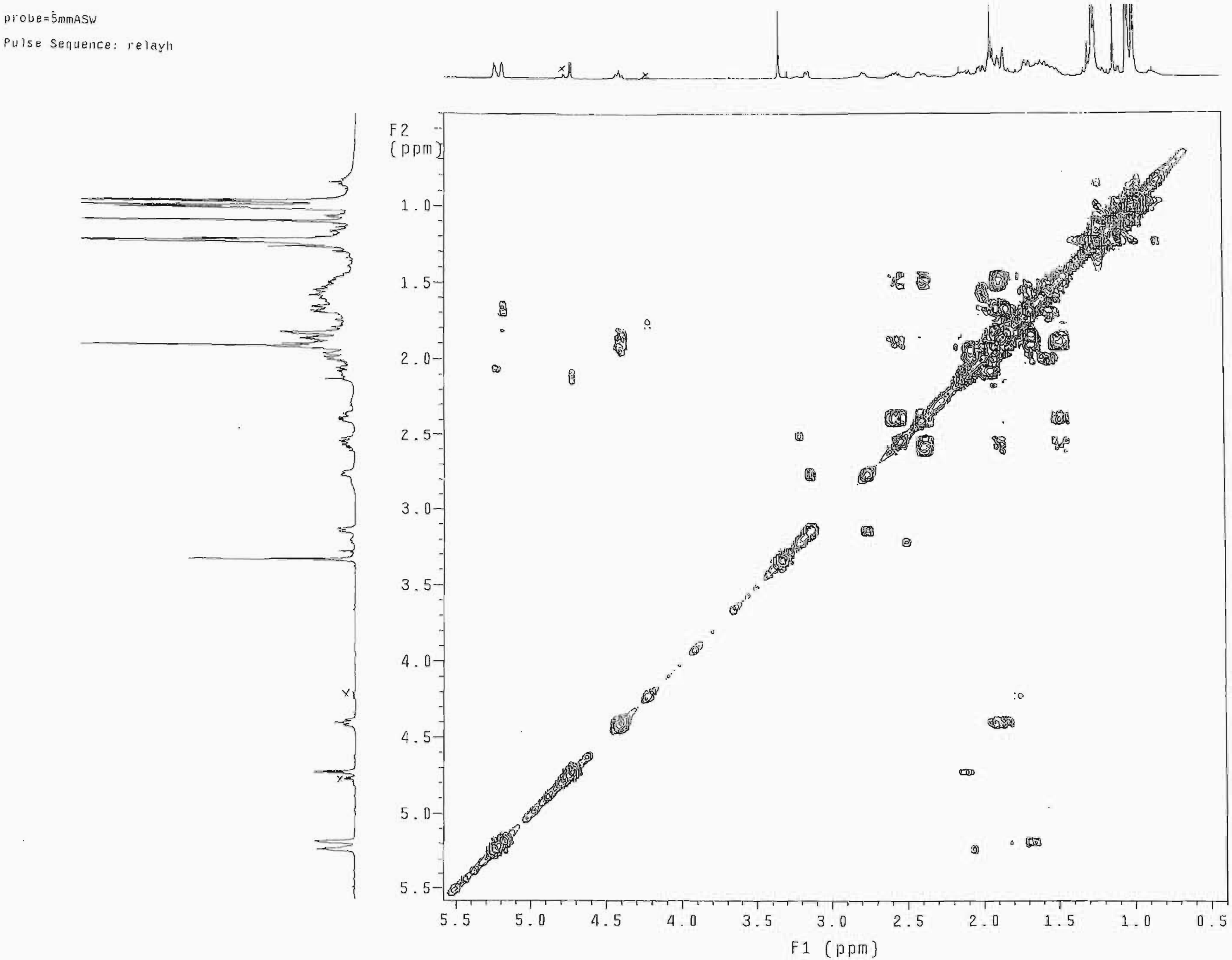
Pulse Sequence: ghmqc_da



HMBC NMR Spectrum of Protosamaderine F (xx)

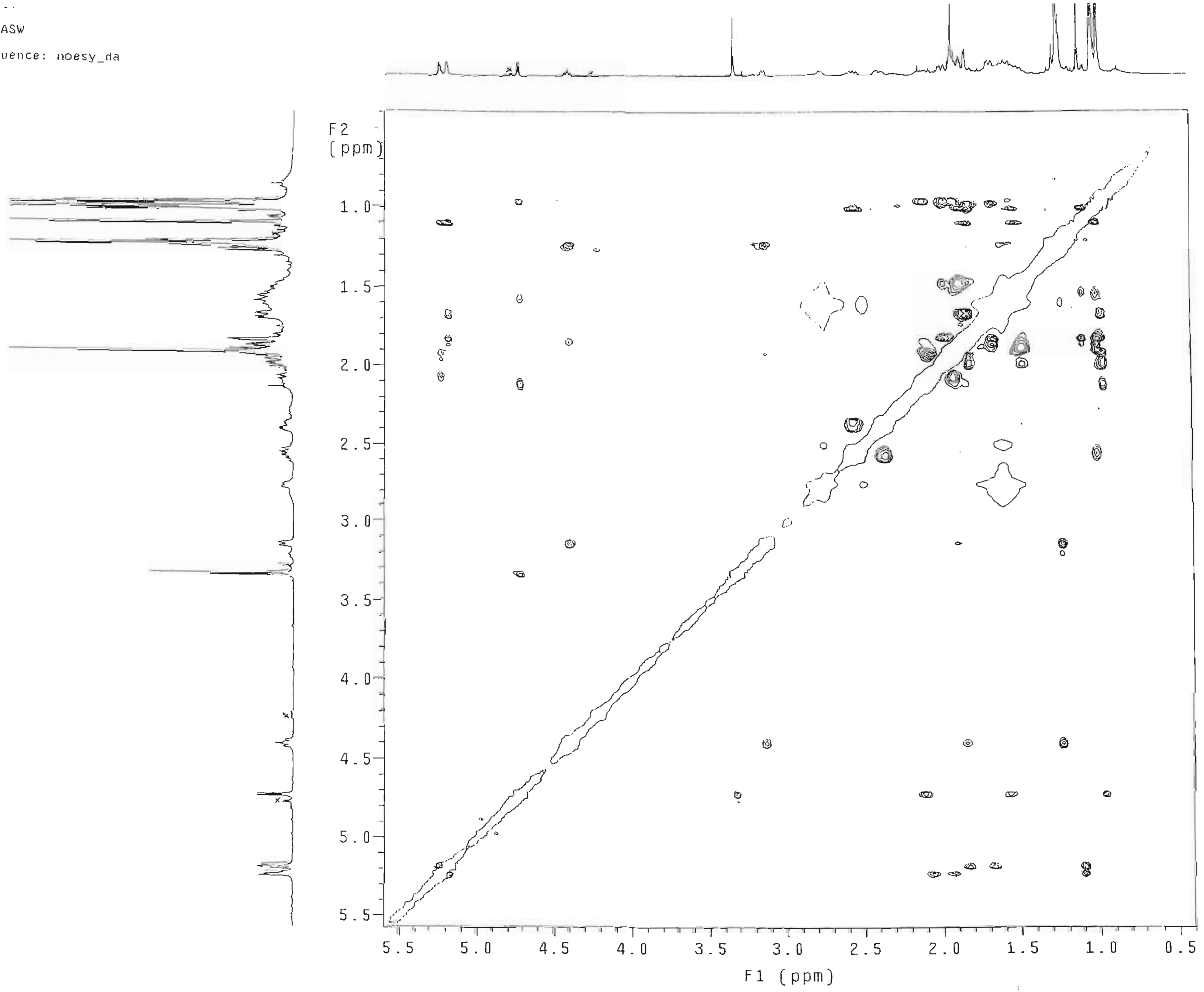
Pulse Sequence: ghmqc_da



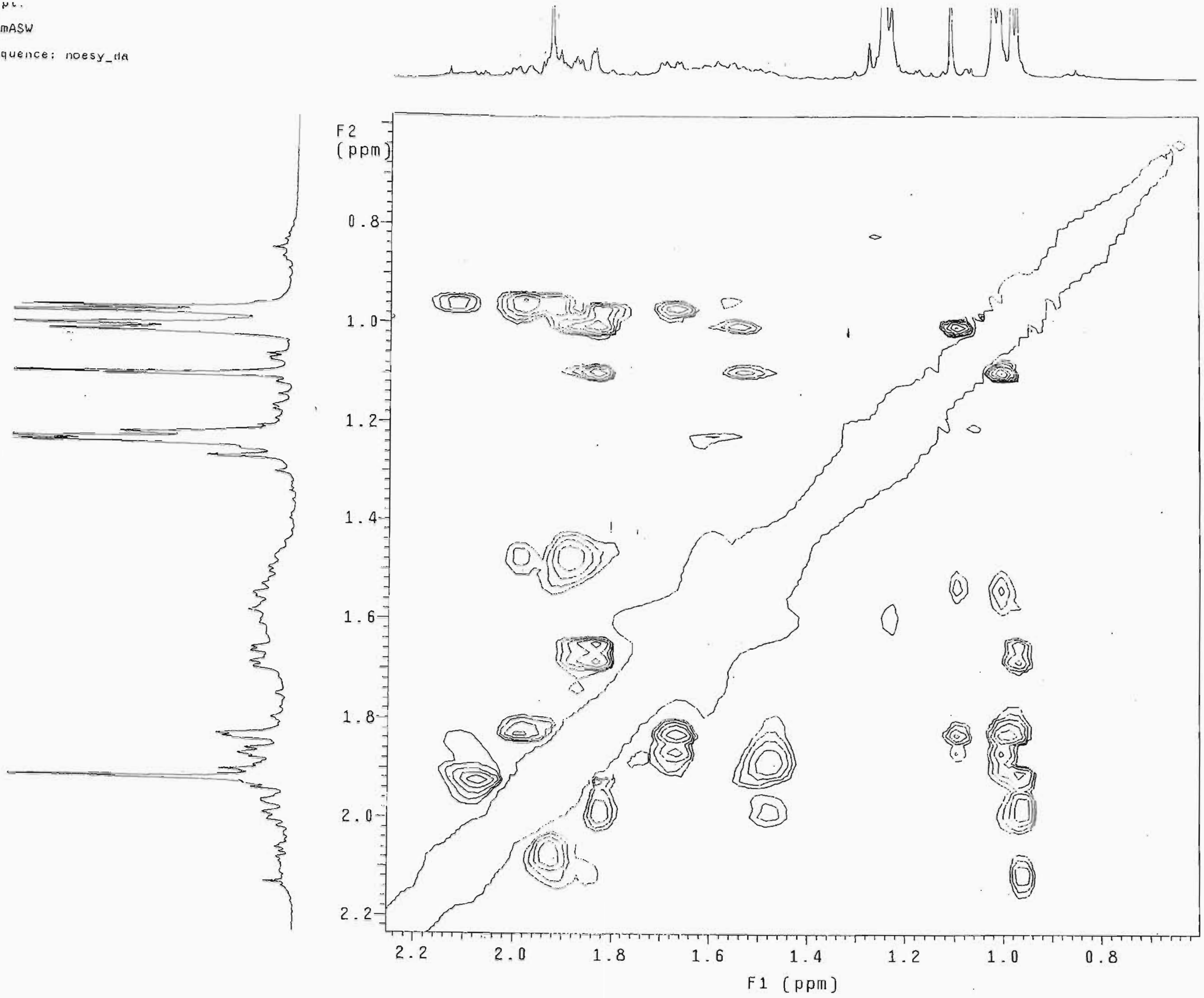

probe=5mmASW
Pulse Sequence: relayh
F2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
F1 (ppm)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
x
x
x
x

mix=1sec
probe=5mmASW

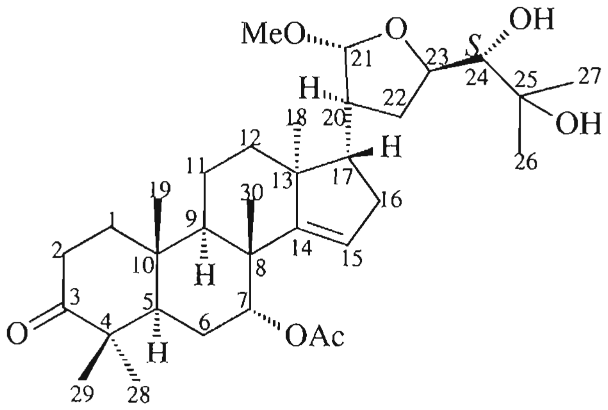
Pulse Sequence: noesy_da



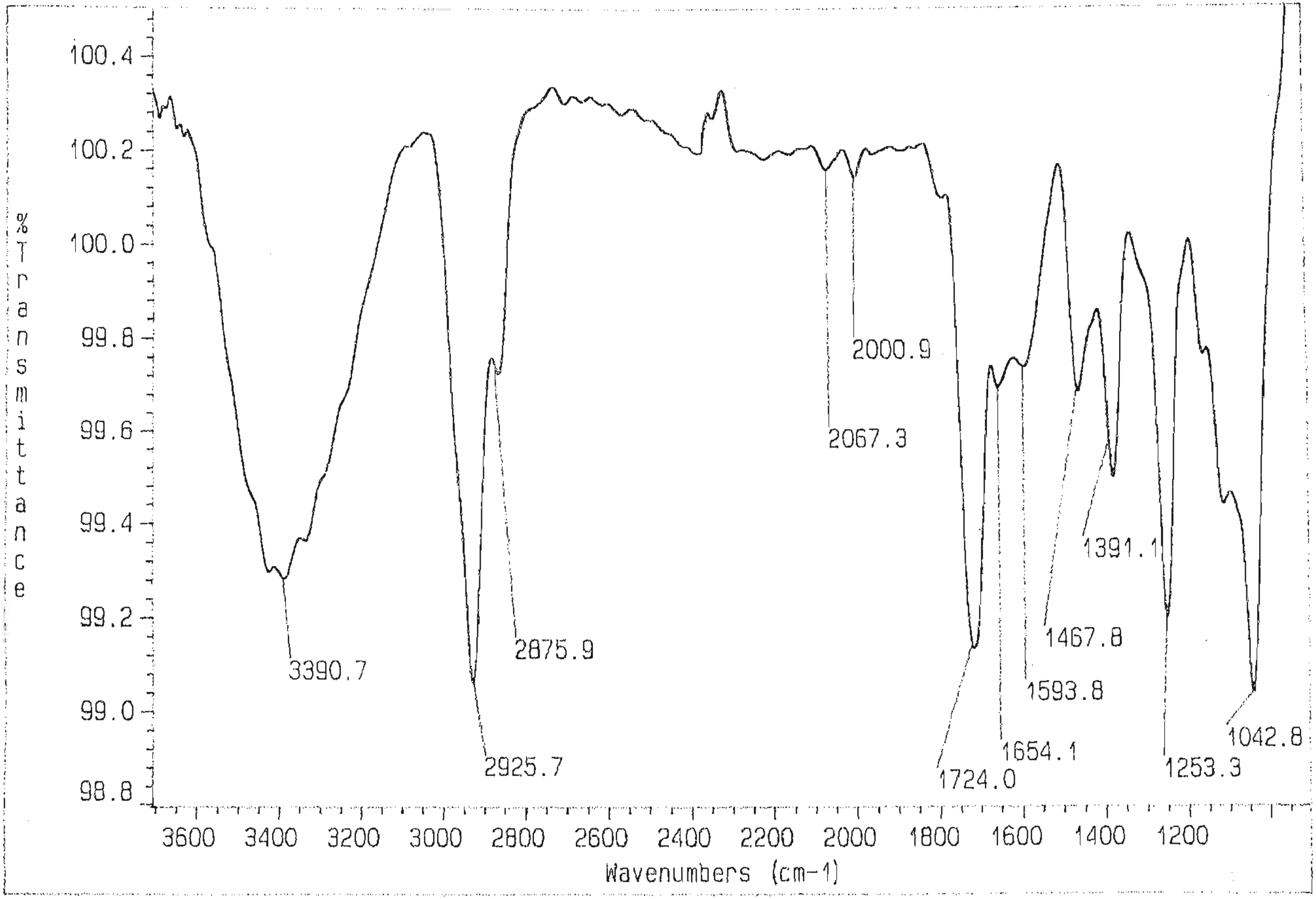
NOESY NMR Spectrum of Protoosmoderine E (xv)


mix=1sec
probe=5mmASW
Pulse Sequence: noesy_da
F2
(ppm)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
F1 (ppm)

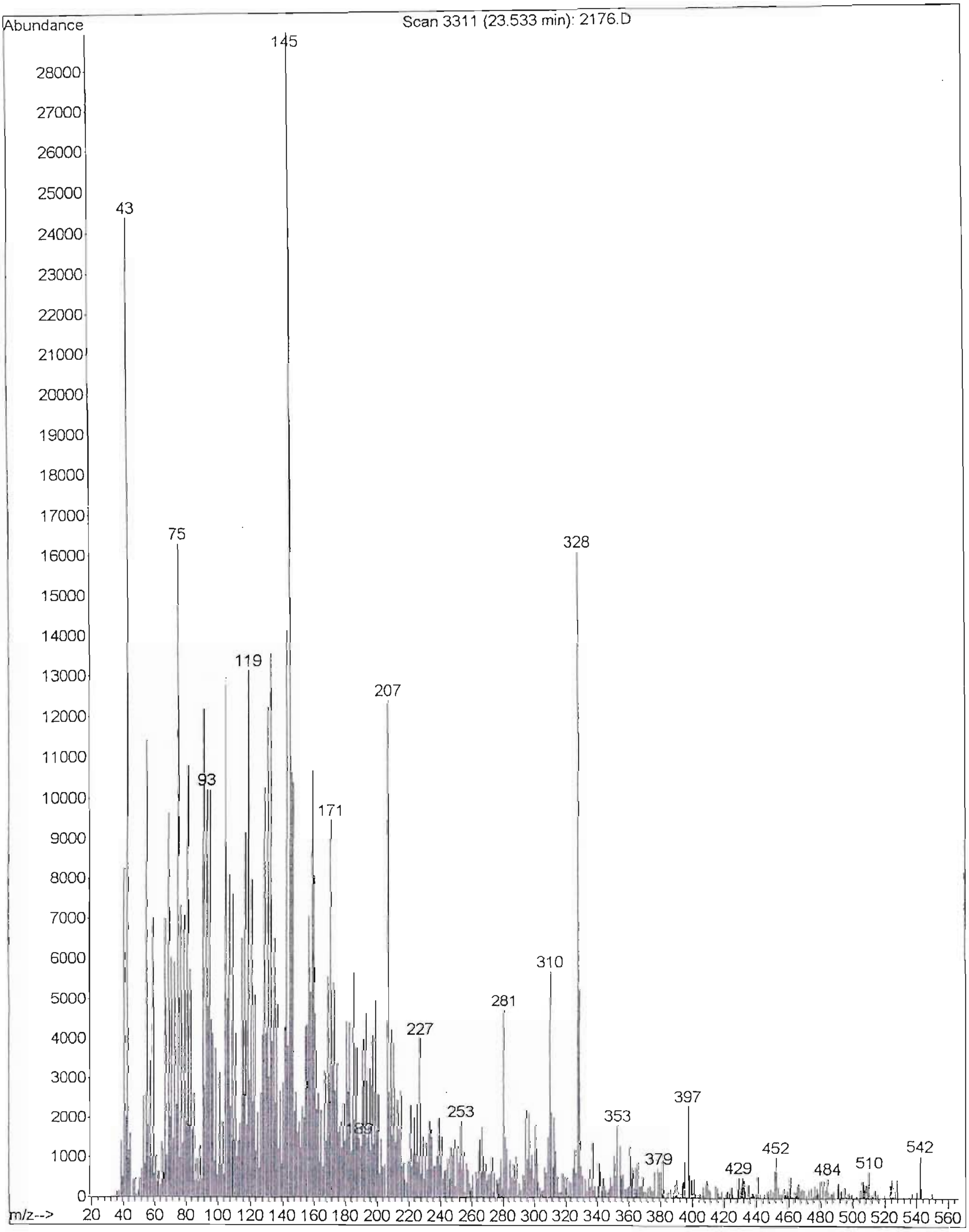
Protosamaderine G (xvi)



(xvi)



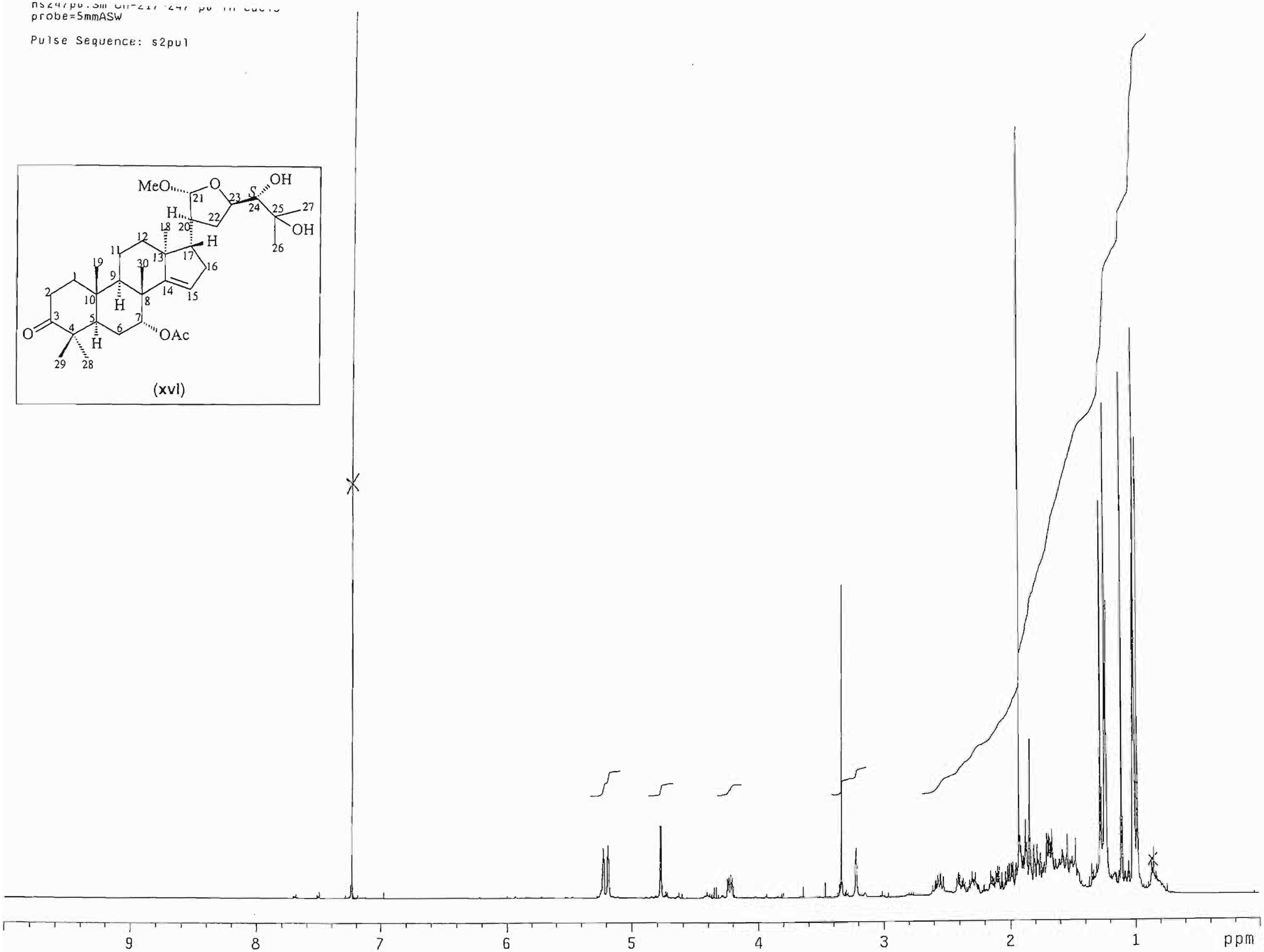
IR Spectrum of Protosamaderine G (xvi)



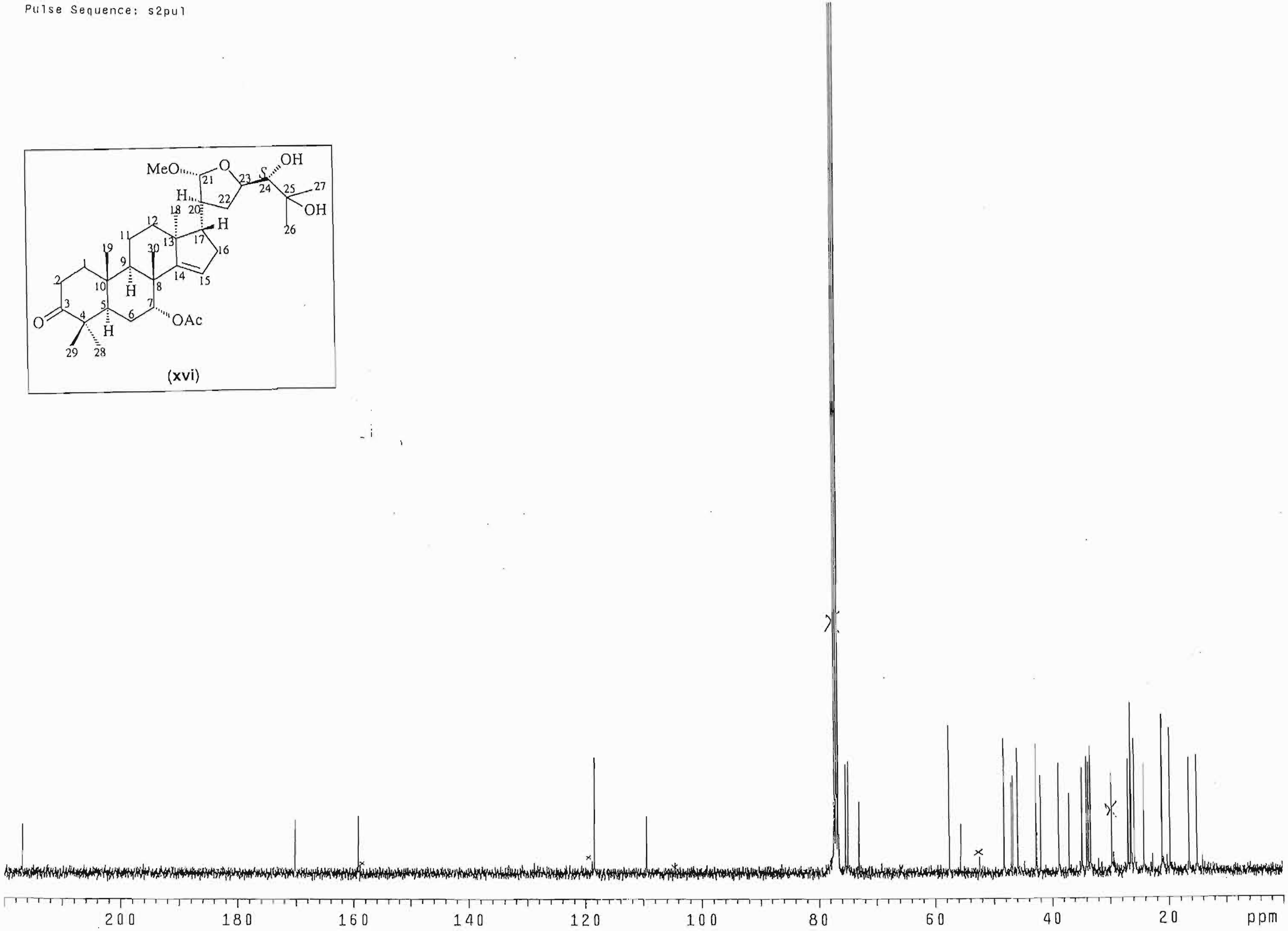
Mass Spectrum of Protosamaderine G (xvi)

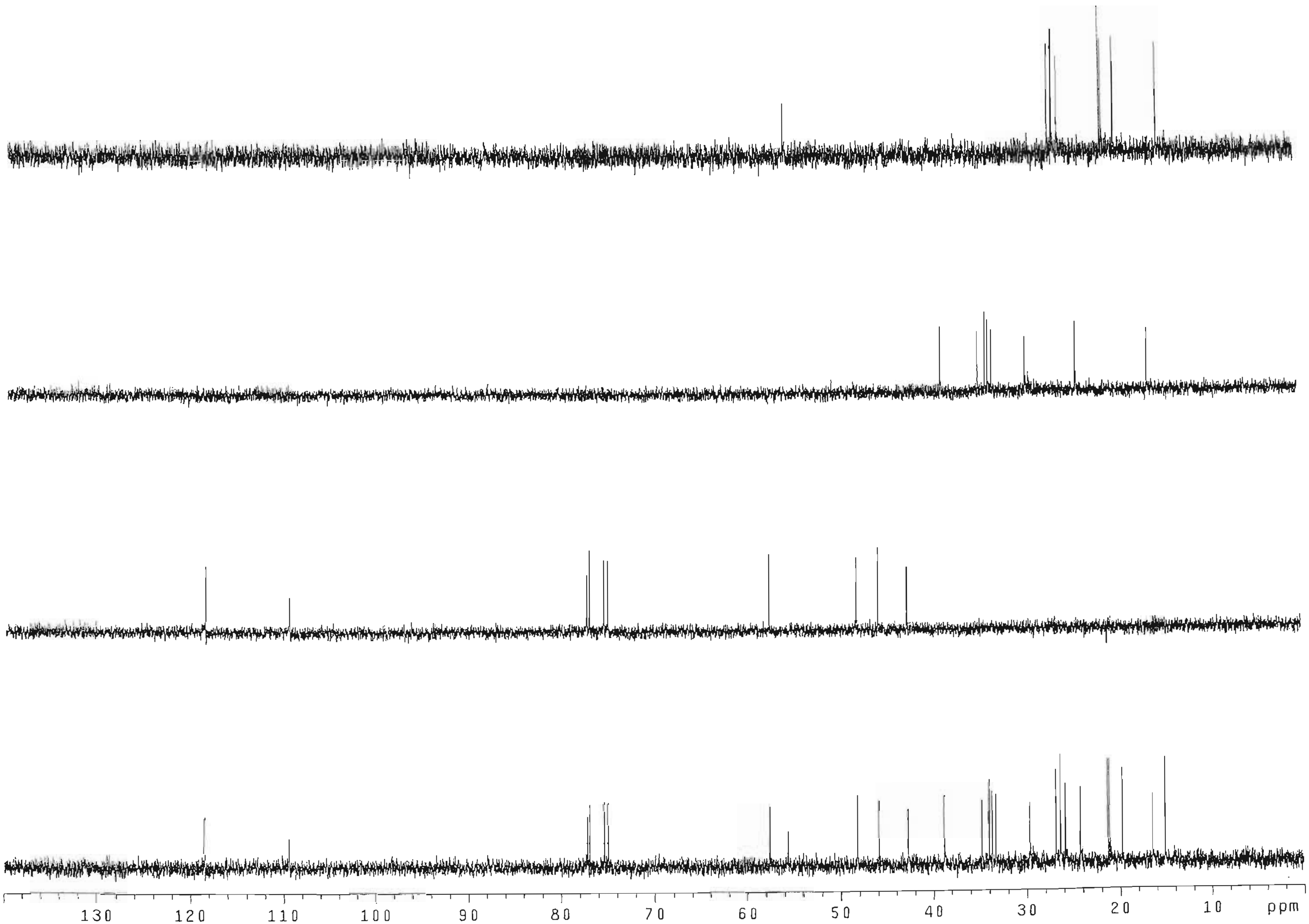
probe=5mmASW

Pulse Sequence: s2pul



G (xvi)

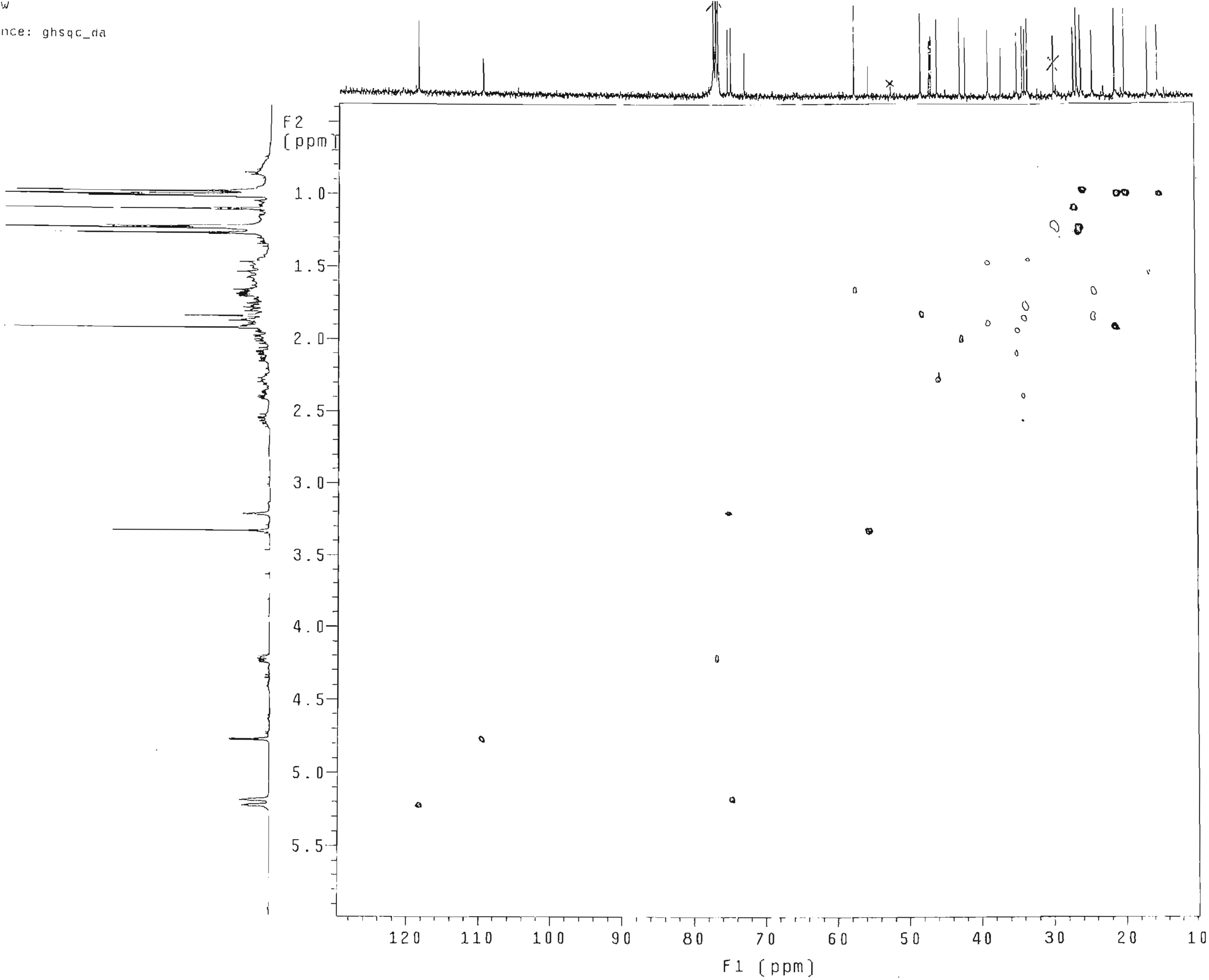

Pulse Sequence: s2pul
MeO
O
OH
OH
H
H
H
H
O
OAc
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
S
(xvi)
200
180
160
140
120
100
80
60
40
20
ppm



DEPT NMR Spectrum of Protoseamaderine G (xvi)

with mult.editing
probe=5mmASW

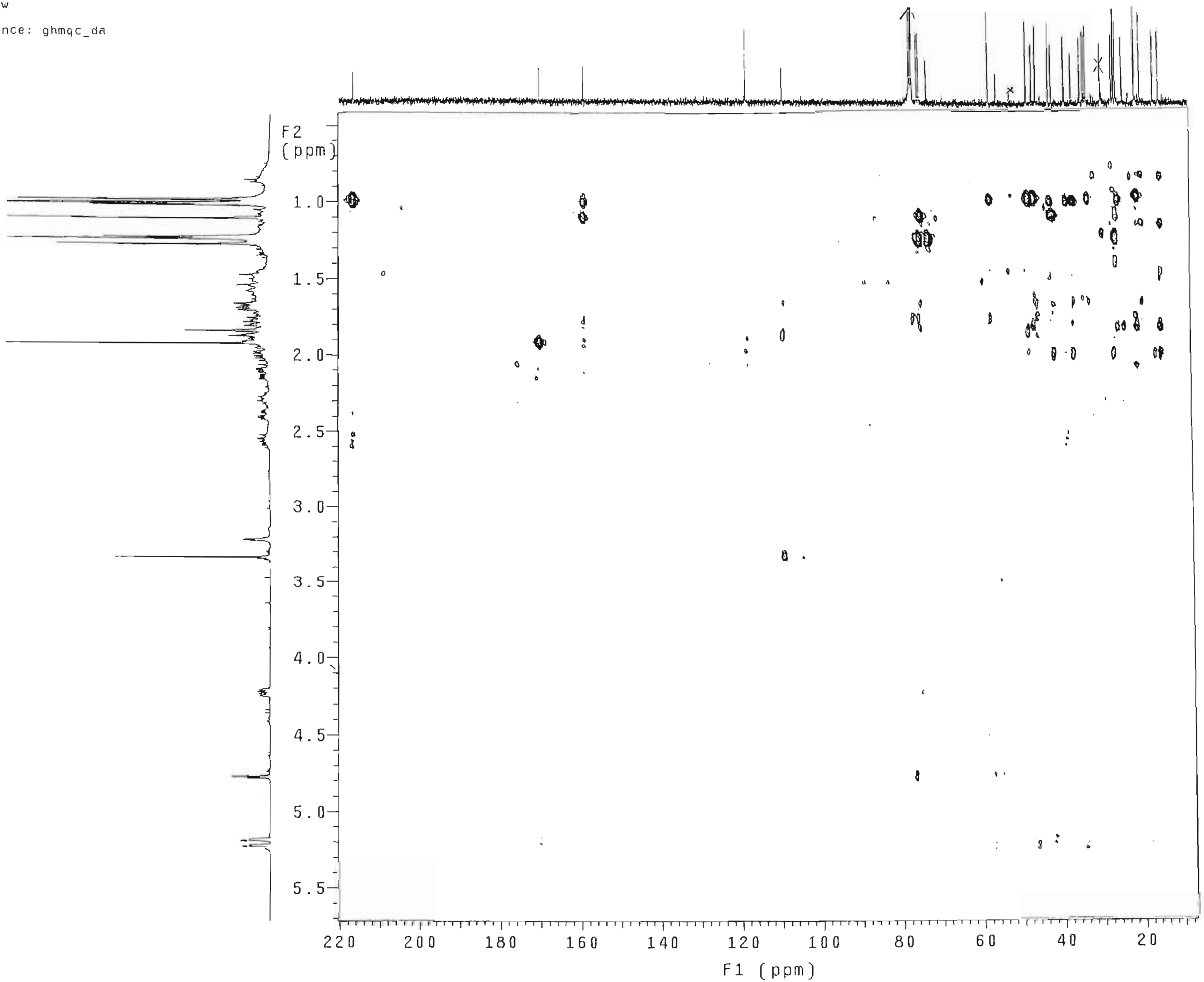
Pulse Sequence: ghsqc_da



HSQC NMR Spectrum of Protosamaderine G (xvi)

probe=5mmASW

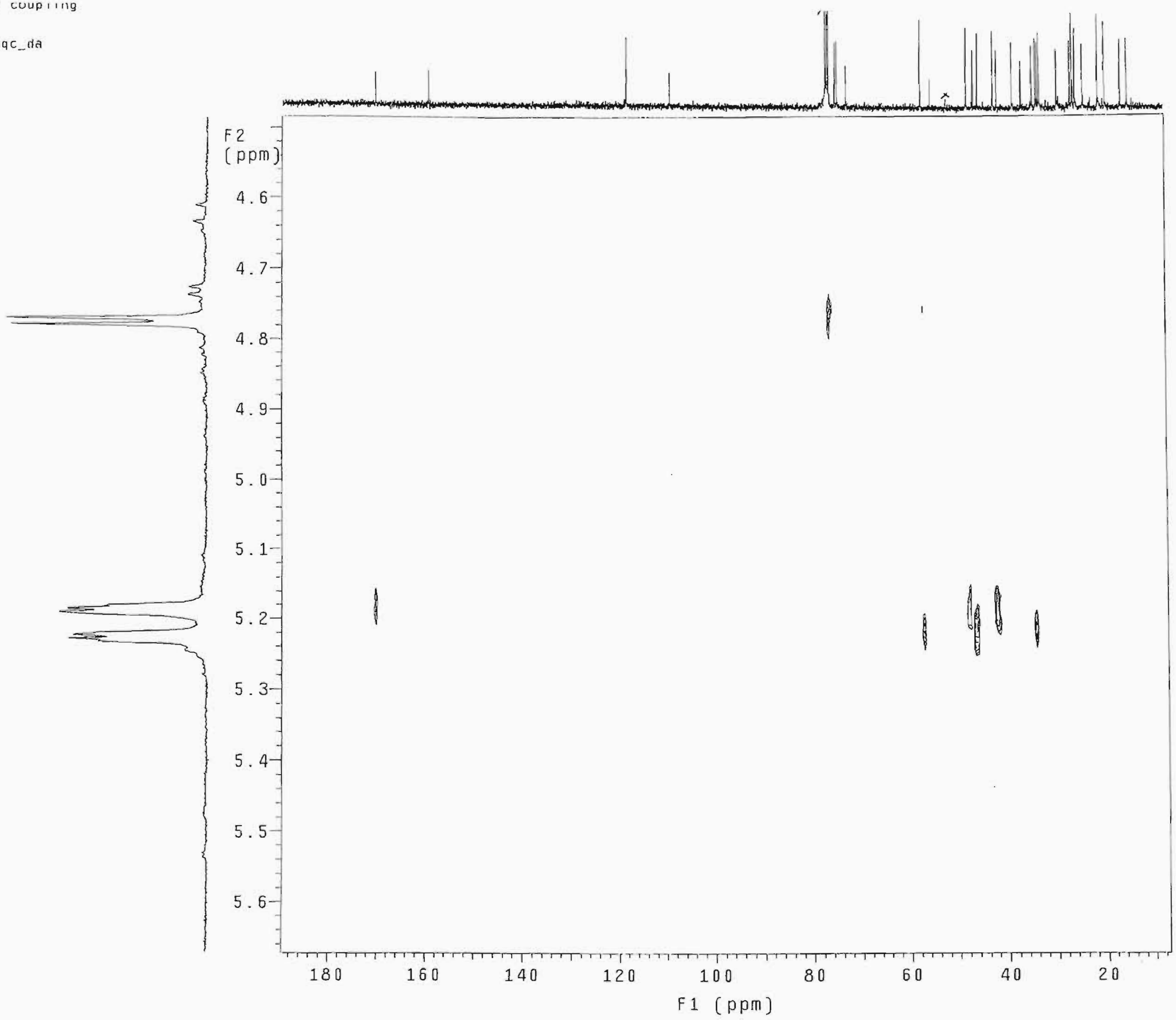
Pulse Sequence: ghmqc_da



HMBC NMR Spectrum of Protosamaderine G (xvi)

