

**ANTIPLASMODIAL ANTHRACENE DERIVATIVES
FROM SOME KENYAN *ALOE* AND *BULBINE*
SPECIES**

BY

JOHN MURABA WANJOHI

DEPARTMENT OF CHEMISTRY

UNIVERSITY OF NAIROBI

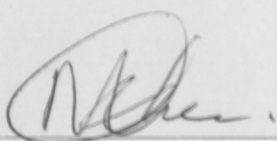


**A THESIS SUBMITTED IN FULFILMENT FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY IN
CHEMISTRY AT THE DEPARTMENT OF CHEMISTRY,
UNIVERSITY OF NAIROBI**

AUGUST 2005

DECLARATION

I hereby declare that all the material in this thesis is my work and has not been presented for a degree in any university

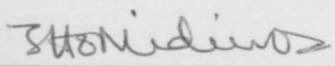
Signed  _____

John Muraba Wanjohi

Department of Chemistry

University of Nairobi

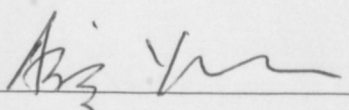
I hereby certify that this thesis has been submitted for examination with my approval as the university supervisor.

Signed  _____

Professor J.O. Midiwo

Department of Chemistry

University of Nairobi

Signed  _____

Dr. Abiy Yenesew

Department of Chemistry

University of Nairobi

DEDICATION

This work is dedicated to my wife Loise Wangui, my children, Richard Wanjohi, Grace Micere, and Stella Wambui, my parents, brothers and sisters.

The beginning of this work, its progress and successful completion could never have been accomplished without the most worthy advice from my supervisors, Professor Jacob O. Njiru and Dr. Abby Yencov. They devoted a lot of their effort and time to my work and my thanks to them is so great that I lack proper words to express myself. I thank the University of Nairobi especially the Department of Chemistry for providing me with space and time to carry out this research and employment through which I could cater for my financial needs. I wish to greatly thank the German Academic Exchange Services (DAAD) for sponsoring the project. My thanks also go to Professor Jörn Heide of the Institut für Organische Chemie, Justus Liebig Universität, Gießen, Germany, for being my host during my six months study visit in Germany. Also acknowledged are Professor Martin Frier and Mathias Heiderich of Potsdam University, Germany, for their assistance in spectroscopic analysis of my samples. The support by the Deutsche Forschungsgemeinschaft, Germany, Grant No. Pe 264/14-2 and by the Bundesministerium fuer Wissenschaften, Grant No. Pe264/14-3, is acknowledged too. Professor Gerhard Bruggemann and Manfred Brömmel of Institut für Organische Chemie, Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany are acknowledged for CD analysis. Dr. Leggett is acknowledged for having carried out the X-Ray crystallographic analysis. Dr. Norman C. Waters, co-workers, P. Bwala & H. Akala and Kenya Industrial Research Institute (KARI) are greatly acknowledged for single-crystal analysis.

ACKNOWLEDGEMENT

The beginning of this work, its progress and successful completion could never have been accomplished without the close, brotherly advice from my supervisors, Professor Jacob O. Midiwo and Dr. Abiy Yenesew. They devoted a lot of their effort and time to my work and my thanks to them is so great that I lack proper words to express myself. I thank the University of Nairobi especially the Department of Chemistry for providing me with space and time to carry out this research and employment through which I could cater for my financial needs. I wish to greatly thank the German Academic Exchange Services (DAAD) for sponsoring the project. My thanks also go to Professor Junes Ipaktsi of the Institut für Organische Chemie, Justus Leibig Universität Gießen, Germany, for being my host during my six months study visit in Germany. Also acknowledged are Professor Martin Peter and Matthias Heiderich of Potsdam University, Germany, for their assistance in spectroscopic analysis of my samples. The support by the Deutsche Forschungsgemeinschaft, Germany, Grant No. Pe 264/14-2 and by the Bundesministerium fuer Zusammenarbeit, Grant No. Pe264/14-3. is acknowledged too. Professor Gerhard Bringmann and Matthias Breuning of Institut für Organische Chemie, Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany are acknowledged for CD analysis. Dr. Lisgarten is acknowledged for having carried out the X-Ray crystallographic analysis. Dr. Norman C.. Waters, co-workers, P. Iiyala & H. Akala and Kenya Medical Research Institute (KEMRI) are greatly acknowledged for antiplasmodial analysis.

The continued support and encouragement from my loved wife, Loise Wangui, was behind the total success of this project. I thank her very very much. My parents, children and other family members had a lot to do with the success of this work and they are greatly acknowledged.

Mr S. G. Mathenge is thanked for identification of the plant materials. Solomon Derese has all along been instrumental to the completion of this work and I highly acknowledge his brotherly assistance. I do also remember all the staff members of Chemistry Department who were all along very cooperative, supportive and generous during the research period. Those whom I did not mention and yet participated in the success of this work are also acknowledged. Most thanks and praise be to the Almighty God for being behind everything good in my life.

LIST OF TABLES.....	XIV
---------------------	-----

LIST OF SCHEMES.....	XVII
----------------------	------

APPENDICES.....	XVIII
-----------------	-------

ABBREVIATIONS.....	XX
--------------------	----

ABSTRACT.....	XXI
---------------	-----

CHAPTER ONE.....	1
------------------	---

INTRODUCTION.....	1
-------------------	---

1.1 GENERAL INTRODUCTION.....	1
-------------------------------	---

TABLE OF CONTENTS

DECLARATION.....	I
SIGNED.....	I
DEDICATION.....	II
ACKNOWLEDGEMENT.....	III
TABLE OF CONTENTS	V
LIST OF FIGURES	XII
LIST OF TABLES	XIV
LIST OF SCHEMES	XVII
APPENDICES	XVIII
ABBREVIATIONS	XX
ABSTRACT	XXI
CHAPTER ONE	1
INTRODUCTION.....	1
1.1 GENERAL INTRODUCTION.....	1

1.2	JUSTIFICATION	4
	CHAPTER TWO	5
	LITERATURE REVIEW	5
2.1	THE FAMILY LILIACEAE	5
2.2	THE GENUS <i>ALOE</i>	5
2.2.1	TROPICAL AFRICAN GROUPS	9
2.2.2	THE SAPONARIAE GROUP	10
2.2.3	<i>ALOE KILIFIENSIS</i> , <i>A. LATERITIA</i> AND <i>A. GRAMINICOLA</i>	11
2.3	THE GENUS <i>BULBINE</i>	12
2.4	COMPOUNDS IDENTIFIED IN <i>ALOE</i>	14
2.4.1	ALKALOIDS	14
2.4.2	STEROIDS	15
2.4.3	FLAVONOIDS	16
2.4.4	ANTHRAQUINONES	18
2.4.5	PRE-ANTHRAQUINONES	24

2.4.6	ANTHRONES	26
2.4.7	DIMERIC ANTHRAQUINONES FROM <i>ALOE</i>	30
2.4.8	PYRONES	31
2.4.9	CHROMONES	33
2.4.10	BENZENE/NAPHTHALENE AND FURAN DERIVATIVES	38
2.5	COMPOUNDS IDENTIFIED IN <i>BULBINE</i>	40
2.6	BIOSYNTHESIS IN GENERAL	47
2.7	BIOSYNTHESIS OF ANTHRAQUINONES	49
2.8	MEDICINAL INFORMATION ON <i>ALOE</i>	52
2.9	<i>ALOE</i> TRADE	54
2.10	INFORMATION ON THE USE OF <i>BULBINE</i>	55
2.11	MALARIA	55
	CHAPTER THREE	57
	RESULTS AND DISCUSSION	57
3.1	PRELIMINARY TEST RESULTS	57

3.1.1. INVESTIGATION OF <i>ALOE</i> SPECIES	57
3.1.2 INVESTIGATION OF <i>BULBINE</i> SPECIES.....	57
3.2 CHARACTERIZATION OF THE COMPOUNDS	58
3.2.1 COMPOUNDS FROM THE SUBTERRANEAN STEMS OF <i>ALOE</i> <i>LATERITIA</i> VAR. <i>LATERITIA</i> , <i>A. KILIFIENSIS</i> , AND <i>A.</i> <i>GRAMINICOLA</i>	58
COMPOUND 1.....	58
COMPOUND 2.....	60
COMPOUND 3.....	61
COMPOUND 3B.....	63
COMPOUND 4.....	65
COMPOUND 5.....	70
COMPOUND 6.....	72
COMPOUND 7.....	74
COMPOUND 8.....	75
COMPOUND 9.....	79

COMPOUND 10.....	81
COMPOUND 11.....	84
COMPOUND 12.....	86
3.2.2 COMPOUNDS FROM THE FRUITS, ROOTS AND STEMS OF	
<i>BULBINE ABYSSINICA</i> AND <i>B. FRUTESCENS</i>	87
COMPOUND 13.....	87
COMPOUND 14.....	91
COMPOUND 15.....	93
COMPOUND 16.....	99
COMPOUND 17.....	100
COMPOUND 18.....	101
COMPOUND 19.....	102
COMPOUND 20.....	103
3.2.3 CHARACTERIZATION OF COMPOUNDS ISOLATED FROM	
ROOTS OF <i>BULBINE FRUTESCENCE</i>	105
COMPOUND 21.....	105

COMPOUND 22.....	106
COMPOUND 23.....	107
COMPOUND 24.....	110
COMPOUND 25.....	110
COMPOUND 26.....	112
COMPOUND 27.....	114
COMPOUND 28.....	114
COMPOUNDS 29, 30, AND 31.....	118
3.3 BIOLOGICAL ACTIVITIES OF CRUDE EXTRACTS AND PURE COMPOUNDS	119
3.3.1 ANTIBACTERIAL TESTS	119
3.3.2 ANTIPLASMODIAL ASSAY	120
3.4 CONCLUSION AND RECOMMENDATIONS.....	123
3.4.1 CONCLUSION	123
3.4.2 RECOMMENDATIONS.....	125
CHAPTER FOUR.....	127

EXPERIMENTAL.....	127
4.1 GENERAL.....	127
4.2 PLANT MATERIAL.....	127
4.3 EXTRACTION AND ISOLATION.....	128
4.3.1 ALOE LATERITIA VAR. LATERITIA.....	128
4.3.2 ALOE KILIFIENSIS.....	129
4.3.3 ALOE GRAMINICOLA	131
4.3.4 BULBINE ABYSSINICA (FRUITS).....	132
4.3.5 BULBINE ABYSSINICA (ROOTS).....	133
4.3.6 BULBINE FRUTESCENS (STEMS AND ROOTS).....	134
4.3.7 BULBINE FRUTESCENS (ROOTS).....	135
4.4 AERIAL OXIDATION	137
4.5 COMPUTATIONAL.....	138
4.6 PHYSICAL AND SPECTROSCOPIC DATA.....	139
4.7 BIOLOGICAL ACTIVITY	149

4.7.1: ANTI-BACTERIAL	149
4.7.2 IN VITRO ANTI-PLASMODIAL ACTIVITY.....	150
REFERENCES.....	153
MASS SPECTRUM.....	228
MASS SPECTRUM.....	228
HMBC EXPANSION.....	237

LIST OF FIGURES

FIGURE 2.1 PHOTOS OF SOME <i>ALOE</i> PLANTS.....	6
FIG. 2.1B PICTURES OF <i>ALOE KILIFIENSIS</i> AND <i>A. GRAMINICOLA</i> TAKEN FROM A BOTANICAL GARDEN BEHIND CHEMISTRY BUILDING, CHIROMO, IN FEBRUARY 2006.....	8
FIGURE 2.2 PICTURES OF SOME <i>BULBINE</i> SPECIES.....	12
FIG 2.2B PHOTOS OF <i>BULBINE ABYSSINICA</i> AND <i>B. FRUTESCENS</i> TAKEN FROM CHEMISTRY GARDEN	13
FIG. 2.3 <i>ALOE</i> MARKET CHAIN IN WEST POKOT, KENYA.....	54

FIGURE 3.1: ASPHODELIN 3D STRUCTURE BY X-RAY CRYSTAL ANALYSIS	62
FIG. 3.2 CD CURVE FOR ASPHODELIN (3).....	62
FIG. 3.2B CD CALCULATIONS FOR COMPOUND 3B USING THE CNDO/ SC-I AND OM2-SCI	64
FIGURE 3.3: HMBC CORRELATION FOR ASPHODELIN-10'- α -L-RHAMNOSIDE (4).....	66
FIGURE 3.3 CMD CNDO/SC-I CALCULATION FOR COMPOUND 4	68
FIGURE 3.3 DBM OM2-SCI AND MD-OM2-SCI CALCULATION FOR COMPOUND 4.....	69
FIG. 3.3E TWO FOLDING TYPES OF THE OCTAKETIDE CHAIN.....	78
FIG. 3.4. ATTRIBUTION OF THE ABSOLUTE CONFIGURATION OF ABYQUINONE A (13) BY COMPARISON OF THE EXPERIMENTAL CD SPECTRUM WITH THE SPECTRA CALCULATED FOR (<i>P</i>)-13 AND (<i>M</i>)-13; A) USING THE CNDO/S AND B) THE OM2 HAMILTONIAN.....	90
FIG. 3.5. ASSIGNMENT OF THE ABSOLUTE CONFIGURATION OF ABYQUINONE B (14) BY COMPARISON OF THE EXPERIMENTAL CD SPECTRUM WITH THE SPECTRA CALCULATED FOR (<i>S</i>)-14	

AND (<i>R</i>)-14 USING THE OM2 HAMILTONIAN WITH A) A SCI AND B) A SDCI CALCULATION.....	92
FIG. 3.6 ATTRIBUTION OF THE ABSOLUTE CONFIGURATION OF ABYQUINONE C (15) BY COMPARISON OF THE EXPERIMENTAL CD SPECTRUM WITH THE SPECTRA CALCULATED FOR A) (10 <i>S</i> ,3' <i>R</i>)-15 AND (10 <i>R</i> ,3' <i>S</i>)-15 AND B) (10 <i>S</i> ,3' <i>S</i>)-15 AND (10 <i>R</i> ,3' <i>R</i>)-15 USING IN BOTH CASES THE OM2 HAMILTONIAN WITH AN SCI CALCULATION.....	95
FIG. 3.7. CENTER-TO-AXIS CHIRALITY TRANSFER FROM ABYQUINONE B (15) TO ABYQUINONE A (13). THE <i>R</i> - CONFIGURATION IN 14 INDUCES A <i>P</i> -CONFIGURED CHIRAL AXIS IN 13 DUE TO THE PRE-ORIENTATION INITIATED BY THE HYDROGEN BOND BETWEEN OH-4 AND OH-8'; BOTH STRUCTURES SHOWN CONSTITUTE GLOBAL AM1 MINIMUM GEOMETRIES.	97

LIST OF TABLES

TABLE 1.1 LEADING CAUSES OF DEATH IN CHILDREN UNDER FIVE YEARS OF AGE, ESTIMATES FOR 2000-2003.....	3
TABLE 2.1: REYNOLD'S GROUPING OF TROPICAL AFRICAN ALOES ..	9
TABLE 2.2: MORPHOLOGICAL FEATURES FOR <i>ALOE KILIFIENSIS</i> , <i>A.</i> <i>LATERITIA</i> AND <i>A. GRAMINICOLA</i>	11

TABLE 2.3: ANTHRAQUINONES OF <i>BULBINE</i>	41
TABLE 2.4 PHENYLANTHRAQUINONES OF <i>BULBINE</i>	41
TABLE 2.5 FURANONAPHTHOQUINONES OF <i>BULBINE</i>	42
TABLE 2.6 MISCELLANEOUS COMPOUNDS OF <i>BULBINE</i>	43
TABLE 3.1: ^1H -NMR DATA FOR COMPOUNDS 1, 3, 4 AND 5	71
TABLE 3.2: ^{13}C -NMR DATA FOR COMPOUNDS 3, 4 & 5.....	72
TABLE 3.3: ^1H NMR DATA FOR COMPOUNDS 6, 7 AND 8.....	77
TABLE 3.4: THE ^{13}C NMR DATA FOR COMPOUNDS 6 AND 8	78
TABLE 3.5: NMR DATA FOR PREANTHRAQUINONES 9, 10 AND 11	85
TABLE 3.6: ^1H NMR DATA (δ) TOGETHER WITH HMBC (2J AND 3J) CORRELATIONS OF FOR COMPOUNDS 13, 14, 15.....	98
TABLE 3.7: ^{13}C NMR DATA FOR COMPOUNDS 13, 14, 15	99
TABLE 3.8: ^1H NMR FOR COMPOUND 17, 18, 19 AND 20.....	104
TABLE 3.9: ^{13}C NMR FOR COMPOUNDS 19 AND 20	104
TABLE 3.10: NMR DATA FOR ELEUTHEROL-9-METHYLETHER (23) ...	108
TABLE 3.11: ^1H AND ^{13}C NMR DATA FOR COMPOUNDS 24 AND 25.....	111

TABLE 3.12: ^1H (500 MHZ) OF 26, 27 AND 28, TOGETHER WITH HMBC (2J AND 3J) CORRELATIONS FOR 26	116
TABLE 3.13: ^{13}C (125 MHZ) OF 26, 27 AND 28.....	117
TABLE 3.14: ^1H NMR DATA FOR COMPOUNDS 30 AND 31	119
TABLE 3.15: ANTIBACTERIAL TEST RESULTS FOR SOME <i>A. KILIFIENSIS</i> CRUDE EXTRACTS AND SOME PURE COMPOUNDS FROM THE EXTRACTS	120
TABLE 3.16: IN-VITRO IC_{50} VALUES AGAINST W2 AND D6 ISOLATES OF <i>PLASMODIUM FALCIPARUM</i> FOR CRUDE EXTRACTS AND PURE SAMPLES FROM <i>ALOE</i> AND <i>BULBINE</i> SPECIES.....	121
TABLE 3.17 : MORE IN-VITRO ANTIPLASMODIAL ACTIVITIES AGAINST W2 ISOLATE OF <i>PLASMODIUM FALCIPARUM</i>	122
TABLE 4.1: PLANT COLLECTION.....	128
TABLE 4.2: ANTIBACTERIAL RESULTS	150
TABLE 4.3: IN VITRO ANTIPLASMODIAL ACTIVITIES AGAINST <i>PLASMODIUM FALCIPARUM</i>	151
TABLE 4.3B: MORE IN-VITRO ANTIPLASMODIAL ACTIVITIES AGAINST W2 ISOLATE OF <i>PLASMODIUM FALCIPARUM</i>	152

LIST OF SCHEMES

SCHEME 2.1: CLASSIFICATION OF THE FAMILY LILIACEAE.....	5
SCHEME 2.1: MAIN STREAMS OF SECONDARY METABOLISM (TORSSEL, 1983)	48
SCHEME 2.2: POLYKETIDE BIOGENETIC PATHWAY LEADING TO SOME ANTHRAQUINONES.....	50
SCHEME 2.3 BIOSYNTHETIC PATHWAY LEADING TO ANTHRAQUINONES IN THE RUBIACEAE.....	51
SCHEME 3.1: EIMS FRAGMENTATION OF CHRYSOPHANOL (1).....	60
SCHEME 3.3 EIMS FRAGMENTATION OF LACCAIC ACID D METHYL ESTER (8).....	77
SCHEME 3.4: MASS FRAGMENTATION PATTERN OF PRECHRYSOPHANOL (9).....	80
SCHEME 3.5: CONVERSION OF PRECHRYSOPHANOL (9) INTO CHRYSOPHANOL (1).....	80
SCHEME 3.5B: ALTERNATIVE MECHANISM FOR THE CONVERSION OF PRECHRYSOPHANOL (9) INTO CHRYSOPHANOL (1).....	81
SCHEME 3.6 EIMS FRAGMENTATION IN ALOESAPONOL I (10)	83

SCHEME 3.8: PROPOSED BIOGENESIS OF

ISOFURANONAPHTHOQUINONES IN <i>BULBINE</i>	109
--	-----

SCHEME 3.8B: PROPOSED PATHWAY FOR THE FORMATION OF 4-

DEMETHYLELEUTHEROL	109
--------------------------	-----

APPENDICES

APPENDIX I: SPECTRA FOR COMPOUND 1	168
APPENDIX II SPECTRA FOR COMPOUND 3	170
APPENDIX III SPECTRA FOR COMPOUND 4	179
APPENDIX IV: SPECTRA FOR COMPOUND 6	191
APPENDIX V SPECTRA FOR COMPOUND 7	196
APPENDIX V SPECTRA FOR COMPOUND 8	200
APPENDIX VI SPECTRA FOR COMPOUND 9	204
APPENDIX VII SPECTRA FOR COMPOUND 11	206
APPENDIX IX SPECTRA FOR COMPOUND 12	208
APPENDIX X SPECTRA FOR COMPOUND 13	210
APPENDIX XI SPECTRA FOR COMPOUND 14	220
APPENDIX XIII SPECTRA FOR COMPOUND 15	229
APPENDIX XIV SPECTRA FOR COMPOUND 16	238
APPENDIX XV SPECTRA FOR COMPOUND 17	239
APPENDIX XVI SPECTRA FOR COMPOUND 18	242
APPENDIX XVII SPECTRA FOR COMPOUND 19	243
APPENDIX XVIII SPECTRA FOR COMPOUND 20	244
APPENDIX XIX SPECTRA FOR COMPOUND 21	246
APPENDIX XX SPECTRA FOR COMPOUND 22	248

APPENDIX XXI SPECTRA FOR COMPOUND 23	250
APPENDIX XXIII SPECTRA FOR COMPOUND 25.....	261
APPENDIX XXIV SPECTRA FOR COMPOUND 26.....	265
APPENDIX XXV SPECTRA FOR COMPOUND 27.....	274
APPENDIX XXVI SPECTRA FOR COMPOUND 28.....	282
APPENDIX XXVIII SPECTRA FOR COMPOUNDS 30 AND 31	291

HMBC	Heteronuclear multiple bond correlation (^1H , ^{13}C)	HMQC	Heteronuclear multiple quantum coherence (^1H , ^{13}C)
NOE	Nuclear overhauser effect	DEPT	Distortionless enhanced polarization transfer
NMR	Nuclear magnetic resonance	λ_{max}	Maximum wavelength of absorption
UV	Ultra violet	δ	Chemical shift
MS	Mass spectrometry	J	Coupling constant
Hz	Hertz	MHz	Mega hertz
D	Doublet	s	Singlet
dd	Doublet, singlet	dd	Doublet of a doublet
t	Triplet	m	Multiplet (multiplicity)
MALDI-TOF	Matrix assisted laser desorption/ionization time-of-flight	$[\alpha]$	Specific rotation measured with sodium D-line light (589 nm) at 25°C
TLC	Thin layer chromatography	PTLC	Preparative thin layer chromatography
DMF	Dimethylformamide	MeOH	Methanol
EtOH	Ethanol	mp	Melting point
NE	Not tested	NA	Not active
ADNOC	Modified Information Neglect of Differential Overlap, Version 1		
MDA-M	Modified Neglect of Differential Overlap		
PA-3	MRCO package for Method Number 3		
CNDO	Complete Neglect of Differential Overlap		
ANI	Amide Ion		

ABBREVIATIONS

m/z	Mass to charge ratio	$[M]^+$	Molecular ion
CD	Circular dichroism	PKR	Polyketide reductase
IC ₅₀	Concentration of 50% inhibition	LC ₅₀	Concentration of 50% lethality
NOESY	Nuclear overhauser and exchange spectroscopy	EIMS	Electron ionization mass spectroscopy
HMBC	Heteronuclear multiple bond correlation ($^2J_{CH}$, $^3J_{CH}$)	HMQC	Heteronuclear multiple quantum coherence ($^1J_{CH}$)
NOE	Nuclear overhauser effect	DEPT	Distortionless enhanced polarization transfer
NMR	Nuclear magnetic resonance	λ_{max}	Maximum wavelength of absorption
UV	Ultra violet	δ	Chemical shift
MS	Mass spectroscopy	J	Coupling constant
Hz	Hertz	MHz	Mega hertz
D	Doublet	s	Singlet
Brs	Broad singlet	dd	Doublet of a doublet
t	Triplet	m	Multiplet (multiplicity)
MALDI-TOF	Matrix assisted laser desorption/ionization-time-of-flight	$[\alpha]_D^{25}$	Specific rotation measured with sodium D-line light (589 nm) at 25°C
TLC	Thin layer chromatography	PTLC	Preparative thin layer chromatography
DCM	Dichloromethane	MeOH	Methanol
EtOAc	Ethylacetate		
		mp	Melting point
NT	Not tested	NA	Not active
MINDO/3	Modified Intermediate Neglect of Differential Overlap, Version 3		
MNDO	Modified Neglect of Diatomic Overlap		
PM3	MNDO Paramagnetic Method Number 3		
CNDO	Complete Neglect of Differential Overlap		
AMI	Austin Model I		

ABSTRACT

The roots and stems of three *Aloe* species (*A. kilifiensis*, *A. lateritia* and *A. graminicola*) and two *Bulbine* species (*Bulbine abyssinica* and *Bulbine frutescens*) were serially extracted by cold percolation with n-hexane, dichloromethane and methanol. Some of the extracts were tested against the chloroquine-sensitive (D6) and the chloroquine-resistant (W2) strains of *Plasmodium falciparum*. A combination of chromatographic methods was used to obtain pure compounds from some of the extracts. This led to the isolation of thirty one compounds which were characterized using spectroscopic techniques such as NMR and MS.

From the subterranean stems of *Aloe lateritia* four anthraquinones, namely chrysophanol (1), aloesaponarin II (6), aloesaponarin I (7), and lacaic acid D-methyl ester (8); three pre-anthraquinones, namely prechrysophanol (9), aloesaponol I (10) and aloesaponol II (11); two anthraquinone dimers namely asphodelin (3b), and 4,7'-dichrysophanol-10'- α -L-rhamnoside (4); and one naphthalene derivative namely isoelutherol (12) were isolated.. Compounds 1, 3, 4, 6, 7, 9, 10, and 11 were also identified in *Aloe kilifiensis*. Similarly, 1, helminthosporin (2), 3b, 4, the bi-chrysophanol glycoside 4,7'-dichrysophanol-10'- β -D-antiarose (5), 7 and 12, were identified in *Aloe graminicola*. Compounds 4 and 5 are reported for the first time in the genus *Aloe*.

The two asphodelin glycosides, 4,7'-dichrysophanol-10'- α -L-rhamnose (4) and 4,7'-dichrysophanol-10'- β -D-antiarose (5) were present in the three *Aloe* species (*A. kilifiensis*, *A. lateritia* var. *lateritia* and *A. graminicola*) belonging to the Saponarica

group. These compounds were absent in *A. secundiflora*, *A. kendongensis* and *A. rabaiensis*, (the three species represent other groups of *Aloe*), which suggested that the asphodelin glycosides could be chemotaxonomic markers of the Saponarica group.

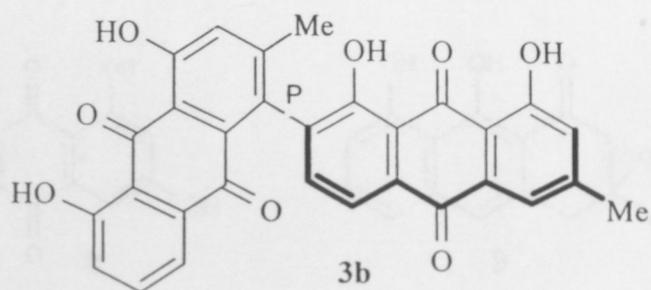
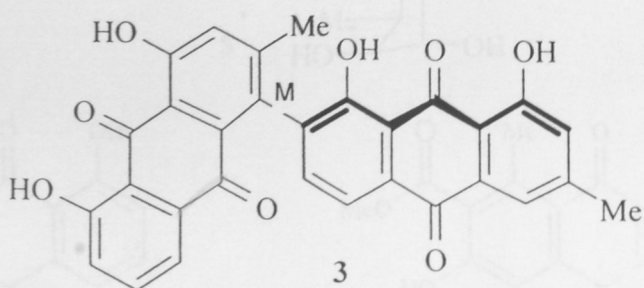
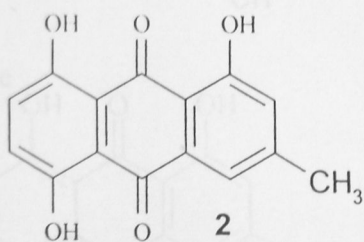
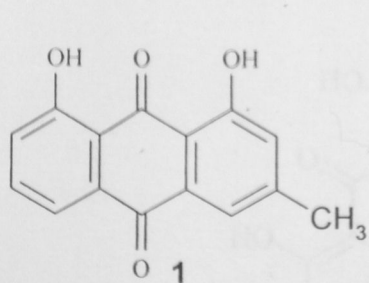
The stereochemistry at the biaryl linkage (C4-C7') for a sample of asphodelin (3) isolated from *Aloe kilifiensis* stems was determined using CD calculations and found to be M (aR). Using this compound as a reference, the absolute configuration of the asphodelin glycoside (4) was determined as P (aS) from CD data. Another sample of asphodelin (3b) isolated from *Aloe lateritia* was found to be P (aS) at the biaryl linkage using CD calculations.

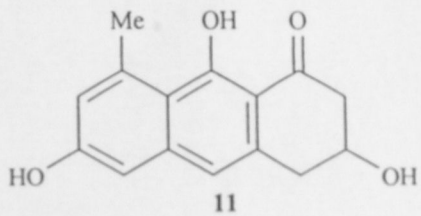
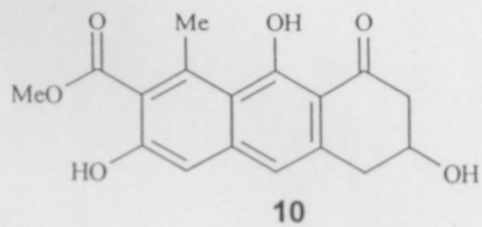
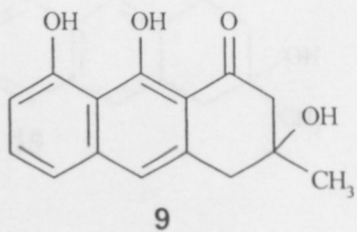
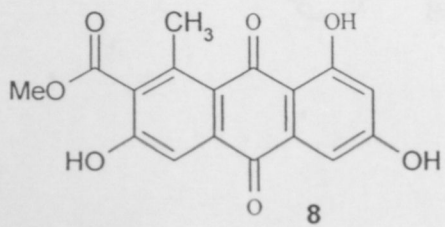
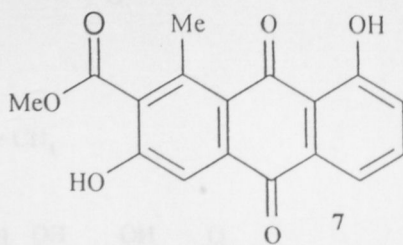
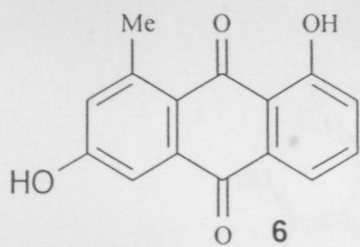
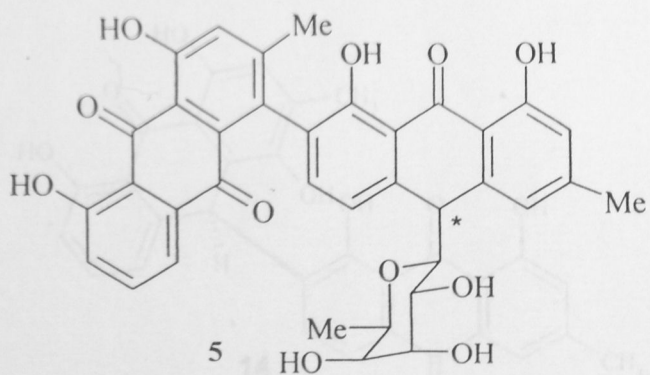
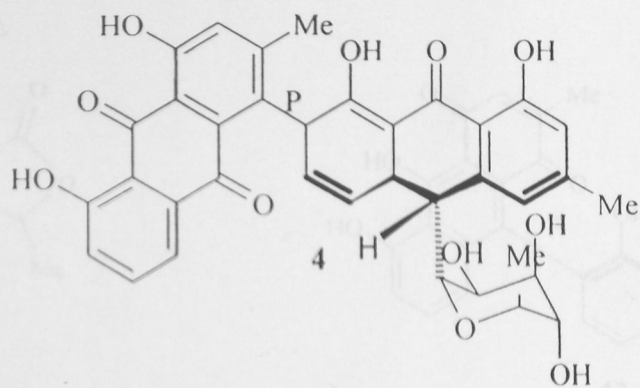
In antiplasmodial assay, aloesaponarin II (6), aloesaponol I (10), aloesaponol II (11), asphodelin (3b) and asphodelin rhamnoside (4) showed appreciable activity against *Plasmodium falciparum*. This suggested that these compounds could be responsible for the activity observed in the crude extracts of *Aloe*. The dichloromethane extract of *Aloe kilifiensis* stems was tested for antibacterial activity. It was found to be active against the gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*. Aloesaponarin I (7) and II (6) isolated from the extract showed activity against both organisms whereas chrysophanol (also from the extract) showed no activity against the two organisms.

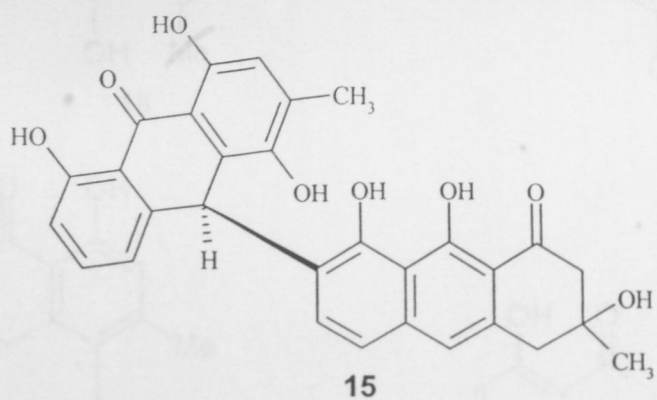
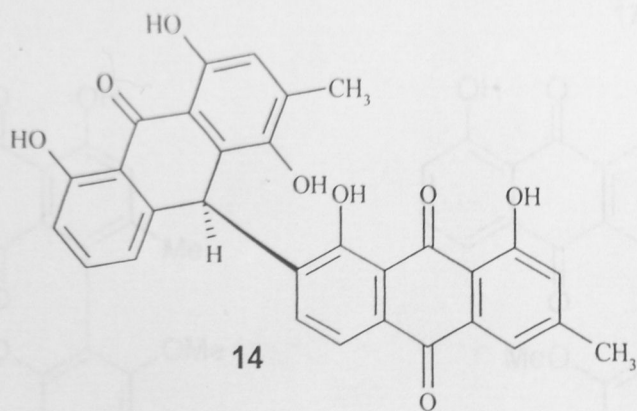
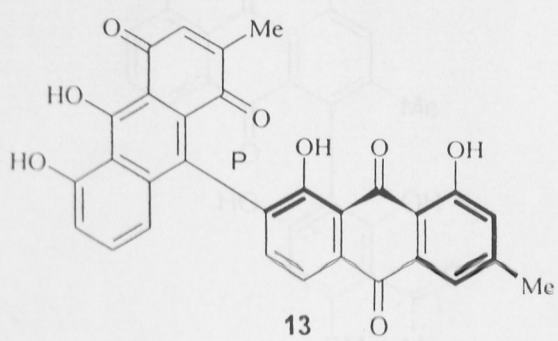
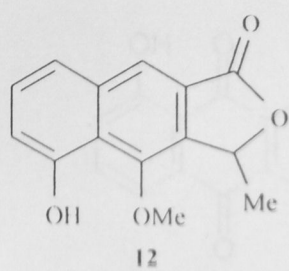
From the fruits of *Bulbine abyssinica* three new dimeric anthracene derivatives, 1',8,8',9-tetrahydroxy-3,3',-dimethyl[7',10-bianthracene]-1,4,9',10'-tetrone (trivial

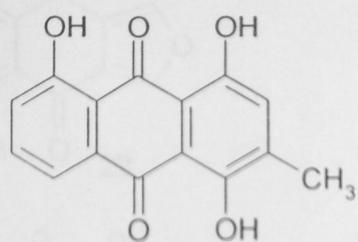
name abyquinone-A) (13), (10*R*)-1,1',4,8,8'-tetrahydroxy-3,3',-dimethyl-[7',10-bianthracene]-9,9',10'(10*H*)-trione (trivial name abyquinone-B) (14) and (3'*R*,10*R*)-3',4'-dihydro-1,3',4,8,8',9'-hexahydroxy-3,3'-dimethyl-[7',10-bianthracene]-1',9(2'*H*,10*H*)-dione (trivial name abyquinone-C) (15) were isolated. The stereochemistry of compounds 13, 14 and 15 was determined using CD calculations. The CD curve obtained experimentally was plotted alongside the CD curve generated mathematically for different conformations of the molecule. The mathematical calculations were performed using different computational techniques (CNDO/S-CI, OM2-S-CI and MD CNDO/S-CI). The conformation whose generated CD curve matched the experimental CD curve was taken to be the correct one for the compound in question. In addition, the known anthraquinones islandicin (16) and chrysophanol (1) were isolated. From the roots of this plant, four anthraquinones containing a phluoroglucinol moiety namely knipholone (17), isoknipholone (18), 6'-*O*-methylknipholone (19) and knipholone anthrone (20) were isolated. Knipholone (17) and isoknipholone (18) were also identified in *B. frutescens* roots. Compounds isolated from the roots of *B. frutescens* were as follows: 5,8-dihydroxy-1-methylnaphtho[2,3-*c*]furan-4,9-dione (21); 1,5,8-trihydroxy-1-methylnaphtho[2,3-*c*]furan-4,9-dione (22); elutherol-9-methyl ether (23); 4'-demethylknipholone (24); 4'-*O*-demethylknipholone-4'-*O*-β-D-glucoside (25); 5,1'-bis-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)1,8,4',5'-tetrahydroxy-6,2'-dimethyl-9'H-[2,9']bianthracenyl-9,10,10'-trione (26a) and its enantiomer (26b); 5,1'-bis-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)1,8,4',5',9'-pentahydroxy-6,2'-dimethyl-9'H-[2,9']bianthracenyl-9,10,10'-trione (27); vanillic acid (28), p-coumeric acid (29); hydro-p-coumaric acid (30).

From *Bulbine*, the compounds, abyquinone-A (**13**), knipholone (**17**), isoknipholone (**18**) and 6'-methoxyknipholone (**19**) showed antiplasmodial activity. The antimalarial activity of knipholone derivatives has been reported. However, this is the first report on the antiplasmodial activity of the derivatives.

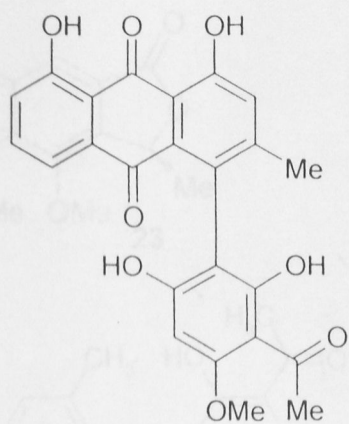




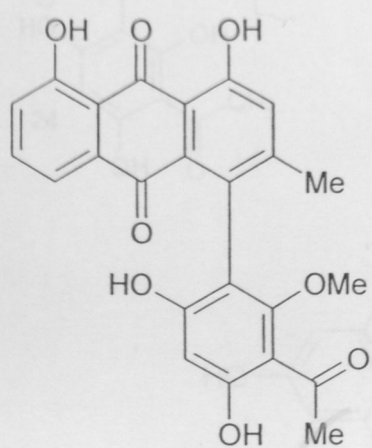




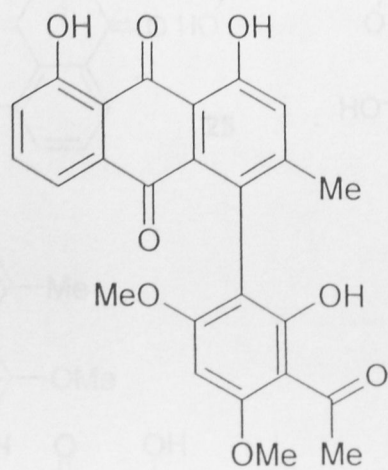
16



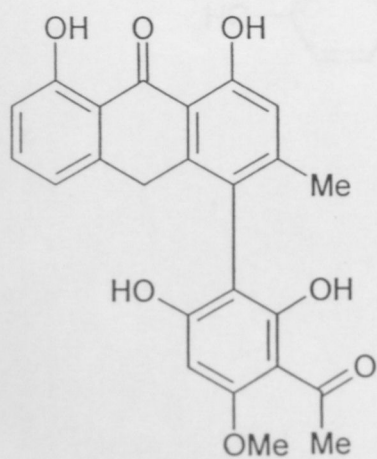
17



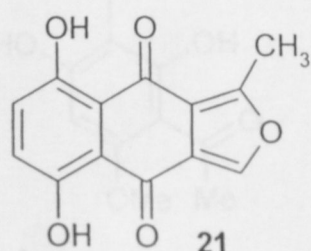
18



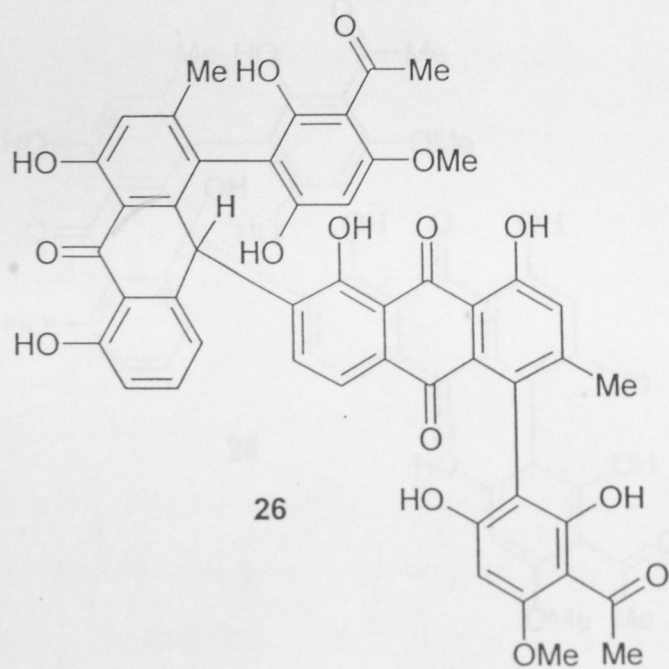
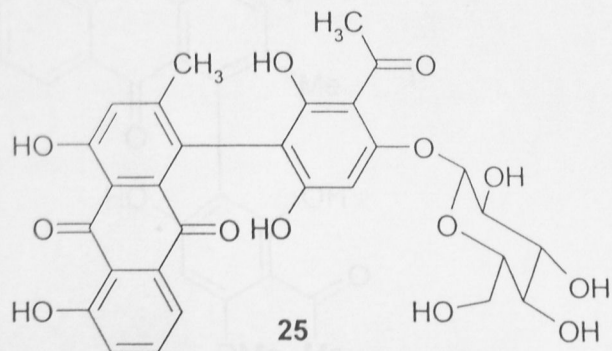
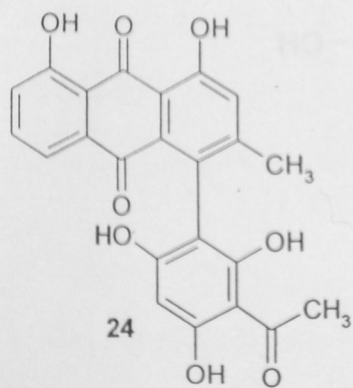
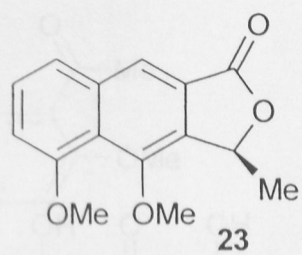
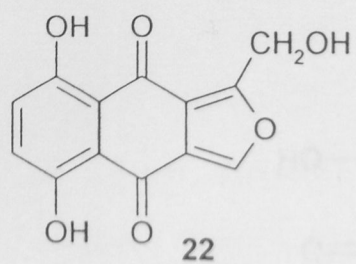
19

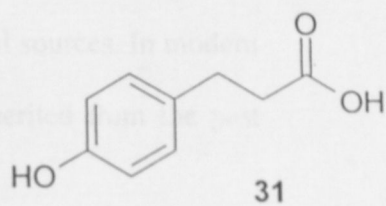
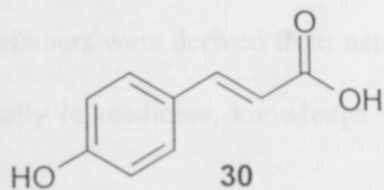
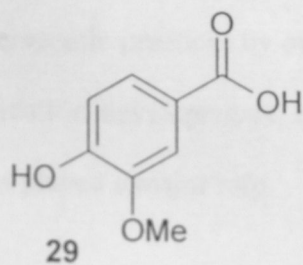
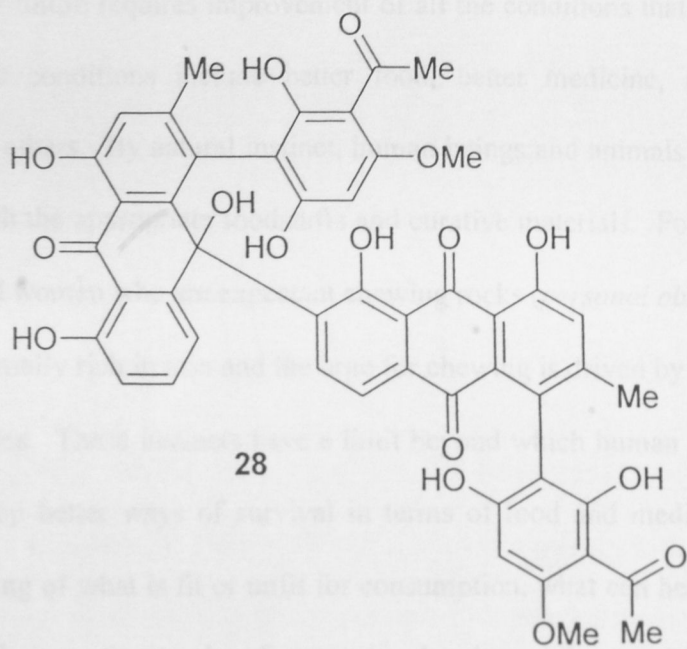
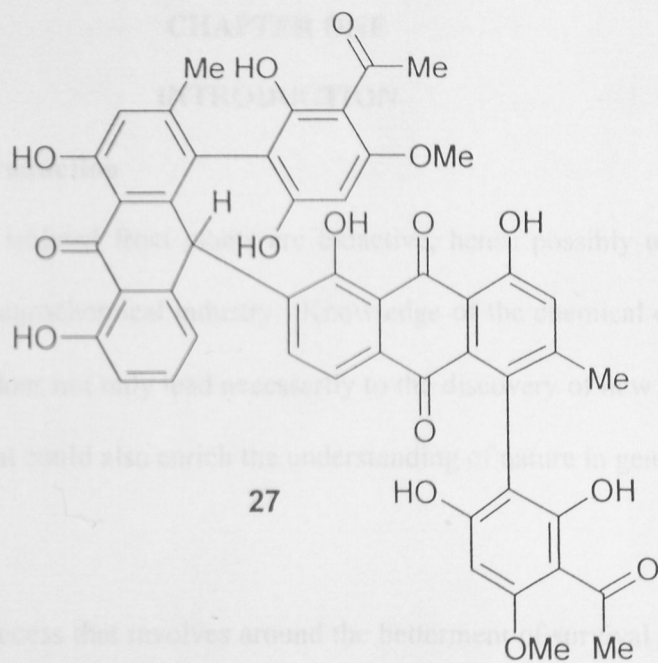


20



21





CHAPTER ONE

INTRODUCTION

1.1 General Introduction

Several compounds isolated from plants are bioactive, hence possibly useful in the pharmaceutical and agrochemical industry. Knowledge of the chemical composition of a natural system does not only lead necessarily to the discovery of new biologically active compounds but could also enrich the understanding of nature in general.

Life is a dynamic process that revolves around the betterment of survival for now and the future. A better future requires improvement of all the conditions that affect life in general. These conditions include better food, better medicine, and better settlement amongst others. By natural instinct, human beings and animals have been able to come up with the appropriate foodstuffs and curative materials. For example, it is common to find women who are expectant chewing rocks (*personal observation*). These rocks are normally rich in iron and the urge for chewing is driven by deficiency of iron in their bodies. These instincts have a limit beyond which human beings had to study and develop better ways of survival in terms of food and medicine. The modern understanding of what is fit or unfit for consumption, what can heal or harm, has taken human beings thousands of years to develop. All materials used in therapeutic practices by our forefathers were derived from natural sources. In modern scientific developments, especially in medicine, knowledge inherited from the past has played a major role.

Modern agricultural practices use chemicals as fertilisers and pesticides. Most of these chemicals are synthetic and not environmentally benign. Solutions to the environmental problems are now being sought through replacement of synthetic agrochemicals with naturally derived materials. Farmers are nowadays being encouraged to use farmyard manure in place of synthetic fertilisers. The use of plant extracts as insecticides is a common practice in some areas of Kenya. The farmers use this method because the synthetic insecticides are extremely expensive and toxic.

Those are just but a few reasons why natural products chemistry has gained much popularity in the recent past and present. A large number of plants are famous for their use in medicine. *Aloe* and *Bulbine* are well known for this. The major compounds in these plants are anthraquinones and related compounds. These compounds could be responsible for the medicinal uses of the plants. Research on the chemical composition of plants is thus a worthy cause..

Malaria is a problem that has far reaching effects in human health, especially, in Africa. The disease accounts for more than half a million clinical cases every year (Krogstand, 1996). It is one of the most severe public health problems worldwide. It is a leading cause of death in many developing countries, where young children and pregnant women are the groups most affected (WHO Health report, 2005). The WHO report on the leading causes of death in children under five years of age is summarized in table 1.1 below.

Table 1.1 Leading Causes of Death in Children Under Five Years of Age, Estimates for 2000-2003

Rank	Cause	Numbers (thousands per year)	% of all deaths
1	Neonatal causes	3,910	37
2	Acute respiratory infections	2,027	19
3	Diarrheal diseases	1,762	17
4	Malaria	853	8
5	Measles	395	4
6	HIV/AIDS	321	3
7	Injuries	305	3
	Other causes	1,022	10
	Total	10,596	100.0

(Source: World Health Organization, The World Health Report 2005)

According to the World Health Organization's World Malaria Report 2005:

At the end of 2004, some 3.2 billion people lived in areas at risk of malaria transmission in 107 countries and territories.

Between 350 and 500 million clinical episodes of malaria occur every year.

At least one million deaths occur every year due to malaria.

About 60% of the cases of malaria worldwide and more than 80% of the malaria deaths worldwide occur in Africa south of the Sahara.

Conventional medicine has turned too expensive for the common man and alternative solutions are needed. As expensive as they are, the drugs turn ineffective because the causal agents mutate and become resistant. There is thus need to seek new cures for malaria and the process has to be repeated consistently because the parasites becomes resistant to the new cure after only a short while. Plants have a good potential for drug discovery for this ailment the drugs

A number of plants in the family Asphodelaceae are known to have anthraquinones (Bringman *et al.*, 2002). *Aloe* and *Bulbine*, both of which are in the family Asphodelaceae are used in traditional medicine for treatment of malaria (Smith and van Wyk (1991). The isolation of compounds from these plants and study of the anti-malarial activity of the isolated compounds is progress towards the discovery of new malaria drugs. A lot of input and concerted effort is needed in order to settle this malaria vice once and for all.

Scheme 1.1: Classification of the Family Liliaceae.

1.2 Justification

Anthracene derivatives such as anthraquinones have been identified as the major compounds in *Aloe* and *Bulbine*. These compounds could be responsible for the potential of the plants in medicine and agriculture.

There are a number of species of *Aloe* and *Bulbine* that grow wildly in Kenya. They are widely used in folk medicine. In several Kenyan communities, one of the major uses of these plants is in the cure of malaria. Information on the chemistry of the Kenyan species of these plants is rarely available if any. There is thus need to generate this information through research. The information would be useful in the classification of the species and also in the identification of the biologically active compounds from the plants. Getting to know which compounds in these plants are responsible for the curing of malaria and other diseases would have far reaching solutions to healthcare in the tropics.

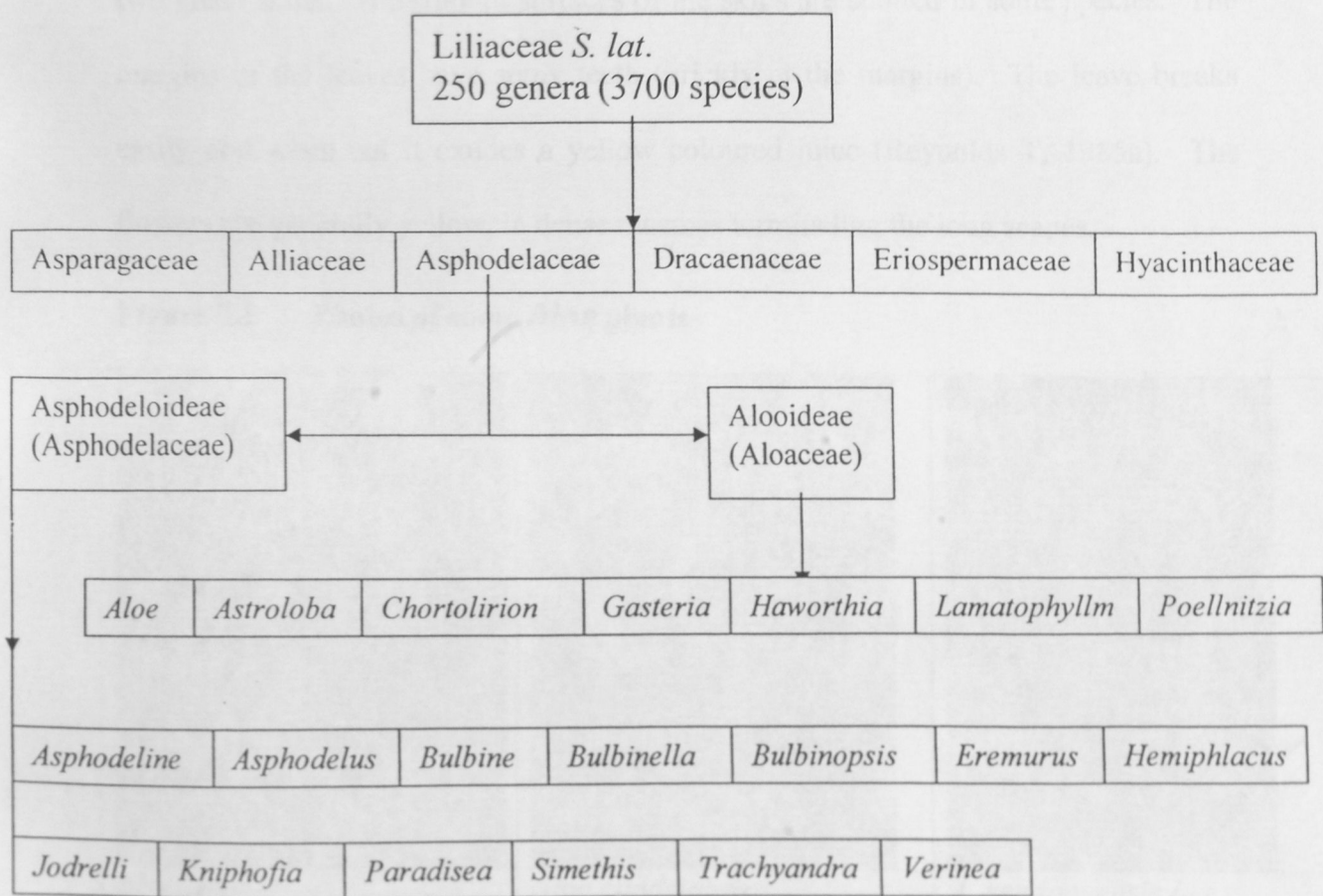
CHAPTER TWO

LITERATURE REVIEW

2.1 The family Liliaceae

Amongst the flowering plants, Liliaceae *S. lat.* is one of the widely distributed families. The family is mostly composed of perennial herbs with rhizomes (Trease and Evans, 1978). The classification of the family Liliaceae is summarized in the flow chart below (Scheme 2.1)

Scheme 2.1: Classification of the family Liliaceae.



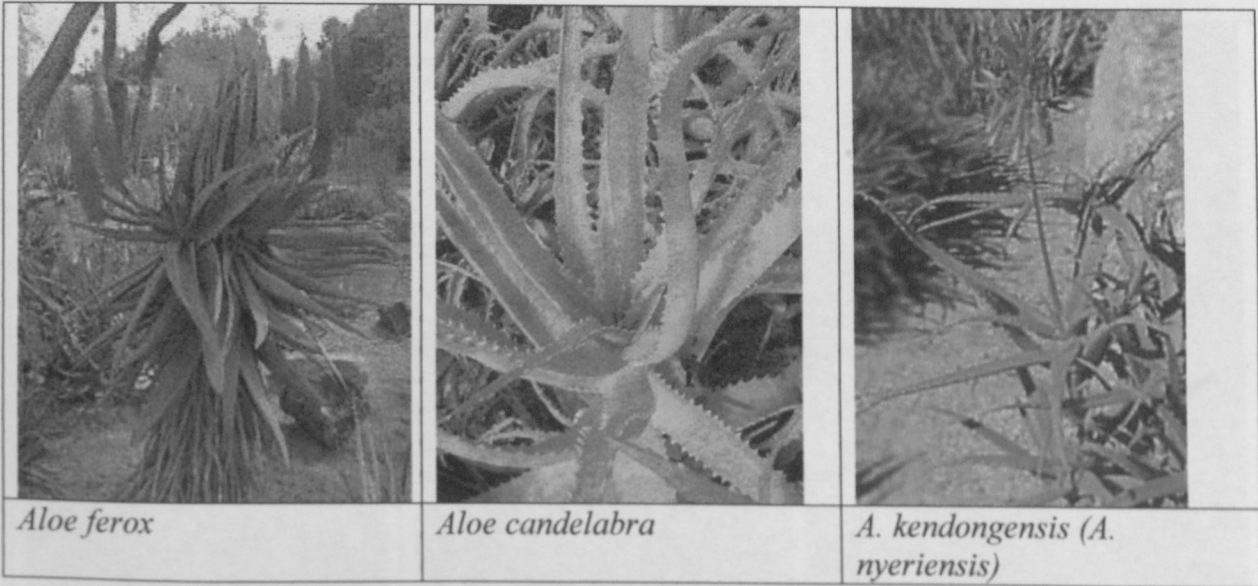
(Yenesew and Dagne. 1993; van Wyk *et al.* 1995a; Smith and van Wyk 1991)

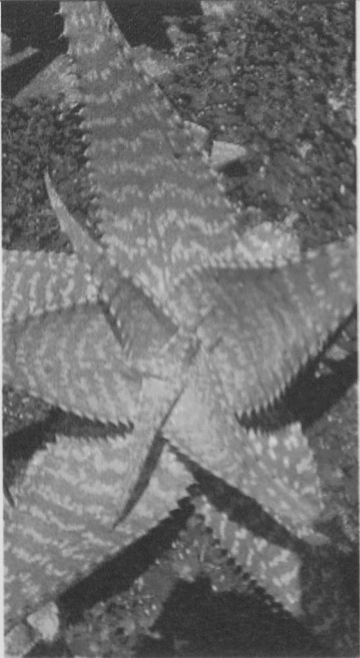
2.2 The Genus Aloe

The genus *Aloe* comprises about 600 species, most of which are found in Africa (Okamura *et al.* 1996). The genus is indigenous to Eastern & Southern Africa and

Madagascar, but has been introduced into the West Indies and many other tropical countries (Mabberley, 1987; Demissew, 1996). Aloes are coarse-looking perennial plants with stem sizes varying with different species. Some species are trees, others are short stemmed, and some have their stems underground. The plants are naturally found in the wild, mostly in semi-arid areas. The common morphological features of all aloes are in the nature of their leaves. The leaves are long, erect and crowded in a basal rosette. They are succulent, containing a clear sticky juice (jelly-like) between two green skins. The smooth surfaces of the skins are spotted in some species. The margins of the leaves have spiny teeth (prickly at the margins). The leave breaks easily and when cut it exudes a yellow coloured juice (Reynolds T, 1985a). The flowers are generally yellow, in dense racemes terminating the long scapes.

Figure 2.1 Photos of some *Aloe* plants



		
<i>Aloe saponaria</i>	<i>Aloe vera</i>	<i>Aloe vera</i> flower

		
<i>Aloe lateritia</i>	<i>Aloe lateritia</i> flowers	<i>Aloe graminicola</i>

Fig. 2.1b Pictures of *Aloe kilifiensis* and *A. graminicola* taken from a botanical garden behind Chemistry building, Chiromo, in February 2006.

Aloe kilifiensis



Aloe graminicola



2.2.1 Tropical African Groups

Aloe has been divided into several groups ranging from grass aloes (*Graminialoe* Reynolds, 1966), to tree aloes (group 20, tall trees, dichotomously branched and re-branched) depending on similarities in morphology (Reynolds, 1966). The groups are tabulated below.

Table 2.1: Reynold's grouping of Tropical African Aloes

Group	Description	Example (group marker)
Group 1	grass aloes	(<i>A. myriacantha</i>)
Group 2	leptoaloe	(<i>A. nuttii</i>)
Group 3	"bulbous" species	(<i>A. Bkettneri</i>)
Group 4	perianth striped	(<i>A. Peckii</i>)
Group 5	-----	<i>A. dorotheae</i> and allies
Group 6	sapanariae	(<i>A. lateritia</i>)
Group 7	hereroenses	(<i>A. Hereroensis</i>)
Group 8	perianth trigonously indented above the ovary	(<i>A. Chabaudii</i>)
Group 9	verae	(<i>A. Barbadosensis</i>)
Group 10	pendet series	(<i>A. Veseyi</i>)
Group 11	laterbracteatae	(<i>A. Cryptopoda</i>)
Group 12	-----	<i>A. Christianii</i> and Allies
Group 13	perianths clavate	(<i>A. Camperi</i>)
Group 14	flowers secund	(<i>A. Secundiflora</i>)
Group 15	racemes bottle brush like	(<i>A. Aculeata</i>)
Group 16	large compact rosettes	(<i>A. percrassa</i>)
Group 17	leaves spreading, canaliculate	(<i>A. Megalacantha</i>)
Group 18	tall stemed species	(<i>A. Volkensii</i>)
Group 19	shrubs	(<i>A. Dawei</i>)
Group 20	trees	(<i>A. Eminens</i>)

There is a total of twenty six *Aloe* species in Kenya, of which six are endemic. The Kenyan species as reported by Reynolds (1966) are: *Aloe erensii*, *A. pirottae*, *A. amudatensis*, *A. graminicola*, *A. kilifiensis* (endemic), *A. lateritia*, *A. rivaie*, *A. otallensis*, *A. calidophila*, *A. turkanensis*, *A. secundiflora*, *A. ruspoliana*, *A. classenii* (endemic), *A. ukambensis* (endemic), *A. tweediae*, *A. marsabitensis*, *A. volkensii*, *A. ballyi*, *A. deserti*, *A. elgonica* (endemic), *A. rabaiensis*, *A. dawei*, *A. kedongensis*, *A. ngobitensis* (endemic), and *A. nyeriensis* (endemic) (Gillett 1968).

2.2.2 The Saponariae group

All the members of this group have perianths with pronounced basal swelling, abruptly constricted above the ovary, thence enlarging to the throat. The group has 38 species, 32 of which are found in South Africa and 10 in Tropical Africa. Four of the species in South Africa are also found in Tropical Africa. The Tropical African species in the group are: *Aloe amudatensis*, *A. graminicola*, *A. kilifiensis*, *A. lateritia*, *A. greatheadii*, *A. swynnertonii*, *A. duckeri*, *A. saponaria* (group marker), *A. zebrina* and *A. macrocarpa*. Reynolds (1966) describes the general group characteristics as follows: Perianths have pronounced basal swelling, abruptly constricted above the ovary, thence enlarging to the throat. Species in the group constitute a heterogeneous and frequently most exasperating group, and it is often impossible to know where one species ends and the other one begins. This heterogeneity has been problematic to the taxonomy of the group (Yenesew, 1991). It was suggested that the easiest way to identify the specimen of *Aloe* belonging to this group, be based on the particular region of collection (Yenesew, 1991). However, Forster (1989) has pointed out that, such kind of identification is far from being ideal, and suggested the importance of generating chemical information in solving the taxonomic problems on this group. The same

2.3 The genus *Bulbine*

The genus *Bulbine* (Asphodelaceae) is represented by about 41 species, some of which include *Bulbine abyssinica*, *B. frutescens*, *B. alooides*, *B. latifolia*, *B. asphodeloides*, *B. annua*, *B. capitata*, *B. bulbosa*, *B. angustifolia*, *B. lagopus*. (van Wyk *et al.*, 1995a). *B. abyssinica* and *B. frutescens* are the only *Bulbine* species found in Kenya. *B. abyssinica* is an indigenous species (Agnew, 1974), and it grows wild in the grasslands. It can only be differentiated from grass after flowering during the rainy season. The plant usually dries out during the dry season. *B. frutescens* is a South African species widely cultivated for aesthetic purposes (Abegaz *et al.*, 2002). One of the commonest plants in the flower gardens of Kenyan cities is *B. frutescens*.

Figure 2.2 Pictures of some *Bulbine* species



B. alooides



B. latifolia



B. frutescens (stalked)



B. frutescens

method had been earlier suggested by Reynolds (1985a). Chemical information has been used by various workers to aid in taxonomic classification of aloes, based on investigation of *Aloe* leaves (Dagne *et al.*, 1994).

2.2.3 *Aloe kilifiensis*, *A. lateritia* and *A. graminicola*

All the three grow in the wild in Kenya. *Aloe kilifiensis* is indigenous to Kenya and is found in the coral reefs of Indian Ocean, Kilifi district, Mombasa. During high tides, the plants are covered by the ocean water and some are sometimes swept away (sometimes an aid to propagation). The racemes are slightly rounded or conic at apex. The leaves of the plant are green with white spots spread throughout the green leaves. The two species, *A. lateritia* and *A. graminicola* also look the same as *A. kilifiensis* physically except for the size of leaves and locality. In Kenya, *A. lateritia* is found in the Rift Valley Province, Central Province, Taita Hills, Kanjiando, Ukambani, and Coast Province. The racemes in *A. lateritia* are similar to those of *A. kilifiensis*. *A. graminicola* is located in the Kenyan Rift Valley Province and Central Province. Unlike the other two, *A. graminicola*'s inflorescence is a branched panicle. All the three species have underground (subterranean) stems. Following is a summary of the morphological features for the three species according to Reynolds (1966).

Table 2.2: Morphological features for *Aloe kilifiensis*, *A. lateritia* and *A. graminicola*

	<i>A. kilifiensis</i>	<i>A. lateritia</i>	<i>A. graminicola</i>
Inflorescence	4-6 branches, 70 cm high	6-10 branches, 100-125 cm high	3-5 branches, 100 cm high
Pedicels	16 mm long	20-25 mm long	20 mm long
Leaves			27 cm
Perianth	Deep wine-red 30 mm long	Orange to scarlet 35-38 mm long	33 mm long
Racemes			20 cm

2.3 The genus *Bulbine*

The genus *Bulbine* (Asphodelaceae) is represented by about 41 species, some of which include *Bulbine abyssinica*, *B. frutescens*, *B. alooides*, *B. latifolia*, *B. asphodeloides*, *B. annua*, *B. capitata*, *B. bulbosa*, *B. angustifolia*, *B. lagopus*. (van Wyk *et al.*, 1995a). *B. abyssinica* and *B. frutescens* are the only *Bulbine* species found in Kenya. *B. abyssinica* is an indigenous species (Agnew, 1974), and it grows wild in the grasslands. It can only be differentiated from grass after flowering during the rainy season. The plant usually dries out during the dry season. *B. frutescens* is a South African species widely cultivated for aesthetic purposes (Abegaz *et al.*, 2002). One of the commonest plants in the flower gardens of Kenyan cities is *B. frutescens*.

Figure 2.2 Pictures of some *Bulbine* species



B. alooides



B. latifolia



B. frutescens (stalked)

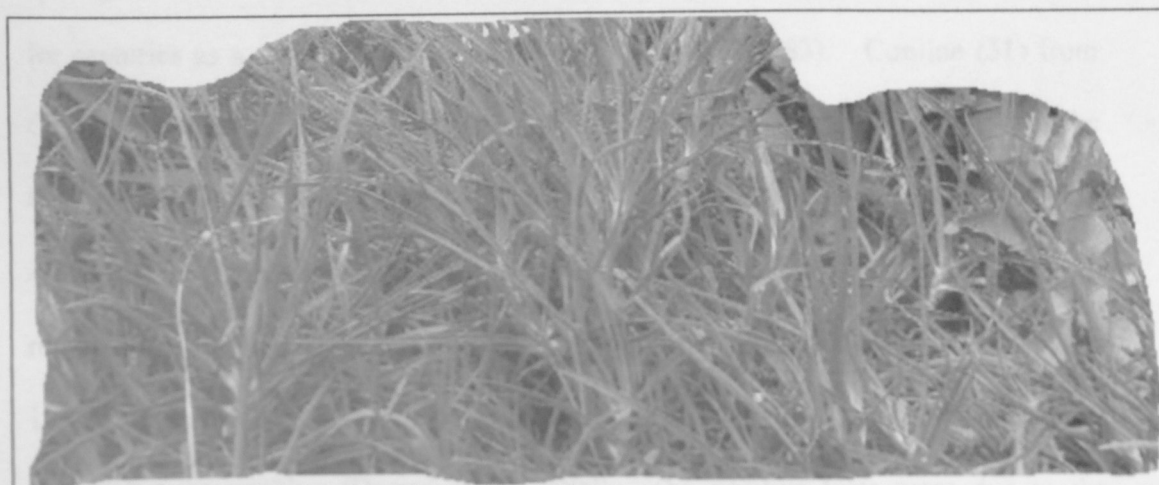


B. frutescens

Fig 2.2b **Photos of *Bulbine abyssinica* and *B. frutescens* taken from chemistry garden**



Bulbine abyssinnica (pictured from Chemistry botanical garden, Chiromo in February 2006)



Bulbine frutescens (pictured from Chemistry flower garden, Chiromo in February 2006)

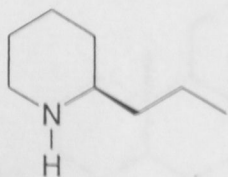
2.4 Compounds identified in *Aloe*

The chemistry of *Aloe* is widely studied, mostly due to its medicinal value and also for chemotaxonomic purposes. In the 1950s and 1960s, research was largely directed towards the physiologically active components, but it has now taken a wider scope (Reynolds, 1985). Compounds have been characterized from the dried exudate prepared for drug purposes but only from a few species (Reynolds, 1985). The leaves and roots of *Aloe* species elaborate many interesting secondary metabolites belonging to different classes of compounds including alkaloids, steroids, anthraquinones, pre-anthraquinones, anthrones, bianthaquinones, chromones, coumarins, pyrones and pyrans (Dagne, 1996). A few flavonoids have been reported from *Aloe* (Dagne *et al.*, 2000). A brief account of each class and a few representative compounds are given in the following subheadings.

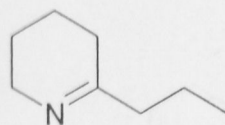
2.4.1 Alkaloids

The term alkaloid refers to naturally occurring basic compounds (apart from simple amines) derived from amino acids and the N-heterocycles of the pyrole, pyrimidine, purine type etc (Torssel, 1983). Majority of alkaloids are found in the plant kingdom, from higher plants down to micro-organisms. Few are found in the animal kingdom. Amongst the alkaloids, there are poisons such as strychnine (35) which has been used for centuries as a rodenticide and vermin killer (Torssel, 1983). Coniine (31) from *Conium maculatum* was used by the ancient Greeks for state executions, one of the most famous victims being Socrates (Torssel, 1983). Coniine has been identified in *Aloe viguieri* (Dring, *et al.*, 1984). γ -coniceine (32) (a piperidine alkaloid) has been reported to occur in six species of *Aloe* with very restricted distribution (Dring, *et al.*, 1984). Screening of 224 species of *Aloe* for alkaloids showed that 21% of the species screened were positive (Dagne *et al.*, 2000). The positive tests were due to the

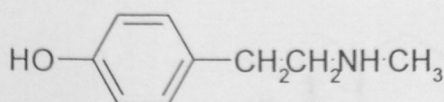
alkaloids N-methyltyramine (33), O,N-dimethyltyramine (34), coniine (31) and γ -coniceine (32). In view of the potential toxicity of alkaloids, it has been pointed out that it is important to screen for alkaloids prior to recommending any *Aloe* species in medicine (Dagne *et al.*, 2000).



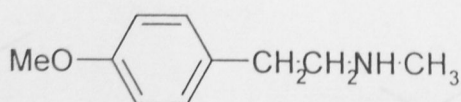
Coniine (31)



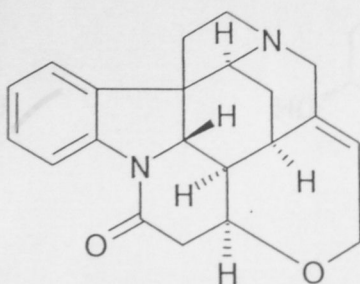
γ -Coniceine (32)



N-Methyltyramine (33)



O,N-Dimethyltyramine(34)

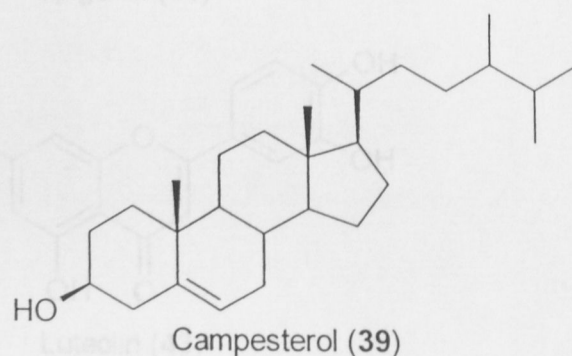
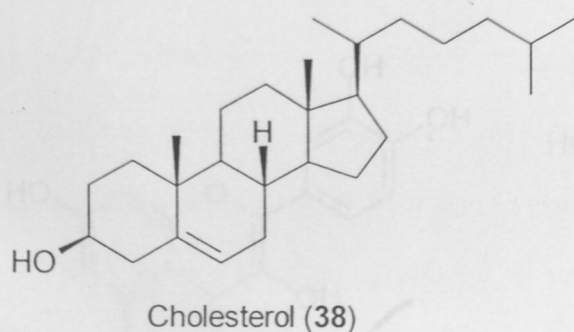
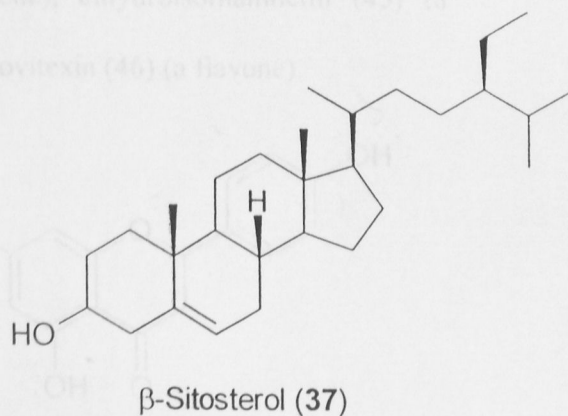
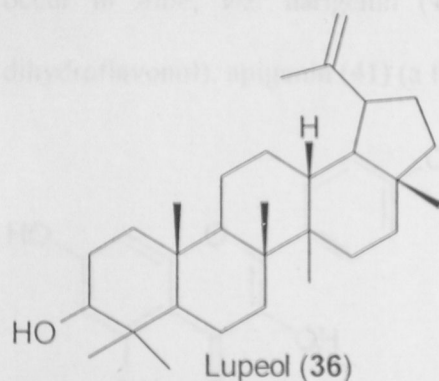


Strychnine (35)

2.4.2 Steroids

The steroids reported in *Aloe* are amongst those that are ever-present in the plant kingdom. These include lupeol (36), β -sitosterol (37), cholesterol (38), campesterol (39) etc. They have been claimed to be responsible for the anti-inflammatory activity of the gel (Davis, 1997). Steroids in general comprise a large number of commonly found compounds that are divided into subgroups, chiefly according to side chain

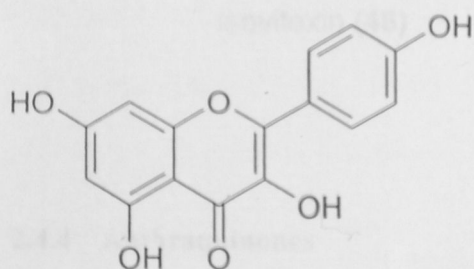
functionality: sterols, sapogenins, cardiac aglycones, bile acids, adrenal steroids and sex hormones, all of vital importance for life (Torssel, 1983).