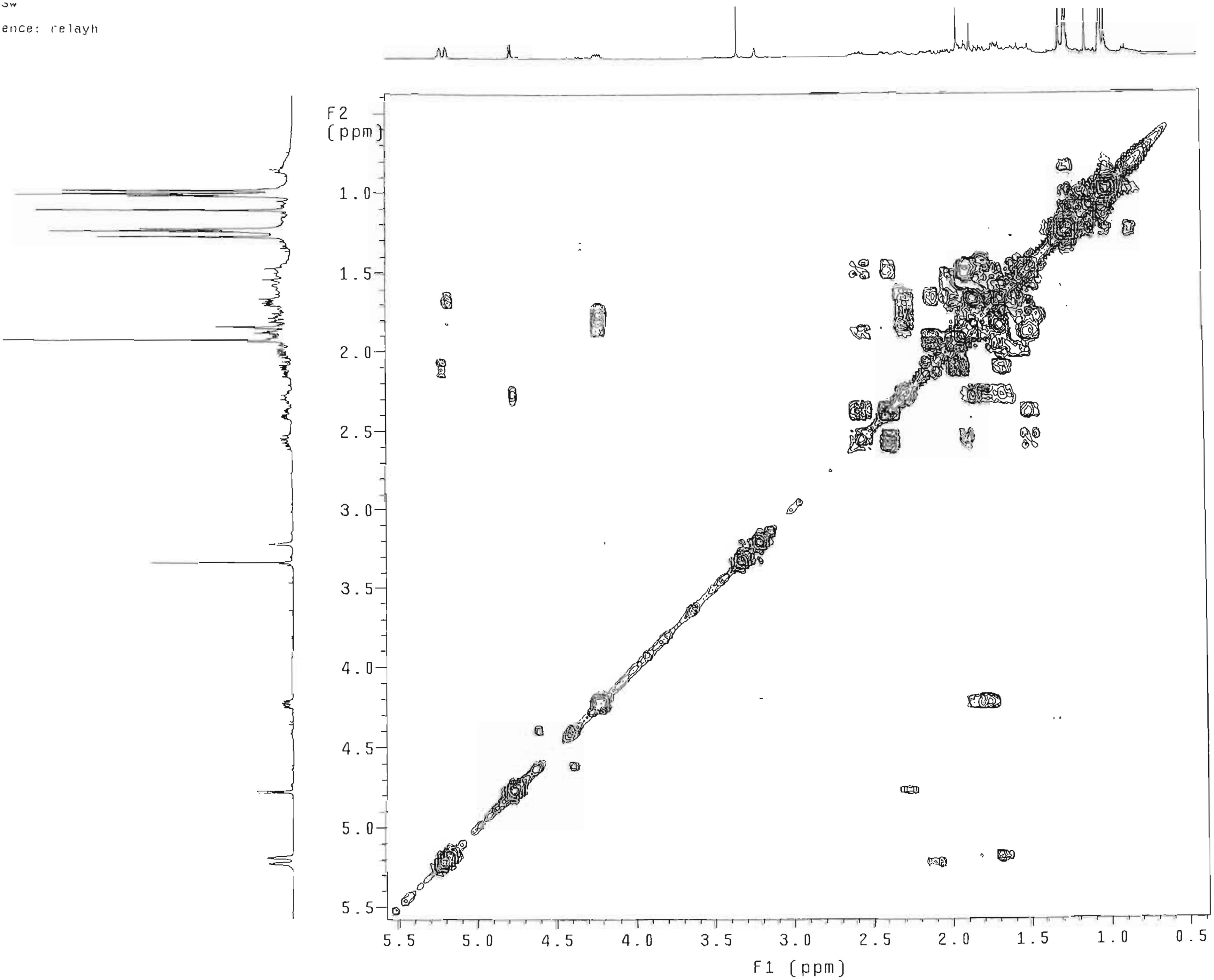
optimized for 10 Hz coupling
probe=5mmASW

Pulse Sequence: ghmqc_da



HMBC NMR Spectrum of Protosamaderine G (xvi)

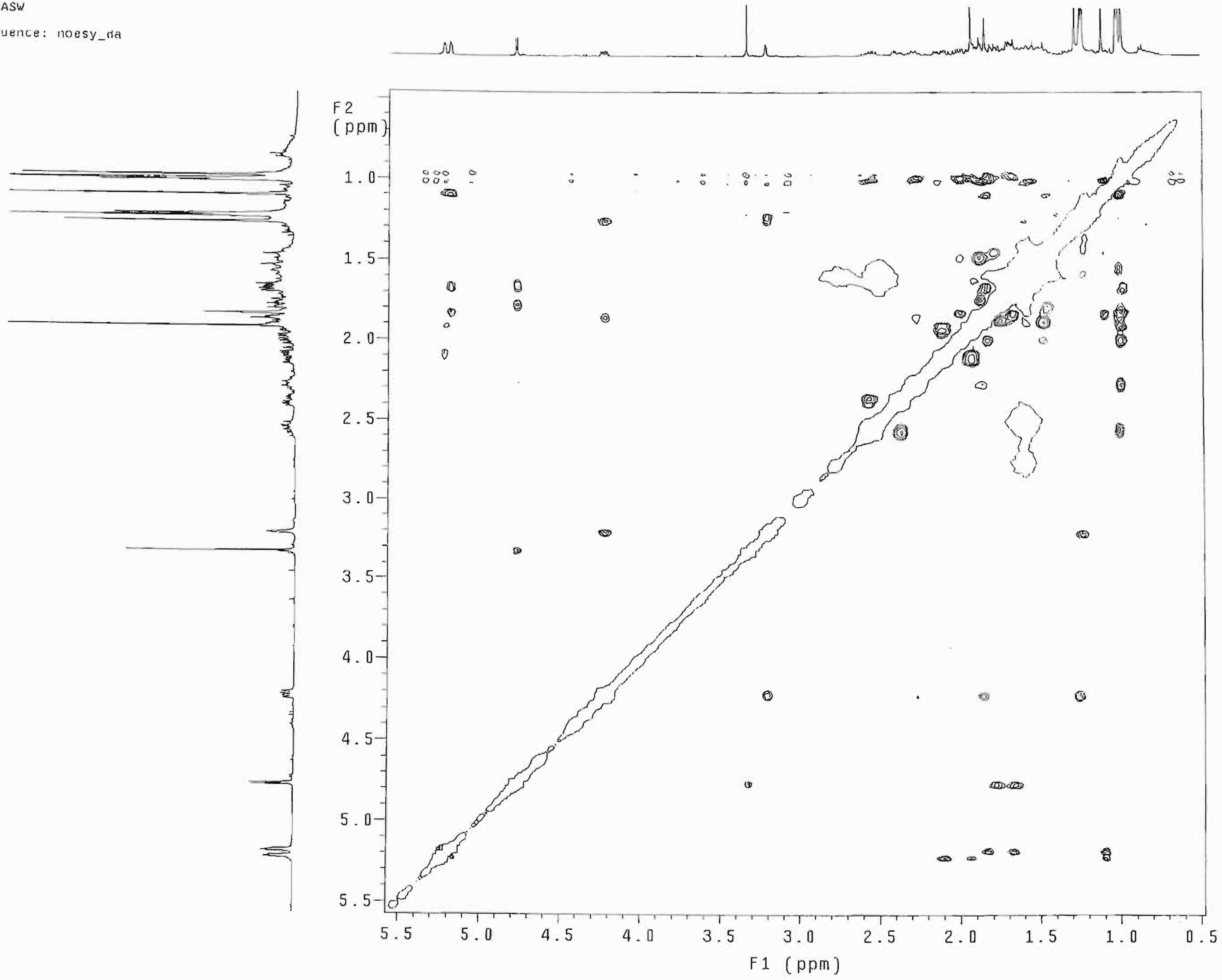
Pulse Sequence: relayh



COSY NMR Spectrum of Protosamaderine G (xvi)

probe=5mmASW

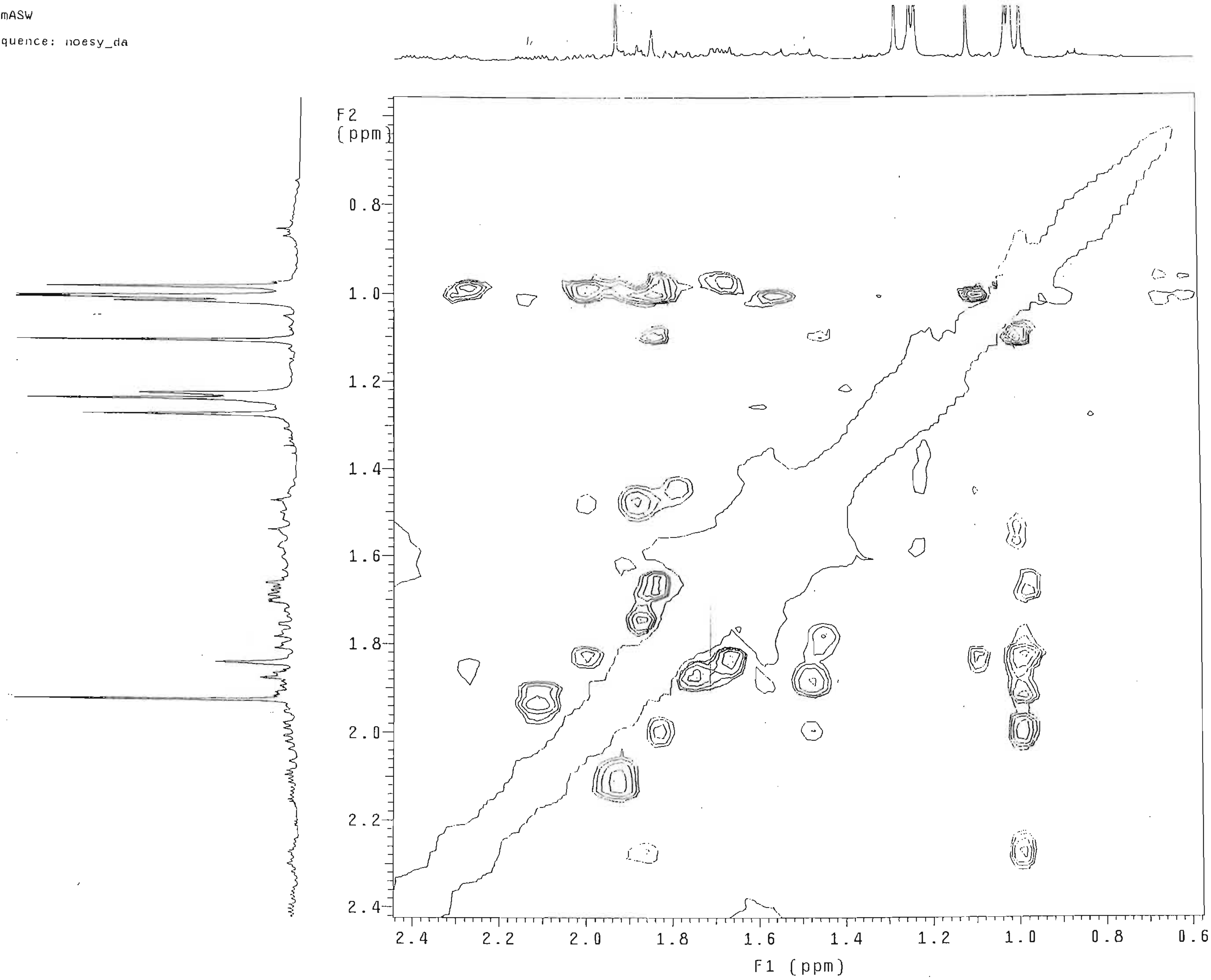
Pulse Sequence: noesy_da



NOESY NMR Spectrum of Protosamaderine G (xvi)

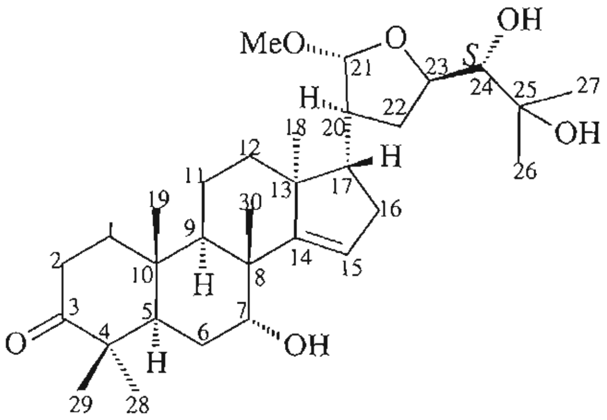
probe=5mmASW

Pulse Sequence: noesy_da

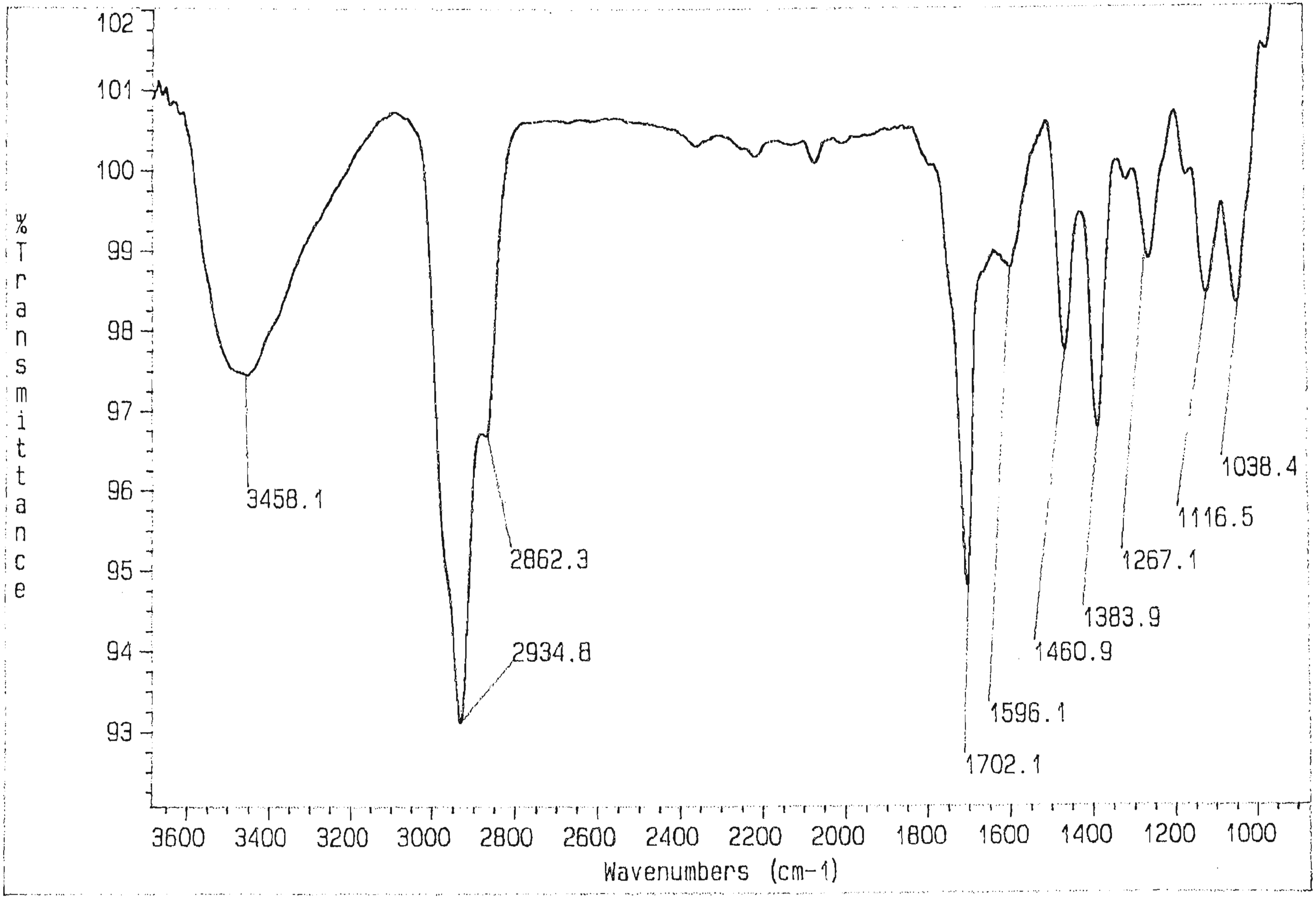


NOESY NMR Spectrum of Protosamaderine G (xvi)

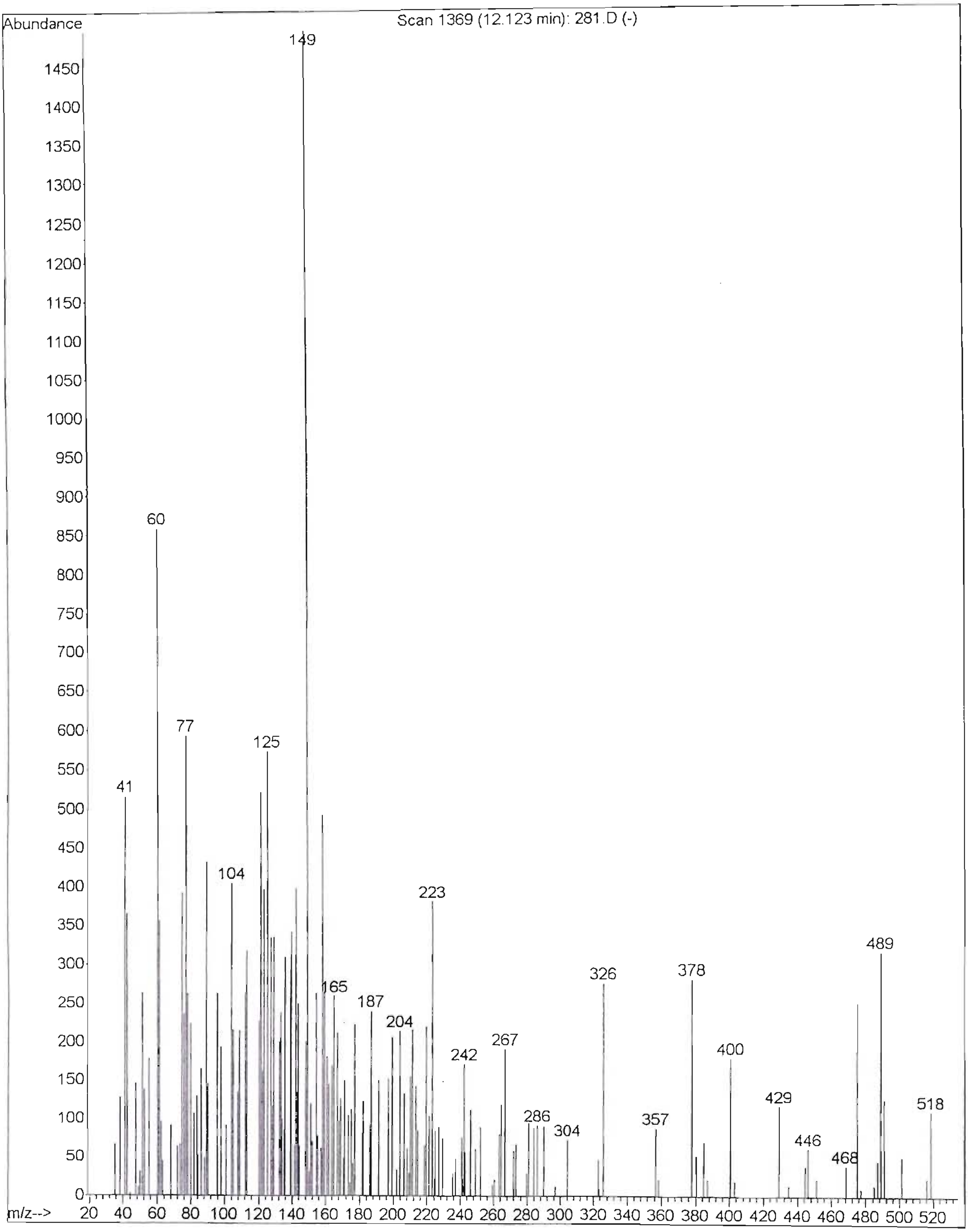
Protosamaderine H (xvii)



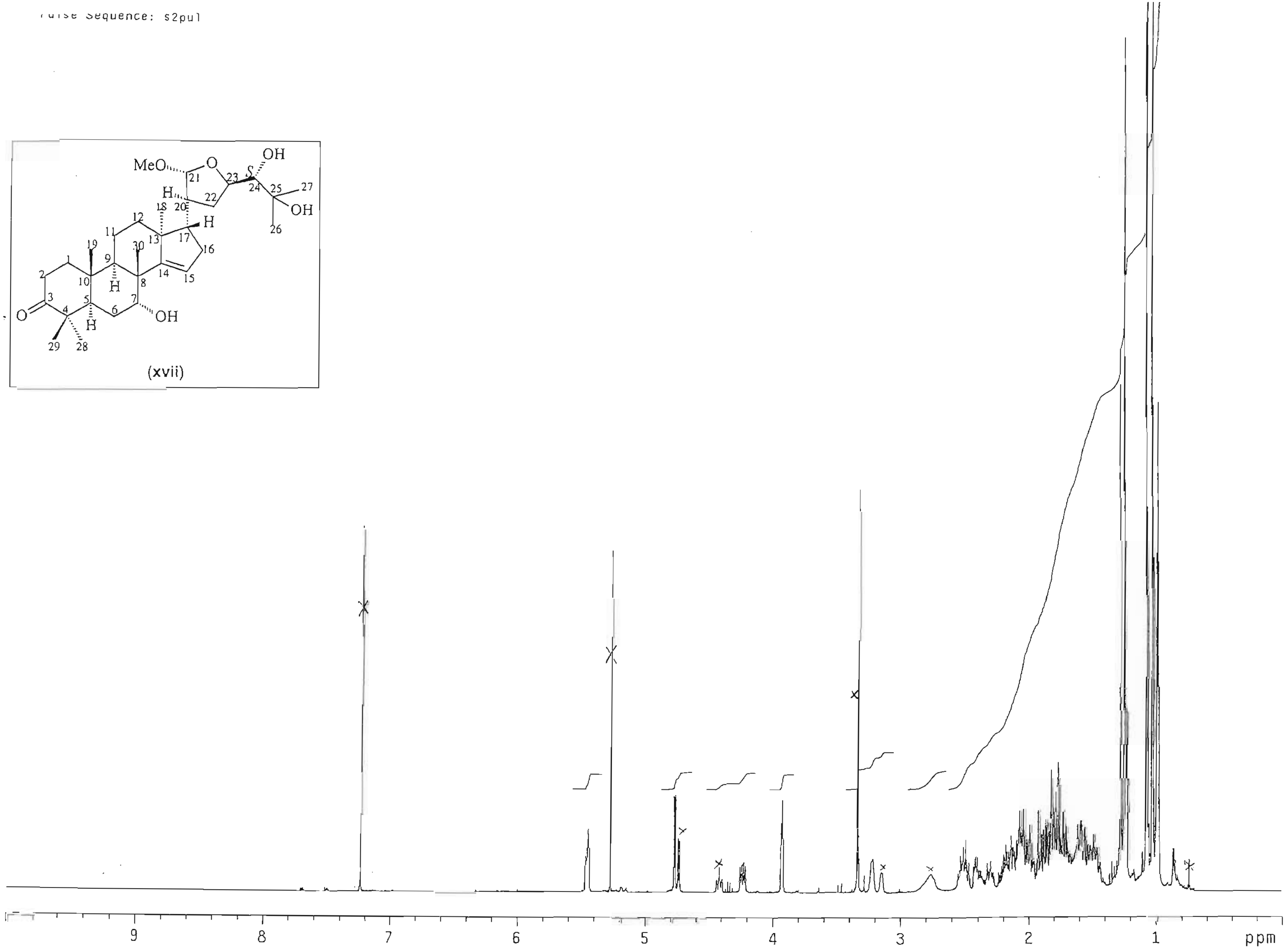
(xvii)



IR Spectrum of Protosamaderine H (xvii)

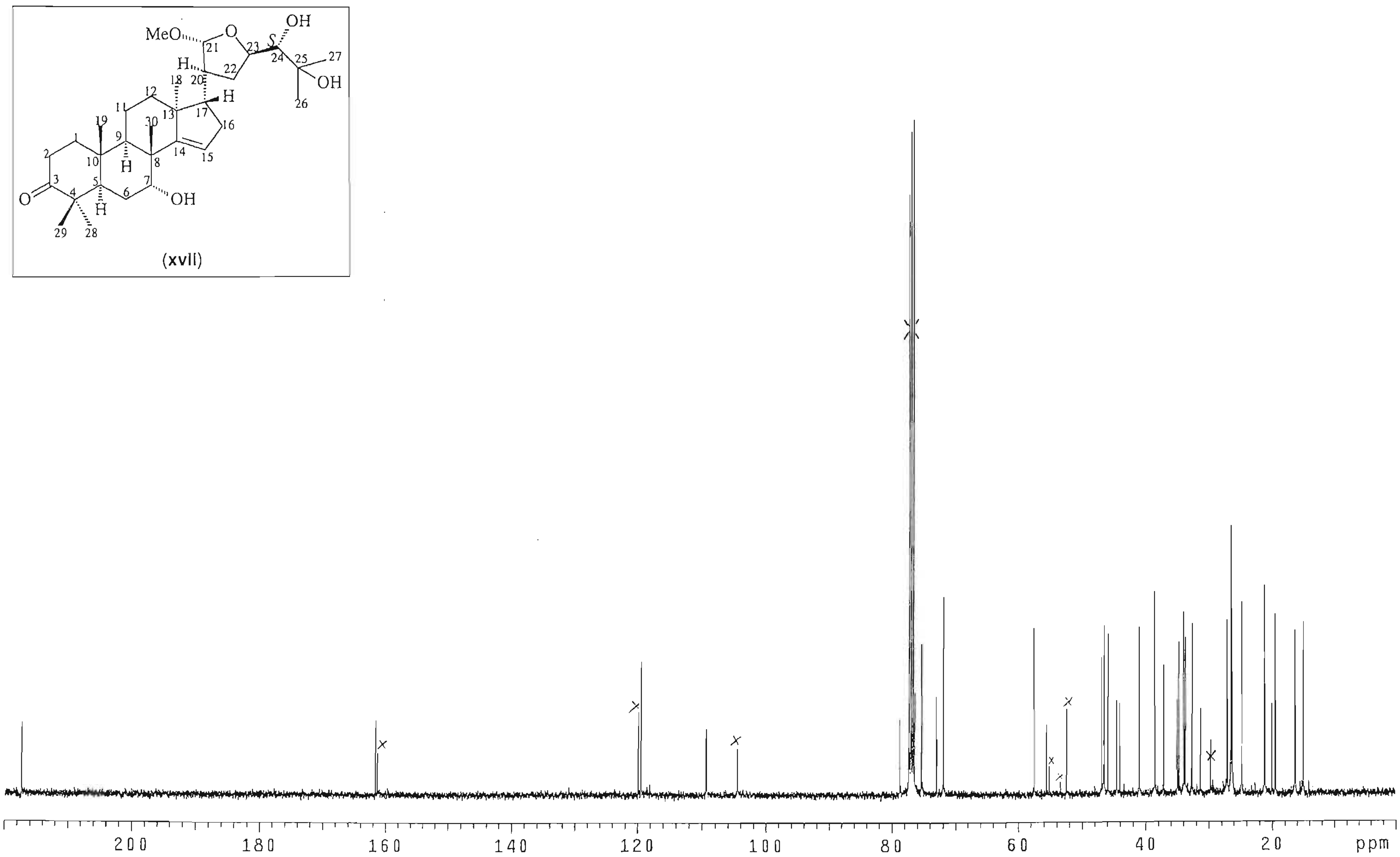


Mass Spectrum of Protosamaderine H (xvii)

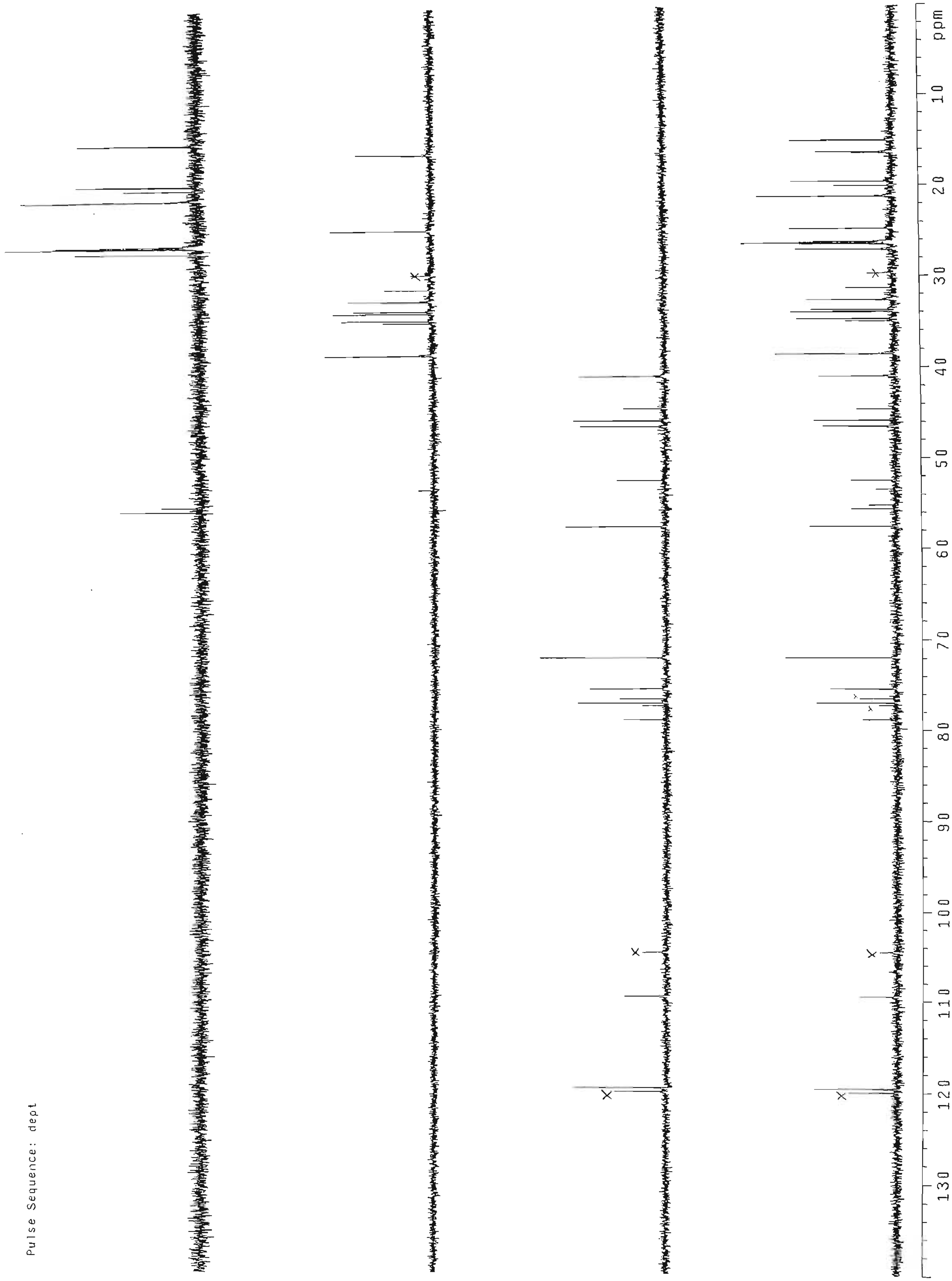


¹H NMR Spectrum of Protosamaderine H (xvii)

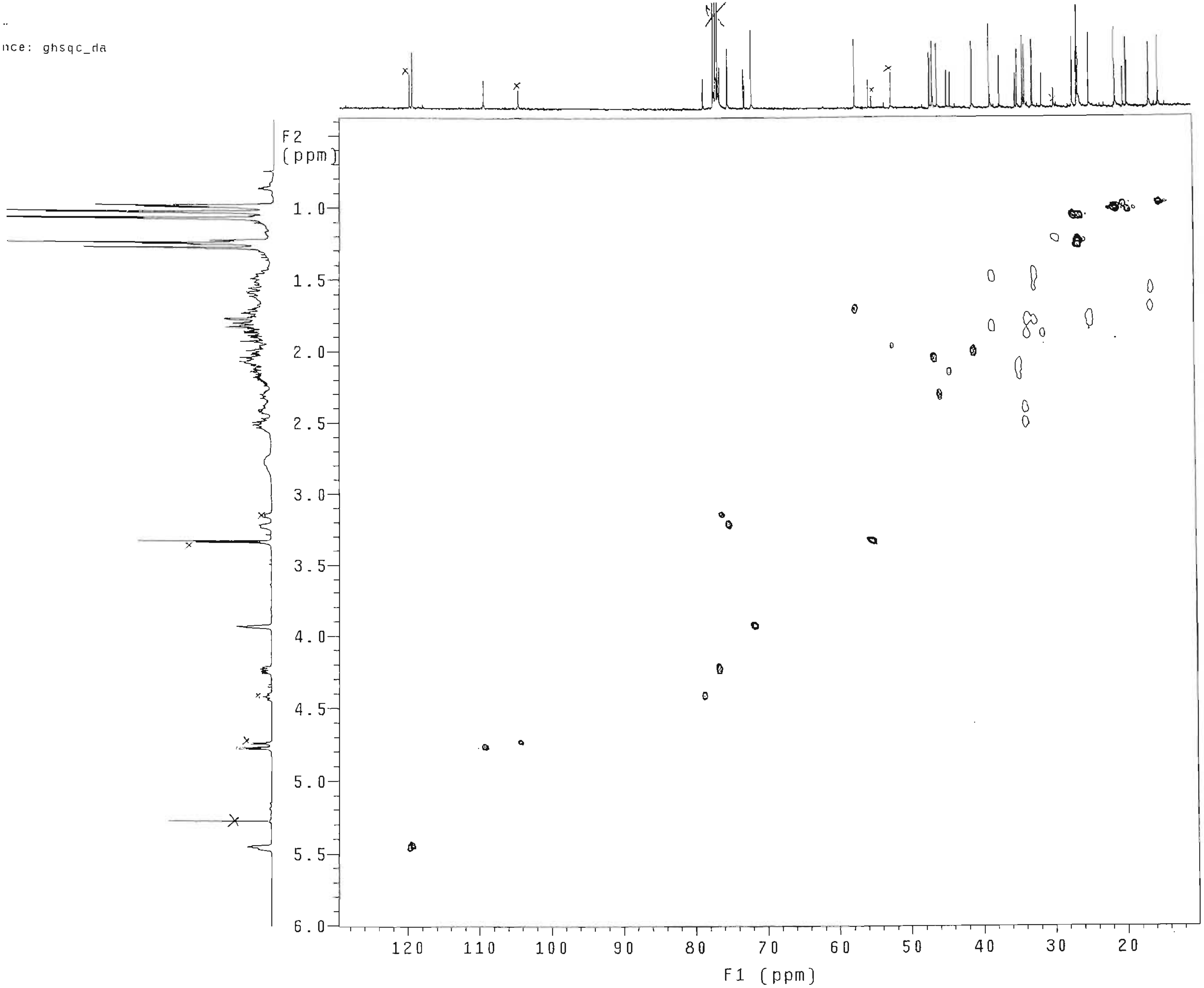
Pulse Sequence: s2pul



^{13}C NMR Spectrum of Protosamaderine H (xvii)



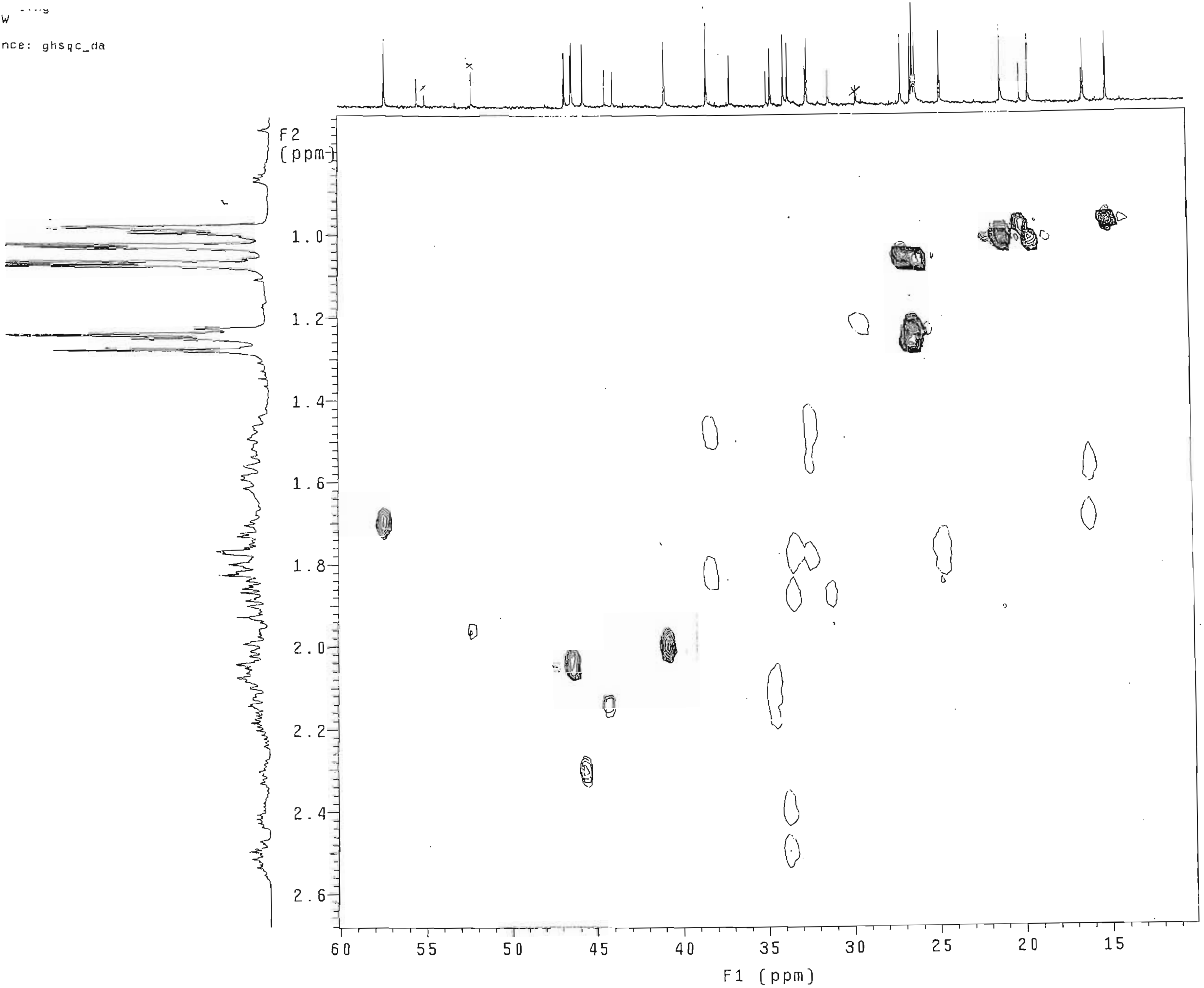
ADEPT NMR Spectrum of



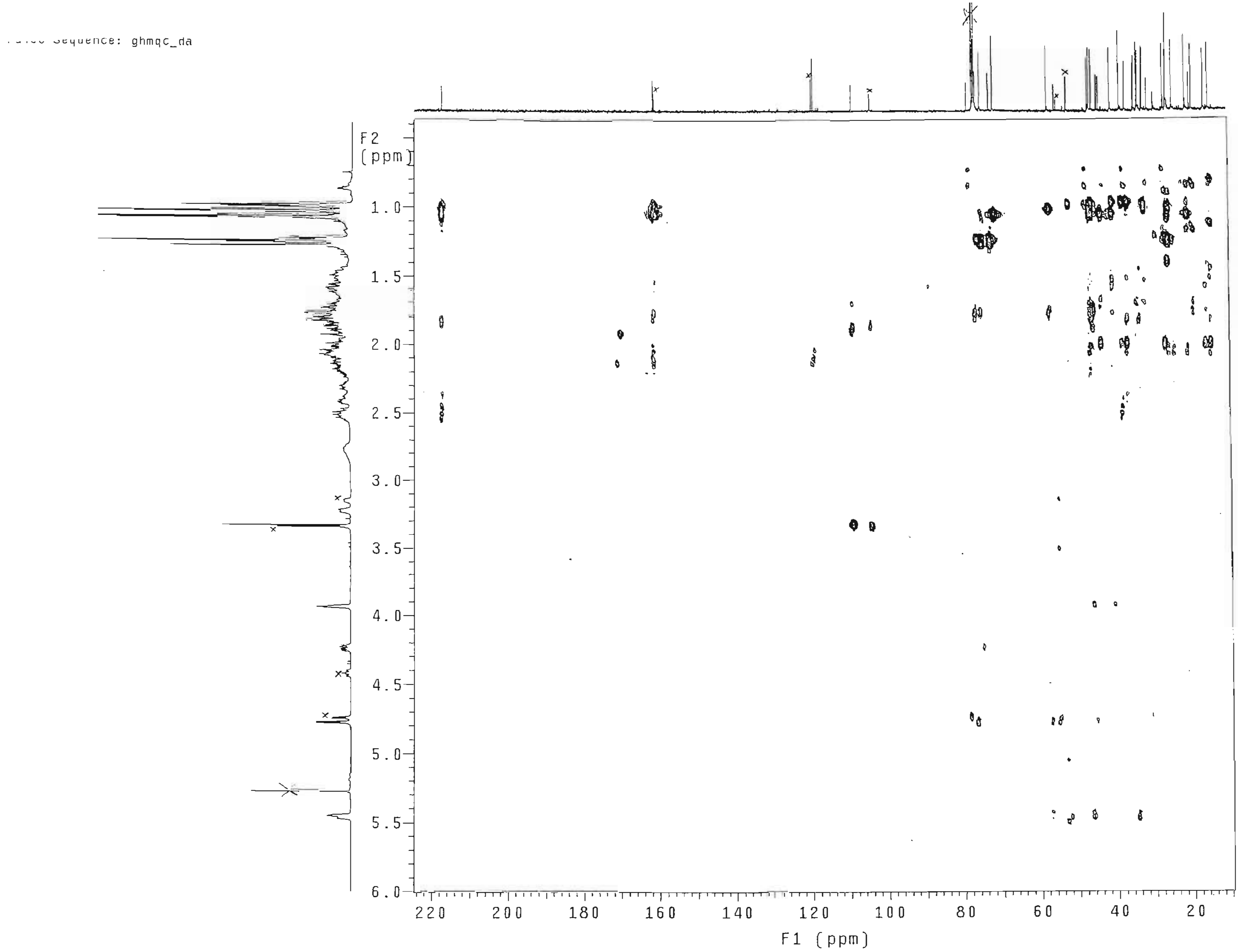
HSQC NMR Spectrum of

probe=5mmASW

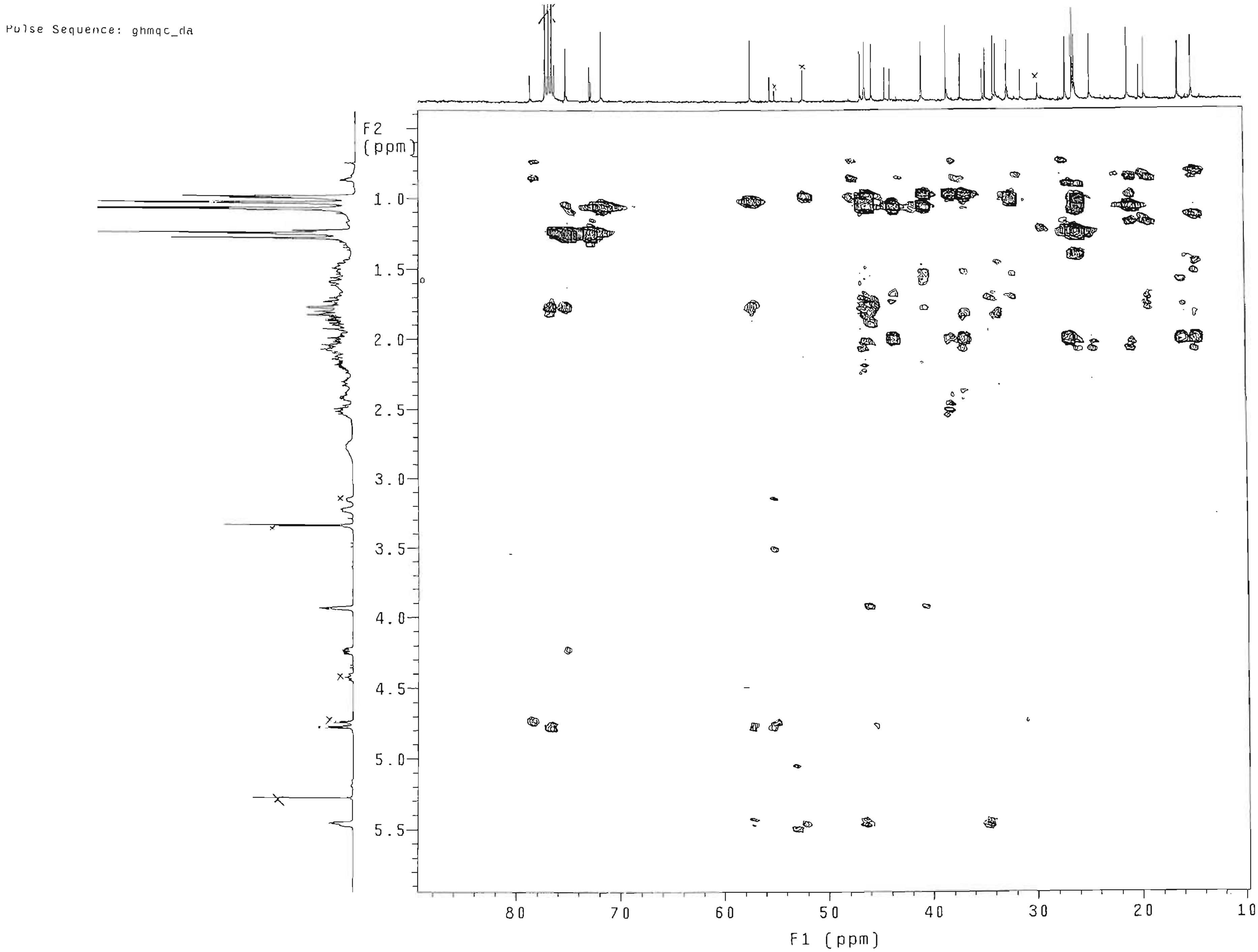
Pulse Sequence: ghsqc_da



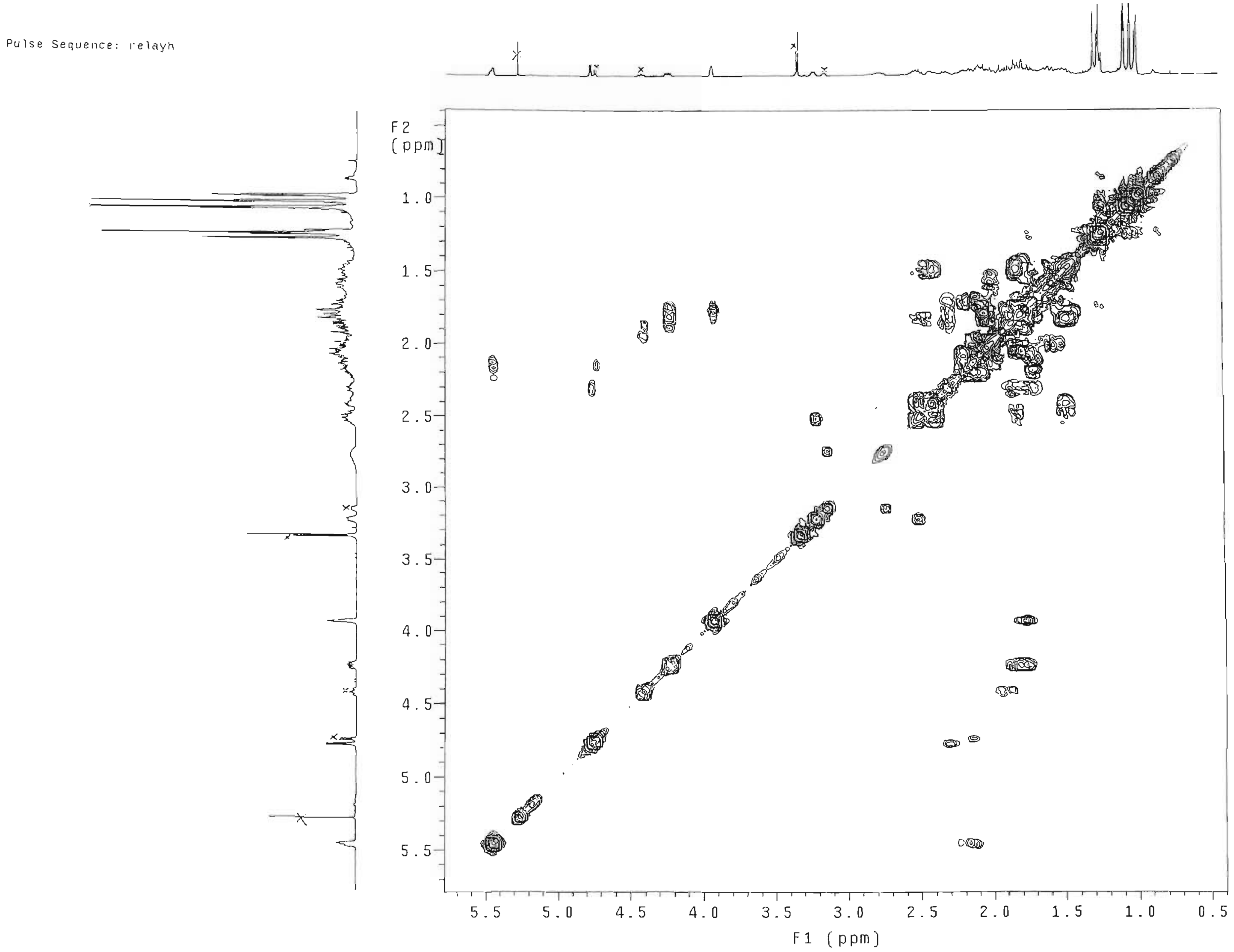
HSQC NMR Spectrum of



HMBC NMR Spectrum of

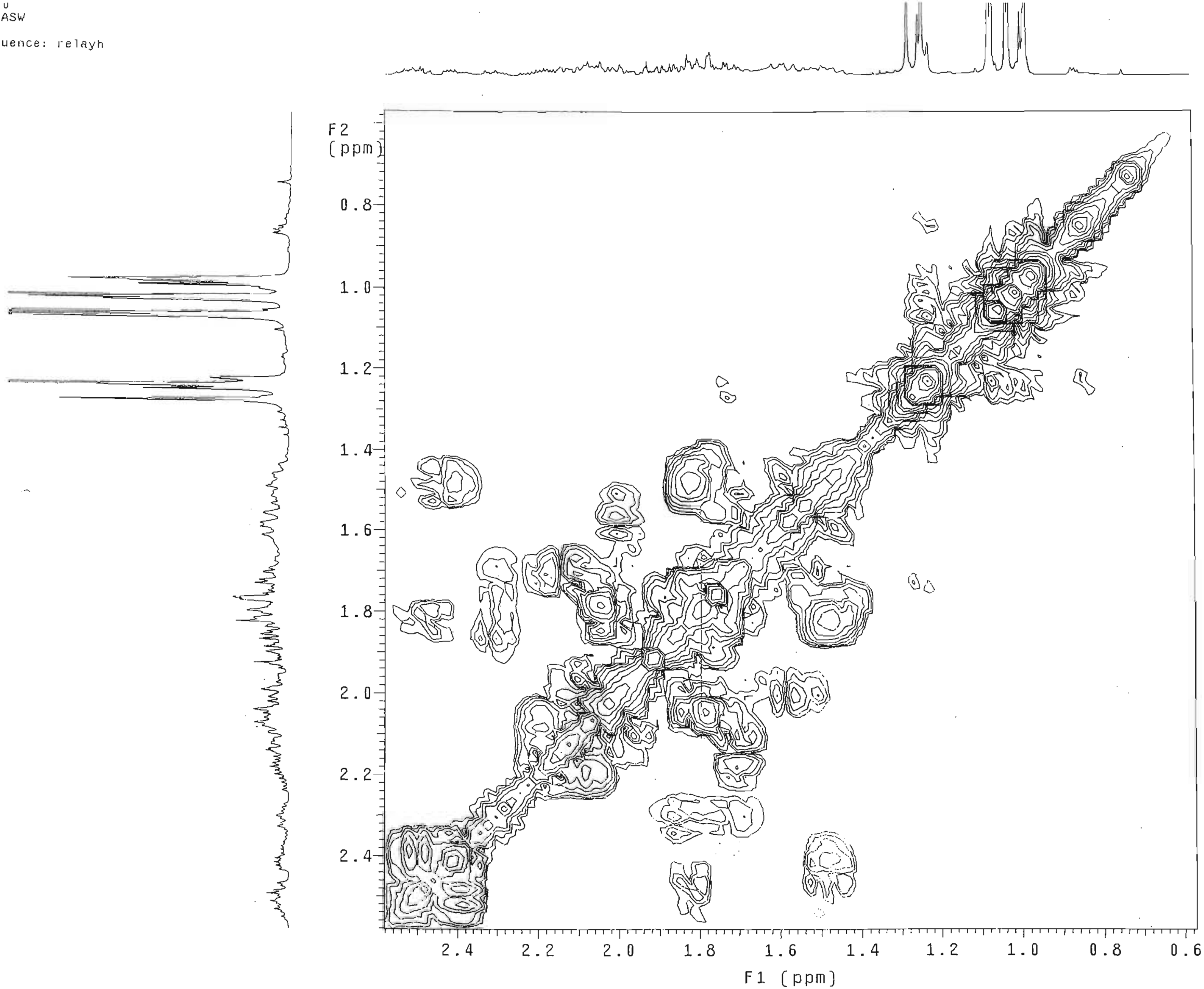


HMBC NMR Spectrum of


Pulse Sequence: relayh
F2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F1 (ppm)

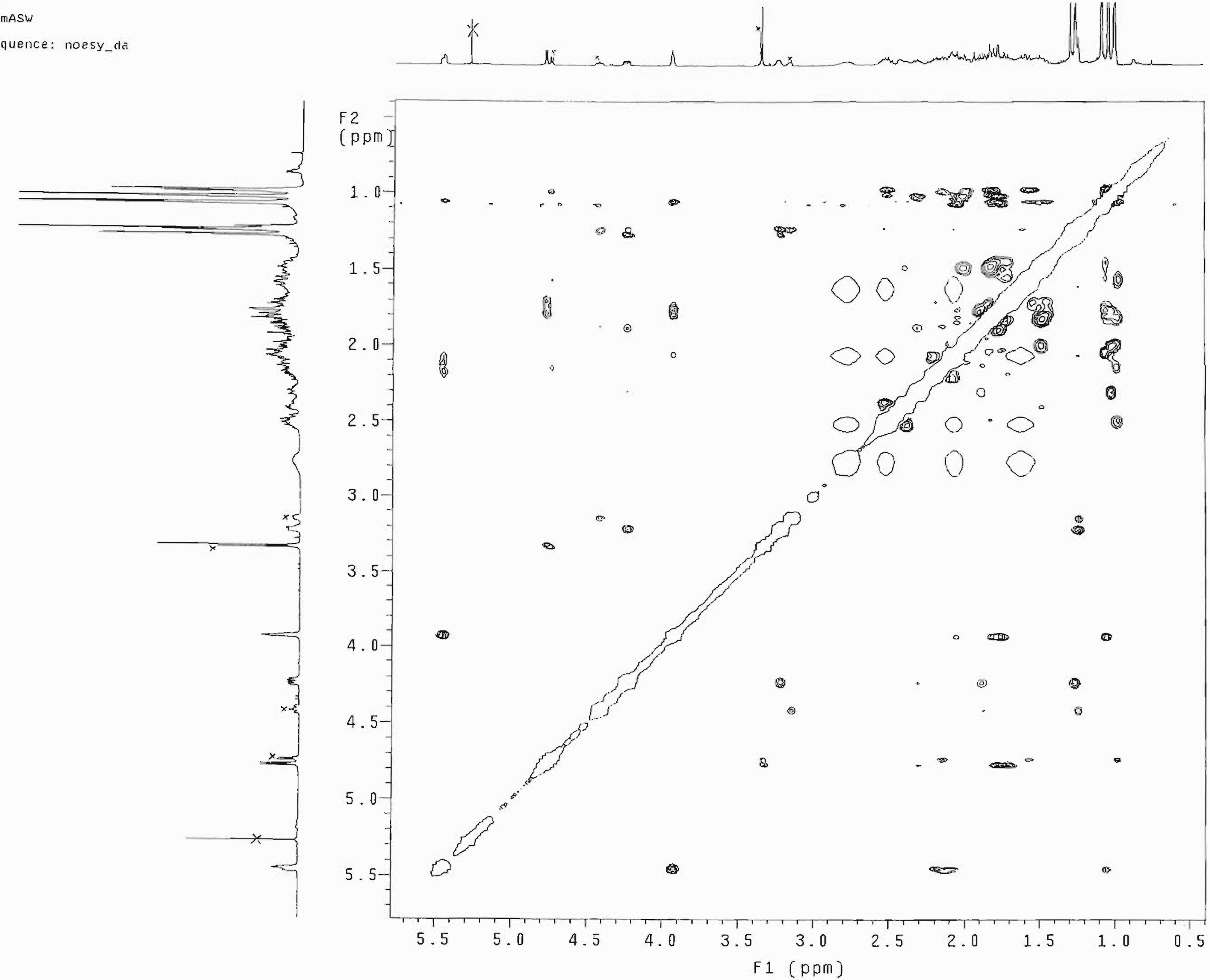
probe=5mmASW

Pulse Sequence: relayh



mix=1sec
probe=5mmASW

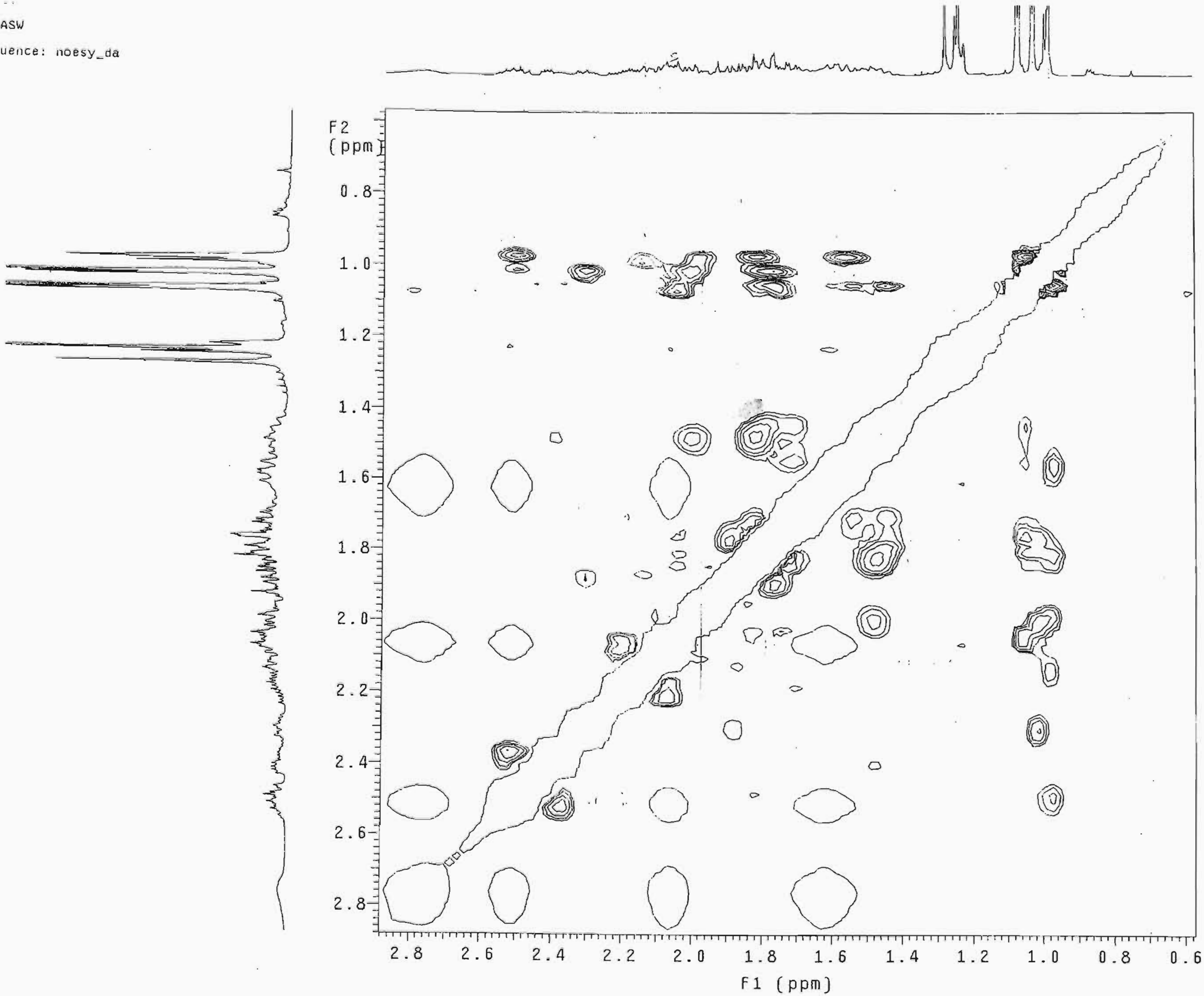
Pulse Sequence: noesy_da



NOESY NMR Spectrum of Protoasmaderine H (xvii)

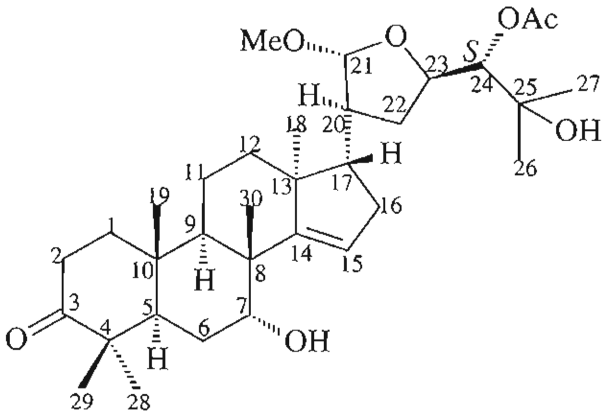
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da

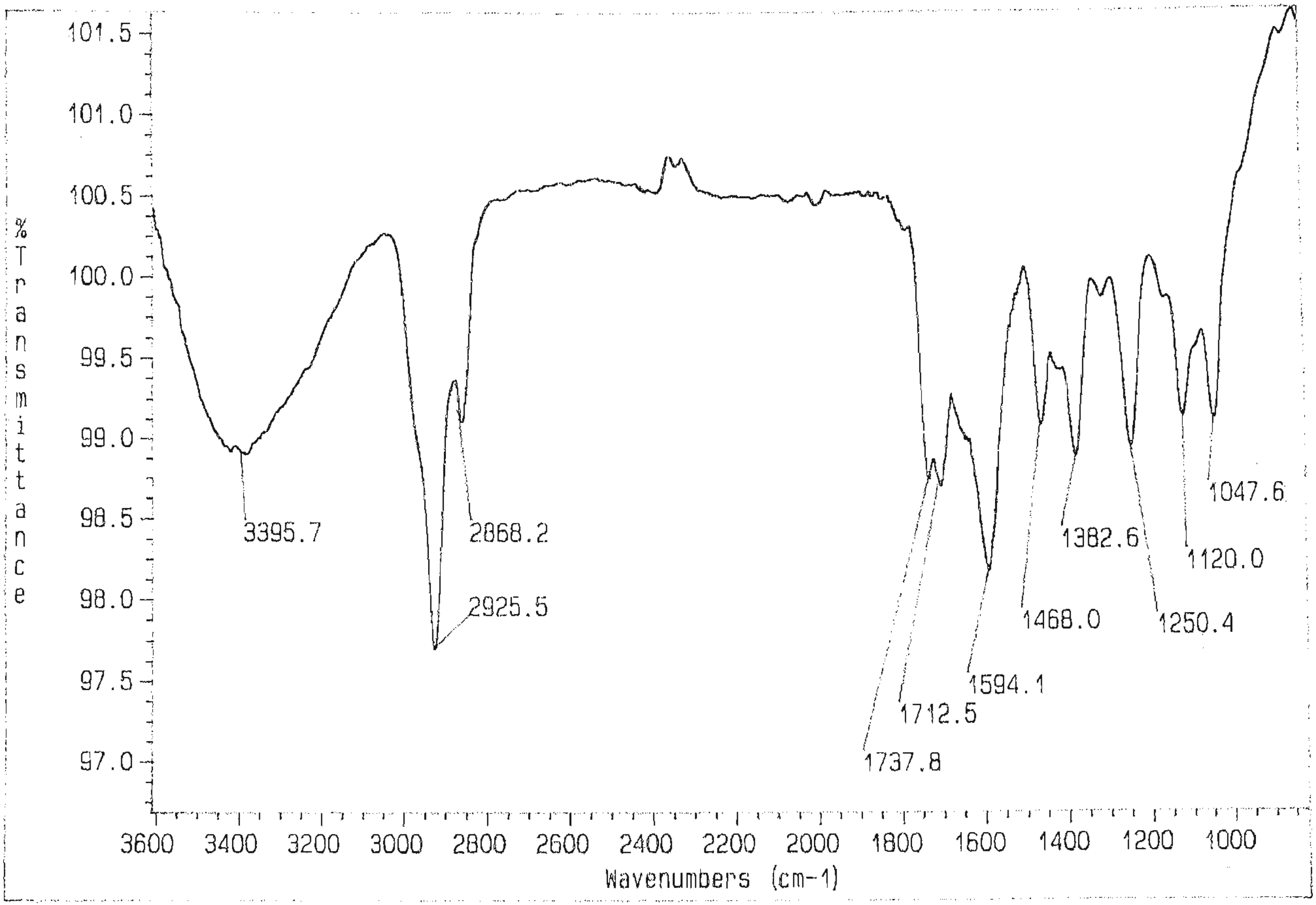


NOESY NMR Spectrum of Protoescamoderine H (xvii)

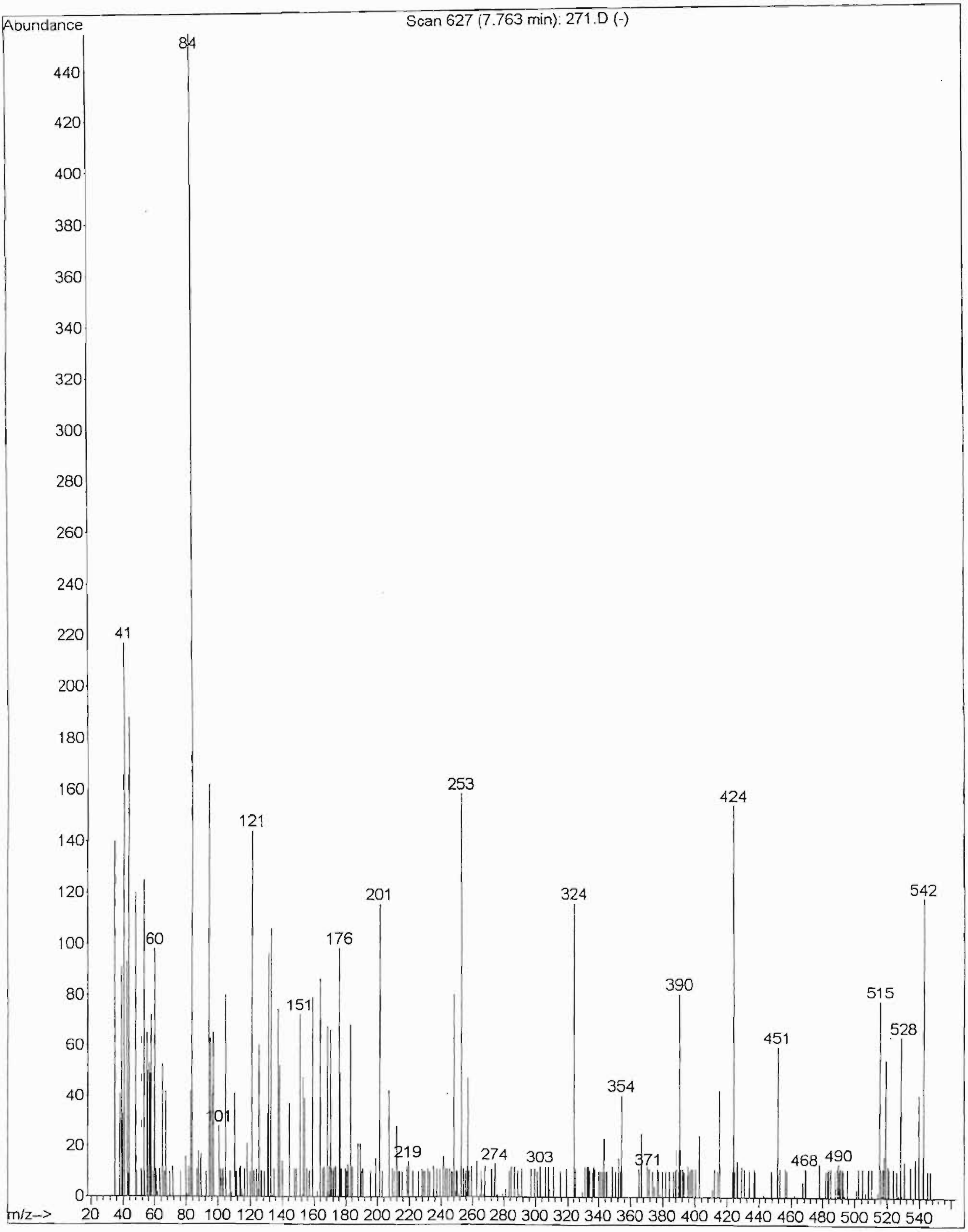
Protosamaderine I (xviii)



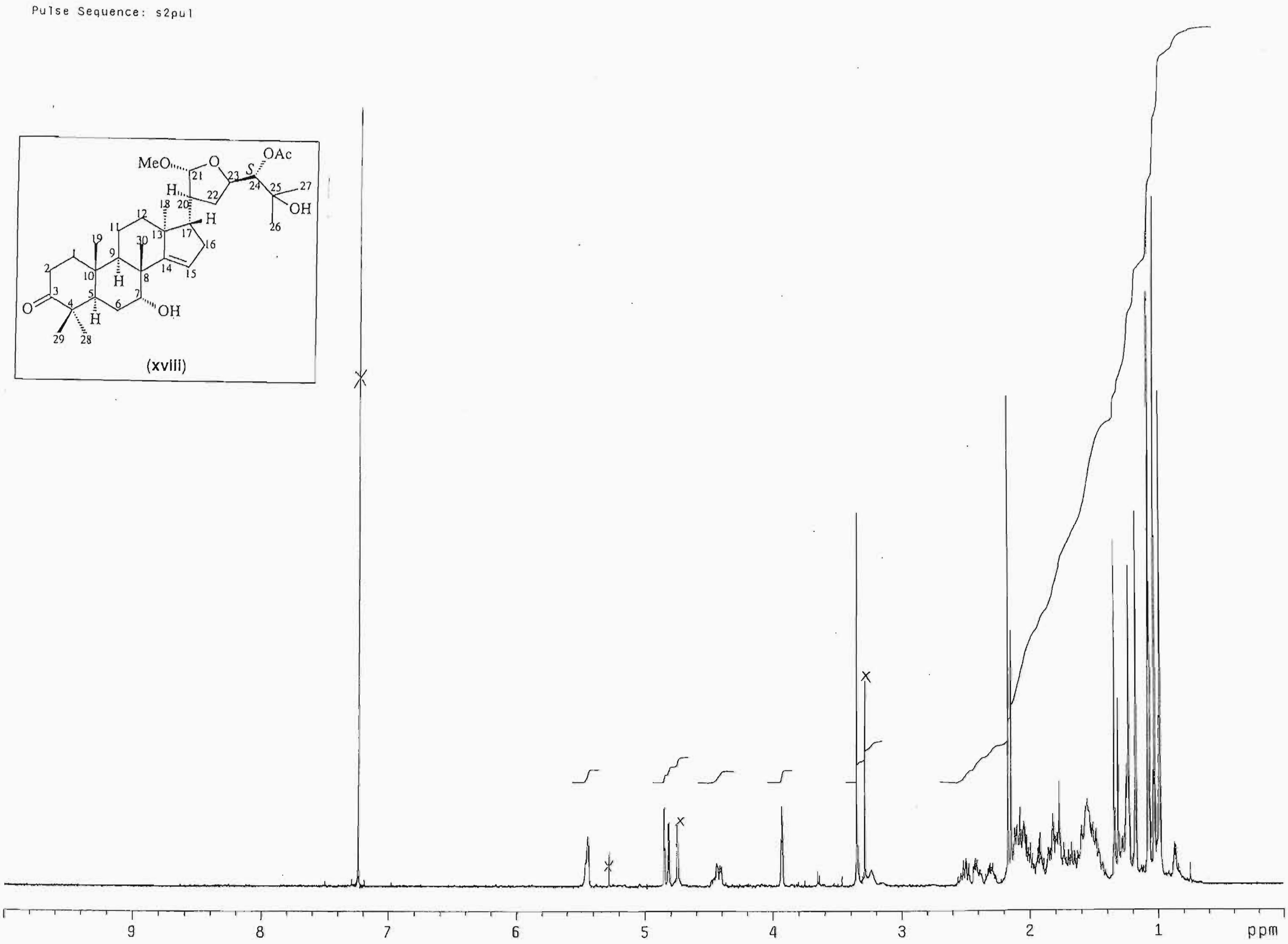
(xviii)