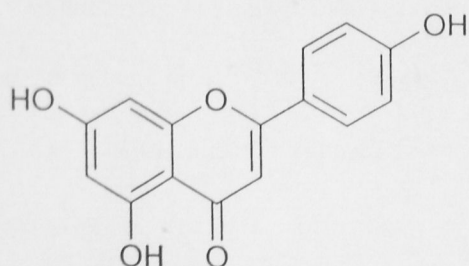
2.4.3 Flavonoids

There has been limited information on the occurrence of flavonoids in the genus *Aloe*. The reason could be due to the fact that most of the flavonoid-containing species are either difficult to obtain (grass-like aloes which are Malagasy endemics) or they do not perform well under cultivation once transplanted from nature (Viljoen *et al.*, 1998). Williams (1975) did a broad-based screening for the flavonoids, kaempferol (40), apigenin (41), quercetin (42) and luteolin (43) in the Liliaceae. Eight out of eighteen species of *Aloe* screened contained trace amounts of these flavonoids. In a chemotaxonomic survey of flavonoids in *Aloe*, significant quantities of the

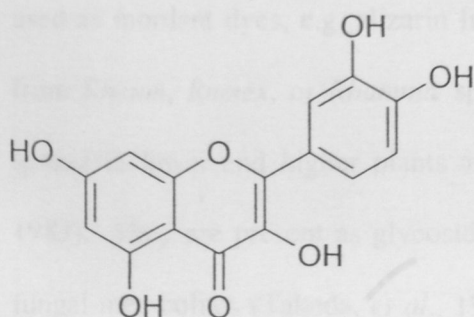
compounds were detected in at least 31 species of the 380 species investigated (Viljoen *op cit*). In the study, it was found out that only four major flavonoid markers occur in *Aloe*, viz: narigenin (44) (a flavanone), dihydroisorhamnetin (45) (a dihydroflavonol), apigenin (41) (a flavone) and isovitexin (46) (a flavone).



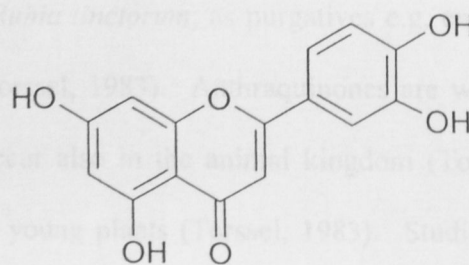
Kaempferol (40)



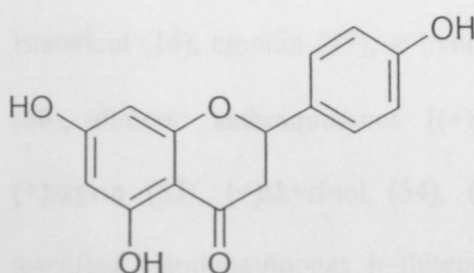
apigenin (41)



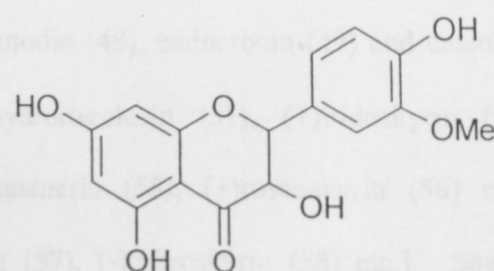
Quercetin (42)



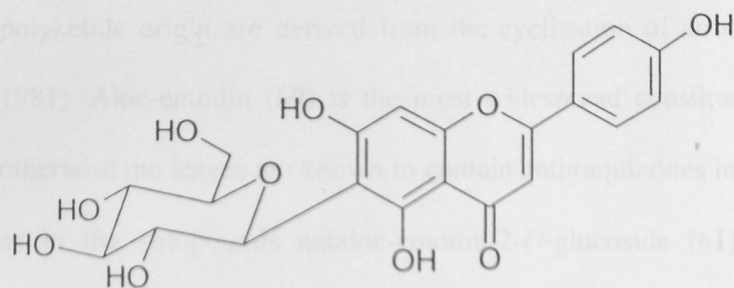
Luteolin (43)



Naringenin (44)



Dihydroisorhamnetin (45)

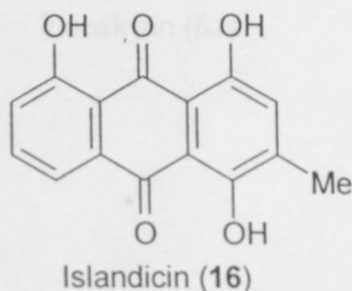
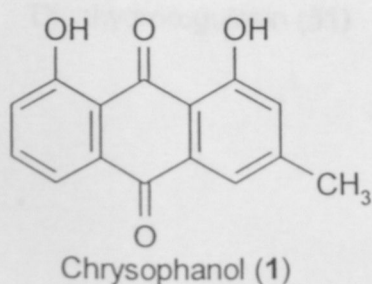


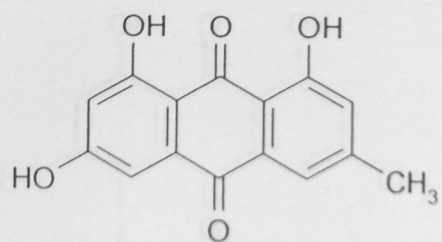
Isovitexin (46)

2.4.4 Anthraquinones

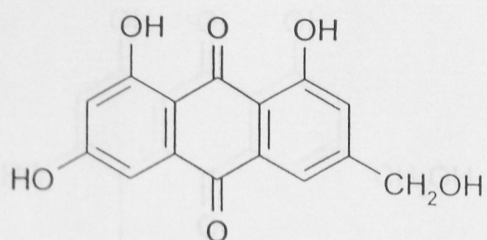
Anthraquinones are the largest group of quinones (Torssel, 1983). They have been used as mordant dyes, e.g. alizarin from *Rubia tinctorum*; as purgatives e.g. emodin from *Rheum*, *Rumex*, or *Rhamnus* spp (Torssel, 1983). Anthraquinones are widely spread in lower and higher plants and occur also in the animal kingdom (Torssel, 1983). They are present as glycosides in young plants (Torssel, 1983). Studies on fungal metabolites (Takeda, *et al.*, 1973) showed that the colouring matter produced by *Penicillium islandicum* Sopp were generally anthraquinones (chrysophanol (1), islandicin (16), emodin (47), ω -hydroxyemodin (48), endocrocin (49) and catenarin (50), dimeric anthraquinones [(+)-dianhydrorugulosin (51), (+)-iridoskyrin (52), (+)-skyrin (53), (+)-skyrinol (54), (+)-dicatenerin (55), (+)-roseoskyrin (56) etc.], modified bianthraquinones [(-)-luteoskyrin (57), (-)-rubroskyrin (58) etc.]. Several anthraquinones and hypericin (59) derivatives have been shown to be the contents of the pigment of pseudococcid insect *Nipaecoccus aurilanatus* (Maskel) (Cameron and Warwick 1976). The taxon *Aloe* is widely associated with the presence of anthraquinone derivatives (Yenesew, 1991). The anthraquinones found in *Aloe* appear to have been derived through the polyketide pathway [Section 2.7] (Yagi, *et al.*, 1978). The anthracene derivatives (anthraquinones and anthrones) of the

polyketide origin are derived from the cyclization of an octaketide chain (Manitto, 1981). Aloe-emodin (60) is the most widespread constituent of the leaves of *Aloe* otherwise the leaves are known to contain anthraquinones in the form of *O*-glycosides as in the compounds nataloe-emodin-2-*O*-glucoside (61) and aloe-emodin-11-*O*-rhamnoside (62) (Dagne *et al.*, 2000). Chrysophanol (1) is a common constituent of both leaves (Dagne, *et al.*, 1991) and roots (Dagne, *et al.*, 2000). Some of the anthraquinones found in the roots of many *Aloe* species, initially isolated from *Aloe saponaria* (Dagne, *et al.*, 2000) include: helminthosporin (2), aloesaponarin I (7), aloesaponarin II (6), isoxanthorin (63), laccaic acid D methyl ester (8) and desoxyerythrolactin (64) (Dagne, *et al.*, 2000). The compounds have now been shown to occur in the roots of many other *Aloe* species (Dagne *et al.*, 1994). The anthraquinones found in *Aloe* are generally of two types, 1,8-dihydroxy-3-methylantraquinones (e.g. ~ chrysophanol (1)) and 1,6-dihydroxy-8-methylantraquinone (e.g. aloesaponarin I (7)). Anthraquinone of the chrysophanol-type are known to occur in both the leaves and roots of *Aloe* but the aloesaponarin-type are confined to the roots (van Wyk *et al.*, 1995a).

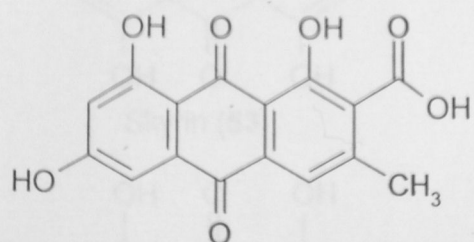




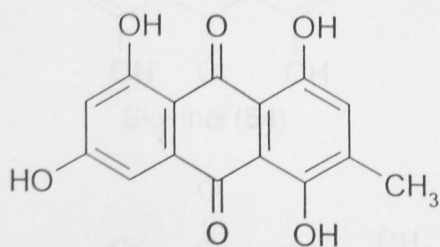
Emodin (47)



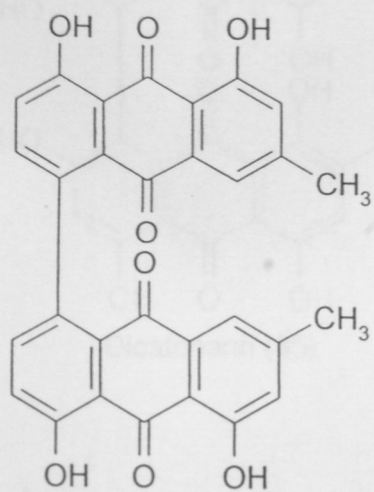
ω-Hydroxyemodin (48)



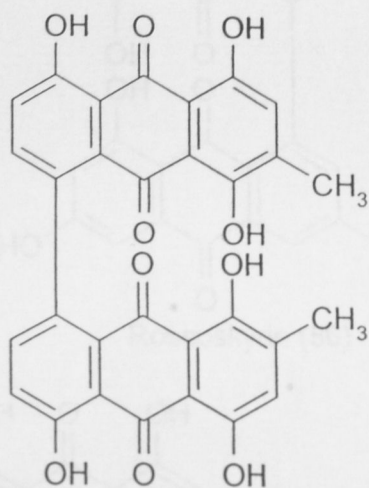
Endocrocin (49)



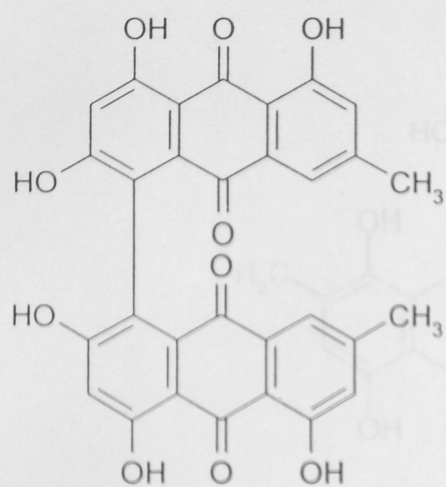
Catenarin (50)



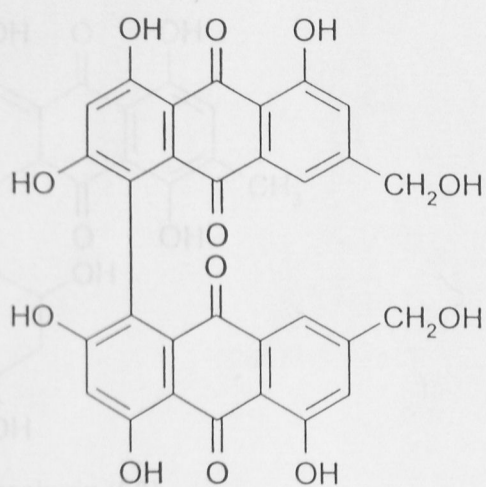
Dianhydrorugulosin (51)



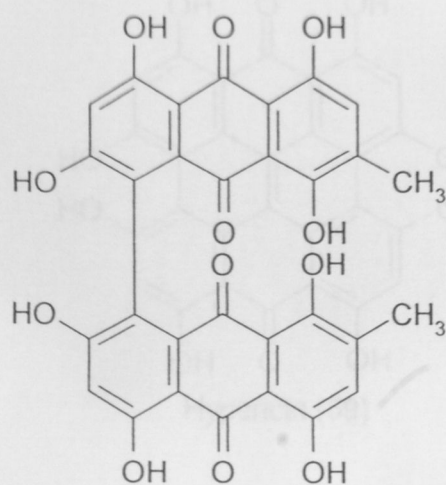
Iridoskyrin (52)



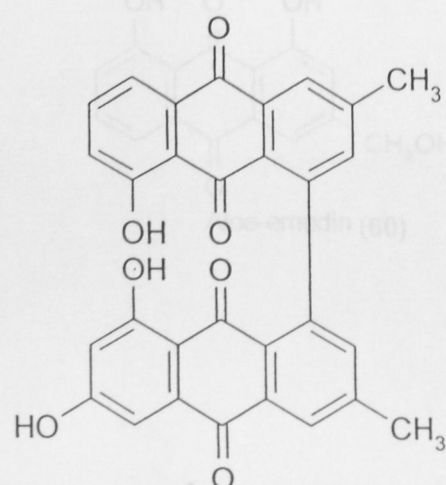
Skyrin (53)



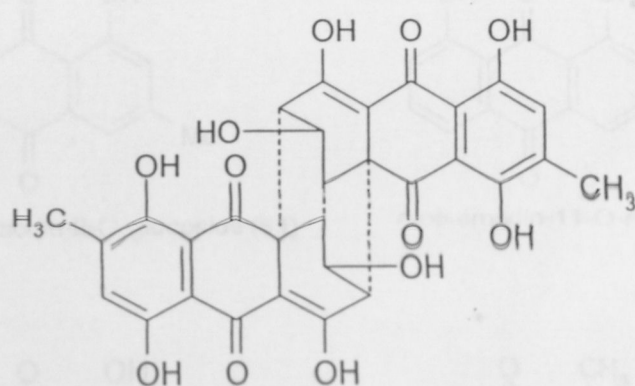
Skyrinol (54)



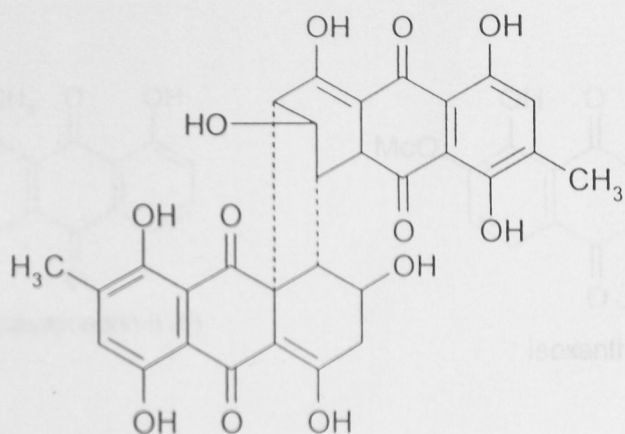
Dicatenarin (55)



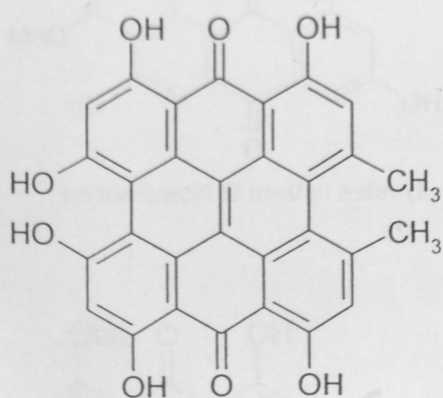
Roseoskyrin (56)



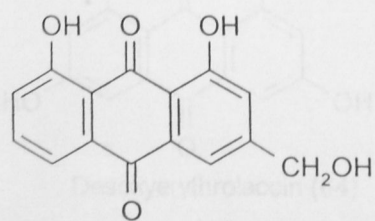
(-)-Luteoskyrin (57)



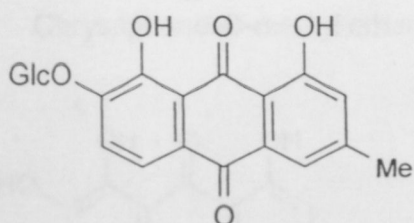
(-)-Rubroskyrin (58)



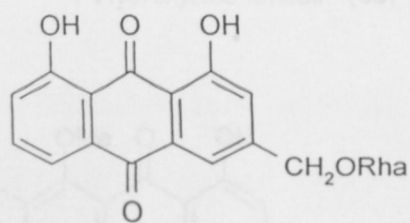
Hypericin (59)



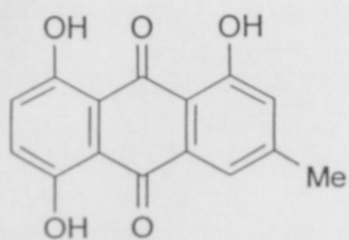
Aloe-emodin (60)



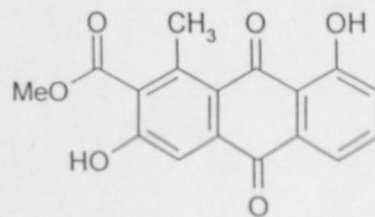
Nataloe-emodin-2-O-glucoside (61)



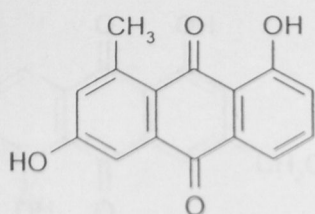
Aloe-emodin-11-O-rhamnoside (62)



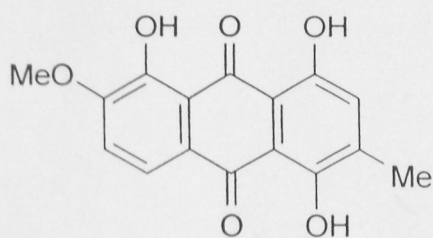
Helminthosporin (2)



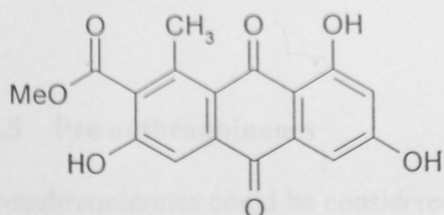
Aloesaponarin I (7)



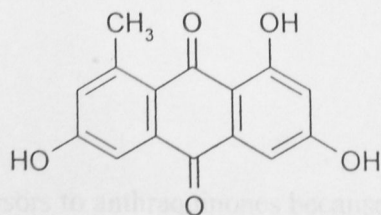
Aloesaponarin II (6)



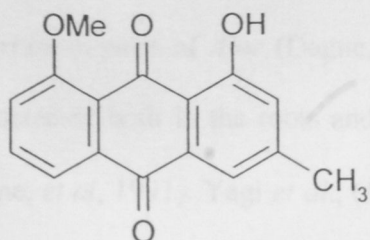
Isoxanthorin (63)



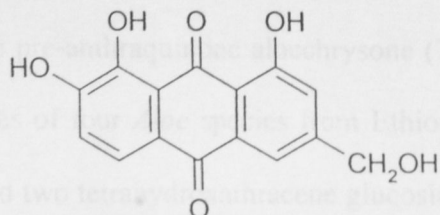
Laccaic acid D methyl ester (8)



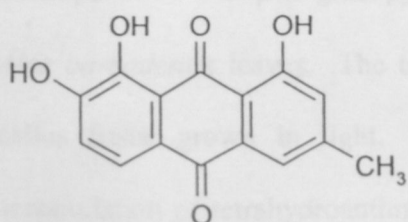
Desoxyerythrolaccin (64)



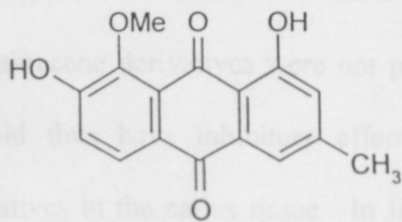
Chrysophanol-8-methyl ether (65)



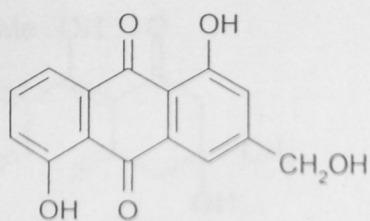
7-Hydroxyaloe-emodin (66)



Nataloe-emodin (67)



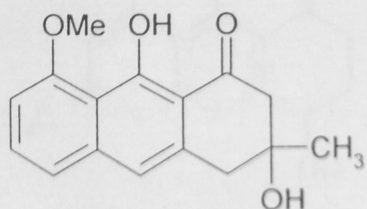
Nataloe-emodin-8-methyl ether (68)



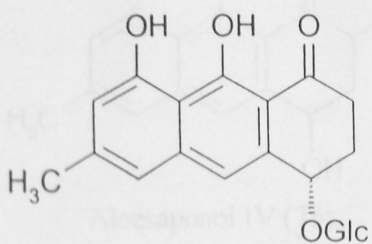
1,5-Dihydroxy-3-hydroxy
methylanthraquinone (69)

2.4.5 Pre-anthraquinones

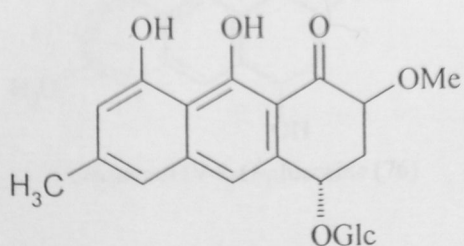
Pre-anthraquinones could be considered as precursors to anthraquinones because they are readily converted to the corresponding anthraquinones by treatment with base (Yenesew *et al.*, 1993). Several pre-anthraquinones have been isolated from the subterranean parts of *Aloe* (Dagne, 1996). The pre-anthraquinone aloechrysone (70) was detected both in the roots and in the leaves of four *Aloe* species from Ethiopia (Dagne, *et al.*, 1991). Yagi *et al.*, (1998) isolated two tetrahydroanthracene glucosides (the preanthraquinones aloesaponol III-4-*O*- β -D-glucopyranoside (71) and aloesaponol IV-4-*O*- β -D-glucopyranoside (72)) from the dark brown callus tissue of *Aloe barbadensis* leaves. The tetrahydroanthracene derivatives were not present in callus tissue grown in light. Light could thus have inhibitory effect on the accumulation of tetrahydroanthracene derivatives in the callus tissue. In fact, these compounds are mainly isolated from subterranean stems (Dagne, *et al.*, 2000) and roots (van Wyk *et al.*, 1995a) of *Aloe* species. The pre-anthraquinone vismione H (73) was shown to be the main contributor to the high levels of antiplasmodium (*Plasmodium falciparum*) activity in the crude extracts of *Vismia guineensis* (Guido, *et al.*, 1999).



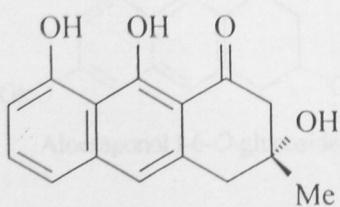
Aloechrysone (70)



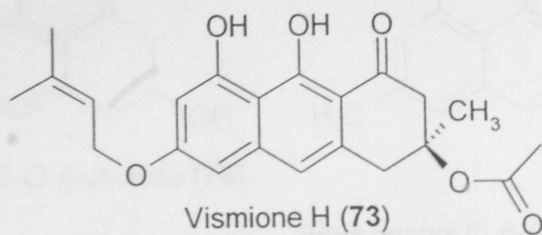
Aloesaponol III-4-O-β-D-glucopyranoside (71)



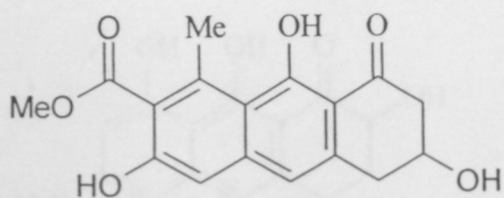
Aloesaponol IV-4-O-β-D-glucopyranoside (72)



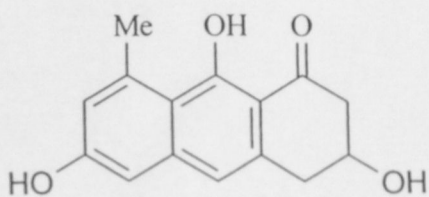
Aloegramonol (9)
(Pre-chrysophanol)



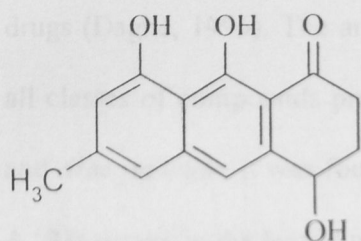
Vismione H (73)



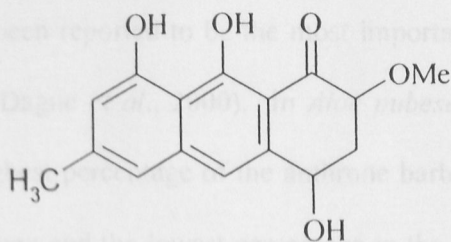
Aloesaponol I (10)



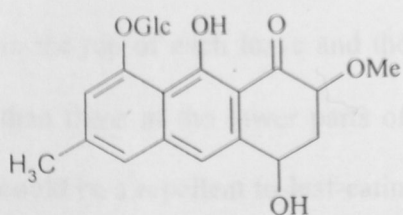
Aloesaponol II (11)



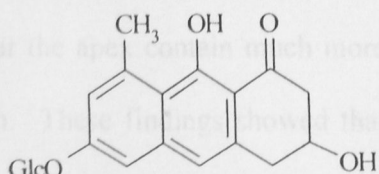
Aloesaponol III (74)



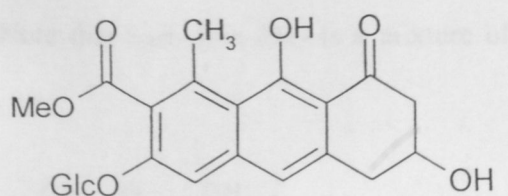
Aloesaponol IV (75)



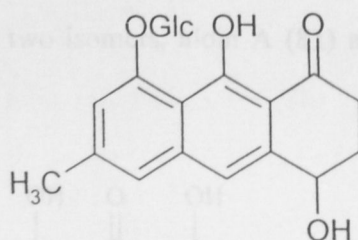
Aloesaponol IV-8-O-glucoside (76)



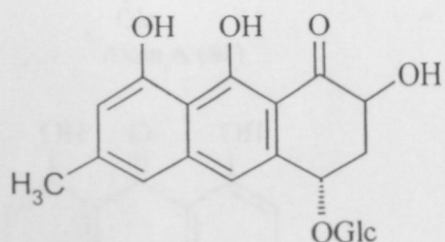
Aloesaponol I-6-O-glucoside (77)



Aloesaponol II-6-O-glucoside (78)



Aloesaponol III-8-O-glucoside (79)

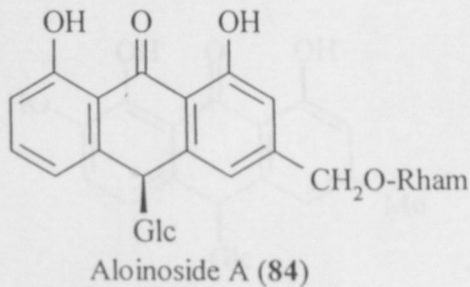
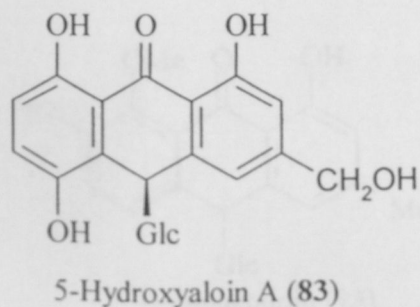
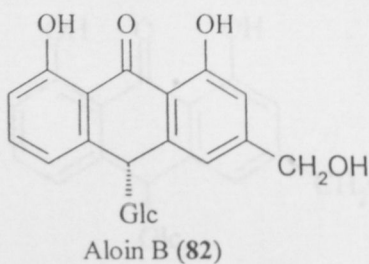
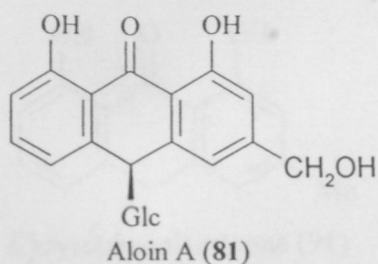


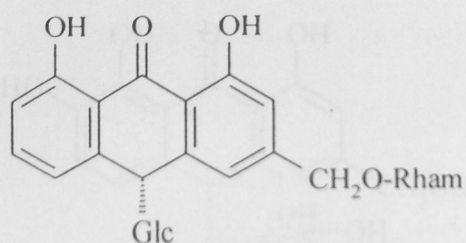
Aloesaponol III-2-hydroxy
4-O- β -D-glucopyranoside (80)

2.4.6 Anthrones

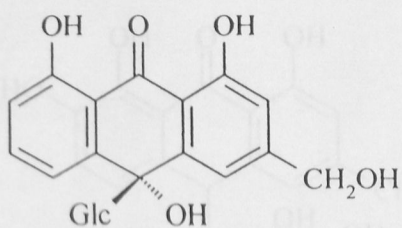
Anthrones are biosynthetic intermediates leading to the anthraquinone structure via simple oxidation. They are responsible for the bitter and purgative properties of *Aloe*

drugs (Dagne, 1996). The anthrones have been reported to be the most important of all classes of compounds present in *Aloe* (Dagne *et al.*, 2000). In *Aloe pubescence* and *Aloe jucunda*, it was found that the highest percentage of the anthrone barbaloin A (92) occurs in the leaves near the plant apex and the lowest percentage in the older and lowest leaves (Groom and Reynolds, 1987). The study of *Aloe aborescens*, by Chauser-Volfson and Gutterman (1996) showed that barbaloin is mostly concentrated in the top of each leaf and the leaves near the apex contain much more barbaloin than those at the lower parts of the branch. These findings showed that barbaloin could be a repellent to leaf-eating animals (it is logical to say that the highest content will be in young leaves and at the top of the leaves, possibly as a defense strategy). The structures of some anthrones obtained from *Aloe* are given below (81 – 99). Note that barbaloin (92) is a mixture of the two isomers, aloin A (81) and aloin B (82).

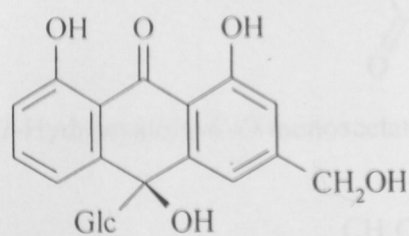




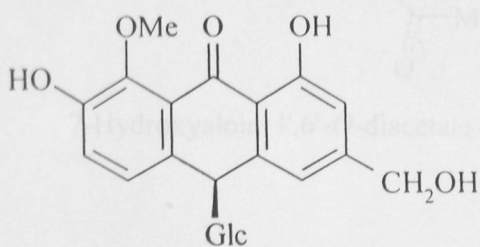
Aloinoside B (85)



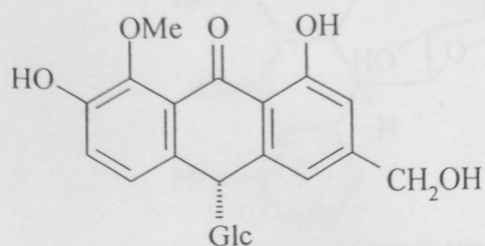
10-Hydroxyaloin A (86)



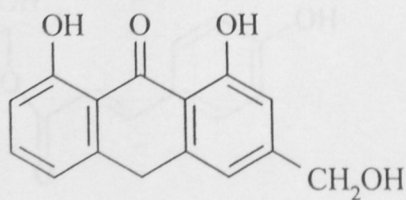
10-Hydroxyaloin B (87)



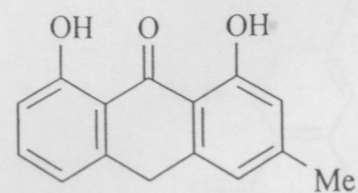
8-O-Methyl-7-hydroxyaloin A (88)



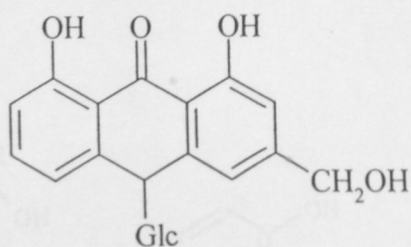
8-O-Methyl-7-hydroxyaloin B (89)



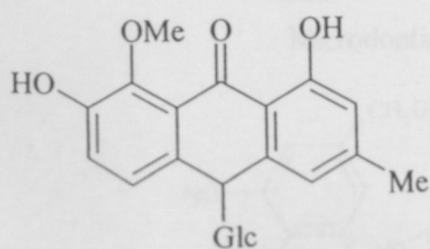
Aloe-emodinanthrone (90)



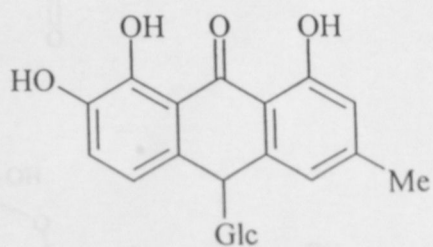
Chrysophanolanthrone (91)



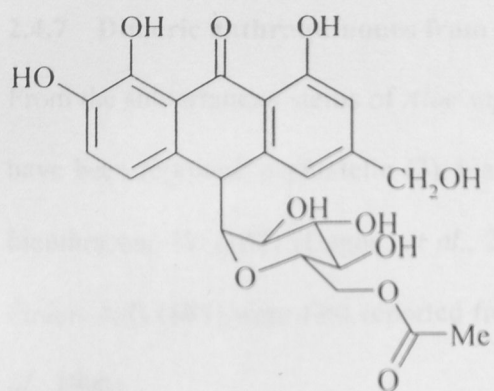
Barbaloin A (92)



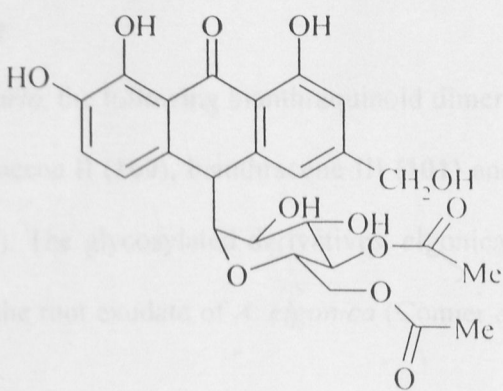
Homonataloin (93)



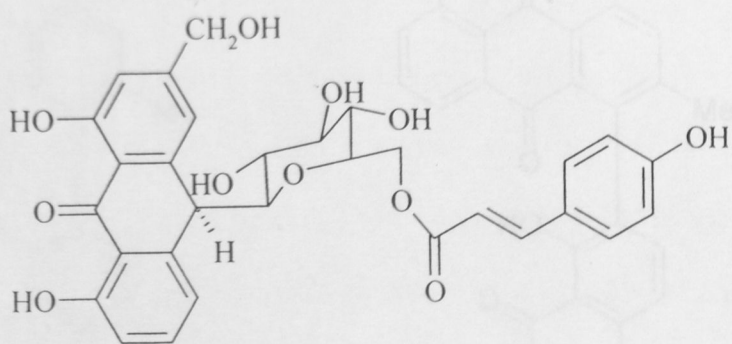
Nataloin (94)



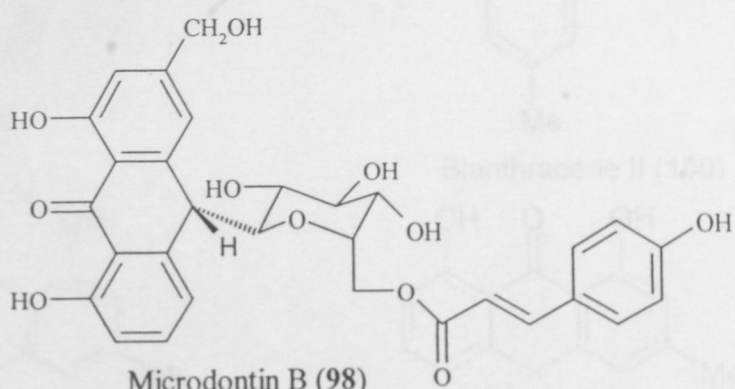
7-Hydroxyaloin-6'-*O*-monoacetate (95)



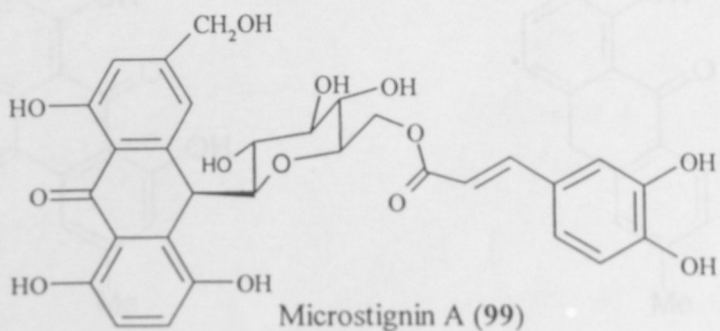
7-Hydroxyaloin-4',6'-*O*-diacetate (96)



Microdentin A (97)



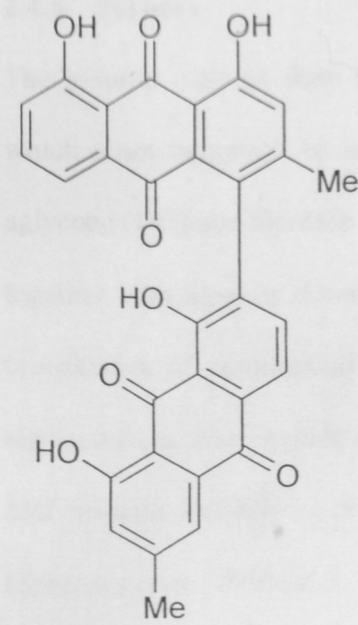
Microdentin B (98)



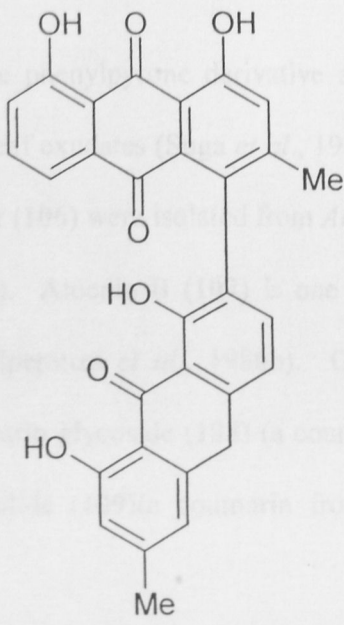
Microstignin A (99)

2.4.7 Dimeric anthraquinones from *Aloe*

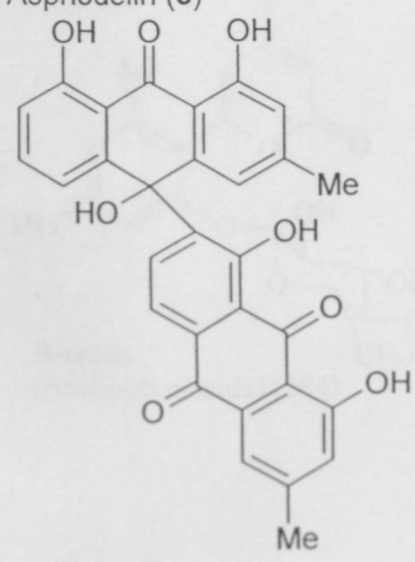
From the subterranean stems of *Aloe saponaria*, the following bianthraquinoid dimers have been reported: asphodelin (3), bianthracene II (100), bianthracene III (101) and bianthracene IV (102) (Dagne, *et al.*, 2000). The glycosylated derivatives, elgonica-dimers A/B (103) were first reported from the root exudate of *A. elgonica* (Conner *et al.*, 1990).



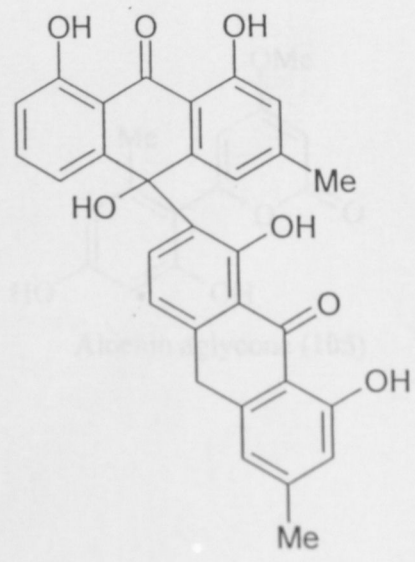
Asphodelin (3)



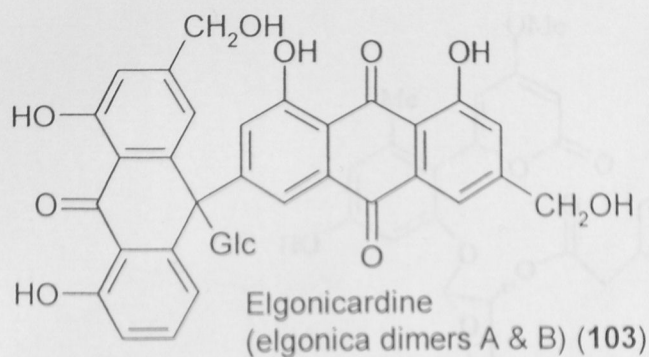
Bianthracene II (100)



Bianthracene III (101)

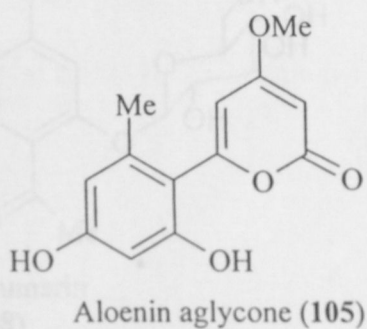
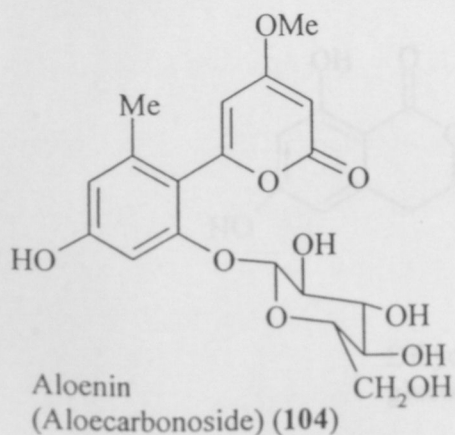


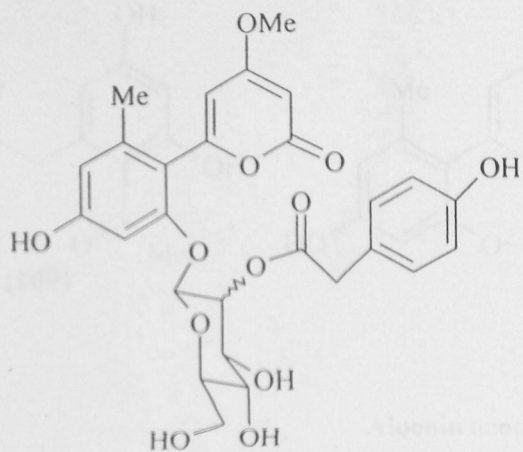
Bianthracene IV (102)



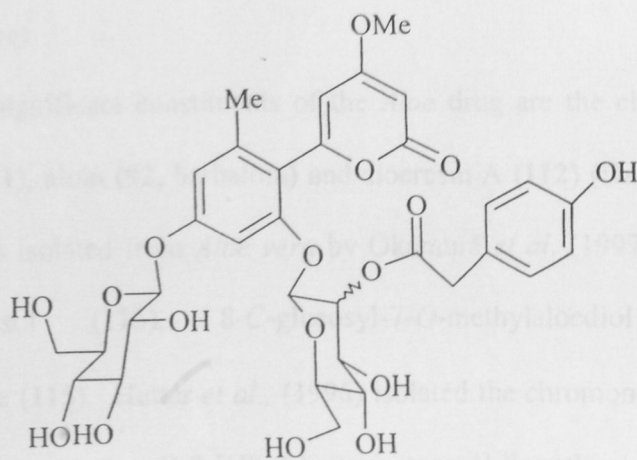
2.4.8 Pyrones

The pyrones isolated from *Aloe* include the phenylpyrone derivative aloenin (104) which is not frequently encountered in the leaf exudates (Suga *et al.*, 1974). Aloenin aglycone (105) and aloenin coumaroyl ester (106) were isolated from *Aloe nyeriensis* together with aloenin (Conner *et al.*, 1987). Aloenin B (107) is one of the major constituents of commercial Kenya *Aloe* (Speranza *et al.*, 1986b). Other pyrones reported from *Aloe* include dihydroisocoumarin glycoside (108) (a coumarin from *A. hildebrandtii* (Veitch *et al.*, 1994), Feralolide (109)(a coumarin from Cape aloe (Speranza *et al.*, 1993) etc.

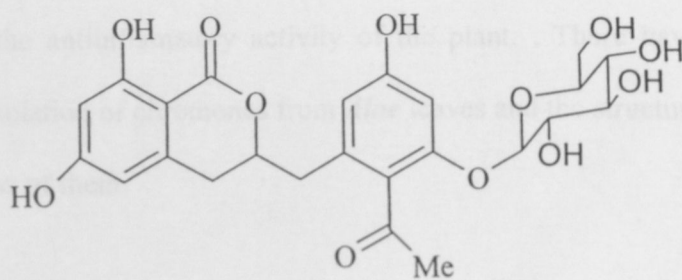




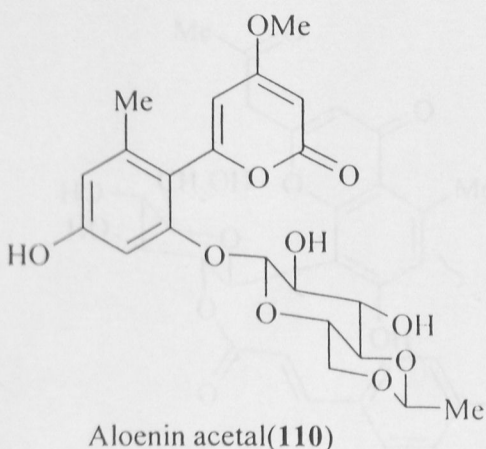
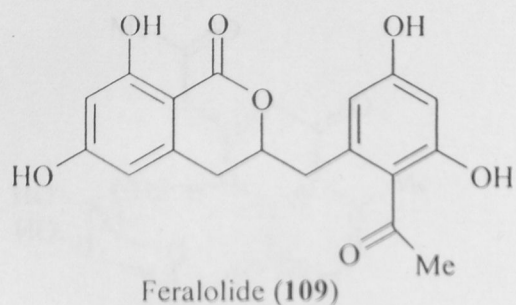
Aloenin-2''-*p*-coumaryl ester (106)



Aloenin B (107)



Dihydroisocoumarin
glucoside (108)



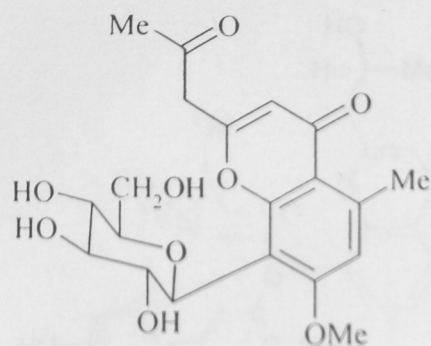
2.4.9 Chromones

The three most significant constituents of the *Aloe* drug are the chromones aloesin (aloeresin B) (111), aloin (92, barbaloin) and aloeresin A (112) (Dagne *et al.*, 2000).

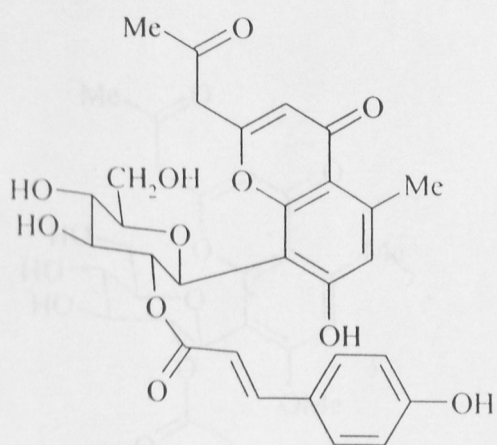
Three chromones isolated from *Aloe vera* by Okamura *et al.* (1997b) included 8-C-glucosyl-(*S*)-aloesol (113), 8-C-glucosyl-7-O-methylaloesol (114) and

isorabaichromone (115). Hutter *et al.*, (1996) isolated the chromone 8-[C-β-D-[2-O-(*E*)-cinnamoyl]glucopyranosyl]-2-[(*R*)-2-hydroxypropyl]-7-methoxy-5-

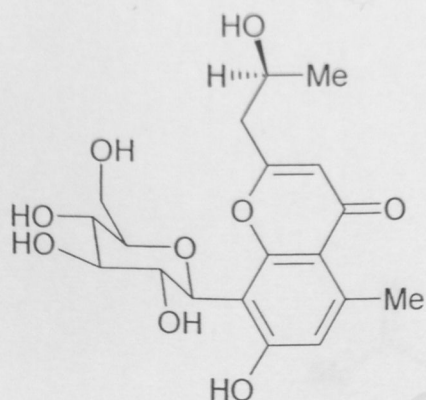
methylchromone (116) from *Aloe barbadensis* and was found to be a significant contributor to the antiinflammatory activity of the plant. . There have been several reports on the isolation of chromones from *Aloe* leaves and the structures given below are just but a few of them.



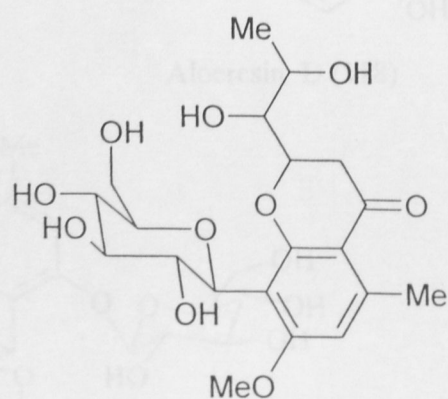
Aloesin (Aloeresin B) (111)



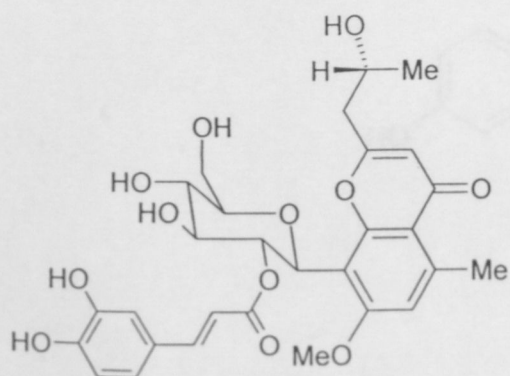
Aloeresin A (112)



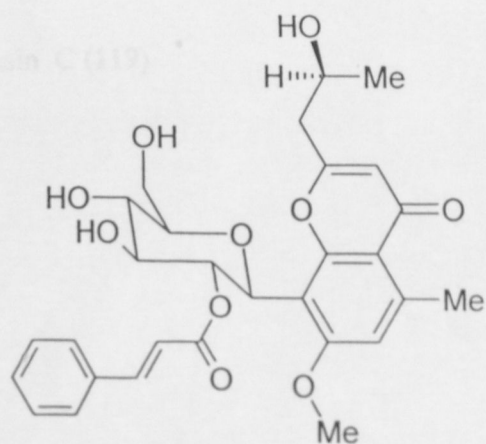
8-C-Glucosyl-(S)-aloesol (113)



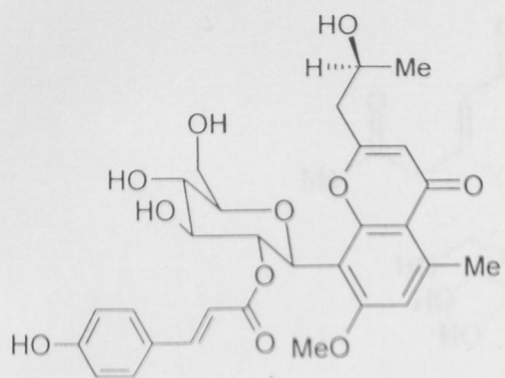
8-C-Glucosyl-7-O-methylaloesol (114)



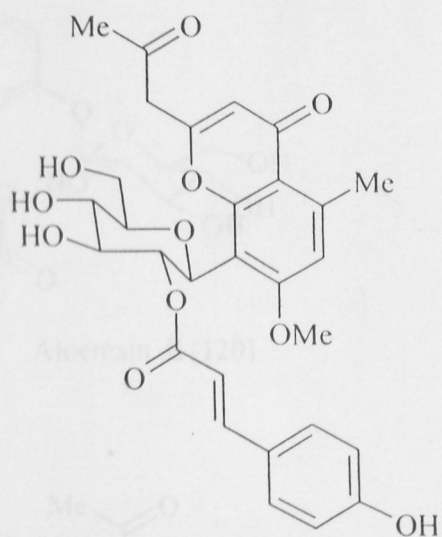
Isorabaichromone (115)



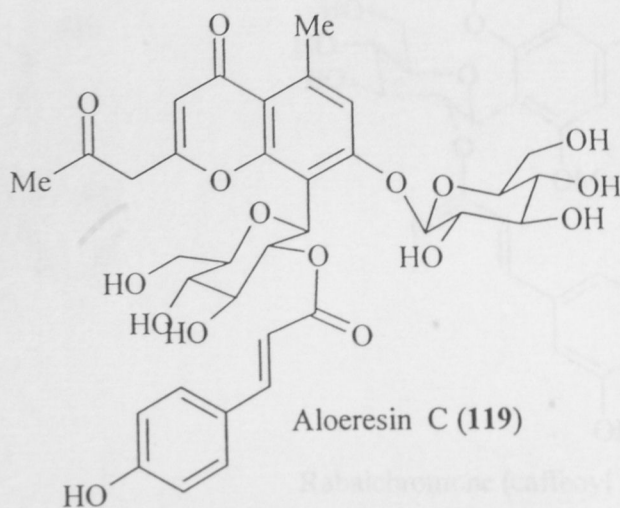
8-[C-b-D-[2-O-(E)-cinnamoyl] glucopyranosyl]-2-[(R)-2-hydroxypropyl]-7-methoxy-5-methylchromone (116)



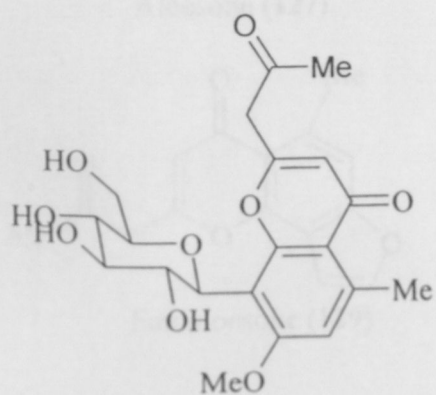
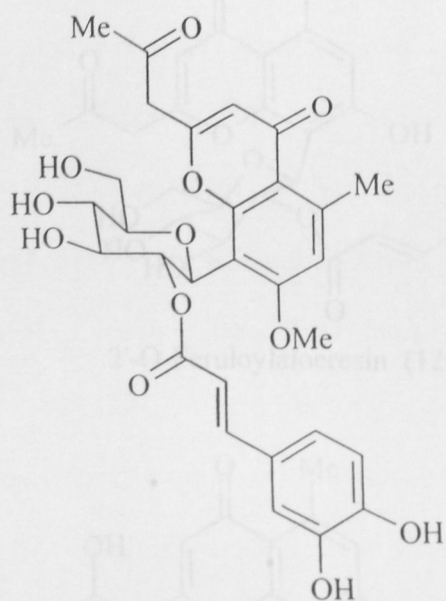
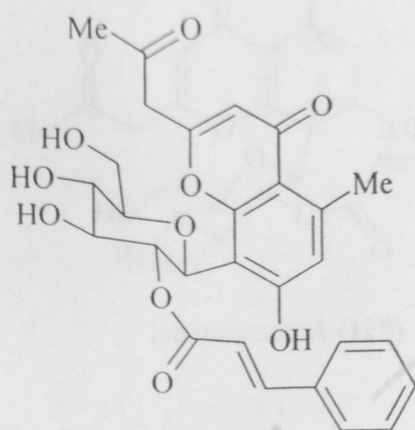
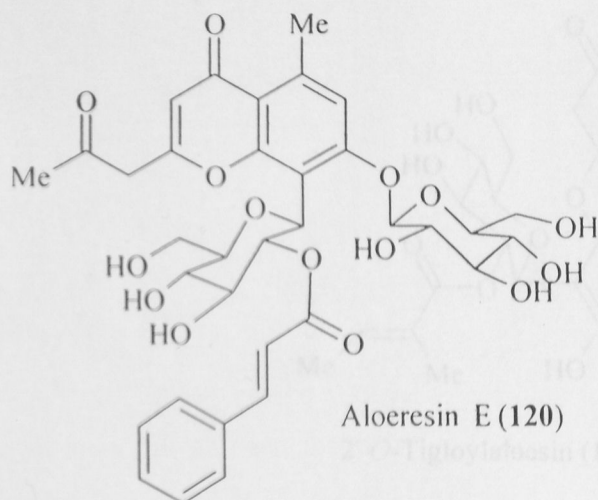
Isoaloeresin D (117)

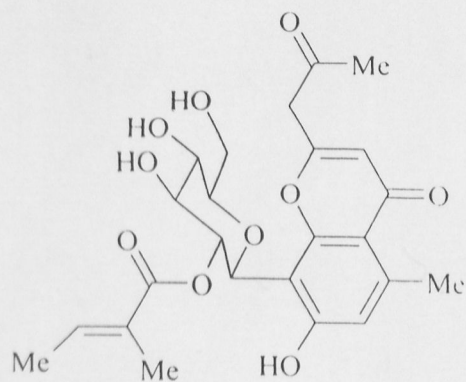


Aloeresin D (118)

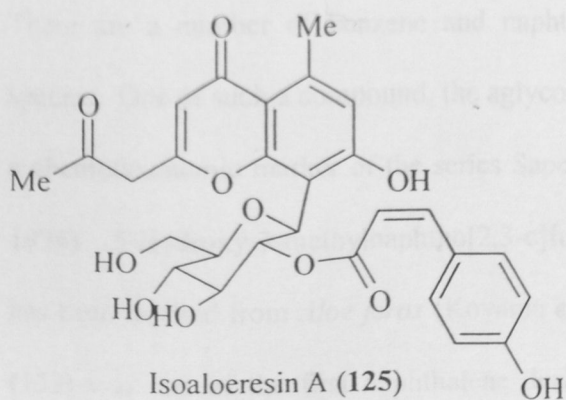


Aloeresin C (119)

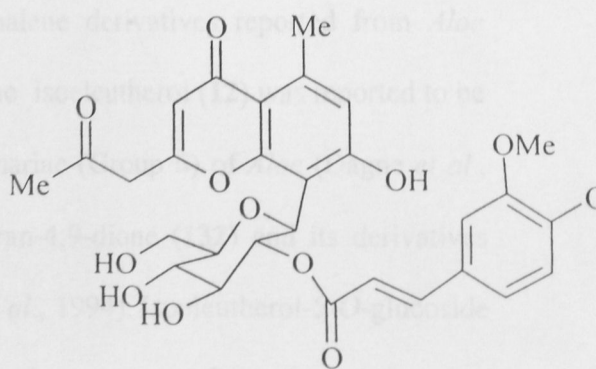




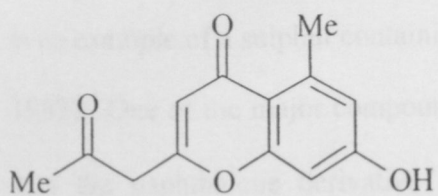
2'-O-Tigloylaloetin (124)



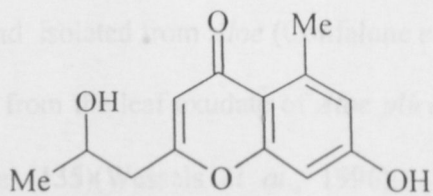
Isoaloeresin A (125)



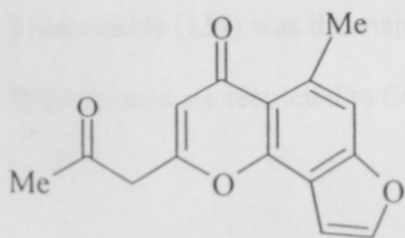
2'-O-Feruloylaloeresin (126)



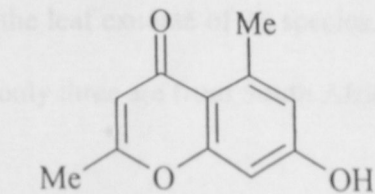
Aloesone (127)



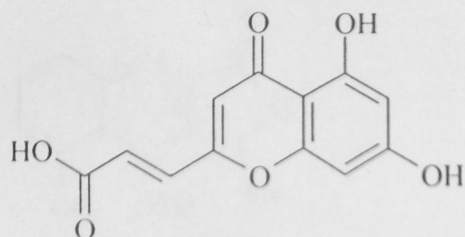
Aloesol (128)



Furoaloesone (129)



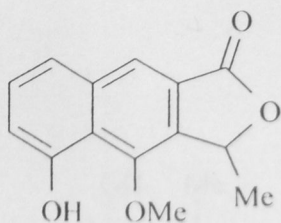
7-Hydroxy-2,5-dimethylchromone (130)



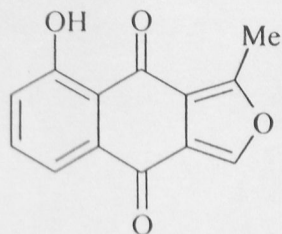
2-(Carboxyethenyl)-5,7-dihydroxychromone (**131**)

2.4.10 Benzene/naphthalene and furan derivatives

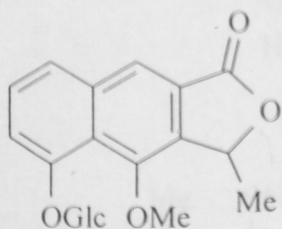
There are a number of benzene and naphthalene derivatives reported from *Aloe* species. One of such a compound, the aglycone isoeleutherol (**12**) was reported to be a chemotaxonomic marker of the series Saponariae (Group 6) of *Aloe* (Dagne *et al.*, 1994). 5-Hydroxy-3-methylnaphtho[2,3-c]furan-4,9-dione (**132**) and its derivatives has been isolated from *Aloe ferox* (Koyama *et al.*, 1994). Isoeleutherol-5-*O*-glucoside (**133**) was one of the first naphthalene derivatives reported from the subterranean stems of *A. saponaria* (Dagne *et al* 2000). Pluridone (**134**) isolated from *A. pluridens* is an example of a sulphur containing compound isolated from *Aloe* (Confalone *et al.*, 1983). One of the major compounds isolated from the leaf exudate of *Aloe plicatilis* was the naphthalene derivative plicataloside (**135**)(Wessels *et al.*, 1996). In a chemotaxonomic survey of 380 taxa of *Aloe*, Viljoen *et al.*, (1999) found that plicataloside (**135**) was the major phenolic in the leaf exudate of 20 species. Most of those species are restricted to East Africa and only three are from South Africa.