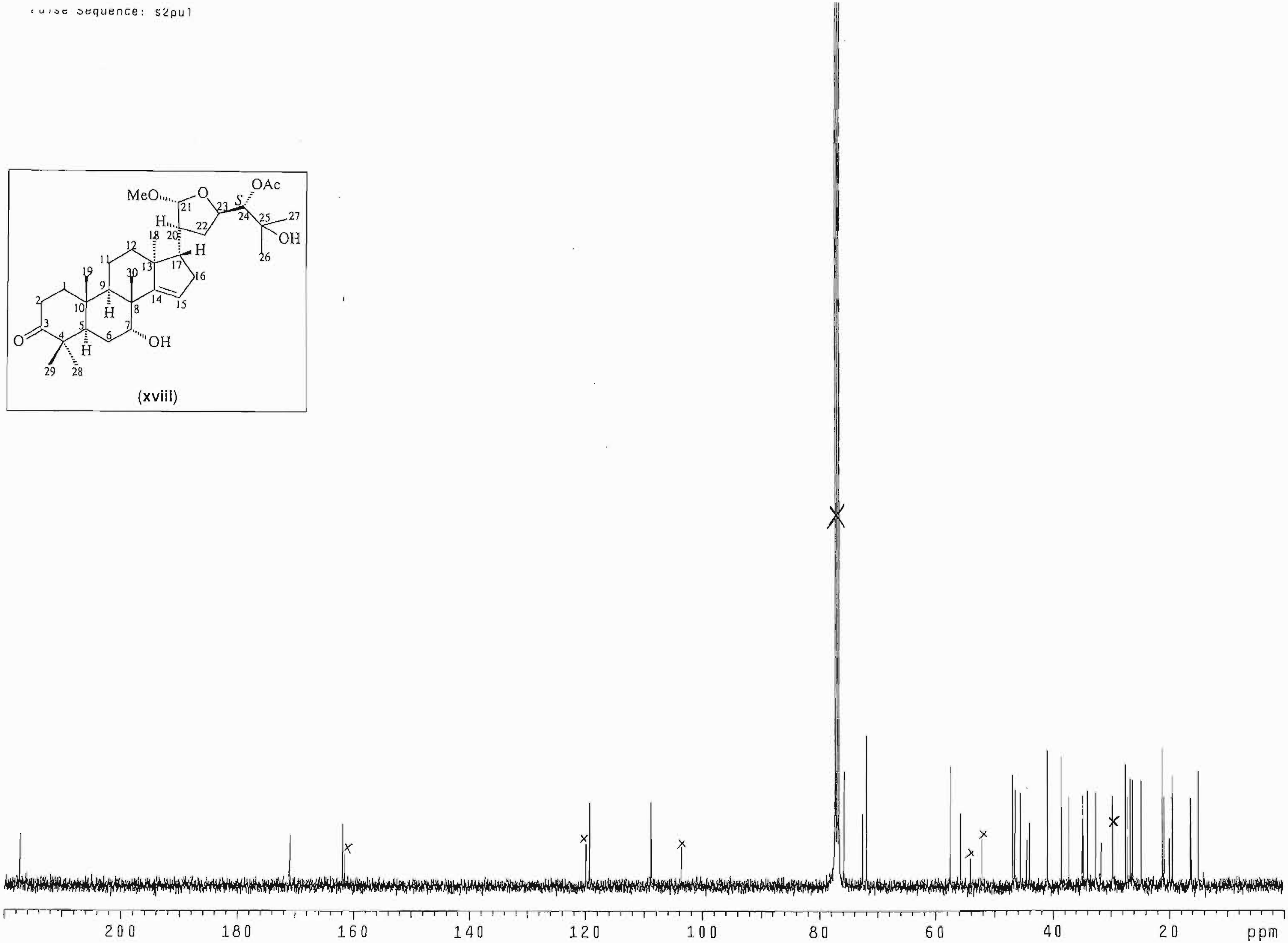
IR Spectrum of Protosamaderine I (xviii)



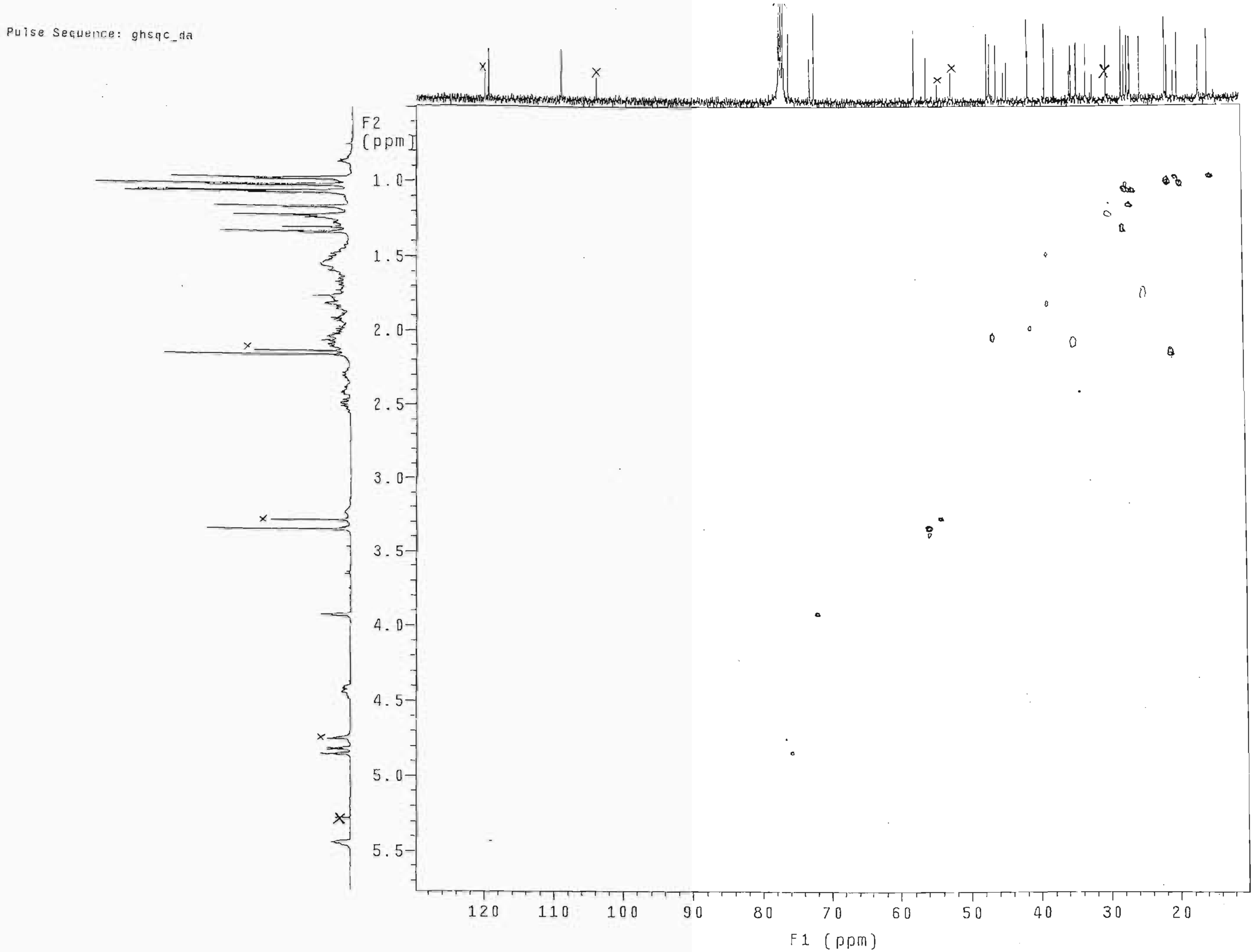
Mass Spectrum of Protosamaderine I (xviii)



^{1}H NMR Spectrum of

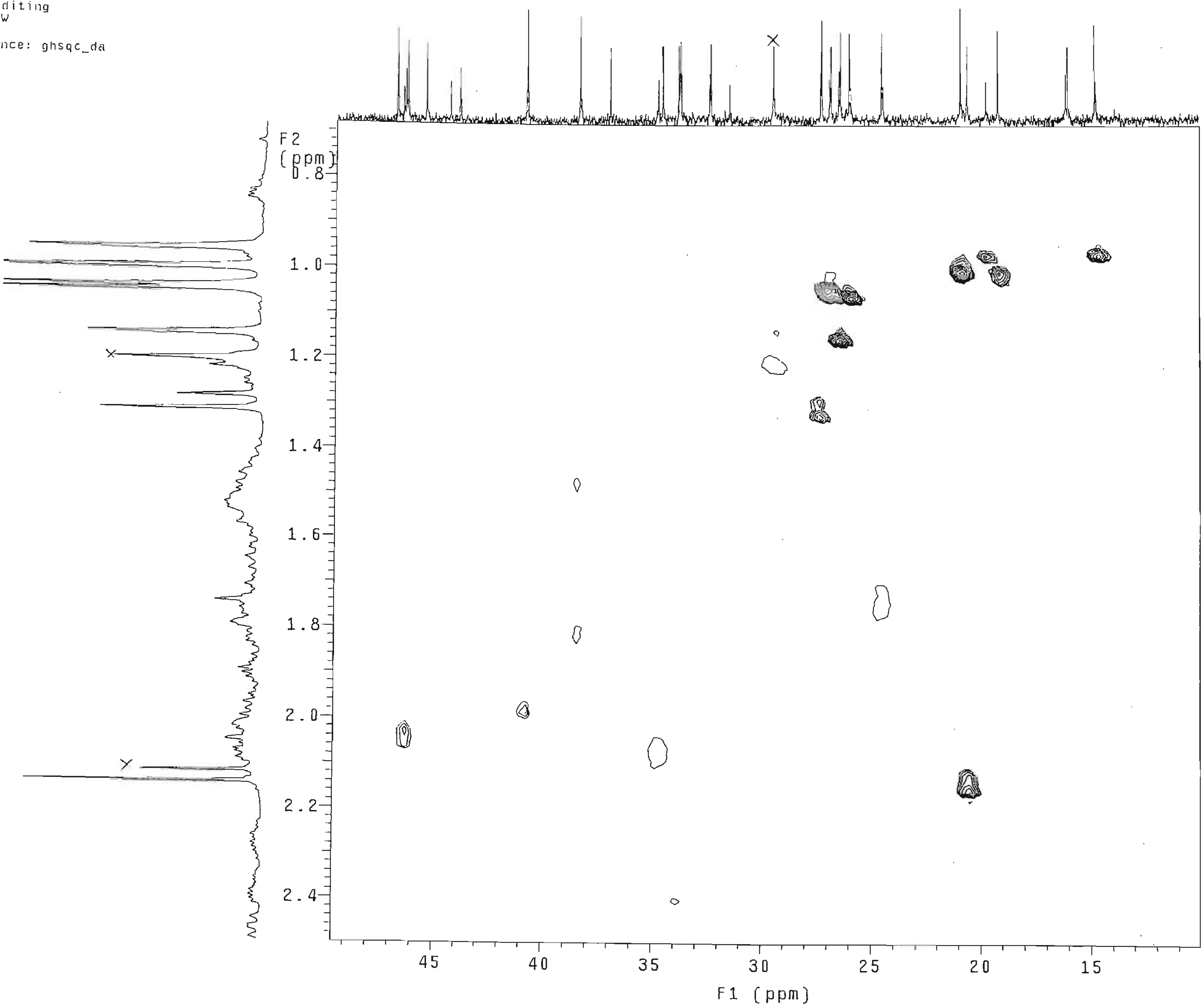


^{13}C NMR Spectrum of Protosamadarine I (xviii)


Pulse Sequence: ghsqc_da
F2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
120
110
100
90
80
70
60
50
40
30
20
F1 (ppm)

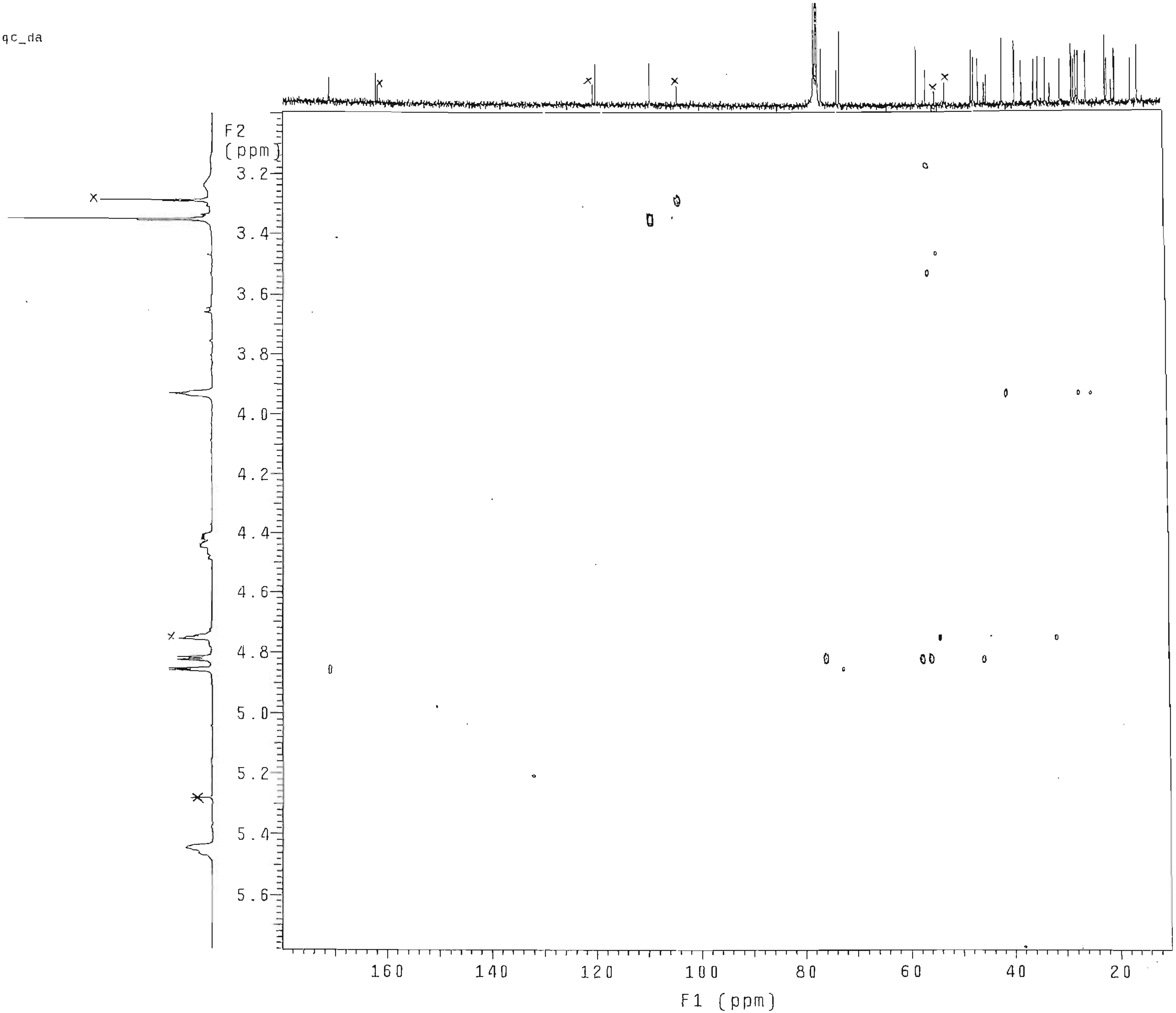
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da

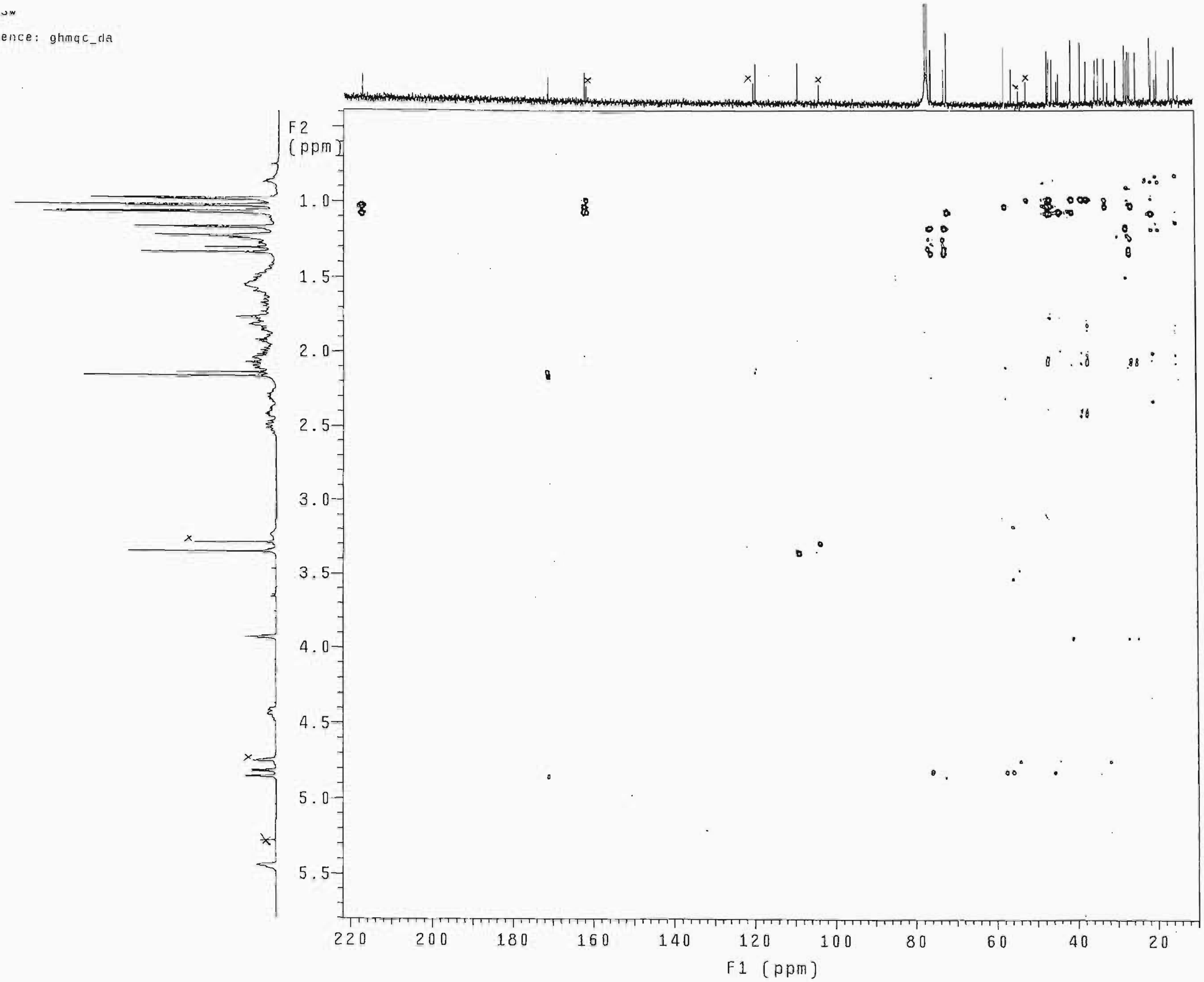


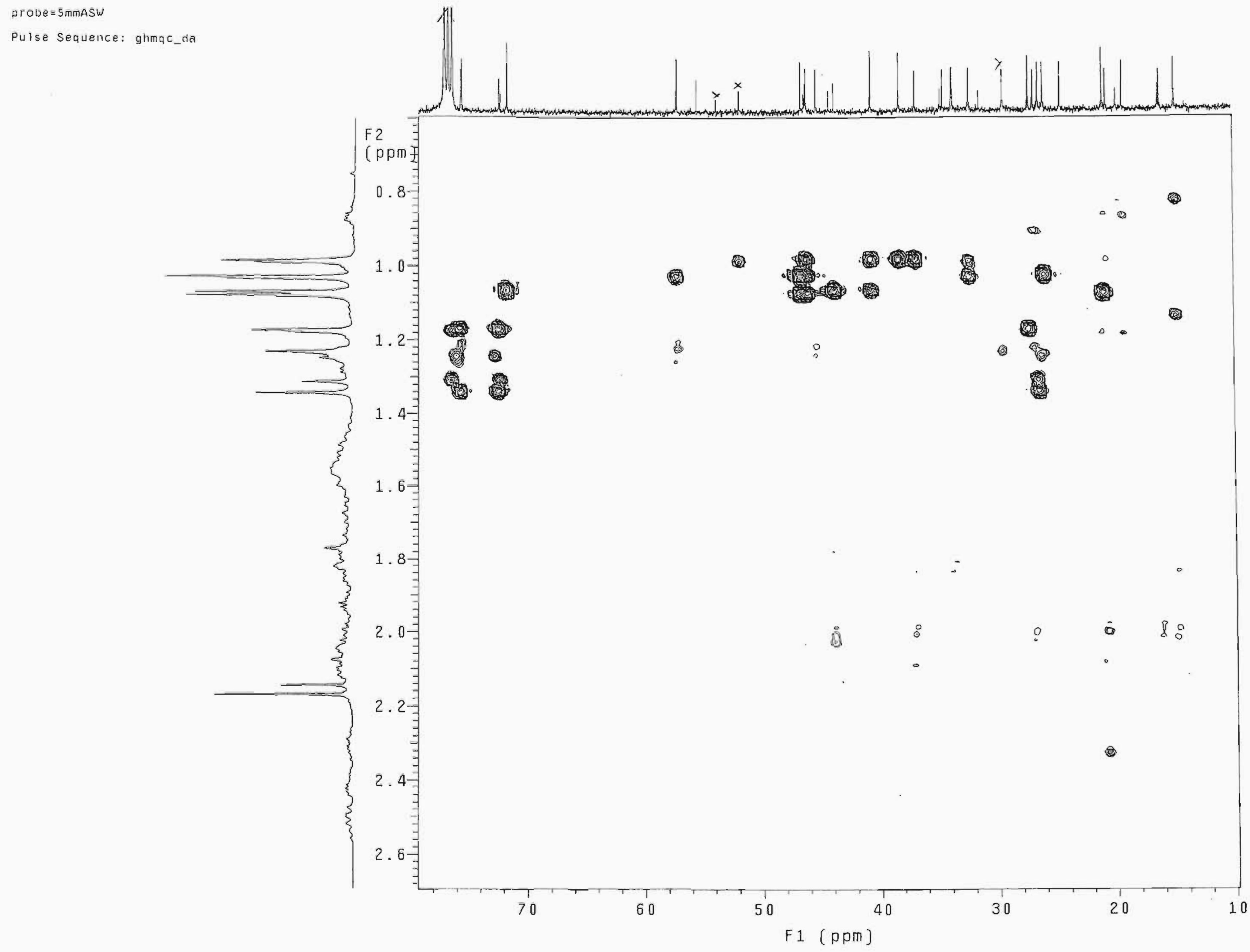
HSQC NMR Spectrum of

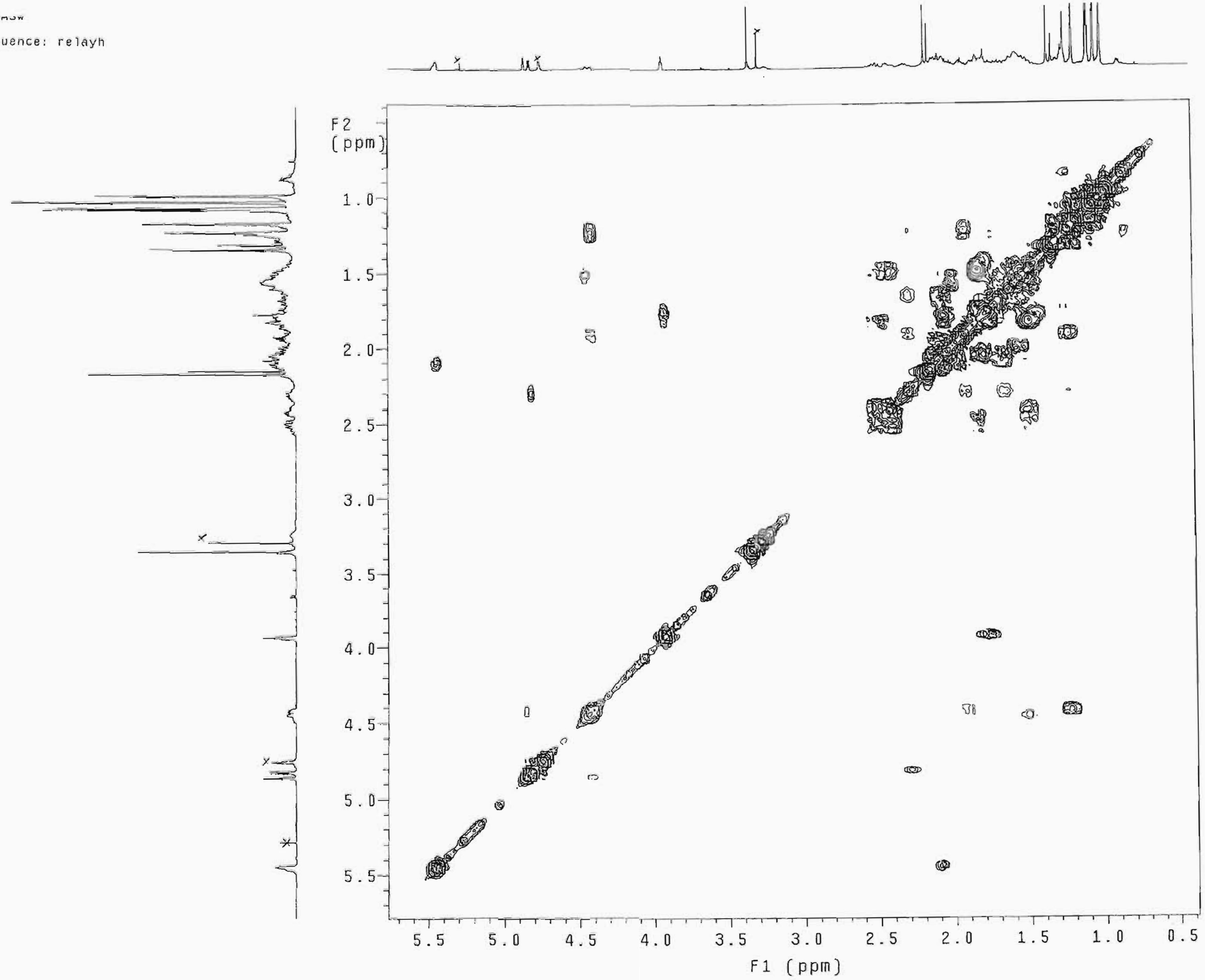
Pulse Sequence: ghmqc_da

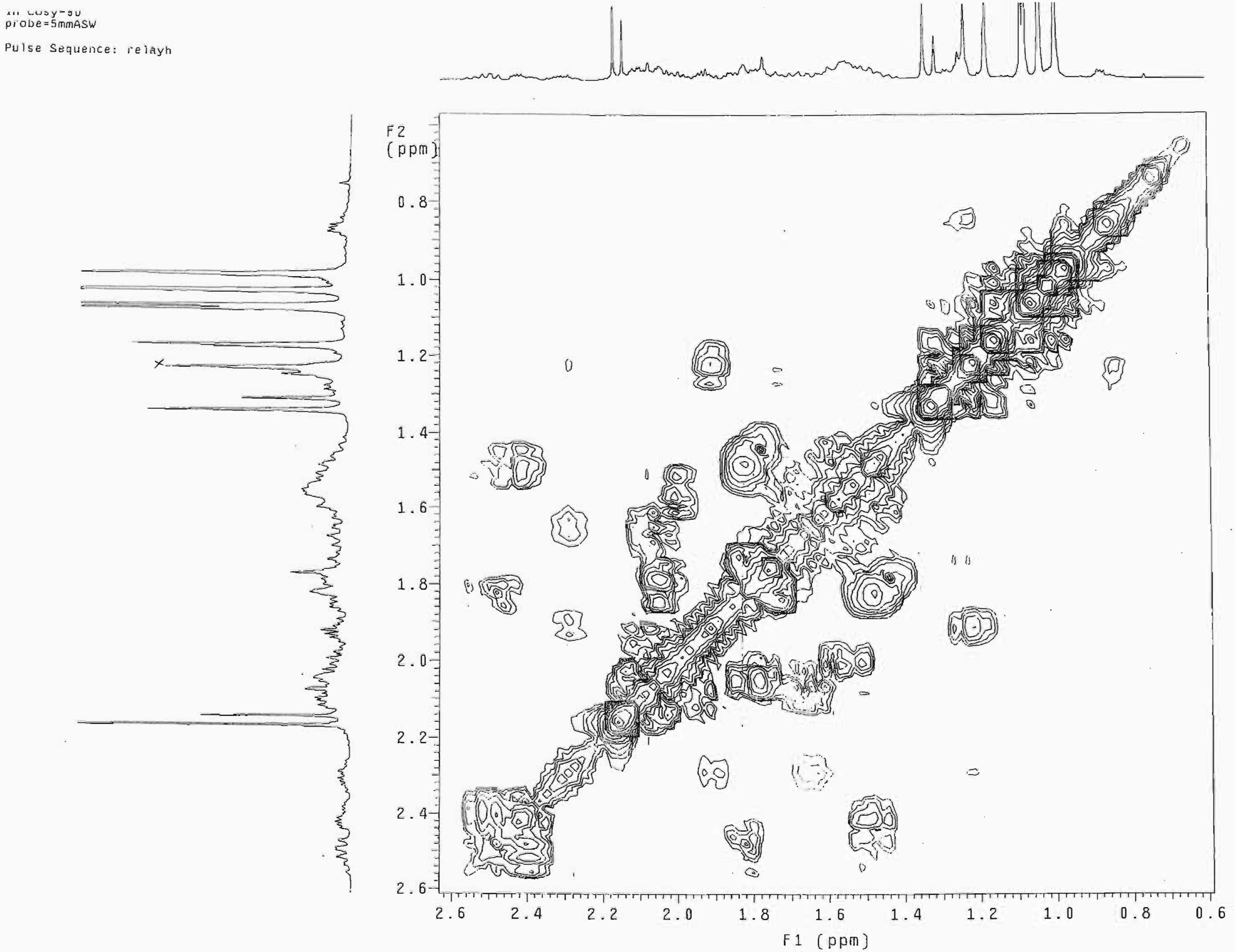


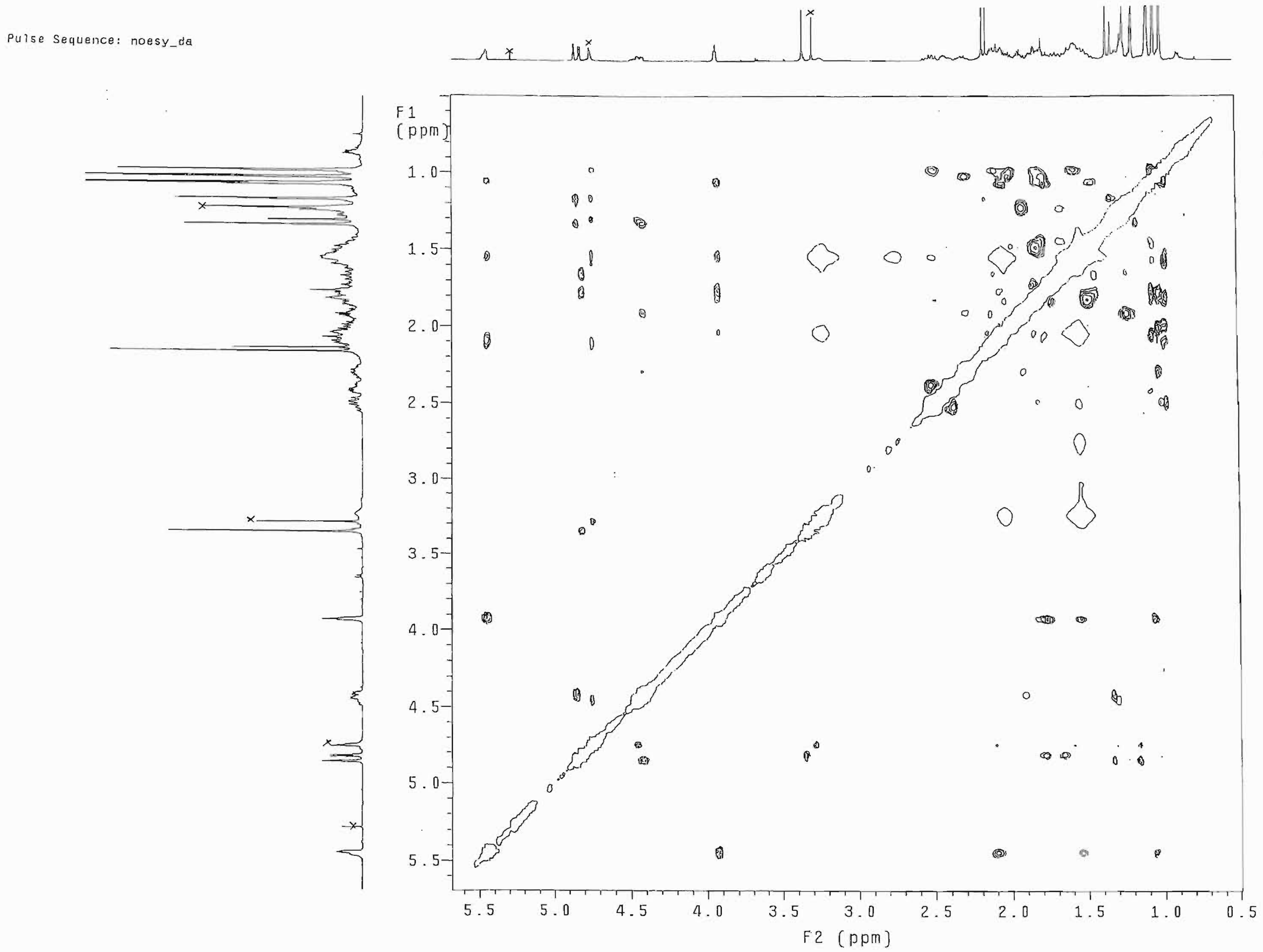
HMBC NMR Spectrum of [illegible]

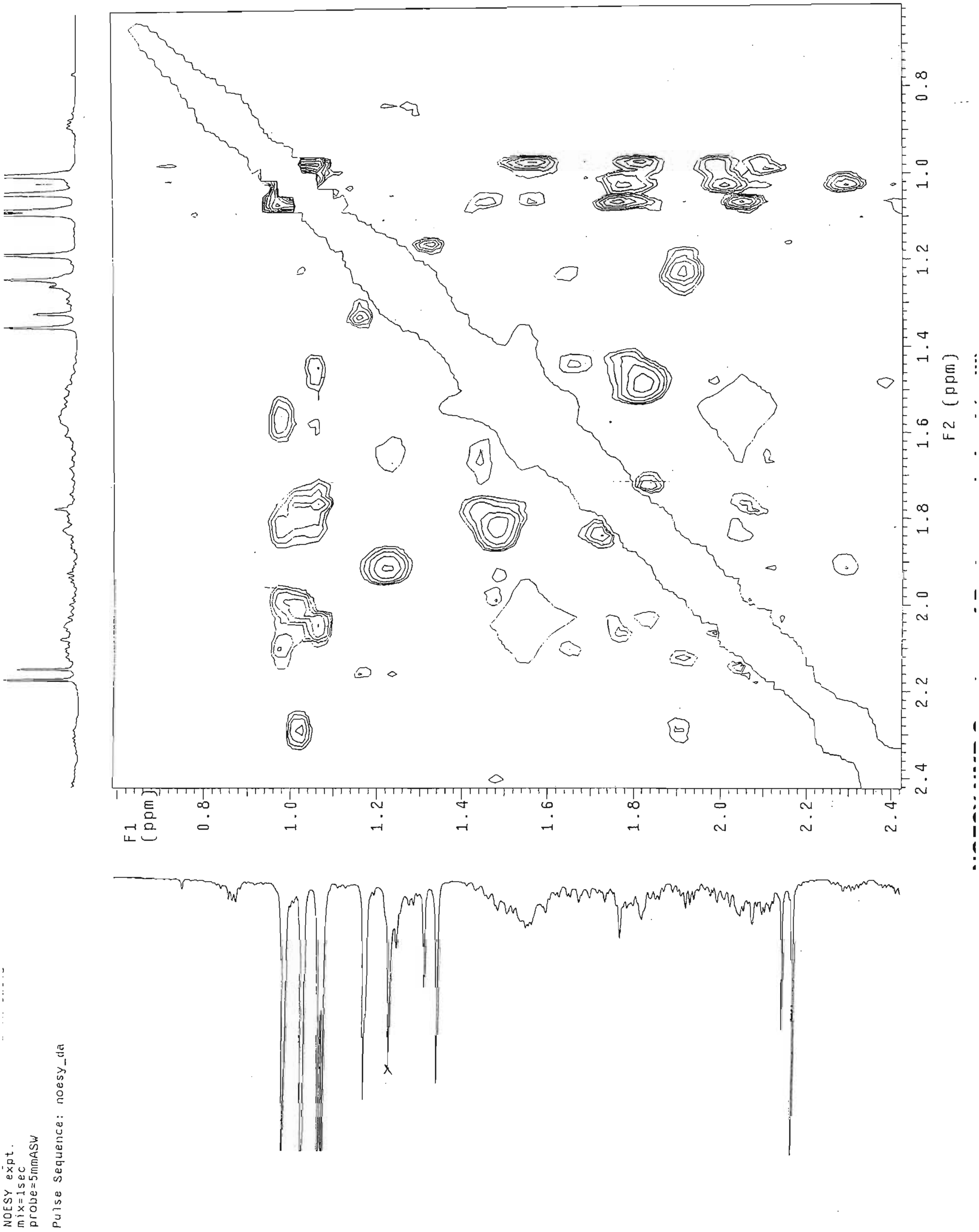

Pulse Sequence: ghmqc_da
F2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
220
200
180
160
140
120
100
80
60
40
20
F1 (ppm)


probe=5mmASW
Pulse Sequence: ghmqc_da
F2 (ppm)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
70
60
50
40
30
20
10
F1 (ppm)


Pulse Sequence: relayh
F2
(ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F1 (ppm)


probe=5mmASW
Pulse Sequence: relayh
F2 (ppm)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
F1 (ppm)

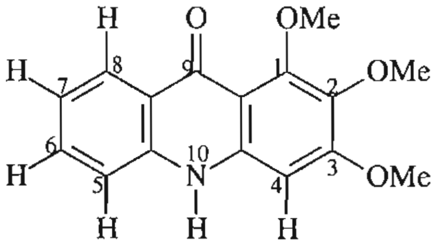

Pulse Sequence: noesy_da
F1 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
F2 (ppm)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5


NOESY expt.
mix=1sec
probe=5mmASW
Pulse Sequence: noesy_da
F1 (ppm)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
F2 (ppm)
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4

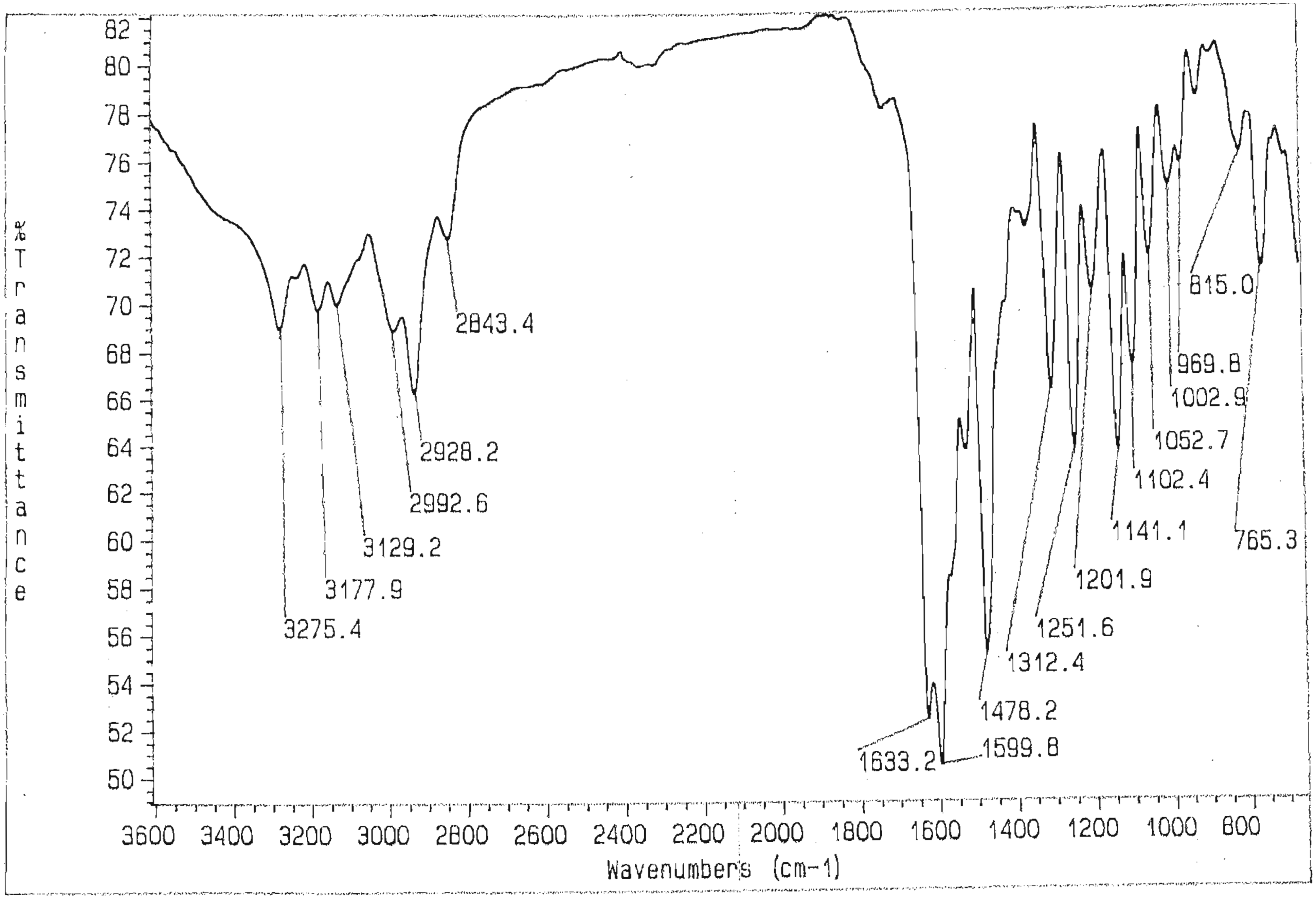
Spectra of Compounds from *Toddaliopsis bremekampii* leaves & branches

Photograph by Dr. Neil Crouch

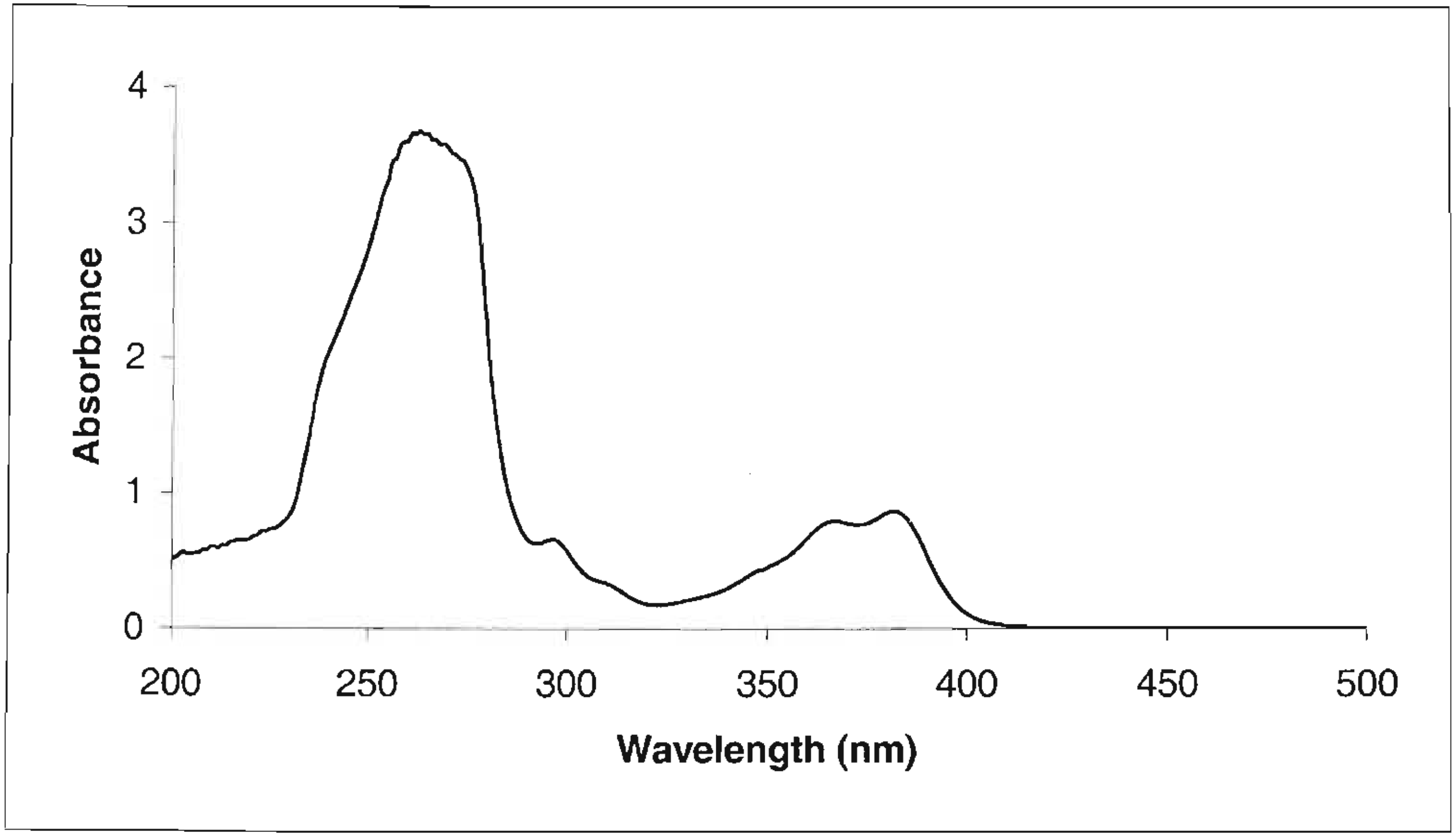
1,2,3-Trihydroxyacridone (i)



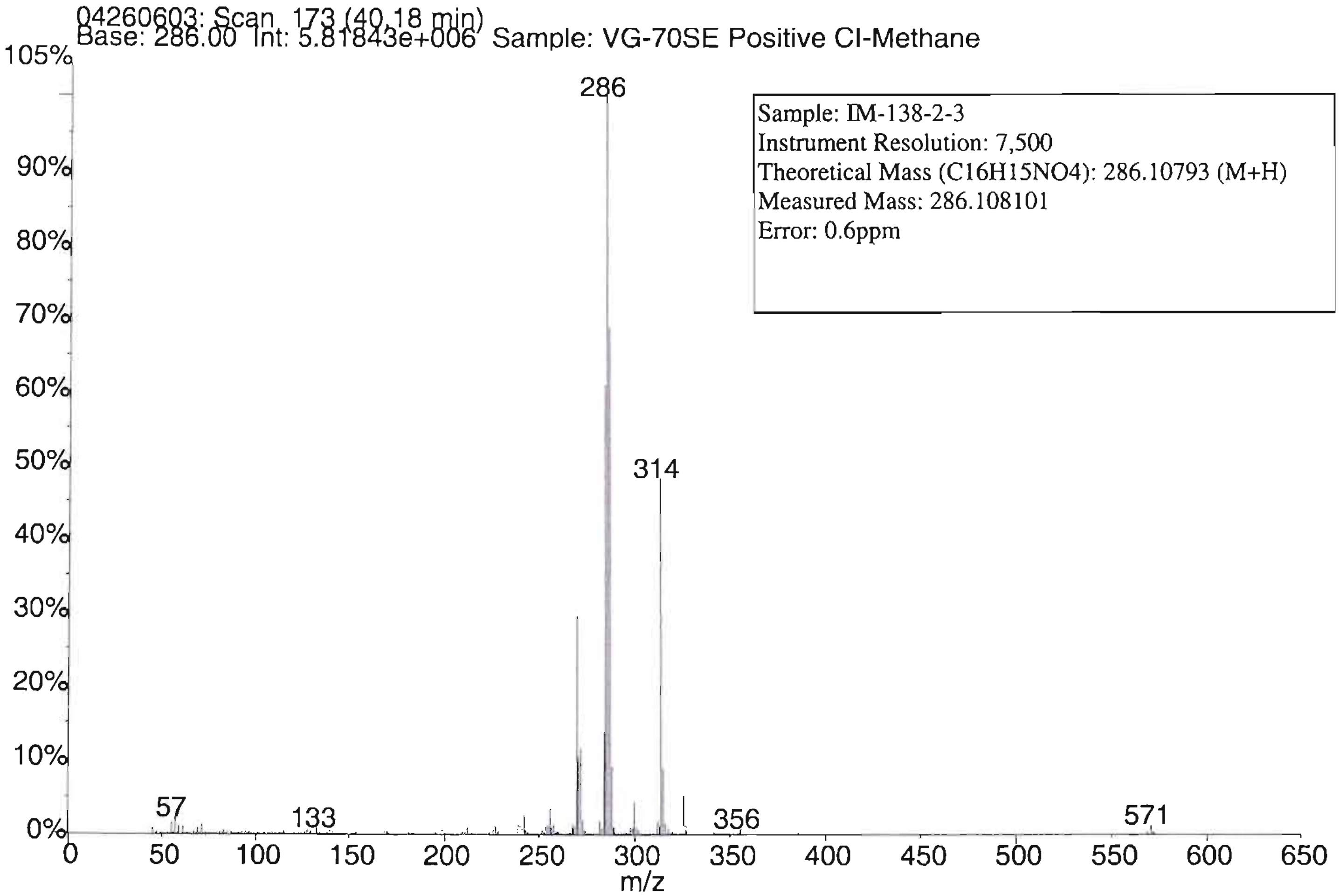
(i)



IR Spectrum of 1,2,3-Trimethoxyacridone (i)



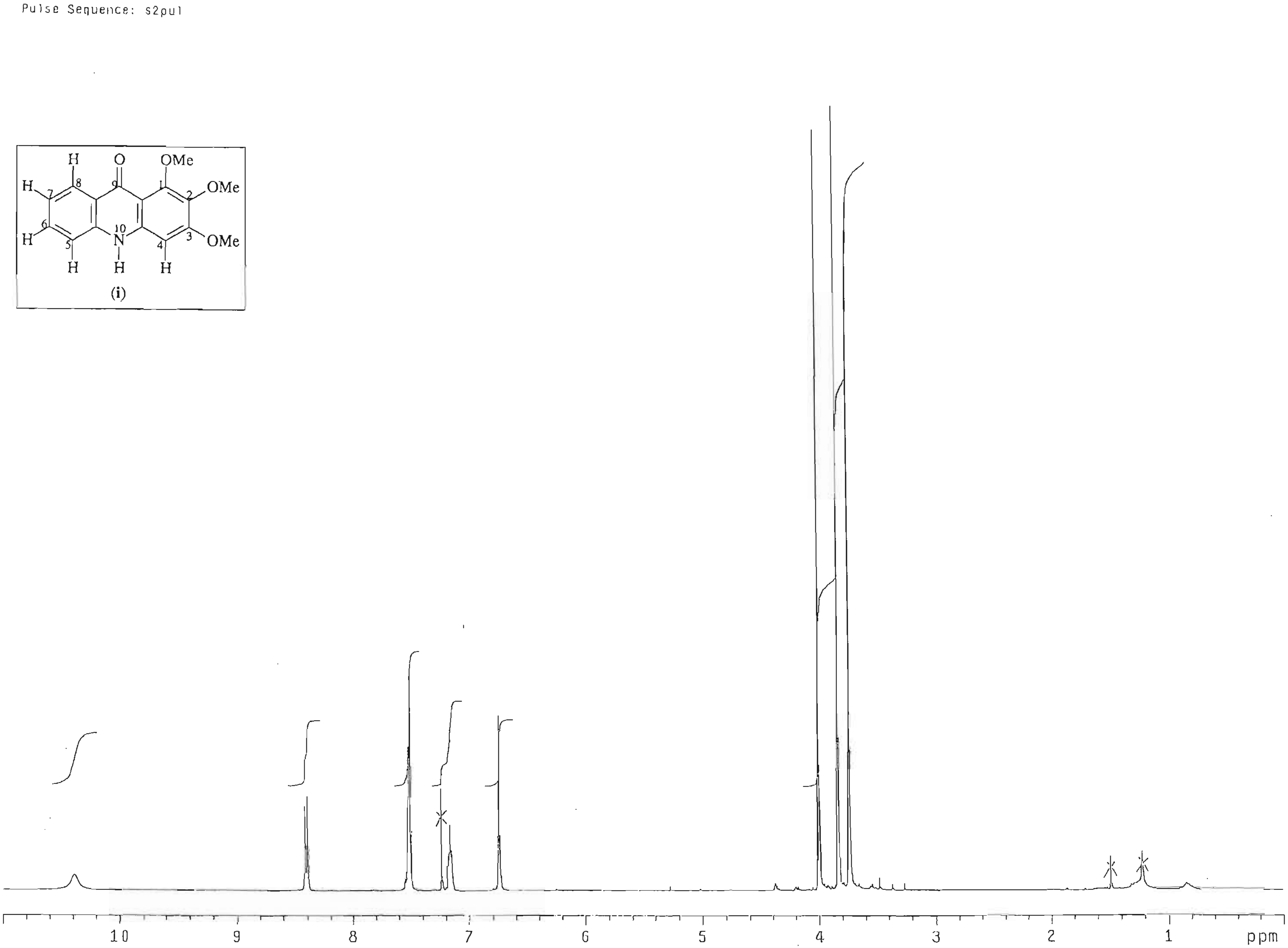
UV Spectrum of 1,2,3-Trimethoxyacridone (i)



04260603: Scan 173 (40.18 min)
Base: 286.00 Int: 5.81843e+006 Sample: VG-70SE Positive CI-Methane

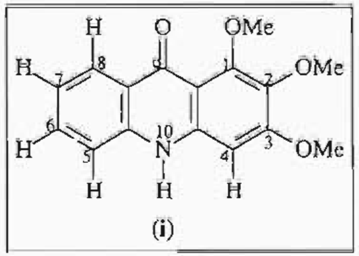
Sample: IM-138-2-3
Instrument Resolution: 7,500
Theoretical Mass (C16H15NO4): 286.10793 (M+H)
Measured Mass: 286.108101
Error: 0.6ppm

Mass Spectrum of 1,2,3-Trimethoxyacridone (i)

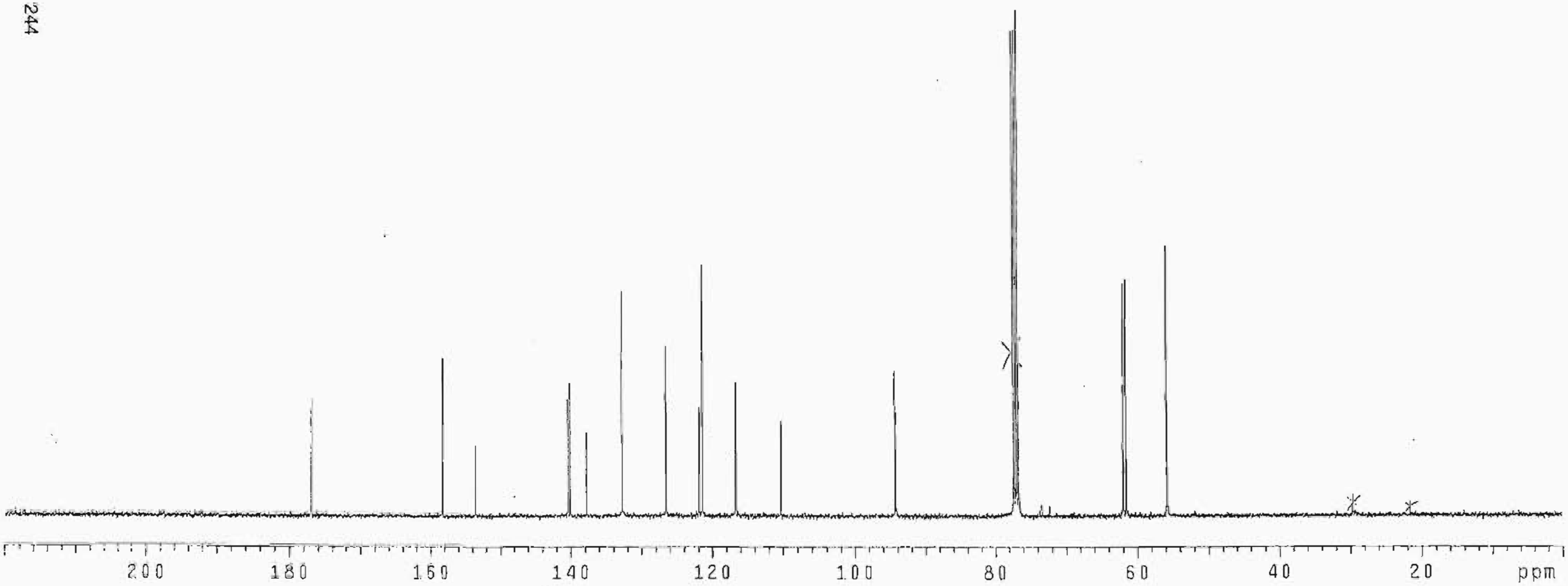


^{1}H NMR Spectrum of 1,2,3-Trimethoxyacridone (i)

Pulse Sequence: s2pul



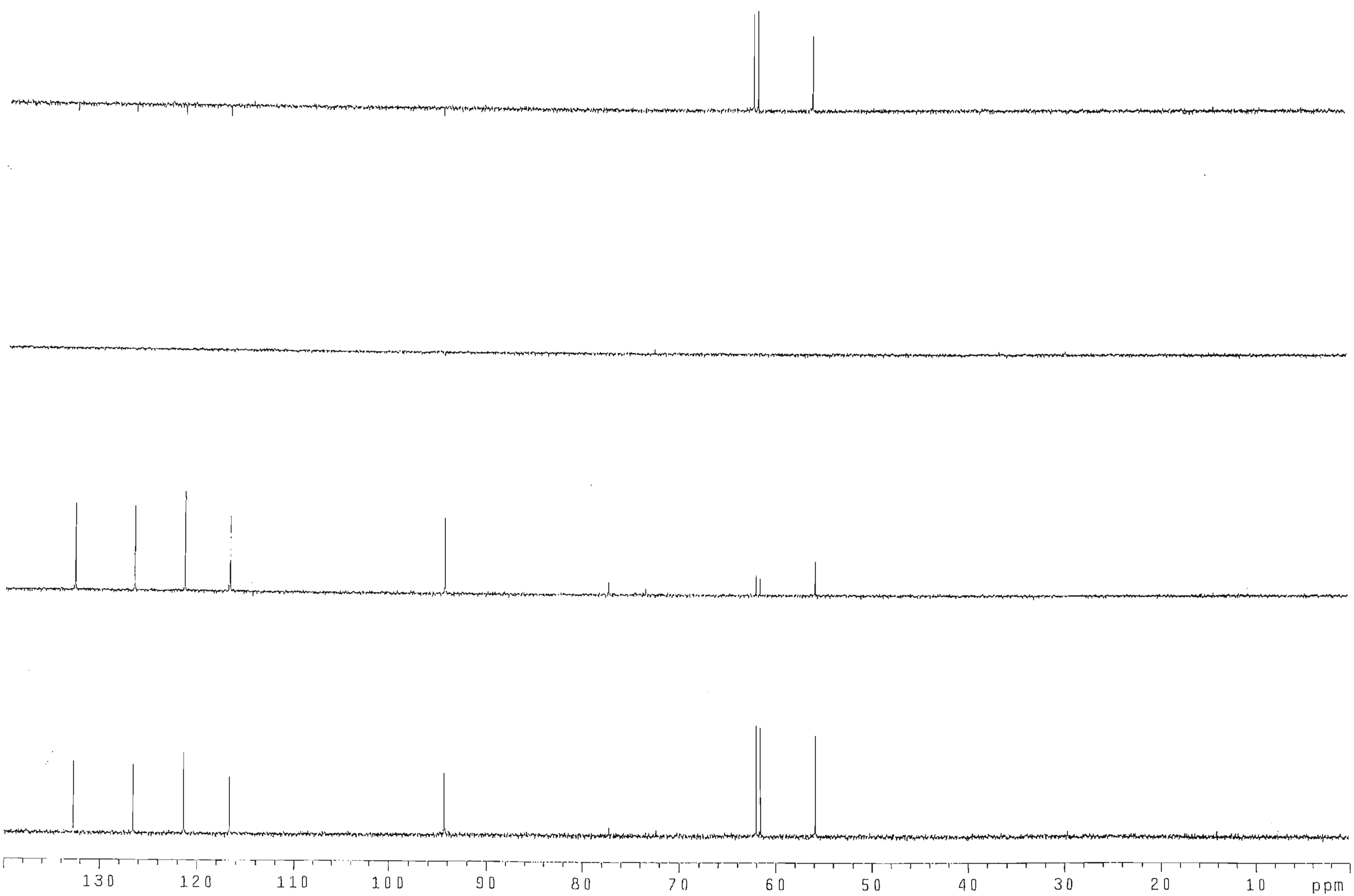
(i)



^{13}C NMR Spectrum of 1,2,3-Trimethoxyacridone (i)

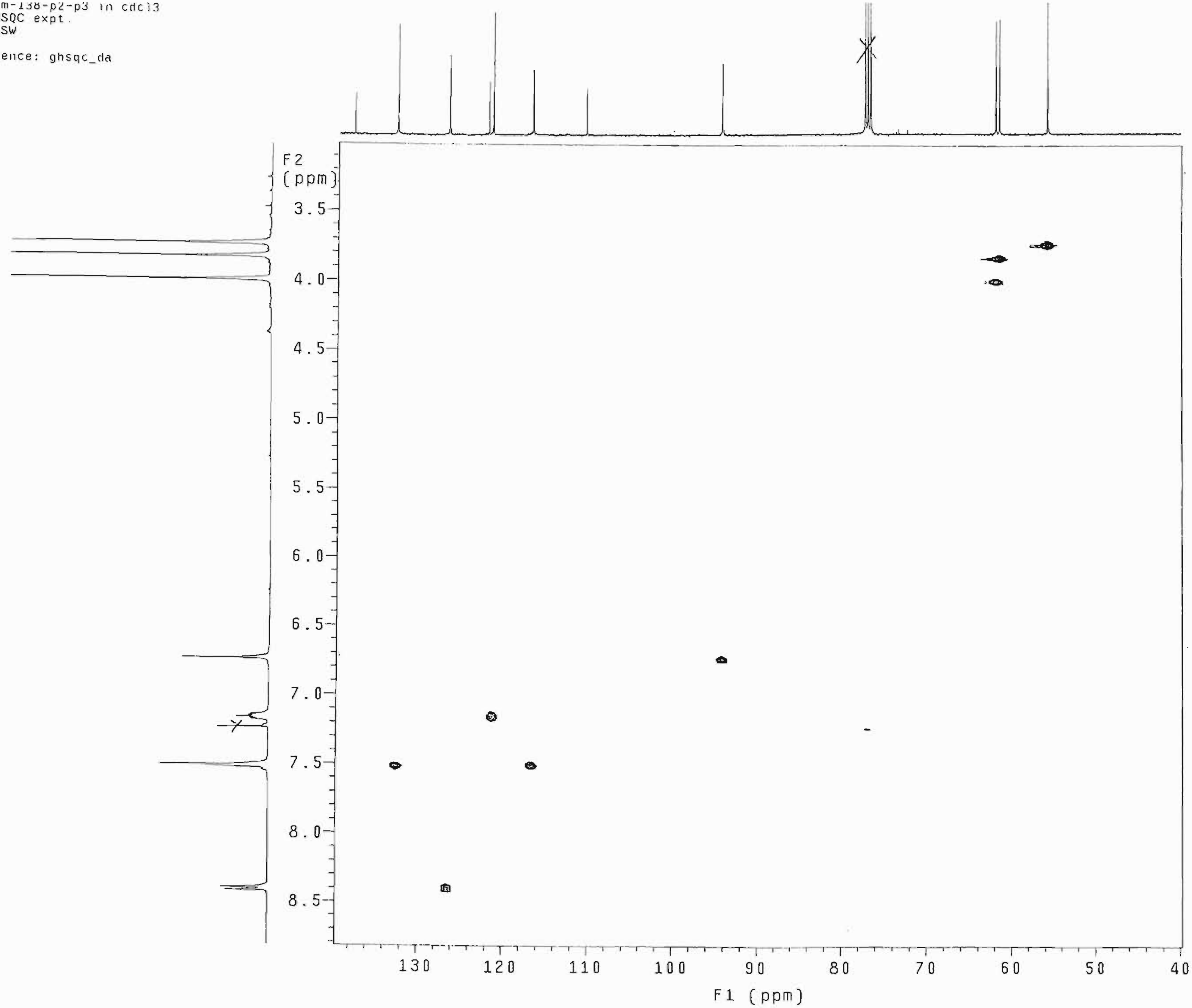
probe=5mmASW

Pulse Sequence: dept



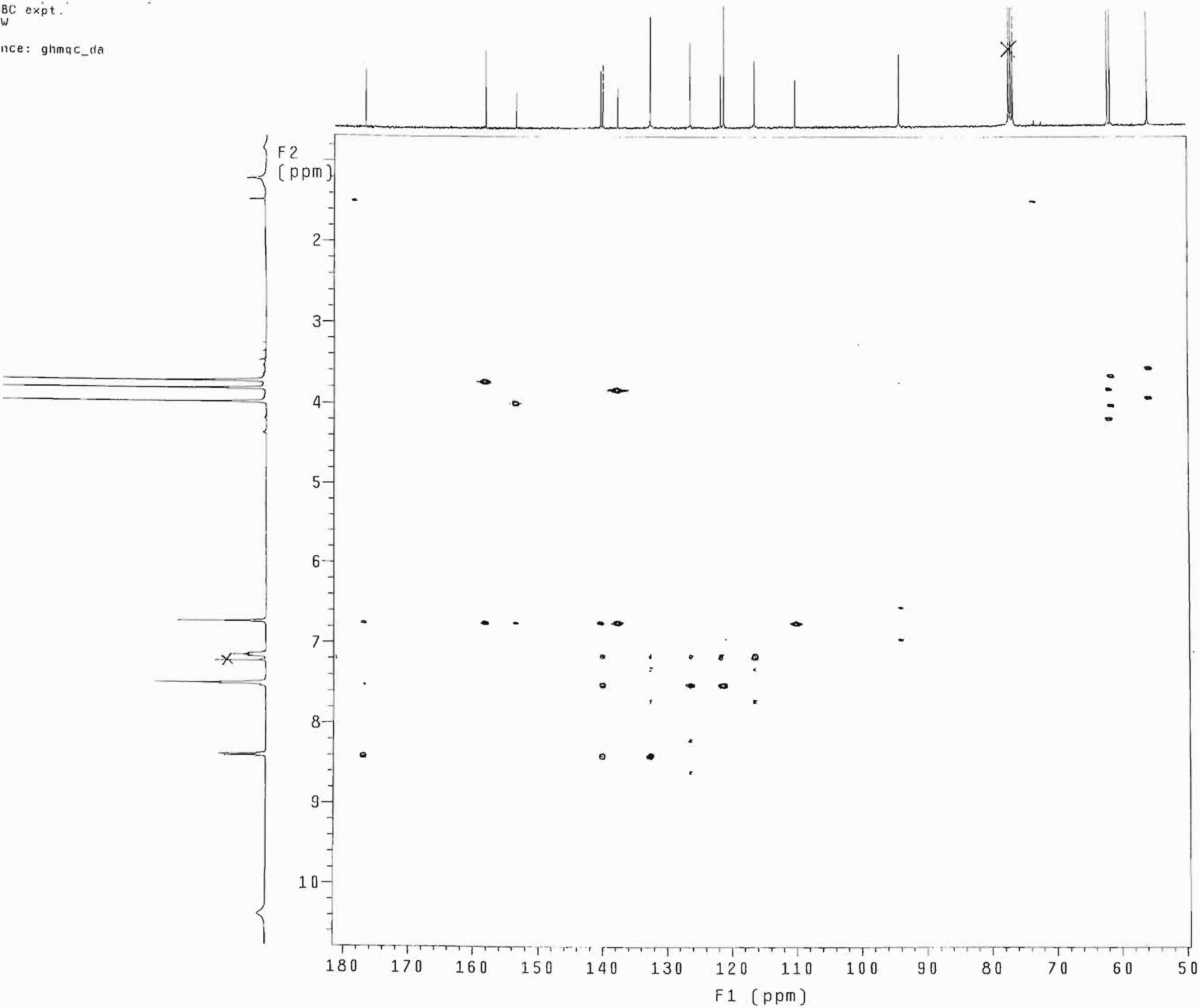
hqtop3.tbim-138-p2-p3 in cdcl3
Gradient HSQC expt.
probe=5mmASW

Pulse Sequence: ghsqc_da



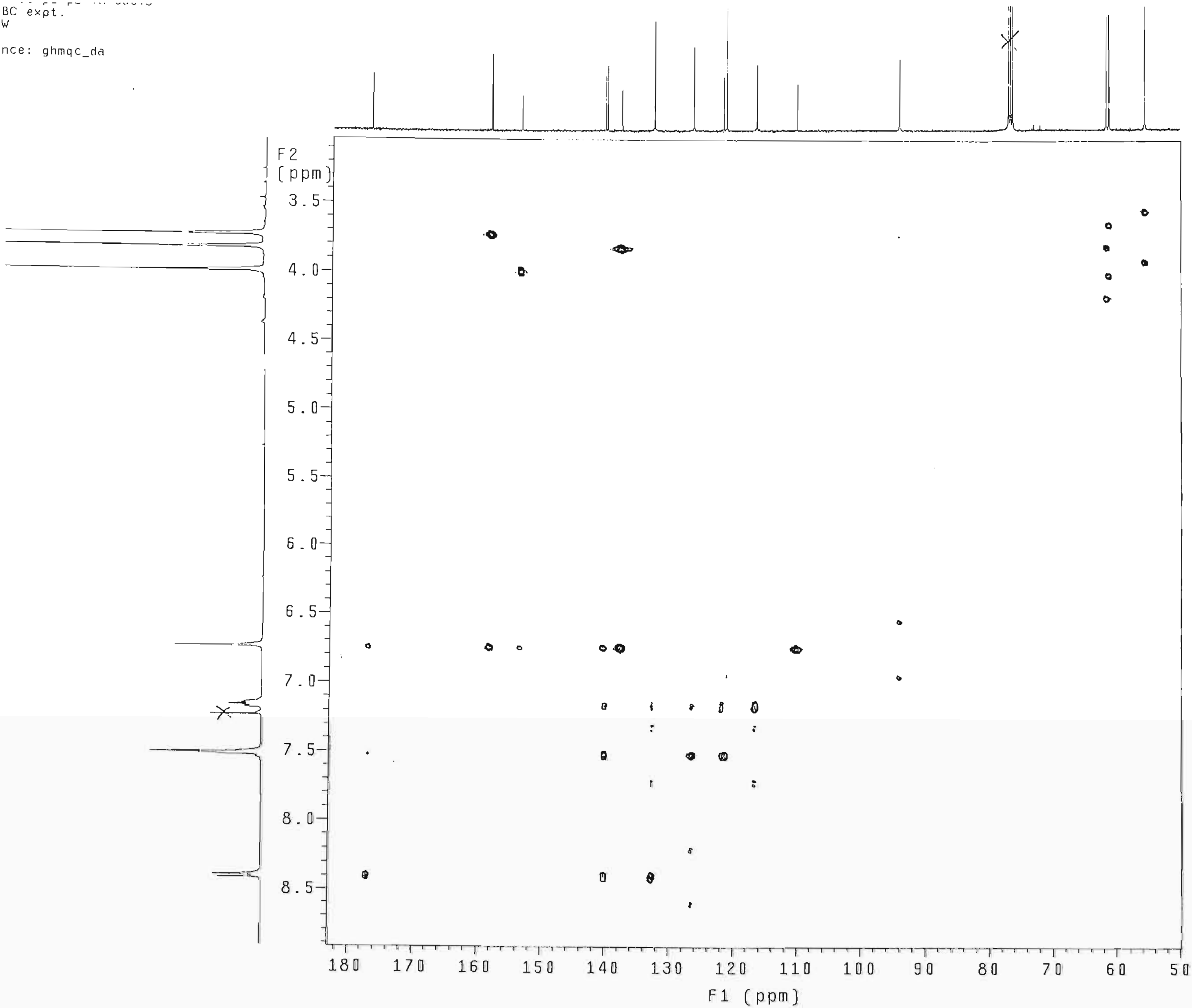
Gradient HMBC expt.
probe=5mmASW

Pulse Sequence: ghmqc_da



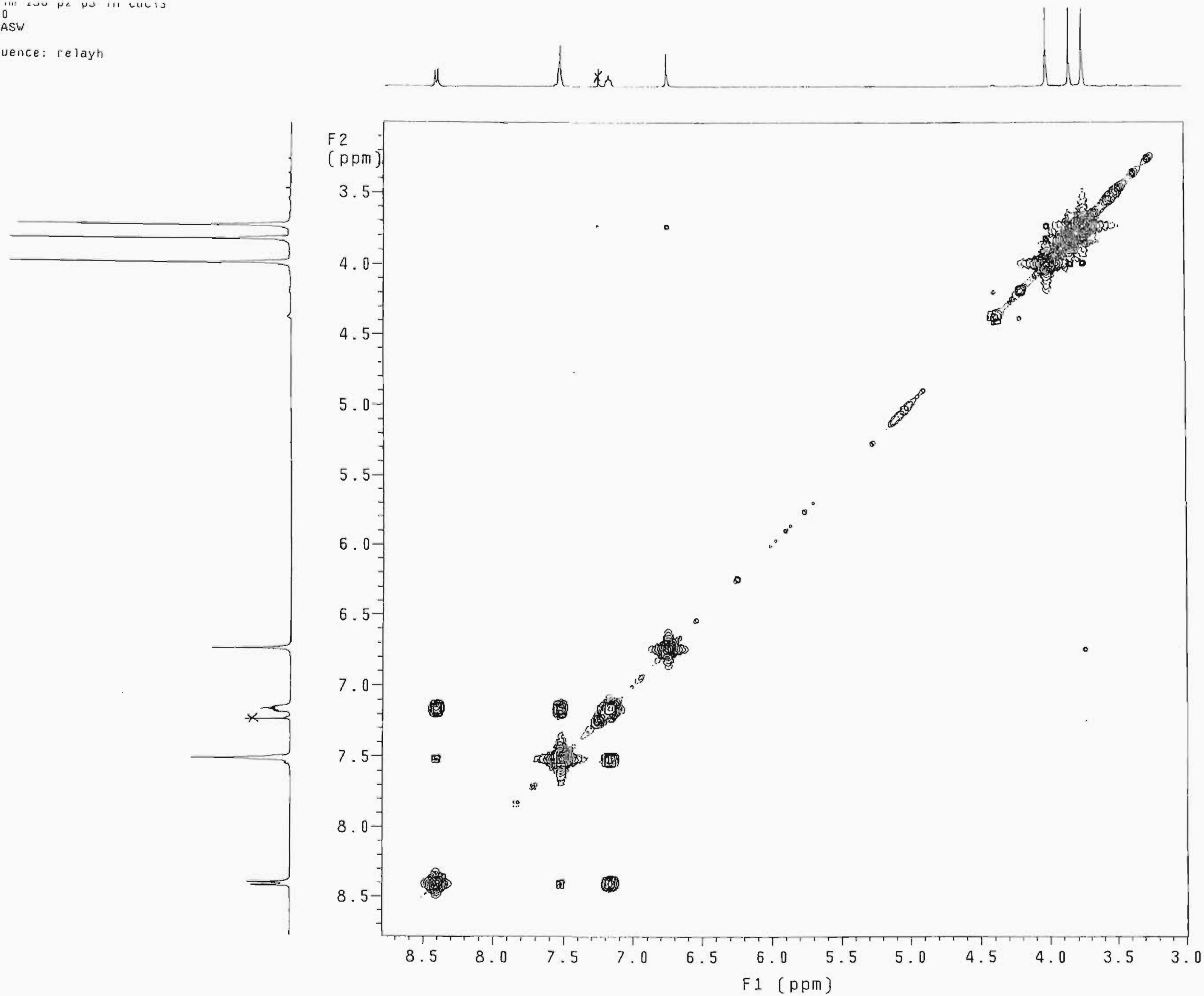
Gradient HMBC expt.
probe=5mmASW

Pulse Sequence: ghmqc_da



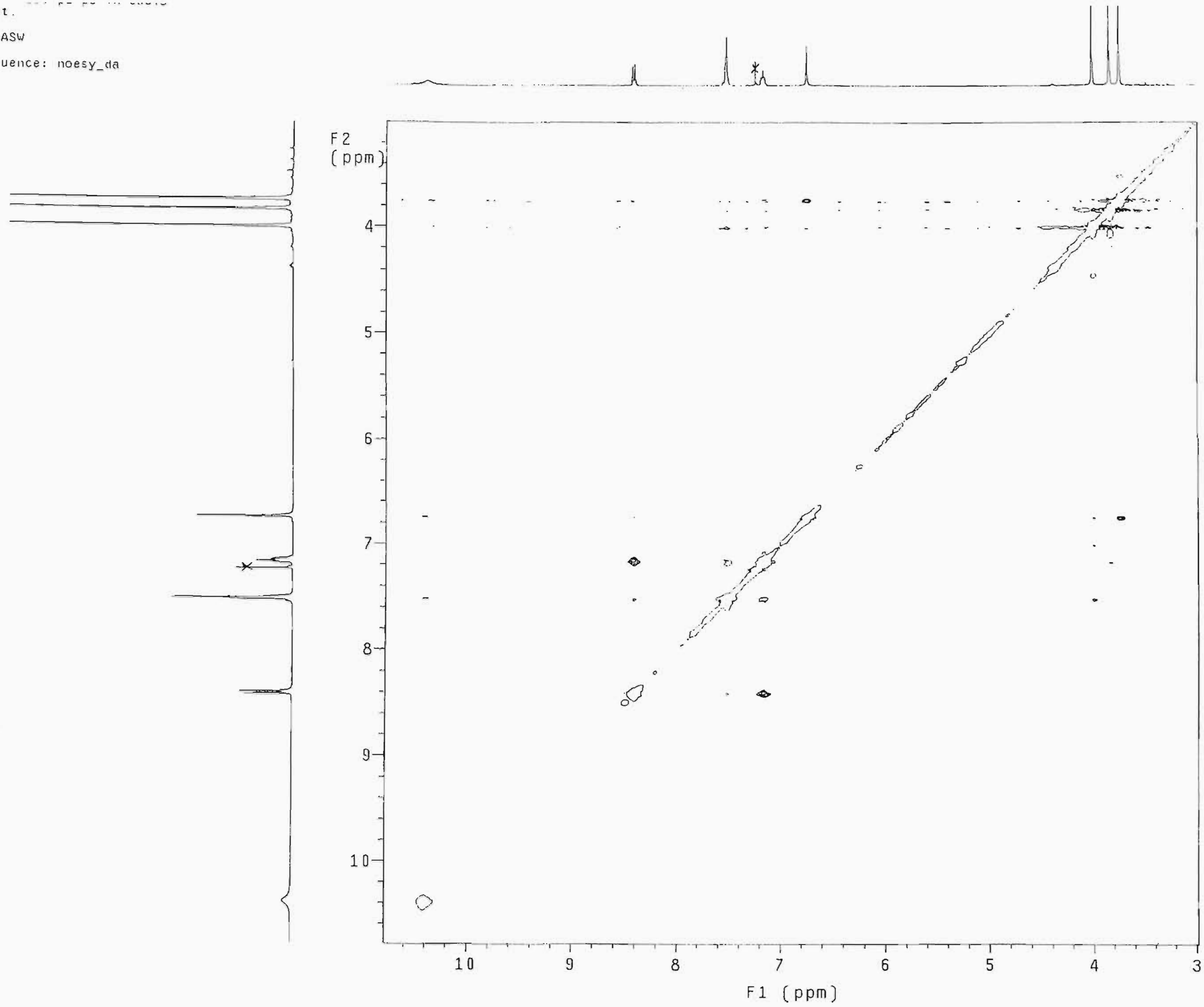
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