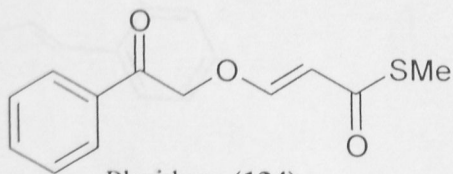
Isoelutherol (12)



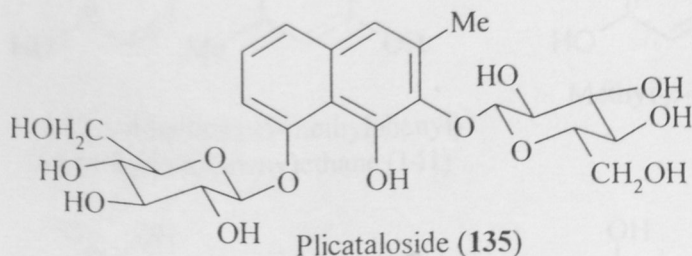
5-Hydroxy-3-methylnaphtho (2,3-c)furan-4,9-dione (132)



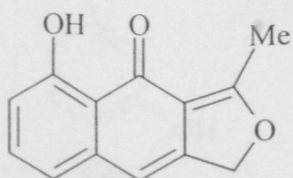
Isoelutherol-5-O-glucoside (133)



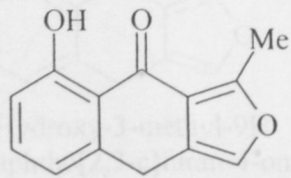
Pluridone (134)



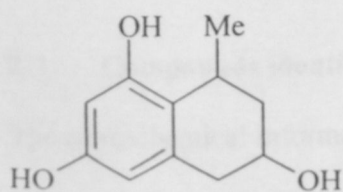
Plicataloside (135)



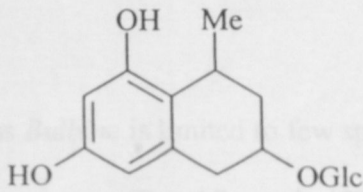
5-Hydroxy-3-methylnaphtho (2,3-c)furan-4(1H)-one (136)



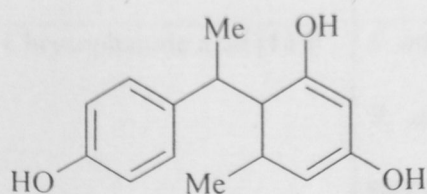
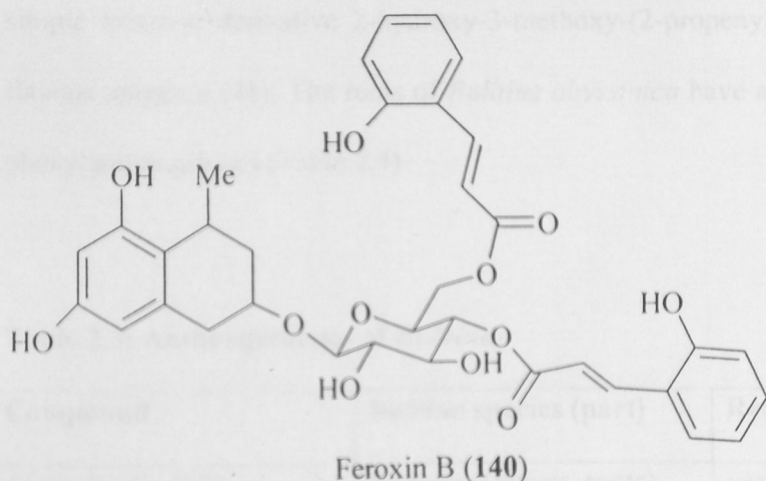
5-Hydroxy-3-methyl-9H-naphtho[2,3-c]furan-4-one (137)



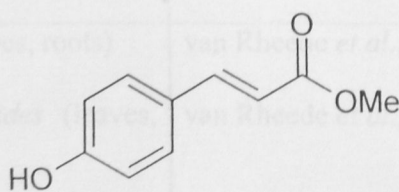
Feroxidin (138)



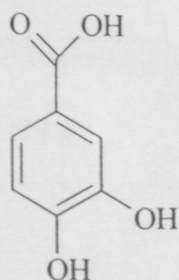
Feroxin A (139)



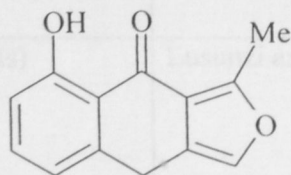
1-(2,3-dihydroxy-5-methylphenyl)-
1-(4-hydroxyphenyl)ethane (141)



Methyl-*p*-coumaric acid (142)



Protocatechuic acid (143)



5-Hydroxy-3-methyl-9H-
-naphtho[2,3-*c*]furan-4-one (144)

2.5 Compounds identified in *Bulbine*

The phytochemical information on the genus *Bulbine* is limited to few species. By far the most extensively investigated is *Bulbine capitata* (Bezabih *et al.*, 1997). From the roots and aerial parts of this plant several anthraquinones have been reported. These include simple anthraquinones (Table 2.3), phenylanthraquinones (Table 2.4) and anthraquinone dimers (Table 2.3). The isofuranonaphthoquinones have been isolated from the roots of *Bulbine capitata* (Table 2.5). This plant has also produced the

simple benzene derivative 2-hydroxy-3-methoxy-(2-propenyl)phenol (**154**) and the flavone apigenin (**41**). The roots of *Bulbine abyssinica* have also produced some five phenylanthraquinones (Table 2.4)

Table 2.3: Anthraquinones of *Bulbine*

Compound	<i>Bulbine</i> species (part)	Reference
Aloe emodin (60)	<i>B. annua</i> (leaves, roots)	van Rheede <i>et al.</i> , 1969
Chrysophanoic acid (145)	<i>B. annua</i> (leaves, roots)	van Rheede <i>et al.</i> , 1969
	<i>B. asphodeloides</i> (leaves, roots)	van Rheede <i>et al.</i> , 1969
	<i>B. capitata</i> (roots)	Lusunzi and Karuso, 2001
	“ (aerial parts)	Bezabih and Abegaz, 1998
7'-10-Bichrysophanoic acid (146)	<i>B. capitata</i> (roots)	Lusunzi and Karuso, 2001

Table 2.4 Phenylanthraquinones of *Bulbine*

Compound	<i>Bulbine</i> species (part)	Reference
Bulbine-knipholone (147)	<i>B. abyssinica</i> (root)	Bringmann <i>et al.</i> , 2002
Knipholone (17)	<i>B. abyssinica</i> (root)	Bringmann <i>et al.</i> , 2002
	<i>B. capitata</i> (aerial part)	Bezabih and Abegaz, 1998
	“ (roots)	Lusunzi and Karuso, 2001
Knipholone anthrone (20)	<i>B. abyssinica</i> (root)	Bringmann <i>et al.</i> , 2002
4'-Demethylknipholone	<i>B. abyssinica</i> (root)	Bringmann <i>et al.</i> , 2002

Compound	<i>Bulbine</i> species (part)	Reference
(24)		
6'- <i>O</i> -Methylknipholone (19)	<i>B. abyssinica</i> (root)	Bringmann <i>et al.</i> , 2002
Foliosone (148)	<i>B. capitata</i> (aerial parts)	Bezabih and Abegaz, 1998
4'-Demethylknipholone (24)	<i>B. capitata</i> (aerial parts)	Bezabih and Abegaz, 1998
4'-Demethylknipholone- 4'- <i>O</i> - β -glucopyranoside (25)	<i>B. capitata</i> (roots)	Lusunzi and Karuso, 2001
Isoknipholone (18)	<i>B. capitata</i> (roots)	Bezabih and Abegaz, 1998
Knipholone-6'-methyl ether (19)	<i>B. capitata</i> (roots)	Bezabih <i>et al.</i> , 1997
Gaboroquinone A (149)	<i>B. capitata</i> (roots)	Bezabih <i>et al.</i> , 2001
Gaboroquinone B (150)	<i>B. capitata</i> (roots)	Bezabih <i>et al.</i> , 2001

Table 2.5 Furanonaphthoquinones of *Bulbine*

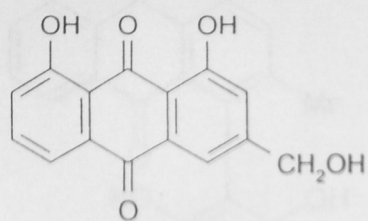
Compound	<i>Bulbine</i> species (part)	Reference
1-Methyl-5-hydroxy-8-methoxynaphtho[2-3- <i>C</i>]-furan-4,9-dione (151)	<i>B. capitata</i> (roots)	Bezabih <i>et al.</i> , 2001
1-Methyl-8-hydroxy-5-methoxy naphtho[2-3- <i>C</i>]-	<i>B. capitata</i> (roots)	Bezabih <i>et al.</i> , 2001

Compound	<i>Bulbine</i> species (part)	Reference
furan-4,9- dione (152)		
5,8-Dihydroxy-1-hydroxy-methylnaphtho[2-3-C]-furan-4,9-dione (22)	<i>B. capitata</i> (roots)	Bezabih <i>et al.</i> , 2001
5,8-Dihydroxy-1-methylnaphtho[2-3-C]-furan-4,9-dione (21)	<i>B. capitata</i> (roots)	Bezabih <i>et al.</i> , 2001
8-hydroxy-1-methylnaphtho[2-3-C]-furan-4,9-dione (153)	<i>B. capitata</i> (roots)	Bezabih <i>et al.</i> , 2001

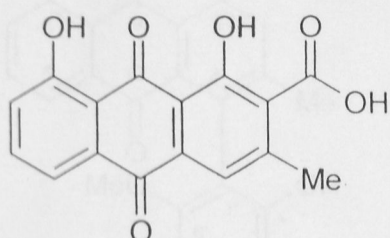
Table 2.6 Miscellaneous compounds of *Bulbine*

Compound	<i>Bulbine</i> species (part)	Reference
Luteolin (43)	<i>B. capitata</i> (roots)	Bezabih and Abegaz, 1998
2-Hydroxy-3-methoxy-5-(prop-phenylpropanoid-2-en)phenol (154)	<i>B. capitata</i> (roots)	Bezabih <i>et al.</i> , 1997

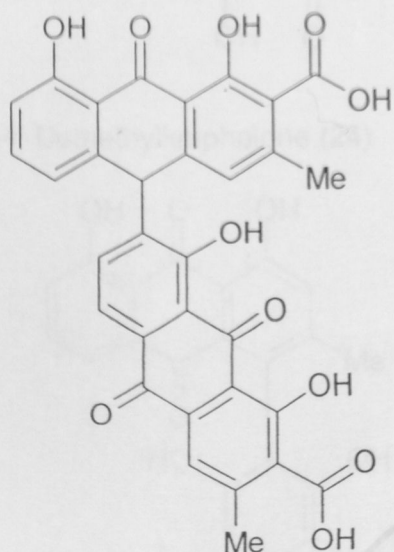
Structures of the compounds identified in *Bulbine*



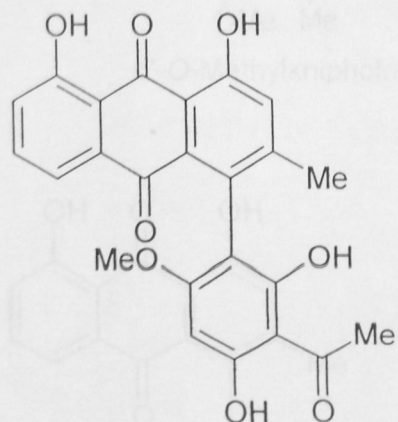
Aloe-emodin (60)



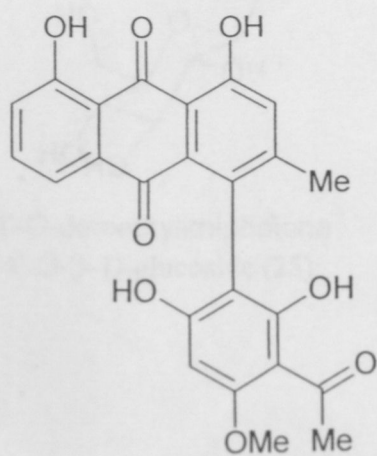
Chrysophanic acid (145)



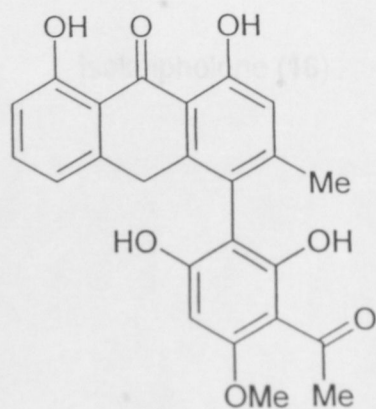
7'-10-Bichrysophanic acid (146)



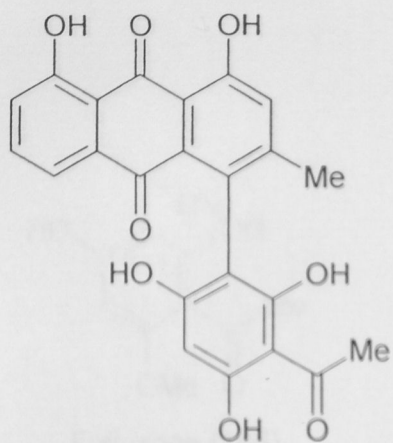
Bulbine knipholone (147)



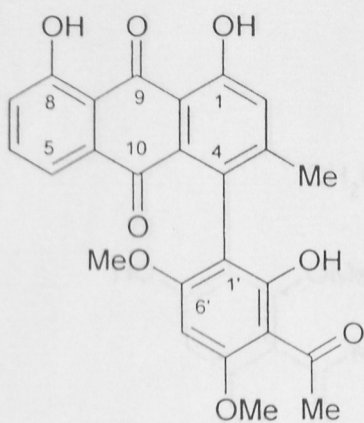
Knipholone (17)



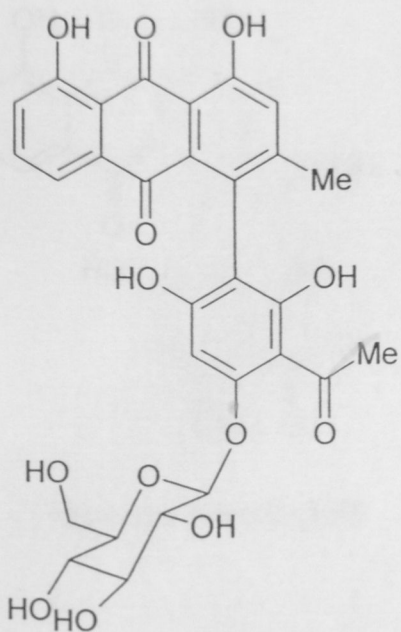
Knipholone anthrone (20)



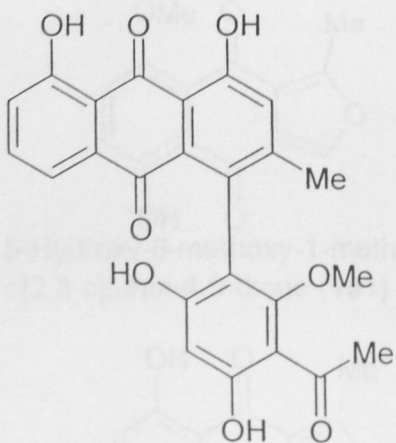
4'-Demethylknipholone (24)



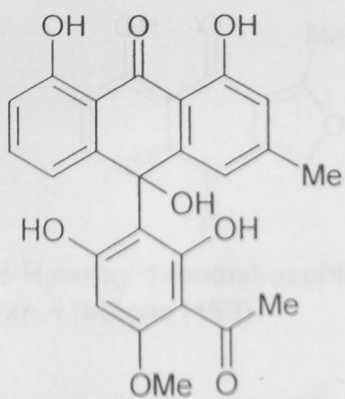
6'-O-Methylknipholone (19)



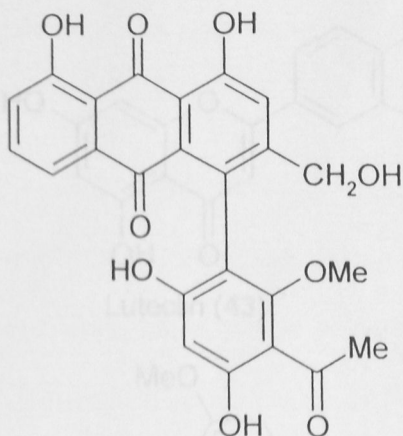
4'-O-demethylknipholone
-4'-O- β -D-glucoside (25)



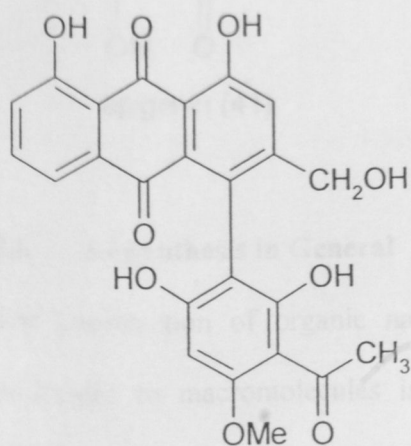
Isoknipholone (18)



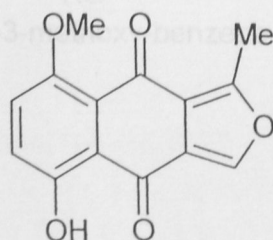
Foliosone (148)



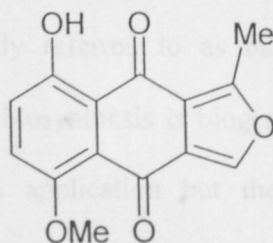
Gaboroquinone A (149)



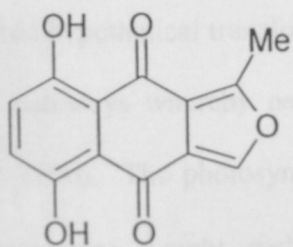
Gaboroquinone B (150)



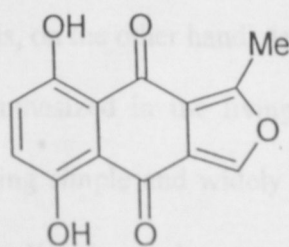
5-Hydroxy-8-methoxy-1-methyl-naphtho[2,3-c]furan-4,9-dione (151)



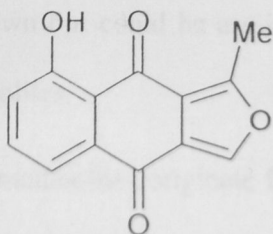
8-Hydroxy-5-methoxy-1-methyl-naphtho[2,3-c]furan-4,9-dione (152)



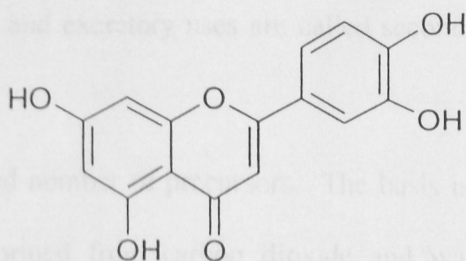
5,8-Dihydroxy-1-methyl-naphtho[2,3-c]furan-4,9-dione (21)



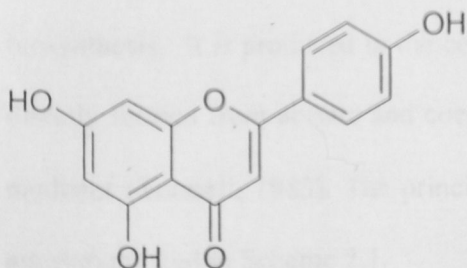
5,8-Dihydroxy-1-methyl-naphtho[2,3-c]furan-4,9-dione (22)



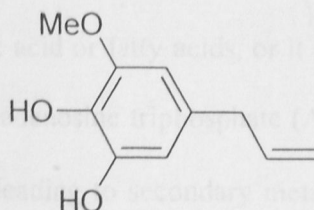
8-Hydroxy-1-methyl-naphtho[2,3-c]furan-4,9-dione (**153**)



Luteolin (**43**)



apigenin (**41**)



5-Allyl-3-methoxy-benzene-1,2-diol (**154**)

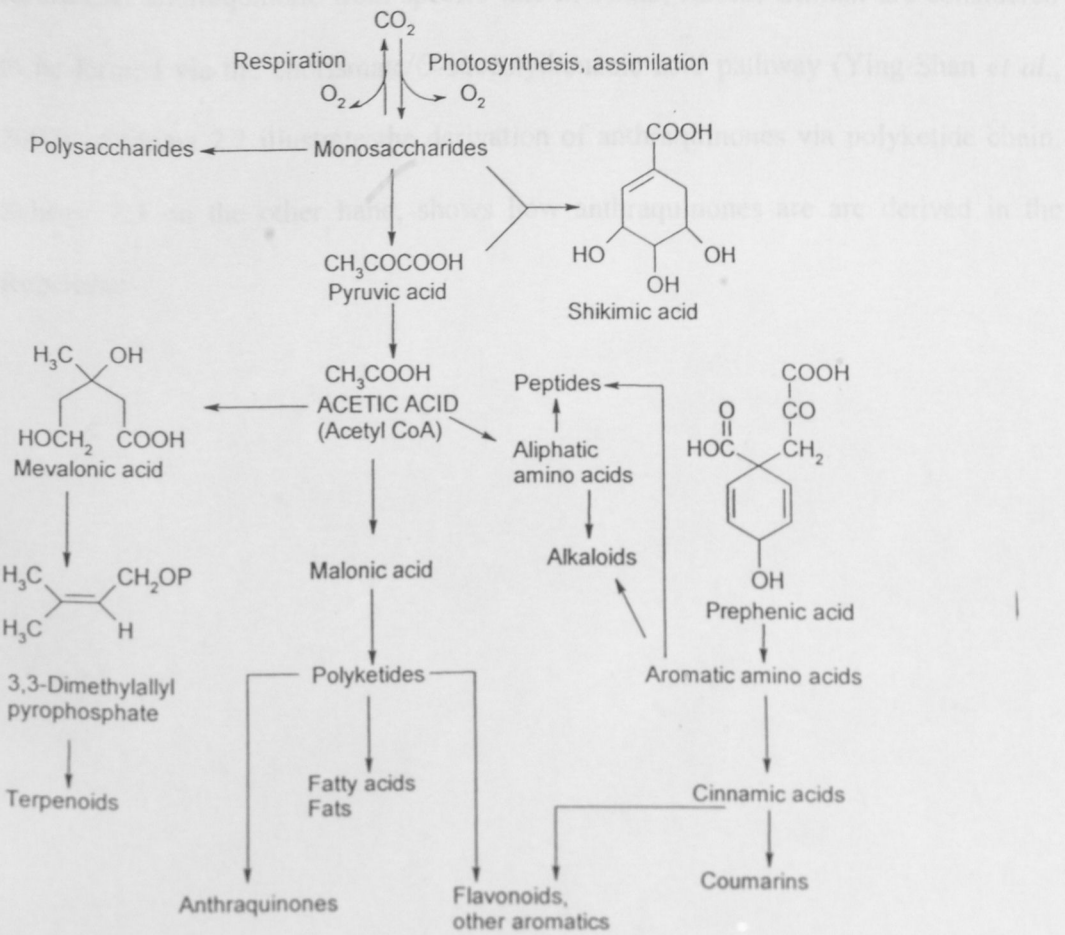
2.6 Biosynthesis in General

The construction of organic natural products by living organisms from simple molecules to macromolecules is more conveniently referred to as biosynthesis. Another term used in connection with the study of biosynthesis is biogenesis. The two words are generally synonymous in normal application but they can be distinguished as follows: biogenesis is a collection of hypotheses which have been proposed to describe the synthesis of natural products in the living organisms; it describes hypothetical transformations. Biosynthesis, on the other hand, describes the actual pathways whereby natural products are synthesized in the living organism (Finar, 2000). The photosynthetic process producing simple and widely distributed low molecular weight carboxylic acids of the Krebs's cycle, α -amino acids, carbohydrates, fats and proteins, is referred to as primary metabolism. The compounds thence derived are known as primary metabolites. The other compounds, synthesized by living organisms, but whose functions to the organisms principally

unknown but could be assigned protective and excretory uses are called secondary metabolites.

Most metabolites originate from very limited number of precursors. The basis is in photosynthesis where carbohydrates are formed from carbon dioxide and water. Acetic acid, in the form of its thioester, acetyl CoA, has a central position in the biosynthesis. It is produced in the cell from pyruvic acid or fatty acids, or it may be directly formed from acetate and coenzyme A with adenosine triphosphate (ATP) as mediator (Torssell, 1983). The principle pathways leading to secondary metabolism are summarized in Scheme 2.1.

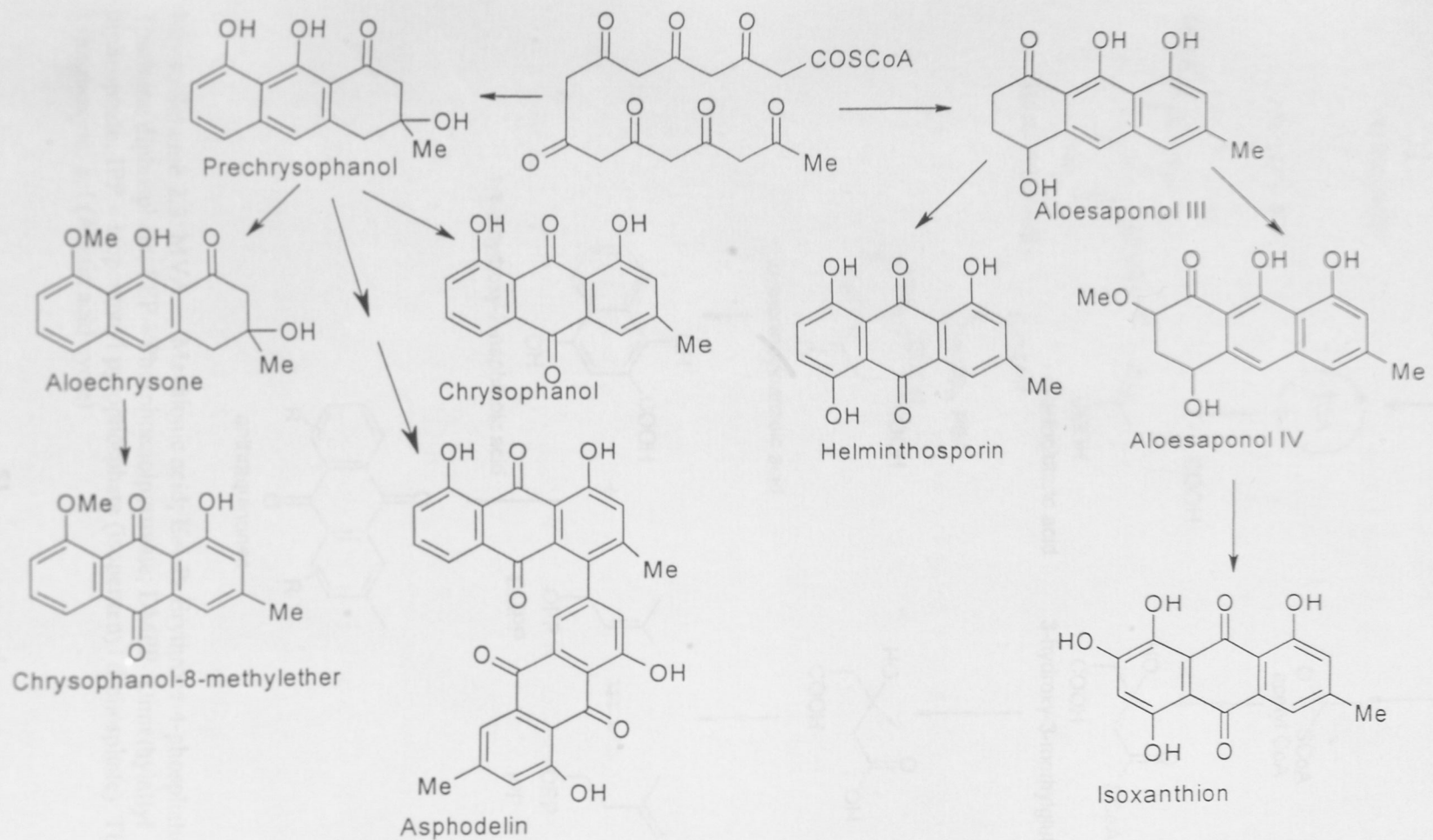
Scheme 2.1: Main streams of Secondary Metabolism (Torssell, 1983)



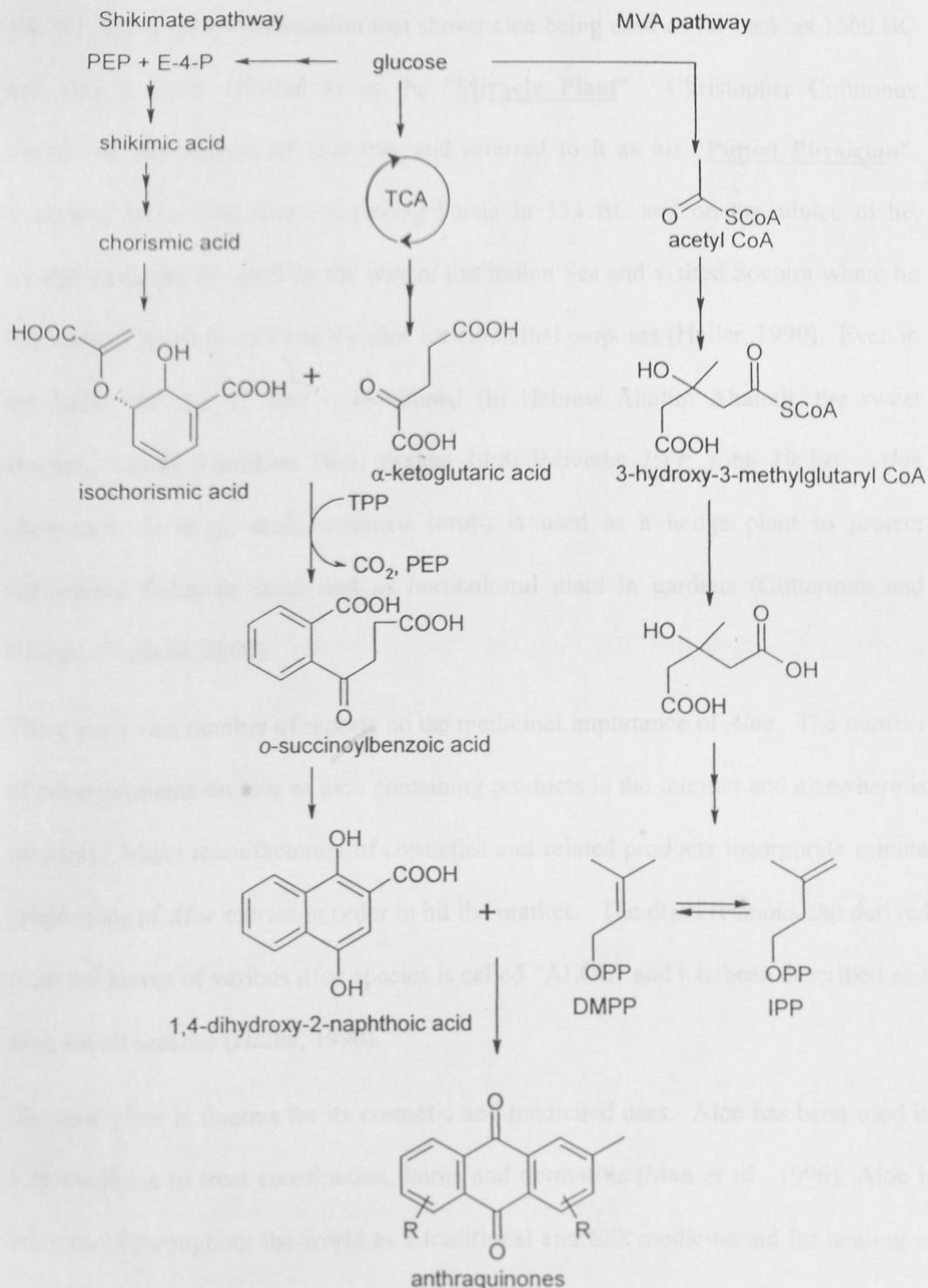
2.7 Biosynthesis of anthraquinones

One of the remarkable features of anthraquinone biosynthesis in higher plants is that they are derived from a variety of different precursors and pathways (Leistner, 1981; Inouye and Leistner, 1988). There are two main biosynthetic pathways leading to anthraquinones in higher plants, the polyketide pathway (van de Berg and Labadie, 1989) and the chorismate/o-succinylbenzoic acid pathway (Leistner, 1985). In the polyketide pathway, anthraquinones are biosynthesized from acetyl-CoA ($\text{CH}_3\text{CO-SCoA}$) and malonyl CoA ($\text{HOOCCH}_2\text{CO-SCoA}$) via an octaketide chain. These types of anthraquinones often exhibit a characteristic substitution pattern, i.e. they are substituted in both rings A and C of the anthraquinone. In some families such as Rubiaceae, anthraquinone from species like *Morinda*, *Rubia*, *Galium* are considered to be formed via the chorismate/O-succinylbenzoic acid pathway (Ying-Shan *et al.*, 2002). Scheme 2.2 illustrates the derivation of anthraquinones via polyketide chain. Scheme 2.3 on the other hand, shows how anthraquinones are derived in the Rubiaceae

Scheme 2.2: Polyketide Biogenetic pathway leading to some Anthraquinones



Scheme 2.3 Biosynthetic pathway leading to anthraquinones in the Rubiaceae



Key to Scheme 2.3: MVA – Mevalonic acid; E-4-P- Erythrose-4-phosphate; TPP – Thiamine diphosphate; PEP – Phosphoenolpyruvate; DMPP- Dimethylallyl diphosphate; IPP – Isopentenyl pyrophosphate (isopentenyl diphosphate); TCA – Tricarboxylic acid (Acetic acid cycle)

2.8 Medicinal Information on *Aloe*

There is historical documentation that shows aloe being used as far back as 1500 BC and aloe is often referred to as the "Miracle Plant". Christopher Columbus discovered the benefits of aloe too, and referred to it as his "Potted Physician". Alexander the Great, after conquering Persia in 333 BC and on the advice of his mentor Aristotle, returned by the way of the Indian Sea and visited Socotra where he left Ionian Greeks to cultivate the aloe for medicinal purposes (Haller, 1990). Even in the Bible, the use of *Aloe* is mentioned (in Hebrew Ahalim Ahaloth, the sweet smelling wood) (Numbers 26:6; Psalms 14:8; Proverbs 7:17; John 19:39). *Aloe aborescens* (a large, multi-stemmed shrub) is used as a hedge plant to protect agricultural fields or stock and as horticultural plant in gardens (Gutterman and Chauser-Volfson, 2000).

There are a vast number of reports on the medicinal importance of *Aloe*. The number of advertisements on aloe or aloe containing products in the internet and elsewhere is limitless. Many manufacturers of cosmetics and related products incorporate minute proportions of *Aloe* extract in order to hit the market. The dried resinous sap derived from the leaves of various *Aloe* species is called "ALOE" and has been described as a drug for all seasons (Haller, 1990).

The aloe plant is famous for its cosmetic and medicinal uses. Aloe has been used in folk medicine to treat constipation, burns and dermatitis (Man *et al.*, 1996). Aloe is recognized throughout the world as a traditional and folk medicine aid for healing of skin injuries, such as cutaneous wounds and sunburns (Bouthet *et al.*, 1995). In Kenya, traditional medicine men have always incorporated *Aloe* leaves (water extract, exudate or succulent fluid) in their herbal concoctions. *Aloe vera* and Cape aloe (*Aloe ferox* Miller) are cultivated in some parts of the world for commercial purposes

(several Internet sites). The *Aloe vera* gel obtained from the inner thin walled mucilaginous parenchyma cells has been used for a host of treatment purposes (Gjerstad and Riner, 1968; Trotter, 1981). The gel differs from the yellow exudate obtained from the pericyclic cells that contain aloe-emodin (60) and other anthracene derivatives and lie beneath the epidermis (Koo, 1994). The plants cape aloe (*A. ferox* Mill.) and *A. marlonthii* Berger have a wide range of traditional uses, of which the production of the laxative drug, Cape aloes, is the most important (van der Bank *et al.*, 1995). Other uses of aloe include cosmetics (skin lotions and hair care products) and food (leaves used for making jam) (van der Bank *et al.*, 1995). Traditional uses of *A. marlonthii* include treatment of roundworm infections, using the powdered leaves in snuff, for stomach troubles, and for hastening weaning of children by rubbing the green leave pulp over the breasts (van der Bank *et al.*, 1995). In South Africa, the leaf juice of *Aloe ferox* is collected and dried by a traditional method to produce a dark brown solid substance known as bitter aloes or Cape Aloes (van Wyk *et al.*, 1995b). The larger part of the produce is exported to Europe (mainly Italy, France, and Germany (van Wyk *et al.*, 1995b). The main purgative principal is the anthrone glycoside barbaloin (92), whose content of the dried leaf exudate is reported to vary between 8.5 and 30% (van Wyk *et al.*, 1995b). In Somalia, the leaf juice of *Aloe microdonta* (vernacular name Dacaar Qaraar) is traditionally used as a remedy for jaundice and skin diseases (Mohamed *et al.*, 1992). A hot glycerine extract of *Aloe barbadensis* was found to inactivate herpes simplex virus type 1 (Sydiskis *et al.*, 1991). When damaged, aloe is known to close any wounds to its leaf almost immediately so that the plant does not lose any of its precious life sustaining fluids. It is probably for this reason that aloe has gained its reputation as a moisturizer in the cosmetic industry.

2.9 Aloe trade

The trade in Aloe products is a major world activity and there are numerous companies dealing in this area. According to the International Aloe Science Council (<http://www.iasc.org/>), the global is reported as 124 million dollars as opposed to a potential of 997 million dollars. According to the same report, the world cultivation of Aloe vera (acreage) is as follows:

American continent	19119 ha
Africa	4170 ha
Australia	300 ha

The report also gives the percentage worldwide cultivation of Aloe vera by country as follows:

Mexico	47%	United States	3%
Dominican Republic	15%,	Costa Rica	2%
Venezuela	15%	India	1%
China	9%	Malysia	1%
Thailand	6%	Guatemala	1%

The case of Kenyan Aloe is presented in the chart (<http://www.itdg.org>) below.

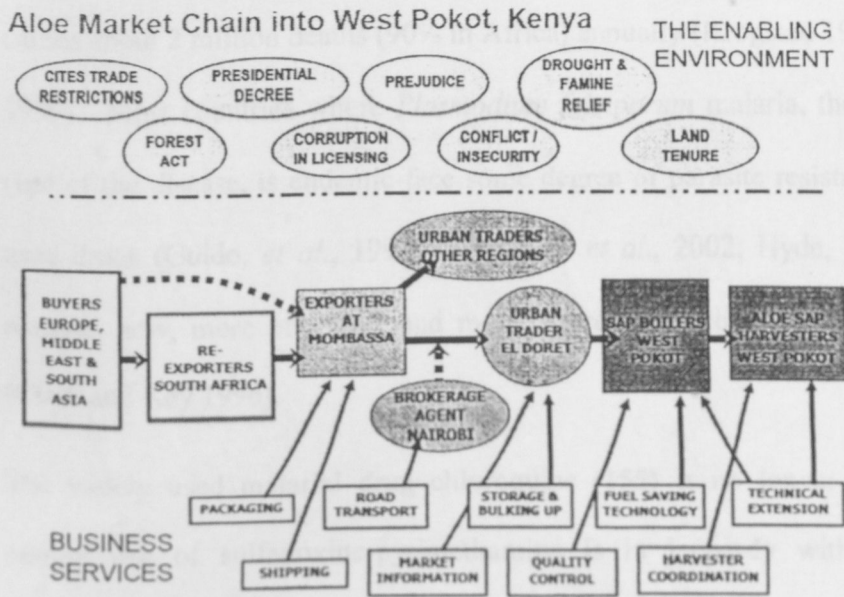


Fig. 2.3 Aloe market chain in West Pokot, Kenya

2.10 Information on the use of *Bulbine*

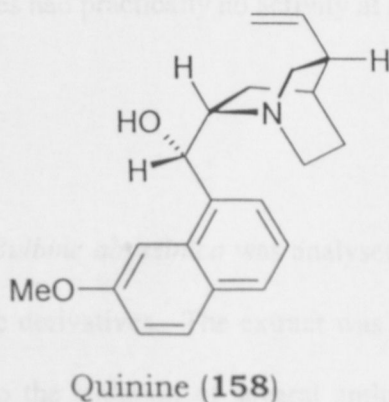
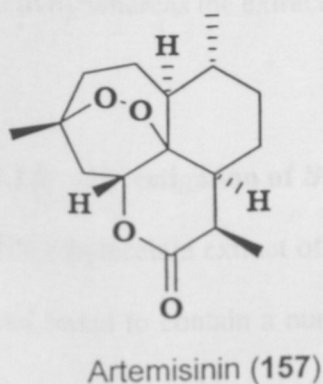
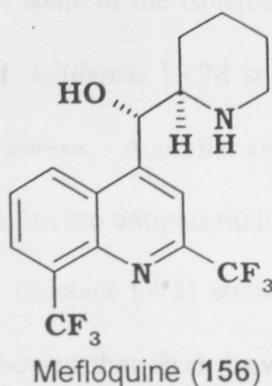
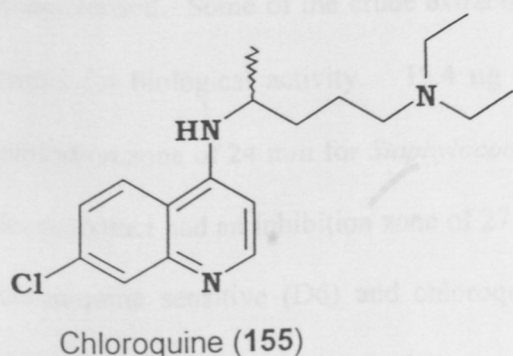
In traditional medicine, *Bulbine* species are used for treatment of various ailments that arise from infections (Watt *et al.*, 1962). In South Africa *B. asphodeloides* is used as a styptic, for scrofula, dehydration and for palpitation (Simon and Lamla., 1991), while *B. latifolia*, is used to treat rheumatism, diarrhea and dysentery and is administered by oral infusion to human adults (van Staden *et al.*, 1994). *B. bulbosa* (Australia) has antimitotic activity (plant cells), active allium cepa root tips. (Barnard, 1949). Mitotic activity has also been reported in the species.

2.11 Malaria

Malaria kills more people than any other communicable disease and is therefore considered the world's most important tropical parasitic disease (François *et al.*, 1999). According to Mishra *et al.*, 1999, the world population living in malaria-endemic areas is close to 2 billion (about 40% of the world population). The disease causes about 2 million deaths (90% in Africa) annually (Krogstad 1996, Mishra *et al.*, 1999). Most countries where *Plasmodium falciparum* malaria, the most dangerous type of the disease, is endemic face some degree of parasite resistance to frequently used drugs (Guido, *et al.*, 1999; Winstanley *et al.*, 2002; Hyde, 2002). For these reasons, new, more effective, and more affordable antimalarials are badly needed (Clark and Key 1996).

The widely used malarial drug chloroquine (155) is no longer effective and the current use of sulfadoxine-pyrimethamine is in jeopardy with the increase in resistance to this combination treatment [Trape *et al.*, 2002; Nzila-Mounda *et al.*, 1998; Sibley *et al.*, 2001].

Therefore, there is need to develop efficacious structurally diverse anti-malarial drugs with a new mode of action to replace the current drugs that are becoming ineffective (Derese, 2004). One of the most important anti-malarials to come from plants is quinine (158) which was isolated from the bark of *Cinchona succuriba* (Rubiaceae) and it served as a template for chloroquine (155) and mefloquine (156) [Balint, 2001; Price, 2002]. The most recent lead drug is artemisinin (157) from the Chinese anti-malarial herb *Artemisia annua* (Astraceae) [Balint, 2001; Price, 2002] which has been used successfully against the parasite that has become resistant to chloroquine (155). *Artemisia annua* has been used for eons by the Chinese to treat malaria (Balint, 2001).



CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Preliminary Test Results

3.1.1. Investigation of *Aloe* species

Extracts of subterranean stems of three *Aloe* species (*Aloe kilifiensis*, *A. lateritia* and *A. graminicola*) were analysed by thin layer chromatography (TLC) and showed the presence of several coloured components. Most of the spots on TLC showed a colour response on exposure on exposure to ammonia or upon being sprayed with methanolic potassium hydroxide. From a chemotaxonomic assumption, these compounds were presumed to be anthraquinone derivatives. The major components were isolated and characterised. Some of the crude extracts and some of the isolated compounds were tested for biological activity. 15.4 µg of *A. kilifiensis* DCM stem extract had an inhibition zone of 24 mm for *Staphylococcus aureus*. A similar amount of the DCM leave extract had an inhibition zone of 27 mm. Invitro antiplasmodial tests against the chloroquine sensitive (D6) and chloroquine resistant (W2) strains of *Plasmodium falciparum* for some of the crude extracts showed that fresh leaves had reasonable activity whereas the extracts of the dried leaves had practically no activity at all.

3.1.2 Investigation of *Bulbine* species

The ethylacetate extract of the dry fruits of *Bulbine abyssinica* was analysed by TLC and found to contain a number of anthracene derivatives. The extract was subjected to chromatographic separation, which led to the isolation of several anthraquinone derivatives. The acetone extract from the roots of *B. abyssinica* was also chromatographed and anthracene derivatives isolated. The same was done to the dichloromethane extracts from stems and roots of *B. frutescens*. A later sample

(collected in January 2004) of *B. frutescens* roots was extracted using acetone and the extract chromatographed yielding a number of compounds. Bioassays were carried out on some of the isolated compounds. Compound 27 showed the highest activity against the chloroquine sensitive (D6) strain of *Plasmodium falciparum*. The other compounds had very low activity in comparison with the control drugs.

3.2 Characterization of the Compounds

3.2.1 Compounds from the subterranean stems of *Aloe lateritia* var. *lateritia*, *A. kilifiensis*, and *A. graminicola*

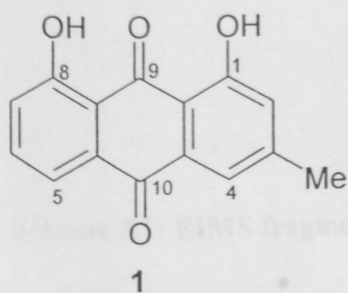
The dried and ground stems of *A. lateritia* var. *lateritia* were extracted using acetone. The extract was chromatographed yielding ten compounds (Compounds 1, 3b, 4, 6, 7, 8, 9, 10, 11 and 12). Powdered stems of *A. kilifiensis* were extracted serially using n-hexane, dichloromethane and acetone. The dichloromethane and acetone extracts were combined on basis of TLC comparisons. The combined extracts were chromatographed yielding eight compounds (compounds 1, 3, 4, 6, 7, 9, 10 and 11). From the tetrahydrofuran extract of *A. graminicola*, compounds 1, 2, 3b, 4, 5, 7 and 12 were identified.

Compound 1

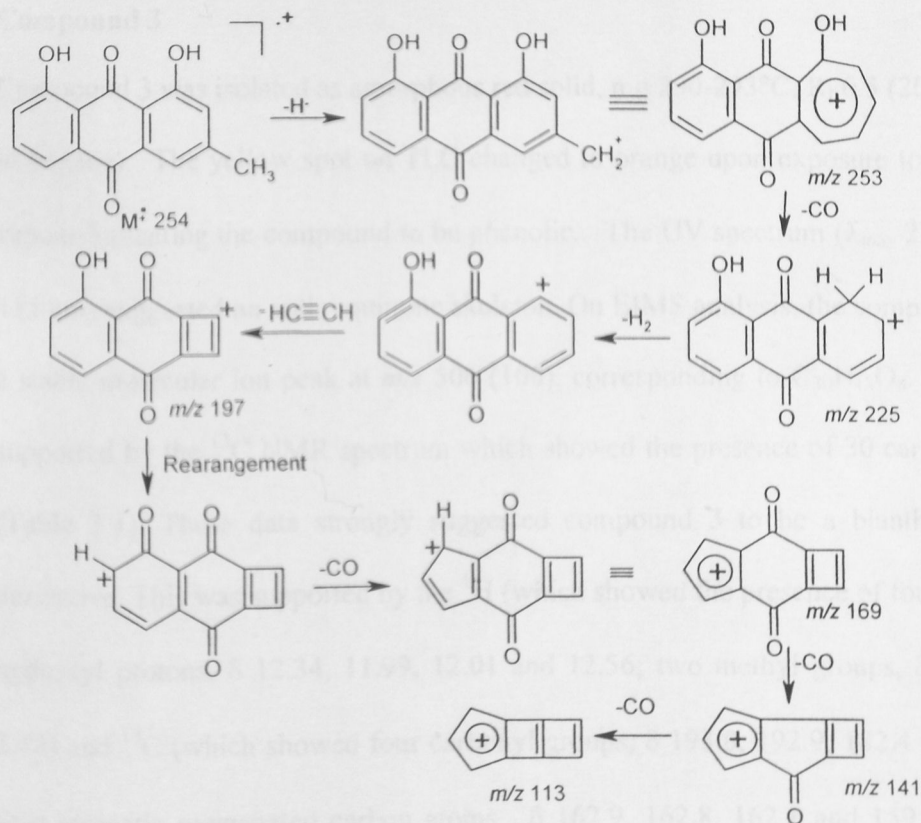
Compound 1 was isolated as yellowish red crystals with melting point of 195 - 197° C. The EIMS spectrum of this compound displayed a molecular ion peak at m/z 254 (100) consistent with a molecular formula $C_{15}H_{10}O_4$. 1H NMR (300 MHz) of this compound showed two downfield shifted hydroxyl protons at δ 12.08 and 11.98, which is consistent with a 1,8-dihydroxyanthraquinone skeleton where the de-

shielding results from chelation. The aromatic protons at C-5, -6 and -7 resonated as mutually coupled protons with an ABC spin system at δ 7.79 (*dd*, $J = 7.5, 1.2$ Hz), δ 7.66 (*t*, $J = 8.1$), and δ 7.27 (*dd*, $J = 7.2, 1.0$ Hz), respectively. The proton on C-4 appeared at δ 7.62 ppm (*d*, 1.2 Hz), while H-2 resonated as a broad singlet at δ 7.07. The broadening resulted from 4J coupling with methyl protons as well as H-4. The methyl group at C-3 resonated at δ 2.45 ppm. A summary of the NMR data is given in Table 3.1. The fragmentation pattern is as given in scheme 3.1 below.

Based on these data, compound **1** was identified as 1,8-dihydroxy-3-methylantraquinone, trivial name chrysophanol (**1**).



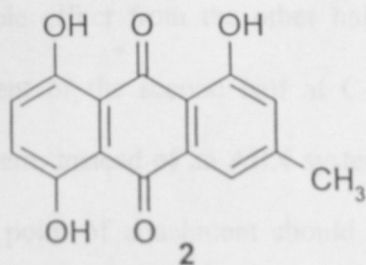
The compound has been reported from several plants in the past. Fairbairn and Shrestha (1967) reported that in the seeds of *Cassia senna* L. plants, no anthraquinones are present but shortly after germination chrysophanol (**1**), then aloe-emodin (**60**) and finally rhein are formed in the young plant. Fairbairn and Muhtadi (1972) established that chrysophanol, aloe-emodin and emodin are biosynthesized via the acetate malonate route in *Rumex obtusifolius*. Leistner (1971) studied the biosynthesis of chrysophanol and emodin in *Rumex alpinus* and reported that these compounds are derived from acetate by linear combination.



Scheme 3.1: EIMS fragmentation of chrysophanol (1)

Compound 2

This compound was isolated as red needle like crystals with a melting point of 215-217° C. The compound was identified as 1,5,8-trihydroxy-3-methylantraquinone (trivial name helminthosporin) upon TLC comparisons with an authentic sample (Yenesew, 1991). Besides from *Aloe*, the compound has been isolated from *Drechslera holmii* (van Eijk and Roeymans (1981).



Compound 3

Compound **3** was isolated as amorphous red solid, mp 290-293°C, R_f 0.5 (20% EtOAc in hexane). The yellow spot on TLC changed to orange upon exposure to ammonia vapour indicating the compound to be phenolic.. The UV spectrum (λ_{max} 258, 291, sh, 435 nm) suggested an anthraquinone skeleton. On EIMS analysis, the compound gave a stable molecular ion peak at m/z 506 (100), corresponding to $C_{30}H_{18}O_8$. This was supported by the ^{13}C NMR spectrum which showed the presence of 30 carbon atoms (Table 3.1). These data strongly suggested compound **3** to be a bianthraquinone derivative. This was supported by the 1H (which showed the presence of four chelated hydroxyl protons, δ 12.34, 11.99, 12.01 and 12.56; two methyl groups, δ 2.13 and 2.48) and ^{13}C (which showed four carbonyl groups, δ 193.0, 192.9, 182.4 and 181.9; four aromatic oxygenated carbon atoms, δ 162.9, 162.8, 162.1 and 159.6 and two methyl groups, δ 22.3 and 21.4) NMR spectra. These data suggested that **3** could be a dimer of chrysophanol (**1**). In the MS the fragment ion at m/z 253 ($C_{15}H_9O_4$) supported that **3** is a bichrysophanol.

The position of linkage was established by comparison of the aromatic protons spin pattern (in the 1H NMR spectrum) of **3** with that of chrysophanol (Table 3.1). In one half of the molecule the signal for H-4 is missing while the doublet for H-2 in chrysophanol is replaced by a singlet in **3**, hence the point of attachment for this half of the molecule is at C-4. The chemical shift position of the methyl at C-3 is up-field shifted due to the anisotropic effect from the other half of the molecule. This is consistent with the placement of the second half at C-4. In the second half, the presence of an AX spin system, instead of an ABX system for ring A (while H-5' is present) suggested that the point of attachment should be at C-7'. Therefore, this

compound is characterized as 4-7'-bichrysophanol, trivial name asphodelin (Yagi et al., 1978; Adinolei *et al.*, 1991). Compound **3** is optically active ($[\alpha]_D = -9.2^\circ$) due to axial chirality at the biaryl linkage. The stereochemistry at the biaryl linkage was established as M (aR) from X-ray crystal structure analysis (Figure 3.1).

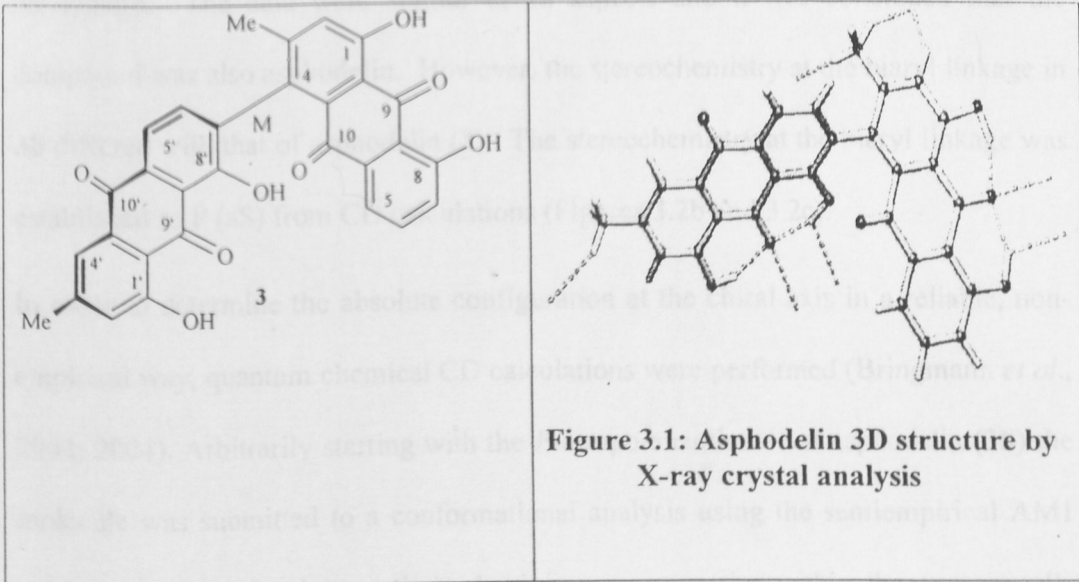


Figure 3.1: Asphodelin 3D structure by X-ray crystal analysis

This atropisomer, on CD analysis, showed a negative cotton effect at 271 nm (figure 3.2). This fact and the negative optical rotation ($[\alpha]_D = -9.2$) indicated that the asphodelin isolated from *A. kilifiensis* has opposite absolute configuration to that reported for asphodelin samples from *Aloe saponaria* (Dagne, 2000) and *Asphodelus* species (Adinolei *et al.*, 1991), both of which were dextrorotatory.

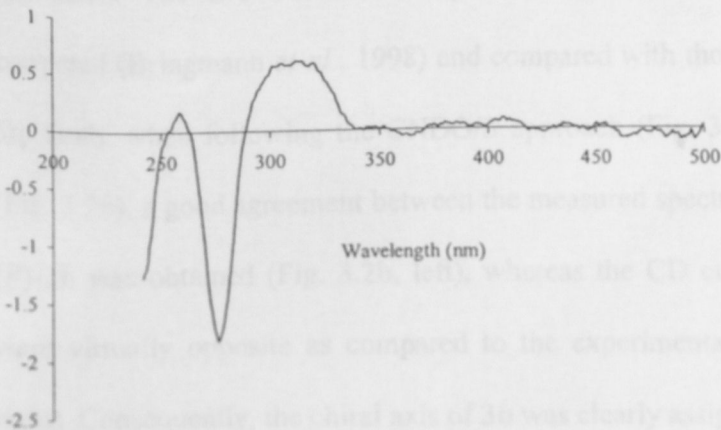


Fig. 3.2 CD curve for asphodelin (**3**)

Compound **3b**

Compound **3b** was isolated as amorphous red solid, mp 290-293°C, R_f 0.5 (20% EtOAc in hexane). TLC of this compound was similar to that of asphodelin (**3**), and hence all its other spectroscopic data (UV, EIMS, NMR) were compared with that of asphodelin. The data were similar in all aspects and it was concluded that the compound was also asphodelin. However, the stereochemistry at the biaryl linkage in **3b** differed with that of asphodelin (**3**). The stereochemistry at the biaryl linkage was established as P (aS) from CD calculations (Figures 3.2b and 3.2c).

In order to determine the absolute configuration at the chiral axis in a reliable, non-empirical way, quantum chemical CD calculations were performed (Bringmann *et al.*, 2003; 2004). Arbitrarily starting with the *P*-atropo-enantiomer of asphodelin (**3b**) the molecule was submitted to a conformational analysis using the semiempirical AM1 method, resulting in eight optimized minimum geometries within the energetically relevant range of 3 kcal/mol above the global minimum. For each single geometry thus obtained, a CD spectrum was calculated using the semiempirical CNDO/S as well as the OM2 Hamiltonian. In both cases the single spectra were then added and weighted following the Boltzmann statistics, i.e. according to the respective heats of formation. The two overall CD spectra, thus obtained, were subsequently UV corrected (Bringmann *et al.*, 1998) and compared with the experimental CD curve of **3b**. Both, when following the CNDO/S approach (Fig. 3.2b) and the OM2 method (Fig. 3.2b), a good agreement between the measured spectrum and those predicted for (*P*)-**3b** was obtained (Fig. 3.2b, left), whereas the CD curves computed for (*M*)-**3b** were virtually opposite as compared to the experimental one (Fig. 3.2b and 3.2c, right). Consequently, the chiral axis of **3b** was clearly assigned to be *P*-configured.

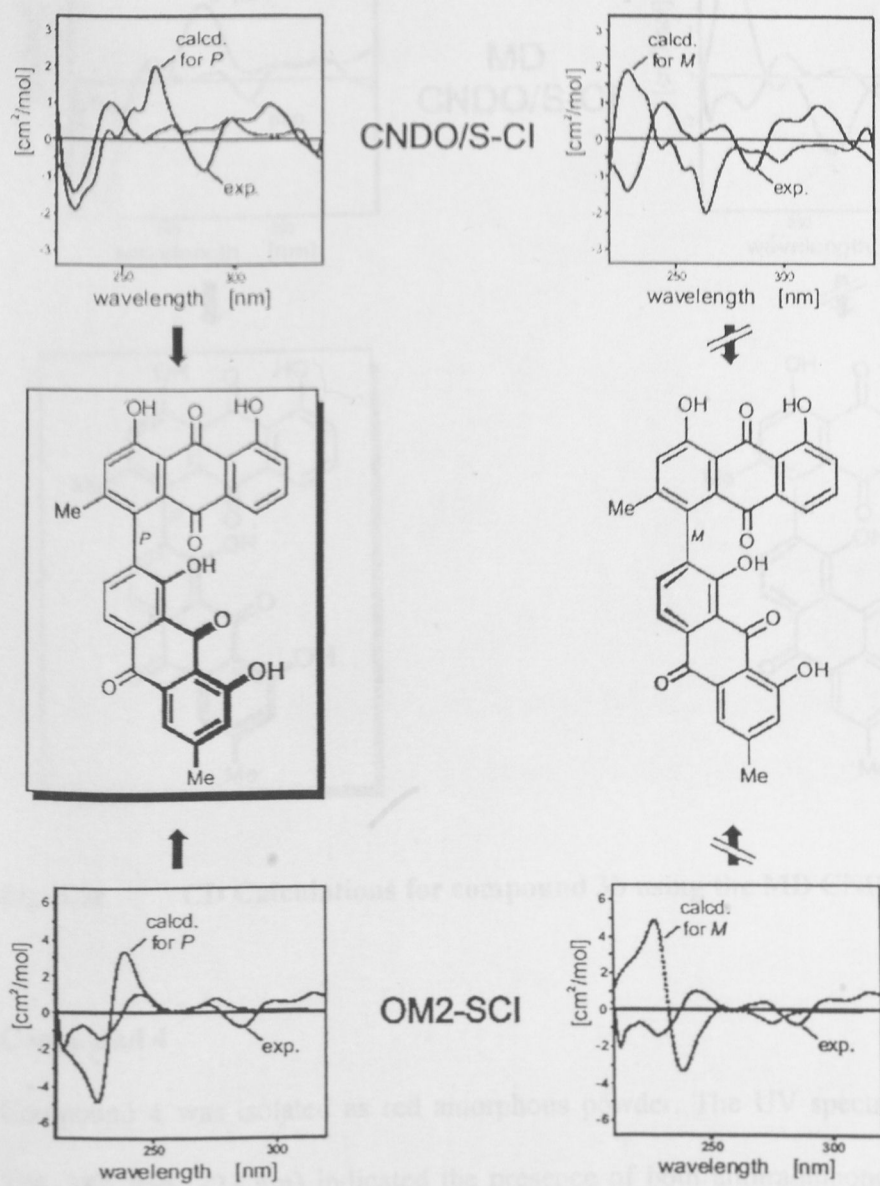


Fig. 3.2b CD Calculations for compound 3b using the CNDO/ SC-I and OM2-SCI

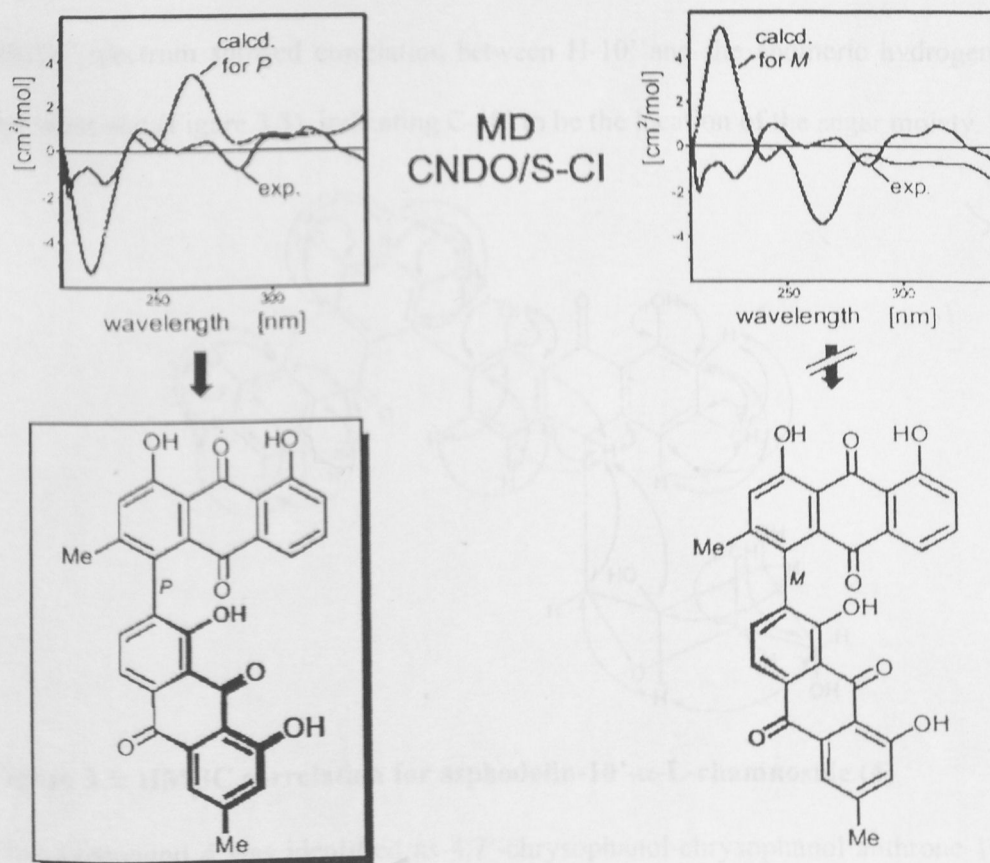


Fig. 3.2c CD Calculations for compound 3b using the MD CNDO/ SC-I

Compound 4

Compound 4 was isolated as red amorphous powder. The UV spectrum (λ_{max} 433, 374, 287, 256, 223 nm) indicated the presence of both anthraquinone and anthrone units. The ^1H and ^{13}C NMR spectra (Table 3.1) of compound 4 showed characteristic features of a bianthrane glycoside (Adinolei *et al.*, 1991). By comparison of the ^1H and ^{13}C NMR spectra (Table 3.1) of this compound with those of asphodelin, it was clear that this compound also has C-4 of one chrysophanol moiety linked to C-7' of another anthracene moiety. The ^1H NMR spectrum of the compound had peaks characteristic of a sugar unit. A thorough observation of the peaks indicated that the

sugar unit was a rhamnoside. This was later confirmed from CD calculations. The HMBC spectrum showed correlation between H-10' and the anomeric hydrogen of the sugar unit (Figure 3.3), indicating C-10' to be the location of the sugar moiety.

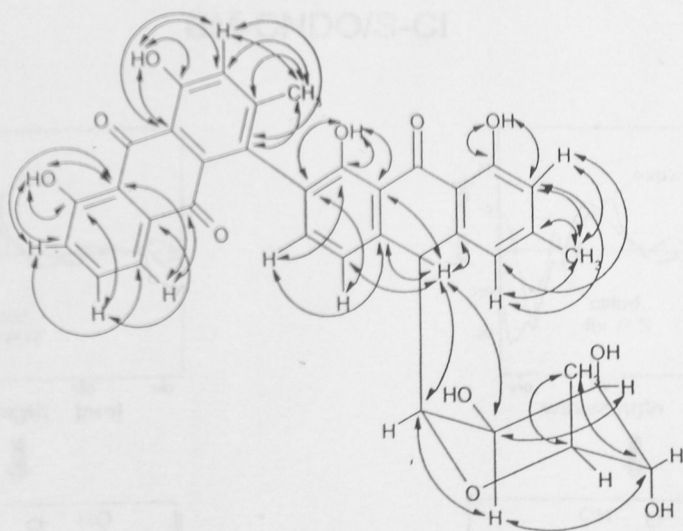
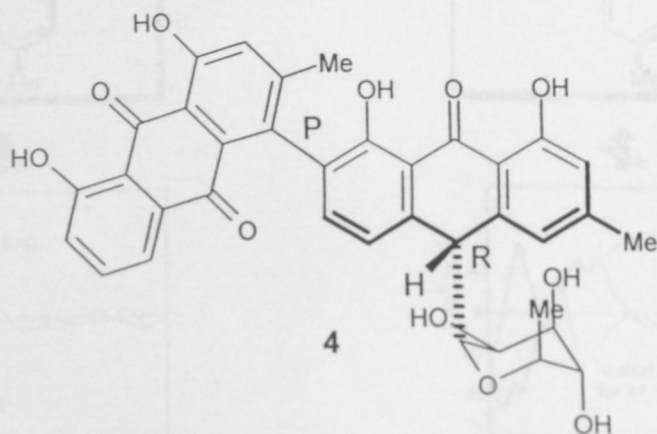


Figure 3.3: HMBC correlation for asphodelin-10'- α -L-rhamnoside (4)

Thus compound **4** was identified as 4,7'-chrysophanol-chrysophanol anthrone-10- α -L-rhamnopyronoside.



This compound has both an axis of chirality at 4-7' and a stereogenic centre at C-10' on the bianthrane skeleton. The CD spectrum of this compound almost resembles that of asphodelin (**3b**) and hence the configuration at the biaryl linkage could be the same [P (αS)]. Further CD calculations were carried out and the configuration at the

biaryl linkage was confirmed to be *P* (*aS*) and the stereochemistry at C-10' as *R* (Figures 3.3b to 3.3d). Computation of the stereochemistry by CD analysis was carried out in a similar to that for compound 3b above.

BM-CNDO/S-CI

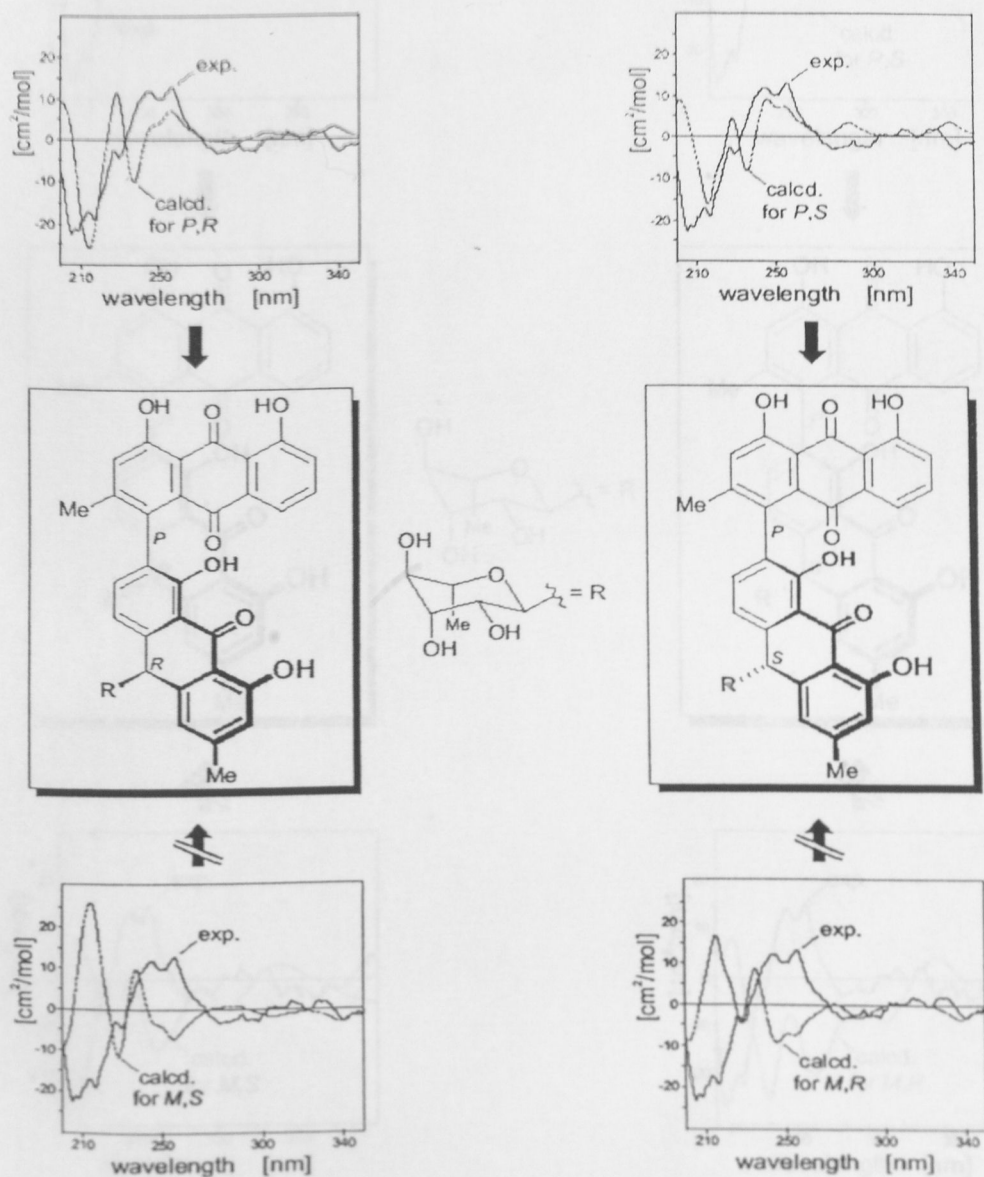


Figure 3.3 b BM CNDO/SC-I calculation for compound 4

MD-CNDO/S-CI

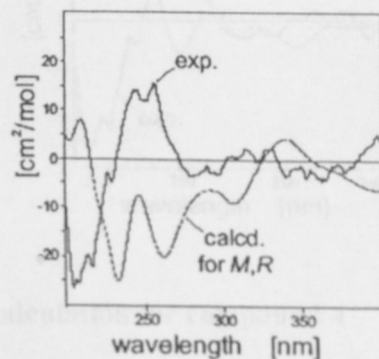
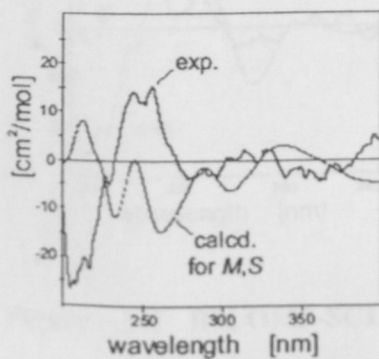
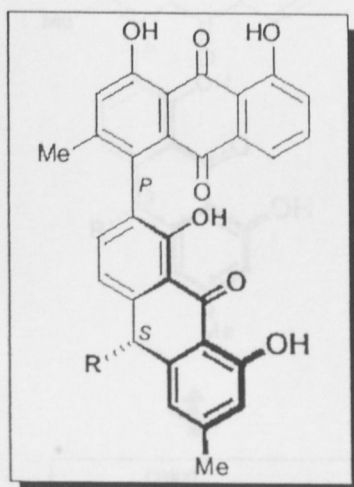
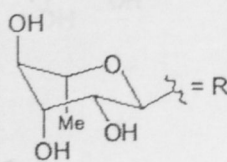
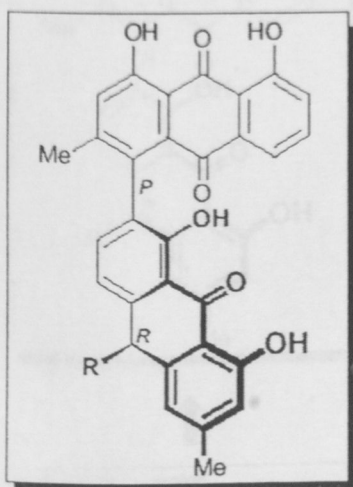
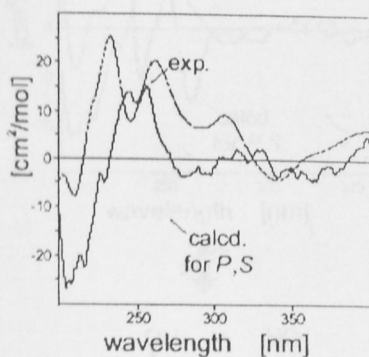
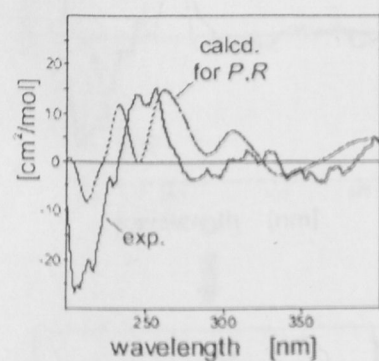


Figure 3.3 c MD CNDO/SC-I calculation for compound 4

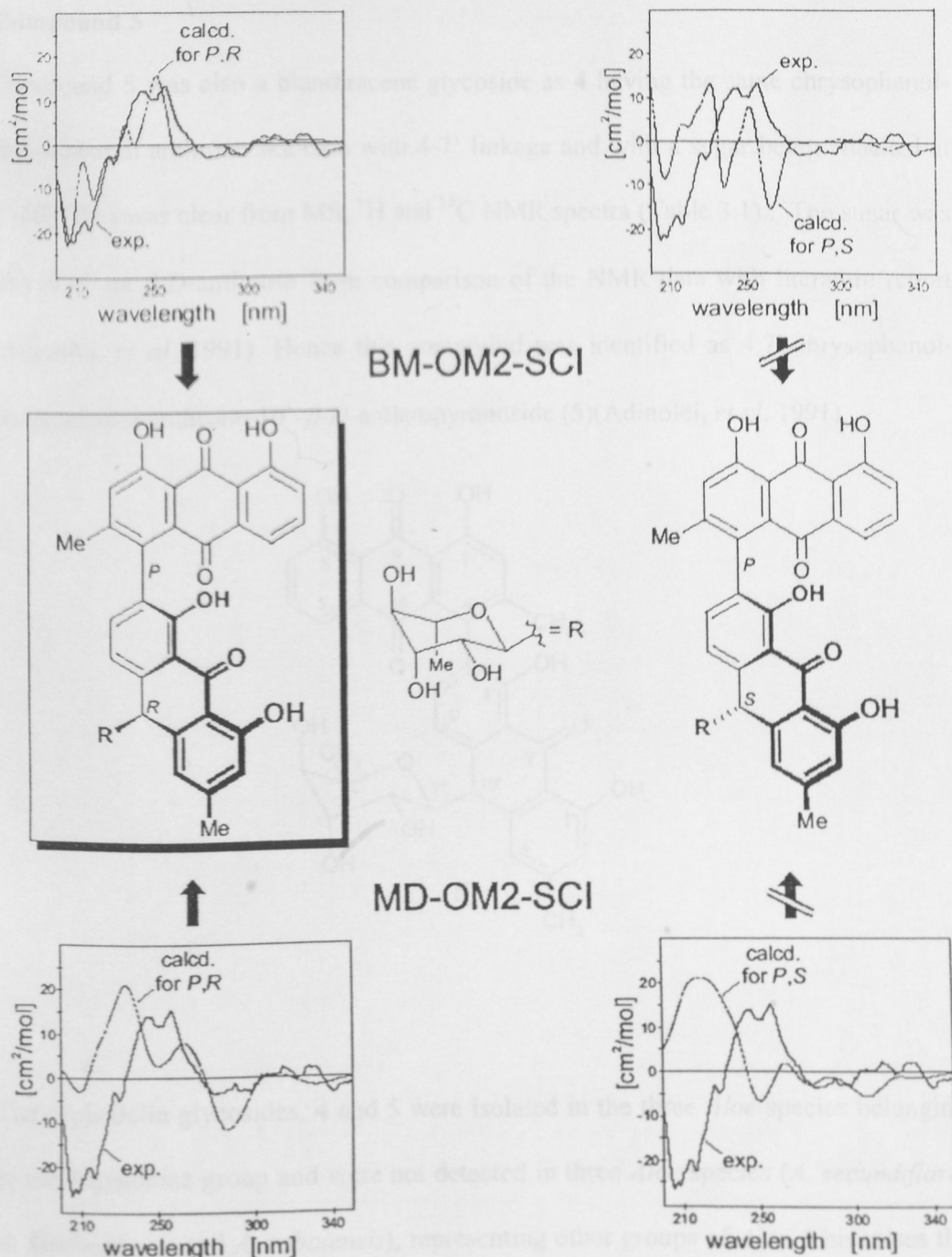
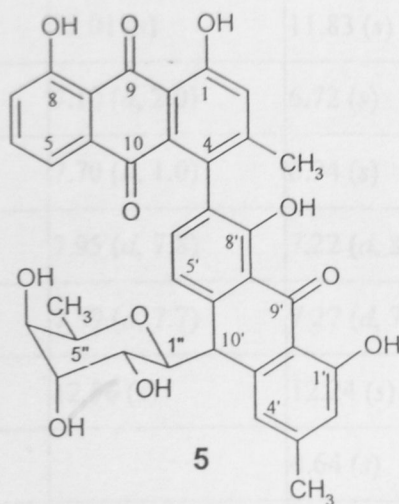


Figure 3.3 d BM OM2-SCI and MD-OM2-SCI calculation for compound 4

Compound 4, along with four other bianthrone glycosides has been reported from the tubers of *Asphodelus ramosus* (Adinolei *et al.*, 1991). However, this is the first report from the genus *Aloe*.

Compound 5

Compound 5 was also a bianthrane glycoside as 4 having the same chrysophanol-chrysophanol anthrone skeleton with 4-7' linkage and with a sugar being attached at C-10'. This was clear from MS, ^1H and ^{13}C NMR spectra (Table 3.1).. The sugar was identified as β -D-antiarose from comparison of the NMR data with literature report (Adinolei, *et al.* 1991). Hence this compound was identified as 4,7'-chrysophanol-chrysophanol anthrone-10'- β -D-antiaropyranoside (5)(Adinolei, *et al.* 1991).



The asphodelin glycosides, 4 and 5 were isolated in the three *Aloe* species belonging to the Saponariae group and were not detected in three *Aloe* species (*A. secundiflora*, *A. kindongensis* and *A. rabaiensis*), representing other groups of *Aloe*. This raises the possibility that compounds 4 and 5 could be chemotaxonomic markers of the Saponariae group, in the same way isoeleutherol is. (Dagne *et al.*, 1994). Therefore it would be interesting to screen other members of this group and representative species from other groups for the presence or absence of these bianthrane glycosides.

Table 3.1: ¹H-NMR data for compounds 1, 3, 4 and 5

C	1	3/3a	4	5
2	7.07 (<i>br s</i>)	7.26 (<i>d</i> , 2.6)	7.33 (<i>s</i>)	7.29 (<i>br s</i>)
4	7.62 (<i>d</i> , <i>J</i> = 1.2)			
5	7.79 (<i>dd</i> , <i>J</i> = 7.5, 1.2)	7.56 (<i>d</i> , 7.2)	7.55 (<i>d</i> , 7.4)	7.30 (<i>dd</i> , 8.2, 0.9)
6	7.66 (<i>t</i> , <i>J</i> = 8.1)	7.59 (<i>t</i> , 7.8)	7.70 (<i>t</i> , 8.0)	7.59 (<i>dd</i> , 8.3, 8.1)
7	7.27 (<i>dd</i> , <i>J</i> = 7.2, 1.0)	7.28 (<i>d</i> , 7.4)	7.28 (<i>d</i> , 8.0)	7.46 (<i>dd</i> , 8.2, 0.9)
1'		12.01 (<i>s</i>)	11.83 (<i>s</i>)	
2'		7.10 (<i>d</i> , 2.0)	6.72 (<i>s</i>)	6.82 (<i>bs</i>)
4'		7.70 (<i>d</i> , 1.0)	6.94 (<i>s</i>)	6.75 (<i>bs</i>)
5'		7.95 (<i>d</i> , 7.8)	7.22 (<i>d</i> , 8.0)	7.08 (<i>d</i> , 7.6)
6'		7.39 (<i>d</i> , 7.7)	7.27 (<i>d</i> , 7.3)	7.13 (<i>d</i> , 7.6)
8'		12.56 (<i>s</i>)	12.24 (<i>s</i>)	
10'			4.64 (<i>s</i>)	4.50 (<i>d</i> , 2.5)
11'		2.48 (<i>s</i>)	2.41 (<i>s</i>)	2.41 (<i>s</i>)
1''			4.00 (<i>t</i> , 7.9)	4.01 (<i>dd</i> , 7.3, 2.4)
2''			3.98 (<i>t</i> , 7.9)	4.10 (<i>dd</i> , 7.3, 4.8)
3''			3.96 (<i>d</i> , 7.6)	3.91 (<i>t</i> , 4.0)
4''			3.44 (<i>s</i>)	3.48 (<i>t</i> , 4.8)
5''			3.36 (<i>m</i>)	3.39 (<i>m</i>)
6''			1.03 (<i>d</i> , 6.7)	1.09 (<i>d</i> , 6.7)
OH-1	12.08	12.38	12.48	
OH-8	11.98	11.99	11.94	
Me-11	2.45	2.13	2.18	2.18

Table 3.2: ^{13}C -NMR data for compounds 3, 4 & 5

Carbon	DEPT	3	4	5	Carbon	DEPT	3	4	5
1	C	162.9	163.3	162.2	4'a	C	134.0	147.7	150.1
1a	C	114.8	115.6	115.3	4'	CH	120.4	120.4	116.1
2	CH	125.5	125.5	124.3	5'a	C		142.3	148.1
3	C	149.4	151.4	148.8	5'	CH	120.0	121.3	119.1
4	C		132.8	131.6	6'	CH	137.2	135.3	134.1
4a	C	132.7	132.2	140.8	7'	C	136.0	128.7	131.1
5a	C		135.5	134.3	8'	C	159.6	160.4	162.1
5	CH	124.3	120.5	125.7	8'a	C		118.3	130.1
6	CH	137.2	138.2	137.2	9'	C	192.9	194.0	192.1
7	CH	124.1	124.4	120.8	10'	CH		46.2	46.4
8	C	162.8	162.7	159.8	11'	CH ₃	21.4	22.1	21.1
8a	C	115.7	116.3	115.5	1''	CH		66.5	79.1
9	C	192.9	195.4	193.8	2''	CH		79.8	66.1
10	C	181.9	182.9		3''	CH		74.6	73.1
11	CH ₃	22.3	21.5	21.5	4''	CH		76.0	73.1
1'	C	162.1	162.9	162.9	5''	CH		74.0	73.1
1'a	C		116.4	128.3	6''	CH ₃		17.9	17.1
2'	CH	121.4	116.5	119.7					
3'	C	149.2	148.8	148.0					

Compound 6

This compound was isolated as orange needles with a melting point of 264-265°C.