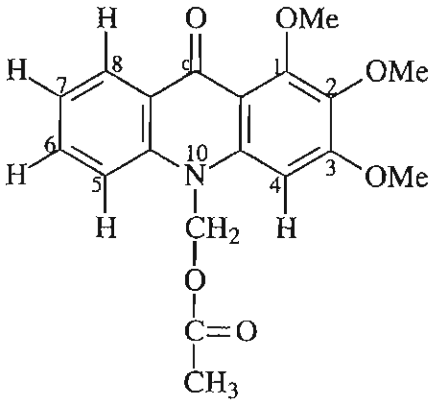


NOESY expt.
mix=1sec
probe=5mmASW

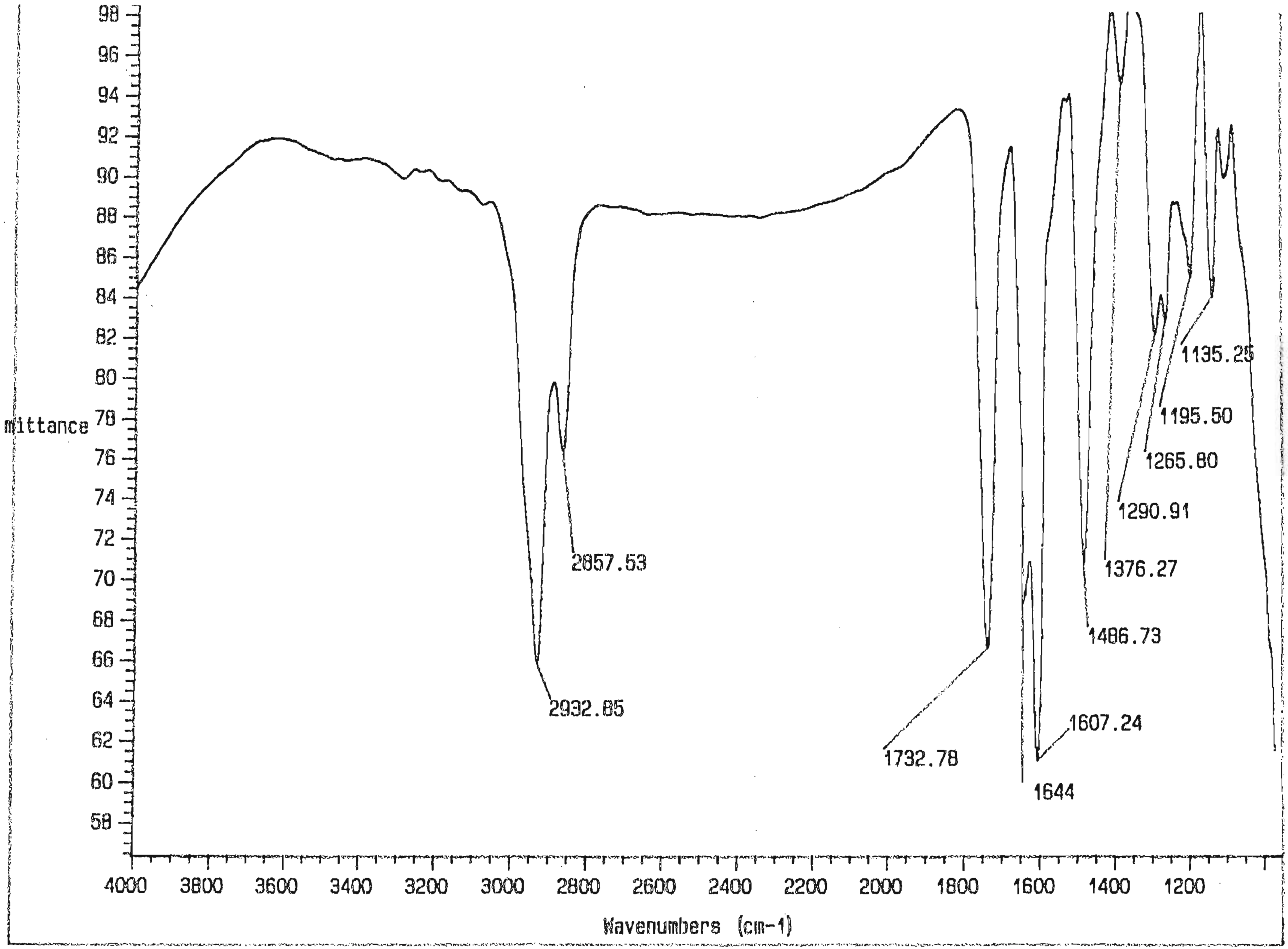
Pulse Sequence: noesy_da



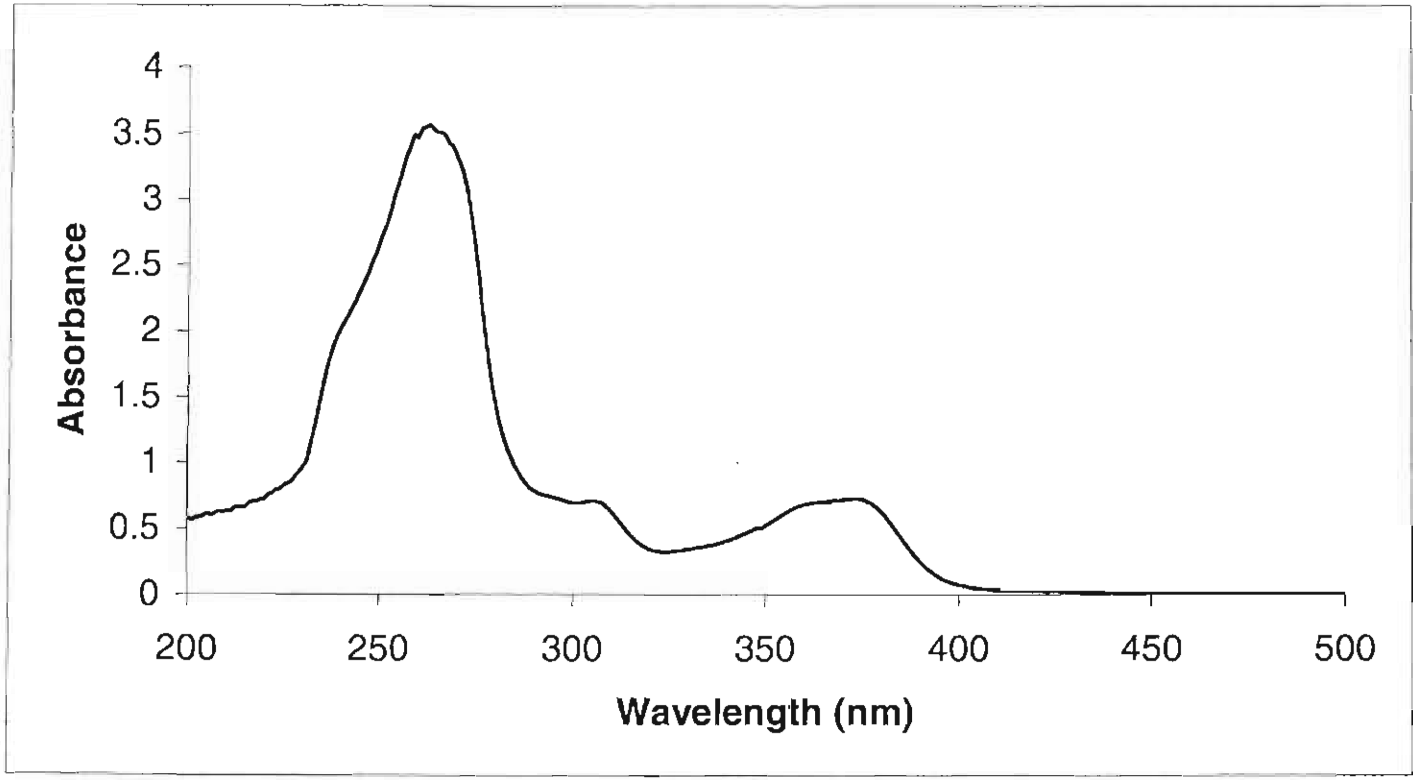
1,2,3-Trimethoxy-10-acetoxymethylacridone (ii)



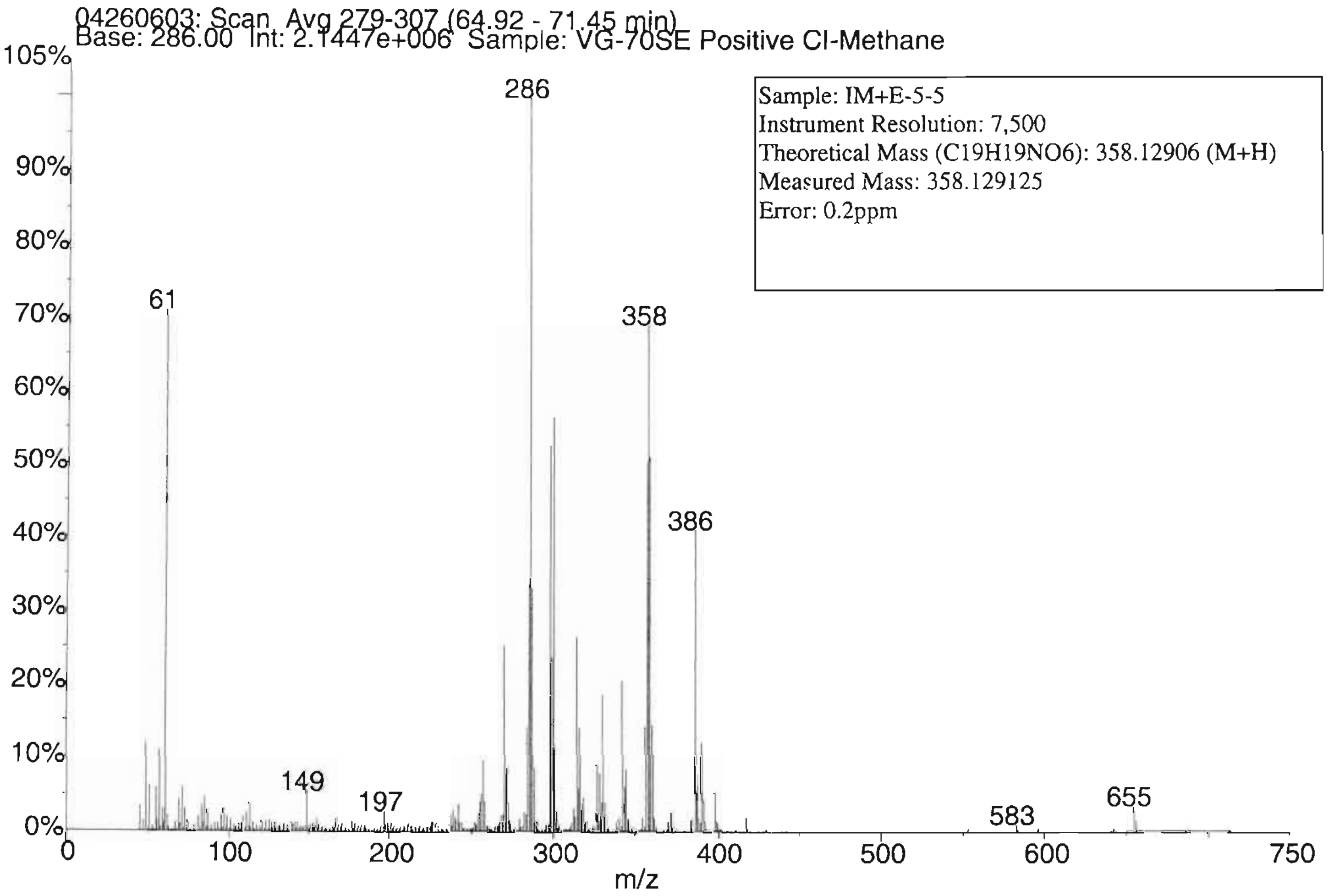
(ii)



IR Spectrum of 1,2,3-Trimethoxy-10-acetoxymethylacridone (ii)

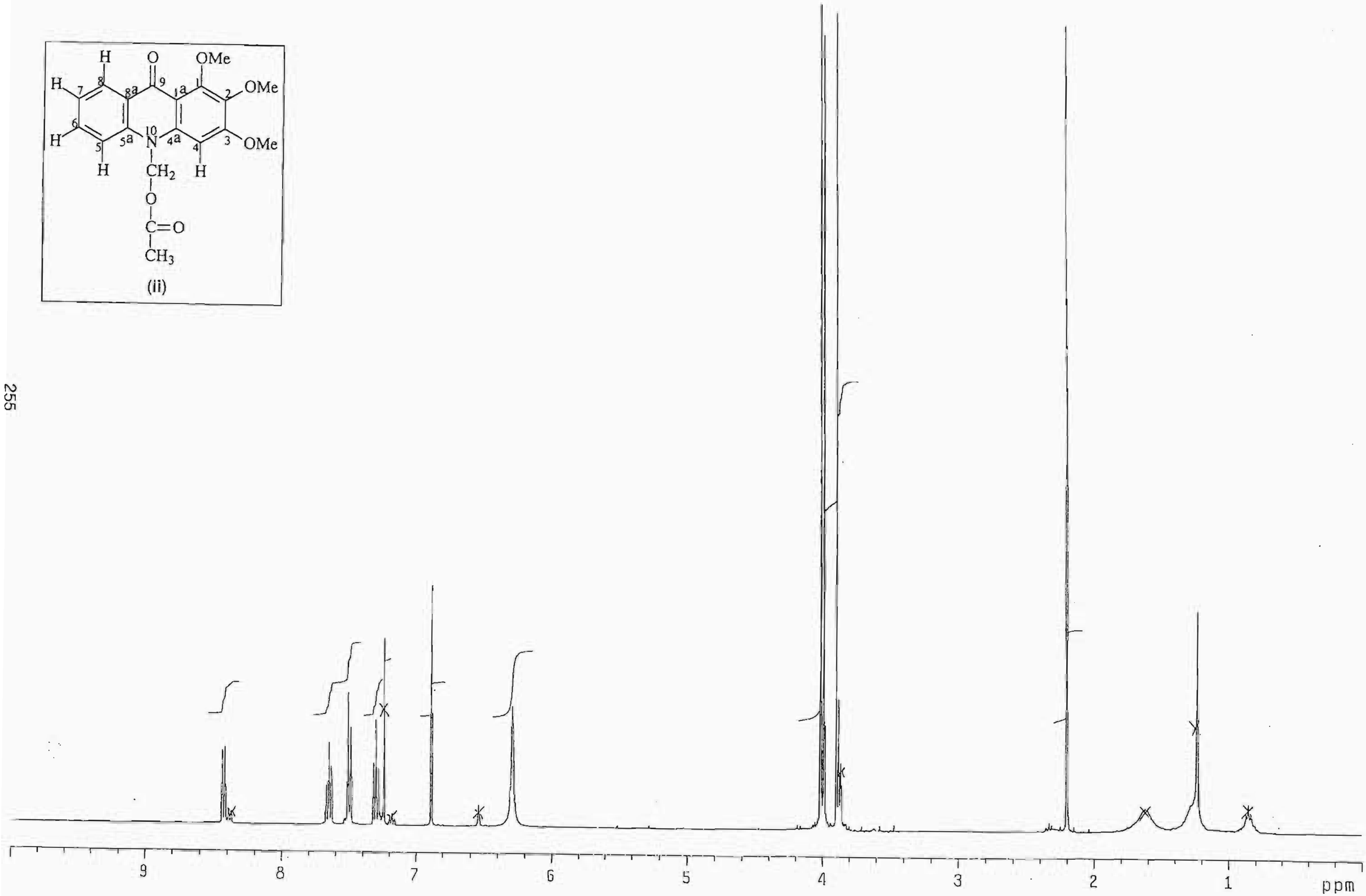


UV Spectrum of 1,2,3-Trimethoxy-10-acetoxymethyleneacridone (ii)

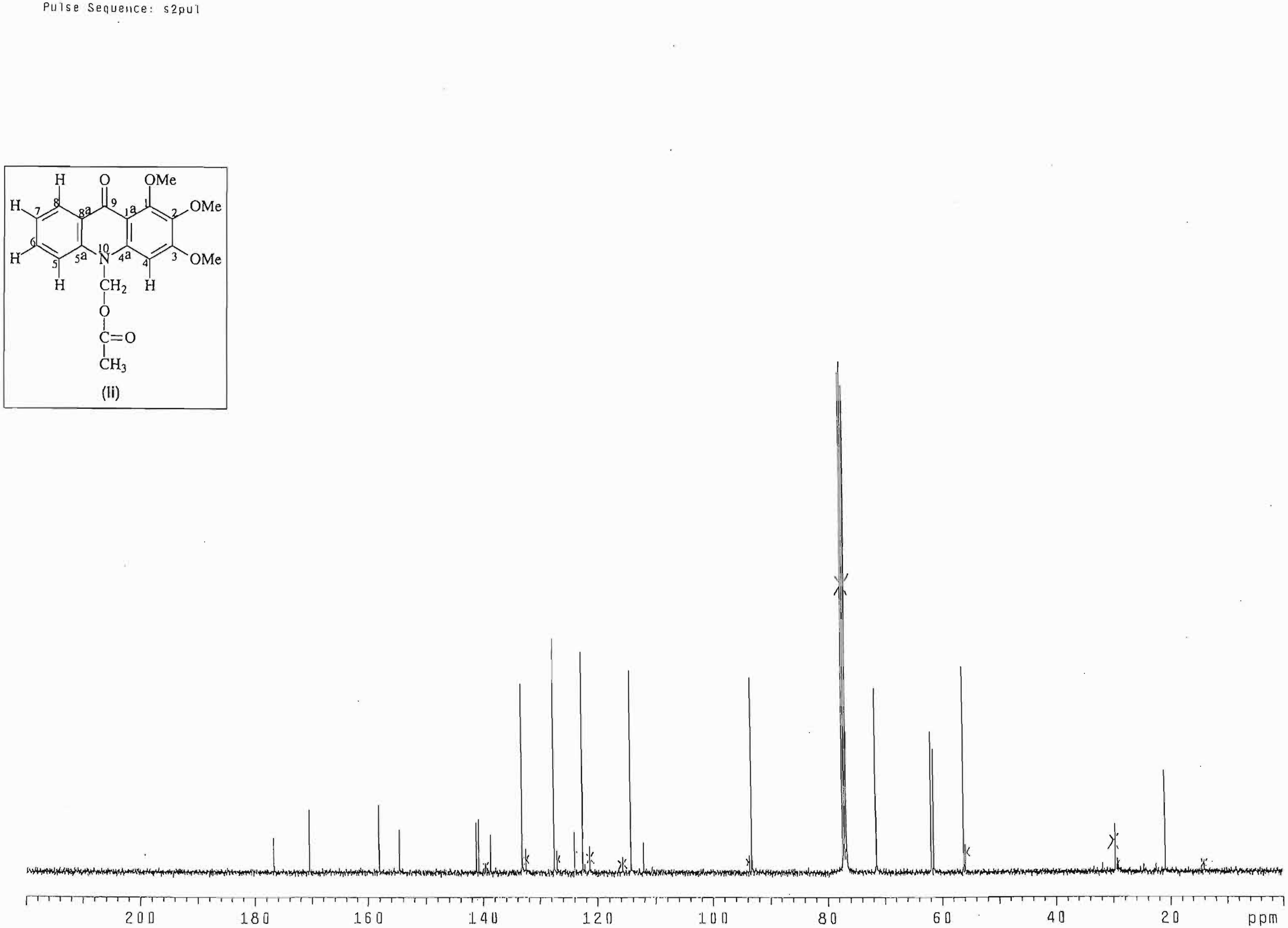


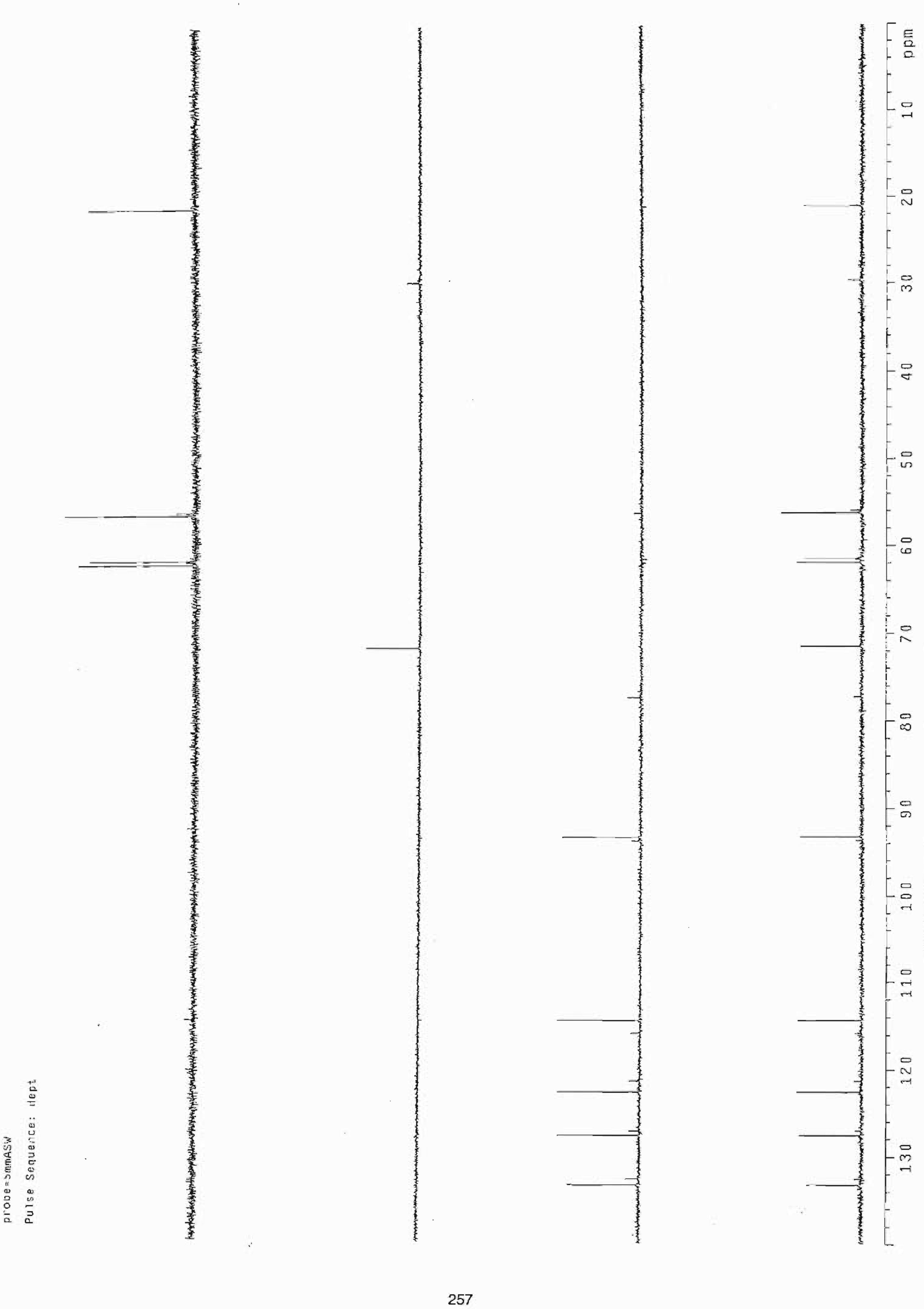
Mass Spectrum of 1,2,3-Trimethoxy-10-acetoxymethylacridone (ii)

Pulse Sequence: s2pul



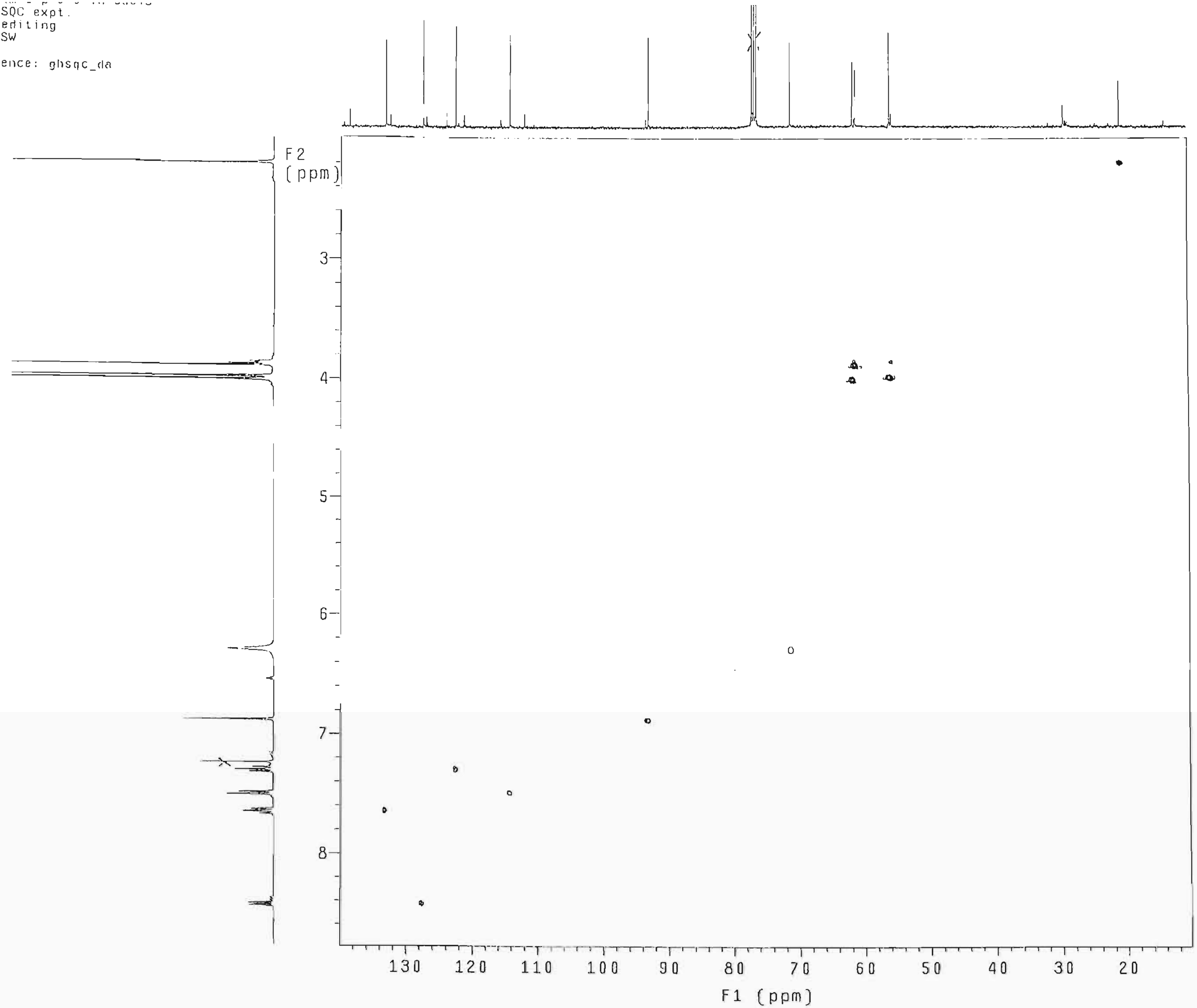
^{1}H NMR Spectrum of 1,2,3-Trimethoxy-10-acetoxymethylacridone (ii)

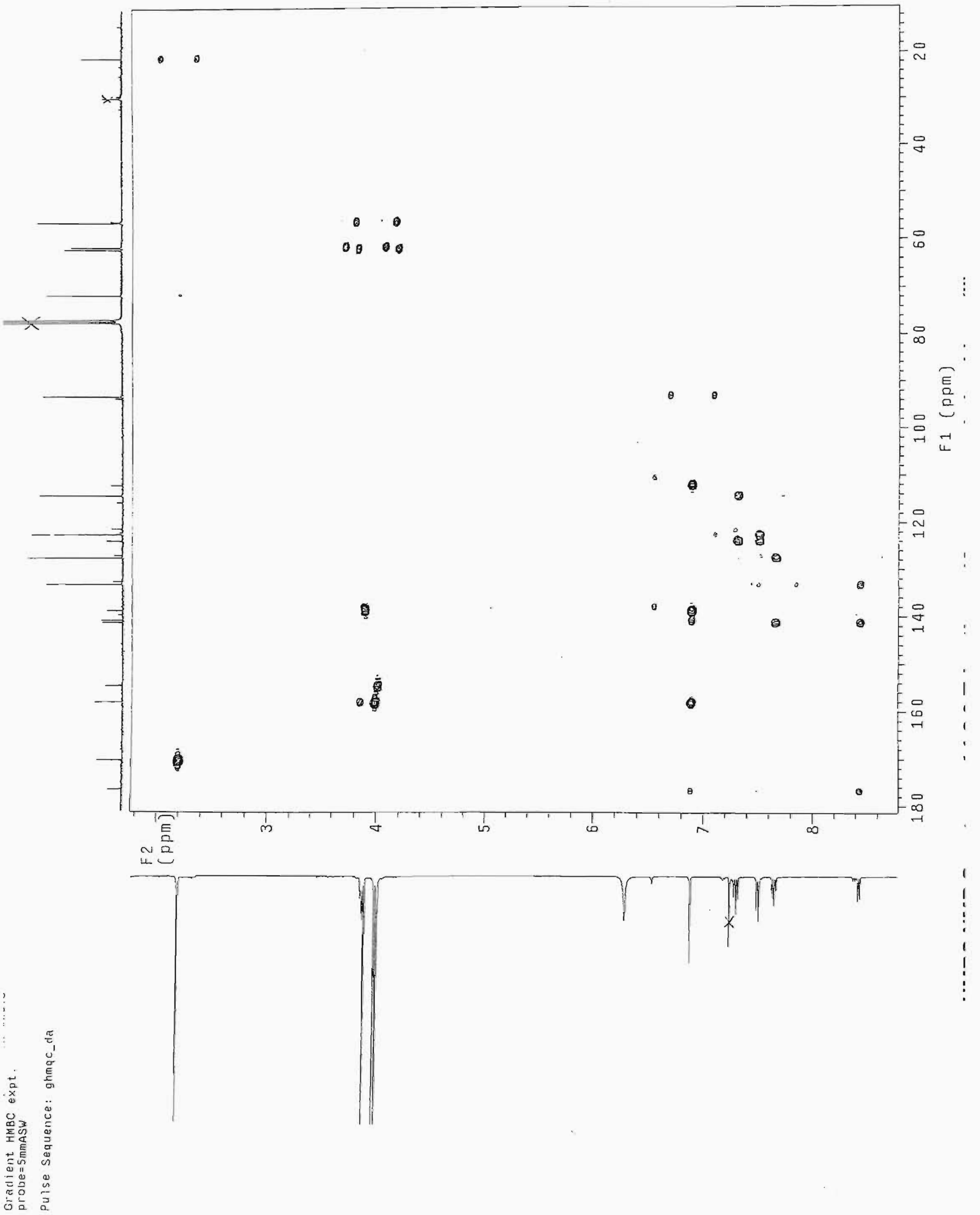

Pulse Sequence: s2pul
H
O
OMe
H
7
8
8a
9
1a
1
2
OMe
6
5a
10
N
4a
4
3
OMe
H
5
H
CH2
H
O
C=O
CH3
(Ii)
200
180
160
140
120
100
80
60
40
20
ppm


probe=5mmASW
Pulse Sequence: dept
ppm
10
20
30
40
50
60
70
80
90
100
110
120
130

Gradient HSQC expt.
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da

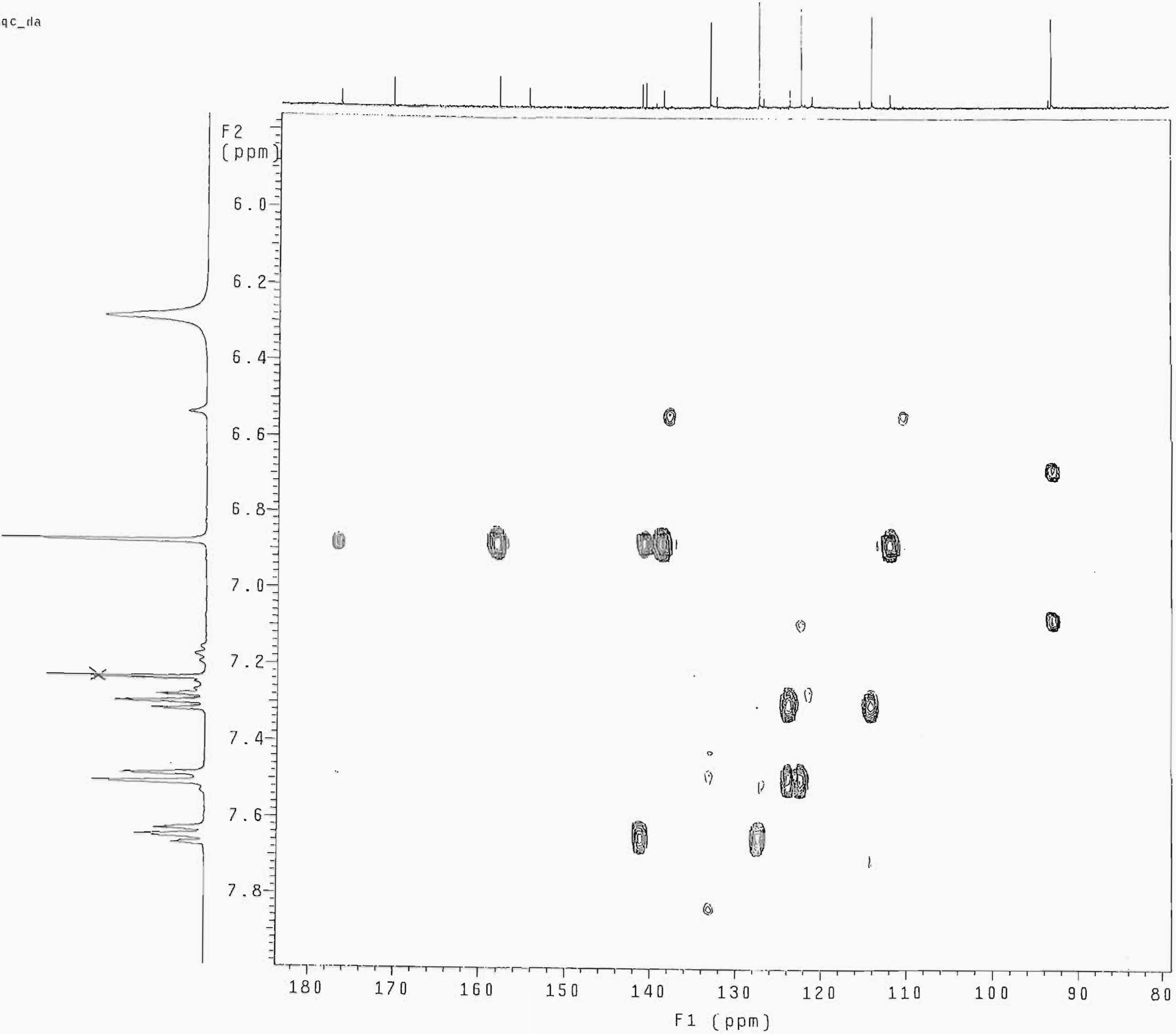




Gradient HMBC expt.
probe=5mmASW
Pulse Sequence: ghmqc_da
F2 (ppm)
3
4
5
6
7
8
F1 (ppm)
20
40
60
80
100
120
140
160
180