EIMS analysis showed a molecular ion peak at m/z 254 (100) corresponding to the molecular formula $\text{C}_{15}\text{H}_{10}\text{O}_4$, suggesting that it is isomeric to chrysophanol (**1**). The

UV spectrum of **6** (λ_{max} 271, 280, 395, 410, 435) is typical of anthraquinones.

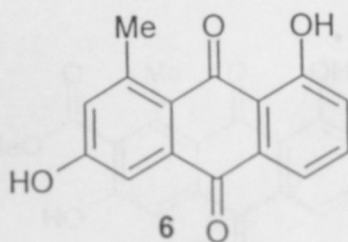
As in chrysophanol (**1**), the ^1H NMR spectrum of compound **6** showed five aromatic protons with an ABX (at δ 7.30 (*dd*, 1H, $J = 7.5, 2.75$ Hz for H-2; 7.73, *t*, $J = 7.5$ Hz for H-3; 7.72, *dd*, 1H, $J = 7.5, 2.75$ Hz for H-4) and an AB (δ 7.61, *d*, 1H, $J = 2.75$ Hz for H-5; 7.12, *bs*, 1H for H-7)) spin systems, suggesting substitution at C-1, C-6 and C-8 on a 9,10-anthraquinone skeleton. The ABX system was confirmed by the H-H COSY spectrum. In the ^{13}C NMR spectrum, the presence of two carbonyl carbons (at C-9

and C-10, δ 190.97 and 183.19, respectively) and two oxygenated aromatic carbon atoms at δ 163.32 is consistent with a 9,10-anthraquinone skeleton.

Unlike in chrysophanol (**1**) the ^1H NMR spectrum of compound **6** (Table 3.3) showed only one chelated hydroxyl group at δ 13.07 indicating that the second hydroxyl group is not chelated. The methyl group in **6** resonated at δ 2.79 ppm (^{13}C 24.13 ppm) which is down field shifted by ca.0.34 ppm compared to that of **1**. The slight deshielding on this group should be due to the peri-effect (anisotropy) from the C-10 carbonyl group and hence the methyl should be placed at C-8 while the second hydroxyl group should then be at C-6. The peak at δ 146.62 (in ^{13}C NMR, Table 3.4) was thus due to the quaternary carbon atom C-8.

The methine carbons at δ 125.07, 136.69, 119.13, 113.16, and 125.49 were due to C-2, C-3, C-4, C-5 and C-7, respectively. These assignments were confirmed by HMQC. The peaks at δ 117.76, 134.04, 138.47 and 124.15 were due to the quaternary carbon atoms at C-1a, C-4a, C-5a and C-8a, respectively.

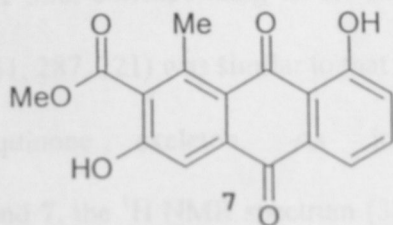
The EIMS (at m/z 197.3 ($\text{M}^+ - \text{CO} - \text{CHO}$) $^+$, 181.3, 169.3, 152.3 ($\text{M}^+ - \text{CO} - \text{CHO} - \text{CHO}_2$) $^+$, 151.3, 139.3 ($\text{M}^+ - \text{CO} - \text{CHO} - \text{C}_2\text{H}_2\text{O}_2$) $^+$, and 115.3) supported the isomeric nature of this compound to chrysophanol. This compound was therefore identified as 1,6-dihydroxy-8-methylanthraquinone, trivial name alosaponarin II (**6**).

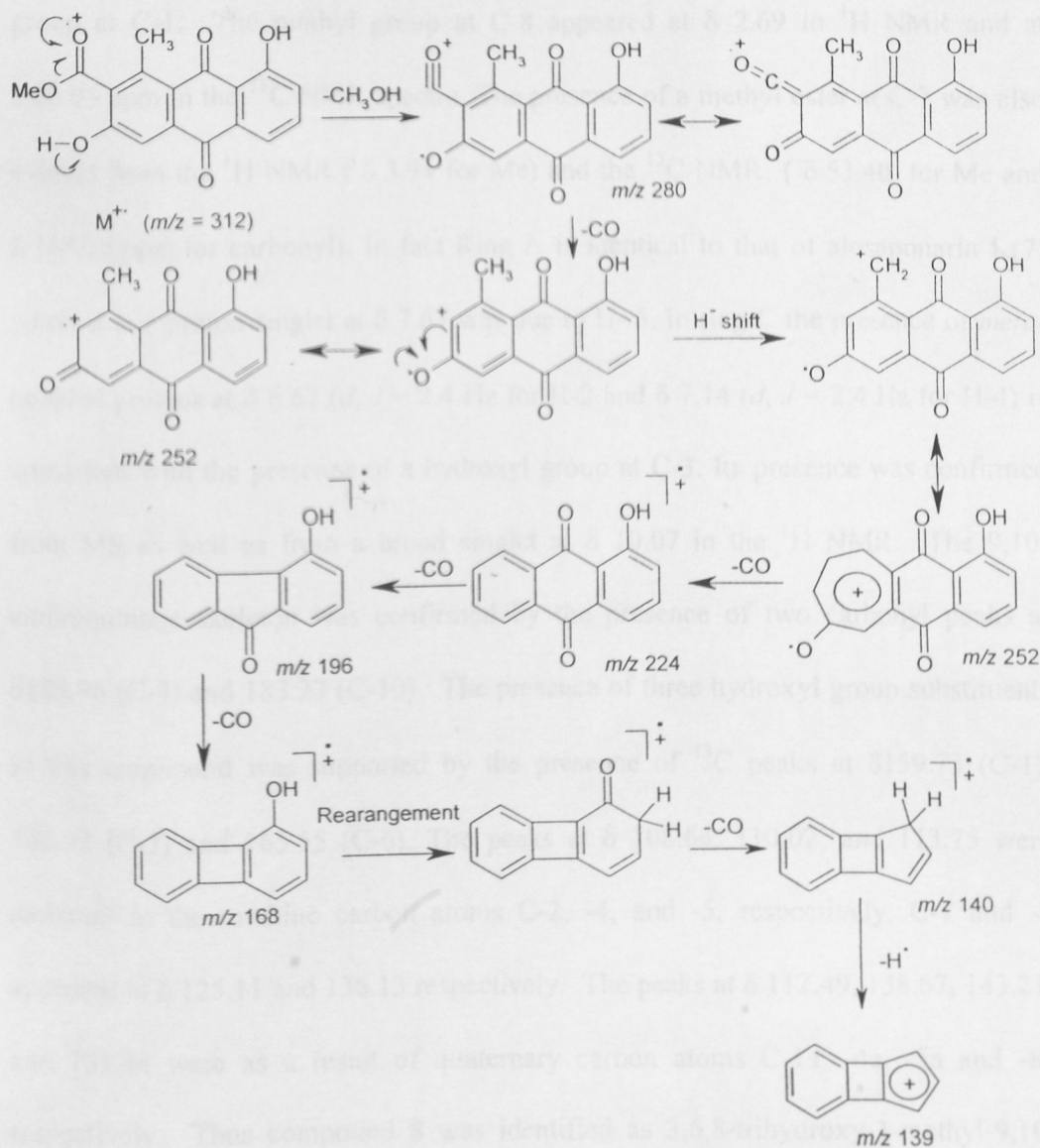


Compound 7

Compound 7 was isolated as orange crystals, melting point 190-192°C. EIMS analysis gave a molecular ion peak at m/z 312 corresponding to the molecular formula of $C_{17}H_{12}O_6$. The UV spectrum showed characteristic absorption bands of anthraquinones ($\lambda_{\max}$ 216.5, 270.0, 408.0 nm). As in compound 6, the 1H NMR spectrum of this compound (Table 3.3) showed one chelated hydroxyl group at δ 12.93 and an aromatic methyl group at δ 2.99 suggesting a 1,6-dihydroxy-8-methylantraquinone skeleton. The 1H NMR spectrum further showed the presence of four aromatic protons of which three constitute an ABX spin system at δ 7.31 (*dd*, 1H, $J = 8.1, 1.2$ Hz), δ 7.69 (*t*, 1H, $J = 8.1$ Hz), and δ 7.80 (*dd*, 1H, $J = 8.1, 1.2$ Hz), corresponding to H-2, -3 and -4, respectively. The fourth aromatic proton at δ 7.78 (*s*, 1H) can then be assigned to H-5 with C-7 being substituted. The substituent at C-7 was identified as a methyl ester from 1H (δ 4.07 ppm for methoxyl) and MS (Scheme 3.2; loss of a MeOH fragment from M^+).

The mass spectrum further showed characteristic fragment peaks at m/z 297 (loss of CH_3 group) and m/z 280 (loss of CH_3OH). Loss of a series of CO molecules resulted in a number of major fragments (Scheme 3.2). Therefore, compound 7 was identified as 3,8-dihydroxy-1-methyl-2-anthraquinone acid methyl ester, trivial name aloesaponarin I (7).





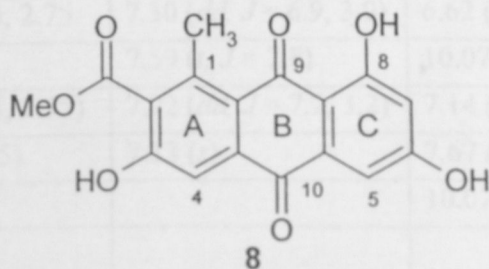
Scheme 3.2: EIMS fragmentation of aloesaponarin I (7)

Compound 8

The compound was isolated as a red crystalline solid which on EIMS analysis gave a molecular ion peak at m/z 328, corresponding to the molecular formula $C_{17}H_{12}O_7$.

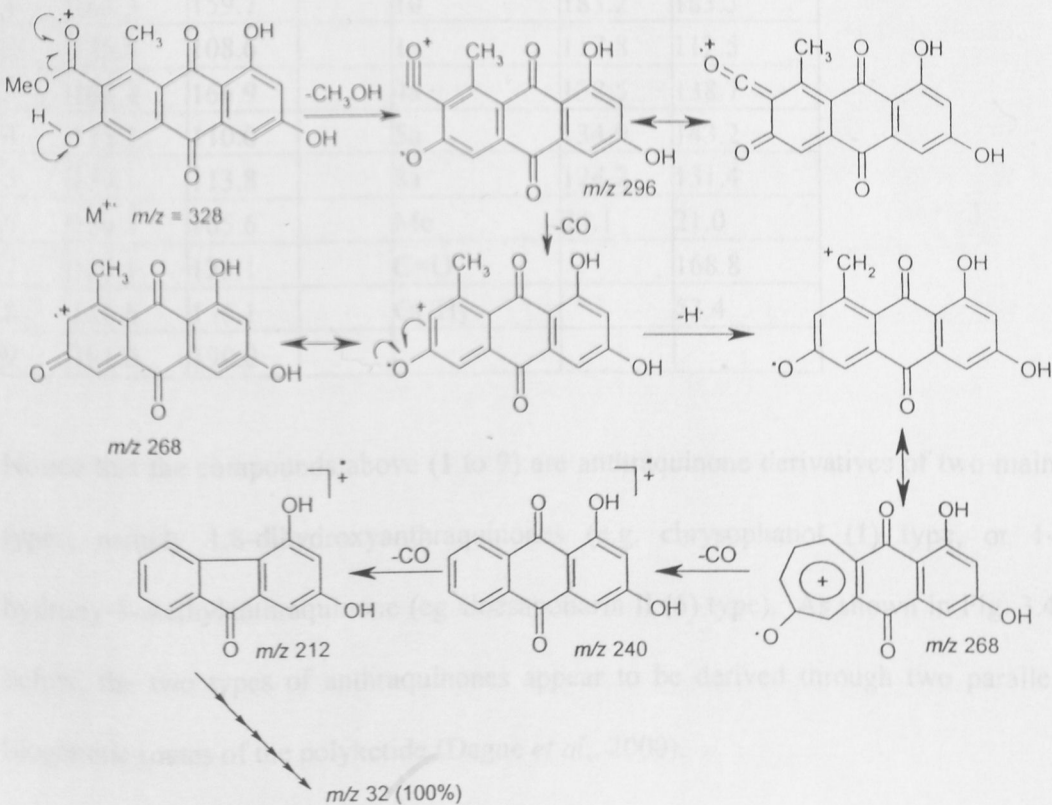
The UV spectrum ($\lambda_{\max}$ 431, 287, 221) was similar to that of compound 7 which again suggested an anthraquinone skeleton. As in the 1,6-dihydroxyl-8-methylantraquinones 6 and 7, the 1H NMR spectrum (300 MHz) of 8 showed a deshielded singlet proton at δ 13.12, due to the hydrogen-bonded proton of the hydroxyl

group at C-1. The methyl group at C-8 appeared at δ 2.69 in ^1H NMR and at δ 20.95 ppm in the ^{13}C NMR spectra. The presence of a methyl ester at C-7 was also evident from the ^1H NMR (δ 3.94 for Me) and the ^{13}C NMR (δ 53.40 for Me and δ 168.78 ppm for carbonyl). In fact Ring A is identical to that of alosaponarin I (7) where a one proton singlet at δ 7.67 was due to H-5. In ring C the presence of *meta*-coupled protons at δ 6.62 (*d*, $J = 2.4$ Hz for H-2 and δ 7.14 (*d*, $J = 2.4$ Hz for H-4) is consistent with the presence of a hydroxyl group at C-3. Its presence was confirmed from MS as well as from a broad singlet at δ 10.07 in the ^1H NMR. The 9,10-anthraquinone skeleton was confirmed by the presence of two carbonyl peaks at δ 189.96 (C-9) and 183.27 (C-10). The presence of three hydroxyl group substituents in this compound was supported by the presence of ^{13}C peaks at δ 159.73 (C-1), 166.92 (C-3) and 165.55 (C-6). The peaks at δ 108.64, 110.02, and 113.75 were assigned to the methine carbon atoms C-2, -4, and -5, respectively. C-7 and -8 appeared at δ 125.11 and 136.13 respectively. The peaks at δ 112.49, 138.67, 143.21, and 131.44 were as a result of quaternary carbon atoms C-1a, -4a, -5a and -8a respectively. Thus compound 8 was identified as 3,6,8-trihydroxy-1-methyl-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid methyl ester, (trivial name laccaic acid D methyl ester (8)).



The fragmentation pattern (see scheme 3.4 below) was consistent with the above structure. Laccaic acid D methyl ester has been previously reported from

subterranean stem of *Aloe graminicola* (Yenesew, *et al.* 1993b), roots of *Aloe berhana* (Dagne *et al.*, 1992) among others.



Scheme 3.3 EIMS fragmentation of Laccaic acid D methyl ester (8)

Table 3.3: ¹H NMR data for compounds 6, 7 and 8

C	6	7	8
1	13.07 (OH)	12.89 (OH)	13.12 (OH)
2	7.30 (dd, <i>J</i> = 7.5, 2.75	7.30 (dd, <i>J</i> = 6.9, 3.0)	6.62 (d, <i>J</i> = 2.4)
3	7.73 (t, <i>J</i> = 7.5)	7.59 (t, <i>J</i> = 7.8)	10.07 (br, OH)
4	7.72 (dd, <i>J</i> = 7.5, 2.75)	7.72 (dd, <i>J</i> = 7.2, 3.2)	7.14 (d, <i>J</i> = 2.4)
5	7.61 (d, <i>J</i> = 2.75)	7.13 (s)	7.67 (s)
6			10.07 (br, OH)
7	7.12 (br s)		
11	2.79 (Me)	2.91 (Me)	2.69 (Me)
13		4.05 (OMe)	3.94 (OMe)

Table 3.4: The ^{13}C NMR data for compounds 6 and 8

C	6	8		C	6	8
1	163.3	159.7		10	183.2	183.3
2	125.5	108.6		1a	117.8	112.5
3	163.3	166.9		4a	138.5	138.7
4	113.2	110.0		5a	134.0	143.2
5	119.1	113.8		8a	124.2	131.4
6	136.7	165.6		Me	24.1	21.0
7	125.1	125.1		C=O		168.8
8	146.6	136.1		OCH ₃		53.4
9	191.0	190.0				

Notice that the compounds above (1 to 9) are anthraquinone derivatives of two main types, namely 1,8-dihydroxyanthraquinones (e.g. chrysophanol (1) type, or 1-hydroxy-8-methylantraquinone (eg aloesaponarin II (6) type). As shown in Fig. 3.4 below, the two types of anthraquinones appear to be derived through two parallel biogenetic routes of the polyketide (Dagne *et al.*, 2000).

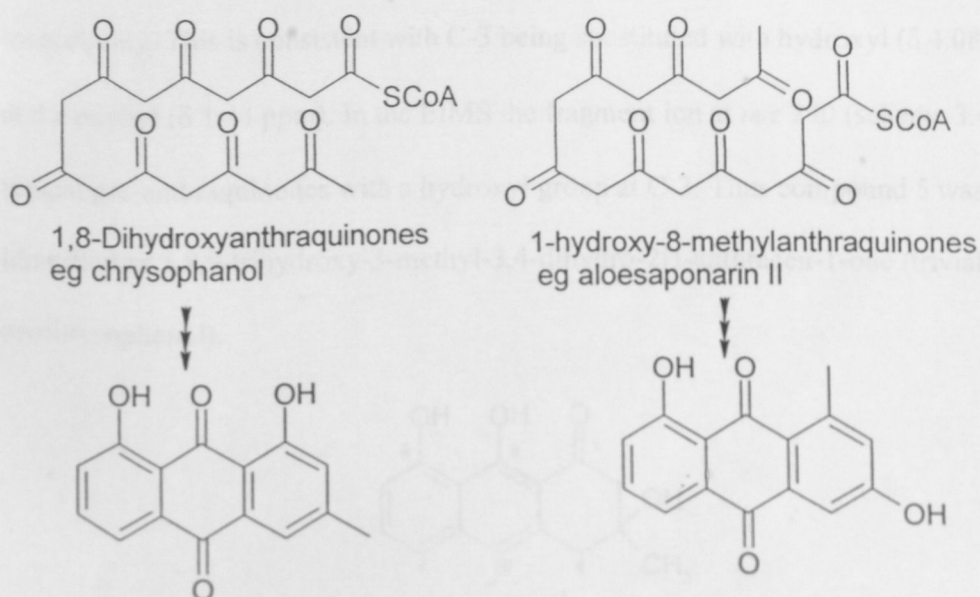
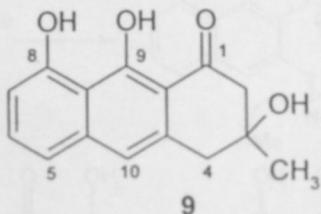
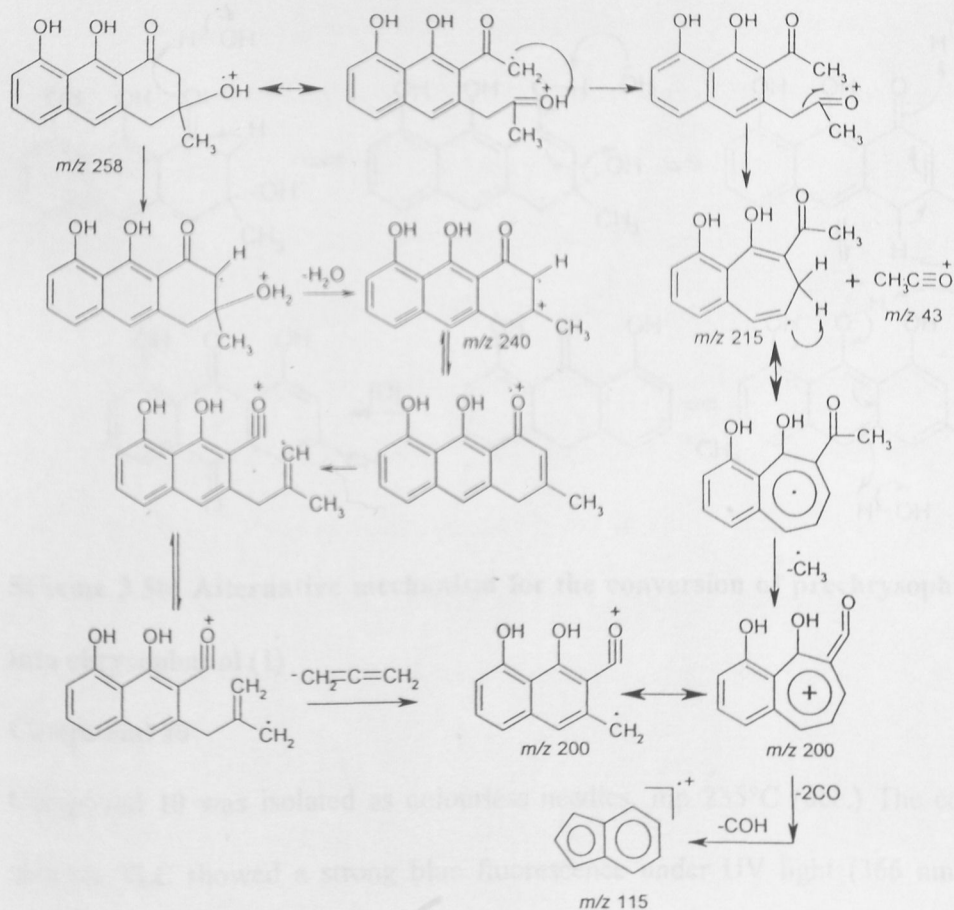


Fig. 3.3e Two folding types of the octaketide chain

Compound 9

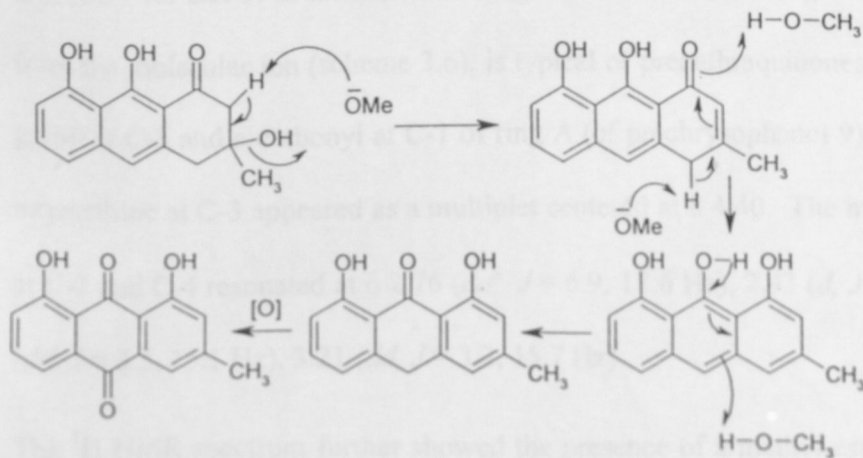
Compound 9 was isolated as light yellow crystals with a melting point of 200-203° C . Mass spectrum (EIMS) of the compound had the molecular ion peak at m/z 258 (66), which was consistent with the molecular formula $C_{15}H_{14}O_4$. The UV spectrum showed absorption bands at λ_{max} 267, 290, 299, 320 and 409 nm which is typical of pre-anthraquinones (Dagne *et al.*, 2000). The 1H NMR (300 MHz, Acetone D_6) spectrum of this compound showed singlets at δ 9.69 and 16.13 which were readily assigned to the two chelated hydroxyl protons (H-8 and H-9, respectively) of pre-anthraquinones. The 1H NMR spectrum also showed four aromatic protons, three of which had an AMX spin system at δ 7.22 ppm (d , $J = 8.0$ Hz for H-5); 7.51 (t , $J = 8.0$ Hz for H-6); and 6.79 (d , $J = 8.0$ Hz for H-7) for ring-C protons. The singlet at δ 7.11 was due to the isolated proton at C-10. In ring A the two methylene groups at C-2 and C-4 appear as two sets of ABqs δ 3.16 ($J = 15.9$ Hz) & 3.06 ($J = 17.4$ Hz) respectively. This is consistent with C-3 being substituted with hydroxyl (δ 4.08, br s) and a methyl (δ 1.44 ppm). In the EIMS the fragment ion at m/z 240 (scheme 3.4.) is typical pre-anthraquinones with a hydroxyl group at C-3. Thus compound 5 was identified as 3,8,9-trihydroxy-3-methyl-3,4-dihydro-2H-anthracen-1-one (trivial name prechrysophanol).



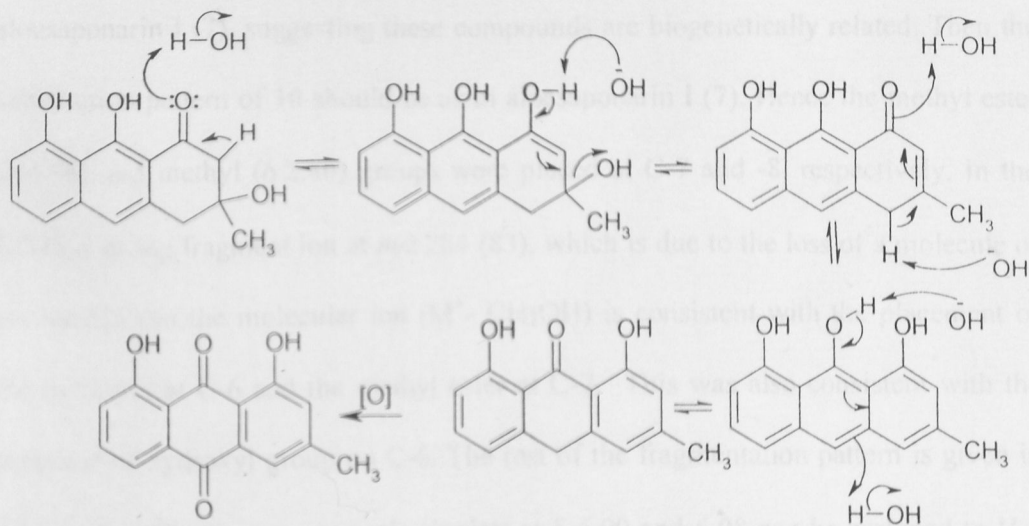


Scheme 3.4: Mass fragmentation pattern of prechrysophanol (9)

Compound 9 was dissolved in basic methanol and left standing overnight upon which chrysophanol (1) was obtained. The postulated mechanism for the conversion is given in scheme 3.5a and 3.5b.



Scheme 3.5: Conversion of prechrysophanol (9) into chrysophanol (1)



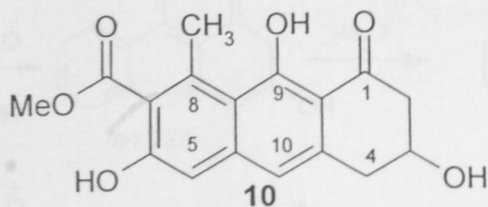
Scheme 3.5b: Alternative mechanism for the conversion of prechrysophanol (9) into chrysophanol (1)

Compound 10

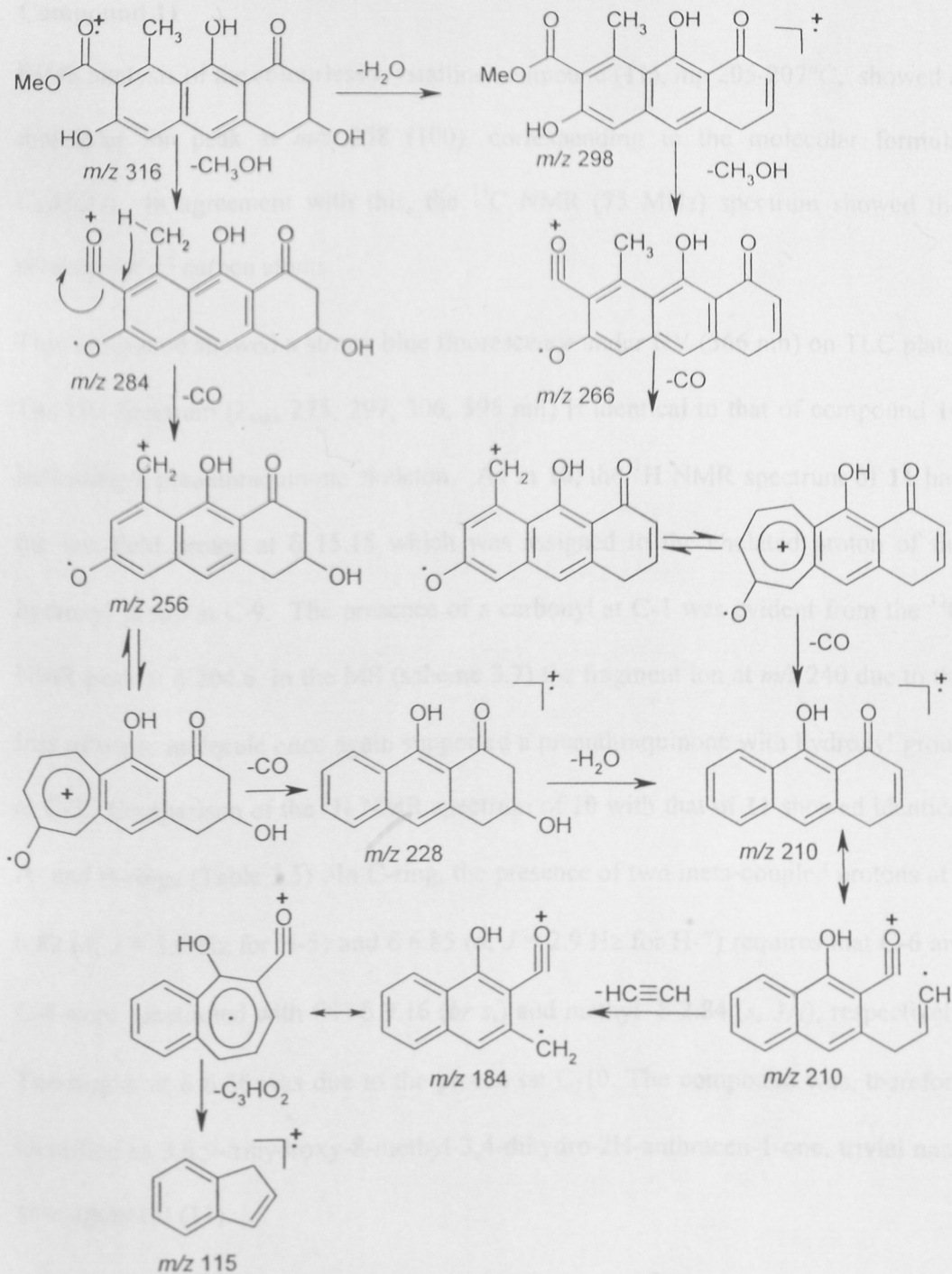
Compound **10** was isolated as colourless needles, mp 235°C (dec.) The colourless spot on TLC showed a strong blue fluorescence under UV light (366 nm). EIMS spectrum showed a molecular ion peak at m/z 316 (7) corresponding to the molecular formula $C_{17}H_{16}O_6$. The UV spectrum ($\lambda_{\max}$ 275, 297, 306, 395 nm) suggested a preanthraquinone skeleton (Dagne *et al.*, 2000). In agreement with this, the 1H NMR (300 MHz) spectrum showed a strongly hydrogen bonded (chelated) hydroxyl group at δ 15.37 for OH-9. In the EIMS the fragment ion at m/z 298 (5), due to loss of water from the molecular ion (scheme 3.6), is typical of preanthraquinones with a hydroxyl group at C-3 and a carbonyl at C-1 of ring A (cf prechrysophanol **9**). In this ring, the oxymethine at C-3 appeared as a multiplet centered at δ 4.40. The methylene protons at C-2 and C-4 resonated at δ 2.76 (*dd*, $J = 6.9, 17.6$ Hz), 2.93 (*d*, $J = 17.7$ Hz), 2.99 (*dd*, $J = 3.5, 15.1$ Hz), 3.21 (*dd*, $J = 3.3, 15.7$ Hz).

The 1H NMR spectrum further showed the presence of a methyl ester (δ 3.90) and a methyl group (δ 2.80). Aerial oxidation of this compound in methanolic KOH gave

aloesaponarin I (7), suggesting these compounds are biogenetically related. Then the substitution pattern of **10** should be as in aloesaponarin I (7). Hence the methyl ester (δ 3.90) and methyl (δ 2.80) groups were placed at C-7 and -8, respectively. In the EIMS a strong fragment ion at m/z 284 (83), which is due to the loss of a molecule of methanol from the molecular ion ($M^+ - CH_3OH$) is consistent with the placement of the hydroxyl at C-6 and the methyl ester at C-7. This was also consistent with the presence of hydroxyl group on C-6. The rest of the fragmentation pattern is given in scheme 3.6. Finally two aromatic singlets at δ 6.90 and 6.98 can be assigned to H-5 and H-10, respectively. The structure of the compound was deduced to be 3,6,9-trihydroxy-1-methyl-8-oxo-5,6,7,8-tetrahydro-anthracene-2-carboxylic acid, trivial name aloesaponol I (**10**).



According to Yagi *et al* (1978) it is possible to show that aloesaponol I (**10**) and laccaic acid D methyl ester (**8**) were biosynthesized in parallel in *Aloe saponaria*

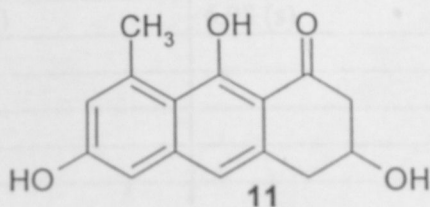


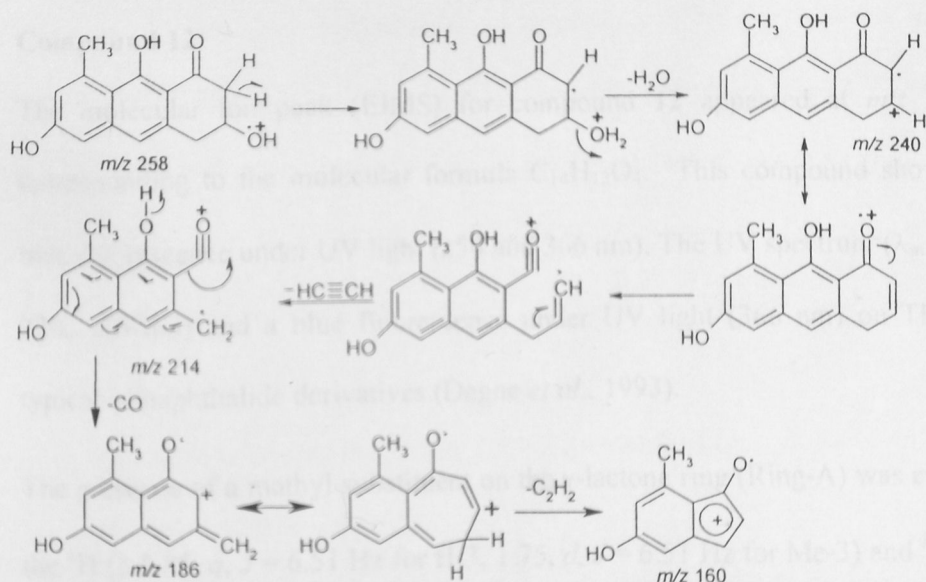
Scheme 3.6 EIMS fragmentation in aloesaponol I (10)

Compound 11

EIMS analysis of the colourless crystalline compound (**11**), mp 205-207°C, showed a molecular ion peak at m/z 258 (100), corresponding to the molecular formula $C_{15}H_{14}O_4$. In agreement with this, the ^{13}C NMR (75 MHz) spectrum showed the presence of 15 carbon atoms.

This compound showed a strong blue fluorescence under UV (366 nm) on TLC plate. The UV spectrum (λ_{max} 275, 297, 306, 395 nm) is identical to that of compound **10** indicating a preanthraquinone skeleton. As in **10**, the 1H NMR spectrum of **11** had the low-field proton at δ 15.15 which was assigned to the chelated proton of the hydroxyl group at C-9. The presence of a carbonyl at C-1 was evident from the ^{13}C NMR peak at δ 204.6. In the MS (scheme 3.7) the fragment ion at m/z 240 due to the loss of water molecule once again supported a preanthraquinone with hydroxyl group at C-3. Comparison of the 1H NMR spectrum of **10** with that of **11** showed identical A- and B-rings (Table 3.5). In C-ring, the presence of two meta-coupled protons at δ 6.82 (d , $J = 3.0$ Hz for H-5) and δ 6.85 (d , $J = 2.9$ Hz for H-7) requires that C-6 and C-8 were substituted with OH δ 9.16 ($br s$), and methyl δ 2.84 (s , $3H$), respectively. The singlet at δ 6.86 was due to the proton on C-10. The compound was, therefore, identified as 3,6,9-trihydroxy-8-methyl-3,4-dihydro-2H-anthracen-1-one, trivial name aloesaponol II (**11**).





Scheme 3.7: Mass Fragmentation pattern for aloesaponol II (11)

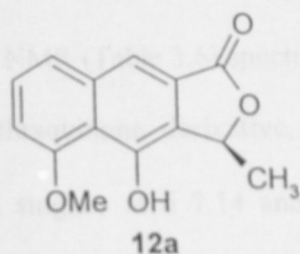
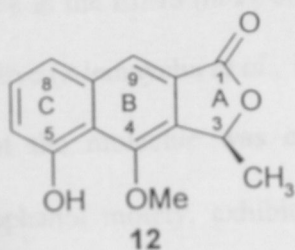
Table 3.5: NMR data for preanthraquinones 9, 10 and 11

	^{13}C	1H δ (m, J Hz)		
C	7	5	6	7
1	204.6			
2	49.2	3.18 (d, J = 15.9) 3.08 (d, J = 16.2)	2.76 (dd, J = 6.9, 17.6); 2.93 (d, J = 17.7)	3.18 (d, J = 13.17) 2.71 (d, J = 17.10)
3	67.0	4.08 (OH) 1.44 (CH_3)	4.40 (m)	4.37 (m)
4	39.7	2.98 (d, J = 17.4) 2.83 (d, J = 16.3)	2.99 (dd, J = 3.5, 15.1); 3.21 (dd, J = 3.3, 15.7)	2.74 (d, J = 17.16) 2.71 (d, J = 17.10)
5	109.1	7.22 (d, J = 8.1)	6.90 (s)	6.82 (d, J = 3.0)
6	168.0	7.51 (dd, J = 7.8, 8.1 Hz)		9.16 (br s, OH))
7	118.4	6.79 (d, J = 7.8)	3.90 (OMe)	6.85 (d, J = 2.9)
8	160.3	9.68 (OH)	2.80 (Me)	
9	167.7	16.13 (OH)	15.37	15.15 (OH)
10	121.1	7.11 (s)	6.98 (s)	6.86 (s)
1a	137.6			
4a	142.8			
5a	143.6			
8a	111.6			
Me-8	25.57			2.84 (s)

Compound 12

The molecular ion peak (EIMS) for compound **12** appeared at m/z 244 (100), corresponding to the molecular formula $C_{14}H_{12}O_4$. This compound showed strong blue fluorescence under UV light (254 and 366 nm). The UV spectrum ($\lambda_{\max}$ 252, 300, 320, 360 nm) and a blue fluorescence under UV light (366 nm) on TLC plate is typical of naphthalide derivatives (Dagne *et al.*, 1993).

The presence of a methyl substituent on the γ -lactone ring (Ring-A) was evident from the 1H (δ 5.86, *q*, $J = 6.51$ Hz for H-3, 1.75, *d*, $J = 6.51$ Hz for Me-3) and ^{13}C (δ 169.4 for C-1, 61.8 for C-3, 150.4 for C-4 and 154.2 for C-5) NMR spectra. The presence of a hydroxyl (δ 9.34, *br s*) and a methoxyl (δ 4.12 in 1H and 61.80 in ^{13}C NMR spectra) substituents on the naphthalide skeleton was also derived from NMR spectra. An ABX spin system in the 1H NMR spectrum, at δ 7.07 (*d*, $J = 7.29$ Hz), δ 7.47 (*dd*, $J = 7.38$ and 8.16 Hz) and δ 7.53 (*d*, $J = 8.01$ Hz) were due to H- 6, -7 and -8 (ring C), respectively. The singlet at δ 8.20 was due to the aromatic proton at C-9 of ring B, which is substituted at C-4 with methoxyl (**12**) or hydroxyl (**12a**) group. The chemical shift value of the methoxyl is downfield shifted (δ 61.8) which indicated that it is di-ortho-substituted (Manfred, 1997), hence has to be placed at C-4 (**12**) rather than C-5 (eleutherol, **12a**). Therefore this compound was characterized as 5-hydroxy-4-methoxy-3-methyl-3H-naphtho[2,3-c]furan-1-one, trivial name isoeleutherol (**12**) (Dagne *et al.*, 1993, Tânia *et al.*, 2003)



Dagne et al 1994 did a comparative TLC analysis of the root extracts of 32 *Aloe* species and found that chrysophanol (1), asphodelin (3), chrysophanol-8-methyl ether, aloechrysone, helminthosporin (2), aloesaponol III, aloesaponarin I (7) aloesaponol I (10), aloesaponarin II (6), aloesaponol II (11) and laccaic acid D-methyl ester (8) are widely distributed in these plants.

3.2.2 Compounds from the fruits, roots and stems of *Bulbine abyssinica* and *B. frutescens*

The ethylacetate extract of the dry fruits of *Bulbine abyssinica* was subjected to chromatographic separation and purification yielding compounds 1, 13, 14, 15 and 16. The acetone extract of the roots of *B. abyssinica* was also chromatographed yielding compounds 17, 18, 19 and 20

Compound 13

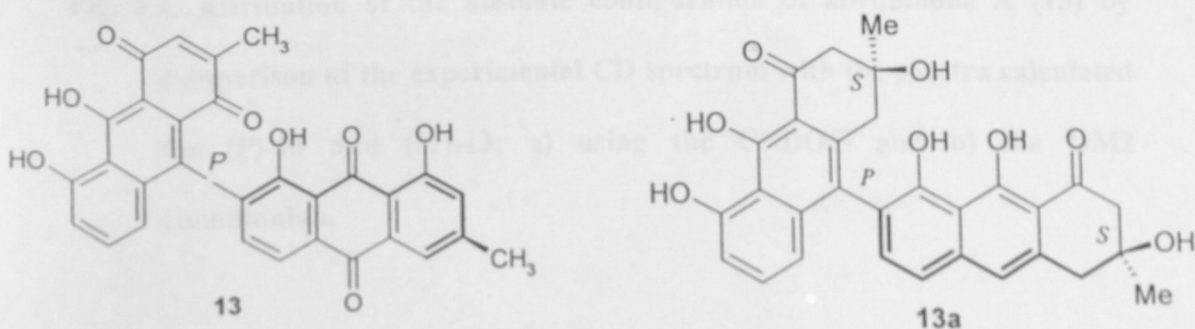
The dark-red amorphous powder of compound 13 showed absorption at $\lambda_{\max}$ 232, 256, 303, 438, 471, 500, 542, and 582 nm in the UV spectrum suggesting an anthraquinone chromophore. The presence of four chelated hydroxy protons (δ 17.04, 12.30, 11.95 and 10.51 in the ^1H NMR spectrum, (see Table 3.6), four carbonyl resonances (δ 193.4, 186.8, 184.3 and 182.2 in the ^{13}C NMR spectrum), two methyl groups (at δ 2.03 and 2.50 in ^1H NMR, 17.3 and 22.5 in ^{13}C NMR), and the molecular ion peak in the EIMS (m/z 506) showed this compound to be a dimeric anthraquinone derivative (Alemayehu *et al.*, 1996a; 1996b). From the NMR (Table 3.6) spectra, one half of the molecule was established as a 9,10-anthraquinone derivative, viz a chrysophanol moiety, exhibiting two broad ^1H NMR singlets at δ 7.14 and 7.72,

assigned to H-2' and H-4', respectively, and with a methyl group (δ 2.50) located at C-3'. Two *ortho*-coupled ($J = 8.0$ Hz) protons at δ 7.95 and 7.43 were attributed to H-5' and H-6', respectively, with C-7' of the chrysophanol moiety being substituted.

For the other half of the molecule, the ^1H NMR signals at δ 17.04 and 10.51 (for two chelated hydroxy protons) and the ^{13}C NMR signals at δ 182.2 and 186.8 (for two carbonyl groups) suggested the presence of a 1,4-anthraquinone skeleton (Alemayehu *et al.*, 1996a; 1996b). The long-wavelength UV absorption maxima (λ_{max} 500, 542, and 582 nm) in compound **13** were in agreement with the presence of such a moiety (Alemayehu *et al.*, 1996a; 1996b). In the 1,4-anthraquinone portion, an AMX spin system at δ 6.93 (*dd*, $J = 1.5, 8.0$ Hz), 7.51 (*t*, $J = 8.0$ Hz), and 7.10 (*dd*, $J = 1.5, 8.0$ Hz) was assigned to H-5, H-6, and H-7, respectively. A one-proton quartet ($J = 1.2$ Hz) was assigned to H-2, which showed a long-range coupling with Me-3 (δ 2.03, *d*, $J = 1.2$ Hz). This assignment was confirmed by a NOESY correlation between H-2 and Me-3. This left C-10 of the 8,9-dihydroxy-3-methyl-1,4-anthraquinone moiety to be the point of attachment to the chrysophanol moiety, thus suggesting a 10,7'-linkage in compound **13**. This connection was confirmed by an HMBC correlation of H-6' with C-10 and a NOESY interaction between H-5 and H-6'. From these data the new natural product was characterized as 8,9,1',8'-tetrahydroxy-3,3',-dimethyl[10,7'-bianthracene]-1,4,9',10'-tetrone, for which the trivial name abyquinone A (**13**) was suggested. The compound was optically active indicating the presence of a non-racemic axially chiral biaryl. In the CD spectrum of **13**, a positive Cotton effect at 276 nm and a negative one at 301 nm, i.e. nearly opposite to those reported for the *P*-configured peroxisomicine A₁ (**13a**) (Pérez *et al.*, 2004), indicated abyquinone A (**13**)

to have the opposite stereoarray at the chiral axis, so that, due to the formalism of the CIP denotation, a *P*-configured axis was expected for **13**, too.

In order to determine the absolute configuration at the chiral axis in a reliable, non-empirical way, quantum chemical CD calculations were performed (Bringmann *et al.*, 2003; 2004). Arbitrarily starting with the *P*-atropenantiomer of abyquinone A (**13**), the molecule was submitted to a conformational analysis using the semiempirical AM1 method, resulting in eight optimized minimum geometries within the energetically relevant range of 3 kcal/mol above the global minimum. For each single geometry thus obtained, a CD spectrum was calculated using the semiempirical CNDO/S as well as the OM2 Hamiltonian. In both cases the single spectra were then added and weighted following the Boltzmann statistics, i.e. according to the respective heats of formation. The two overall CD spectra, thus obtained, were subsequently UV corrected (Bringmann *et al.*, 1998) and compared with the experimental CD curve of **13**. Both, when following the CNDO/S approach (Fig. 3.4a) and the OM2 method (Fig. 3.4b), a good agreement between the measured spectrum and those predicted for (*P*)-**13** was obtained (Fig. 3.4, left), whereas the CD curves computed for (*M*)-**13** were virtually opposite as compared to the experimental one (Fig. 3.4, right). Consequently, the chiral axis of **13** was clearly assigned to be *P*-configured.



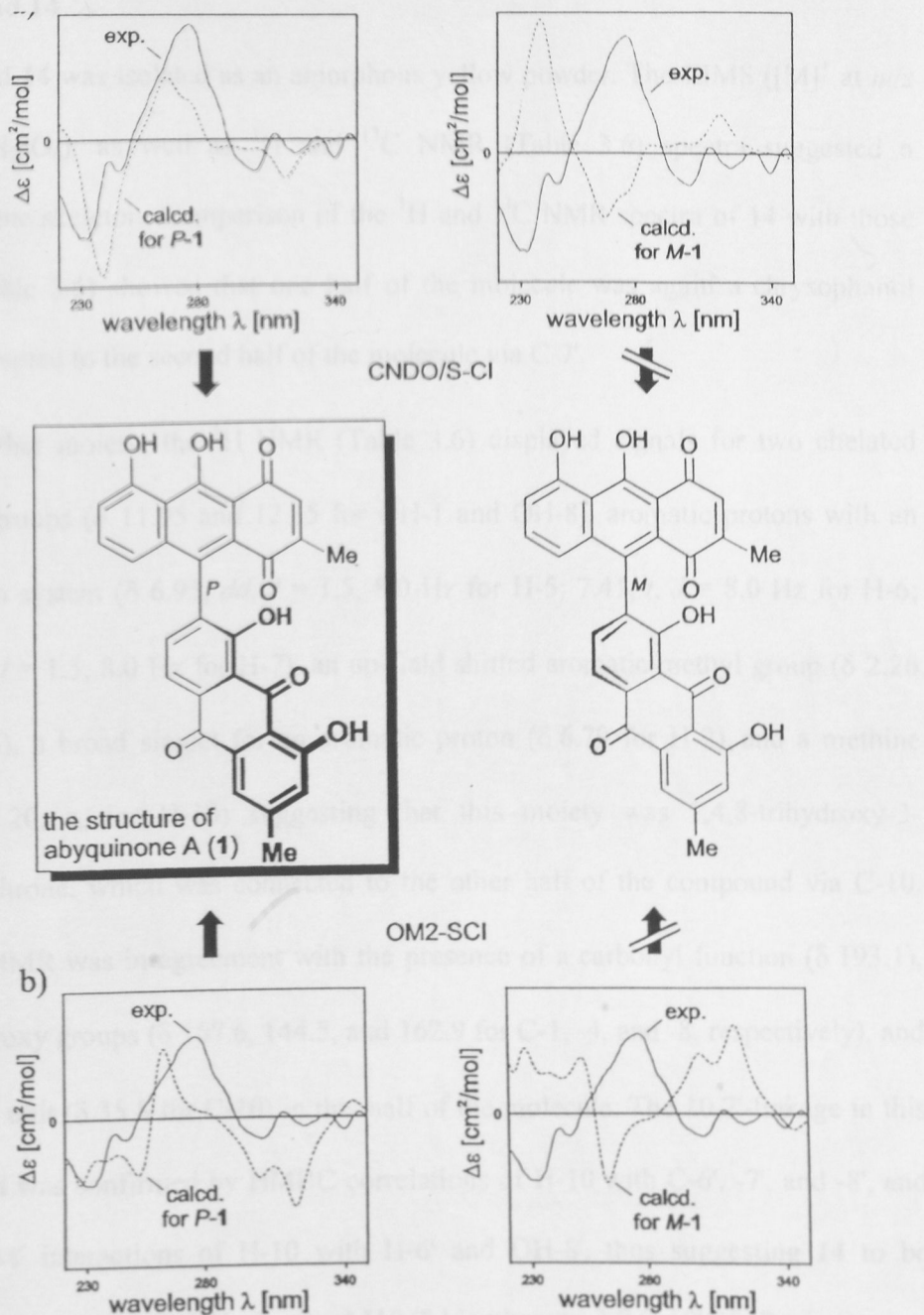


Fig. 3.4. Attribution of the absolute configuration of abyquinone A (13) by comparison of the experimental CD spectrum with the spectra calculated for *(P)*-13 and *(M)*-13; a) using the CNDO/S and b) the OM2 Hamiltonian.

Compound 14

Compound **14** was isolated as an amorphous yellow powder. The EIMS ($[M]^+$ at m/z 508, $C_{30}H_{20}O_8$), as well as 1H and ^{13}C NMR (Table 3.6) spectra suggested a bianthracene skeleton. Comparison of the 1H and ^{13}C NMR spectra of **14** with those of **13** (Table 3.6) showed that one half of the molecule was again a chrysophanol moiety coupled to the second half of the molecule via C-7'.

For the other moiety, the 1H NMR (Table 3.6) displayed signals for two chelated hydroxy groups (δ 11.95 and 12.35 for OH-1 and OH-8), aromatic protons with an AMX spin system (δ 6.95, *dd*, $J = 1.5, 8.0$ Hz for H-5; 7.41, *t*, $J = 8.0$ Hz for H-6; 6.88, *dd*, $J = 1.5, 8.0$ Hz for H-7), an up-field shifted aromatic methyl group (δ 2.26 for CH_3 -3), a broad singlet for an aromatic proton (δ 6.79 for H-2), and a methine proton (6.20, *s*, for H-10) suggesting that this moiety was 1,4,8-trihydroxy-3-methylantrone, which was connected to the other half of the compound via C-10. The ^{13}C NMR was in agreement with the presence of a carbonyl function (δ 193.1), three hydroxy groups (δ 157.6, 144.5, and 162.9 for C-1, -4, and -8, respectively), and a methine unit (δ 35.8 for C-10) in this half of the molecule. The 10,7'-linkage in this compound was confirmed by HMBC correlations of H-10 with C-6', -7', and -8', and by NOESY interactions of H-10 with H-6' and OH-8', thus suggesting **14** to be 1,4,8,1',8'-pentahydroxy-3,3',-dimethyl-[10,7'-bianthracene]-9,9',10'(10*H*)-trione, which was henceforth given the trivial name abyquinone B.

The configurational assignment of abyquinone B (**14**) was again achieved by means of the AM1-Boltzmann approach as described above for compound **13**. In this case, the conformational analysis resulted in two conformers within the range of 3 kcal/mol above the global minimum, and the overall CD spectra received with the OM2

approach on the SCI (Fig. 3.5a) and the SDCI (Fig. 3.5b) levels, led to the unambiguous attribution of the chiral center of **14** to be *R*-configured (Fig. 3.5, right).

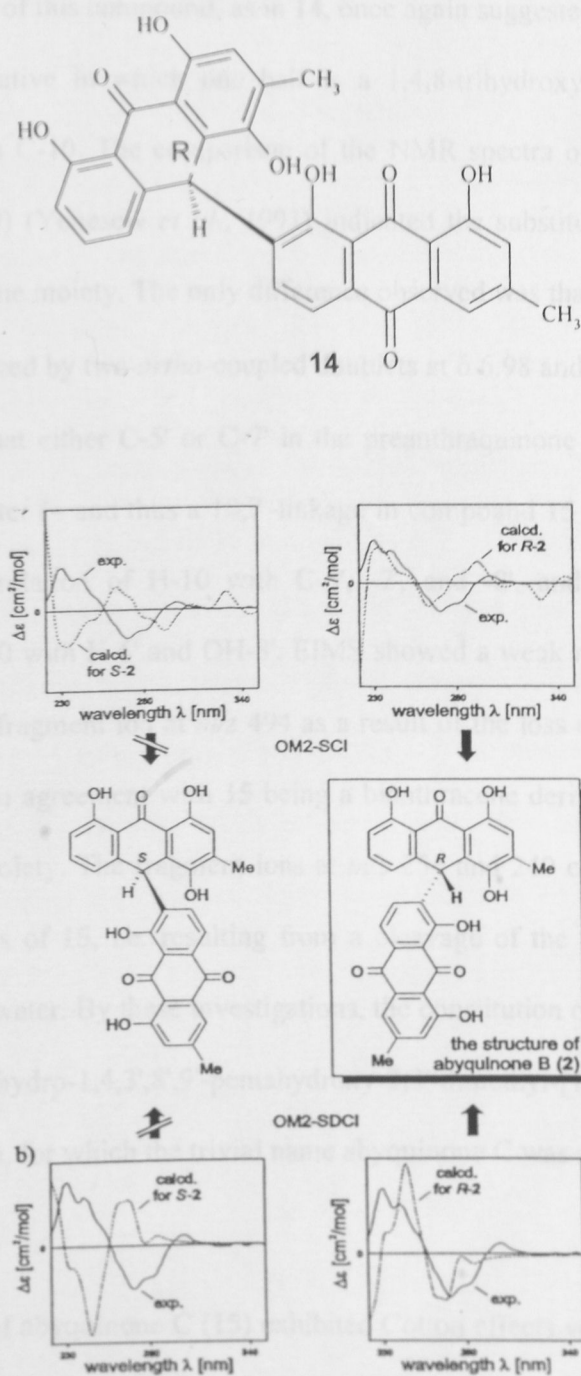


Fig. 3.5. Assignment of the absolute configuration of abyquinone B (**14**) by comparison of the experimental CD spectrum with the spectra calculated for (*S*)-**14** and (*R*)-**14** using the OM2 Hamiltonian with a) a SCI and b) a SDCI calculation.

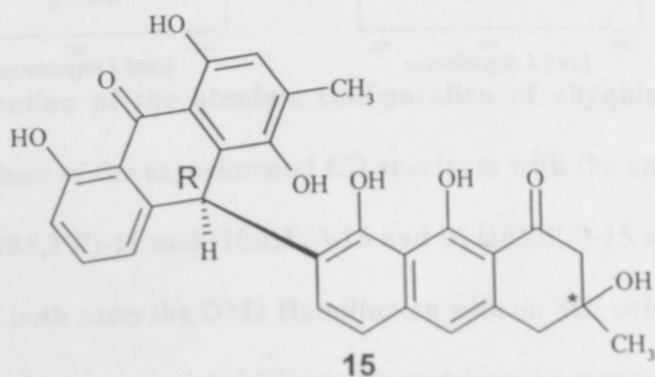
Compound 15

Compound **15** was isolated as a yellowish brown amorphous powder. The NMR spectra (Table 3.6) of this compound, as in **14**, once again suggested the presence of a bianthrane derivative in which one half is a 1,4,8-trihydroxy-3-methylanthrone moiety coupled via C-10. The comparison of the NMR spectra of **15** with those of prechrysophanol (**9**) (Yenesew *et al.*, 1993) indicated the substituent at C-10 to be such an anthracenone moiety. The only difference observed was that the AMX system in **9** was now replaced by two *ortho*-coupled doublets at δ 6.98 and 6.84 ($J = 8.0$ Hz), which suggested that either C-5' or C-7' in the preanthraquinone moiety of **15** was substituted. The latter (– and thus a 10,7'-linkage in compound **15** –) was confirmed by an HMBC correlation of H-10 with C-6', -7', and -8', and also by NOESY correlations of H-10 with H-6' and OH-8'. EIMS showed a weak molecular ion peak at m/z 512, with a fragment ion at m/z 494 as a result of the loss of one molecule of water, which was in agreement with **15** being a bianthrane derivative containing a prechrysophanol moiety. The fragment ions at m/z 254 and 240 corresponded to the monomeric portions of **15**, i.e. resulting from a cleavage of the 10,7'-linkage, with subsequent loss of water. By these investigations, the constitution of **15** was identified as 3',4'-dihydro-1,4,3',8',9'-pentahydroxy-3,3'-dimethyl-[10,7'-bianthrane]-9,1'(10*H*,2'*H*)-dione, for which the trivial name abyquinone C was suggested.

The CD spectrum of abyquinone C (**15**) exhibited Cotton effects very similar to those obtained for abyquinone B (**14**) at 230 nm, 270 nm, and 300 nm. Hence the absolute configuration at C-10, as the chiral center dominating the CD behavior of **15**, should also be *R*, whereas a reliable conclusion about the absolute stereostructure at C-3',

which has a much lower influence on the molecular CD, was not possible at that stage.

For the determination of the absolute configurations of **15** at its two stereogenic centers, C-10 and at C-3', again quantum chemical CD calculations were performed. Since not even the relative configuration of these two chiral centers was known, two independent conformational analyses, for the (10*S*,3'*R*)- and the (10*S*,3'*S*)-diastereomer of **15**, were launched, resulting in both cases in 28 minimum structures within the range of 3 kcal/mol above the global minimum. While both overall simulated CD spectra for (10*S*,3'*R*)-**15** and (10*S*,3'*S*)-**15** behaved virtually oppositely compared with the experimental curve (Fig. 3.15, left), a good agreement for the measured CD spectrum with both spectra computed for (10*R*,3'*S*)-**15** and (10*R*,3'*R*)-**15** was obtained (Fig. 3.15, right), thus, at least, assigning the stereocenter at C-10 to be *R*-configured. This result was in accordance with the above-determined *R*-configuration of **14** and the easy transformation of **15** into **14** by dehydration and oxidation (see below).



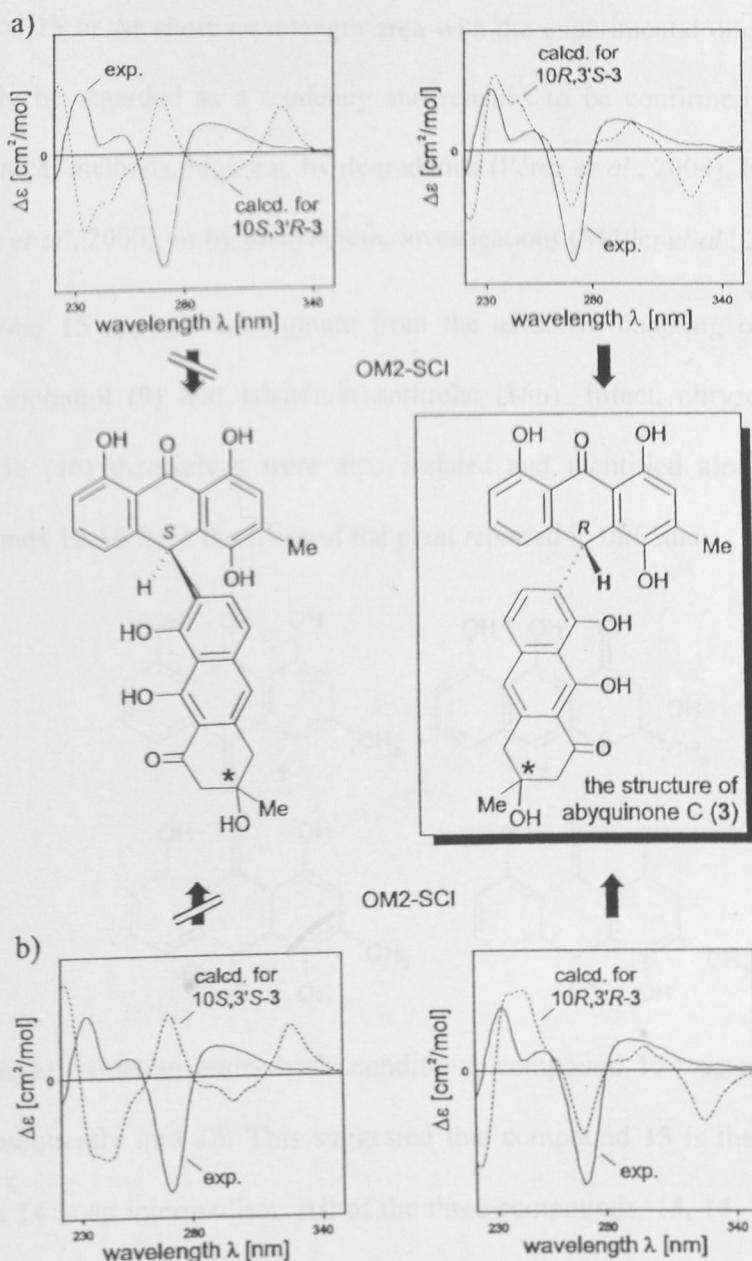
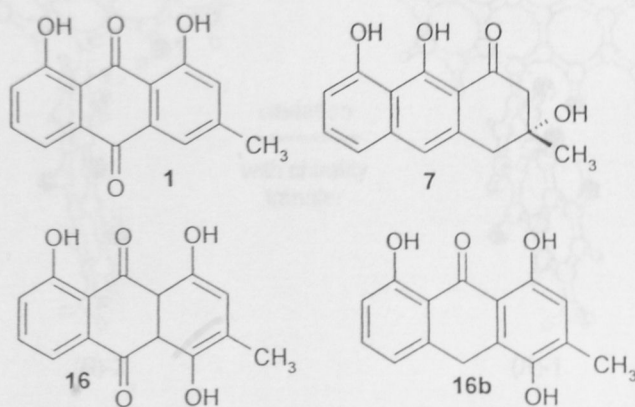


Fig. 3.6 Attribution of the absolute configuration of abyquinone C (15) by comparison of the experimental CD spectrum with the spectra calculated for a) $(10S,3'R)-15$ and $(10R,3'S)-15$ and b) $(10S,3'S)-15$ and $(10R,3'R)-15$ using in both cases the OM2 Hamiltonian with an SCI calculation.

A closer look at the two calculated CD spectra matching the measured one (Fig. 3.6, right) might suggest the speculation that the absolute configuration at C-3' of abyquinone C (15) is *S*, due to the slightly better agreement of the computed curve for

(10*R*,3'*S*)-**15** in the short wavelength area with the experimental one. This, however, can only be regarded as a tendency and remains to be confirmed by chemical or biochemical methods, like, e.g. by degradation (Pérez *et al.*, 2004), by total synthesis (Müller *et al.*, 2000), or by biosynthetic investigations (Müller *et al.*, 2004).

Compound **15** appears to originate from the oxidative coupling of the precursors prechrysophanol (**9**) and islandicin anthrone (**16b**). Infact, chrysophanol (**1**) and islandicin (**16**) themselves were also isolated and identified along with the new compounds **13-15** from the fruits of the plant reported in this study.



Upon aerial oxidation under basic conditions, compound **15** was converted into **14** and subsequently into **13**. This suggested that compound **15** is the precursor to **13** whereas **14** is an intermediate. All of the three compounds, **13**, **14**, and **15** were also detected (by TLC) in the crude ethyl acetate extract, clearly indicating that these substances are genuine natural products. There is still a possibility that dissolved oxygen could have transformed **15** to **14** and subsequently to **12** suggesting that only **15** could be a true natural product.

Besides the smooth course of the iterative reactions, the stereochemical implications are of particular interest. While the dehydration of **15** to give **14** simply implies the loss of one stereogenic center, with the remaining one being expectedly the same in **15** and **14**, as confirmed by the quantum chemical CD calculations, the formation of

13 from **14** involves the loss of the only stereogenic center and the new formation of a rotationally hindered biaryl axis. This reaction occurs with a remarkable center-to-axis chirality transfer. The direction of the asymmetric induction – (*R*)-**14** → (*P*)-**13** – can be explained in terms of the global minimum structure of **14** shown in Fig. 3.15 (left), in which the 'Southern' molecular portion is firmly locked in an array with the 'trioxy front' directed above the plane, fixed by a strong hydrogen bond of the proton of OH-4 with the oxygen of OH-8'. This pre-orientation is apparently fully retained during the oxidative conversion of **14** to abyquinone A (**13**), thus leading to a *P*-configuration at the originating chiral axis (Fig. 3.7, right).

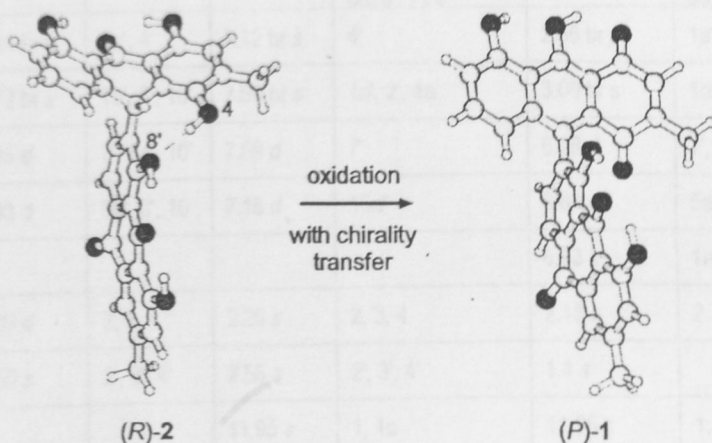


Fig. 3.7. Center-to-axis chirality transfer from abyquinone B (**15**) to abyquinone A (**13**). The *R*-configuration in **14** induces a *P*-configured chiral axis in **13** due to the pre-orientation initiated by the hydrogen bond between OH-4 and OH-8'; both structures shown constitute global AM1 minimum geometries.

The conversion is likely to be highly efficient in terms of chirality transfer, since (– as already the precursors **15** and **14** –) the final product **13** also appeared as enantiomerically pure by chromatographic analysis on a chiral phase, giving rise to only one peak each, with no CD difference at either of the left and right slopes of the respective peaks.

Table 3.6: ^1H NMR data (δ) together with HMBC (2J and 3J) correlations of for compounds 13, 14, 15

	13		14		15	
C	^1H	HMBC	^1H	HMBC	^1H	HMBC
2	6.97 <i>q</i>	4, 9a	6.79 <i>br s</i>	1a, 4, 3-Me	6.72 <i>br s</i>	3, 3-Me
5	6.93 <i>dd</i>	6, 8a, 10	6.95 <i>dd</i>	8a	6.87 <i>dd</i>	7, 10
6	7.51 <i>t</i>	5a, 8	7.41 <i>t</i>	5a, 8	7.38 <i>t</i>	5a, 8
7	7.10 <i>dd</i>	5, 8, 8a	6.88 <i>dd</i>	5	6.84 <i>dd</i>	5, 8
10			6.20 <i>s</i>	1a, 4, 4a, 5, 5a, 8a, 6', 7', 8'	6.13 <i>s</i>	1a, 4, 4a, 5, 5a, 8a, 6', 7', 8'
2'	7.14 <i>br s</i>	1a', 4'	7.12 <i>br s</i>	4'	2.86 <i>br s</i>	1a', 1'
4'	7.72 <i>br s</i>	1a', 2', 10'	7.60 <i>br s</i>	1a', 2', 4a	3.06 <i>br s</i>	1a', 10'
5'	7.95 <i>d</i>	7', 8a', 10'	7.59 <i>d</i>	7'	6.98 <i>d</i>	7', 10'
6'	7.43 <i>d</i>	5a', 8', 10	7.18 <i>d</i>	10a'	6.84 <i>d</i>	5a', 8'
10'					6.93 <i>s</i>	1a', 4', 5', 5a', 8a'
Me-3	2.03 <i>d</i>	2, 3, 4	2.26 <i>s</i>	2, 3, 4	2.18 <i>s</i>	2
Me-3'	2.50 <i>s</i>	2', 3', 4'	2.55 <i>s</i>	2', 3', 4'	1.4 <i>s</i>	
OH-1			11.95 <i>s</i>	1, 1a	11.96 <i>s</i>	1, 1a, 2
OH-8	10.51 <i>s</i>		12.35 <i>s</i>	8, 8a	12.36 <i>s</i>	7, 8
OH-9	17.04 <i>s</i>	1a				
OH-1'	11.95 <i>s</i>	1', 2'	11.87 <i>s</i>	1', 1a', 2'		
OH-8'	12.30 <i>s</i>	7', 8', 8a'	13.16 <i>s</i>		10.74 <i>s</i>	
OH-9'					16.12 <i>s</i>	

$J^{5,6} = J^{6,7} = J^{5,6'} = 8.0 \text{ Hz}$; $J^{5,7} = 1.5 \text{ Hz}$; In 13 $J^2, \text{Me-3} = 1.2 \text{ Hz}$.

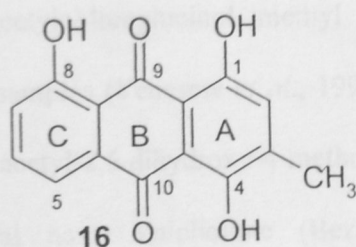
Table 3.7: ^{13}C NMR data for compounds 13, 14, 15

C	13	14	15
1	182.2	157.6	157.5
1a	108.3	113.2	113.0
2	134.8	118.5	118.2
3	152.5	137.4	138.1
4	186.8	144.5	145.2
4a	134.0	127.0	127.1
5	120.6	120.0	120.5
5a	138.1	144.4	145.9
6	134.4	137.1	137.0
7	116.7	116.5	116.1
8	160.1	162.9	162.9
8a	116.2	115.5	115.7
9	170.1	193.1	194.0
10	132.3	35.8	35.0
1'	163.2	163.0	204.4
1a'	114.3	113.8	110.7
2'	124.7	124.6	51.6
3'	150.2	150.8	71.4
4'	121.7	121.8	43.5
4a'	134.9	133.5	136.5
5'	120.3	120.5	120.5
5a'	133.5	132.5	138.7
6'	137.3	136.8	132.0
7'	136.3	138.4	123.6
8'	160.9	158.4	152.1
8a'	115.8	116.8	112.9
9'	193.4	194.2	164.8
10'	184.3	181.5	118.1
Me-3	17.3	17.5	17.5
Me-3'	22.5	22.4	29.4

Compound 16

Compound 16 was isolated as glittering red crystals, melting point 217-219°C. On TLC analysis, the compound showed a magenta spot R_f 0.5 (5% EtOAc in hexane). The spot changed to purple on exposure to NH_3 , a characteristic common to 1,4,8-trihydroxy or 1,5,8-trihydroxyanthraquinones. In support of this the ^1H NMR spectrum showed three chelated hydroxyl protons, δ 13.49, 12.33 and 12.28 ppm. In addition, the presence of an aromatic methyl group was evident from a singlet integrating to

three protons at δ 2.38. Also present was an ABX spin system of three mutually coupled protons, δ 7.30 (*d*, $J = 8.6$ Hz), 7.69 (*t*, $J = 7.8$ Hz, 7.8 Hz and 7.89 (*d*, $J = 7.7$ Hz) corresponding to C-ring aromatic protons at C-7, -6 and -5, respectively, with C-8 having one of the three chelated hydroxyl groups. A broad singlet at δ 7.16 is due to H-2 with the biogenetically expected methyl group at C-3. Then the remaining two hydroxyl groups can be placed at C-1 and C-4 of A-ring. Therefore, this compound was identified as 1,4,8-trihydroxy-3-methylantraquinone, trivial name islandicin (Karrer, 1976).



The compound has been previously isolated from the stem bark of *Ventilago bombaiensis* (Pepalla *et al.*, 1992). Cumming and Thomson (1970) identified the compound in *Maesopsis eminii*.

Compound 17

Compound 17, isolated as deep red needles; showed an orange spot on TLC, R_f 0.2 (30% EtOAc in hexane). EIMS analysis showed an $M+1$ peak at m/z 435 corresponding to the molecular formula of $C_{24}H_{19}O_8$. The UV absorption (λ_{max} 224, 246, 254, 271, 289, 369 and 430 nm) was characteristic of an anthraquinone chromophore. Comparison of the 1H NMR spectrum (Table 3.8) of this compound with chrysophanol (1) showed that it is a chrysophanol derivative. Thus two downfield shifted singlets at δ 12.47 and 11.96 were due to the chelated hydroxyl groups at C-1 and C-8; the C-ring aromatic protons at C-5, -6 and -7 constitute an

ABX spin system at δ 7.55 (*dd*, $J = 8.0, 1.0$ Hz), 7.75 (*t*, $J = 8.0$ Hz), and 7.29 (*dd*, $J = 8.0, 1.0$ Hz). In ring A the signal for H-4 is missing with H-2 appearing as a singlet at δ 7.32, while the methyl at C-3 was up-field shifted δ 2.17 relative to that of chrysophanol. These NMR features suggested that this compound has an aromatic substituent at C-4 on a chrysophanol skeleton. The substituent at C-4 was identified as acetylphloroglucinol methyl ether from MS (m/z 253 and 181 for $M^+ - C_9H_9O_4$ and $M^+ - C_{15}H_9O_4$) and 1H NMR (δ 14.19 for OH-2'; 6.24, *s*, for H-5'; 2.63, 3H, *s*, for the acetyl Me on C-3'; 3.98, 3H, *s* for OMe at C-4'). Finally reductive cleavage of **17** gave chrysophanol and acetylphloroglucinol methyl ether (identified by TLC comparison with authentic samples (Yenesew *et al.*, 1994). Therefore compound **17** was identified as 1-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methylantraquinone, trivial name knipholone (Bezabih and Abegaz, 1998). Knipholone-type compounds appear to be characteristic constituents for the genera *Bulbine*, *Bulbinella* and *Kniphofia* (van Wyk, *et al.*, 1995a). Knipholone isolated from *Kniphofia foliosa* was found to be a selective inhibitor of leukotriene metabolism in a human blood assay with an IC_{50} value of 4.2 μM (Wube, 2006). Inhibition of leukotriene formation is one of the approaches to the treatment of asthma and other inflammatory diseases (Wube, 2006). Besides from *Bulbine* and *Kniphofia* species, Knipholone has also been reported from the pods of *Senna didymobotrya* (Alemayehu, *et al.*, 1996a).