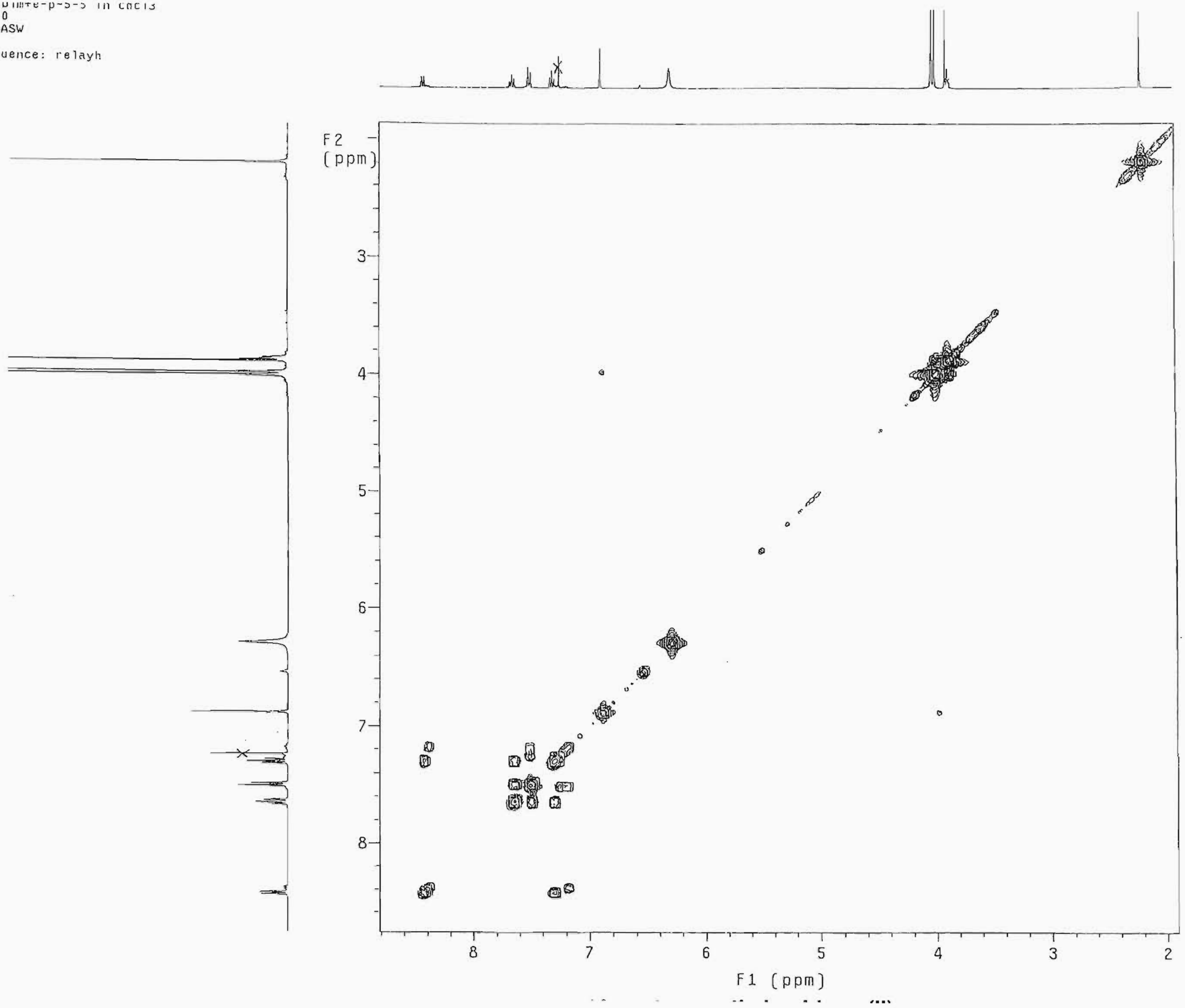
Gradient HMBC expt.
probe=5mmASW

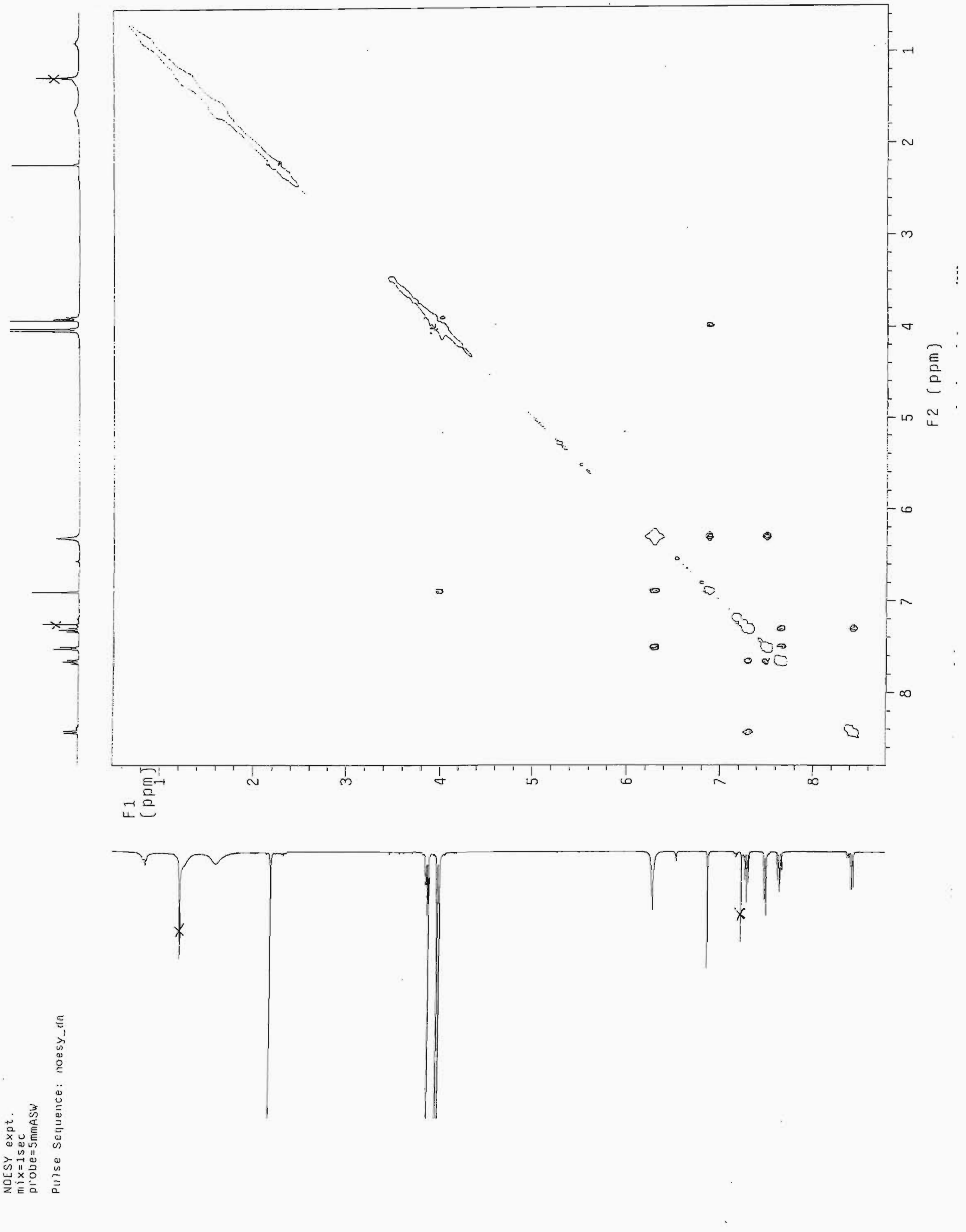
Pulse Sequence: ghmqc_da

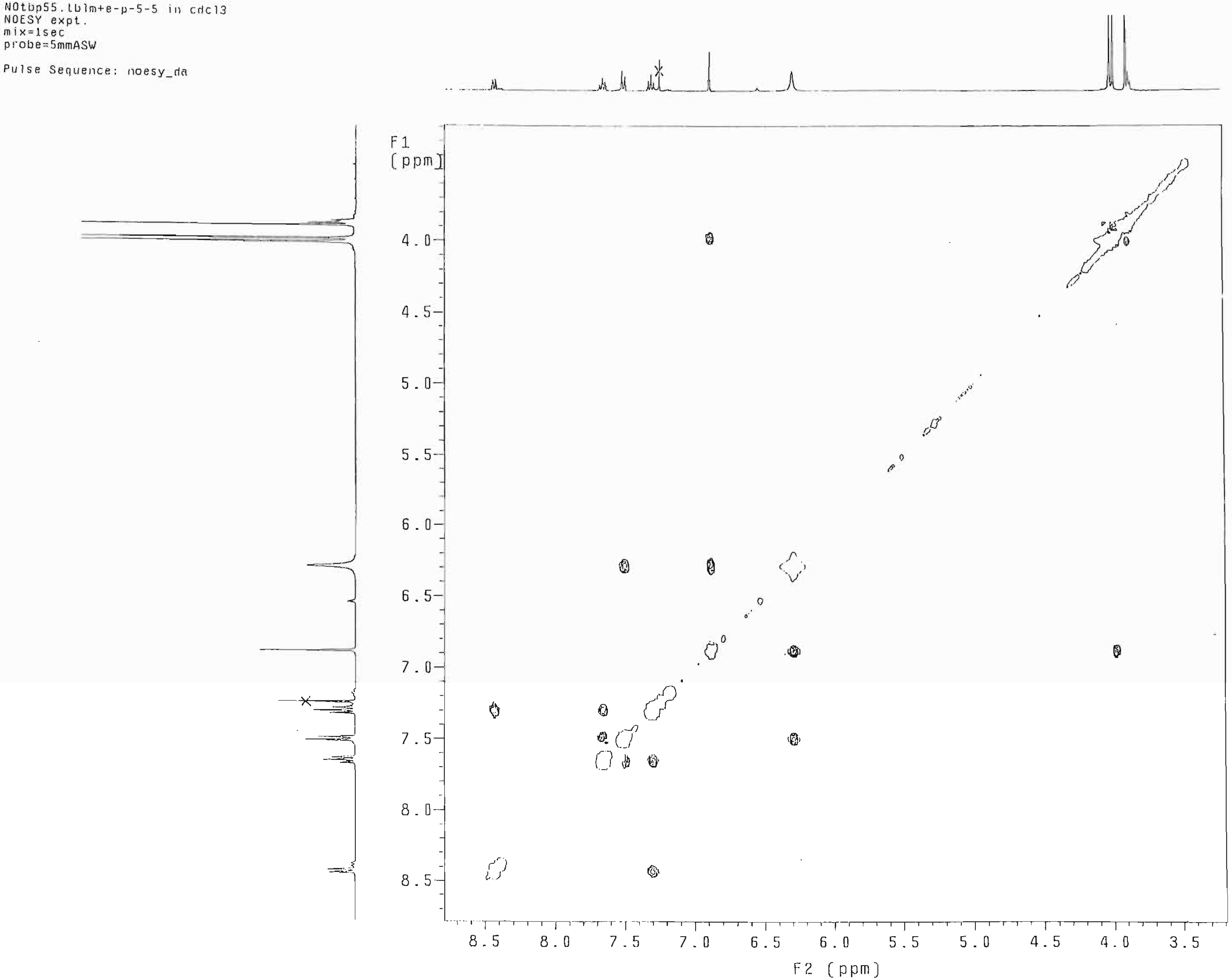


cytbp55.tbimte-p-5-5 in cdcl3
1H Cosy-90
probe=5mmASW

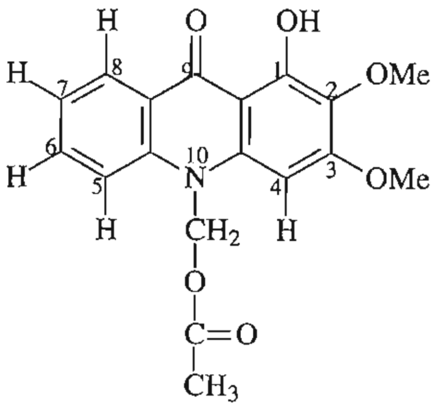
Pulse Sequence: relayh



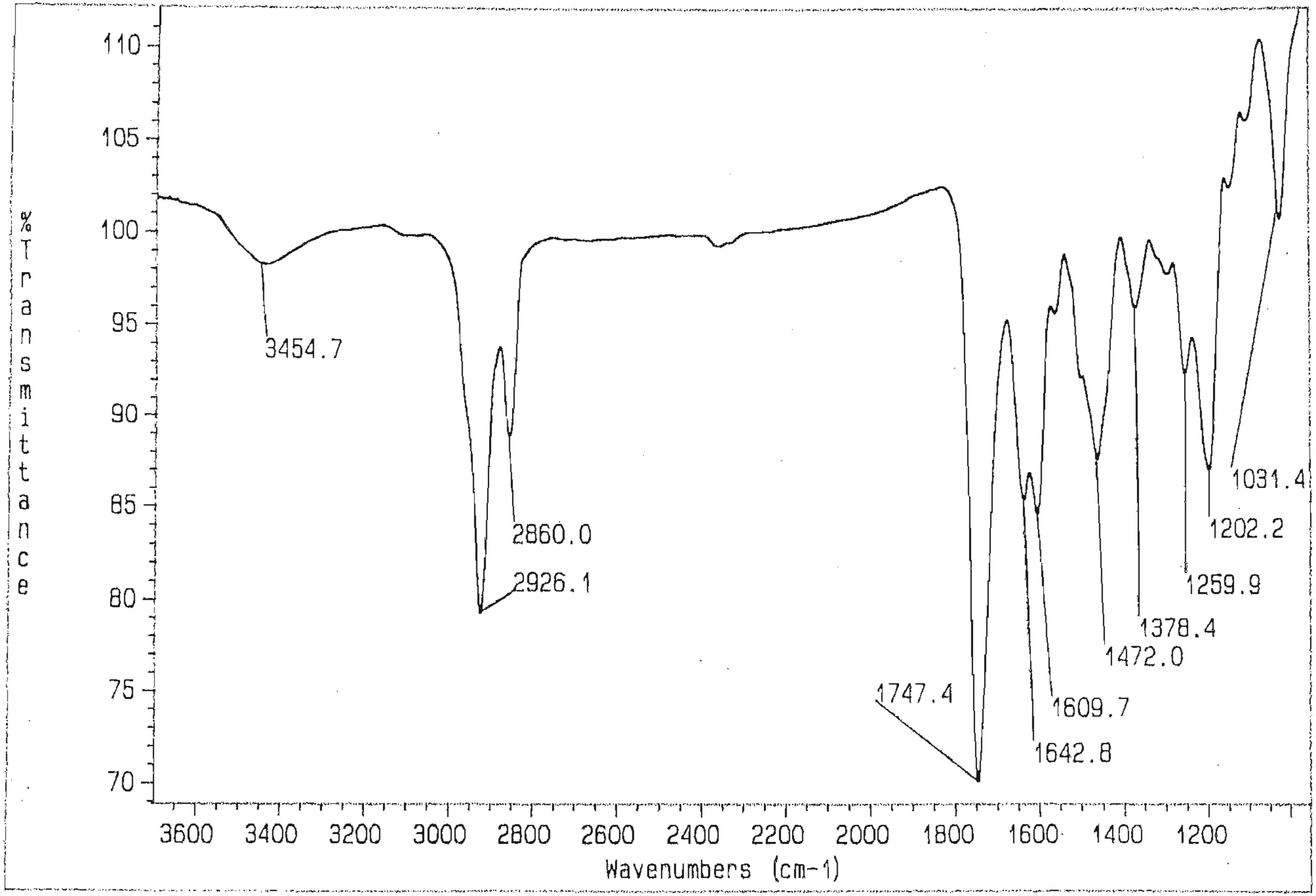

NOESY expt.
mix=1sec
probe=5mmASW
Pulse Sequence: noesy_da
F1 (ppm)
F2 (ppm)
1
2
3
4
5
6
7
8


NOtbp55.lblm+e-p-5-5 in cdcl3
NOESY expt.
mix=1sec
probe=5mmASW
Pulse Sequence: noesy_da
F1
(ppm)
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
F2 (ppm)

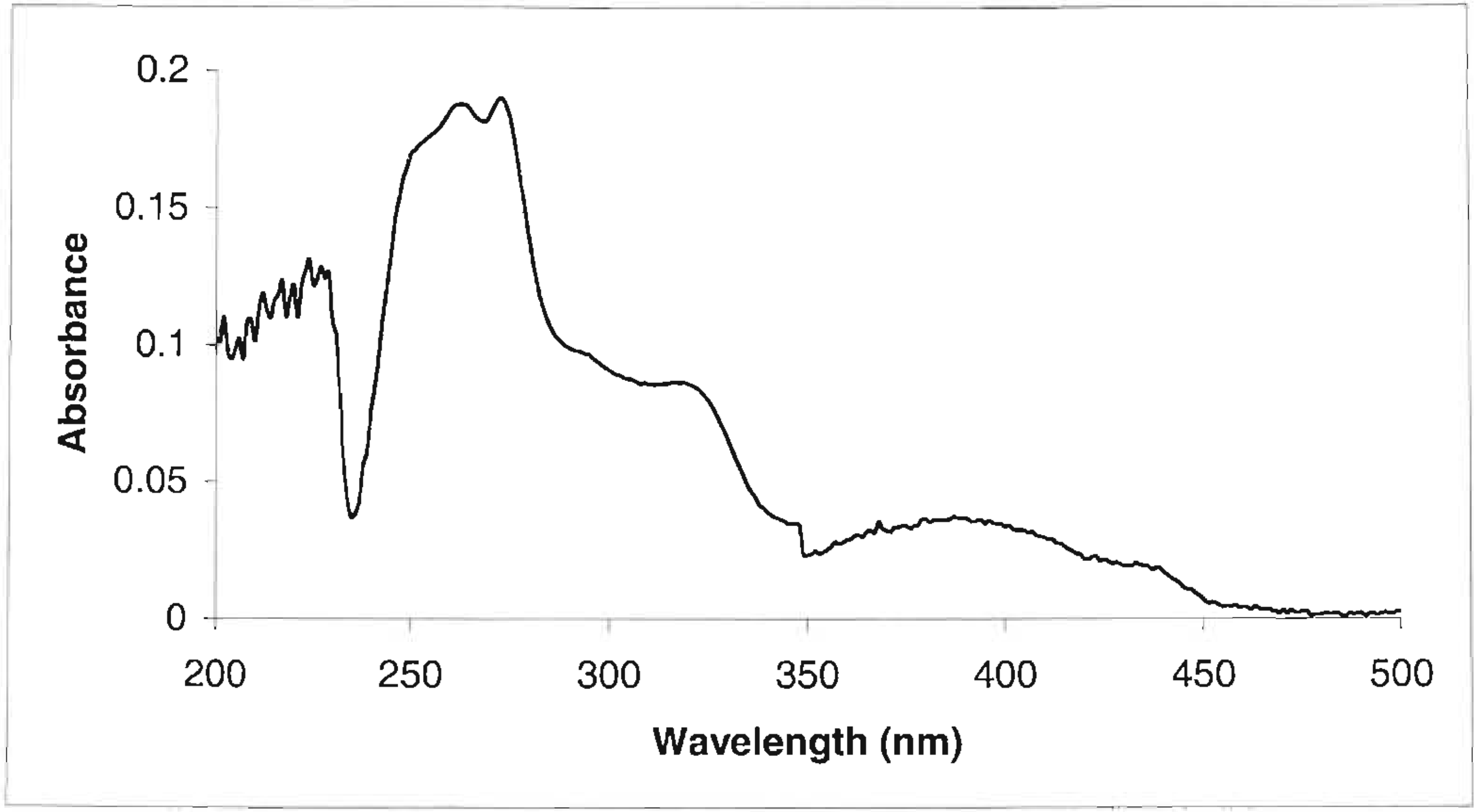
1-Hydroxy-2,3-dimethoxy-10-acetoxymethylacridone (iii)



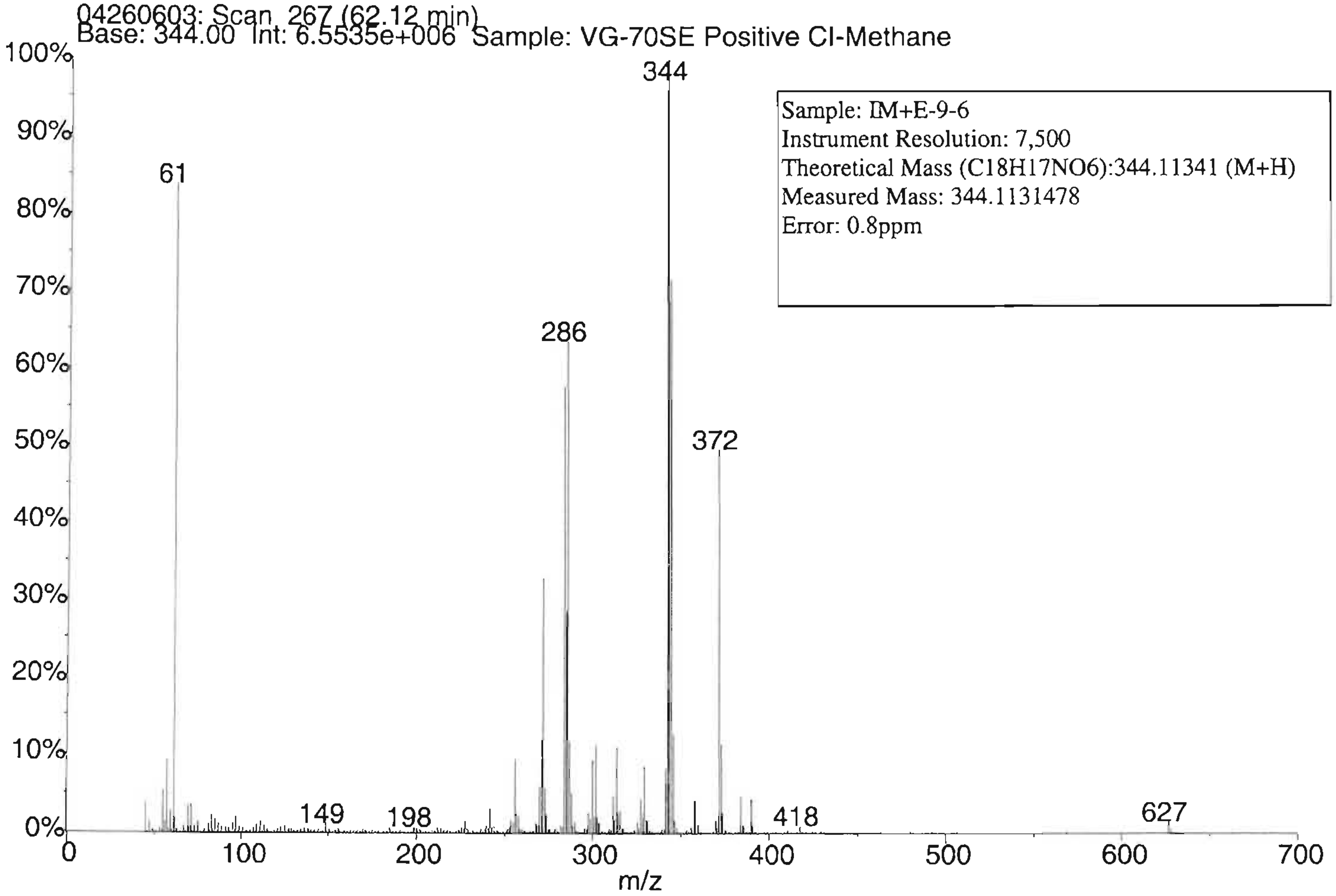
(iii)



IR Spectrum of 1-Hydroxy-2,3-dimethoxy-10-acetoxymethylacridone (iii)

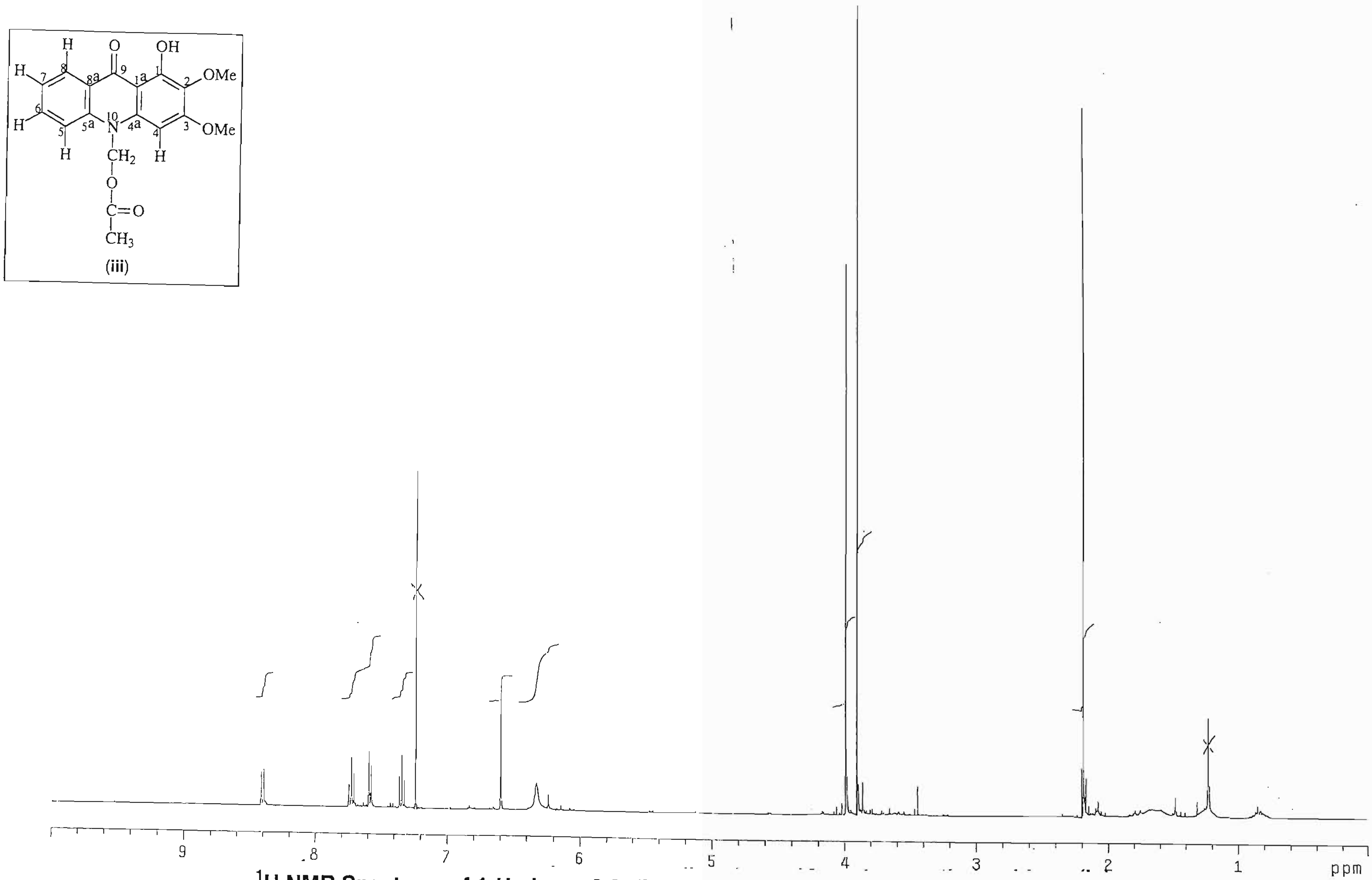


UV Spectrum of 1-Hydroxy-2,3-dimethoxy-10-acetoxymethyleneacridone (iii)

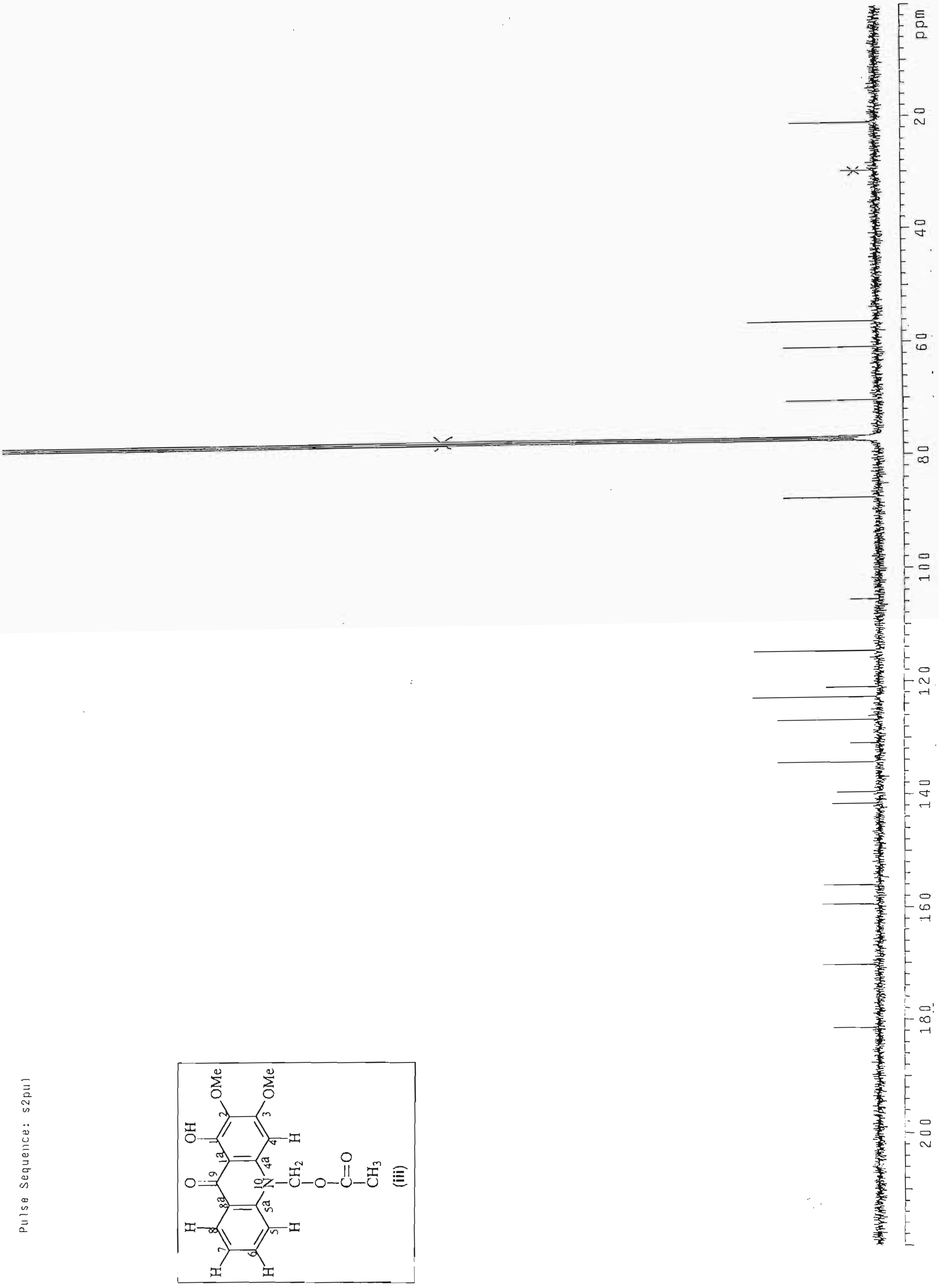


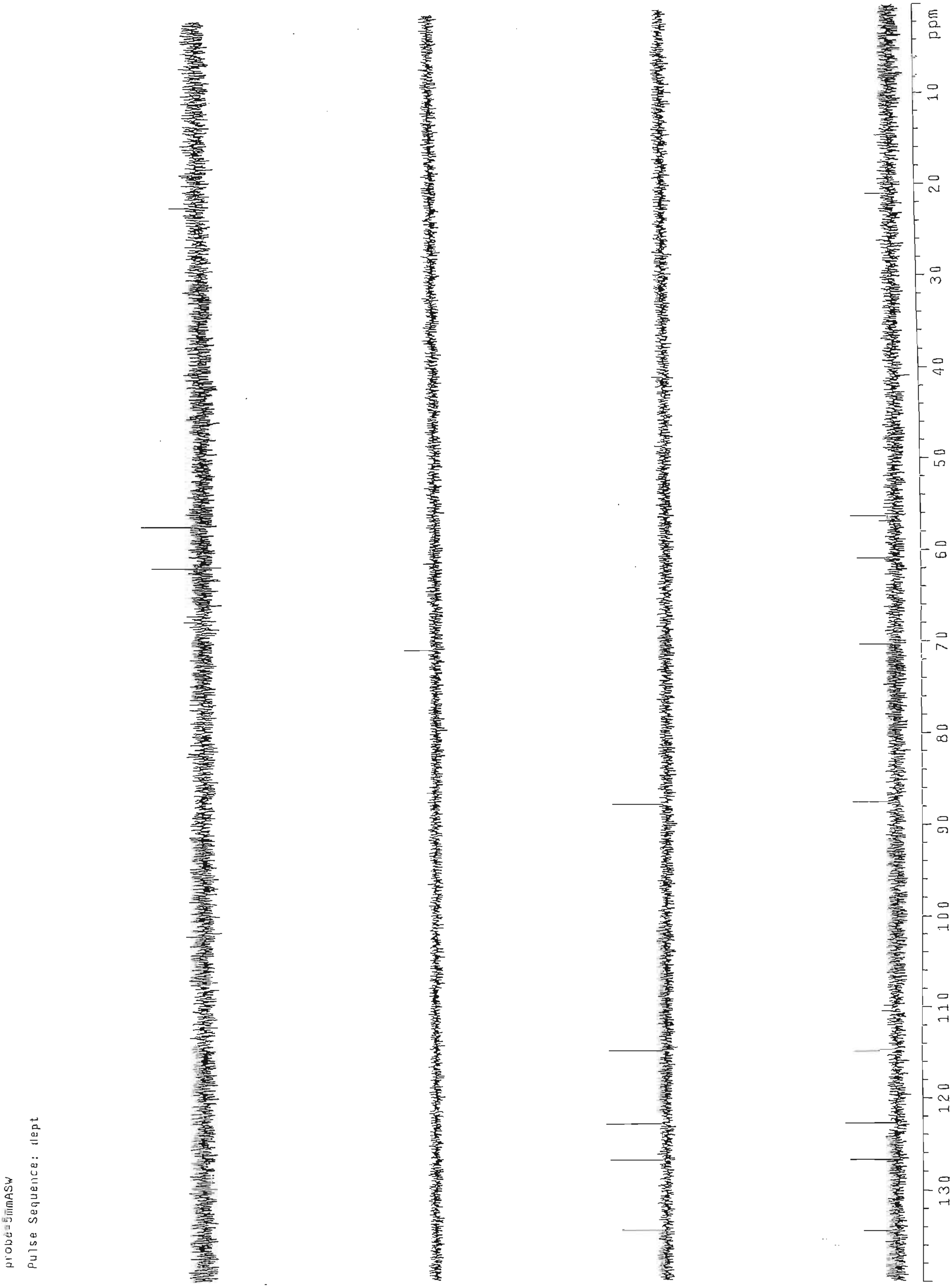
Sample: IM+E-9-6
Instrument Resolution: 7,500
Theoretical Mass (C18H17NO6):344.11341 (M+H)
Measured Mass: 344.1131478
Error: 0.8ppm

Mass Spectrum of 1-Hydroxy-2,3-dimethoxy-10-acetoxymethylacridone (iii)



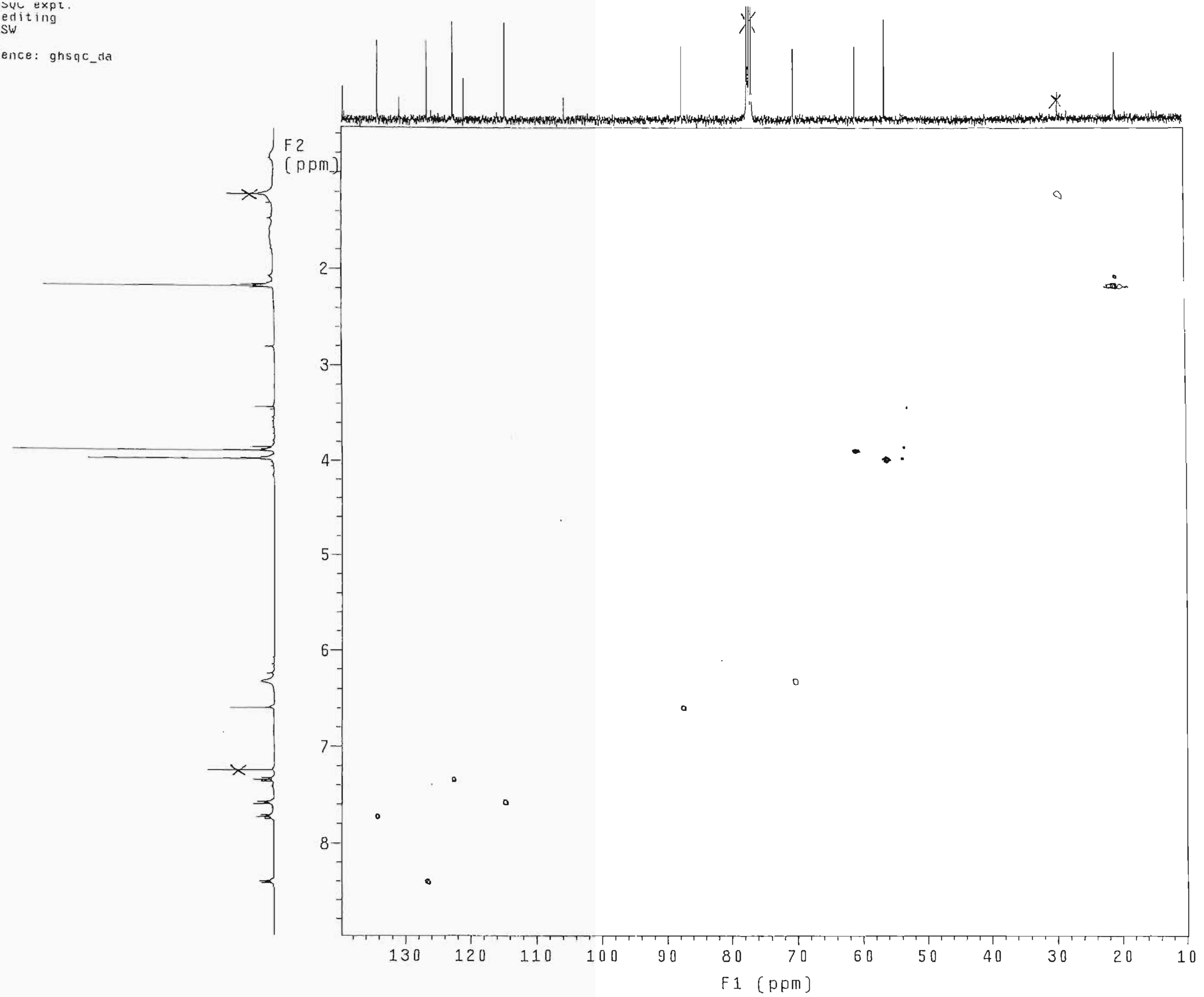
^{1}H NMR Spectrum of 1-Hydroxy-2,3-dimethoxy-10-acetoxymethylacridone (iii)

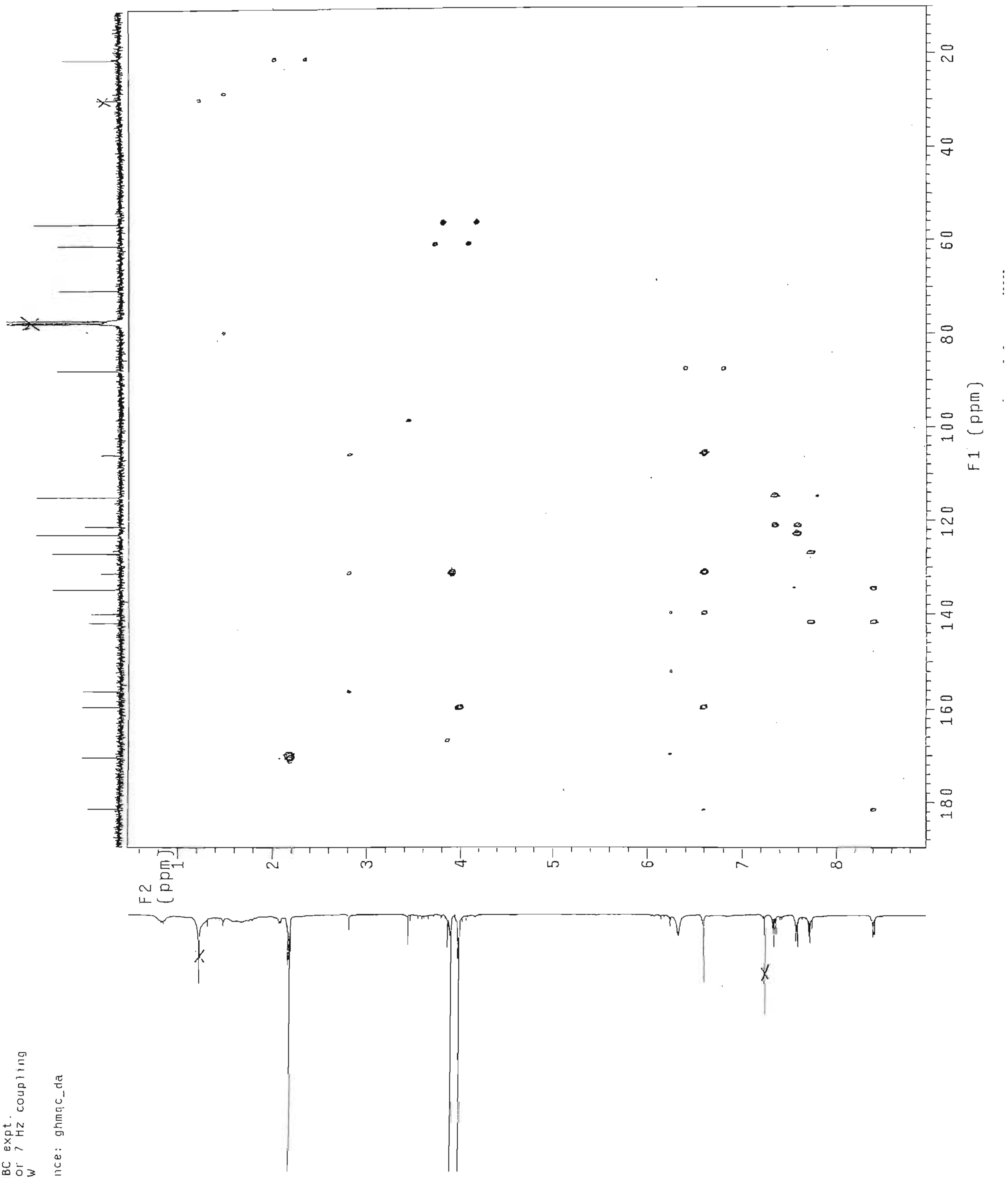

Pulse Sequence: s2pul
ppm
20
40
60
80
100
120
140
160
180
200
O
OH
OMe
OMe
H
H
H
H
H
N
CH₂
O
C=O
CH₃
1
1a
2
3
4
4a
5
5a
6
7
8
8a
9
10
(iii)


probe=5mmASW
Pulse Sequence: dept
ppm
10
20
30
40
50
60
70
80
90
100
110
120
130

Gradient HSQC expt.
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da



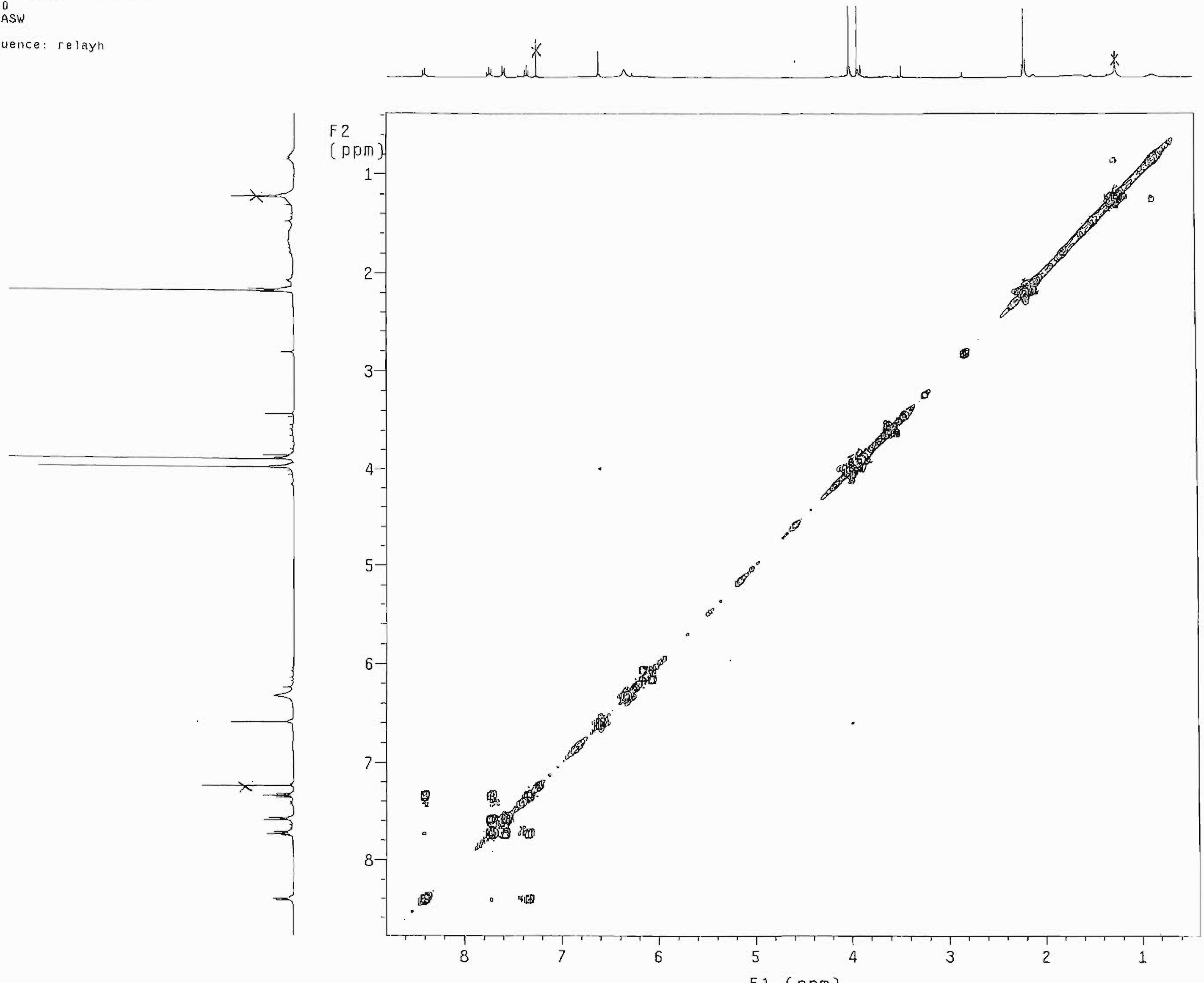


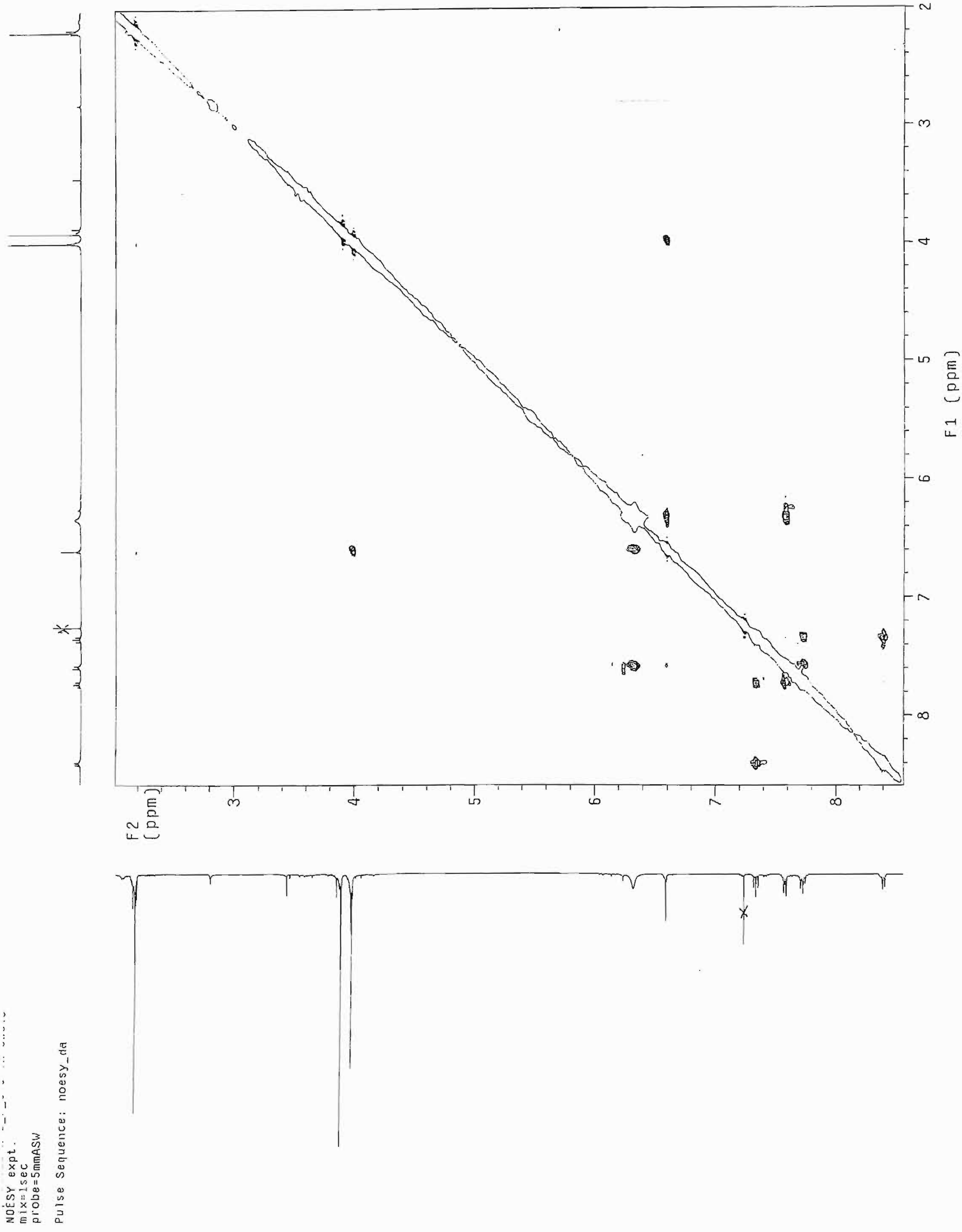
Gradient HMBC expt.
optimized for 7 Hz coupling
probe=5mmASW

Pulse Sequence: ghmqc_da

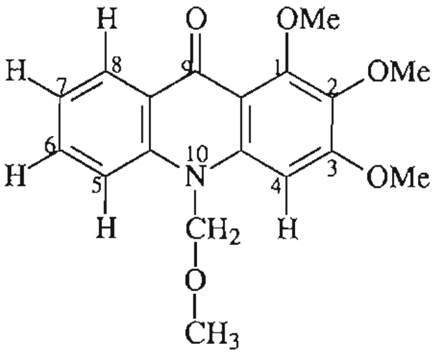
cyp50.1bb M+L_P_5-6 in CDCl3
1H Cosy-90
probe=5mmASW

Pulse Sequence: relayh

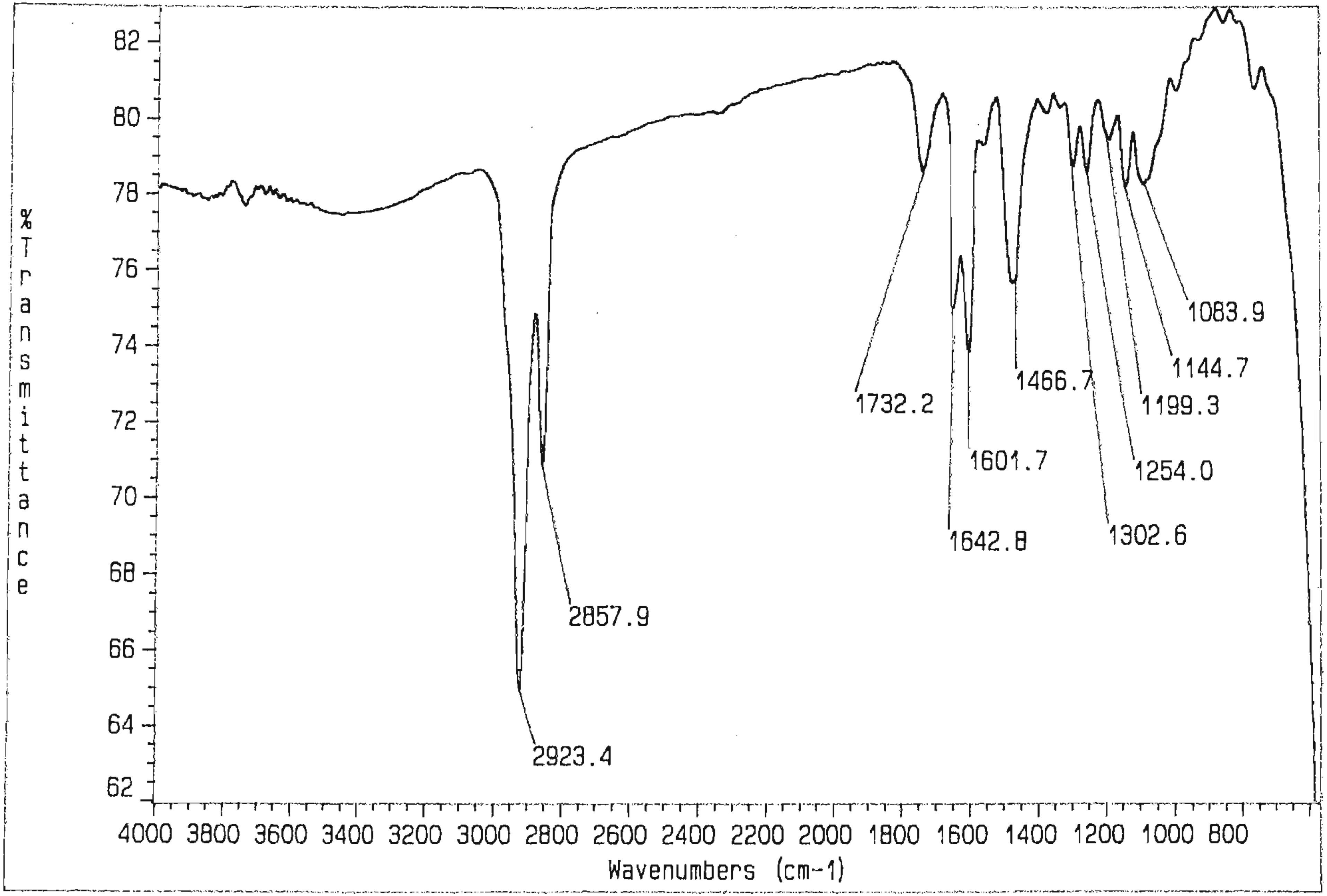



NOESY expt.
mix=1sec
probe=5mmASW
Pulse Sequence: noesy_da
F1 (ppm)
2
3
4
5
6
7
8
F2 (ppm)
3
4
5
6
7
8

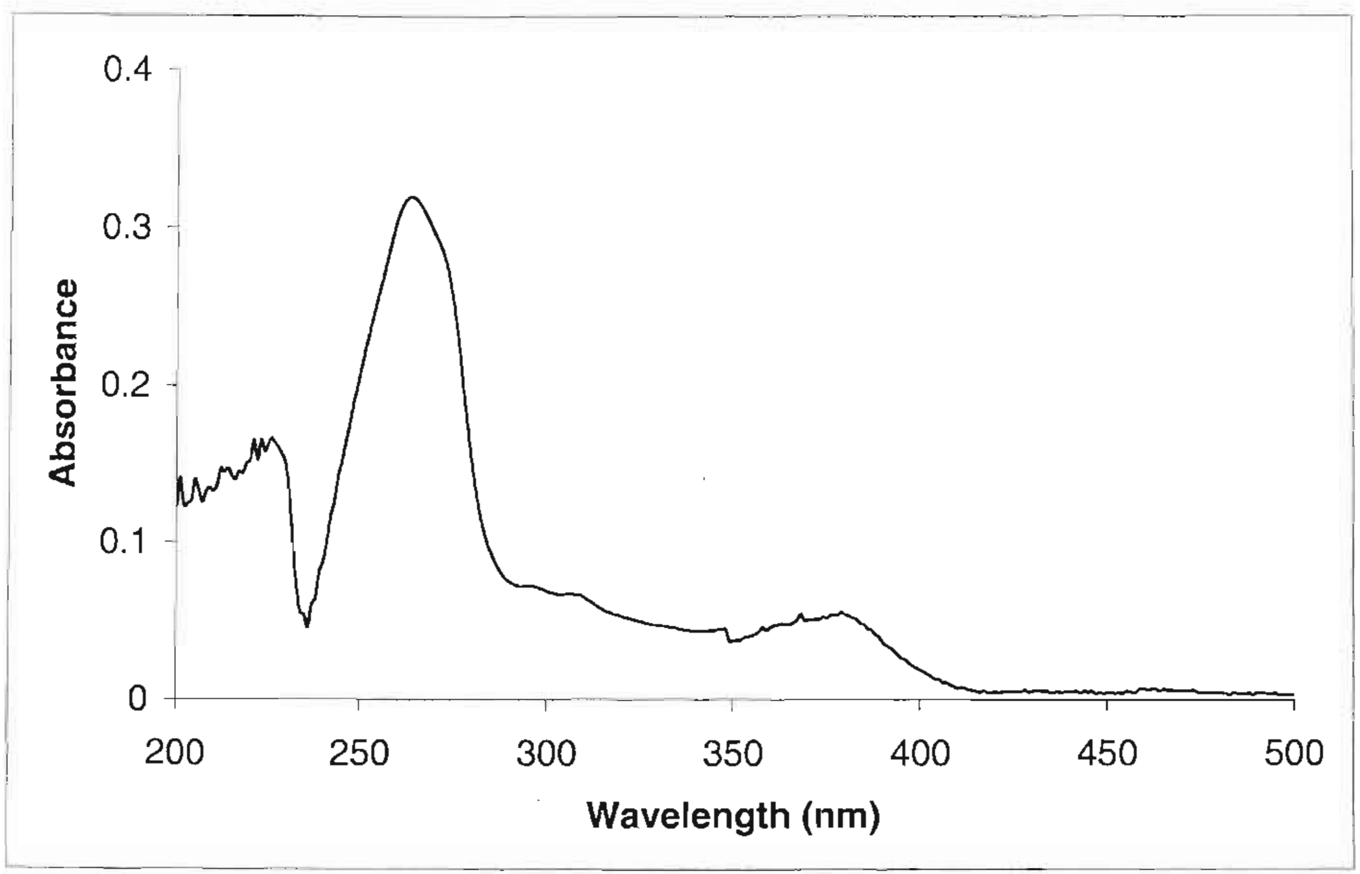
1,2,3-Trimethoxy-10-methoxymethylacridone (iv)



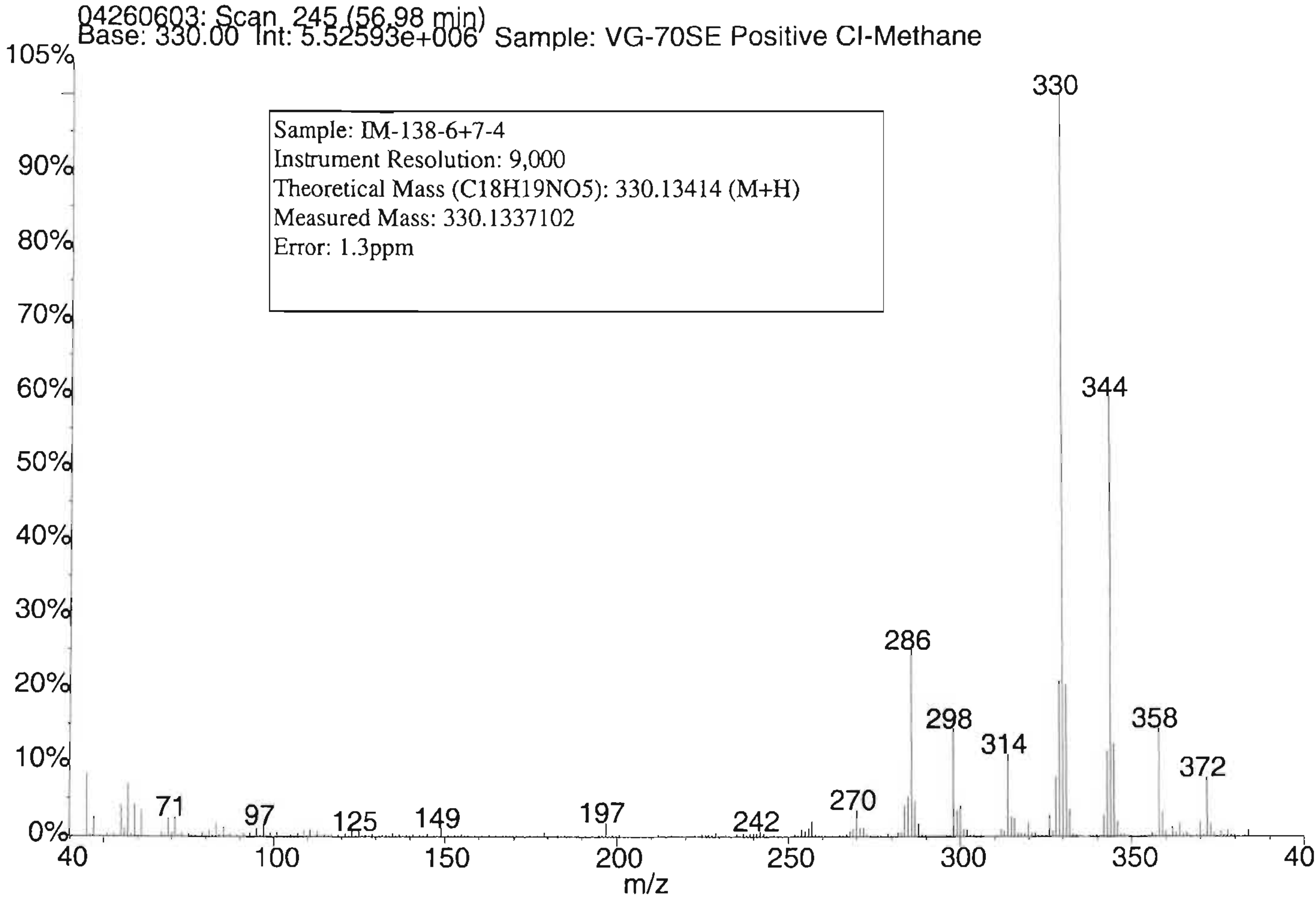
(**iv**)



IR Spectrum of 1,2,3-Trimethoxy-10-methoxymethylacridone (iv)



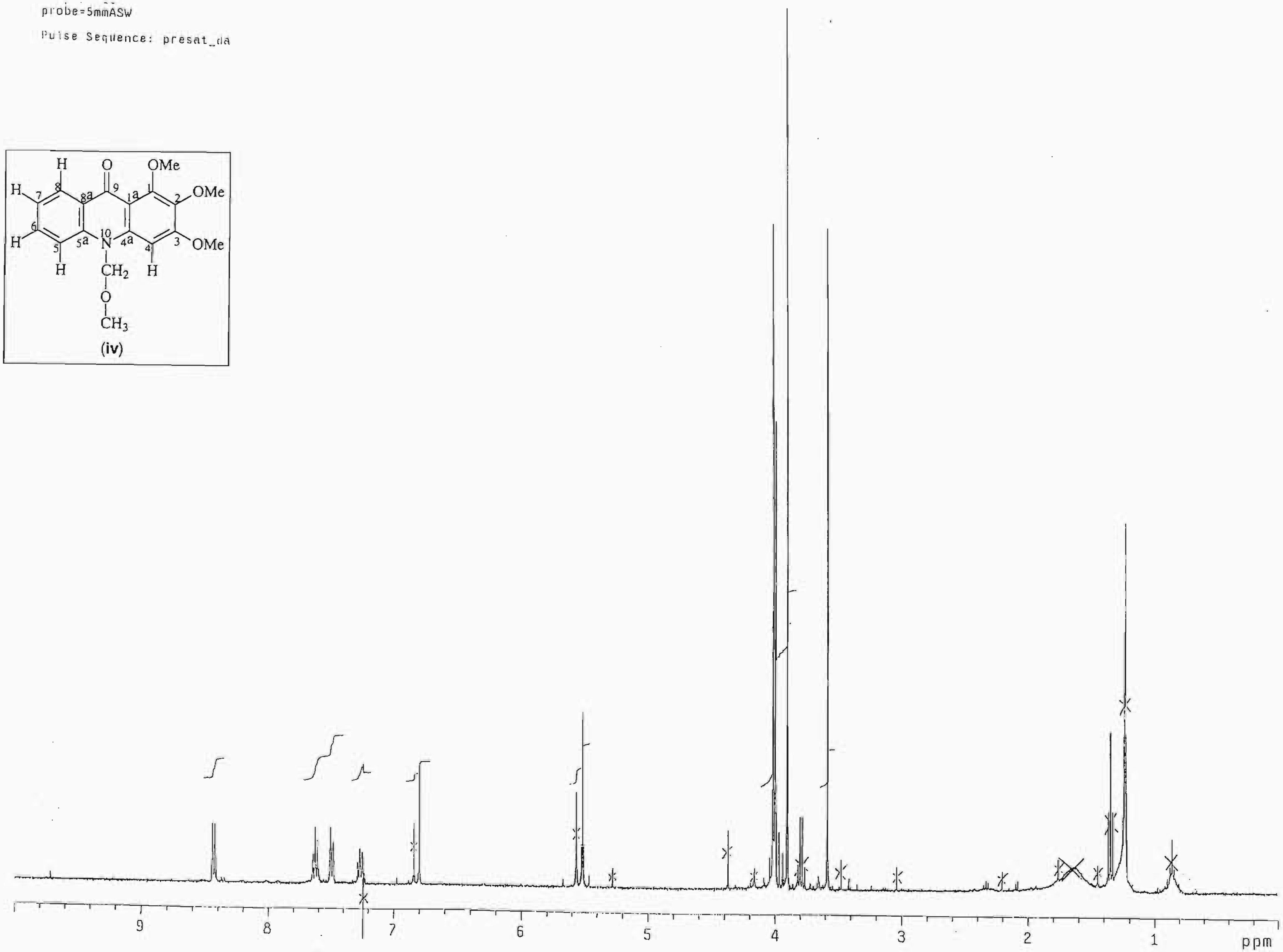
UV Spectrum of 1,2,3-Trimethoxy-10-methoxymethylene acridone (iv)



Sample: IM-138-6+7-4
Instrument Resolution: 9,000
Theoretical Mass (C18H19NO5): 330.13414 (M+H)
Measured Mass: 330.1337102
Error: 1.3ppm

Mass Spectrum of 1,2,3-Trimethoxy-10-methoxymethylacridone (iv)

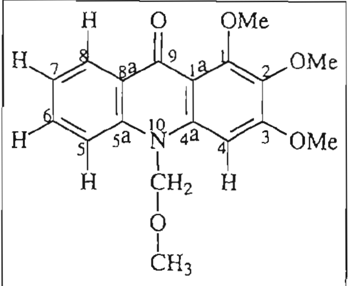
probe=5mmASW
Pulse Sequence: presat_da



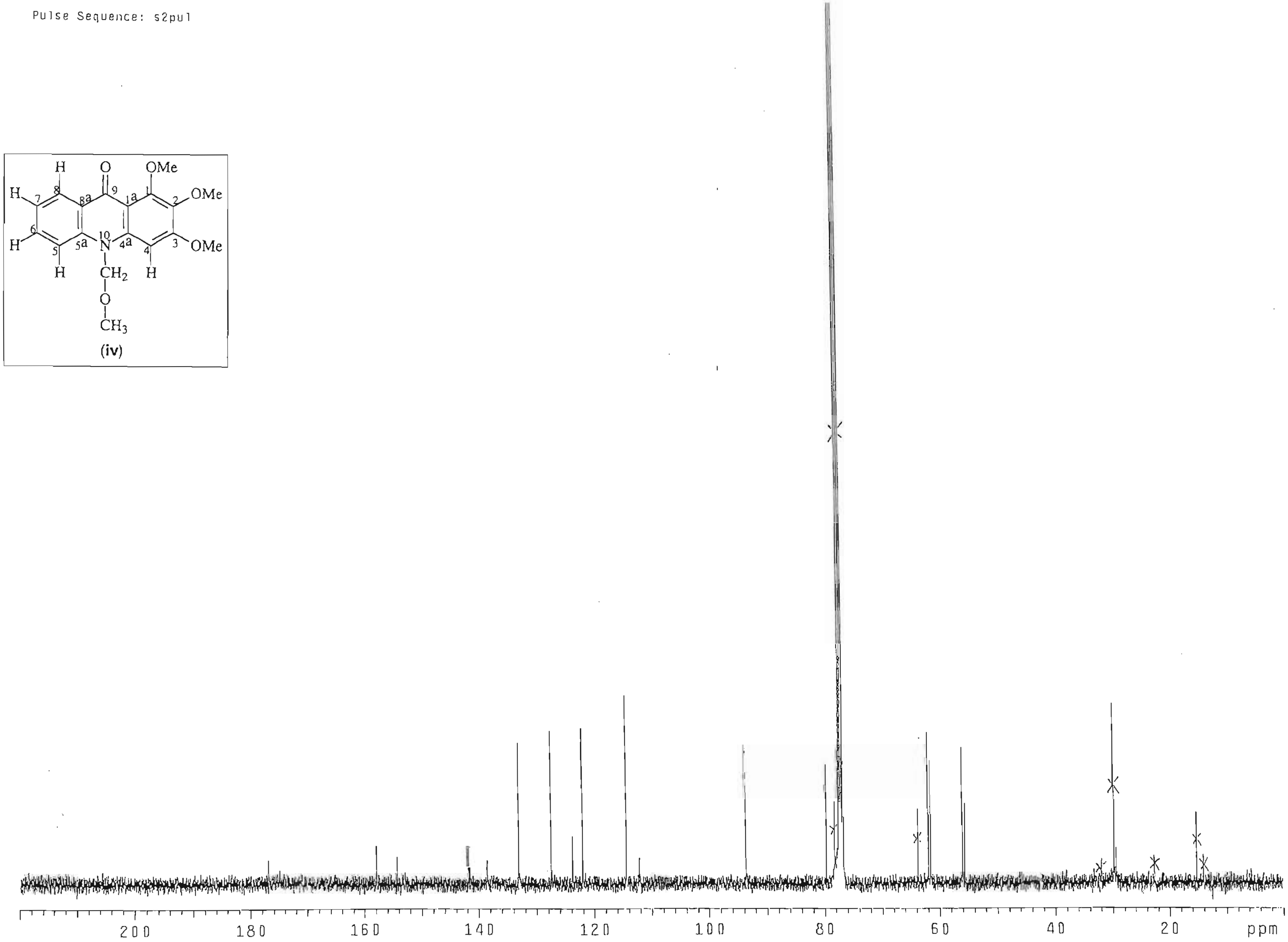
(iv)

^{1}H NMR Spectrum of 1,2,3-Trimethoxy-10-methoxymethylacridone (iv)

Pulse Sequence: s2pul

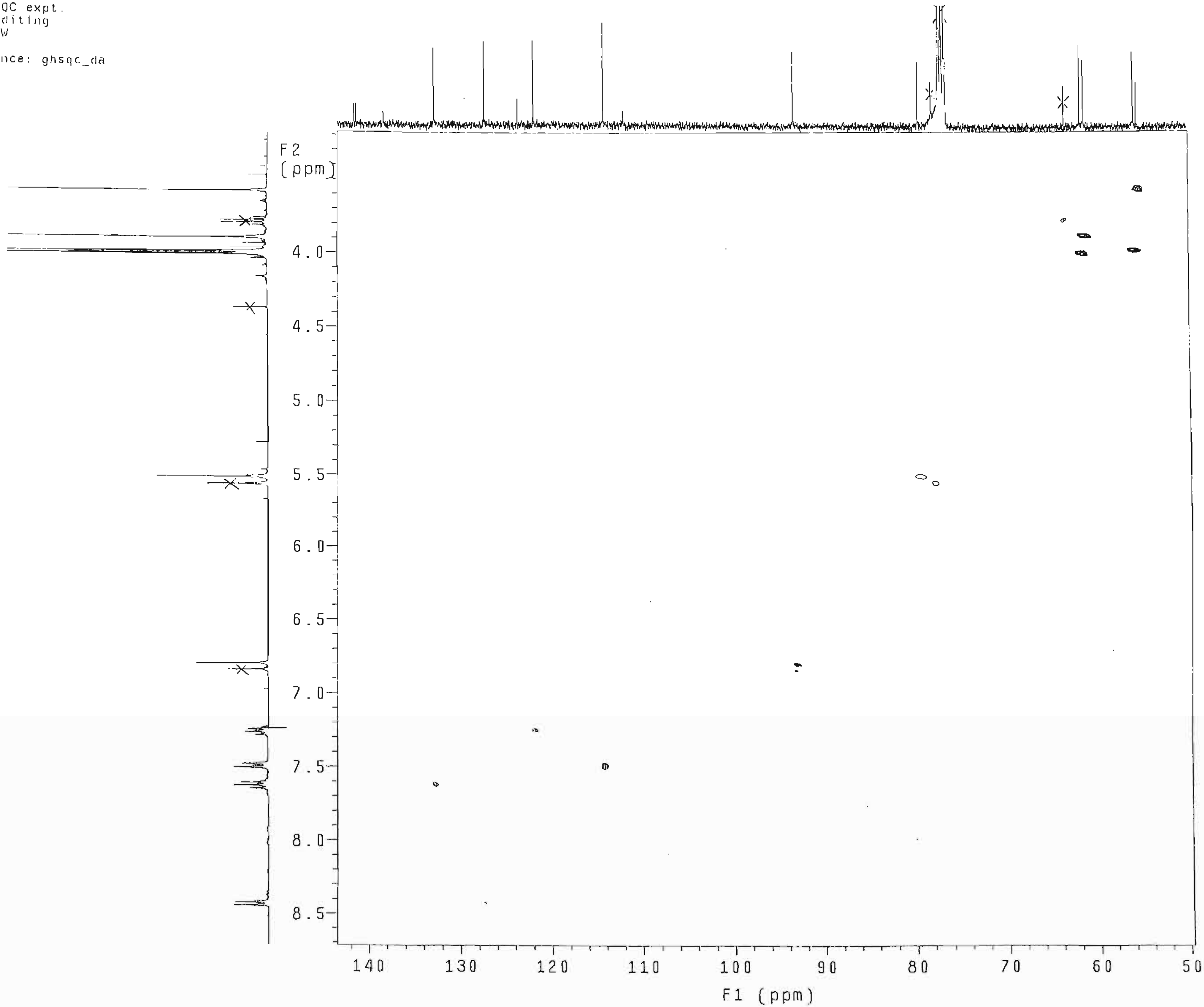


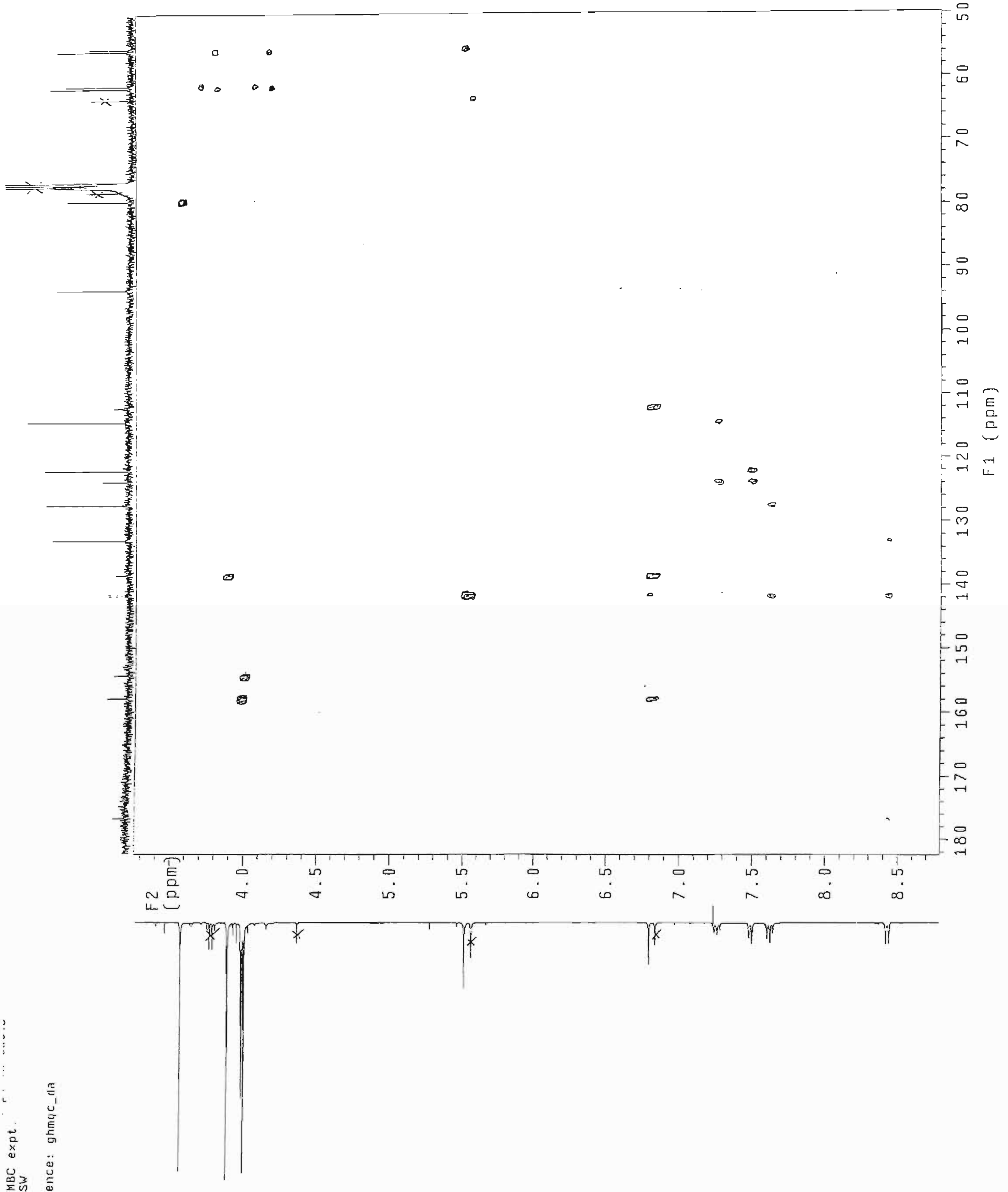
(iv)

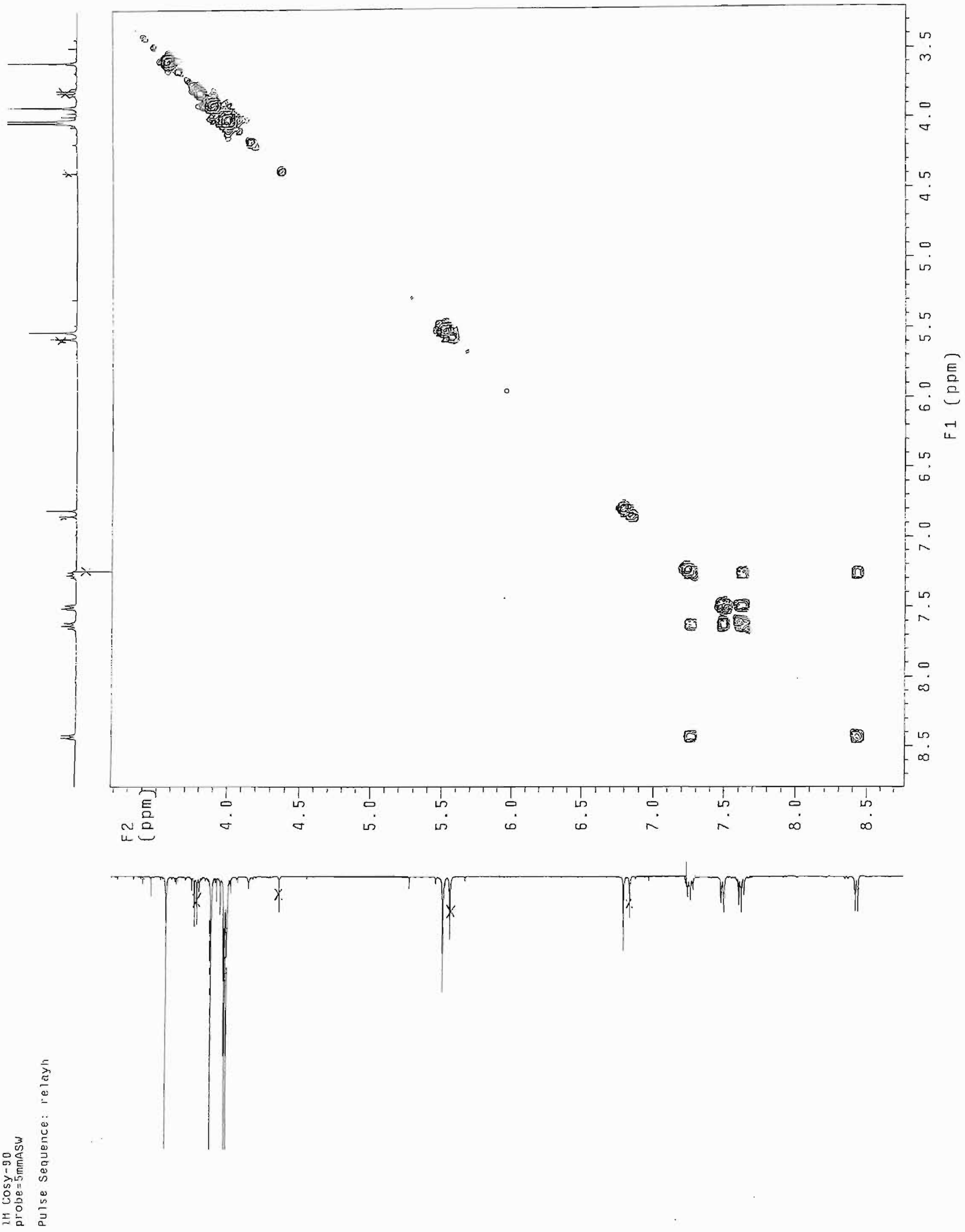


Gradient HSQC expt.
with mult.editing
probe=5mmASW

Pulse Sequence: ghsqc_da




Gradient HMBC expt.
probe=5mmASW
Pulse Sequence: ghmqc_da
F2 (ppm)
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
F1 (ppm)
50
60
70
80
90
100
110
120
130
140
150
160
170
180


1H Cosy-90
probe=5mmASW
Pulse Sequence: relayh
F1 (ppm)
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
F2 (ppm)
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5

[illegible] in chcl3
NOESY expt.
with presat_chcl3
mix=1sec
probe=5mmASW

Pulse Sequence: noesy_da