Compound 18

The red crystals of compound **18** showed a yellow spot on TLC, R_f 0.3 (20% EtOAc in hexane). Comparison of the 1H NMR data (Table 3.8) with those of knipholone

(17) suggested that **18** is an isomer of knipholone (17) having the same chrysophanol skeleton with acetylphloroglucinol methyl ether substituent at C-4. The only difference between these two compounds is on the chemical shift values of the methoxyl and the chelated hydroxyl groups in the acetylphloroglucinol methyl ether moiety. The methoxyl in **18** is up-field shifted (δ 3.31) compared to that of knipholone (δ 3.98) suggesting that these two compounds differ on the position of attachment of this group. Thus the methoxyl in **18** should be placed at C-2' with the hydroxyl groups being at C-4' (δ 13.51) and C-6'. The group being close to the shielding region of the chrysophanol moiety causes the up-field shift of the methoxyl group. Hence this compound is isomeric to knipholone and hence identified as 1-(3-acetyl-4,6-dihydroxy-4-methoxyphenyl)-4,5-dihydroxy-2-methylanthraquinone, trivial name isoknipholone (Yenesew *et al.*, 1994). The compound has been previously reported from the roots of *Bulbine capitata* (Bezabih, *et al.*, 1997) and from *Kniphofia foliosa* (Yenesew *et al.*, 1994).

Compound 19

The dark red crystals of compound **19** gave a yellow spot, R_f 0.4 (20% acetone in hexane) upon TLC analysis. The ^1H NMR (Table 3.8) spectrum of this compound was similar to that of knipholone (17), suggesting that this compound is also a chrysophanol derivative having an acetylphloroglucinol substituent at C-4. In fact the only difference between compounds **17** and **19** is the presence of an additional methoxyl, in **19**, as deduced from the ^1H NMR (at δ 3.74) and ^{13}C NMR (δ 56.37) spectra. This showed compound **19** is a methyl ether derivative of knipholone (17). The additional methoxyl group was placed at C-6' since the three chelated hydroxyl protons at C-1, -8 and -1' appeared at δ 13.89 ppm, 12.58 ppm and 12.06 in the ^1H

NMR spectrum. Therefore compound **19** was identified as 6'-O-methylknipholone.

The knipholone 6'-methyl ether has been reported previously from *Bulbine capitata* (Bezabih *et al* 1997)

Compound 20

The compound was isolated as yellowish-brown solid, R_f 0.4 (Hexane:EtOAc; 1:1).

The ^1H and ^{13}C NMR spectra are similar to those of knipholone (**17**) (Table 3.8 and 3.9). The major difference between these compounds is the appearance of mutually coupled protons at δ 4.03 (*d*, $J = 23.3$) and δ 3.81 (*d*, $J = 23.3$) in the ^1H NMR spectrum, and the appearance of a methylene carbon at δ 31.4 in the ^{13}C NMR spectrum instead of a carbonyl at C-10 (as in **17**), suggesting that **20** is an anthrone of knipholone (**17**). Finally aerial oxidation of **20** gave knipholone (**17**) and hence compound **20** was identified as 4-(3-acetyl-2,6-dihydroxy-4-methoxyphenyl)-1,8-dihydroxy-10H-anthracen-9-one, trivial name knipholone anthrone. It has been previously reported from the stem of *Kniphofia foliosa* (Dagne and Yenesew 1993).

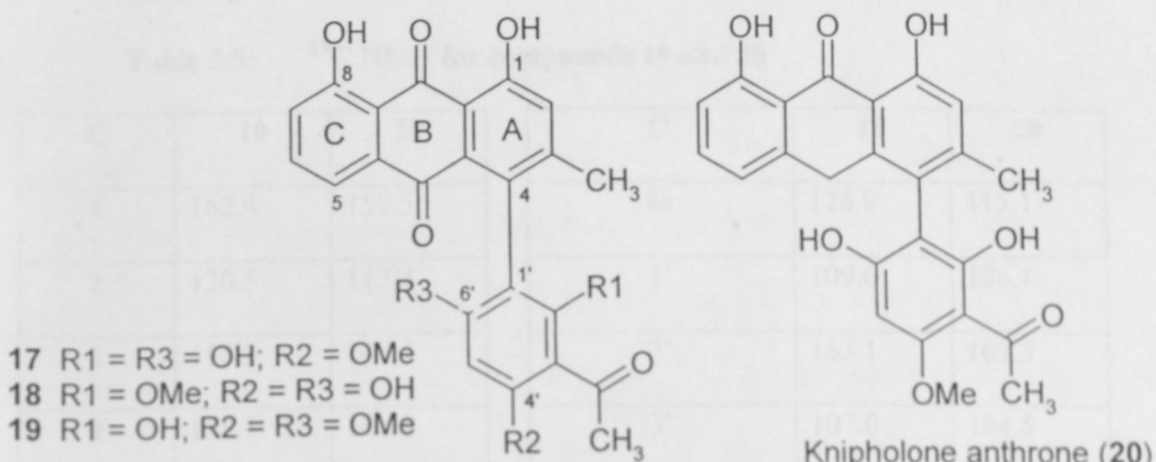


Table 3.8: ¹H NMR for compound 17, 18, 19 and 20

C	17	18	19	20
2	7.32 (br s))	7.23 (br s)	7.22 (br s)	6.91 (br s)
5	7.55 (dd, $J = 8.0$, 1.0)	7.57 *	7.53*	6.80 (d, $J = 8.4$)
6	7.75 (t, $J = 8.0$)	7.60 *	7.56*	7.36 (t, $J = 7.7$)
7	7.29 (dd, $J = 8.0$, 1.0)	7.27*	7.17*	6.69 (d, $J = 7.4$)
2'	14.19 (OH)	3.31 (OMe)	13.89 OH)	14.25 (OH)
4'	3.98 (OMe)	13.51 (OH)	3.97 (OMe)	3.94
5'	6.24	6.30	6.09	6.15
6'			3.74 (OMe)	
10				4.03 (d, $J = 22.2$); 3.81 (d, $J = 24.4$)
OH-1	12.47	12.58	12.58	12.53
Me- 3	2.17	2.16	2.08	2.11
OH-8	11.96	11.97	12.06	12.19
C(O)- Me-3'	2.63	2.61	2.64	2.66

* Coupling not well resolved

Table 3.9: ¹³C NMR for compounds 19 and 20

C	19	20
1	162.4	159.5
2	120.5	117.4
3	152.2	149.5
4	135.4	141.8
5	116.4	118.8

C	19	20
8a	126.0	115.1
1'	109.6	106.1
2'	163.1	162.3
3'	107.0	104.5
4'	163.2	163.8

6	132.0	135.9
7	115.7	115.3
8	162.9	152.5
9	193.6	193.4
10	183.2	31.4
1a	124.2	114.5
4a	137.5	142.9
5a	129.1	119.4

5'	87.0	90.3
6'	163.7	163.2
Me-3	22.1	21.0
CO-3'	204.2	203.3
Me-3'	34.4	33.3
OMe-4'	56.6	55.8
OMe-6'	56.4	

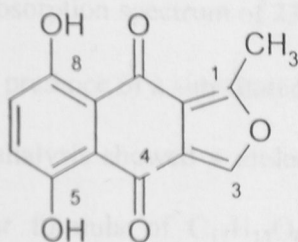
3.2.3 Characterization of compounds isolated from roots of *Bulbine frutescence*

The acetone extract of the roots of *Bulbine frutescence* was subjected to a combination of chromatographic techniques that led to the isolation of compounds **21** to **28** and knipholone (**17**). Knipholone (**17**) was identified as described in section 3.2.2 (page 97). The characterization of the rest of the compounds (**21** to **28**) will be discussed below.

Compound **21**

The compound was isolated as an orange amorphous solid. EIMS analysis of the compound showed a molecular ion peak at m/z 244 corresponding to the molecular formula of $C_{13}H_8O_5$. The UV spectrum (λ_{max} 226, 240 sh, 295, 349 and 440 nm) suggested an isofuranonaphthoquinone skeleton (Bezabih *et al.*, 1997). In the 1H NMR spectrum, two singlets at δ 12.98 and 12.82 were assigned to the two chelated hydroxyl groups at C-5 and -8. Two singlets at δ 7.27 and 7.26 can then be assigned to H-6 and H-7. In the furan ring a singlet at δ 8.07 was assigned to H-3, C-1 being substituted with methyl group (δ 2.80). Hence this compound was identified as 5,8-

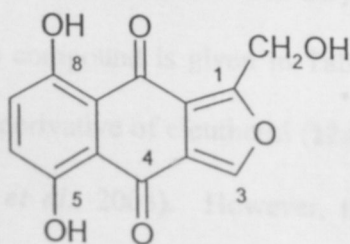
dihydroxy-1-methylnaphtho[2,3-C]furan-4,9-dione (**21**). This compound has been previously reported from *Bulbine capitata* (Bezabih *et al.*, 1997).



21

Compound 22

Just as in compound **21**, the UV spectrum ($\lambda_{\max}$ 226, 240*sh*, 295, 349 and 440 nm) of compound **22** again suggested an isofuranonaphthoquinone skeleton. Comparison of the ^1H NMR of this compound with that of **21** showed similar pattern (two singlets at 7.27 and 7.21 for H-6 and H-7, one singlet at δ 8.15 for H-3), except that the three proton singlet at δ 2.80 (Me-1) in compound **21** is replaced by a two proton singlet at δ 4.93 in compound **22**. This strongly suggested that **22** is a derivative of **21** where the methyl at C-1 is oxidized to oxymethylene. In agreement with this the EIMS of **22** showed a molecular ion peak at m/z 260 ($\text{C}_{13}\text{H}_8\text{O}_6$). Hence this compound was identified as 5,8-dihydroxy-1-hydroxymethylnaphtho[2,3-C]furan-4,9-dione (**22**). This compound has also been reported from *Bulbine capitata* (Bezabih *et al.*, 1997).



22

Compound 23

Compound 23, which fluoresce blue in solution, was isolated as a colourless amorphous powder. The UV absorption spectrum of 23 (λ_{max} 215, 249, 271, 302, 313, 328 and 357 nm) indicated the presence of a substituted naphthoquinone chromophore (Tânia *et al.*, 2003). HRMS analysis showed a molecular ion peak at m/z 258.0885 corresponding to a molecular formula of $\text{C}_{15}\text{H}_{14}\text{O}_4$. The presence of a methyl substituent on the γ -lactone ring (Ring-A) was evident from the ^1H (δ 5.72, q , $J = 6.5$ Hz for H-3; 1.71, d , $J = 6.5$ Hz for Me-3) and ^{13}C (δ 170.0 for C-1, 77.0 for C-3, 151.4 for C-4 and 156.1 for C-5) NMR spectra. While ring-B and -C each contains one methoxyl (δ 3.99 and 3.87 in ^1H NMR; 62.6 and 56.2 in ^{13}C NMR) group.

An ABX spin system in the ^1H NMR spectrum at δ 6.95 (d , $J = 7.5$ Hz), δ 7.41 (t , $J = 8.0$ Hz) and δ 7.54 (d , $J = 8.0$ Hz) were due to H- 6, -7 and -8 respectively, of ring C with a methoxyl substituent at C-8. The singlet at δ 8.11 was due to the aromatic proton at C-9 of ring B, which is substituted at C-4 with the second methoxyl group (δ 3.99 in ^1H and 62.7 in ^{13}C NMR). Therefore this compound was characterized as elutherol-9-methyl ether (23) (Tânia *et al.*, 2003). This was also confirmed by the presence of characteristic fragment peaks on the MS spectrum *viz.* m/z 258 (M^+), 243 ($\text{M}^+ - \text{Me}$), 228 ($\text{M}^+ - 2\text{Me}$), 215 (100%, $\text{M}^+ - \text{Me} - \text{CO}$), 200 ($\text{M}^+ - 2\text{Me} - \text{CO}$). The complete NMR data for this compound is given in Table 3.10. This compound has been reported as a synthetic derivative of eleutherol (12a) isolated from the leaves of *Eleutherine bulbosa* (Tânia *et al.*, 2003). However, this is the first report on its occurrence in nature. Isoelutherol has been reported from *Aloe* species (section 3.2.1). The co-occurrence of compound 23 along with 21 and 22, suggested that demethyleleutherol (23b) could be the likely precursor to 21 and 22 (Scheme 3.8).

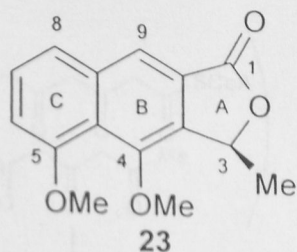
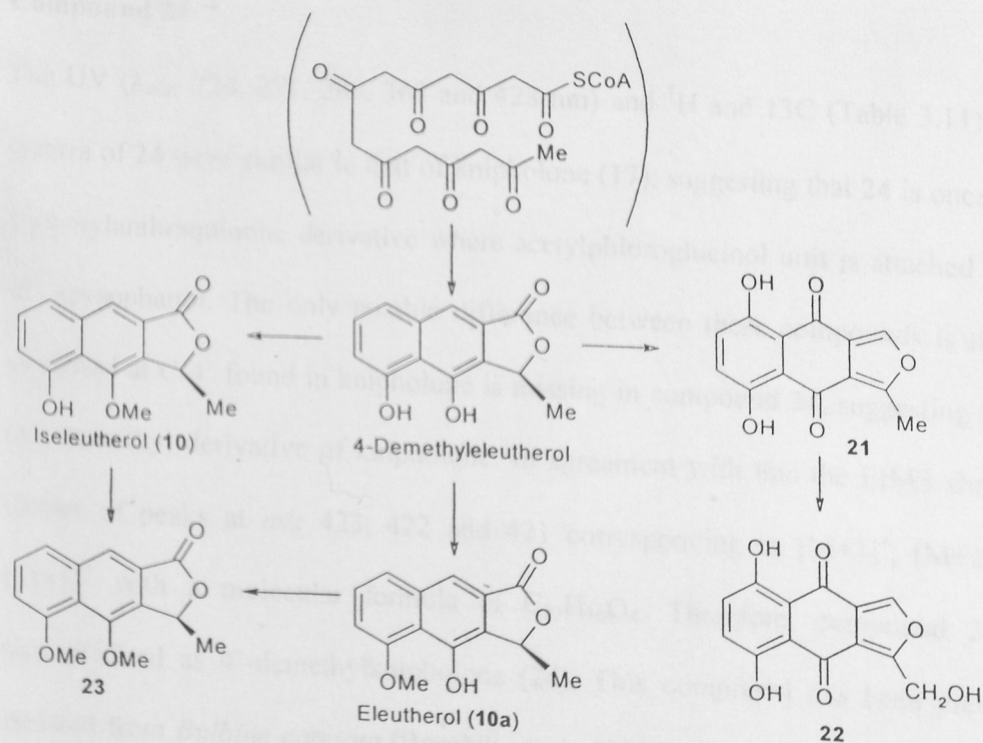
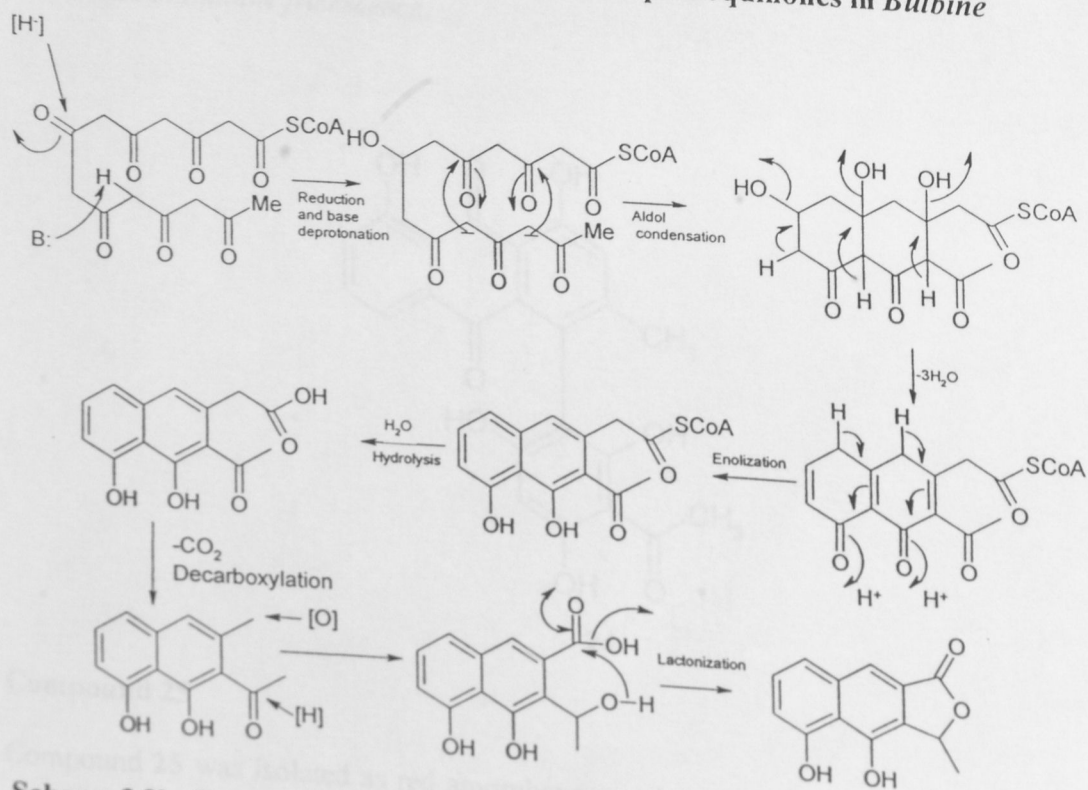


Table 3.10: NMR data for eleutherol-9-methylether (23)

C	^1H NMR (δ)	^{13}C NMR
1		170.0
3	5.72 (q, $J = 6.5$)	77.0
4		151.4
5		156.1
6	6.95 (d, $J = 7.5$ Hz)	108.1
7	7.41 (t, $J = 8.0$ Hz)	127.2
8	7.54 (d, $J = 8.0$ Hz)	122.9
9	8.11 (s)	122.6
1b		138.1
3b		125.1
5b		123.1
8b		135.9
Me-3	1.71 (3H, d, $J = 6.5$)	20.1
OMe-4	3.99 (3H, s)	56.2
OMe-5	3.87 (3H, s)	62.6



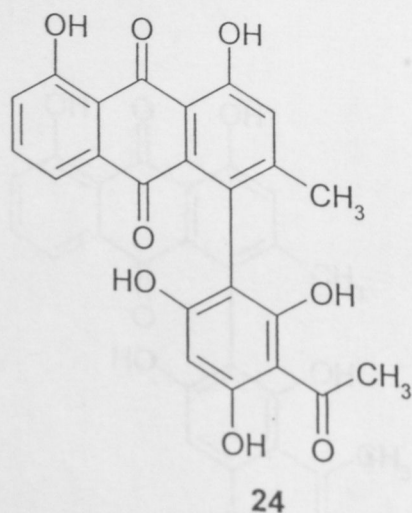
Scheme 3.8: Proposed biogenesis of isofuranonaphthoquinones in *Bulbine*



Scheme 3.8b: Proposed pathway for the formation of 4-demethyleleutherol

Compound 24

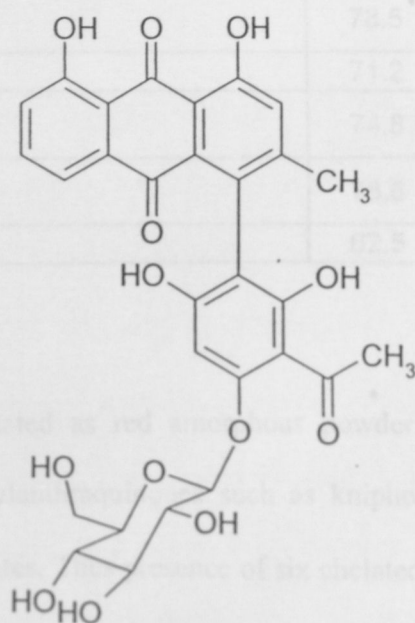
The UV (λ_{max} 224, 271, 289, 365 and 428 nm) and ^1H and ^{13}C (Table 3.11) NMR spectra of **24** were similar to that of knipholone (**17**), suggesting that **24** is once again a phenylanthraquinone derivative where acetylphloroglucinol unit is attached at C-4 of chrysophanol. The only notable difference between these compounds is that the methoxyl at C-4' found in knipholone is missing in compound **24**, suggesting that **24** is a demethyl derivative of knipholone. In agreement with this the EIMS showed a cluster of peaks at m/z 423, 422 and 421 corresponding to $[\text{M}+3]^+$, $[\text{M}+2]^+$ and $[\text{M}+1]^+$ with a molecular formula of $\text{C}_{23}\text{H}_{16}\text{O}_8$. Therefore, compound **24** was characterized as 4'-demethylknipholone (**24**). This compound has been previously isolated from *Bulbine capitata* (Bezabih *et al.*, 1998) but this is the first report on its occurrence in *Bulbine frutescence*.



Compound 25

Compound **25** was isolated as red amorphous powder. The UV (λ_{max} 226, 271, 287, 369 and 429 nm) again suggested a phenylanthraquinone skeleton. Comparison of the ^1H and ^{13}C NMR (Table 3.11) spectra of **25** with that of **24** suggested **25** to be a

glycoside of **24**. A doublet at δ 5.03 ($J = 7.5$ Hz) confirmed the *O*- β -D-glucosidic nature of the linkage. The glucose unit was placed at the oxygen on C-4' based on comparison of NMR spectra (Table 3.10) with literature report (Abegaz *et al.*, 2002). Therefore, compound **25** was identified as 4'-*O*-demethylknipholone-4'-*O*- β -D-glucoside (**25**). Signal overlapping (duplication) in the spectrum of this compound was evident in that the proton on C-1'' appeared as two doublets at δ 5.03 ($J = 7.5$) and δ 4.99 ($J = 8$), while the proton at C5' appeared as two peaks (δ 6.288 and 6.291) instead of a singlet. This was an indication that the compound could be a mixture of M and P atropisomers. Since the compound is a glycoside of compound **24**, compound **24** could also be an enantiomeric mixture of M and P atropisomers. This fact would not be seen from the NMR spectra of compound **24** because enantiomers normally have identical NMR patterns. Upon glycosilation of the aglycone, a chiral center is introduced making the enantiomeric aglycones diastereomers (overlapping peaks in compound **25**).



25

Table 3.11: ^1H and ^{13}C NMR data for compounds **24** and **25**

C-	24		25	
	¹³ C	¹ H	¹³ C	¹ H
1	163.1	12.38 (OH)	164.0	
2	125.2	7.17 (<i>br s</i>)	125.7	7.17 (<i>br s</i>)
3	153.0		153.0	
4	129.4		129.5	
5	119.9	7.41 (<i>d</i> , <i>J</i> = 7.0)	120.3	7.56 (<i>dd</i> , <i>J</i> = 2.5, 8.5)
6	137.7	7.61 (<i>t</i> , <i>J</i> = 7.5, 8)	138.1	7.42 (<i>t</i> , <i>J</i> = 8)
7	123.9	7.15 (<i>d</i> , <i>J</i> = 8)	124.4	7.16 (<i>dd</i> , <i>J</i> = 7, 8.5)
8	162.9	11.84 (OH)	162.5	
9	193.9		194.4	
10	183.6		183.8	
1a	115.6		116.0	
4a	133.0		136.1	
5a	135.7		138.1	
8a	116.3		116.8	
3Me	20.6	2.05 (3H, <i>s</i> Ar-Me)	20.9	2.03 (3H, <i>d</i> , <i>J</i> = 6.5)
1'	107.4		106.8	
2'	162.4	13.14 (<i>br s</i> OH)	163.6	
3'	105.5		102.3	
4'	162.3	10.11 (<i>br s</i> OH)	162.9	
5'	95.2	6.07 (<i>br s</i>)	95.3	6.29 (<i>d</i> , <i>J</i> = 3)
6'	162.3	8.76 (<i>br s</i> OH)	161.8	
3'C(O)Me	204.4		205.0	
3'C(O)Me	32.5	2.54 (3H, <i>s</i> , Ar-COMe)	33.7	2.67 (<i>s</i>)
1"			102.2	5.03 (<i>d</i> , <i>J</i> = 7.5)
2"			78.5	3.66 (<i>m</i> , <i>J</i> _{ratios} = 8.0:10:8.0:6.5)
3"			71.2	3.34 (<i>dd</i> , <i>J</i> = 9.5, 2.0)
4"			74.8	3.51 (<i>m</i> , <i>J</i> _{ratios} = 4.5:4.5:4.5::3.5:4.5)
5"			78.6	3.87 (<i>m</i> , <i>J</i> _{ratios} = 2.0:5.0:2.0:3.0:2.0:5.0:2.0)
5"CH ₂ OH			62.5	3.42 (2H <i>d</i> , <i>J</i> = 13.0)

Compound 26

Compound 26 was isolated as red amorphous powder. The ¹H NMR spectrum resembles that of phenylanthraquinones such as knipholone (17), except that the signals appear as duplicates. Thus presence of six chelated hydroxy protons (δ 12.05, 12.23, 12.51, 12.58, 14.23 and 14.45), two aromatic methyls (δ 1.99 and 2.13), two methoxyls (δ 3.69 and 3.92), two acetyl groups (δ 2.71 and 2.74) and nine aromatic

protons (Table 3.12) suggested that this compound could be a dimer of knipholone-type anthraquinones. In support of this the TOF-EIMS spectrum showed a $[M+1]^+$ peak at m/z 853.2117 corresponding to the molecular formula of $C_{48}H_{37}O_{15}$. The UV (λ_{max} 275, 294, 385, 414, 446 nm) and ^{13}C NMR (δ 194.29, 192.98 for C-9 and C-9', 182.35 for C-10 or C-10', 203.29 and 203.99 for two acetyl carbonyl) showed the presence of five carbonyl groups and is consistent with **26** being a dimer of phenylanthraquinone and phenylanthrone. The phenylanthraquinone moiety was identified as knipholone. Thus the acetylphloroglucinol is attached at C-4 of A-ring with C-3 being substituted with methyl group. This was established from HMBC spectrum where correlation of H-2 with C-4, 9a and Me-3 as well as correlation of the methyl protons at C-3 with C-2 and C-4. The C-ring protons which appear as an ABX system in knipholone (Yenesew *et al.*, 1994), now appear as two sets of mutually coupled doublets at δ 7.23 and 6.89 ($J = 8.0$ Hz) for H-5 and H-6, respectively; which suggested that the site of linkage in this moiety is at C-7.

Comparison of the 1H and ^{13}C NMR spectra of the other half of the molecule with those of knipholone anthrone moiety (Dagne and Yenesew, 1993) showed identical features except that the signals for methylene group at C-10 (C-10' in **26**) in knipholone anthrone [δ 4.03 (*d*, $J = 23.3$), δ 3.81 (*d*, $J = 23.3$) in 1H NMR and δ 31.4 in ^{13}C NMR] are now replaced with a methine (δ 6.03 in 1H and 36.8 in ^{13}C NMR) group in **26**. This suggested that the knipholone half is attached at C-10' of knipholone anthrone moiety; and hence 7-10' linkage in **26**. This compound was therefore identified as 7,10'-kinpholone knipholone anthrone (**26**). The positions of the two methoxyls were confirmed to be at C-4'' and C-4''' from ROESY experiment

where correlation of the methoxyls with H-5'' and H-5', respectively, were observed. Furthermore, the chemical shift position of the methoxyl carbon atoms are in the normal range (δ 55.82 and 55.92) ruling out the possibility of any of these being at C-2'' and C-2''' (as in isoknipholone, Yenesew *et al.*, 1994). In the same way, the chemical shift value of the chelated hydroxyl groups in the two acetylphloroglucinol units (δ 14.23 and 14.45) were similar to what has been reported for knipholone (Yenesew *et al.*, 1994), rather than as in bulbine-knipholone (Bringmann *et al.*, 2002).

Compound 27

The TOF-EIMS spectrum of the second compound 27 showed an $[M+1]^+$ peak at m/z 853.2117 corresponding to the molecular formula of $C_{48}H_{37}O_{15}$, is in agreement to this compound being isomeric with 26. In agreement with this the UV ($\lambda_{\max}$ 275, 294, 385, 414, 446 nm) showed the presence of phenylanthraquinone and phenylanthrone chromophore. Comparison of the 1H and ^{13}C NMR (Table 3.12) of this compound with those of 26 showed identical pattern again suggesting a dimer composed of knipholone and knipholone anthrone with 7-10' linkage. Hence compounds 26 and 27 could be diastereomers differing in the configuration at C-10'.

Compound 28

The compound was isolated as red amorphous powder. TOF-EIMS spectrum showed a $[M+1]^+$ peak at m/z 869.2048 corresponding to the molecular formula of $C_{48}H_{37}O_{16}$, which again suggested dimer of knipholone-type anthraquinone. The UV ($\lambda_{\max}$ 278, 293, 385, 412, 444 nm) spectrum showed both phenylanthraquinone and phenylanthrone chromophore. Comparison of the 1H and ^{13}C NMR (Table 3.121) with

26 R = H
27 R = Me

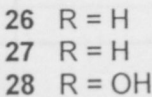


Table 3.12: ¹H (500 MHz) of 26, 27 and 28, together with HMBC (²J and ³J)

correlations for 26			27	28
Carbon	¹ H	HMBC	¹ H	¹ H
2	7.30 br <i>s</i>	4, 9a, 3-Me	7.19 br <i>s</i>	7.23 br <i>s</i>
5	7.23 br <i>d</i>	7, 8a, 10	7.13 <i>d</i>	7.14 <i>d</i>
6	6.89 <i>d</i>		6.71 <i>d</i>	7.67 <i>d</i>
2'	6.96 br <i>s</i>	4', 9'a, 3'-Me	6.88 br <i>s</i>	7.02 br <i>s</i>
5'	6.92 br <i>d</i>	7', 8'a, 10	6.77 <i>bd</i>	6.59 br <i>d</i>
6'	7.33 <i>t</i>	8', 8'a, 10'a	7.26 <i>t</i>	7.36 <i>t</i>
7'	6.80 <i>dd</i>		6.71 <i>dd</i>	6.92 <i>dd</i>
10'	6.03 <i>s</i> *		6.06*	
5''	6.10 <i>s</i>	3'', 4'', 6''	6.02 <i>s</i>	6.17 <i>s</i>
5'''	5.57 <i>s</i>		5.48 <i>s</i>	6.09 <i>s</i>
Me-3	2.13 <i>s</i>	2', 4'	2.04 <i>s</i>	2.09 <i>s</i>
Me-3'	1.99 <i>d</i>	2, 4	1.90 <i>d</i>	1.86 <i>d</i>
COMe-3''	2.74 <i>s</i>	COMe-4'''	2.63 <i>s</i>	2.66 <i>s</i>
COMe-3'''	2.71 <i>s</i>	COMe-4''	2.52 <i>s</i>	2.38 <i>s</i>
OMe-4''	3.92 <i>s</i>	4'''	3.82 <i>s</i>	3.97 <i>s</i>
OMe-4'''	3.69 <i>s</i>	4''	3.62 <i>s</i>	3.90 <i>s</i>
OH-1	12.51 <i>s</i>	2, 8, 9a	12.13 <i>s</i>	12.38 <i>s</i>
OH-8	12.01 <i>s</i>	8, 8a	11.86 <i>s</i>	11.88 <i>s</i>
OH-1'	12.58 <i>s</i>	2', 8', 9'a	11.49 <i>s</i>	12.97 <i>s</i>
OH-8'	12.23 <i>s</i>	8', 8'a	12.37 <i>s</i>	12.42 <i>s</i>
OH-2''	14.45 <i>s</i>	1'', 2''	14.36 <i>s</i>	13.04 <i>s</i>
OH-2'''	14.23 <i>s</i>		13.96 <i>s</i>	14.10 <i>s</i>
OH-6''	5.30 br <i>s</i>			
OH-6'''	6.00 br <i>s</i>			

9	194.3	194.3	193.2
9'	115.4	115.4	113.2
10	16.80*	16.8*	77.0
10'a	146.6	146.3	146.5
1'	105.2	105.3	105.2
2'	163.2	163.2*	163.8*
2''	106.3	105.8	106.5
3'	163.3	164.2	163.8
5'	90.3	90.8	91.6
6'	129.7	129.4	129.8
1''	107.3	107.4	108.0
2''	163.8*	163.2*	163.7*

Table 3.13: ^{13}C (125 MHz) of 26, 27 and 28

Carbon	26	27	28
1	163.5	163.5	163.3
2	125.8	125.6	125.7
3	153.0	152.9	152.8
4	125.9	126.1	125.1
4a	133.0	133.0	133.1
5	120.1	120.0	117.8
6	135.9	136.1?	132.2
7	139.3	139.3	139.3
8	158.8	158.8	158.7
8a	115.6	115.7	115.6
9	193.0	192.9	192.7
9a	115.6	115.3	115.4
10	182.4	182.5	182.4
10a	132.9	132.9	133.1
1'	163.2	163.1	163.1
2'	118.4	118.4	120.2
3'	150.7	150.5	150.2
4'	122.0	121.7	122.8
4a'	145.9	145.3	145.0
5'	119.6	119.7	118.9
6'	137.1	137.0	137.3
7'	115.9	115.9	117.8
8'	162.9	162.8	162.5
8a'	114.4	114.5	113.3
9'	194.3	194.3	193.2
9a'	115.4	115.4	113.3
10'	36.80*	36.9*	77.0
10' a	146.6	146.3	146.5
1''	105.2	106.5	105.2
2''	163. ^a	163.2 ^a	163.8 ^a
3''	106.5	105.4	106.5
4''	163.3	164.2	163.8
5''	90.8	90.8	91.6
6''	159.7	159.8	159.8
1'''	107.3	107.4	108.0
2'''	163.45 ^a	163.2 ^a	163.7 ^a

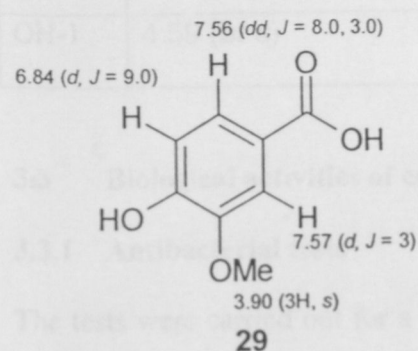
3'''	106.2	106.1	106.0
4'''	164.5	164.4	164.3
5'''	89.9	90.0	91.1
6'''	160.8	160.9	161.0
Me-3	20.9	20.9	20.9
Me-3'	20.8	20.8	21.2
OMe-4	55.9	55.9	55.9
OMe-4'	55.8	55.8	56.0
COMe-3''	33.2 ^b	33.2 ^b	33.5
COMe-3'''	33.2 ^b	33.1 ^b	33.2
COMe-3''	203.3 <i>s</i>	203.7 <i>s</i>	204.5 <i>s</i>
COMe-3'''	204.0 <i>s</i>	203.8 <i>s</i>	203.8 <i>s</i>

$J_{ortho} = 8.0 \text{ Hz}$; $J_{5,7}$

- observed at 238 K

Compounds 29, 30, and 31

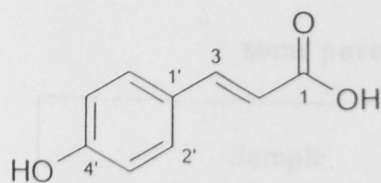
From the roots of *Bulbine frutescens*, the compounds vanillic acid (**29**), *p*-Coumaric acid (**30**) and hydro-*p*-Coumaric acid (**31**) were identified..



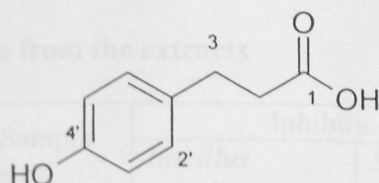
$M^+ 168$

Melting point 211 – 212° C

Compounds **30** and **31** were isolated as a mixture but the ^1H NMR pattern was quite well resolved and the peak assignments were possible. The ^1H NMR data for the two compounds is given in Table 3.14 below



30



31

Table 3.14: ¹H NMR data for compounds 30 and 31

C	30	31
2	6.39 (1H, <i>d</i> , <i>J</i> = 15.6)	3.47 (2H, <i>t</i> , <i>J</i> = 7.2, 7.5)
3	7.45 (1H, <i>d</i> , <i>J</i> = 16.2)	2.76 (2H, <i>t</i> , <i>J</i> = 7.8, 6.9)
2' & 6'	7.40 (2H, <i>dd</i> , <i>J</i> = 1.8, 6.9)	7.07 (2H, <i>dd</i> , <i>J</i> = 2.1, 6.8)
3' & 5'	6.80 (2H, <i>dd</i> , <i>J</i> = 1.8, 6.9)	6.72 (2H, <i>dd</i> , <i>J</i> = 2.1, 6.6)
OH-1	4.59 (br s)	4.59 (br s)

3.3 Biological activities of crude extracts and pure compounds

3.3.1 Antibacterial tests

The tests were carried out for a few of the crude extracts and some pure compounds against two bacterial strains viz. *Bacillus subtilis* and *Staphylococcus aureus*. The inhibition zones were measured for known concentrations of sample. The dichloromethane extracts of *A.kilifiensis* stems and leaves showed no activity against *Bacillus subtilis* but both had some activity against *Staphylococcus aureus*. Compounds 6 and 7 were active against both strains of bacteria. These results are presented in Table 3.13 below.

Table 3.15: Antibacterial test results for some *A. kilifiensis* crude extracts and some pure compounds from the extracts

Sample	Sample size	Inhibition zone	
		<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>
Dichloromethane stem extract of <i>A. kilifiensis</i>	15.4 µg	Nil	24 mm
Dichloromethane leave extract of <i>A. kilifiensis</i>	15.4 µg	Nil	27 mm
Aloesaponarin I (7)	11.4 µg	26 mm	25 mm
Aloesaponarin II (6)	10.4 µg	28 mm	25 mm

3.3.2 Antiplasmodial assay

The extracts and some pure compounds were tested against the chloroquine-sensitive (D6) and the chloroquine-resistant (W2) strains of *Plasmodium falciparum*. Chloroquine, mefloquine and quinine were used as control drugs. As the results show (Table 3.16), the crude acetone extract of *A. kilifiensis* fresh leaves had the highest activity against both strains W2 and D6 in comparison with all the other crude extracts. Considering that this was the only extract of fresh plant parts, the possibility is that the activity gets lost in the process of drying. Dichloromethane crude extract of the dry stems of *A. kilifiensis* also had some appreciable activity against W2 but non-against D6. Two of the compounds (compounds 3 and 6) isolated from this extract (*A. kilifiensis* stems DCM crude) were tested and found to have a much higher activity than the crude extracts. It is thus possible that the two compounds are part of those responsible for the activity of the crude extract.

Table 3.16: In-vitro IC₅₀ values against W2 and D6 isolates of *Plasmodium falciparum* for crude extracts and pure samples from *Aloe* and *Bulbine* species.

Control drugs	IC ₅₀ (µg/ml)	
	W2	D6
Chloroquine	0.0207	0.0050
Mefloquine	0.0036	0.0309
Quinine	0.0527	0.0465
Crude extracts		
<i>Aloe kilifiensis</i> stems hexane crude	>50.0	>50.0
<i>A. kilifiensis</i> stems dichloromethane crude	22.5	>50.0
<i>A. kilifiensis</i> fresh leaves acetone crude	10.6	14.7
<i>A. graminicola</i> dry leaves THF crude extract	>50.0	>50.0
Pure compounds		
Asphodelin (3)	6.0	8.0
4,7'-Dichrysophanol-10'-α-L-rhamnoside (4)	10.8	13.5
Aloesaponarin II (6)	5.0	NT
Aloesaponol I (10)	8.0	NT
Aloesaponol II (11)	8.5	NT
Abyquinone A (13)	9.0	12.3
Abyquinone B (14)	NT	24.1
Knipholone (17)	6.2	10.2
Isoknipholone (18)	7.9	NT
Knipholone 6'-methyl ether (19)	10.8	NT

NT NOT TESTED

Table 3.17: More in-vitro antiplasmodial activities against W2 isolate of

Plasmodium falciparum

Control drugs	IC ₅₀ (µg/ml)	Compound	IC ₅₀ (µg/ml)
Chloroquine	0.0182	Aloesaponarin II (6)	4.9695
Mefloquine	0.0083	Laccaic acid D-methyl ester (8)	1.0501
Quinine	0.0821	Knipholone (17)	2.798
Pure compounds		Isoknipholone (18)	7.8756
Asphodelin (3)	5.7058	Knipholone 6'-methyl ester19	10.7557
4,7'-Dichrysophanol-10'- α-L-rhamnoside (4)	2.3642	27	0.2631

As seen in the above Tables 4.3 and 4.3b, all the samples tested inhibited the growth of *P. falciparum* isolates W2 and D6 in *vitro* to some extent. The most potent was compound 27 with an IC₅₀ value of 0.263 µg/ml. In comparison with the conventional antimalarial agent quinine (0.082 µg/ml), the activity of compound 27 is relatively high. The activity of the other compounds tested was low in relation to the conventional malarial drugs.

Knipholone anthrone (20) has been reported as the most active phenylanthraquinone (IC₅₀ = 0.149 µg/ml cf. 0.141 µg/ml for chloroquine) against *P. falciparum* (K1 strain) (Bringman *et al.*, 2002). In the same report, knipholone (17) had an IC₅₀ of 0.627 µg/ml against the K1 strain. In the current study, knipholone had an IC₅₀ of 27.98 µg/m) against the strain W2 of *P. falciparum*. The activity observed in this study is much lower in comparison with that reported by Bringman *et al.* (2000), probably due to the difference in the strains used. It is also possible that the

knipholone tested here has the opposite configuration at the biaryl axis from that tested by Bringman *et al.* (2002)

3.4 CONCLUSION AND RECOMMENDATIONS

3.4.1 Conclusion

In this research, the isolation and characterization of a total of thirty one compounds was accomplished. Twelve of the compounds were from *Aloe* species while the rest were from *Bulbine* species. Most of these compounds were anthraquinone derivatives which have been derived through the polyketide pathway. A few of the compounds obtained were simple propanoid derivatives.

Some of the compounds screened for biological activity showed an appreciable activity. Some crude extracts and a few pure compounds from *Aloe* showed antibacterial activity against two bacterial strains viz. *Bacillus subtilis* and *Staphylococcus aureus*. A number of the compounds isolated showed antiplasmodial activity. The pure compounds tested had a higher antiplasmodial activity than the crude extracts from which they were isolated.

Amongst the compounds obtained from *Aloe*, isoelutherol (12) and the asphodelin glycosides (3 and 4) were present in the subterranean stems of all the *Aloe* species (belonging to the *Saponariae* group) analyzed. Isoelutherol has been previously described as a chemotaxonomic marker in the *Saponariae* group (Dagne, *et al.*, 2000). The two asphodelin glycosides were identified in all the species belonging to the *Saponariae* group, and hence they could be chemotaxonomic markers of the group.

From *Bulbine frutescens*, knipholone and knipholone derivatives were amongst the major compounds obtained. Furanonaphthoquinones were also isolated from this

plant. Three new compounds obtained from the fruits of *B. abyssinica*, abyquinone A, B and C appear to be biogenetically interrelated. Thus oxidation of abyquinone C produced abyquinone B which on further oxidation resulted in the formation of abyquinone A. The crude extracts also showed the presence of the three compounds leading to the conclusion that all the three are true natural products. However, one cannot fully rule out the possibility of compound 15 being the only true natural product amongst the three. This would be so in consideration that dissolved oxygen could be responsible for the conversion of 15 into 14 and subsequently to 13

The isolation of the aglycone 4'-demethylknipholone (compound 24) and its glycosilated derivative (compound 25) from the same crude extract was a clear indication that under proper conditions, it is possible to obtain compounds at the various stages of biosynthesis. This could lead to a better understanding of the biosynthetic routes.

The stereochemistry of asphodelin (3), asphodelin-10'- α -L rhamnoside (4) and the abyquinones A-C (13, 14 and 15) was determined. This is the first time that the stereochemistry at the biaryl linkage of anthraquinones is determined. This information can thus be used as a reference for future determination of stereochemistry of other bi-anthraquinones through CD comparisons. It is also noted that the atropisomerism at the biaryl linkage of asphodelin from *Aloe kilifiensis* was different from that of asphodelin from *A. lateritia*.

3.4.2 Recommendations

There are many species of *Aloe* growing in the wild in Kenya. Reynolds, 1966 reported there to be a total of twenty six species in Kenya of which six were endemic. The geographical distribution of *Aloe* in Kenya needs to be documented and the chemical constitution of all Kenyan *Aloe* species needs to be studied. This will help in identification of medicinally and commercially important *Aloe* species.

In this study, the biological activity of the control drugs was many times higher than that of the compounds tested. Considering the claims on the curative nature of the plants (*Aloe* and *Bulbine*) it is possible that something could have happened between the harvesting and the testing. If possible, the extraction of the plants should be done in a method similar to what the medicine men do. The activity of the extract obtained using the traditional method should be compared with the activity of the extracts obtained using organic solvents.

More samples of *A. kilifiensis* stems need to be obtained and asphodelin isolated. The stereochemistry of the asphodelin should be thoroughly studied and compared with that of *A. lateritia*. Stereochemistry of abyquinone C was not completely determined due to lack of enough samples. More of the fruits of *Bulbine abyssinica* are needed so as to obtain more of abyquinone C for stereochemical studies.

The sample size in all cases needs to be increased so that the minor compounds can also be isolated and studied. A study should be carried out for the variation of chemical composition with age.

In this study, an attempt was made to isolate compounds from flesh leaves of *Aloe graminicola*. Column chromatography was conducted in the dark and at a reduced temperature. The compounds obtained decomposed before being analyzed. To prevent such decomposition, methods need be developed such that fresh samples can be extracted, chromatographed and analyzed without any exposure to the environment.

Bulbine abyssinica grows in the wild in Kenya. The areas from which the samples in this study were obtained are arable and have so far been cultivated for food crops. The plant is considered a weed and could soon get extinct. To preserve the plant, it would be important to have a plantation of the plant in its original geographical location and also in a botanical garden elsewhere. This should apply to *Aloe* species as well.

It would be interesting to separate the enantiomers in 4'-demethylknipholone (compound 24) and compare the biological activity of each compound with that of the mixture. The separation could be achieved by the use of a chiral column. It would be easier to introduce a sugar molecule (glycosilate) and the enantiomers end up having a chiral center each, hence becoming diastereomers. The diastereomers can then be separated and each hydrolysed resulting in separate atropisomers.

The antiplasmodial activity of knipholone (17) requires thorough investigation. The axial-chirality of the molecule being investigated should be known well in advance. The bioassays should include antiplasmodial activity against all the strains of *Plasmodium falciparum* mentioned in section 3.3.2.

CHAPTER FOUR

EXPERIMENTAL

4.1 General

Melting points were determined on a Kofler hot-stage microscope and are uncorrected. infra-red (IR) spectra were taken on a Jasco Fourier transform/infra-red (FT/IR-410) spectrometer. Ultraviolet (UV) spectra were recorded on a Varian Cary 50 probe spectrophotometer. Analytical thin layer chromatography (TLC): Merck pre-coated silica gel 60 F₂₅₄ plates. Column chromatography (CC) on oxalic acid impregnated silica gel 60 (70-230 mesh). CC on Sephadex-LH 20 gel. PTLC: Merck 60 F₂₅₄ silica gel, (25 g silica gel per 20 X 20 cm glass plate). Solvents: laboratory grade, further purified by distillation. Electron impact mass spectroscopy (EIMS): direct inlet, 70 eV on a SSQ 710, Finnigan MAT spectrometer. ¹H NMR (300 MHz) and ¹³C NMR (75 MHz) on Bruker spectrometer using TMS as internal standard. ¹H NMR (200 MHz Varian). HMQC and HMBC spectra were acquired using the standard Bruker software. CD spectra were recorded on a Jasco J-715 spectropolarimeter and optical rotations on a Jasco P-1020 polarimeter (using a sodium D line and a quartz cuvette with 1 cm path length and 1 cm internal diameter).

4.2 Plant material

Plant materials were collected from different regions of the country (Table 4.1). The plants were identified at the University of Nairobi Herbarium where voucher specimen samples were deposited.

Table 4.1: Plant collection

Plant	Part	Date of collection	Source area	Voucher specimen
<i>Aloe kilifiensis</i>	Whole	29/8/1996	Veterinary Hospital of Kilifi District, Coast Province in the coral reefs of Indian Ocean	SMJMW001
<i>Aloe lateritia</i> var. <i>lateritia</i>	Whole	30/8/1996	Bushes on the roadside of Mombasa-Nairobi road (about 60 Km away from Mombasa)	SMJMW002
<i>Aloe graminicola</i>	Whole	15/5/1999	Roadside of the Naivasha/Gilgil road (about 60 km from Nairobi)	SMJMW003
<i>Bulbine abyssinica</i>	Fruits	August 1994	Near Thika town (towards Gacharage) along the Nairobi-Thika highway	SGM-AYT-1994-06
	Roots	September 2001	Near Thika town (towards Gacharage) along the Nairobi-Thika highway	SMJMW004
<i>Bulbine frutescens</i>	Roots	November 2001	Flower gardens of Chiromo Campus, University of Nairobi	SMJM005
<i>Bulbine frutescens</i>	Roots	January 2004	Flower gardens of Chiromo Campus, University of Nairobi	-

4.3 Extraction and isolation

4.3.1 *Aloe lateritia* var. *lateritia*

The subterranean stems (red in colour) were air dried, ground into powder (995 g) and extracted by percolation with acetone (2 X 24 hours). The acetone concentrate (16 g) was subjected to chromatographic separation on silica gel, eluting with petroleum ether (60 – 80) containing increasing amounts of ethylacetate. 60 eluents (about 250 ml each) were collected and combined into 26 fractions (A to Z).

Fraction B was purified by eluting over a Sephadex LH 20 column using 25% ethylacetate in petroleum ether as eluent. This yielded 25 mg orange crystals (R_f 0.52 on petroleum ether: chloroform 1:1) of compound 1. Fraction G was eluted via Sephadex LH 20 using 1:1 petroleum ether:chloroform and two bands separated. The concentrate from the first band was subjected to preparative thin layer

chromatography using chloroform as the eluent. 30 mg of a red amorphous solid (compound **3b**), R_f 0.42 (1:1 petroleum ether:chloroform). Concentrate of the second band was re-crystallized on petroleum ether/chloroform producing 9 mg of yellowish orange crystals (compound **6**), R_f 0.8 on 30% ethylacetate/chloroform. Fraction I was eluted via Sephadex using acetone: methanol 1:1. The major band was purified further by PTLC using petroleum ether:ethylacetate 4:1. 4 mg of a light yellow amorphous powder (compound **12**), R_f 0.3 (Petroleum ether: ethylacetate 3:1) was obtained. Fraction L was purified by CC over Sephadex eluting with ethylacetate. 6 mg orange crystals of compound **7** (R_f 0.82 on CHCl_3 :EtOAc 1:1) were obtained. Fraction Q was also eluted over Sephadex (LH 20) with EtOAc as solvent. This yielded 5 mg light yellow amorphous powder of compound **9** (R_f 0.73, CHCl_3 :EtOAc 1:1). Fraction R was similarly treated yielding 17 mg yellow powder of compound **8** (R_f 0.6 on Petroleum ether: ethylacetate 2:3). Fraction T was recrystallized on ethylacetate/petroleum ether. 10 mg of light yellow needles (compound **7**), R_f 0.4 on petroleum ether:ethylacetate 1:1, were obtained. Fraction W was left to stand in the eluting solvent (30% ethylacetate/ petroleum ether) for several hours. 6 mg of near colourless needles (compound **10**), R_f 0.6 (10% methanol in dichloromethane) were obtained. On 10% methanol in dichloromethane, the compound (**6**) had an R_f of 0.5. Fraction Y was purified by elution over Sephadex using acetone as eluent. The yellow solid obtained was re-crystallized on chloroform/methanol yielding yellow crystals (R_f 0.3 on 10% methanol in dichloromethane) of compound **4** (16 mg).

4.3.2 *Aloe kilifiensis*

The fine dry powder of subterranean stems (701 g) was extracted by percolation with hexane, dichloromethane and methanol successively. The extraction was repeated

three times per solvent, each extraction taking 24 hours. The methanol extract was partitioned on ethylacetate/water. The extractions yielded 7.53 g of hexane extract, 11.74 g dichloromethane extract and 16.27 g of ethylacetate partition.

The dichloromethane extract and the ethylacetate partition of the methanol extract were combined on basis of TLC comparison. 25.96 grams of the mixture were chromatographed on silica gel (deactivated using 3% oxalic acid). The column was washed on a polarity gradient of n-hexane in dichloromethane through ethylacetate and washed with methanol. The 68 eluents collected were combined into 16 fractions (A to O) on the basis of TLC similarity.

550 mg of Fraction E was dissolved in dichloromethane : methanol 1:1 and the soluble portion eluted over Sephadex LH 20, yielding E1 to E6. E3 (80 mg) was developed on PTLC using CH_2Cl_2 . This yielded 26 mg of compound 3 (yellow spot, R_f 0.4 on 20% ethylacetate in hexane).

240 mg of fraction G (eluent of 90% dichloromethane in hexane) was dissolved in ethylacetate (hot) and developed on PTLC plates using hexane:ethylacetate:acetic acid 14:5:1. Four fractions G1 to G4 were collected. G2 and G3 were each developed in PTLC plates using Hexane: dichloromethane 8:2. The major portion of the combined eluents was further purified by PTLC (20% dichloromethane in hexane) and cc on silica gel (20 g SiO_2 , 50% ethyl acetate in hexane). 10 mg of compound 7 (R_f 0.5 on 50% EtOAc in hexane) were obtained. G4 was developed on PTLC using 50% EtOAc in hexane. This yielded 6.6 mg of compound 9 (R_f = 0.4 50% ethylacetate/hexane) and 10 mg of compound 1 (R_f 0.6 50% ethylacetate/hexane).

Fraction H (eluted using 100% CH_2Cl_2) formed a precipitate that was dissolved in dichloromethane and eluted via a silica gel (20 g, deactivated) column. The product

was re-crystallized on 50% dichloromethane in hexane giving 10 mg of compound **1** (R_f 0.7 on hexane:dichloromethane 2:8). The mother liquor of fraction H (186 mg) was dissolved in CH_2Cl_2 :MeOH 1:1 and eluted via a Sephadex (LH 20) column. 20 portions were collected and combined into 6 fractions H1 to H6. 40 mg of H5 was column chromatographed via silica gel and the major fraction developed on PTLC (15% ethylacetate in dichloromethane). This yielded 10 mg of compound **6** (R_f 0.5 CH_2Cl_2 :EtOAc 9:1). Fraction J was developed on PTLC using CH_2Cl_2 :EtOAc 1:1. on ethylacetate/hexane yielding 6 mg of compound **11** (R_f 0.6, CH_2Cl_2 :EtOAc 1:1) and 9 mg of compound **10** (R_f 0.3 CH_2Cl_2 :EtOAc 1:). Fraction M was recrystallized on dichloromethane/methanol and 4 mg of compound **4** (R_f 0.4, 3% methanol in dichloromethane).

4.3.3 *Aloe graminicola*

The subtarennean stems were sun dried and ground. The powder (500 g) extracted by cold percolation with tetrahydrofuran (2 X 24 hours) yielding 20 g of concentrate. The concentrate was packed on a silica gel column and eluted starting with hexane followed by hexane/EtOAc mixtures of different ratios (increased ethyl acetate concentrations). Twelve fractions (A to L) were collected. All the fractions were analysed by comparison of their TLCs with authentic samples. Fraction B contained compound **1**; Fraction E compound **2** (R_f 0.7, EtOAc: CH_2Cl_2 1:9); fraction F compound **3b** (R_f 0.3, EtOAc: CH_2Cl_2 1:9); fraction H compound **7**; fraction J compound **4**. Fraction J (77 mg) was eluted via Sephadex (LH 20) using CH_2Cl_2 :MeOH 1:1 and the major band (yellow) developed on PTLC (4% MeOH in dichloromethane). This yielded 5 mg of compound **4** (R_f 0.6 15% methanol in dichloromethane).

dichloromethane) and 14 mg of compound 5 (R_f 0.3 15% methanol in dichloromethane).

4.3.4 *Bulbine abyssinica* (fruits)

The dried and ground powder (150 g) of the fruits of *Bulbine abyssinica* was extracted with ethyl acetate by cold percolation. The crude extract (3.5 g) was chromatographed on oxalic acid impregnated silica gel (100 g), eluting with increasing polarities of mixtures of *n*-hexane / dichloromethane and then ethyl acetate / dichloromethane. A total of 23 fractions, each ca. 200 ml, were collected and combined into seven major fractions (A to G).

The methanol soluble portion of fraction C (eluted with 20% dichloromethane in *n*-hexane) was crystallized from dichloromethane / *n*-hexane to give compound 16 (3 mg) as red crystals (R_f 0.5 on 5% ethyl acetate in *n*-hexane). PTLC (5% ethyl acetate in *n*-hexane) separation of fraction D (eluted with 30% dichloromethane in *n*-hexane), gave further amounts of compound 16 (4 mg) and compound 1 (16 mg) as a yellow crystalline compound (R_f 0.4 on 5% ethyl acetate in *n*-hexane). Fraction E (eluted with 40% dichloromethane in *n*-hexane) was crystallized from dichloromethane / *n*-hexane to give compound 13 (55 mg) as dark red amorphous powder (R_f 0.7, dichloromethane). Fraction F (eluted with 50% dichloromethane in *n*-hexane) showed two spots on TLC (10% ethyl acetate in dichloromethane). PTLC separation (oxalic acid impregnated silica gel, solvent dichloromethane) and subsequent crystallization from dichloromethane / *n*-hexane yielded compound 14 (43 mg) as a yellowish brown

powder. Fraction G (eluted with 5% ethyl acetate in dichloromethane) was purified by CC on Sephadex LH 20 (CH_2Cl_2 :MeOH; 1:1) to give compound 15 (33 mg).

4.3.5 *Bulbine abyssinica* (roots)

125 g of the dry powder was extracted by cold percolation in acetone (three times: 1 Hr, 12 hrs, 12 hrs). 10 g of the combined extract was chromatographed on Sephadex gel using CH_2Cl_2 : MeOH 1:1 giving 62 portions that were combined into 15 fractions A to O. Fraction O was eluted via Sephadex and three portions collected. The main portion was dissolved in a little dichloromethane (very soluble). On addition of hexane to the dark red solution, a brown precipitate formed immediately. ^1H NMR of the precipitate suggested that the compound could be a dimer of compound 17. The quantity was too low for further analysis. Fraction M was dissolved in dichloromethane and hexane added. After some hours, two solids formed together (brown and red). The solids were separated physically after filtration and drying. The red compound was pure (R_f 0.2 on 100% CH_2Cl_2). The mother liquor of M was dissolved in dichloromethane and hexane added. The precipitate that formed was filtered and the residues run over Sephadex (50% methanol in dichloromethane). This yielded more of the red solid (R_f 0.2 on 100% dichloromethane). The solid was recrystallized on dichloromethane/methanol and 17 mg of red needles (compound 17) were obtained. Fractions G and H were combined and recrystallized on dichloromethane/hexane. Four days later, dark red needles had formed together with a light red precipitate. The crystals and the precipitate showed similar TLC pattern (yellow spot R_f 0.4 on 20% acetone in hexane). This was identified as 6'-methoxyknipholone (19). Fraction J was purified by eluting via Sephadex using 50% methanol in dichloromethane. Red crystals of compound 20 (R_f 0.4 on 50%

ethylacetate in hexane) were obtained. Fraction K was passed through Sephadex and 8 portions collected. Fraction L was also passed over Sephadex (CH_2Cl_2 :MeOH 1:1) and the major compound obtained was knipholone (17). A compound having yellow fluorescence (R_f 0.5 on 3% methanol in dichloromethane) on (UV_{254}) and colourless on visible light was detected in J, K, and L. The Sephadex eluents of these fractions with this compound were combined and eluted over a silica gel column. Of the four fractions collected, one was almost pure (R_f 0.3 on 100% dichloromethane). This was purified further by PTLC (100% CH_2Cl_2) and sharp needles of compound 18 were obtained. Fraction O was passed through Sephadex (CH_2Cl_2 :MeOH 1:1) and the major eluent dissolved in a little dichloromethane. Hexane was added and a brown precipitate formed immediately. The precipitate was once more eluted over Sephadex (CH_2Cl_2 :MeOH 1:1) and of the 15 eluents collected, the third one was developed on PTLC (2% MeOH in DCM). The product was further purified by PTLC using CHCl_3 :EE 4:1. The major band resulted in 7 mg of compound 27.

4.3.6 *Bulbine frutescens* (stems and roots)

500 g powder of *Bulbine frutescens* roots were extracted using 70% dichloromethane in hexane. 400 g powder of the stems was also extracted similarly. The two extracts had similar TLC patterns and hence were combined. 32 g of the combined extract were column chromatographed via de-activated silica gel eluting with hexane followed by mixtures of hexane and acetone. 46 eluents were obtained and combined into 15 fractions (A to P). Fraction I was run through Sephadex and the major band developed on PTLC (15% ethylacetate in hexane). The product was re-developed on PTLC and compound 18 (R_f 0.3 on 20% ethylacetate in hexane) was obtained upon re-crystallization. The mother liquor of fraction I was combined with fractions J, K

and a Sephadex eluent of fraction L. The combined fractions (5.2 g) were packed on a de-activated silica gel column and 30 eluents obtained. These were combined into five fractions of which the one with R_f 0.2 (yellow spot) on 30% ethylacetate/hexane was identified as knipholone (17). Combined fractions M and N (2 g) were subjected to column chromatography on de-activated silica gel. 17 portions were collected and combined into 12 parts, M1 to M12. M4 and M5 were pure and identified as isoknipholone (18). M11 was cleaned by passing it over a silica gel column and eluting with 20% ethylacetate in hexane. 3 mg of a red compound (R_f 0.5 50% EtOAc in hexane) was obtained. ^1H NMR (200 MHz) of this compound suggested a dimer of knipholone and 6'-*O*-methylknipholone but the sample was too little for generating enough spectra to have the compound fully identified.

4.3.7 *Bulbine frutescens* (roots)

200 g of powdered roots of *B. frutescens* were extracted with acetone (2 X 24 hours X 1 litre) by cold percolation. 15 grams of crude extract were obtained. The extract was chromatographed over Sephadex (LH 20) using CHCl_3 :MeOH 1:1. Three fractions (I, II and III) were obtained. Fraction II (3.5 grams) was column chromatographed over 100 grams of silica gel, using a chloroform/methanol polarity gradient. The 25 eluents obtained were combined into 13 fractions (A to M). Fraction K (173 mg) was subjected to HPLC using the conditions: solvent-MeOH:H₂O 7:3; flow rate-5 ml per minute; detector-240 nm. Two bands were collected as follows: K1 (R_f 19.63 – 24.12), K2 (R_f 34.82 – 43.21). MS, ^1H NMR and other spectroscopic analyses of K2 showed that the compound was 4'-*O*-demethylknipholone-4'-*O*- β -D-glucoside (25). Fraction J was eluted via Sephadex (LH 20) using the solvent CHCl_3 :MeOH 1:3. Nine eluents were collected and the fourth one J4 (the only UV active one) further purified

by PTLC (CHCl_3 :EtOAc 1:1). This yielded 4-hydroxy-3-methoxybenzoic acid (vannilic acid **29**). Fraction I was passed over Sephadex (LH 20) using the solvent mixture chloroform:methanol 1:3 and eight eluents (I1 to I8) collected. I4 (the only one with a UV active spot) was further purified by PTLC (CHCl_3 :EtOAc 1:1) and a mixture of (E)-3-(4-hydroxyphenyl)-acrylic acid (**30**) and 3-(4-hydroxyphenyl)-propionic acid (**31**) obtained. Half of fraction G was fractionated on Sephadex (LH 20) eluting with methanol. This resulted into 27 eluents that were combined into 12 portions (G1 to G12). G2 was further analysed and found to be knipholone (**17**). G5 was found to be 4-O-demethylknipholone (**24**). The other half of fraction G was also applied on Sephadex and eluted with a mixture of chloroform:methanol 1:1. Five eluents (G13 to G17) were obtained. G7 to G10 were combined and eluted with a mixture of chloroform:methanol 1:1 over Sephadex (LH 20) resulting in four portions (G7i to G7iv). G7iii and G7iv were combined and run over flash (N_2) silica gel eluting with a mixture of dichloromethane:methanol 97:3. The product was further purified by PTLC (CHCl_3 :MeOH 15:1). More of compound **24** was obtained in the process. G7i and G13 were combined and labeled fraction N. The combined fraction was run over Sephadex LH 20 (CHCl_3 :MeOH 1:1) and the major eluent filtered over CC SiO_2 gel (2% MeOH in DCM) resulting in six portions that were combined into two fractions N1 and N2. N1 was developed on PTLC (CHCl_3 :MeOH 20:1) yielding fractions N3 to N6. N3 and N4 inter-converted in acetone. N3 and N4 were combined developed on PTLC (CHCl_3 :MeOH 20:1) yielding N10 and N11. N11 was pure and further analysis showed it to be compound **26**. N5 and N6 also inter-converted in acetone. The two were combined and developed on PTLC (CHCl_3 :EE 4:1) yielding N7, N8 and N9. N8 was pure and spectroscopic analysis showed it to be compound **27** (10 mg). N9 was pure and further analysis showed it to be compound

28. Fraction B was eluted with a mixture of chloroform:methanol 1:1 over Sephadex (LH 20) giving rise to B1-B4. The eluents were dried and stored for future analysis. Fraction E was purified by PTLC using the solvent mixture dichloromethane:hexane 2:3. Three bands were collected (E1 to E3). The process was repeated for fraction C yielding bands C1 to C4. C3 and E3 were similar and hence combined. Spectroscopic analysis showed the compound to be 5,8-dihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**21**). Fraction D formed a deep red precipitate that was filtered and the mother liquor eluted with a mixture of chloroform:methanol 1:1 over Sephadex (LH 20). D1 to D5 were obtained in the process. D1 was purified by PTLC using chloroform and the compound elutherol-9-methyl ether (**23**) obtained. D5 was also purified by PTLC (CHCl₃:EtOAc 9:11). This yielded the compound 1,5,8-trihydroxy-1-methylnaphtho[2,3-c]furan-4,9-dione (**22**).

4.4 Aerial oxidation

Abyquinone C (**15**) (10 mg) was dissolved in 5% methanolic KOH and the solution was stirred for two hours. The solution was acidified and extracted with dichloromethane. Preparative TLC purification of the dichloromethane layer gave compound **13** (trace), **14** (5 mg) and unreacted **15** (3 mg). In a separate experiment compound **15** (5 mg) was completely converted to **13** (3 mg) when the methanolic solution was stirred for three days. Compound **14** was also converted to **13** in a similar experiment. The reactions were monitored by TLC and the identity of the products was confirmed by ¹H NMR analysis.

Compound **9** (5 mg) was dissolved in 5% methanolic potassium hydroxide and the solution allowed to stand for 12 hours. The solution was neutralized with dilute sulphuric acid and extracted with dichloromethane. The organic layer was co-spotted on TLC with chrysophanol and found to be the same.

4.5 Computational

The conformational analyses were performed on a Linux AMD MP 2400+ workstation by means of the semi-empirical AM1 method as implemented in the program package Gaussian 98, starting from pre-optimised geometries generated by the TRIPOS force field as implemented in the molecular modeling package SYBYL 6.9. The wave functions required for the computation of the rotational strengths for the electronic transitions from the ground state to excited states were obtained by CNDO/S calculations followed by SCI computations including 625 singly occupied configurations and the ground state determinant, and by OM2 calculations followed by SCI and SDCI calculations including 900 singly and 400 doubly occupied configurations, respectively, and the ground state determinant. These computations were also carried out with a Linux AMD MP 2400+ workstation using the BDZDO/MCDSPD program package, and by the use of the MNDO99 software package. The single CD spectra were summed up and weighted following the Boltzmann statistics, i.e., according to the respective heats of formation. The rotational strengths were transformed into $\Delta\epsilon$ values and for a better visualization superimposed with a Gaussian band shape function.

4.6 Physical and spectroscopic data

Compound 1

Melting point 195-197° C; ^1H NMR: 12.08 (OH-1), 7.07 (br s, H-2), 7.62 (d, $J = 1.2$, H-4), 7.79 (dd, $J = 7.5, 1.2$, H-5), 7.66 (t, $J = 8.1$, H-6), 7.27 (dd, $J = 7.2, 1.0$, H-7), 11.98 (OH-8), 2.45 (Me-3); **EIMS** 254.2 (100); 226.2 (14.2); 198.3 (10.5); 197.3 (13.8); 152.3 (17.2); 151.2 (12.0); 141.3 (10.3); 139.2 (10.4); 127.2 (15.93); 115.3 (19.5); 77.2 (14.81); 76.2 (16.9); 75.3 (13.4); 63.2 (17.0); 51.2 (10.2); 32.2 (33.86).

Compound 2

(R_f 0.7, EtOAc:CH₂Cl₂ 1:9). The compound was identified from a CC fraction of *Aloe graminicola* by TLC comparison with authentic sample (Yenesew, 1991).

Compound 3

Red prisms, **melting point** 161-163° C, ^1H NMR, (500 MHz) 12.50, 12.38, 12.01, 11.99, 7.95 (d, $J = 7.8$), 7.70 (d, $J = 1.0$), 7.59 (t, $J = 7.8$), 7.56 (d, $J = 7.2$), 7.39 (d, $J = 7.7$), 7.28 (d, $J = 7.4$), 7.26 (d, $J = 2.6$), 7.10 (d, $J = 2.0$), 2.48, 2.13; ^{13}C NMR 192.85, 192.85, 181.90, 162.89, 162.76, 162.05, 159.64, 149.40, 149.18, 137.24, 137.15, 135.95, 134.00, 132.66, 125.54, 124.30, 124.14, 121.36, 120.35, 120.01, 115.72, 114.80, 22.30, 21.41. **X-ray crystallographic data** (appendix 3). **CD data** (appendix) 3 **EIMS** 32.3 (17), 39.3 (8), 43.3 (7), 65.3 (9), 77.3 (20), 89.3 (7), 91.3 (34), 92.3 (12), 93.3 (12), 105.3 (9), 106.3 (11), 107.3 (10), 113.2 (10), 115.3 (12), 119.3 (13), 120.2 (9), 121.3 (36), 125.3 (8), 130.9 (7), 132.0 (8), 135.2 (17), 138.2 (15), 139.2 (13), 143.6 (9), 144.9 (12), 149.2 (6), 150.3 (14), 151.2 (13), 152.2 (10), 153.1 (7), 163.3 (8), 165.1 (14), 166.6 (7), 176.3 (10), 179.4 (7), 187.4 (9), 189.2 (9),

193.3 (7), 202.3 (14), 213.3 (9), 225.9 (9), 253.4 (36), 358.5 (8), 461.1 (12), 488.2 (13), 490.2 (26), 491.2 (10), 506.1 (100), 507.2 (25),

Compound 4

EIMS m/z 639 ($M^+ + H$, $C_{36}H_{30}O_{11}$), 491 ($M^+ - C_6H_{12}O_4$) **1H NMR** (300 MHz) 12.48, 12.24, 11.94, 11.83, 7.70, (t, $J = 8.0$), 7.55, (d, $J = 7.4$), 7.33, 7.28, (d, $J = 8.0$), 7.27, (d, $J = 7.3$), 7.22, (d, $J = 8.0$), 6.94, 6.72, 4.64, 4.00, (t, $J = 7.9$), 3.98, (t, $J = 7.9$), 3.96, (d, $J = 7.6$), 3.44, 3.36, (m), 2.41, 2.18, 1.03, (d, $J = 6.7$), **^{13}C NMR** (125 MHz), 195.4, 194.0, 182.9, 163.3, 162.9, 162.7, 160.4, 151.4, 148.8, 147.7, 142.3, 138.2, 135.5, 135.3, 132.8, 132.2, 128.7, 125.5, 124.4, 121.3, 120.5, 120.4, 118.3, 116.5, 116.4, 116.3, 115.6, 79.8, 76.0, 74.6, 74.0, 66.5, 46.2, 22.1, 21.5, 17.9;

Compound 5

Melting point 149-153° C ($CDCl_3$), **EIMS** m/z 638 (M^+ , $C_{36}H_{30}O_{11}$), 491 ($M^+ - C_6H_{12}O_4$); **1H NMR** (500 MHz) 7.59 (dd, $J = 8.3, 8.1$), 7.46 (dd, $J = 8.2, 0.9$), 7.30 (dd, $J = 8.2, 0.9$), 7.29 (bs), 7.13 (d, $J = 7.6$), 7.08 (d, $J = 7.6$), 6.82 (bs), 6.75 (bs), 4.50 (d, $J = 2.5$), 4.10 (dd, $J = 7.3, 4.8$), 4.01 (dd, $J = 7.3, 2.4$), 3.91 (t, $J = 4.0$), 3.48 (t, $J = 4.8$), 3.39 (m), 2.41, 2.18, 1.09 (d, $J = 6.7$); **^{13}C NMR** (125 MHz) 162.2, 193.8, 192.7, 162.9, 162.1, 159.8, 150.2, 148.8, 148.0, 148.0, 140.8, 137.2, 134.3, 134.0, 131.9, 131.6, 130.9, 128.3, 125.7, 124.3, 120.8, 119.9, 119.7, 116.5, 115.5, 115.3, 79.5, 73.9, 73.8, 73.0, 66.9, 46.4, 21.7, 21.5, 17.0.

Compound 6

Melting point 264-265° C ; ^1H NMR (300 MHz) 13.07 (OH), 7.76 (*dd*, $J = 6.3, 3.2$), 7.72 (*t*, $J = 6.3$), 7.61 (*d*, $J = 7.65$), 7.31 (*dd*, $J = 6.3, 3.3$), 7.13 (*d*, $J = 2.41$), 2.79; ^1H NMR (500 MHz) δ 13.07 (OH), 7.72 (*dd*, $J = 7.5, 2.9$ Hz), 7.73 (*t*, $J = 7.5$), 7.30 (*dd*, $J = 7.5, 2.9$ Hz), 7.61, (*d*, 2.5 Hz), 7.12 (*s*), 2.79 (*s*); ^{13}C NMR (125 MHz) δ 190.97, 183.19, 163.32, 146.62, 125.49, 113.16, 119.13, 136.69 and 125.07, 117.76, 134.04, 138.47, 124.15; **H-H COSY** (500 MHz): δ 7.7X7.3, 7.6X7.1; **C-H COSY** 7.1187X125.488, 7.605X113.166, 7.715X119.138, 7.730X136.700, .301X125.08. **Dept 90**: δ 136.700, 125.448, 119.138, 113.166. **Dept 135**: δ 136.701, 125.482, 125.078, 119.13, 113.151, +24.142; **EIMS** 255.3 (15), 254.2 (100), 237.3 (7), 236.3 (53), 197.3 (13), 169.3 (8), 152.3 (12), 151.3 (8), 141 (8), 139.3 (11), 115.3 (22), 76.2 (11), 63.2 (15), 32.2 (63).

Compound 7

Melting point 190-192° C; ^1H NMR (300 MHz) δ 12.89, δ 7.72 (*dd*, 1H, $J = 7.5, 3.2$ Hz), δ 7.593 (*t*, 1H, $J = 7.8$ Hz), δ 7.30 (*dd*, 1H, $J = 6.9, 3.0$), δ 7.13 (*s*, 1H), δ 4.05, δ 2.91 (3H) ppm; **EIMS** 312.3 (5), 281.3 (2), 280.3 (7), 252.3 (1), 224.3 (2), 196.3 (1), 192.2 (2), 168.3 (2), 150.2 (1), 149.2 (3), 140.3 (2), 139.2 (4), 126.3 (2), 115.3 (1), 113.3 (1), 111.3 (1), 105.3 (1), 99.3 (1), 98.3 (1), 97.3 (1), 93.3 (1), 89.3 (1), 85.3 (2), 84.3 (2), 83.3 (2), 81.3 (1), 77.2 (2), 76.2 (1), 75.3 (2), 73.2 (1), 71.2 (2), 70.2 (2), 69.3 (3), 67.2 (1), 63.2 (1), 60.2 (1), 57.3 (3), 56.3 (1), 55.2 (3), 51.2 (1), 45.2 (1), 44.2 (5), 43.2 (4), 41.2 (2), 40.2 (7), 39.2 (1), 32.2 (100), 31.3 (7), 30.2 (1).

Compound 8

^1H NMR (300 MHz) δ 13.12 (1H) 10.07 (b, 1H), 7.67 (bs, 1H), 6.62 (*d*, $J = 2.4$ Hz), 7.14 (*d*, $J = 2.4$ Hz), 3.94 (*s*, 3H), 2.69 (*s*, 3H); ^{13}C NMR (75 MHz) δ 168.78, 189.96, 183.27, 166.92, 165.55, 159.73, 143.21, 138.67, 136.13, 131.44, 125.11, 113.75, 112.49, 110.02, 108.64; EIMS 328.3 (25), 296.3 (35), 268.2 (9), 240.2 (13), 212.2 (7), 181.3 (11), 143.3 (15), 115.3 (24), 113.3 (25), 85.3 (25), 83.3 (29), 69.2 (44), 55.2 (44), 32.2 (100).

Compound 9

Light yellow crystals, melting point 200-203° C. ^1H NMR (300 MHz, Acetone D_6) δ 16.13 (*s*, OH), 9.69 (b, OH), 7.51 (t, $J = 8.0$), 7.22 (*d*, $J = 8.0$), 7.11 (*s*, 1H), 6.79 (*d*, $J = 8.0$ Hz), 4.08 (*s*, OH), 3.14 ($J = 15.9$), 2.98 ($J = 17.4$), 2.91 ($J = 17.4$), 1.44, 3.18 ($J = 15.9$),. EIMS 258.3 (M^+ , 66), 240.3 (48), 216.3 (11), 215.3 (6), 201.3 (16), 200.3 (27), 198.2 (9), 197.3 (11), 173.3 (7), 165.2 (3), 141.3 (5), 116.3 (9), 115.3 (27), 77.2 (4), 63.2 (4), 43.2 (15), 32.2 (100).

Compound 10

Colourless needles, melting point >235° C. ^1H NMR (300 MHz) δ 15.37 (*s*, 1H), 6.98 (*s*), 6.90 (*s*), 4.40 (t, $J_{\text{vic}} = 6.6$ Hz), 3.90 (*s*, 3H), 2.99 (*d*, $J = 17.7$ Hz), 2.99 (*d*, $J = 13.5$ Hz), 2.98 (*d*, $J = 13.8$ Hz), 2.93 (*d*, $J = 17.7$ Hz), 2.80 (*s*, 3H). EIMS 316.3 (27), 298.3 (5), 285.3 (18), 284.3 (83), 266.3 (10), 256.3 (17), 228.3 (14), 210.3 (15), 184.3 (6), 181.3 (10), 152.3 (9), 128.3 (19), 127.2 (12), 119.3 (12), 115.3 (10), 106.4 (13), 76.2 (11), 32.2 (100)

Compound 11

Colourless crystals, **melting point** 205° - 207° C; $^1\text{H NMR}$ δ 15.150, 9.16 (b, OH), 6.819, 6.864, 4.368 (t, 2H, $J = 3.6$), 3.18 ($J_{\text{gem}} = 13.71$ Hz), 3.014, ($J_{\text{gem}} = 12.81$ Hz), 2.74 ($J_{\text{gem}} = 17.16$), 2.71 ($J_{\text{gem}} = 17.10$ Hz); $^{13}\text{C NMR}$ (75 MHz) 204.60, 66.98, 167.99, 167.68, 160.33, 49.21, 39.71, 109.13, 118.37, 121.09, 137.65, 142.81, 143.63, 111.61, 25.57; **EIMS** 259.3 (18), 258.3 (100), 240.3 (34), 239.3 (16), 225.2 (20), 215.3 (8), 214.2 (22), 211.3 (8), 197.3 (8), 186.3 (5), 185.3 (7), 165.3 (6), 158.2 (9), 157.3 (12), 139.2 (7), 129.3 (16), 128.3 (23), 127.2 (13), 115.3 (24), 91.3 (7), 77.2 (12), 63.2 (8), 43.2 (15), 32.2 (37).

Compound 12

$^1\text{H NMR}$ 300 MHz, CDCl_3) δ 9.34 (bs), 8.20 (bs), 7.53 (d, $J = 8.01$ Hz), 7.47 (dd, $J = 7.38, 8.16$ Hz), 7.07 (d, $J = 7.29$ Hz), 5.86 ($J = 6.3, 6.48, 6.45$ Hz), 4.12, 1.75 (d, $J = 6.51$ Hz); $^{13}\text{C NMR}$ (75 MHz) 169.44, 154.2, 150.4, 136.98, 131.2, 128.72, 123.49, 123.49, 121.39, 119.54, 113.61, 61.81, 61.8, 19.74; **EIMS** 245.3 ($\text{M}^+ + 1$, 15), 244.2 (M^+ , $\text{C}_{14}\text{H}_{12}\text{O}_4$, 100), 230.2 (12), 229.2 (81), 214.2 (10), 202.2 (7), 201.3 (51), 187.2 (12), 186.2 (12), 173.3 (23), 159.2 (14), 158.2 (26), 129.3 (7), 128.3 (9), 127.3 (12), 120.3 (10), 115.2 (33), 114.3 (16), 113.3 (13), 102.3 (28), 101.1 (28), 85.2 (9), 79.2 (7), 77.3 (11), 76.3 (7), 75.2 (11), 74.2 (7), 63.2 (11), 51.2 (8), 43.2 (14), 32.2 (29),

Compound 13

Dark red amorphous powder; mp 161 °C (CH_2Cl_2); $\alpha_D^{20} = -248^\circ$ (c 0.02, CH_2Cl_2 , mean value; the measured α_D values varied from -200 to -300 due to the long-wavelength UV absorbance; IR (NaCl): 3452, 1667, 1618, 1427, 1381, 1269, 1132,

1073, 1025, 1004, 965, 885, 754 cm^{-1} ; UV/Vis (MeOH): λ_{max} ($\log \epsilon$) = 232 (3.83), 256 (3.60), 303 (3.00), 438 (3.26), 471 (3.20), 500 (3.01), 542 (3.02), 582 (2.60) nm; CD (CH_2Cl_2): $\Delta\epsilon_{229}$ -12.4, $\Delta\epsilon_{276}$ 10.5, $\Delta\epsilon_{301}$ -2.5; ^1H NMR (Table 1); ^{13}C NMR (Table 1); EIMS m/z (rel. int.): 506 (M^+ , 100), 489 (61), 461 (7), 281 (22), 260 (12), 219 (6), 207 (15), 191 (5), 149 (13), 59 (20); HREIMS m/z 506.0993 (M^+ ; calcd for $\text{C}_{30}\text{H}_{18}\text{O}_8$, 506.1002).

Compound 14

Yellowish brown amorphous powder; mp 184 °C (MeOH); $\alpha_D^{20} = -25.2^\circ$ (c 0.02, MeOH); IR (NaCl): 3431, 1662, 1621, 1420, 1379, 1259, 1122, 1069, 1025, 885, 754 cm^{-1} ; UV/Vis (MeOH): λ_{max} ($\log \epsilon$) = 232 (3.92), 256 (3.60), 303 (3.00), 438 (3.18), 467 (2.78) nm; CD (CH_2Cl_2): $\Delta\epsilon_{230}$ 10.1, $\Delta\epsilon_{237}$ 7.0, $\Delta\epsilon_{240}$ 7.7, $\Delta\epsilon_{270}$ -6.7, $\Delta\epsilon_{300}$ 1.6; ^1H NMR (Table 1); ^{13}C NMR (Table 1); EIMS m/z (rel. int.): 508 (M^+ , 20), 489 (8), 281 (24), 254 (25), HREIMS m/z 508.1153 (M^+ ; calcd for $\text{C}_{30}\text{H}_{20}\text{O}_8$, 508.1158).

Compound 15

Yellow amorphous powder; mp 193 °C (MeOH); $\alpha_D^{20} = -63.5^\circ$ (c 0.1, MeOH); IR (NaCl): 3428, 1633, 1620, 1600, 1485, 1284, 1124, 775 cm^{-1} ; UV/Vis (MeOH): λ_{max} ($\log \epsilon$) = 232 (4.09), 276 (3.91), 398 (3.48) nm; CD (MeOH): $\Delta\epsilon_{194}$ 2.8, $\Delta\epsilon_{208}$ -2.4, $\Delta\epsilon_{212}$ -1.5, $\Delta\epsilon_{218}$ -2.6, $\Delta\epsilon_{230}$ 1.8, $\Delta\epsilon_{241}$ -0.1, $\Delta\epsilon_{251}$ 1.2, $\Delta\epsilon_{273}$ -4.8, $\Delta\epsilon_{323}$ 1.1; ^1H NMR (Table 1); ^{13}C NMR (Table 1); EIMS m/z (rel. int.): 512 (M^+ , 3), 494 (6), 493, (7), 474 (16), 254 (100), 240 (67); HREIMS m/z 512.1465 (M^+ ; calcd for $\text{C}_{30}\text{H}_{24}\text{O}_8$, 512.1471).

Compound 16

Glittering red crystals, R_f 0.5 (5% EtOAc/hexane), ^1H NMR (200 MHz)

δ 13.49, 12.33, 12.28, 7.30 (*d*, 8.6 Hz), 7.69 (*t*, 7.8 Hz, 7.8 Hz), δ 7.89 (*d*, 7.7 Hz), 7.16. (*bs*), 2.38 (*s*)

Compound 17

Deep red needles, orange spot R_f 0.2 (30% EtOAc/hexane). . UV absorption (λ_{max} nm) 224.0, 245.5, 254.0, 271.5, 289.0, 368.5 and 430.0) ^1H NMR (200 MHz, CDCl_3), δ 14.14, 12.52, 11.87; 7.52, 7.23, 7.16, 7.14, 6.11 (*s*) 3.91 (3H) 2.62, 2.12 ^1H NMR (300 MHz, acetone D_6) δ 7.55 (*dd*, $J = 8.0, 1.0$ Hz), 7.48 (*t*, $J = 8.0$ Hz), 7.29 (*dd*, $J = 8.0, 1.0$ Hz), 7.316 (*bs*), 6.24, 3.98 (3H), 2.63 (3H) 2.17 (3H); **EIMS** m/z 436 ($\text{M}^+ + 2$, 66%) 434 (M^+ , 3%, $\text{C}_{24}\text{H}_{18}\text{O}_8$, 421 (55), 420 (34), 401 (11), 387 (6), 355 (10), 322 (6), 294 (4), 281 (11), 269 (12), 243 (7), 239 (7), 221 (10), 207 (8), 183 (12), 167 (34), 149 (30), 129 (12), 125 (31), 104 (22), 98 (33), 43 (53), 36 (100),

Compound 18

Red crystals, yellow spot R_f of 0.3 (20% EtOAc/hexane). ^1H NMR (200 MHz, CDCl_3) δ 13.51, 12.58, 11.97, 7.60, 7.57, 7.27, 7.23, 6.30, 2.61, 2.16, 3.31 (3H)

Compound 19

Dark red crystals, yellow spot $R_f = 0.4$ (20% acetone/hexane). ^1H NMR (200 MHz, CDCl_3) δ 13.89, 12.58, 12.06, 7.56, 7.53, 7.17, 7.22 (1H), 6.09 (*bs*, 1H), 3.97 (3H), 2.08 (3H), 3.97, (3H), 3.74, (3H), (3.98, 3H); ^{13}C NMR 204.17, 193.56, 183.15,

163.67, 163.22, 163.06, 162.94, 162.42, 152.17, 120.54, 116.40, 132.02, 115.69, 86.99, 135.35, 109.64, 106.95, 124.24, 137.54, 129.07, 126.00

Compound 20

Reddish brown crystals, R_f 0.4 (50% EtOAc/Hexane); $^1\text{H NMR}$ (200 MHz, CDCl_3) δ 14.25, 12.53, 12.19, 3.94 (3H), 2.66 (3H), 2.11 (3H), 6.91 (s), 6.80 (d, $J = 8.4$), 7.36 (dd, $J = 7.6, 7.8$ Hz), 6.69 (d, $J = 7.4$ Hz), 6.15 (s), 4.03 (d, $J = 22.2$ Hz), 3.81 (d, $J = 24.4$ Hz), $^{13}\text{C NMR}$ δ 203.26, 193.40, 31.36, 159.52, 149.46, 141.76, 159.52, 106.05, 162.30, 104.53, 163.82, 163.16, 117.44, 118.79, 135.94, 115.29, 90.25, 114.54, 142.89, 119.36, 115.12, 55.80, 33.33, 20.98.

Compound 21

EIMS m/z 244 ($\text{M}^{+\bullet}$, 100%, $\text{C}_{13}\text{H}_8\text{O}_5$); 228 (17), 215 (24), 199 (9), 187 (9), 170 (5), 160 (8), 149 (4) UV (λ_{max} 226, 240 sh, 295, 349 and 440 nm); $^1\text{H NMR}$ δ 12.98 (s, OH), 12.82 (s, OH), 8.07 (s), 7.27 (s), 7.26 (s), 2.80 (3H, s).

Compound 22

UV (λ_{max} 226, 240 sh, 295, 349 and 440 nm); 8.15 (s), 7.27 (s), 7.21 (s), 4.93 (2H, s) m/z EIMS.260 ($\text{M}^{+\bullet}$, 100%, $\text{C}_{13}\text{H}_8\text{O}_6$), 245 (10), 242 (38), 231 (12), 214 (44), 187 (14), 186 (21), 170 (3), 167 (30), 158 (5), 149 (53), 57 (19), 43 (11)

Compound 23

Colourless amorphous powder, blue fluorescence in solution. UV ($\lambda_{\max}$ 215, 249, 271, 302, 313, 328 and 357 nm); HRMS m/z 258.0885 ($M^{+\bullet}$, $C_{15}H_{14}O_4$). EIMS m/z 258 (M^+ , 88), 243 (96%, M^+-Me), 228 (7%, M^+-2Me), 215 (100%, $M^+-Me-CO$), 200 (4%, $M^+-2Me-CO$), 199 (8), 187 (31), 171 (21), 127 (22), 115 (25), 43 (32), 1H NMR δ 8.11 (*s*), 5.72, *q*, $J = 6.5$ Hz), 1.71, (*d*, $J = 6.5$ Hz), 6.95 (*d*, $J = 7.5$ Hz), δ 7.41 (*t*, $J = 8.0$ Hz) and δ 7.54 (*d*, $J = 8.0$ Hz), 3.99 (OMe), ^{13}C NMR (δ 169.4, 61.8, 150.4, 154.2, 3.99, 3.87, 62.7, 56.2).

Compound 24

UV ($\lambda_{\max}$ 224, 271, 289, 365 and 428 nm); 1H NMR, 13.14 (*br s* OH), 12.38 (OH), 11.84 (OH), 10.11 (*br s* OH), 8.76 (*br s* OH), 7.61 (*t*, $J = 7.5, 8$), 7.41 (*d*, $J = 7.0$), 7.17 (*br s*), 7.15 (*d*, $J = 8$), 6.07 (*br s*), 2.54 (3H, *s*, Ar-COMe), 2.05 (3H, *s* Ar-Me), ^{13}C NMR δ 204.4, 193.9, 183.6, 163.1, 162.9, 162.4, 162.3, 162.3, 153.0, 137.7, 135.7, 133.0, 129.4, 125.2, 123.9, 119.9, 116.3, 115.6, 107.4, 105.5, 95.2, 32.5, 20.6; EIMS a cluster of peaks at 423, (100) 422 (64) and 421 (30) ($[M+3]^+$, $[M+2]^+$ and $[M+1]^+$, $C_{23}H_{16}O_8$), 407 (38), 406 (31), 405 (25), 389 (12), 361 (9), 334 (7), 321 (17), 306 (3), 295 (16), 281 (5), 269 (34), 242 (14), 214 (18), 211 (33), 190 (19), 167 (27), 164 (33), 153 (40), 149 (19), 112 (33), 104 (46), 98 (53), 82 (98), 66 (89), 46 (67), 43 (88).

Compound 25

Red amorphous powder, EIMS m/z 605 ($M^+ + Na$), UV ($\lambda_{\max}$ 226, 271, 287, 369 and 429 nm); 1H NMR 7.56 (*dd*, $J = 2.5, 8.5$), 7.42 (*t*, $J = 8$), 7.17 (*br s*), 7.16 (*dd*, $J = 7, 8.5$), 6.29 (*d*, $J = 3$), 5.03 (*d*, $J = 7.5$), 3.87 (*m*, $J_{ratios} = 2.0:5.0:2.0:3.0:2.0:5.0:2.0$),

3.66 (m, $J_{ratios} = 8.0:10:8.0:6.5$), 3.51 (m, $J_{ratios} = 4.5:4.5:4.5::3.5:4.5$), 3.42 (2H d, $J = 13.0$), 3.34 (dd, $J = 9.5, 2.0, 2.67$ (s), 2.03 (3H, d, $J = 6.5$), ^{13}C NMR 205.0, 194.4, 183.8, 164.0, 163.6, 162.9, 162.5, 161.8, 153.0, 138.1, 138.1, 136.1, 129.5, 125.7, 124.4, 120.3, 116.8, 116.0, 106.8, 102.3, 102.2, 95.3, 78.6, 78.5, 74.8, 71.2, 62.5, 33.7, 20.9

Compound 26

Red amorphous powder. ^1H NMR spectrum (Table 3.12), ^{13}C NMR (Table 3.13) TOF-EIMS spectrum $[\text{M}+1]^+$ peak at m/z 853.2117 corresponding to the molecular formula of $\text{C}_{48}\text{H}_{37}\text{O}_{15}$. UV (λ_{max} 275, 294, 385, 414, 446 nm)

Compound 27

TOF-EIMS spectrum $[\text{M}+1]^+$ peak at m/z 853.2117 corresponding to the molecular formula of $\text{C}_{48}\text{H}_{37}\text{O}_{15}$, UV (λ_{max} 275, 294, 385, 414, 446 nm), ^1H NMR (Table 3.12), ^{13}C NMR (Table 3.12).

Compound 28

Red amorphous powder. TOF-EIMS spectrum $[\text{M}+1]^+$ peak at m/z 869.2048 corresponding to the molecular formula of $\text{C}_{48}\text{H}_{37}\text{O}_{16}$, UV (λ_{max} 278, 293, 385, 412, 444 nm), ^1H NMR (Table 3.12), ^{13}C NMR (Table 3.13).

Compound 29

Melting point 211 – 212° C, EIMS m/z 169 (100%, $M^{++} + 1$), 168 (66%, M^+), 154 (33), 126 (8), 98 (9). $^1\text{H NMR}$ 7.57 (d , $J = 3$), 7.56 (dd , $J = 3.0, 8.0$), 6.84 (d , $J = 9.0$), 3.90 (3H, s).

Compound 30

$^1\text{H NMR}$ 7.45 (1H, d , $J = 16.2$), 7.40 (2H, dd , $J = 1.8, 6.9$), 6.80 (2H, dd , $J = 1.8, 6.9$), 6.39 (1H, d , $J = 15.6$), 4.59 ($br s$), EIMS m/z 164 (M^+)

Compounds 31

7.07, (2H, dd , $J = 2.1, 6.8$), 6.72 (2H, dd , $J = 2.1, 6.6$), 4.59 ($br s$), 3.47 (2H, t , $J = 7.2, 7.5$), 2.76 (2H, t , $J = 7.8, 6.9$), EIMS m/z 166 (M^+)

4.7 Biological activity

4.7.1: Anti-bacterial

This was done by dissolving a given quantity of compound in acetone, placing it on a filter paper (diameter 2.1 cm), allowing the acetone to dry and putting the disc on a petri-dish. On the petri-dish was agar inoculated with the bacterial strain. The petri-dish was covered and placed in an incubator. The inhibition zone was then measured afterwards. The results obtained were as in Table 4.2 below.

Table 4.2: Antibacterial results

Compound	Sample size	Inhibition zone	
		<i>Bacillus Subtilis</i>	<i>Staphylococcus aureus</i>
CH ₂ Cl ₂ stem extract of <i>A. kilifiensis</i>	15.4 µg	Nil	24 mm
CH ₂ Cl ₂ leaves extract of <i>A. kilifiensis</i>	15.4 µg	Nil	27 mm
Aloesaponarin I	11.4 µg	26 mm	25 mm
Aloesaponarin II	10.4 µg	28 mm	25 mm
4,5-Dialoesaponarin II	23.0 µg	Nil	23 mm

4.7.2 In vitro anti-plasmodial activity

The antiplasmodial assay on the crude extracts and pure compounds was performed by means of the microculture radioisotope technique as described by Desjardins (Desjardins *et al.*, 1979; Jenett-Siems *et al.*, 2000, Chulay *et al.*, 1983). The concentration at which growth was inhibited by 50% (IC₅₀) was estimated by interpolation. Two different strains of *Plasmodium falciparum* parasite cultures were used in this study. They are commonly used in drug sensitivity assays. The chloroquine-sensitive Sierra Leone I (D6) and chloroquine-resistant Indochina I (W2) strains were maintained in a continuous culture supplemented with mixed gas (90% nitrogen, 5% oxygen, 5% carbon dioxide), 10% human serum, and 6% haematocrit of A⁺ red blood cells. Once cultures reached a parasitemia of 3% with at least a 70% ring developmental stage present, parasites were transferred to a 96-well microtiter culture plate with wells pre-coated with compound. The samples were serially diluted across the plate to provide a range of concentrations to be used in the calculation of IC₅₀ values (by graphical interpolation). Plates were incubated in a mixed gas incubator for

24 hours. Following the specified incubation time, ³H-hypoxanthine was added and parasites allowed to grow for an additional 18 hours. Cells were harvested with a plate harvester (TomTec) onto filter paper and washed to eliminate unincorporated isotope. Filters were measured for activity in a microtiter plate scintillation counter (Wallac). Data from the counter was imported into a Microsoft Excel spreadsheet, which was then imported into an Oracle database/program to determine IC₅₀ values. The results obtained are tabulated below (Table 4.3)

Table 4.3: In vitro antiplasmodial activities against Plasmodium falciparum

Control drugs	IC ₅₀ (µg/ml)	
	W2	D6
Chloroquine	20.7 X 10 ⁻³	5.0 X 10 ⁻³
Mefloquine	3.6 X 10 ⁻³	30.9 X 10 ⁻³
Quinine	52.7 X 10 ⁻³	46.5 X 10 ⁻³
Crude extracts		
Aloe kilifiensis stems hexane crude	>50.0	>50.0
A. kilifiensis stems DCM crude	22.5	>50.0
A. kilifiensis fresh leaves acetone crude	10.6	14.7
A. graminicola dry leaves THF crude extract	>50.0	>50.0
Pure compounds		
	6.0	8.0
3	10.8	13.5
4	5.0	
6	8.0	
10	8.5	
11	9.0	12.3
13		24.1
14		10.2
17	6.2	
18	7.9	
19	10.8	

Table 4.3b: More in-vitro antiplasmodial activities against W2 isolate of

Plasmodium falciparum

Control drugs	IC ₅₀ (ng/ml)
Chloroquine	18.2
Mefloquine	8.3
Quinine	82.1
Pure compounds	
3	5705.8
4	2364.2
6	4969.5
8	1050.1
17	2798.0
18	7875.6
19	10755.7
27	263.1

REFERENCES

- Abegaz, B.M., Bezbihi, M.T., Msuta, T., Brun, R., Menche, D., Muhlbacher, J., and Bringman G. (2002). Gaboroquinones A and B and 4'-O- β -D-glucopyranoside, phenylanthraquinones from *Bulbine frutescens*. *J. Nat. Prod.* **65**, 1117-1121
- Adinolei Matteo, Lanzetta Rosa, Marciano Carmela Elsa, Parrilli Michelangelo (1991). A new class of anthraquinones-anthrone-c-glycosides from *asphodelus ramosus* tubers. *Tetrahedron*, **47**, 25, 4435-4440.
- Agnew, A.D.Q. (1974). Upland Kenya wild flowers. A flora of the ferns and herbaceous flowering plants of Upland Kenya. Oxford University Press, pp 674-675.
- Alemayehu, G. and Abegaz, B. M. (1996a). Bianthraquinones from the seeds of senna multiglandulosa. *Phytochemistry*, **41**, 919-921.
- Alemayehu, G.; Hailu, A.; Abegaz, B. M. (1996b). Bianthraquinones from Senna didymobotrya. *Phytochemistry*, **42**, 1423-1425.
- Ando, N. and Yamaguchi, I. (1990). Kankyu Kiyo-Tokyo Kasei Daigaku, **30**, 15; Chemical Abstracts, 1990, **113**, 148952
- Balint, G. A. (2001). Artemisinin and its derivatives: an important new class of anti-malarial agents. *Pharmacology and Therapeutics* **90**, 261.
- Barnard, C. (1949) The C-mitotic activity of cryptopleurine. *Aust J Sci* **12** 30-31
- Bezabih, M, Abegaz, B.M. (1998). 4'-Demethylknipholone from aerial parts of *Bulbine capitata*. *Phytochemistry* **48** 6: 1071-1073

- Bezabih, M. Motlhagodi, S., Abegaz, B. M. (1997). Isofuranonaphthoquinones and phenolics and knipholone derivatives from the roots of *Bulbine capitata*. *Phytochemistry* **46**, 6 1063-1067.
- Bezabih, M., Abegaz, B. M., Dufall, K., Croft, K., Skinner Adams, T., Davis, T. M. E. (2001). Antiplasmodial and antioxidant isofuranonaphthoquinones from the roots of *Bulbine capitata*. *Planta Med.* **67**, 4 340-344.
- Bouthet, Catherine F., Schirf, Virgil R. and Winters, Wendell D. (1995). Stimulation of Neuron-like Cell Growth by *Aloe* Substances. *Phytochemistry Research*, **9**, 185-188.
- Bringmann, G., Menche, D., Brun, R., Msuta, T., Abegaz, B. M., (2002). Bulbine-knipholone, a new axially chiral phenylanthraquinone from *Bulbine abyssinica* (Asphodelaceae): Isolation, structural elucidation, synthesis and antiplasmodial activity. *Eur. J. Org. Chem.* **6**, 1107-1111
- Bringmann, G.; Busemann, S. In *Natural Product Analysis*; Schreier, P., Herderich, M., Humpf, H. U., Schwab, W., Eds.; Vieweg: Wiesbaden, (1998); pp 195–212.
- Bringmann, G.; Dreyer, M.; Faber, J. H.; Dalsgaard, P. W.; Stærk, D.; Jaroszewski, J. W.; Ndangalasi, H.; Mbago, F.; Brun, R.; Reichert, M.; Maksimenka, K.; Christensen, S. B. (2003). Ancistrotanzanine A, the first 5,3'-coupled naphthylisoquinoline alkaloid, and two further, 5,8'-linked related compounds from the newly described species *Ancistrocladus tanzaniensis*. *J. Nat. Prod.*, **66**, 1159–1165.
- Bringmann Gerhard; Mühlbacher Joerg ; Reichert, Matthias; Dreyer Michael.; Kolz, Juergen; Speicher, Andreas. (2004). Stereochemistry of Isoplagiochin C, a Macrocyclic Bisbibenzyl from Liverworts *J. Am. Chem. Soc.*, **126**, 30 9283–9290.

- Cameron Donald W. and Warwick D. Raverty (1976). Pseudohypericin and other phenanthroperylene quinines. *Aust. J. Chem.* **29**, 1521-1533
- Capon, R. J.; Groves, D. R.; Urban, S.; Watson, R. G. (1993) Spongiaquinone Revisited: Structural and Stereochemical Studies on Marine Sesquiterpene/Quinones From a Southern Australian Marine Sponge, *Spongia* sp. *Aust. J. Chem.*, **46**, 1245-1253
- Chauser-Volfson Elena and Gutterman Y. Tzchak, (1996). The barbaloin content and distribution in *Aloe aborescens* leaves according to the leave part, age, position and season. *Israel Journal of Plant Sciences*, **44**, 289-296
- Cheney, R.H. (1970). "Aloe Drug in Human Therapy." *Quarterly Journal Crude Drug Res*, 10:1523-15.
- Clark, C. and Key, S. W. (1996). WHO weekly releases of revised facts on malaria, April 8, 15, 7-9.
- Confalone, P. N., Huie, E. M., Patel, N. G. (1983). The isolation, structure determination, and synthesis of pluridone, a novel insecticide from *Aloe pluridens* *Tetrahedron Lett.*, **24**, 5563-5566.
- Conner, J. M., Gray, A. I., Waterman, P. G., Kioy, D. (1990). *J. Nat. Prod.* **53**, 1362.
- in Dagne Ermias, Bisrat Daniel, Viljoen Alvaro and van Wyk Ben-Erik (2000). Chemistry of *Aloe* species. *Current Organic Chemistry*, **4**, 1055-1078
- Conner, John M., Gray Alexander I., Reynolds Tom and Waterman, G. (1989). Anthracene and Chromone derivatives in the exudate of *Aloe rabaiensis*. *Phytochemistry*, **28**, 12, 3551-3553

- Conner, John M., Gray Alexander I., Reynolds Tom and Waterman, G. (1987). Anthraquinone, anthrone and phenylpyrone components of *Aloe nyeriensis* var. *kedongensis* leaf exudates. *Phytochemistry*, **26**, 2995-2997
- Cuhlay, J.D., Haynes, J.D. and Diggs, C.L. (1983): Plasmodium falciparum: assessment of in vitro growth by [³H]hypoxanthine incorporation. *Experimental Parasitology* **55**, p.138.
- Cumming A. M. and Thomson R. H (1970) Anthraquinones in *Maesopsis eminii* *Phytochemistry*, **9** 11 2399-2400
- Dagne Ermias and Steglich Wolfgang (1984). Knipholone: a unique anthraquinone derivative from *Kniphofia foliosa*. *Phytochemistry* **23** 8 1729-1731
- Dagne Ermias, Casser Ingrid and Steglich Wolfgang (1992). Aloechrysone, a dihydroanthracenone from *Aloe berhana*. *Phytochemistry* **31** 5 1791-1793
- Dagne Ermias and Yenesew Abiy (1993). Knipholone anthrone from *Kniphofia foliosa*. *Phytochemistry* **34** 5 1440-1441
- Dagne Ermias (1996). Review of the chemistry of *Aloes* of Africa. *Bull. Chem. Soc. Ethiop.* **10**(1), 89 – 103.
- Dagne Ermias, Bisrat Daniel, Viljoen Alvaro and van Wyk Ben-Erik (2000). Chemistry of *Aloe* species. *Current Organic Chemistry*, **4**, 1055-1078
- Dagne, E. Yenesew, A., Asmellash, S., Demissew, S. and Mavi, S. (1994). Anthraquinones, Pre-anthraquinones and isoeleutherol in the Roots of *Aloe* species. *Phytochem*, **35** 2, 401.
- Dagne E, Mammo W, Bekele A, Odyek O and Byaruhanga MA, (1991). Flavonoids of *Millettia dura*. *Bull Chem Soc Ethiop* **5**: 81-86

- Dagne, E.; Yenesew, A. (1994). Anthraquinones and chemotaxonomy of the Asphodelaceae. *Pure Appl. Chem.*, **66**, 2395–2398.
- Davis, R. H. (1997). *Aloe vera. A scientific approach*. Vantage Press, New York.
- Demissew Sebsebe, (1996). Botanical Aspects of Aloes of North East Africa. *Bull. Chem. Soc. Ethiop.* **10**(1), 74-88
- Derese Solomon (2004). Anti-plasmodial flavonoids from some Kenyan Papilionoideae species. Ph.D. Thesis, University of Nairobi.
- Desjardins, R. E., Canfield, C. J., Haynes, J. D., Chulay, J. D. (1979). *Antimicrob. Agents Chemother.* **16**, 710.
- Downing, J. W. Program Package BDZDO/MCDSPD, Department of Chemistry and Biochemistry, University of Colorado: Boulder, USA; modified by Fleischhauer, J.; Schleker, W.; Kramer, B.; ported to Linux by Gulden, K.-P.
- Dring, J. V., Nash, R. J., Roberts, M. F.. and Reynolds, T. (1984). Hemlock alkaloids in Aloes. Occurrence and distribution of γ -coniceine. *Planta Med.*, **50**, 442-443
- Fairbairn J W. and Shrestha A. B. (1967). The distribution of anthraquinone glycosides in *Cassia senna* L. *Phytochemistry* **6** 9 1203-1207
- Fairbairn, J. W. and Muhtadi F. J. (1972) The biosynthesis and metabolism of anthraquinones in *Rumex obtusifolius*. *Phytochemistry* **11**, 1 215-219
- Finar I. L. (2000). Organic Chemistry. Vol. 2. Stereochemistry and the chemistry of natural products. 5th ed. Longman Scientific & Technical. Pp 454
- Forster Pl. 1989. *Gynura drymophila* (Asteraceae : Senecioneae), a succulent daisy from Australia. *Anacampseros* **5**. (3): 52-54

- Gillett, J. D. (1968). The Identification of Aloes in East Africa. *J. E. Afr. Nat. Hist. Soc.* Vol. XXVI No. 2(114) 65-73
- Gjerstad, G., and Riner, T. D. (1968). Current status of aloes as a cure-all. *Am. J. Pharm.* **140**, 58 – 64
- Groom, Q. J. and Reynolds, T. (1987). Barbaloin in *Aloe* species. *Planta Med.* **53**, 345-358
- Guido François, Steenackers Tania, Ake Assi Laurent, Steiglich Wolfgang, Lamottke Kai, Holenz Jorg and Bringman Gerhard (1999). Vismone H and structurally related anthranoid compounds of natural and synthetic origin as promising drugs against the human malaria parasite *Plasmodium falciparum*: structure-activity relationships. *Parasitol Res* **85**: 582-588. © Sringer-Verlag.
- Guttermann Yitzchak and Chauser-Volfson Elena (2000). The distribution of phenolic metabolites barbaloin, aloesin and aloenin as a peripheral defense strategy in the succulent leaf parts of *Aloe aborescens*. *Biochemical Systematics and Ecology* **28**, 825 - 838
- Haller John, S. (1990). A drug for all seasons medical and pharmacological history of aloe. *Bull. N. Y. Acad. Med.* **66**, 6 647 - 669
- Hutter John A., Salman Mohammad, Stavinoha, William B., Satsangi Neera, Williams Robert F., Streeper Robert T. and Weintraub Susan T. (1996). Antiinflammatory C-glucosyl chromone from *Aloe barbadensis*. *J. Nat. Prod.*, **59**, 541-543

- Hyde, J.E. (2002). Mechanisms of resistance of *Plasmodium falciparum* to anti-malarial drugs. *Microbes Infect* **4**, 165.
- Inouye, H. and Leistner, E., (1988). The Chemistry of Quinoid Compounds, vol. 2. John Wiley & Sons, New York, 1293 – 1349.
- Ismail Nor Hadiani, Ali Abdul, M., Aimi Norio, Kitajima Mariko, Takayama Hiromitsu and Lajis Nordin, H. (1997). Anthraquinones from *Morinda Elliptica*. *Phytochemistry*, **45**, 8 1723-1725
- Jenett-Siems K., Jakupovic, J., Solis, P. N., Gupta, M. P., Mockenhaupt, F. P., Bienzle, U. and Eich E. (2000). Sipandinolide. A butenolide including a novel type of carbon skeleton from *Sipurina andina*. *Planta Med.* **66**, 384
- Karrer Walter (1976). Konstitution und Vorkommen der organischen Pflanzenstoffe. Birkhäuser Verlag Basel und Stuttgart. Pp 515
- Koo, M. W. L. (1994). *Aloe vera*: Antiulcer and Antidiabetic effects. *Phytotherapy research* **8**, 465 - 469
- Koyama Junko, Ogura Tamaki and Tagahara Kiyoshi (1994). Naphtho[2,3-c]furan-4,9-dione and its derivatives from *Aloe ferox*. *Phytochemistry* **37**, 4 1147-1148.
- Krogstad D.J. (1996). Malaria as a re-emerging disease. *Epidemiol. Rev.* **18**: 77-79.
- Leistner, E. (1971). A second pathway leading to anthraquinones in higher plants. *Phytochemistry* **10** 12 3015-3020
- Leistner, E. (1981). Biosynthesis of Plant Quinones. In: Conn, E. E. (Ed.), *Biochemistry of plants*, 7. Academic London. 403 – 423

- Leistner, E. (1985). Biosynthesis of chorismate-derived Quinones in plant cell cultures. In: Neumann, K. H., Barz, W., Reinhard, E. (Eds.). Primary and secondary metabolism of plant cell cultures. Springer, Berlin, 215 – 224.
- Lusunzi Qhotsokoane, M. A., Karuso, P. (2001). Secondary metabolites from Basotho medicinal plants. II. *Bulbine capitata*. *Asian J. Chem.* 54, 7 427-430
- Mabberley, D. J. (1987). The plant book: A portable dictionary of Higher plants. Cambridge University press, Cambridge.
- Man Ki Park, Jeong Hill Park, Young Geun Shin, Wang Yu Kim, Jong Ho Lee and Kyeong Ho Kim (1996). Neoaloesin A: a new C-glucofuranosyl chromone from the *Aloe barbadensis*. *Planta Med.* 62, 363 – 365.
- Manfred Hesse, Herbert Meier and Bernd Zeeh (1997). Spectroscopic methods in organic chemistry. D. Enders, R. Noyori and B. M. Trost (ed). Georg Thieme Verlag Stuttgart . New York
- Manitto Paulo 1981. Biosynthesis of natural products. Ellis Horwood Limited, New York. PP 169-212
- Mishra, S.K., Satpathy, S.K. and Mohanty, S. (1999): Survey of malaria treatment and deaths. *Bull. World Health Organ.* 77 12, 1020.
- Mohamed H. Farah, Rolf Anderson and Gunnar Samuelsson (1992). Microdentin A and B: Two new aloin derivatives from *Aloe microdonta*. *Planta Med.* 58, 88 – 93.
- Müller, M.; Lamottke, K.; Löw, E.; Magor-Veenstra, E.; Steglich, W. (2000). Stereoselective total syntheses of atrochrysone, torosachrysone and related 3,4-dihydroanthracen-1(2H)-ones. *J. Chem. Soc., Perkin Trans. 1*, 2483–2489.
- Müller, M.; Lamottke, K.; Steglich, W.; Busemann, S.; Reichert, M.; Bringmann, G.;

- Mutanyatta Joan, Bezabih Merhatibeb, Abegaz M Berhanu., Dreyer Michael, Brun Reto, Kocher Nikolaus and Bringmann Gerhard (2005). The first 6'-O-sulfated phenylanthraquinones: isolation from *Bulbine frutescens*, structural elucidation, enantiomeric purity, and partial synthesis *Tetrahedron*, **61**, 35, 8475-8484
- Spiteller, P.(2004). Biosynthesis and Stereochemistry of Phlegmacin-Type Fungal Pigments. *Eur. J. Org. Chem.*, **2004**, Issue 23, 4850-4855
- Nzila-Mounda, A, Mberu, E.K., Sibley, C.H., Plowe, C.V., and Winstanley, P.A. (1998): Kenyan *Plasmodium falciparum* field isolates: correlation between pyrimethamine and chlorcycloguanil activity *in vitro* and point mutations in the dihydrofolate reductase domain. *Anti-microbial Agents and Chemotherapy* **42**, 164.
- Okamura Nobuyuki, Hine Noriko, Harada Satomi, Fujioka Toshihiro, Mihashi Kunihide, Nishi Masatoshi, Miyahara Kazumoto and Yagi (1997). Diastereomeric C-Glycosilanthrones of *Aloe vera* leaves. *Phytochemistry* **45**, 7 1519-1522
- Okamura Nobuyuki, Hine Noriko, Tateyama Yukiko, Motoko Nakazawa, Fujioka Toshihiro, Mihashi Kunihide and Yagi Akira (1997b). Three chromones of *Aloe vera* leaves. *Phytochemistry* **45**, 7 1511-1513
- Okamura Nobuyuki, Masami Asai, Noriko Hine, Akira Yagi (1996). High Performancer Liquid Chromatographic Determination of Phenolic Compounds in *Aloe* Species. *Journal of Chromatography A*. **746** 225-231
- origins of incorporative pharmacopoeial processes among xhosa healers in Southern Africa. *j Ethnopharmacol* **33** 3: 237-242.

- Pepalla Sridhara Babu, Jammula Subba Rao, Rao K. V. Jagannadha and Thomson H Ronald (1992). Quinones and tetracosanolide in *Ventilago bombaiensis*. *Phytochemistry*, **31** 6 2103-2104
- Pérez, A.; Ramírez-Durón, R.; Piñeyro-López, A.; Waksman, N.; Reichert, M.; Bringmann, G. (2004). The absolute configuration of peroxisomicines A1 and A2. *Tetrahedron*, **60**, 8547–8552.
- Price, R.N. (2002): Artemisinin drugs: novel anti-malarial agents. *Expert Opinion on Investigational Drugs*. **9**, 1815.
- Reynolds, G.W. (1966). "The *Aloes* of Tropical Africa and Madagascar". Mbabane, Swaziland, The *Aloes* Book Fund.
- Reynolds, T. (1985). The compounds in *Aloe* Leaf Exudates: a Review. *Bot J. Linn. Soc.*, **90**, 157-177.
- Reynolds, T. (1985a). Observations on the Phytochemistry of the *Aloe* Leaf-exudate Compounds. *Botanical Journal of the Linnean Society*, **90** 179-199.
- Rubeena Saleem, Shaheen Faizi, Farhat Deebe, Bina Shaheen Siddiqui and Mahmood Qazi, (1997). Anthrones from *Aloe Barbadensis*. *Phytochemistry*, **45**, 6 1279-1282
- Sibley, C.H., Hyde, J.E., Sims, P.F., Plowe, C.V., and Kublin, J.G. (2001): Pyrimethamine-sulfadoxine resistance in *Plasmodium falciparum*: what next? *Trends in Parasitology* **17**, 582
- Simon, C., Lamla, M., (1991). Merging pharmacopoeia: understanding the historical origins of incorporative pharmacopoeial processes among xhosa healers in Southern Africa. *J Ethnopharmacol* **33** 3: 237-242

- Smith, Gideon F. & van Wyk, 1991. Generic relationships in the Aloooideae (Asphodelaceae). *Taxon* **40** 557-581
- Speranza, G., Dada, G., Lunazzi, L., Gramatica, P. and Manitto, P. (1986b). Aloenin B., a new diglucosylated 6-phenyl-2-pyrone from Kenya *Aloe. J. Nat. Prod.* **49**, 800-805.
- Speranza, G., Dada, G., Lunazzi, L., Gramatica, P. and Manitto, P. (1986). A C-glucosylated 5-methylchromone from Kenya aloe *Phytochemistry* **25**, 2219
- Speranza, G., Manitto, P., Cassara, P., Monti, D. (1993). Feralolide, a dihydroisocoumarin from cape aloe *Phytochemistry* **33**, 175-178
- Suga, T., Hirata, T. and Tori, K. (1974). Structure of aloenin, a bitter glucoside from *Aloe* species. *Chemistry letters* 715-718
- Sydiskis, R. J., Owen, D. G., Lohr, J. L., Rosler K. H. A. and Blomster, R. N. (1991). Inactivation of enveloped viruses by anthraquinones extracted from plants. *Antimicrobial agents and chemotherapy* **35**, 12, 2463 – 2466.
- Takeda, N., Seo, S., Ogihara, Y., Sankawa, U., Iitaka, I., Kitagawa, I. And Shibata, S. (1973). Anthraquinonoid colouring matters of *Penicillium islandicum* Sopp and some other fungi (-)luteoskyrin, (-)rubroskyrin, (+)rugolosin and their related compounds. Studies on fungal metabolites-XXXI¹ : *Tetrahedron*, **29** 3703-3719
- Tânia Maria Almeida Alves, Helmut Kloos, Carlos Leomar Zani (2003). Eleutherinone, anovel fungitoxic naphthoquinone from *Eleutherine bulbosa* (Iridaceae) *Memórias do Instituto Oswaldo Cruz* vol. 98 no. 5 Rio de Janciro

- Thiel, W. Software (2000). Package MNDO99, Version 6.0, Max-Planck-Institut für Kohlenforschung, Kaiser-Wilhelm-Platz 1, D-45470 Mülheim, Germany.
- Torssell Kurt, B. G. (1983). Natural Products Chemistry. A Mechanistic and biosynthetic approach to secondary metabolism. John Wiley & Sons Ltd. pp 4 – 18.
- Trape, J.F., Pison, G., Spiegel, A., Enel, C. and Rogier, C. (2002): Combating malaria in Africa. *Trends in Parasitology* **18**, 224.
- Trease, G.E. and Evans, W. C. (1978). Pharmacognosy, 11th edition, Baillier Tindall, London.
- Triter, R. T., (1981). Folk remedies as indicators of common illnesses: Examples from the United States-Mexico border. *J. Ethnopharmacol.* **4**, 207 – 221.
- van den Berg, A. J. J., Labadie, R. P., (1989). Quinones. In Harborne, J. B. (Eds.), Plant Biochemistry, vol. 1. Academic Press, London, pp451 – 491.
- van der Bank Herman, van Wyk Ben-Erik and van der Bank Michelle (1995). Genetic variation of two economically important *Aloe* species (Aloaceae). *Biochemical Systematics and Ecology* **23**, 3 251-256
- van Eijk W. Gijsbertus and Roeymans J. Henry (1981) Revenelin, chrysophanol, and helminthosporin, pigments from *Drechslera holmii* and *Drechslera ravenelii* Experimental Mycology **5** 4 373-375
- van Rheede, van Oudtshoorn, MCB,. (1964) Chemotaxonomic investigations in *Asphodeleae* and *Aloineae* (Liliaceae). *Phytochemistry* **3** 383-390
- van Staden, L.F, Drewes, S.E (1994). Knipholone from *bulbine latifolia* and *Bulbine frutescens*. *Phytochemistry* **35** 3: 685-686

- van Wyk, Ben-Erik, van Rheede van Oudtshoorn, M. C. B., and Smith, Gideon F. (1995b). Geographical variation in the major compounds of *Aloe ferox* leaf exudate. *Planta Med.* **61** 250-253
- van Wyk Ben-Erik, Yenesew Abiy, Dagne Ermias, (1995a). Chemotaxonomic Significance of Anthraquinones in the Roots of Asphodeloideae (Asphodelaceae).. *Biochemical Systematics and Ecology*, **23**, 3 227-281
- Veitch C.Nigel, Simmonds S.J. Monique, Blaney M. Wally and Reynolds Tom (1994). A dihydroisocoumarin glucoside from *Aloe hildebrandtii*. *Phytochemistry* **35**, 1163-1166
- Viljoen, A. M., van Wyk, B-E. and van Heeden, F. R. (1998). Distribution and chemotaxonomic significance of flavonoids in *Aloe* (Asphodelaceae). *Pl. Syst. Evol.* **211**: 31-42.
- Viljoen Alvaro M., van Wyk Ben-Erik, Newton Len E. (1999). Plicataloside in *Aloe* – a chemotaxonomic appraisal. *Biochemical Systematics and Ecology* **27**, 507-517.
- Watt, J.M., Breyer-Brandwijk, M.G., 1962. The medicinal and poisonous plants of Southern and Eastern Africa, 2nd edition, Livingstone Ltd., Edinburgh and London, pp 695-696
- Weber Wolfgang, Thiel Walter. Theoretical Chemistry Accounts: Theory, Computation, and Modeling. *Theor. Chem. Act.*, **103**, 6 495–506.
- Wessels, Philippus L., Cedric W. Holzapfel, Ben-Erik van Wyk, Wilhelmina Marais. 1996. Plicataloside, an *O,O*-Diglycosilated naphthalene Derivative from *Aloe plicatilis*. *Phytochemistry* **41**, 6 1547 – 1551

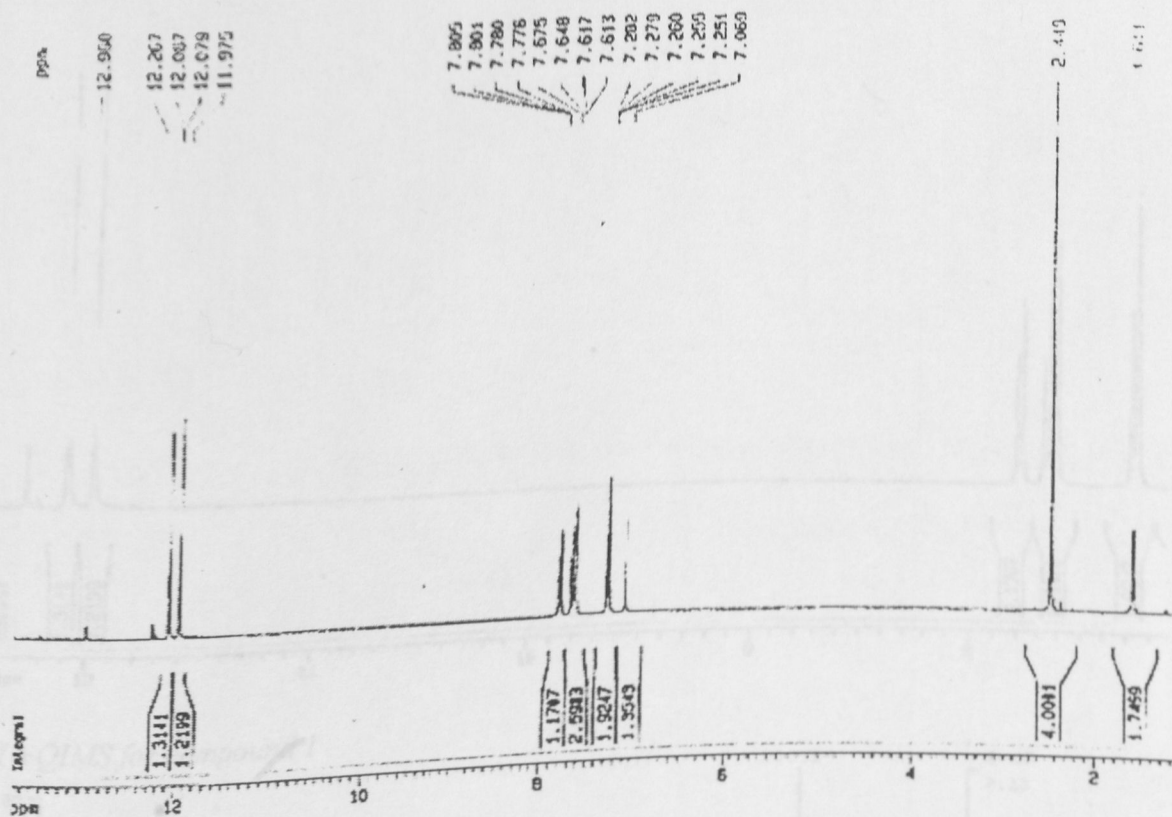
- Williams, C. A., (1975). Biosystematics of *Monocotyledoneae*-flavonoid patterns in leaves of *Lilicieae*. *Biochem. Syst. Ecol.* **3**, 229-244
- Winstanley, P.A., Ward, S.A., and Snow, R.W. (2002): Clinical status and implications of anti-malarial drug resistance. *Microbes Infect* **4**, 157.
- World Health Organization's World Malaria Report 2005. (LEE Jong-wook, Director-General, World Health Organization)
- Wube, A.A., Bucar, F. Asres, K. Gibbons, S. Adams, M. Streit, B. Bodensieck, A. and Baue, R. (2006). Knipholone, a selective inhibitor of leukotriene metabolism. *Phytomedicine*, **13**, 6 452-456
- Yagi Akira, Yamanouch Mayumi i and Nishioka Itsuo (1978). Biosynthetic relationship between tetrahydroanthracene and anthraquinone in *Aloe saponaria* *Phytochemistry*, **17** 5 895-897
- Yagi, A., Hine N., Asai, M., Nakasawa, M., Tateyama, Y., OKamura, N., Fujioka, T., Mihashi, K. and Shimomura K. (1998). Tetrahydroanthracene glycosides in callus tissue from *Aloe barbadensis* leaves. *Phytochemistry*, **47** (7). 1267-1270
- Yenesew Abiy, Dagne Ermias, Müller Michael and Steglich Wolfgang (1994). An anthrone, an anthraquinone and two oxanthrones from *Kniphofia foliosa*. *Phytochemistry*, **37** 2 525-528
- Yenesew A. and Dagne E. (1993). Contribution to the Chemotaxonomy of the Asphodelaceae. 5th Napreca Symposium on Natural Product, Antananarivo, Madagascar.

- Williams, C. A., (1975). Biosystematics of *Monocotyledoneae*-flavonoid patterns in leaves of *Lilicieae*. *Biochem. Syst. Ecol.* **3**, 229-244
- Winstanley, P.A., Ward, S.A., and Snow, R.W. (2002): Clinical status and implications of anti-malarial drug resistance. *Microbes Infect* **4**, 157.
- World Health Organization's World Malaria Report 2005. (LEE Jong-wook, Director-General, World Health Organization)
- Wube, A.A., Bucar, F. Asres, K. Gibbons, S. Adams, M. Streit, B. Bodensieck, A. and Baue, R. (2006). Knipholone, a selective inhibitor of leukotriene metabolism. *Phytomedicine*, **13**, 6 452-456
- Yagi Akira, Yamanouch Mayumi i and Nishioka Itsuo (1978). Biosynthetic relationship between tetrahydroanthracene and anthraquinone in *Aloe saponaria* *Phytochemistry*, **17** 5 895-897
- Yagi, A., Hine N., Asai, M., Nakasawa, M., Tateyama, Y., OKamura, N., Fujioka, T., Mihashi, K. and Shimomura K. (1998). Tetrahydroanthracene glycosides in callus tissue from *Aloe barbadensis* leaves. *Phytochemistry*, **47** (7). 1267-1270
- Yenesew Abiy, Dagne Ermias, Müller Michael and Steglich Wolfgang (1994). An anthrone, an anthraquinone and two oxanthrones from *Kniphofia foliosa*. *Phytochemistry*, **37** 2 525-528
- Yenesew A. and Dagne E. (1993). Contribution to the Chemotaxonomy of the Asphodelaceae. 5th Napreca Symposium on Natural Product, Antananarivo, Madagascar.

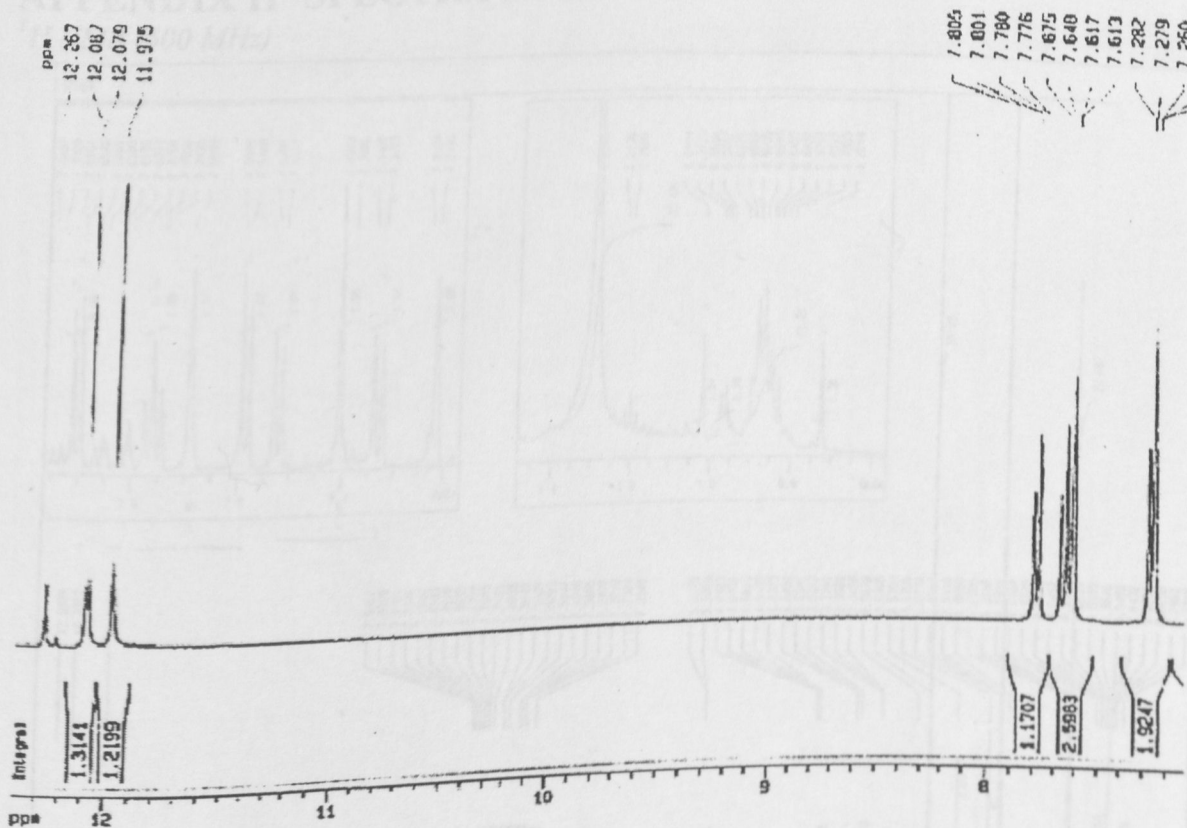
- Yenesew, A. (1991). Phytochemical studies of some *Aloe* species of the *Sapotariae* group. MSC, Thesis, University of Nairobi.
- Yenesew, A., Ogur, J. A. and Duddeck, H. (1993b). Prechrysophanol from *Aloe graminicola*. *Phytochemistry*, **34**, 5 1442-1444.
- Ying-Shan Han, Robert van der Heijden, Alfons W. M. Lefeber, Cornelis Erkelens, Robert Verpoorte, (2002). Biosynthesis of anthraquinones in cell cultures of *Cinchona* 'Robusta' proceeds via the methylerythritol 4-phosphate pathway. *Phytochemistry* **59** 45 – 55

APPENDIX I: SPECTRA FOR COMPOUND 1

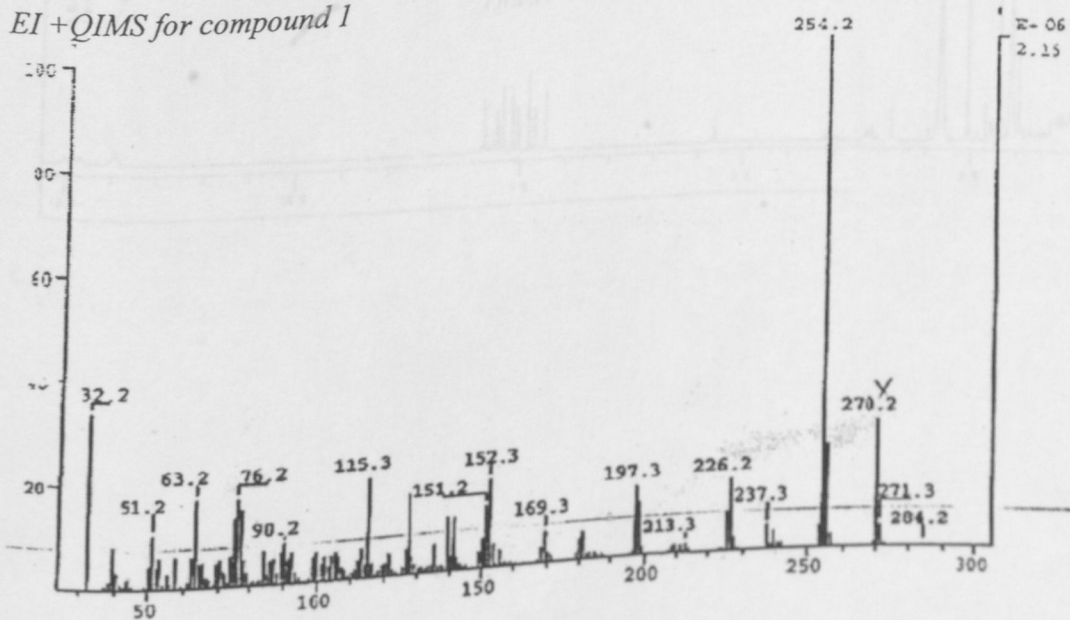
^1H NMR: Solvent CDCl_3 ; SF: 300.1333673 MHz



¹H NMR for Compound 1 expansion

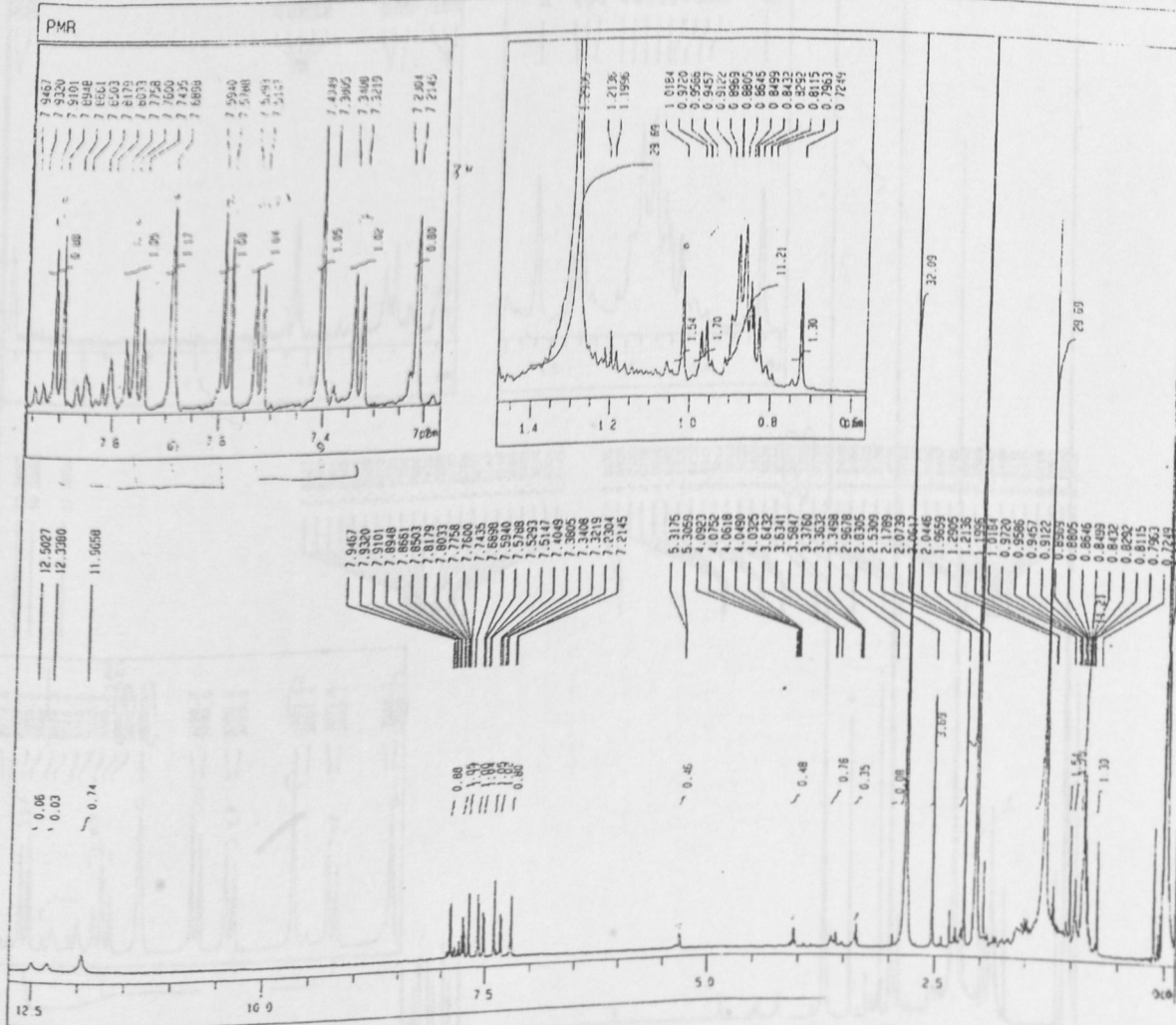


EI + QIMS for compound 1

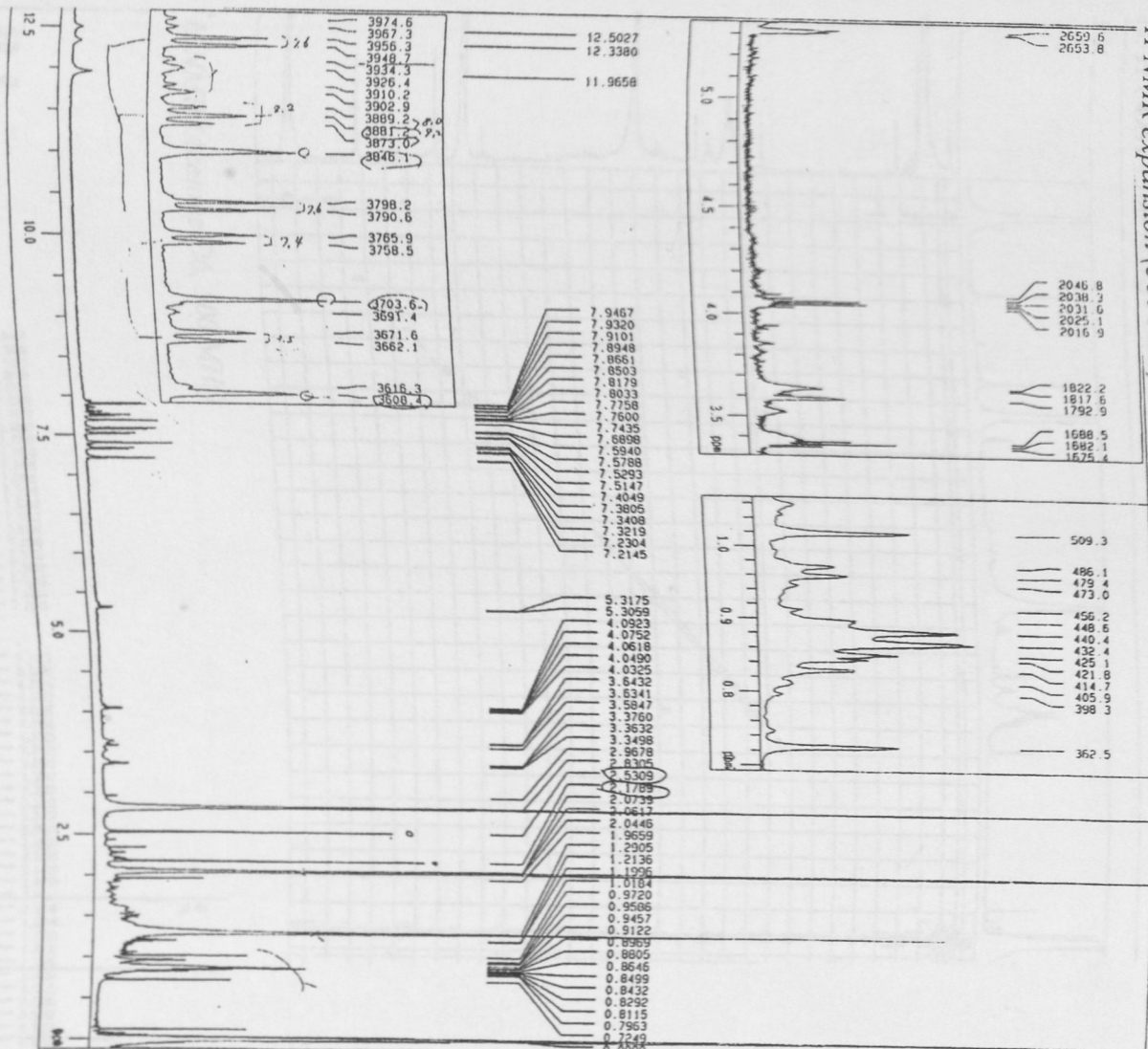


APPENDIX II SPECTRA FOR COMPOUND 3

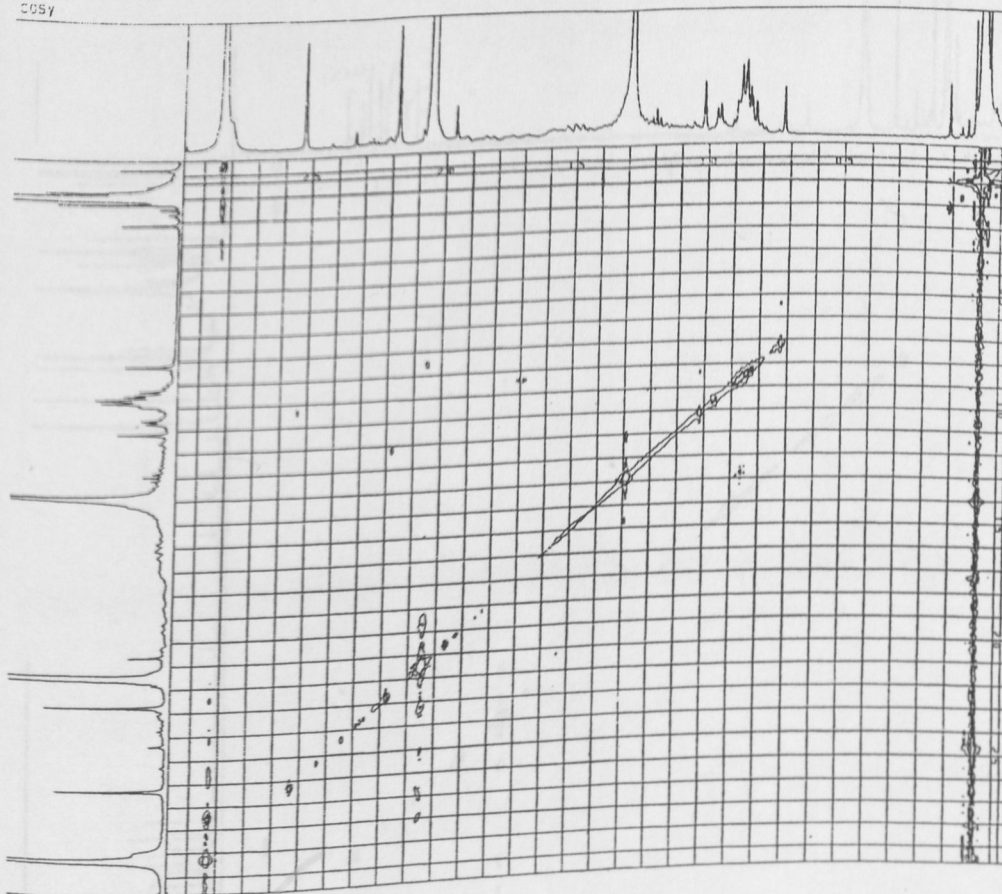
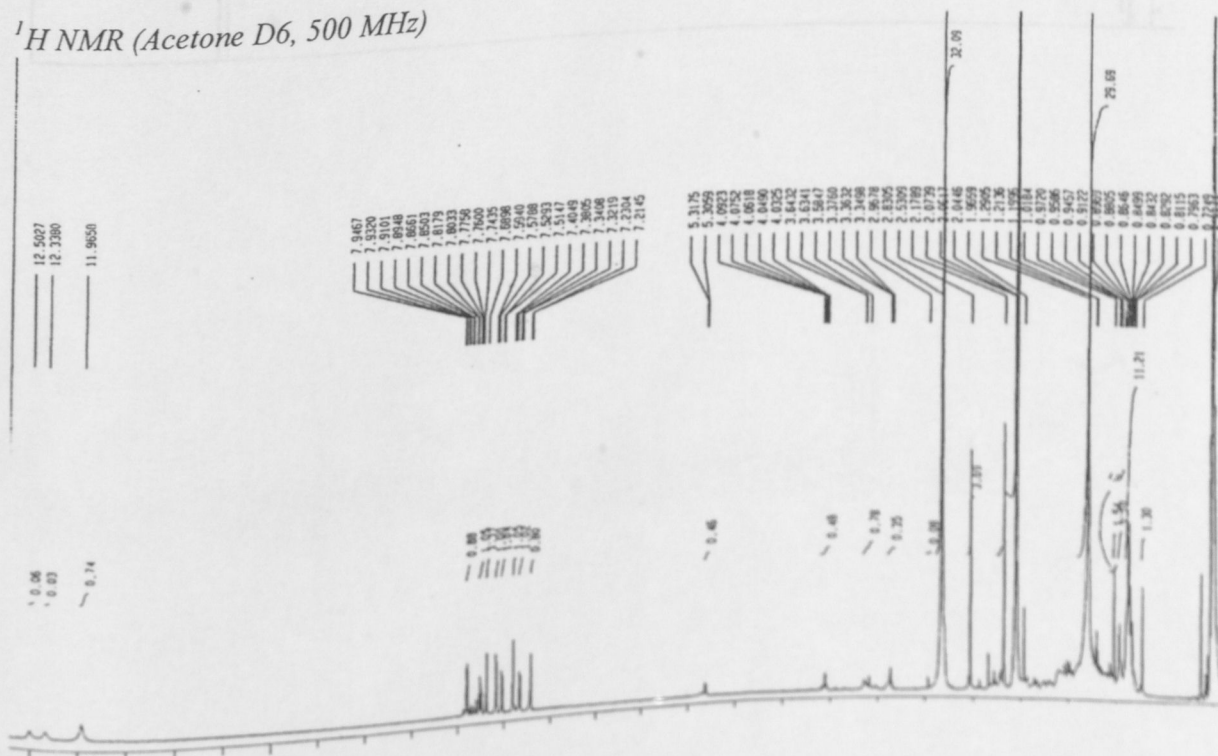
¹H NMR (500 MHz)



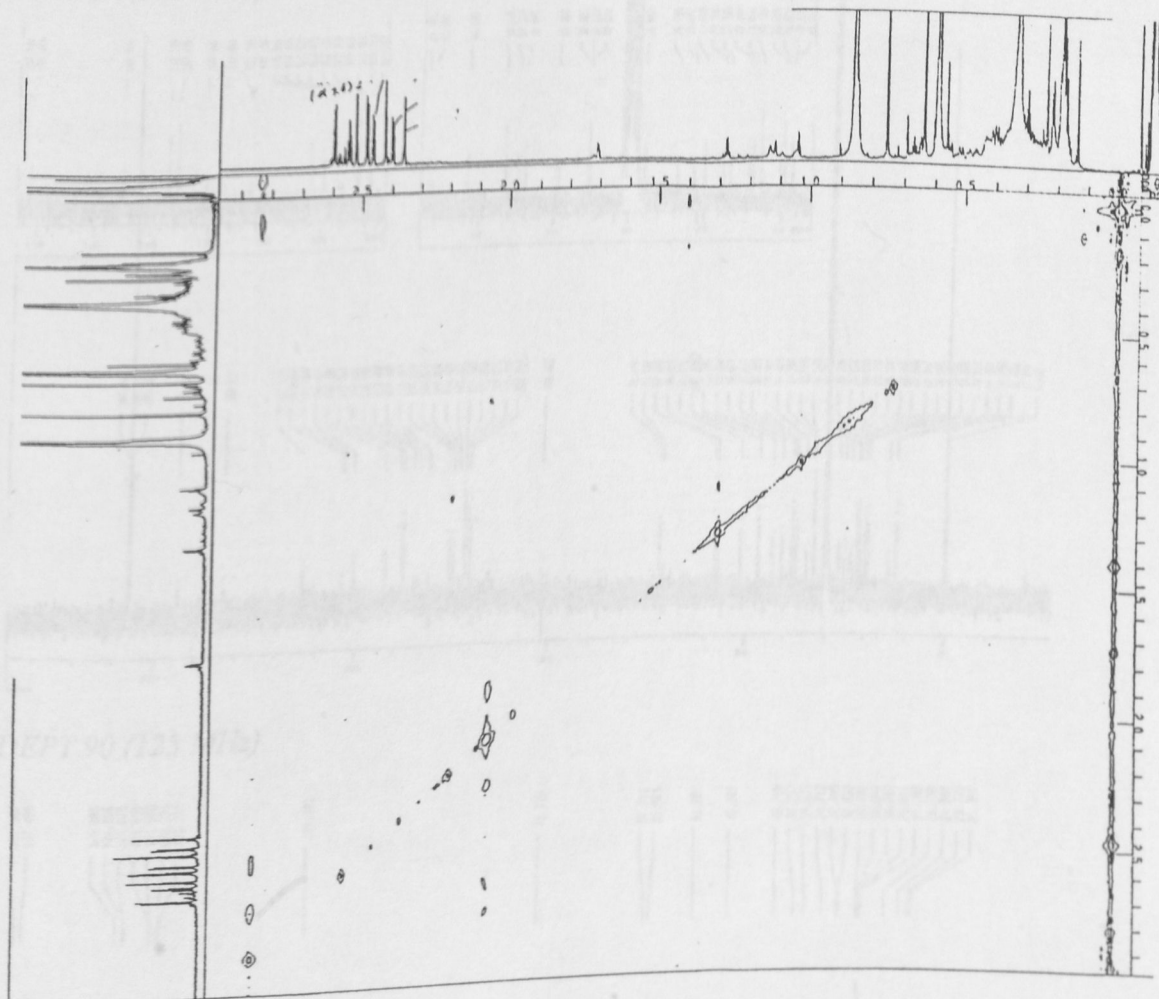
¹H NMR expansion (500 MHz)



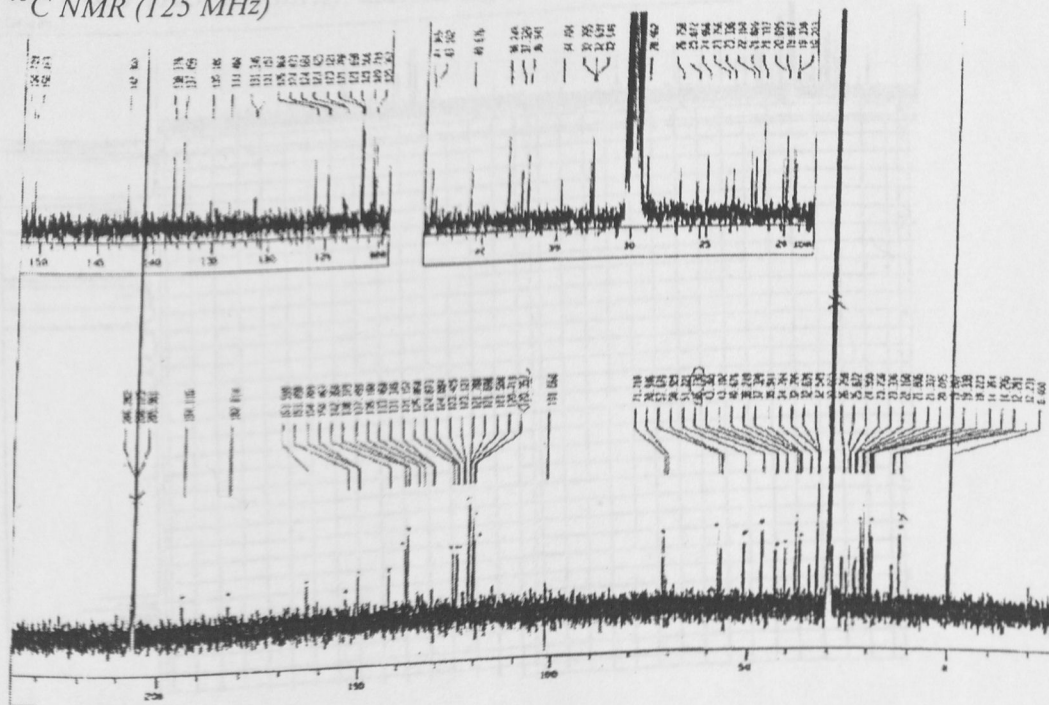
COSY

 ^1H NMR (Acetone D6, 500 MHz)

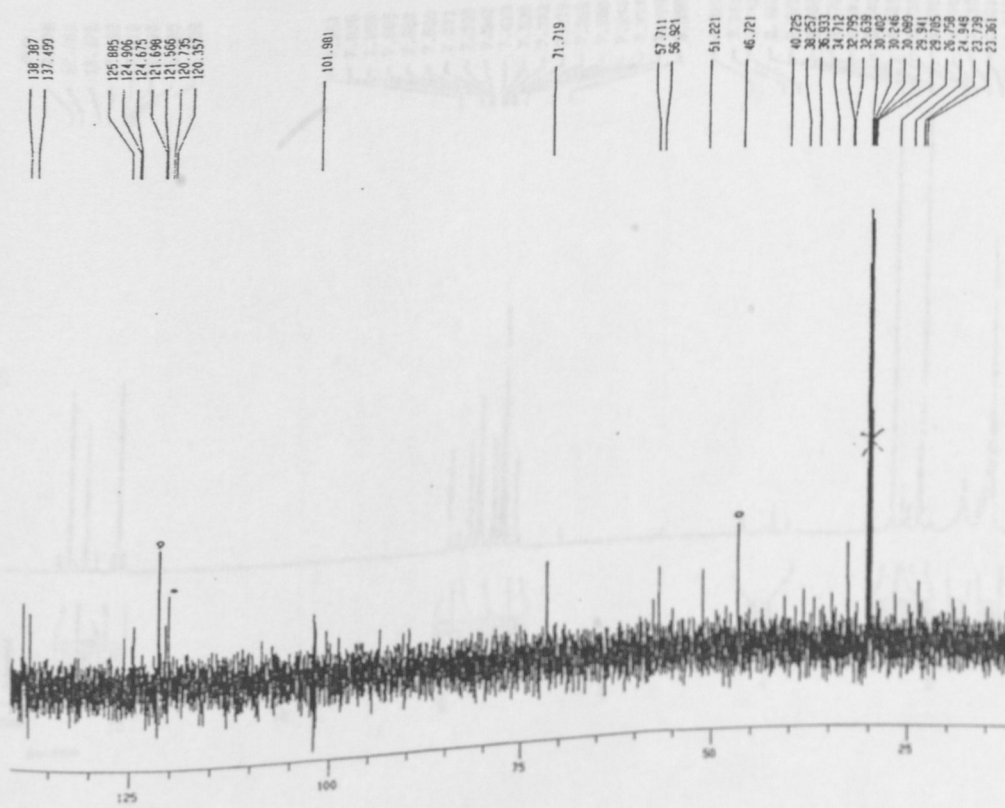
H, H COSY (acetone D₆, 500 MHz)



^{13}C NMR (125 MHz)

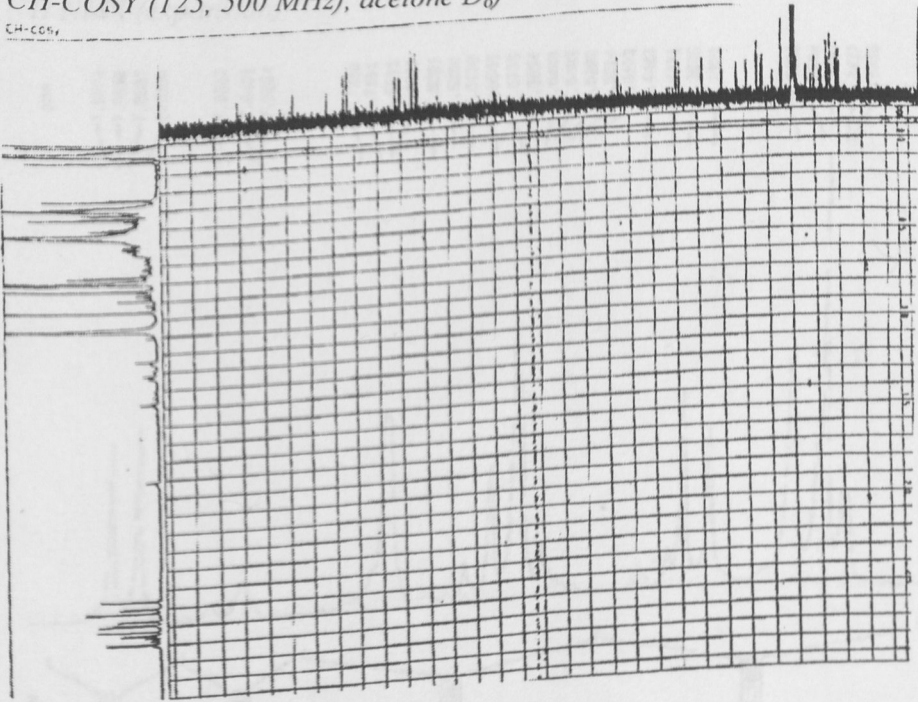


DEPT 90 (125 MHz)

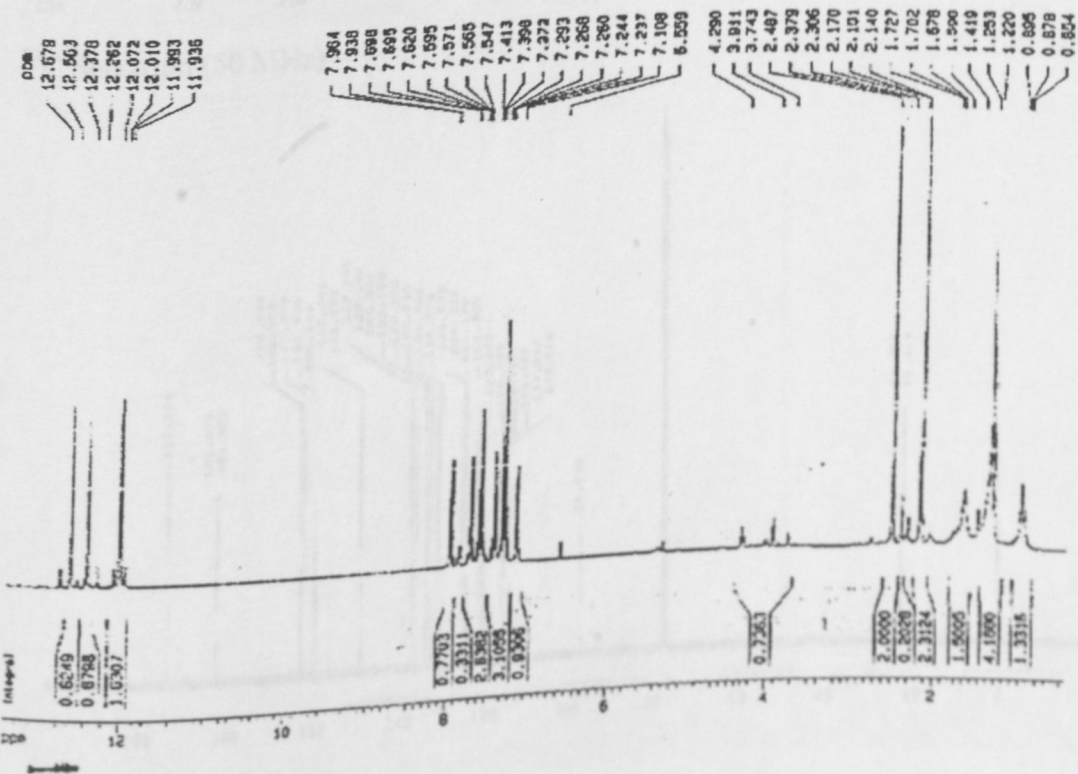


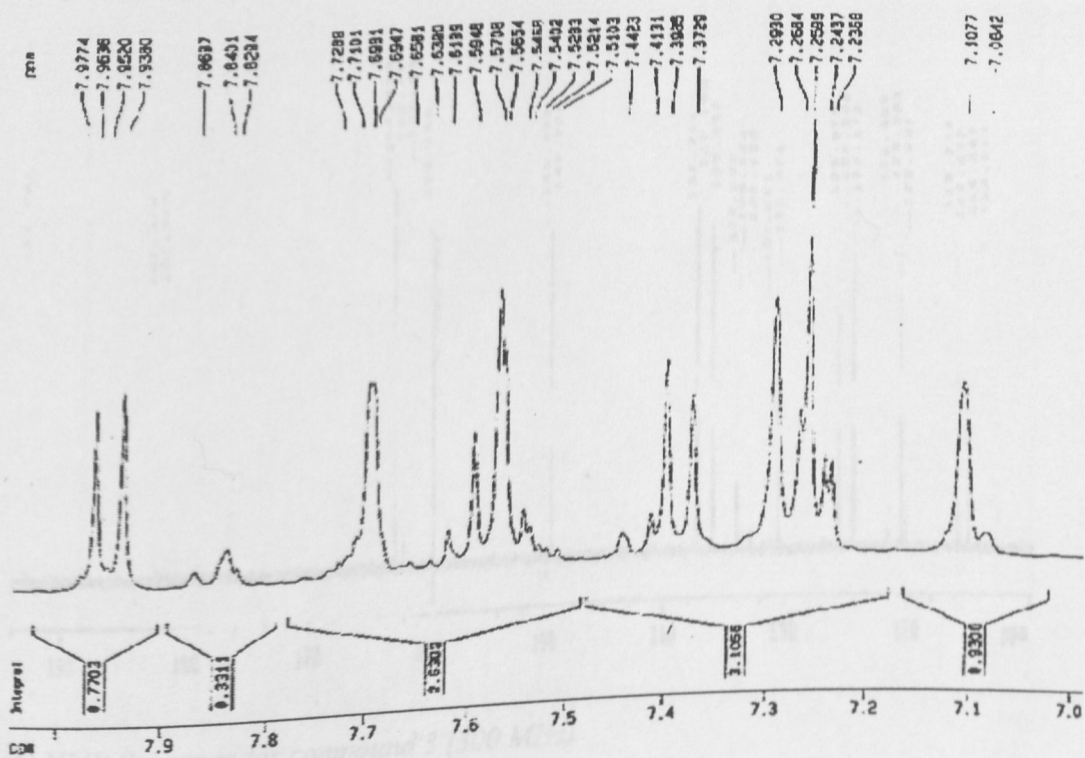
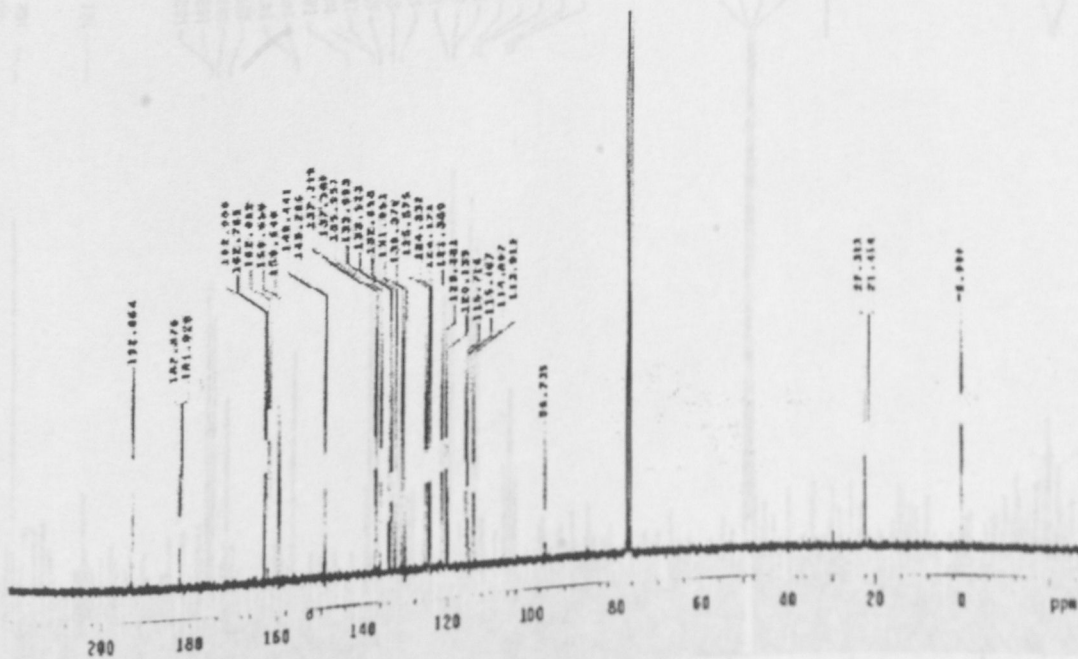
CH-COSY (125, 500 MHz), acetone D₆)

CH-COSY

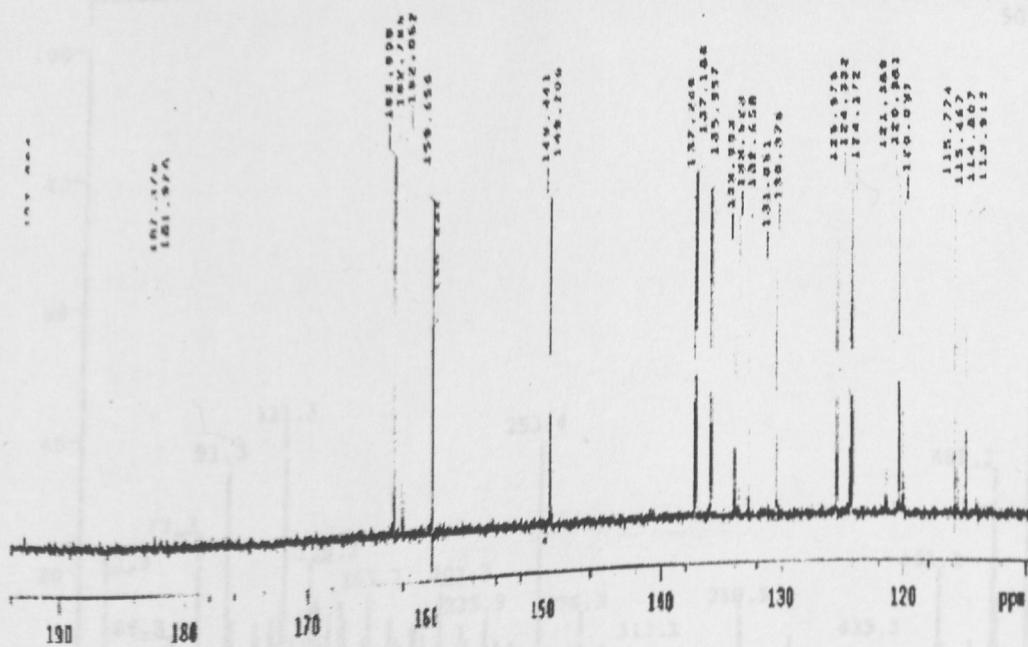


¹H NMR (300 MHz)

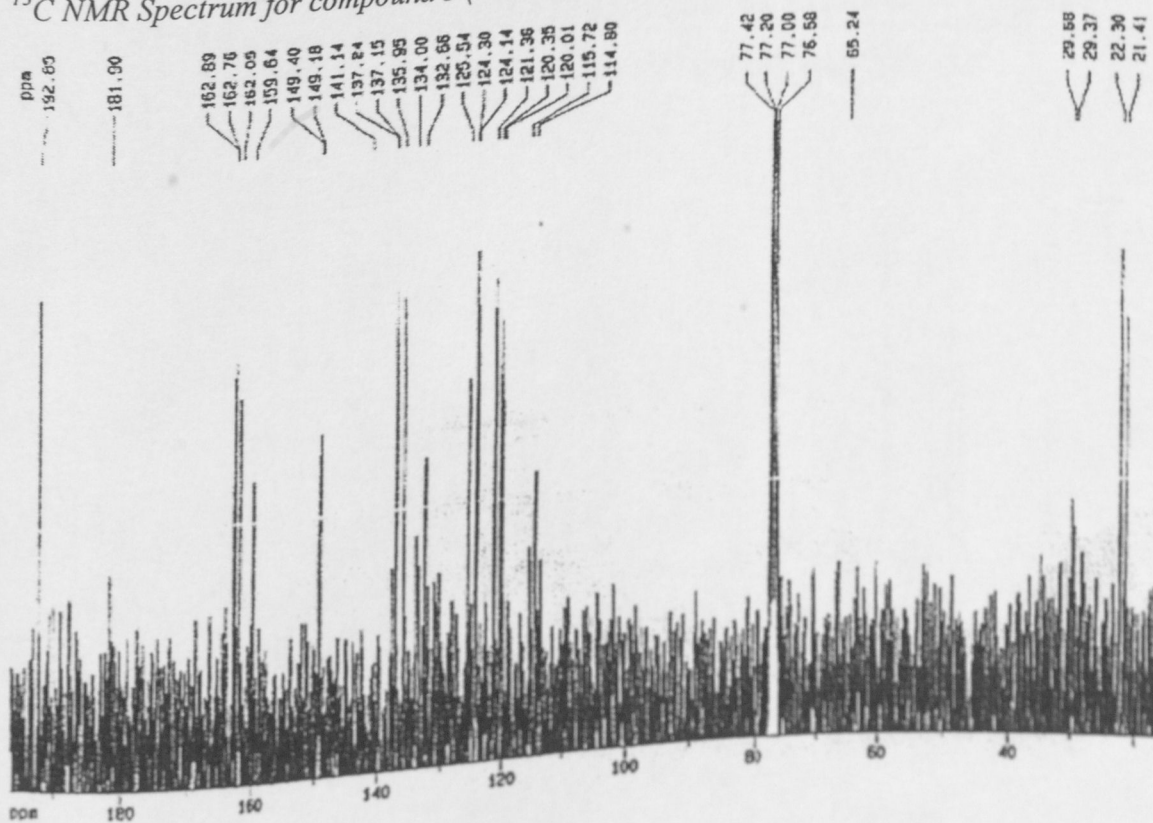


^1H NMR (expansion) ^{13}C Spectrum (50 MHz)

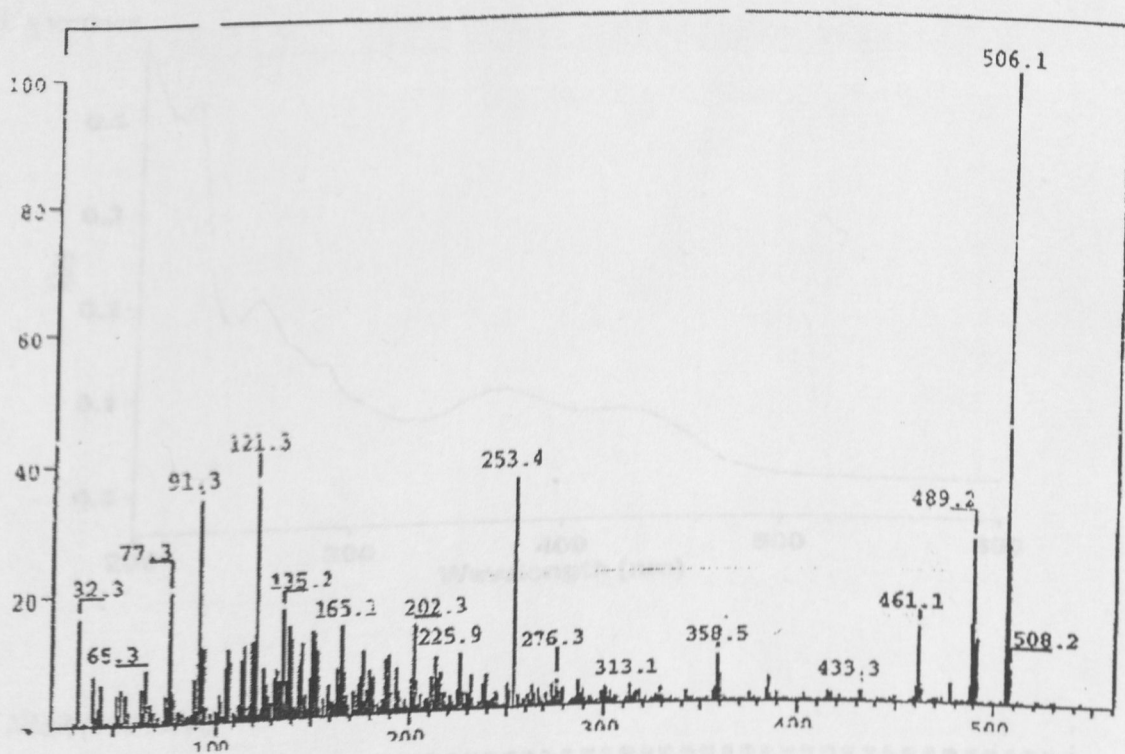
^{13}C Spectrum (expansion)



^{13}C NMR Spectrum for compound 3 (300 MHz)

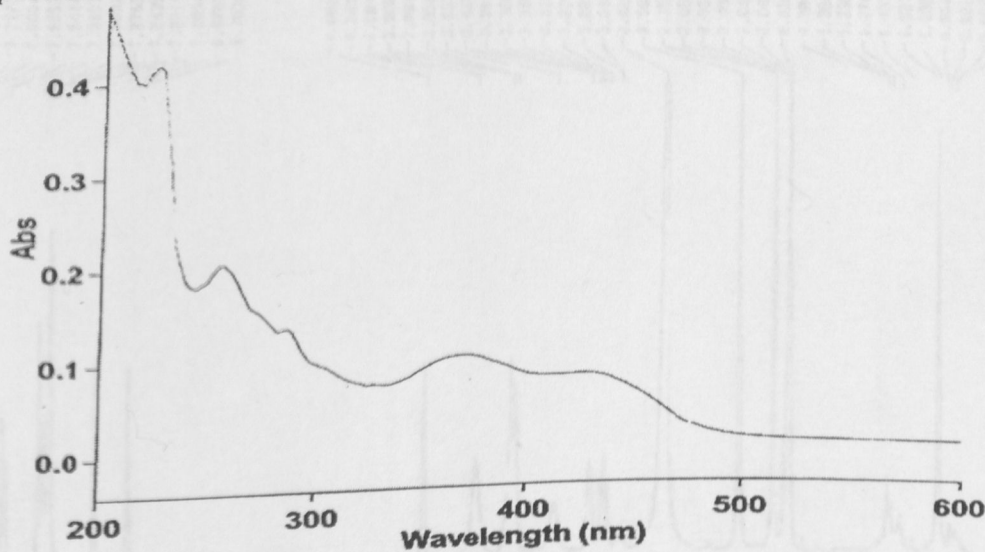


Mass spectrum for compound 3

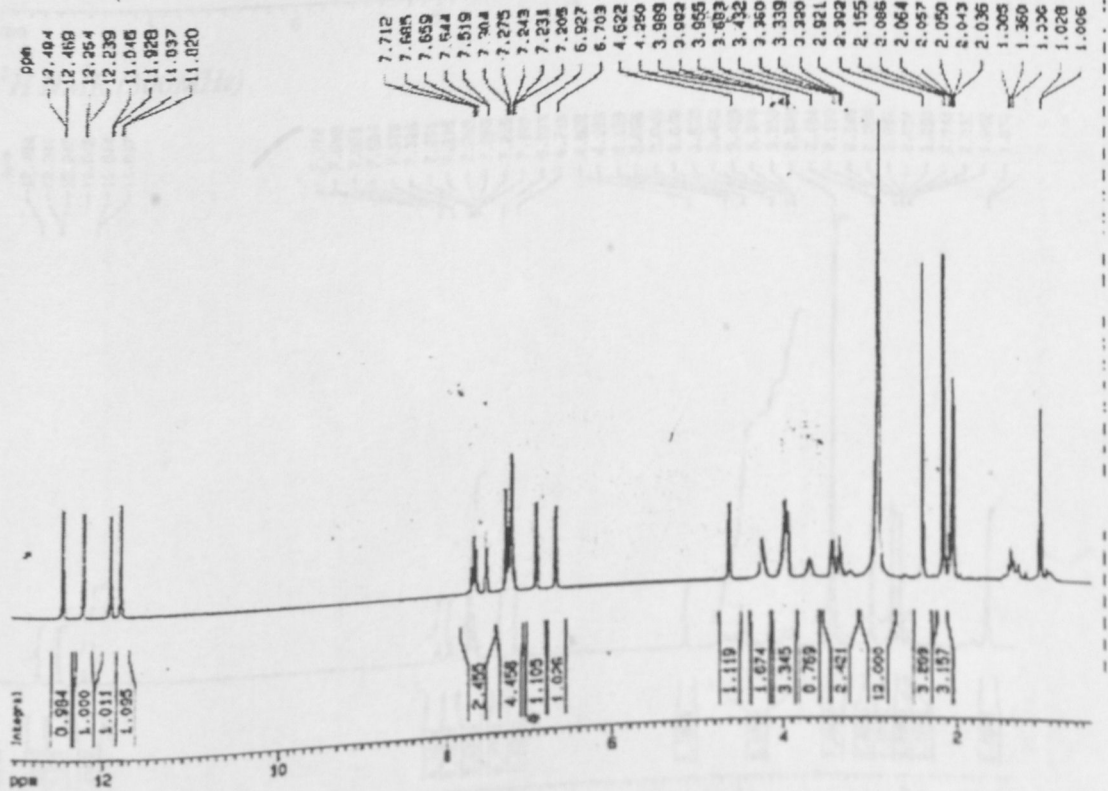


APPENDIX III SPECTRA FOR COMPOUND 4

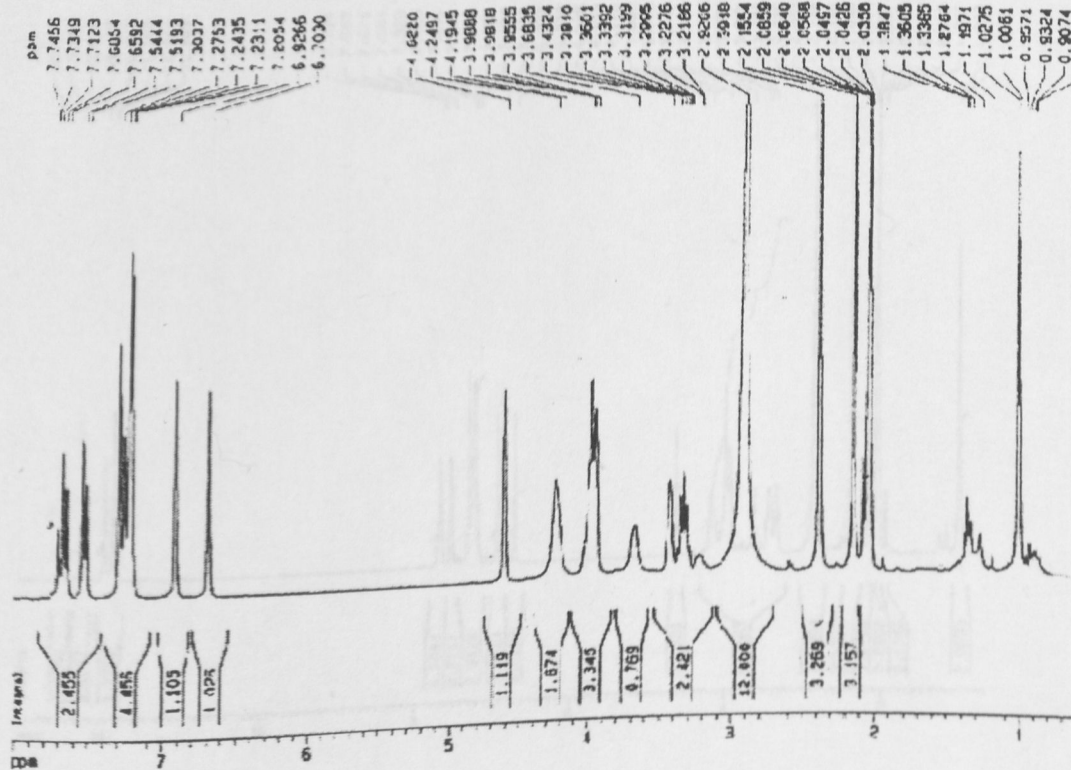
UV spectrum



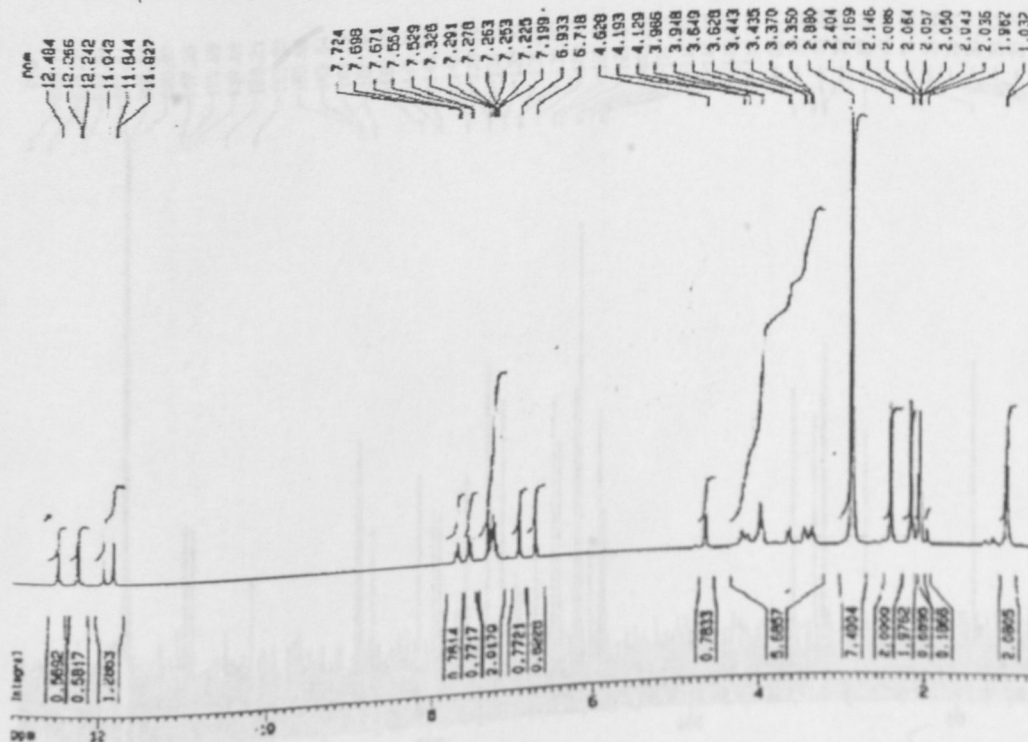
^1H NMR (300 MHz)



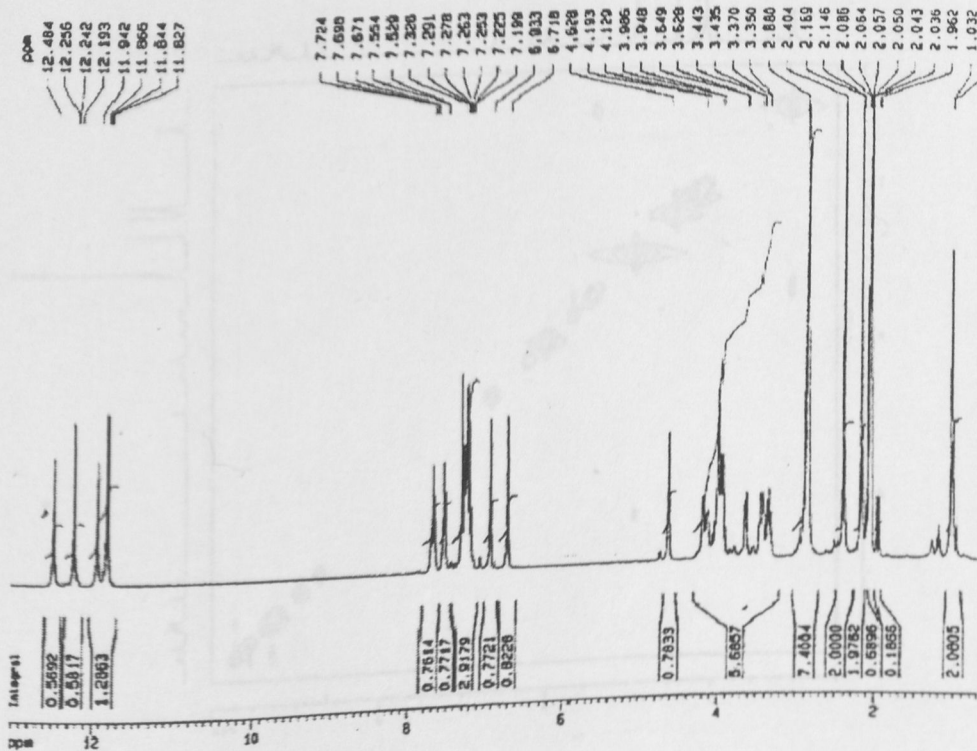
^1H NMR (expansion)



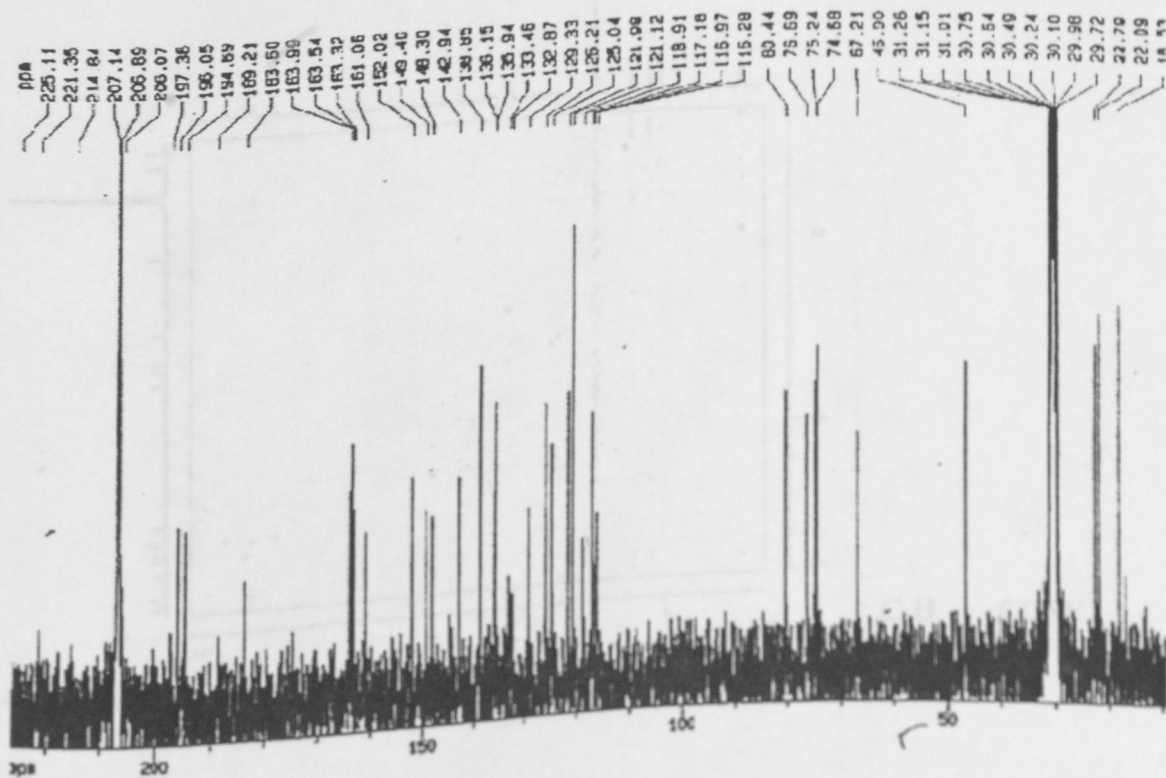
^1H NMR (300MHz)



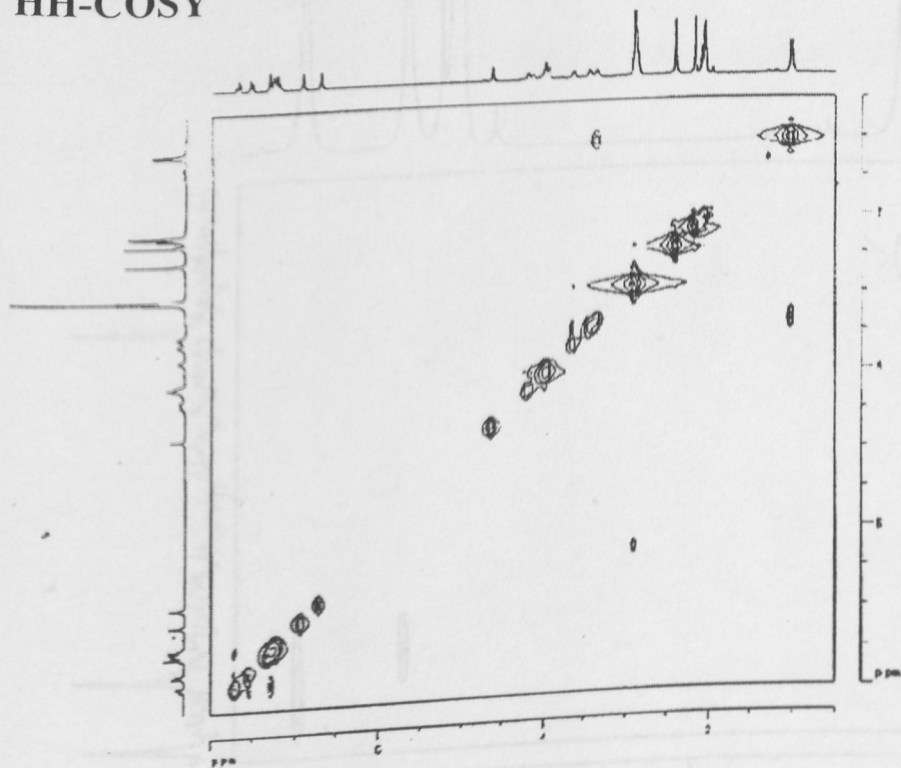
^1H NMR (expansion)



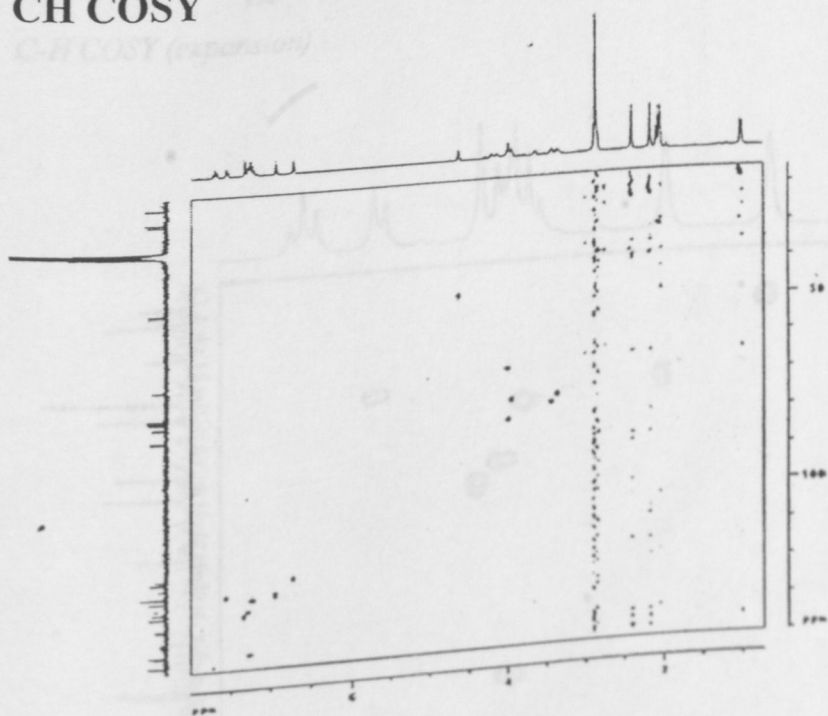
^{13}C NMR (75 MHz)



HH-COSY



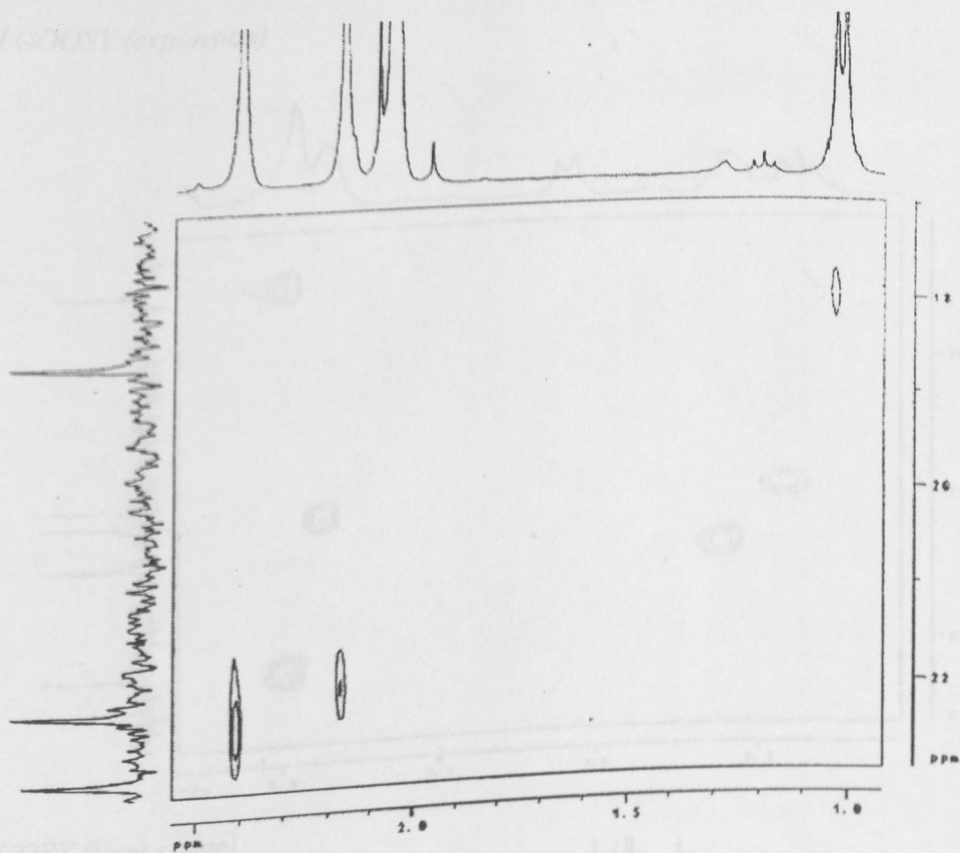
CH COSY



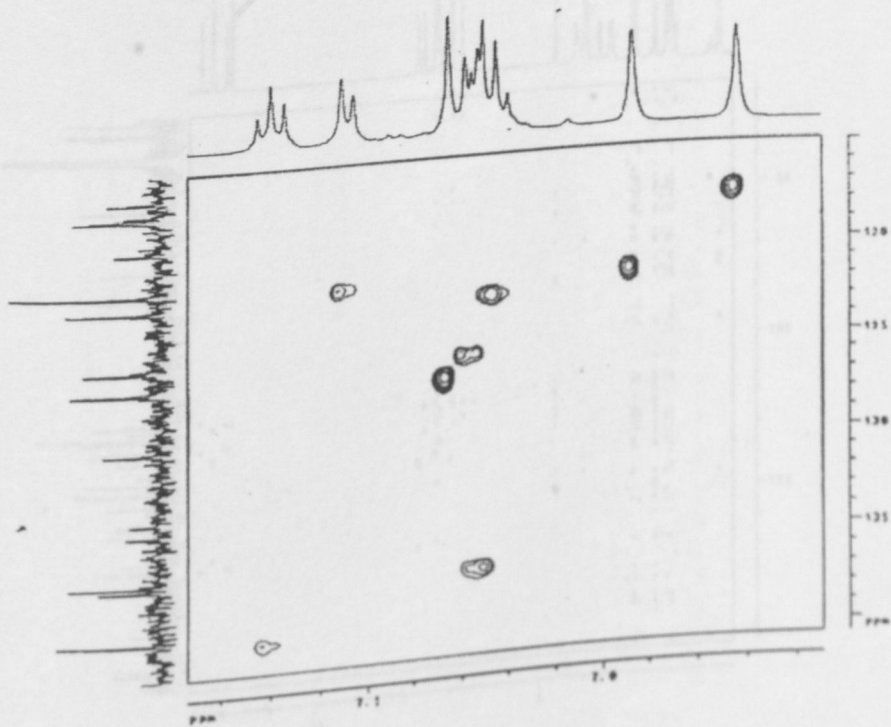
C-H COSY

(expansion)

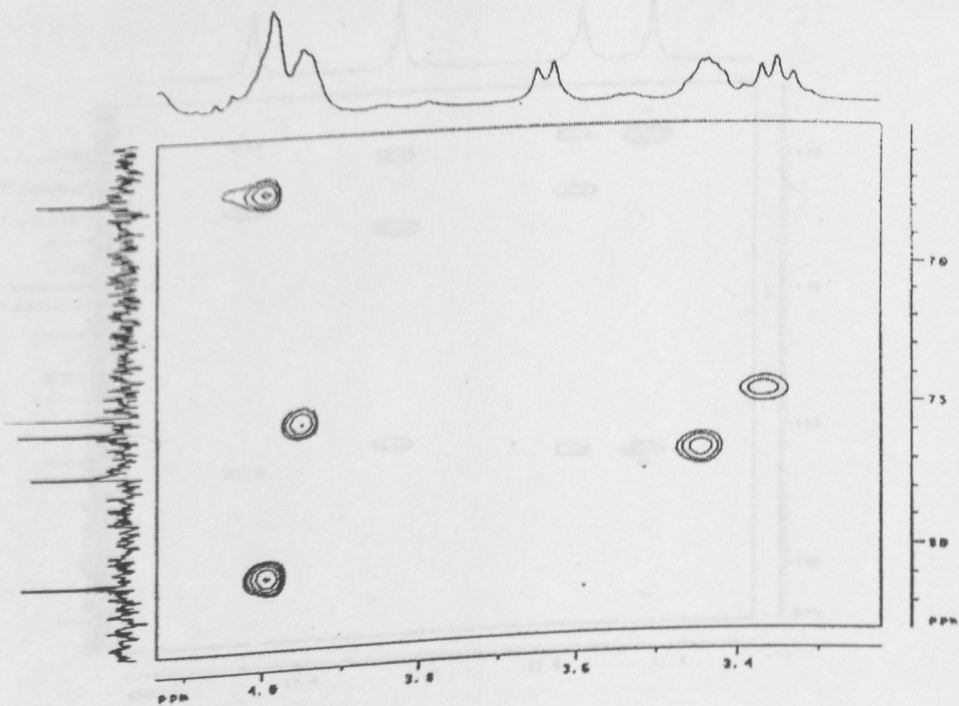
C-H COSY (expansion)



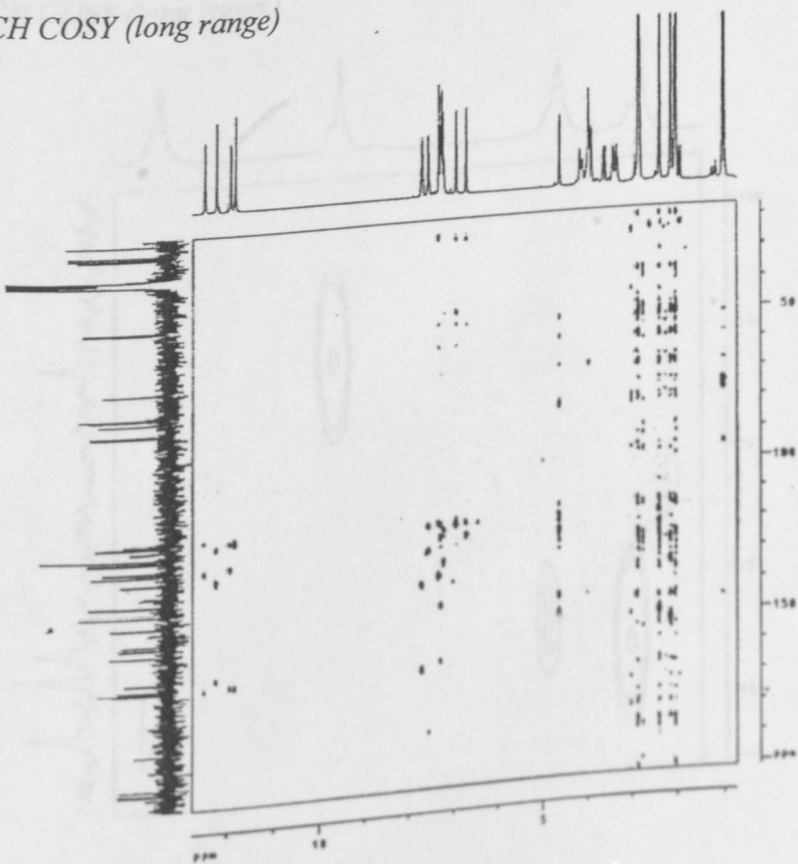
C-H COSY (expansion)



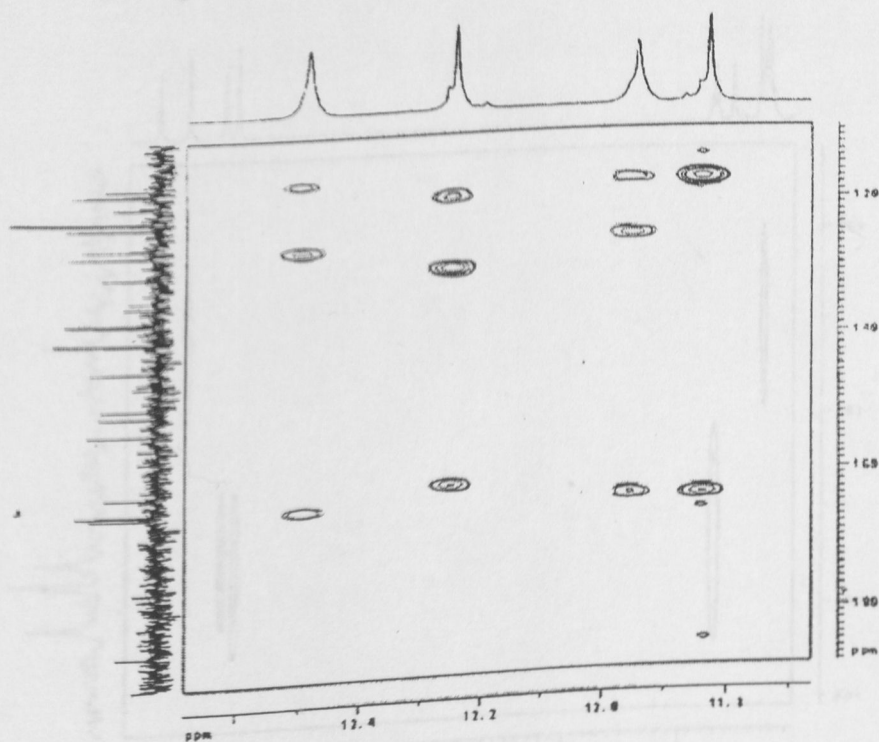
C-H COOSY (expansion)



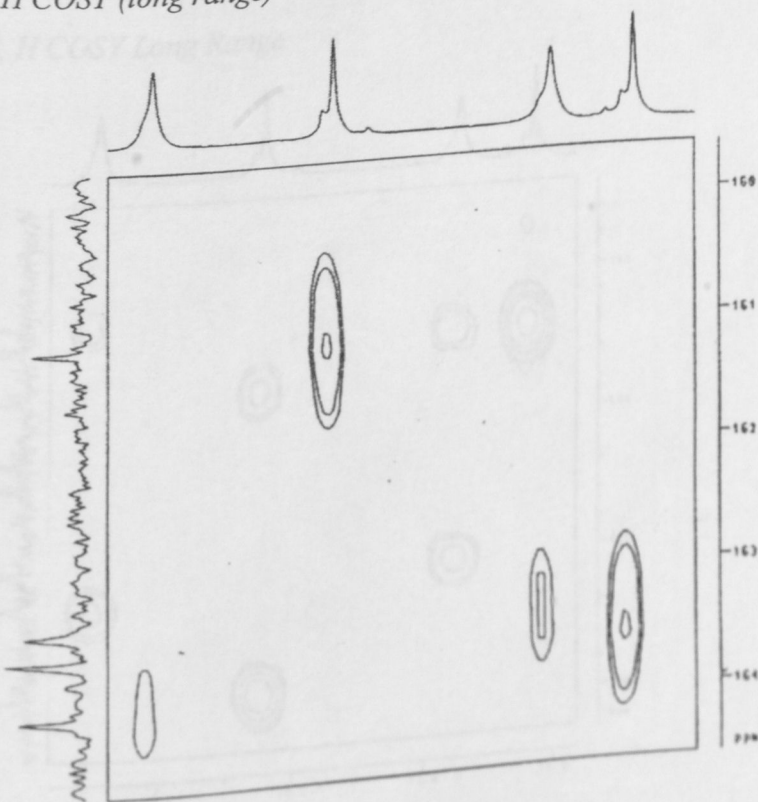
CH COSY (long range)



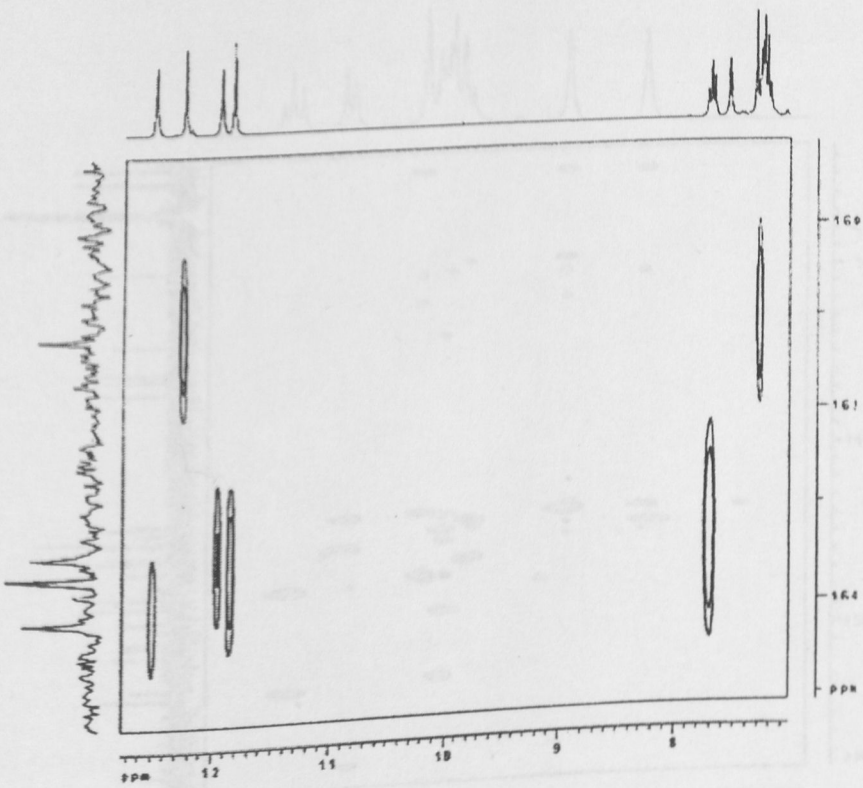
CH COSY (long range)



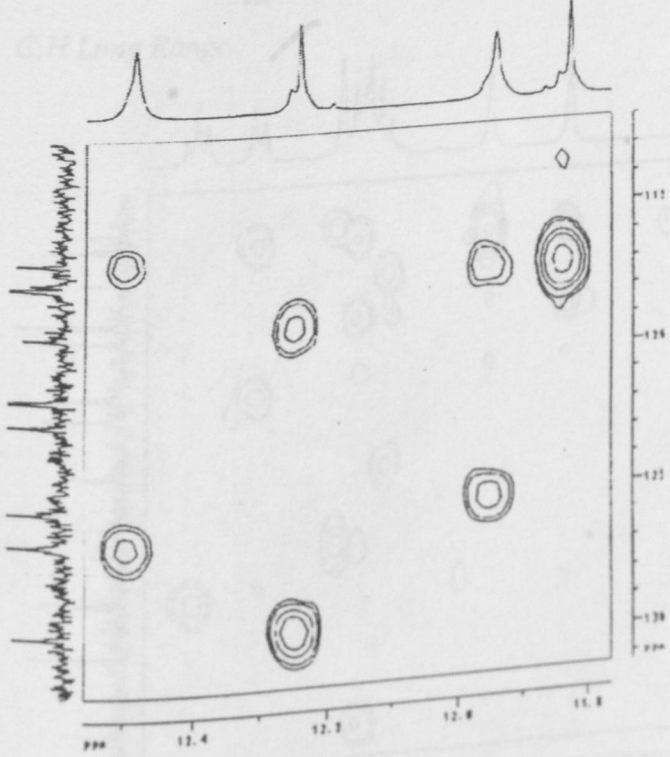
CH COSY (long range)



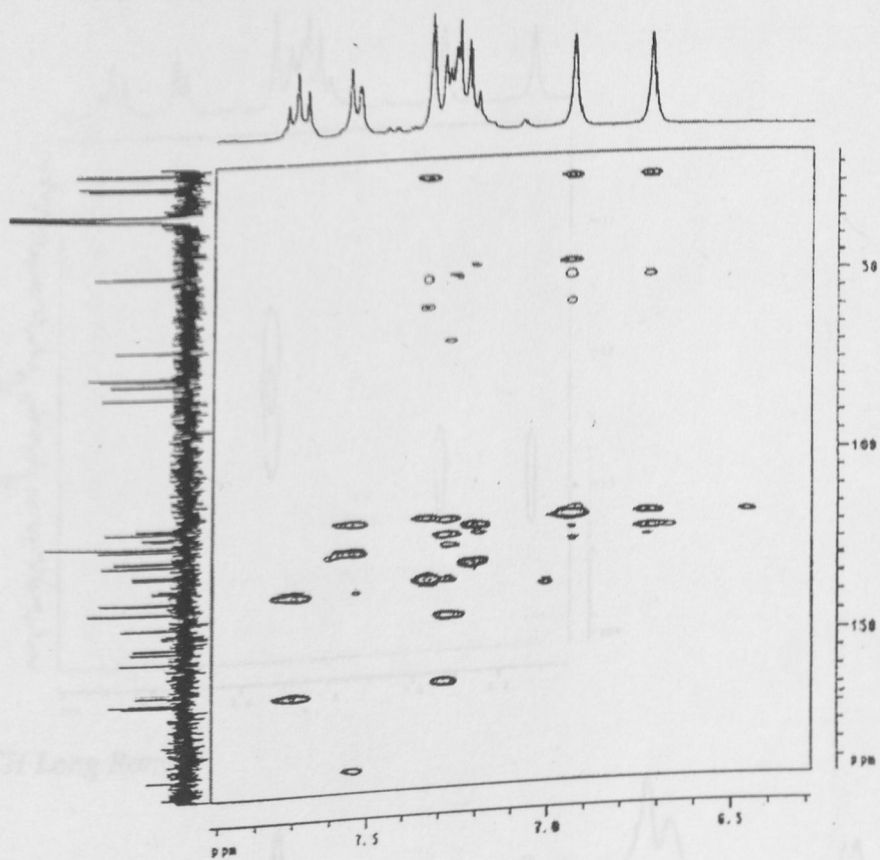
C,H Long Range



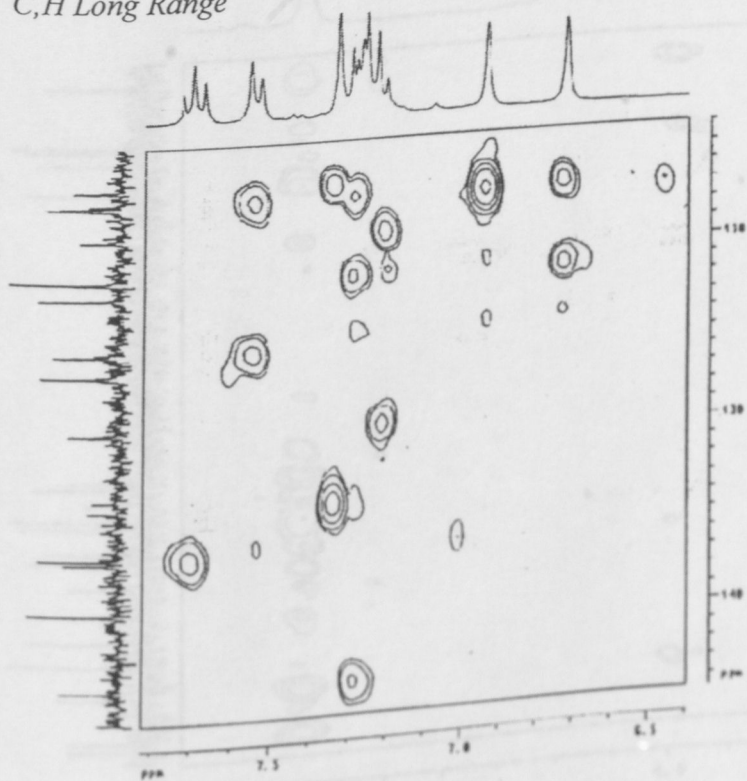
C, H COSY Long Range



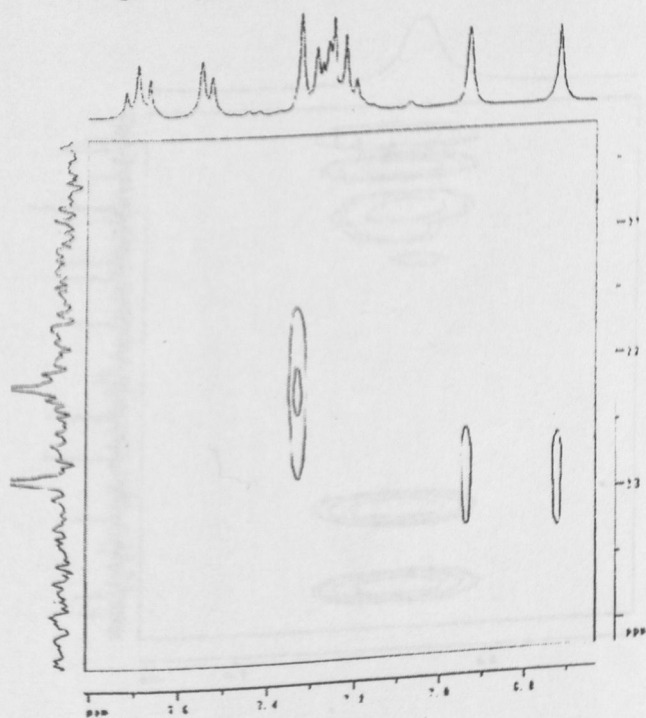
C, H Long Range



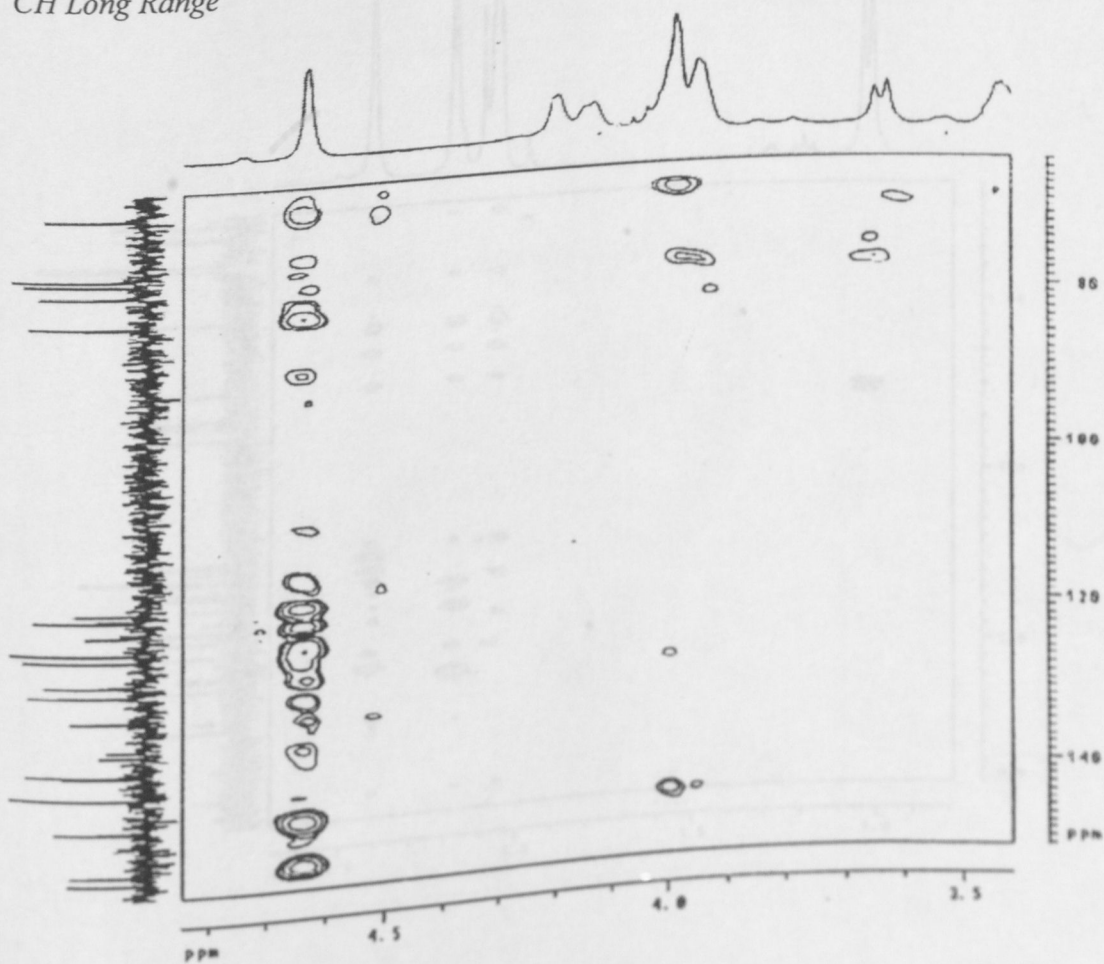
C,H Long Range



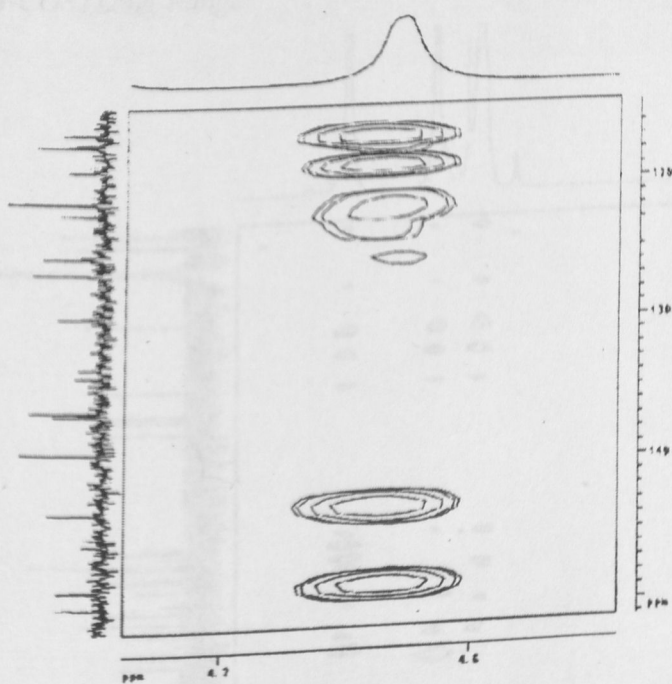
CH Long Range



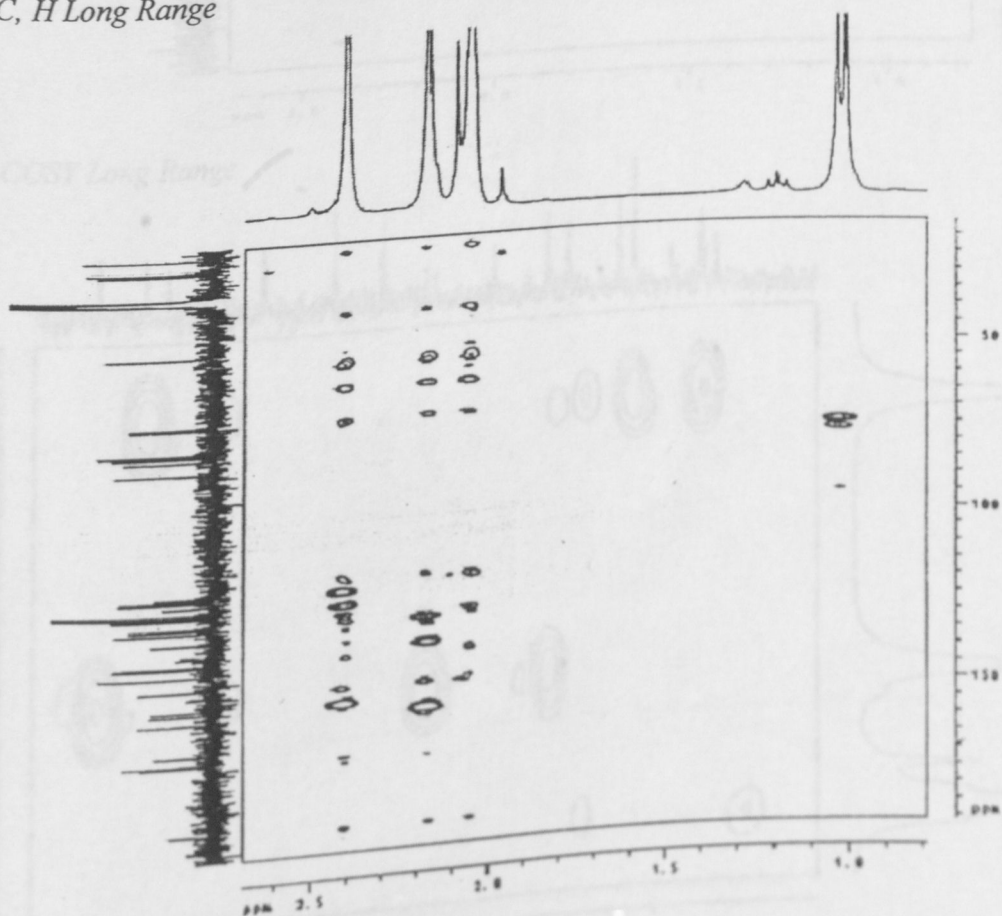
CH Long Range



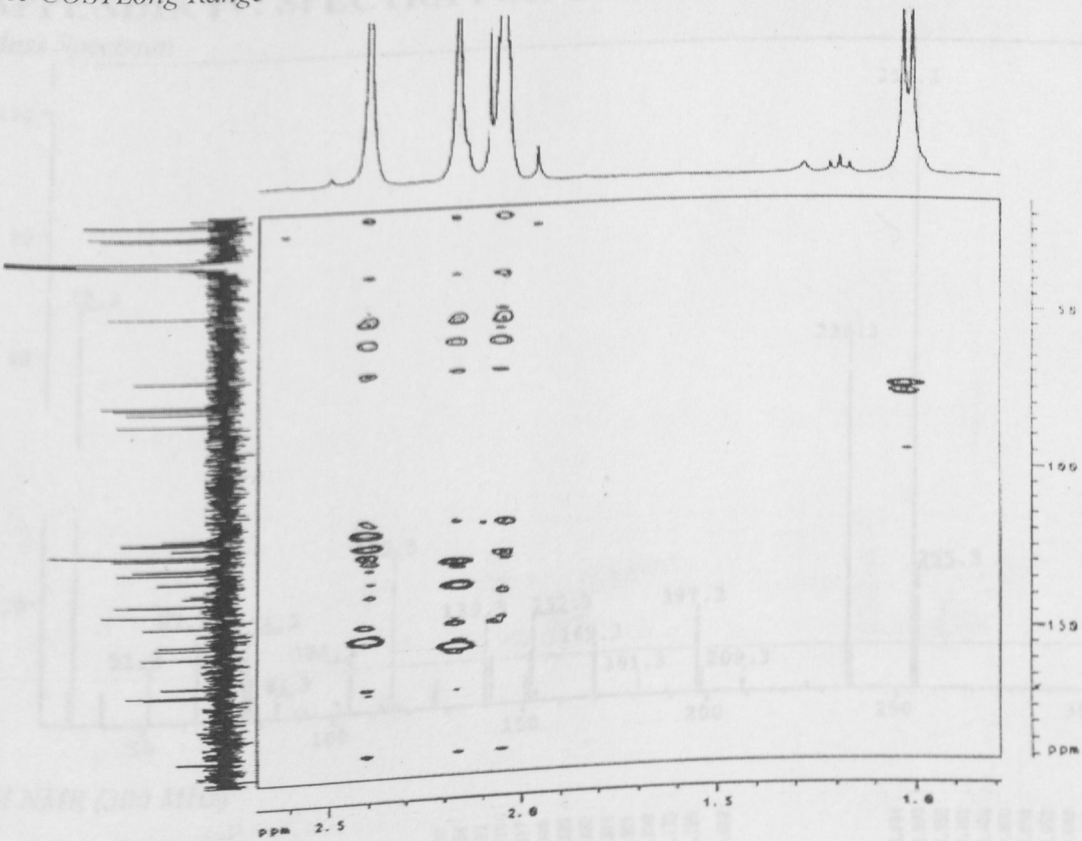
CH Long Range



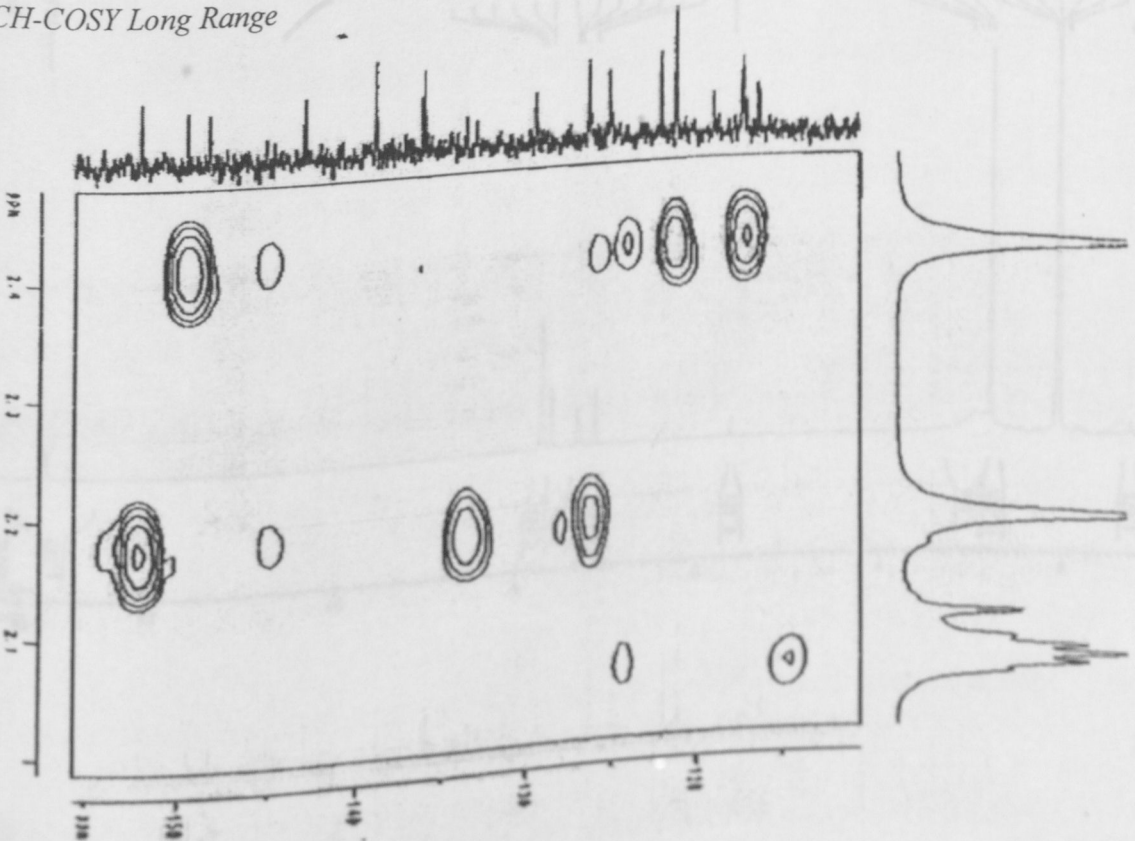
C, H Long Range



CH-COSY Long Range

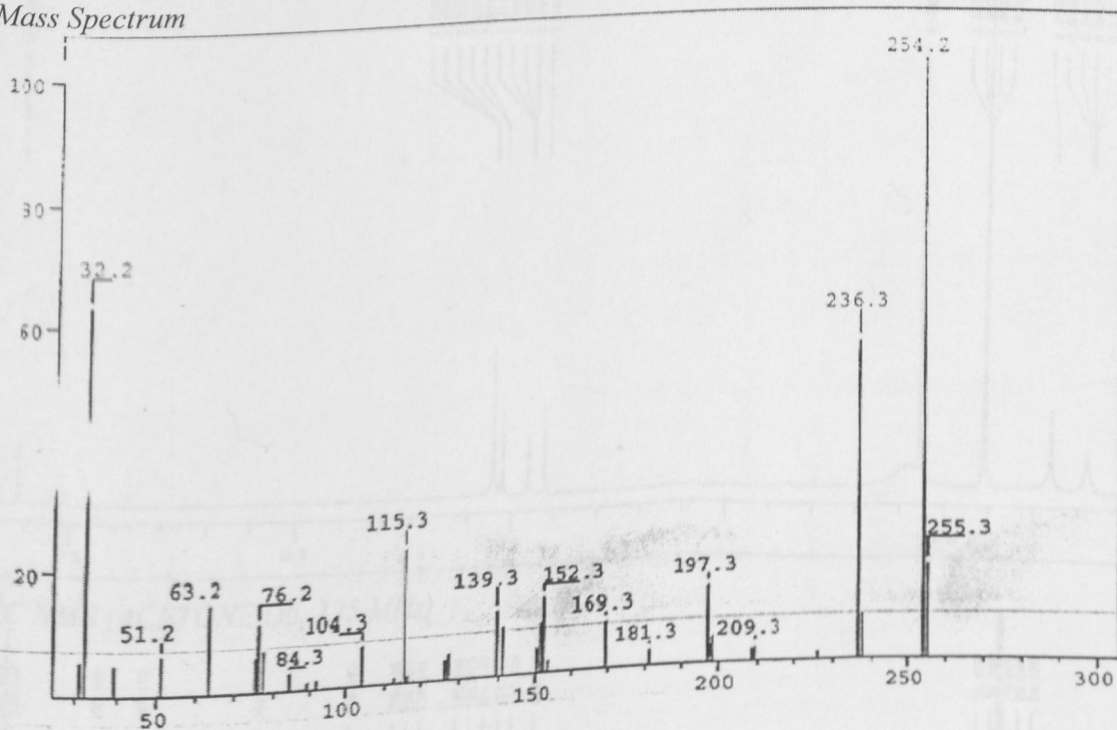


CH-COSY Long Range

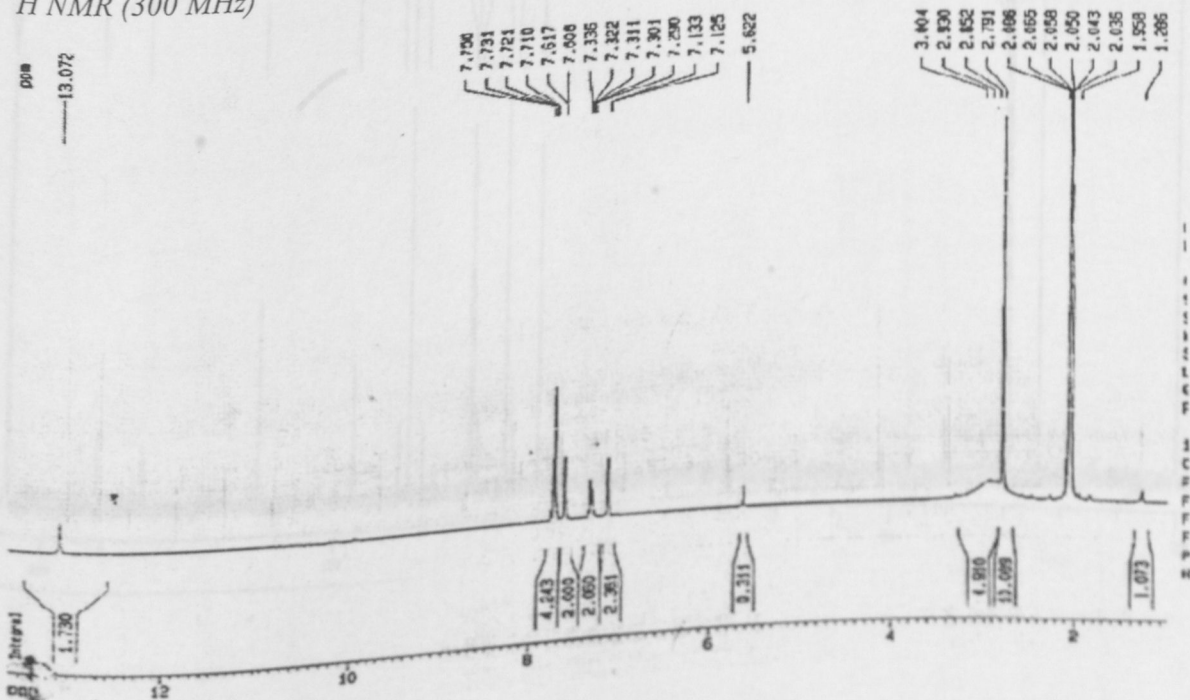


APPENDIX IV: SPECTRA FOR COMPOUND 6

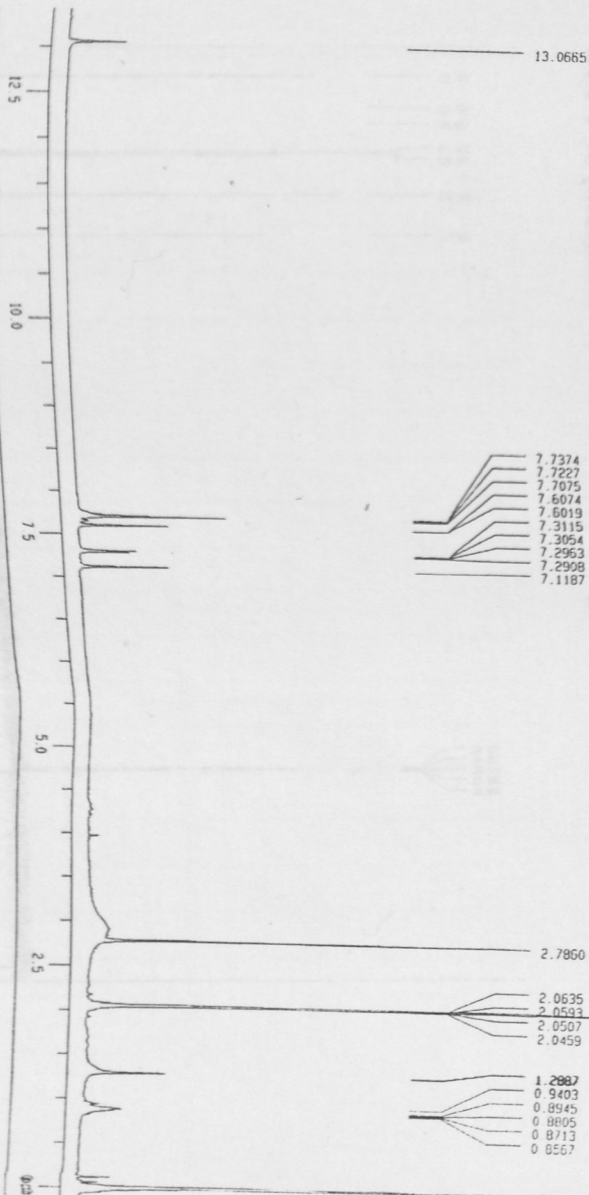
Mass Spectrum



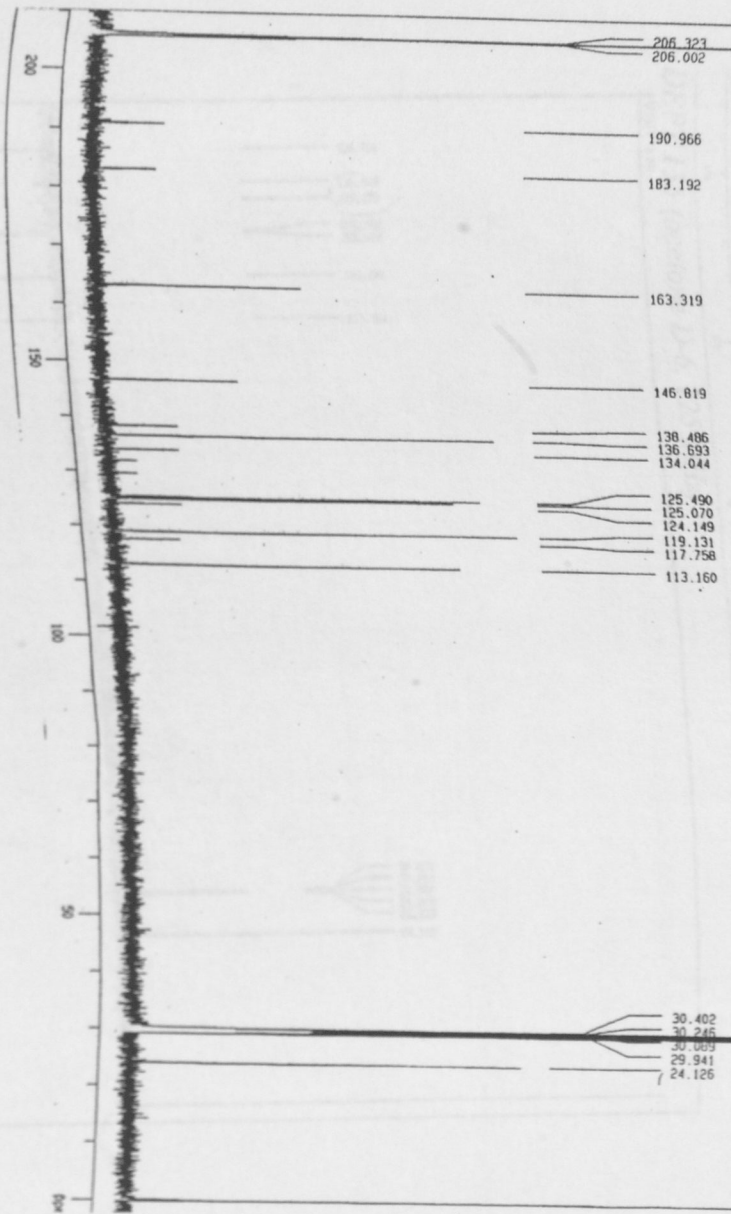
^1H NMR (300 MHz)



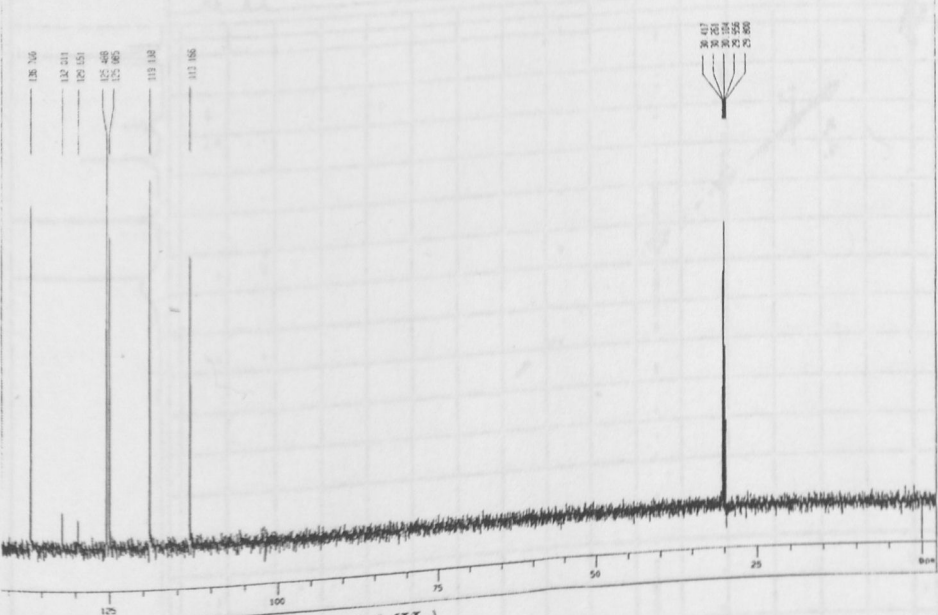
¹H NMR (acetone D₆, 500 MHz)



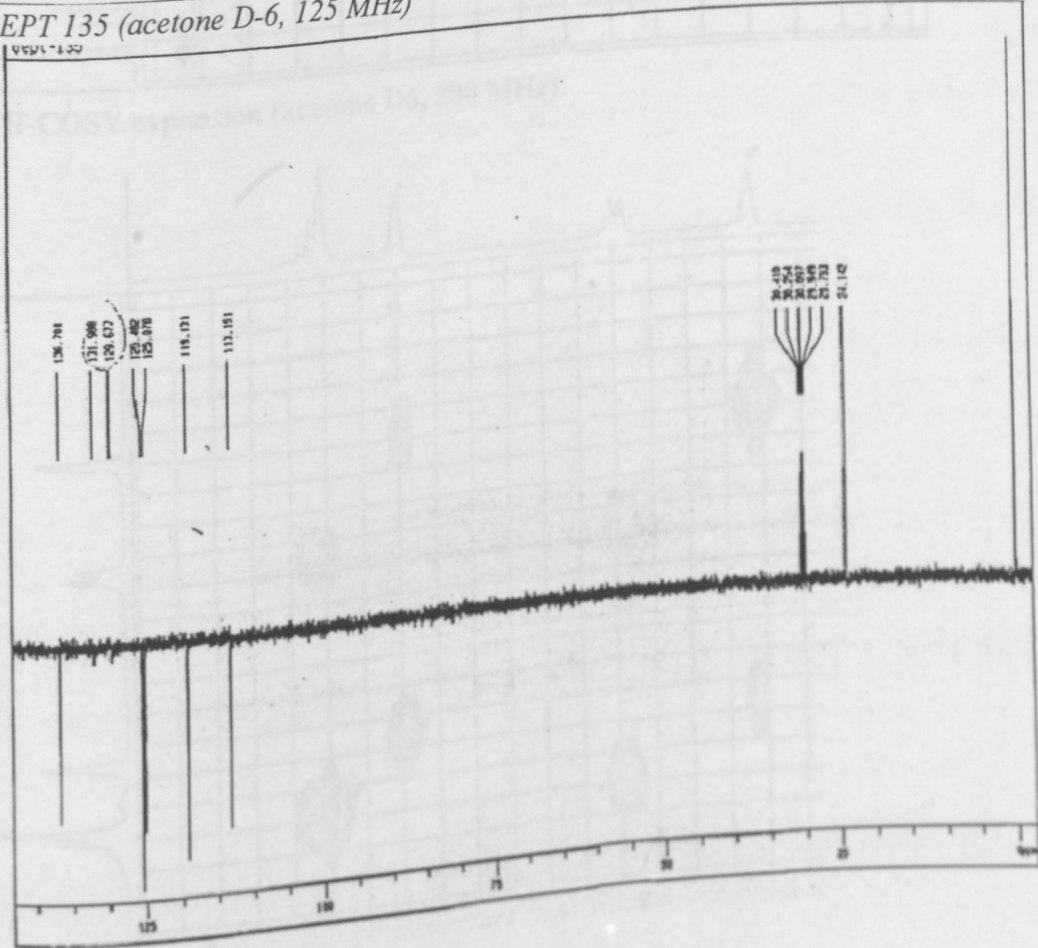
¹³C NMR (ACETONE D₆, 125 MHz)



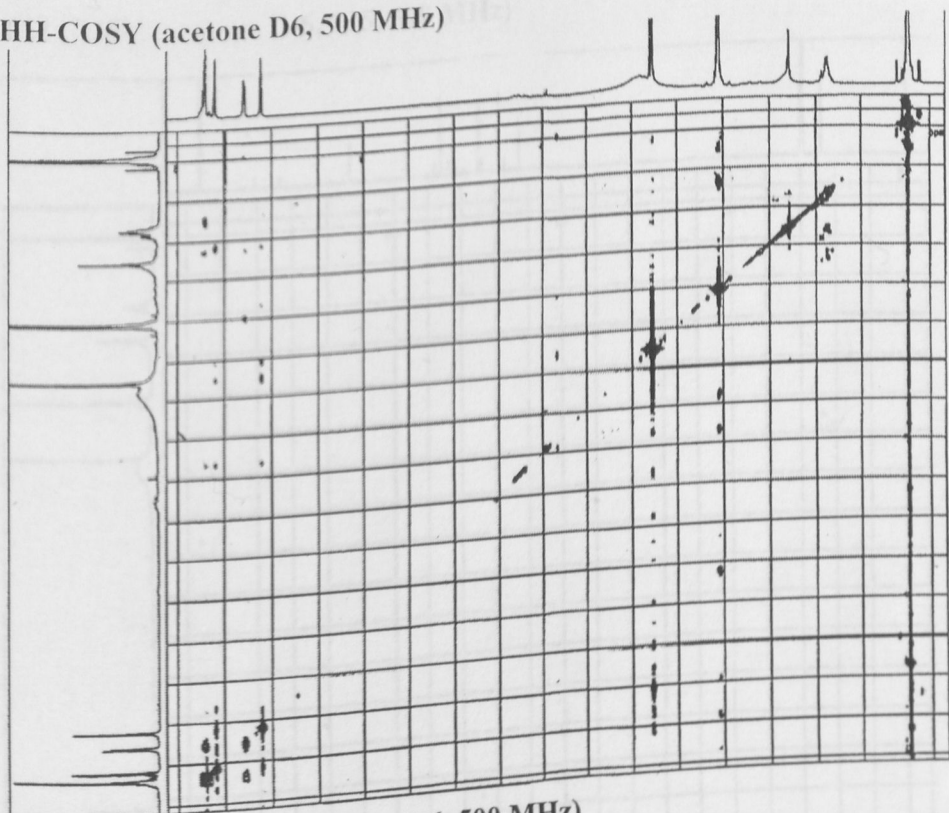
DEPT 90 (acetone D-6, 125 MHz)



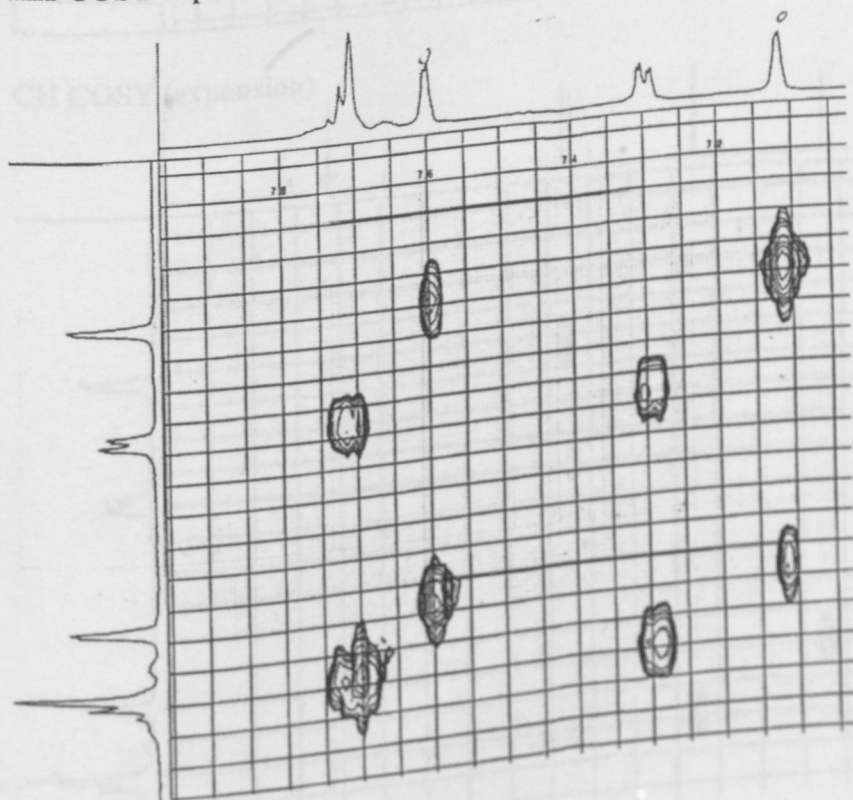
DEPT 135 (acetone D-6, 125 MHz)



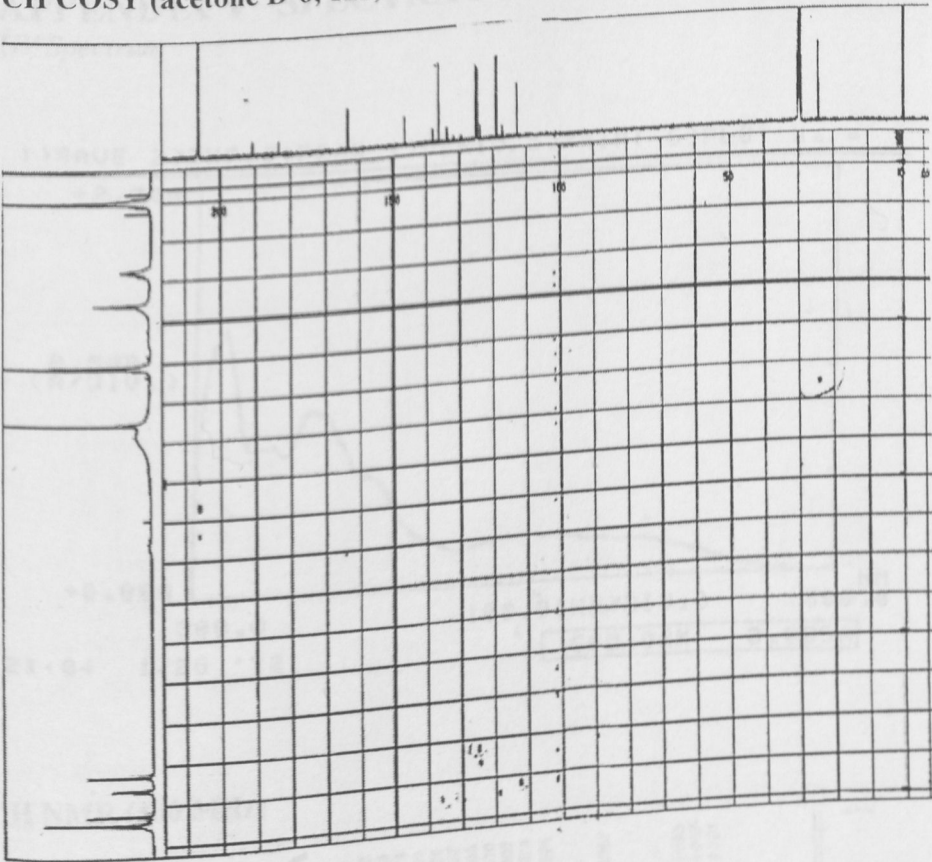
HH-COSY (acetone D6, 500 MHz)



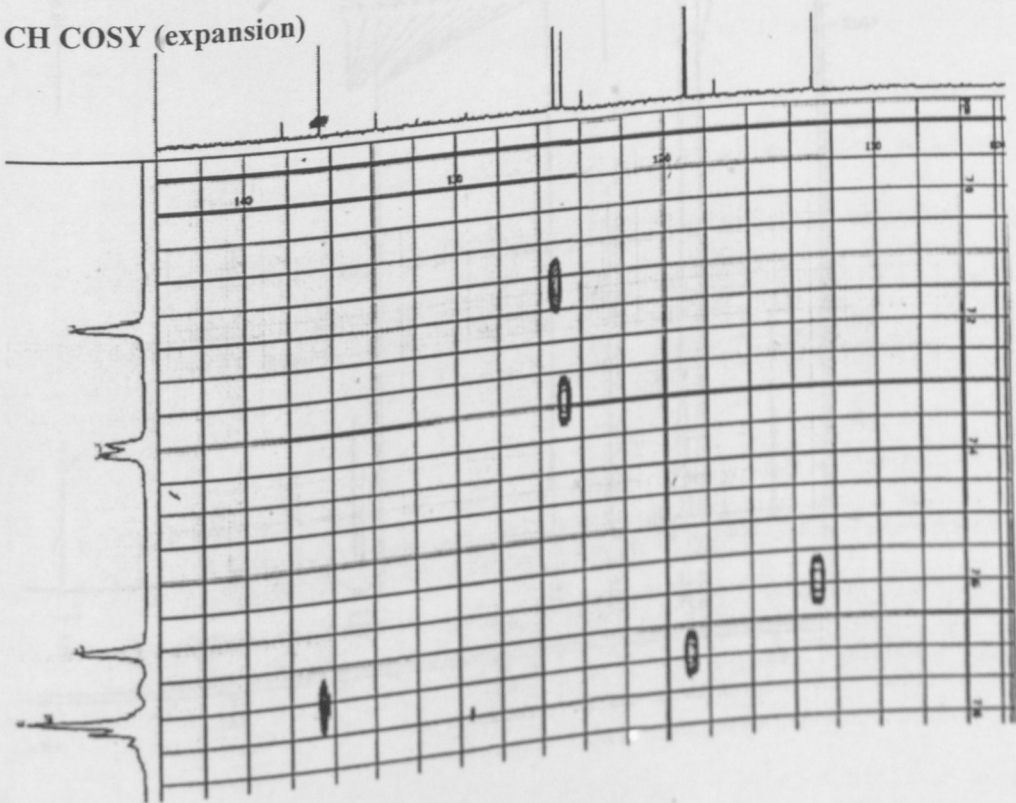
HH-COSY expansion (acetone D6, 500 MHz)



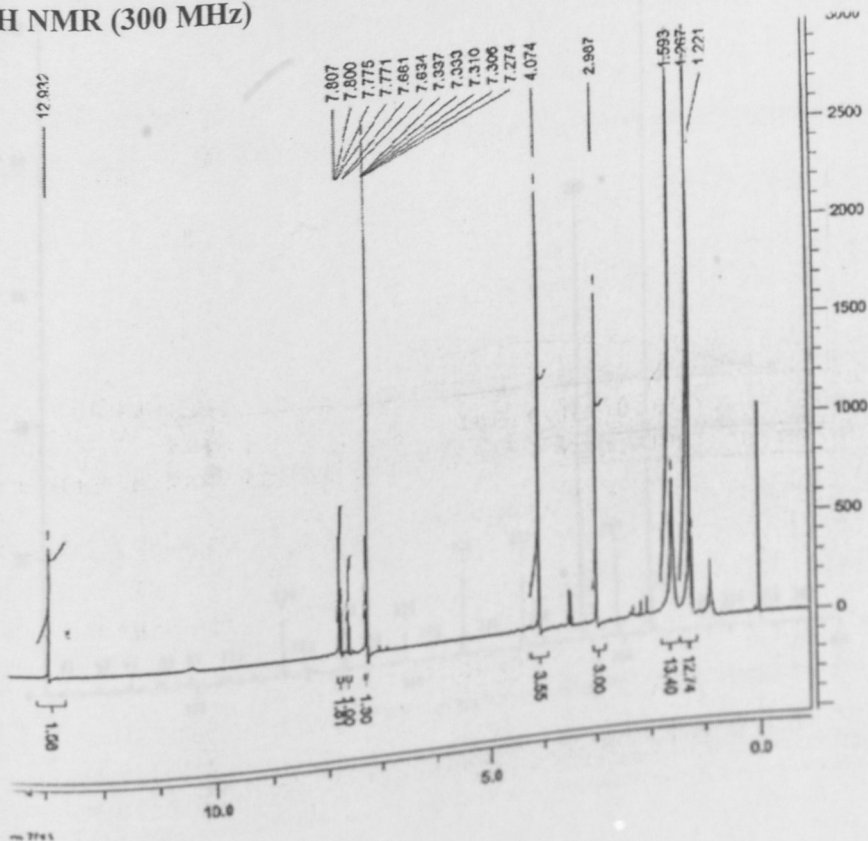
CH COSY (acetone D-6, 125, 500 MHz)



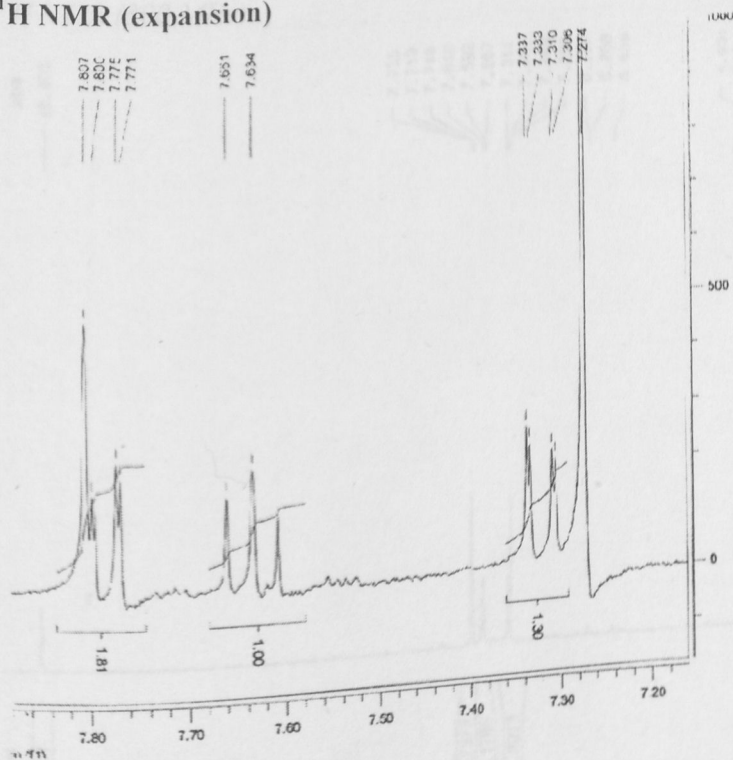
CH COSY (expansion)



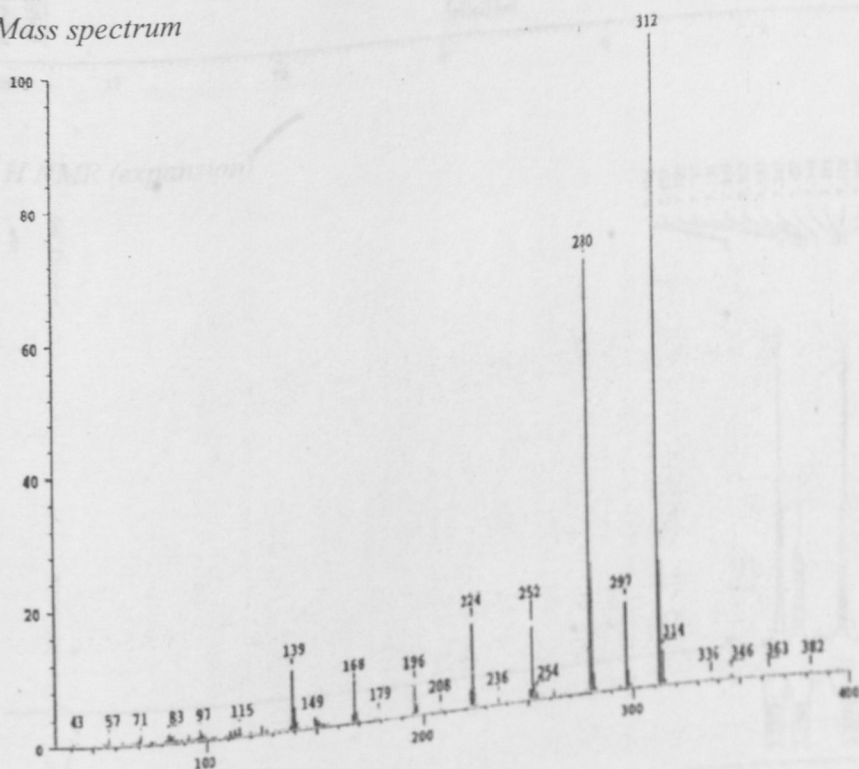
UV Spectrum

[illegible]¹H NMR (300 MHz)

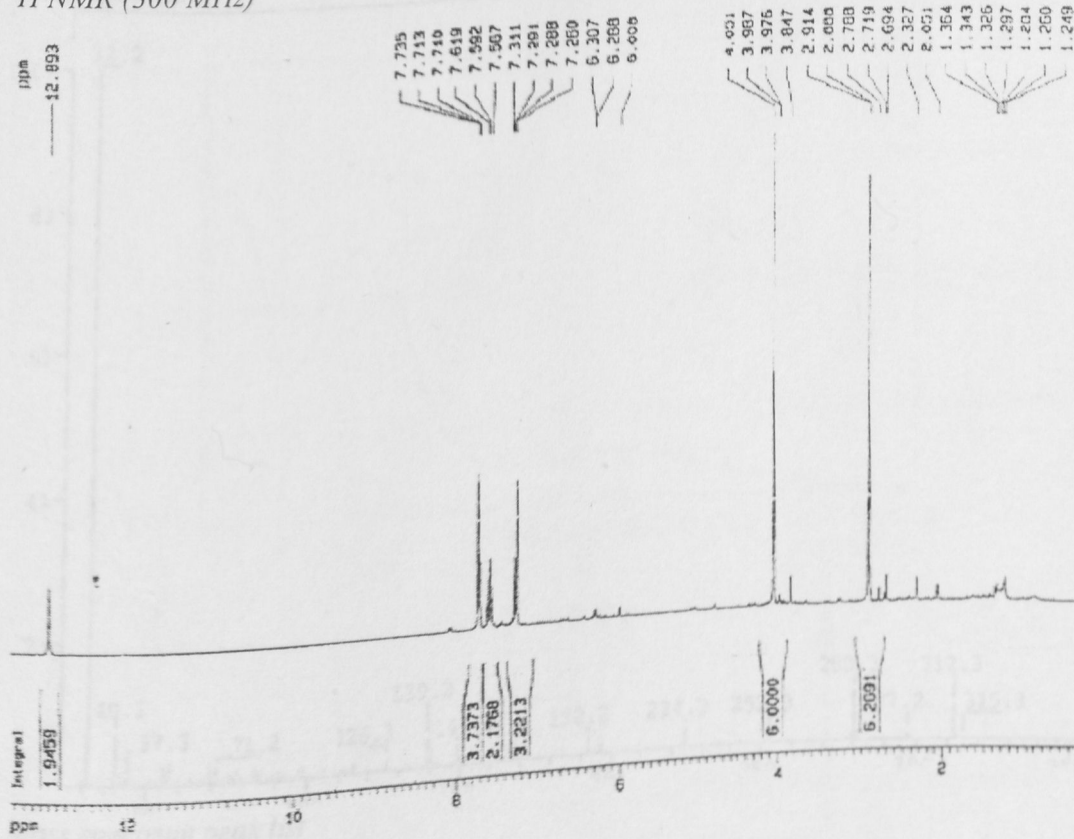
¹H NMR (expansion)



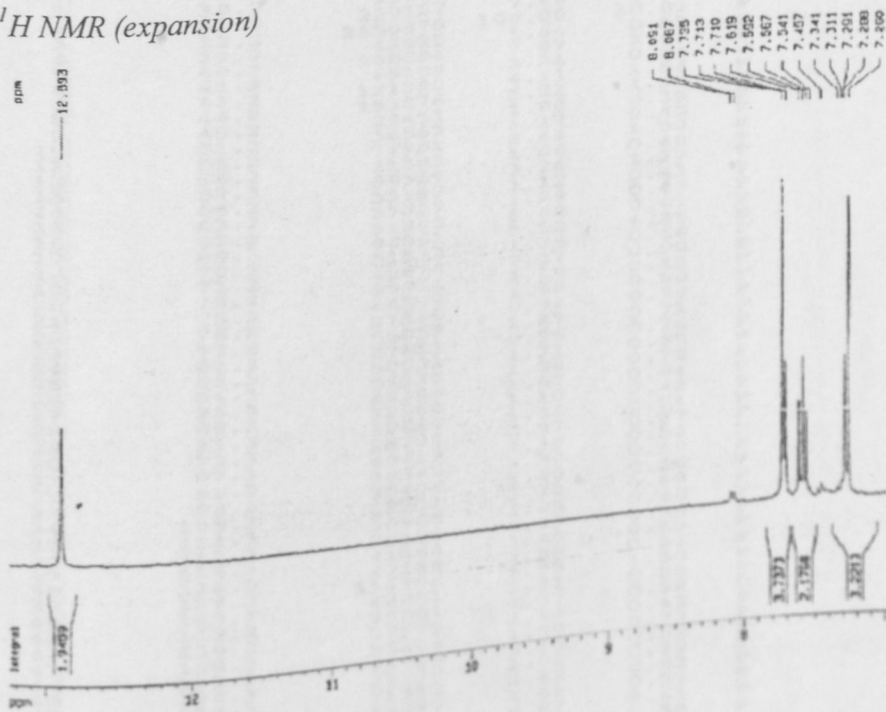
Mass spectrum



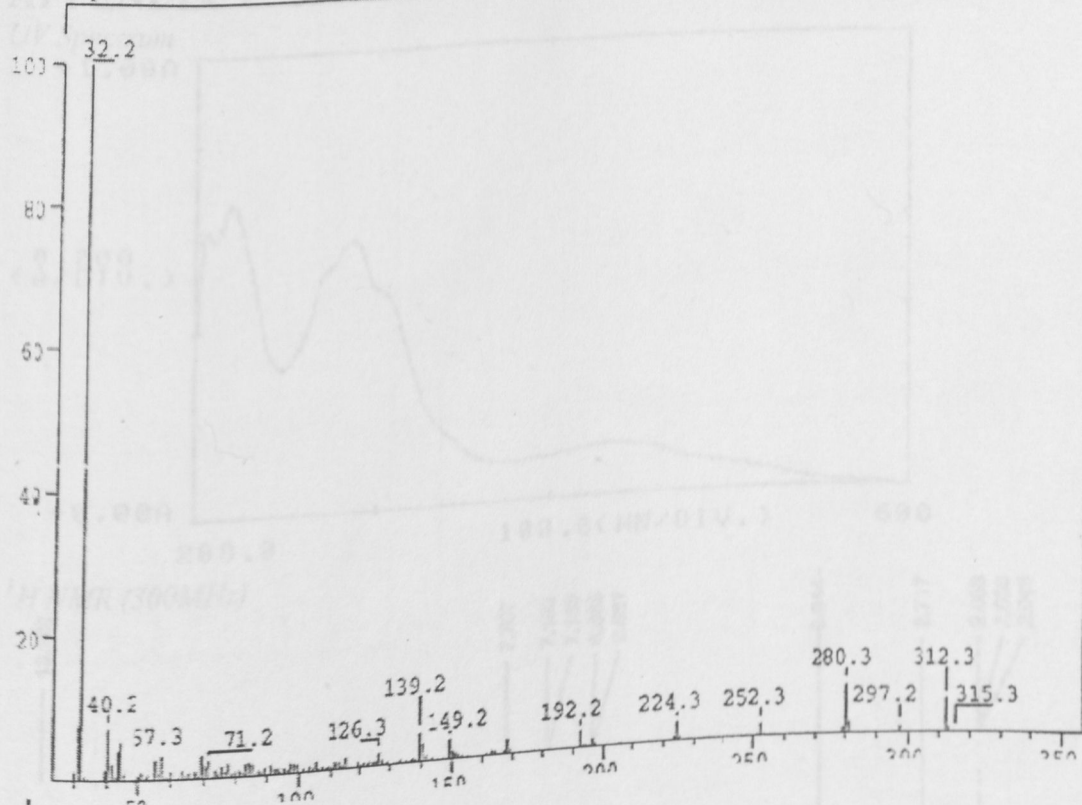
^1H NMR (300 MHz)



^1H NMR (expansion)



Mass spectrum

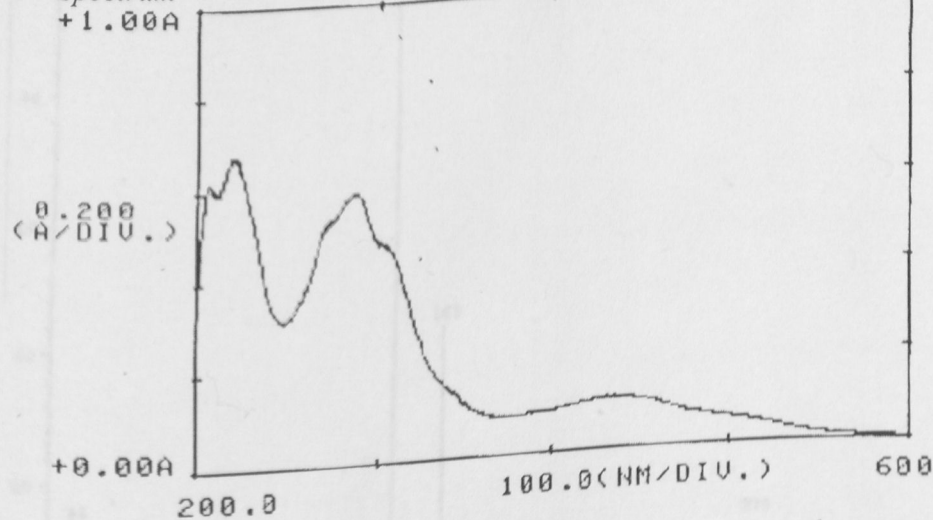


Mass spectrum peak list

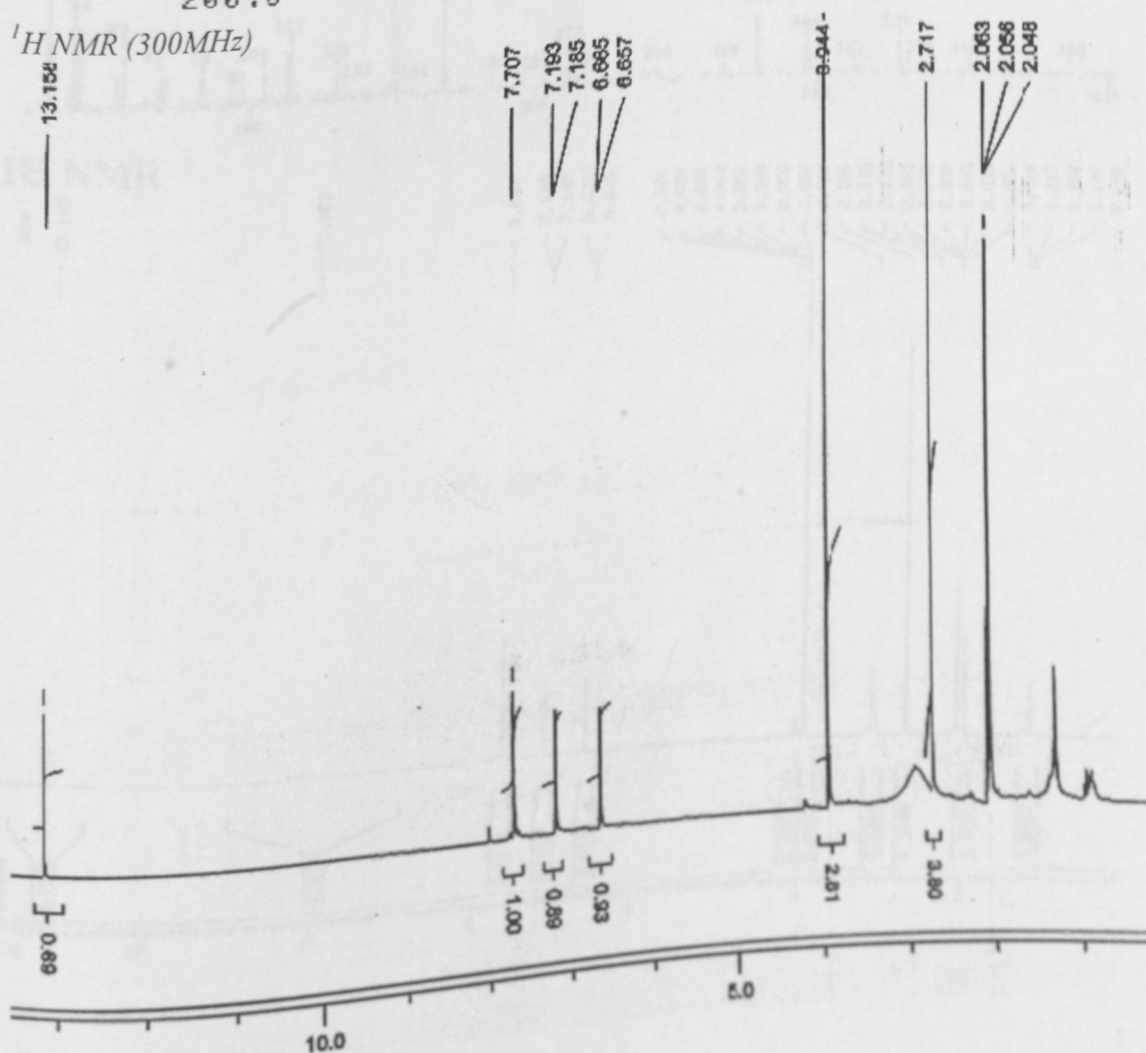
No.	Mass	Intensity	%RA	%RIC	Flags
1	27594		1.20	0.46	FM
2	30.2	35450	7.46	2.84	F
3	31.3	220127	100.00	38.02	F
4	32.2	2950875	1.44	0.55	F
5	39.2	42425	7.07	2.69	F
6	40.2	208654	2.21	0.84	F
7	41.2	65199	4.11	1.56	F
8	43.2	121390	5.21	1.98	F
9	44.2	153876	1.00	0.38	F
10	45.2	29451	1.17	0.45	F
11	51.2	34563	2.64	1.00	F
12	55.2	77951	1.06	0.40	F
13	56.3	31383	3.34	1.27	F
14	57.3	98559	1.12	0.43	F
15	60.3	33195	1.29	0.49	F
16	63.3	37927	1.08	0.41	F
17	67.3	31883	1.28	1.25	F
18	69.3	96861	1.75	0.66	F
19	70.3	51522	2.17	0.90	F
20	71.3	69874	1.19	0.45	F
21	73.3	35051	1.58	0.60	F
22	75.3	46549	0.94	0.36	F
23	76.3	27595	1.87	0.71	F
24	77.3	55057	0.95	0.36	F
25	81.3	27911	0.71	0.65	F
26	83.3	20521	1.88	0.72	F
27	84.3	55525	1.67	0.64	F
28	85.3	49298	1.10	0.42	F
29	89.3	32568	1.34	0.51	F
30	91.3	39688	1.97	0.37	F
31	93.3	28722	0.38	0.52	F
32	97.3	40619	1.30	0.49	F
33	98.3	38345	1.20	0.46	F
34	99.3	35406	1.30	0.49	F
35	105.3	38231	1.05	0.40	F
36	111.3	30965	0.99	0.38	F
37	113.3	29159	1.40	0.53	F
38	115.3	41360	1.60	0.61	F
39	126.3	47138	3.95	1.50	F
40	139.2	116702	3.31	1.88	F
41	140.3	68081	2.37	0.98	F
42	149.2	75861	1.12	0.42	F
43	150.2	32979	1.10	0.72	F
44	168.3	56203	2.15	0.82	F
45	192.2	63558	1.02	0.39	F
46	196.3	29970	2.42	0.92	F
47	224.3	71312	1.49	0.57	F
48	252.3	44097	6.87	2.61	F
49	280.3	202750	1.52	0.58	F
50	312.3	44895	4.79	1.82	F

APPENDIX V SPECTRA FOR COMPOUND 8

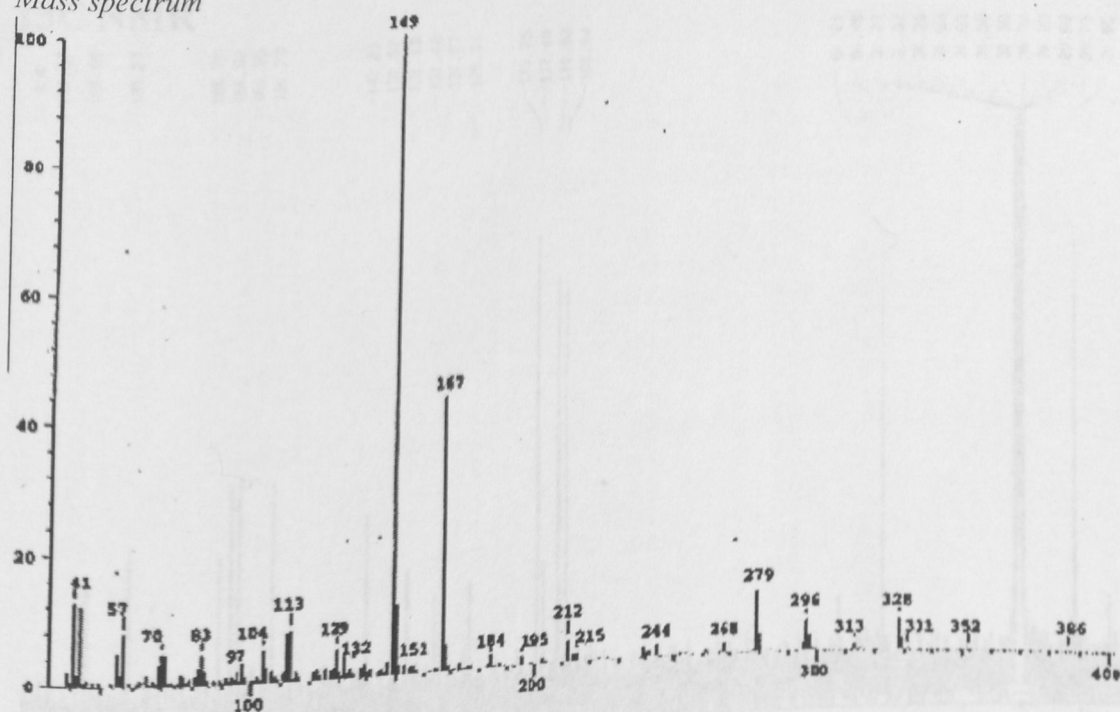
UV Spectrum



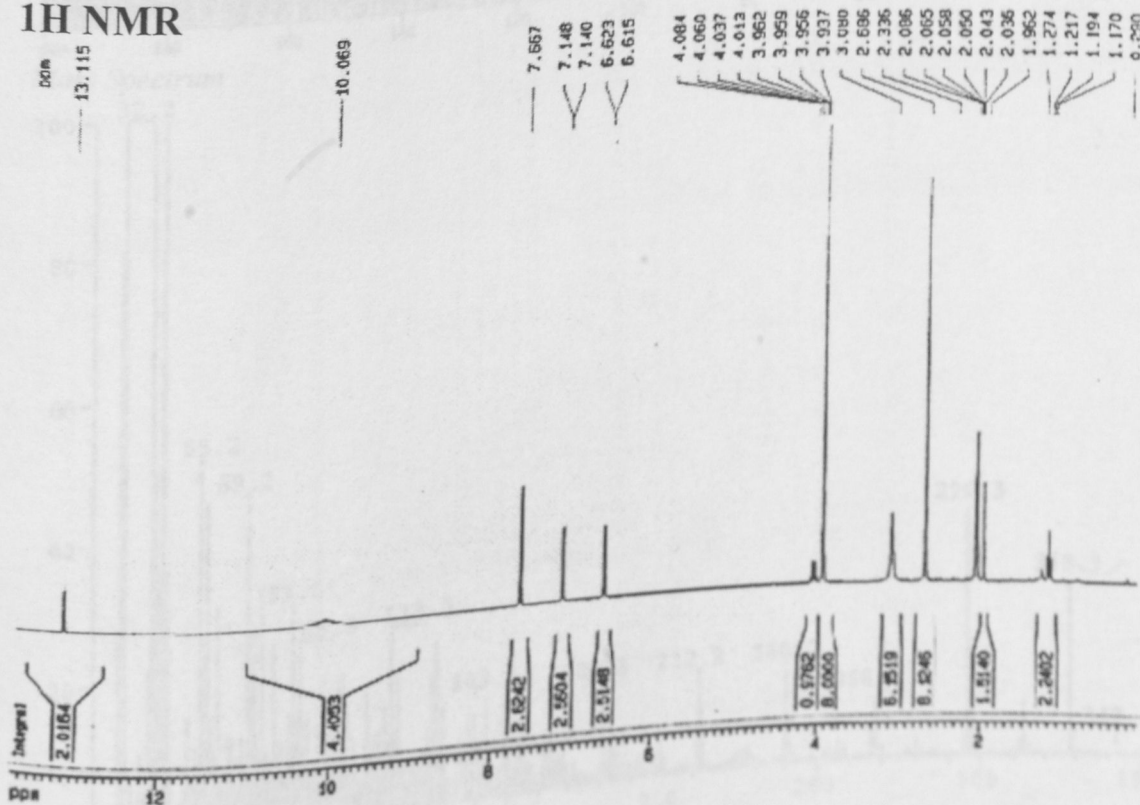
^1H NMR (300MHz)



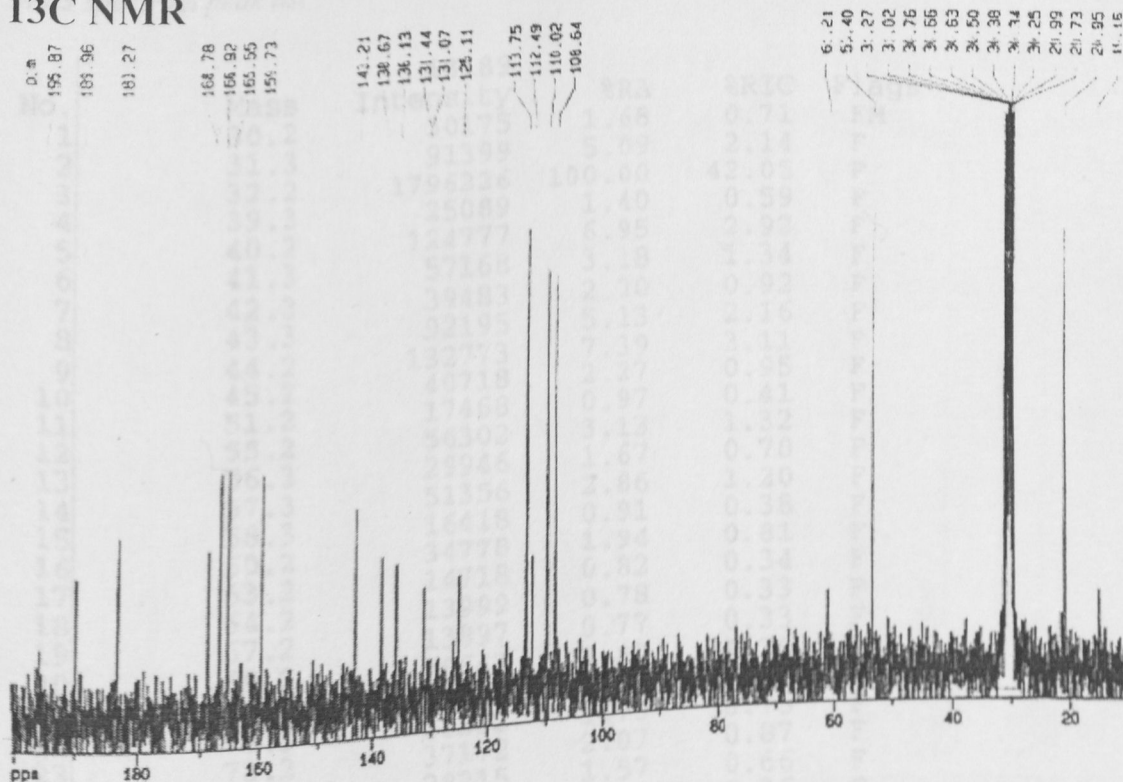
Mass spectrum



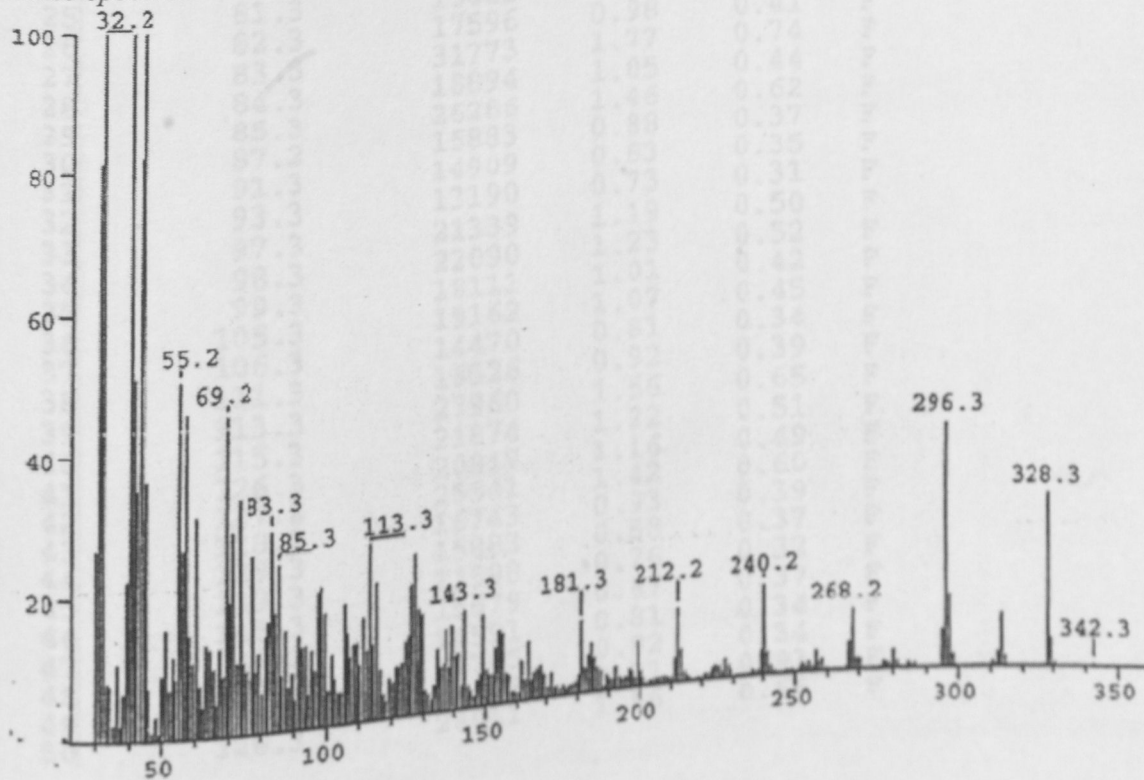
¹H NMR



¹³C NMR



Mass Spectrum

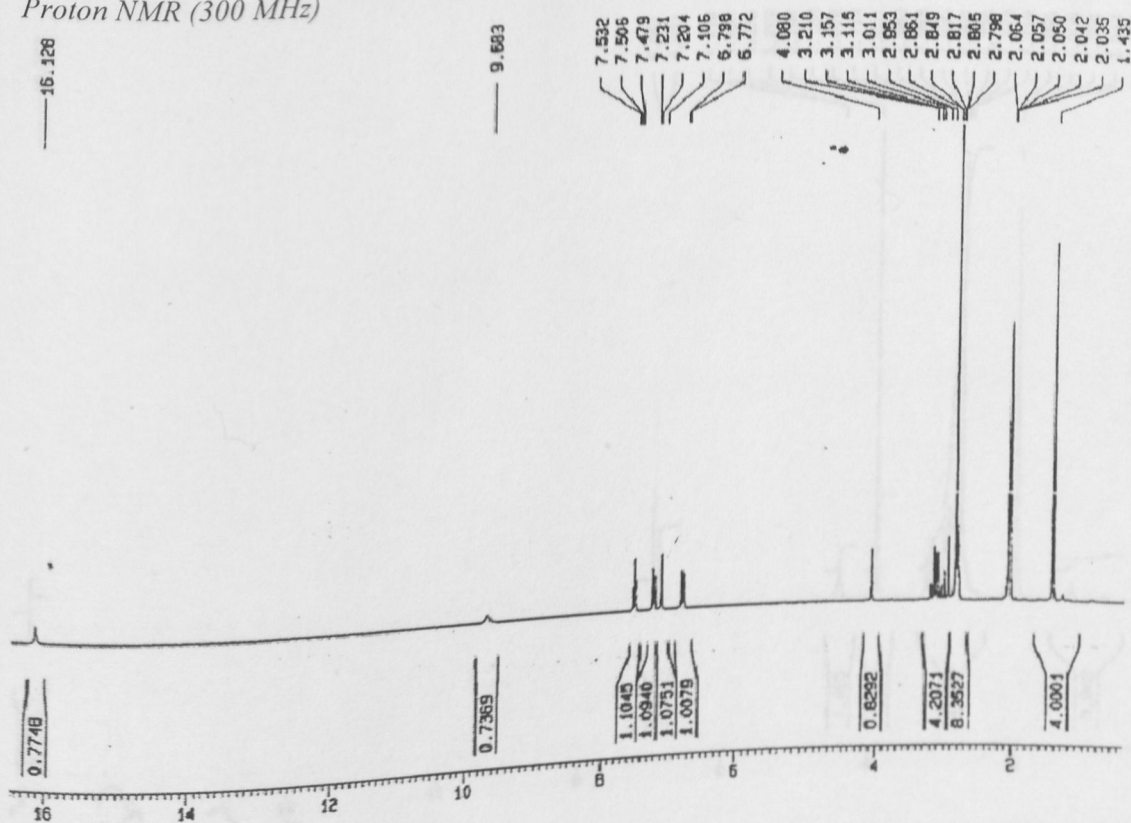


Mass spectrum peak list

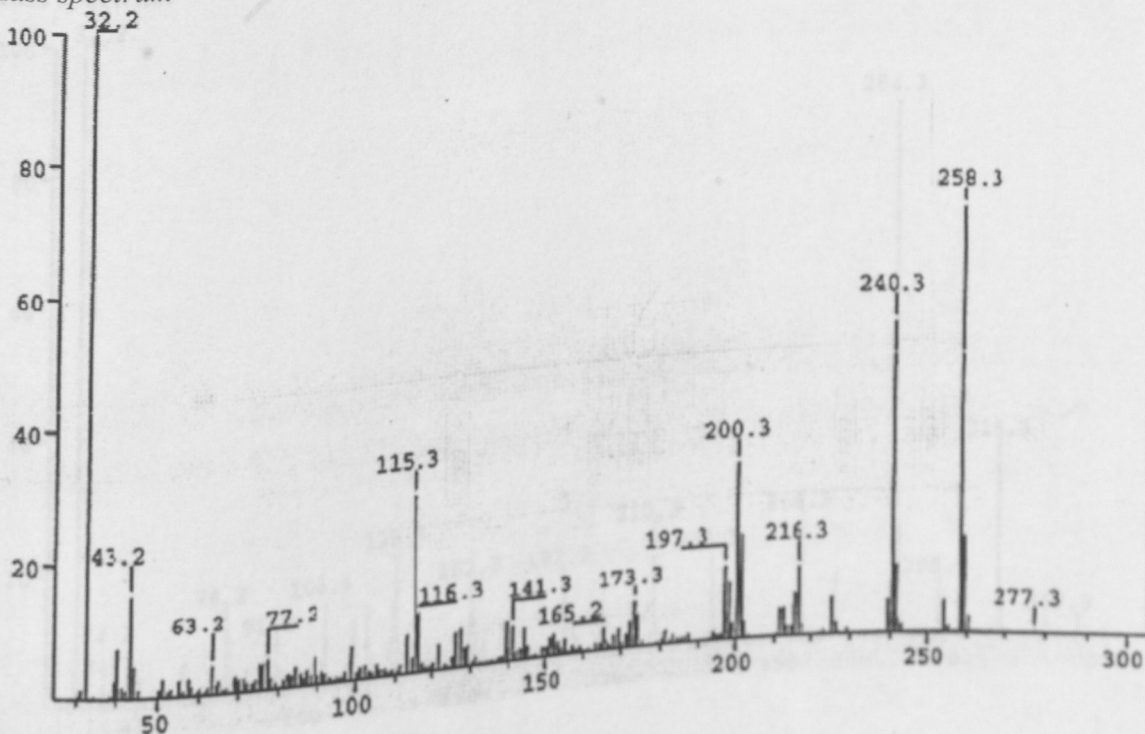
No.	Mass	Intensity	%RA	%RIC	Flags
1	30.2	13189	1.68	0.71	FM
2	31.3	30175	5.09	2.14	F
3	32.2	91399	100.00	42.05	F
4	39.3	1796226	1.40	0.59	F
5	40.2	25089	6.95	2.92	F
6	41.3	124777	3.18	1.34	F
7	42.3	57168	2.20	0.92	F
8	43.3	39483	5.13	2.16	F
9	44.2	92195	7.39	3.11	F
10	45.2	132773	2.27	0.95	F
11	51.2	40718	0.97	0.41	F
12	55.2	17468	3.13	1.32	F
13	56.3	56302	1.67	0.70	F
14	57.3	29946	2.86	1.20	F
15	58.3	51356	0.91	0.38	F
16	60.2	16418	1.94	0.81	F
17	63.2	34778	0.82	0.34	F
18	64.2	14718	0.78	0.33	F
19	67.2	13999	0.77	0.33	F
20	69.2	13897	2.82	1.19	F
21	70.2	50643	1.18	0.49	F
22	71.3	21129	1.79	0.75	F
23	73.2	32232	2.07	0.87	F
24	77.2	37172	1.57	0.66	F
25	81.3	28215	0.86	0.36	F
26	82.3	15412	0.98	0.41	F
27	83.3	17596	1.77	0.74	F
28	84.3	31773	1.05	0.44	F
29	85.3	18894	1.46	0.62	F
30	87.2	26286	0.88	0.37	F
31	91.3	15883	0.83	0.35	F
32	93.3	14909	0.73	0.31	F
33	97.3	13190	1.19	0.50	F
34	98.3	21339	1.23	0.52	F
35	99.3	22090	1.01	0.42	F
36	105.3	18111	1.07	0.45	F
37	106.3	19162	0.81	0.34	F
38	111.3	14470	0.92	0.39	F
39	113.3	16526	1.56	0.65	F
40	115.3	27960	1.22	0.51	F
41	126.3	21874	1.16	0.49	F
42	127.2	20817	1.42	0.60	F
43	128.2	25541	0.93	0.39	F
44	129.3	16743	0.88	0.37	F
45	139.3	15883	0.76	0.32	F
46	143.3	13598	0.87	0.37	F
47	149.2	15679	0.81	0.34	F
48	240.2	14591	0.82	0.34	F
49	296.3	14705	2.21	0.93	F
50	328.3	39642	1.56	0.66	F
		28011			

APPENDIX VI SPECTRA FOR COMPOUND 9

Proton NMR (300 MHz)



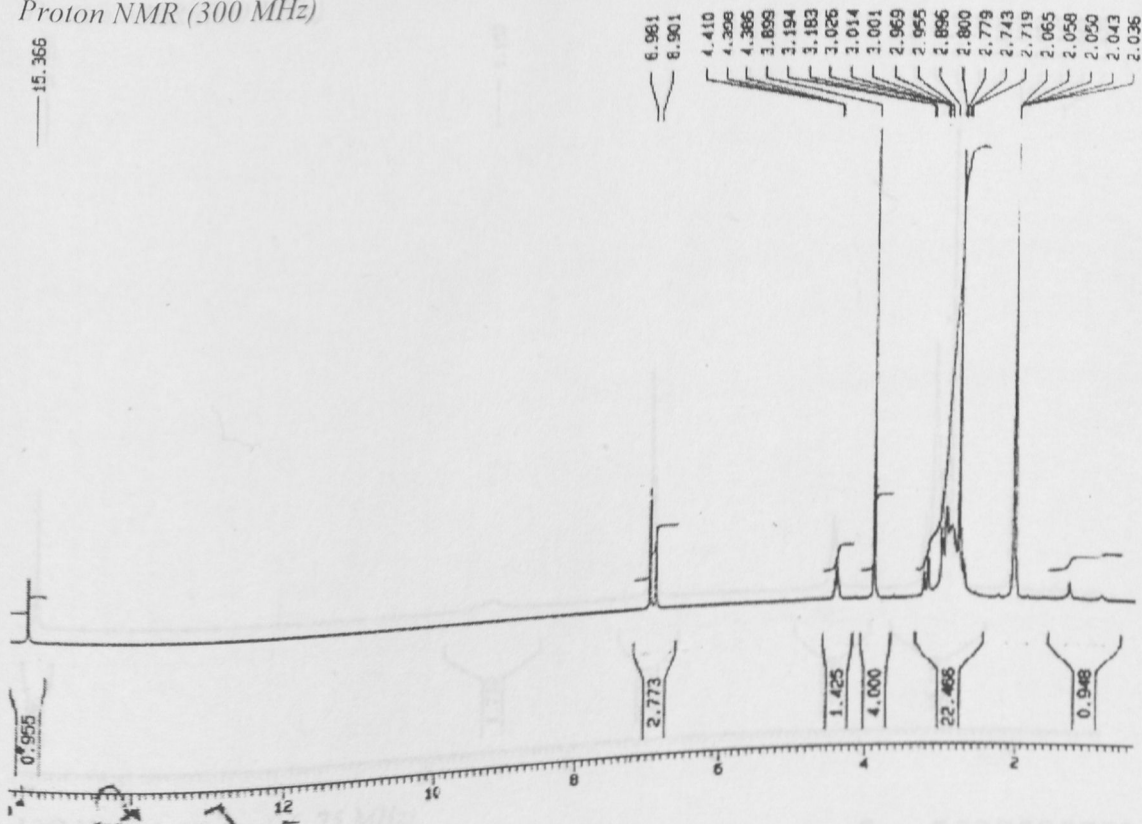
Mass spectrum



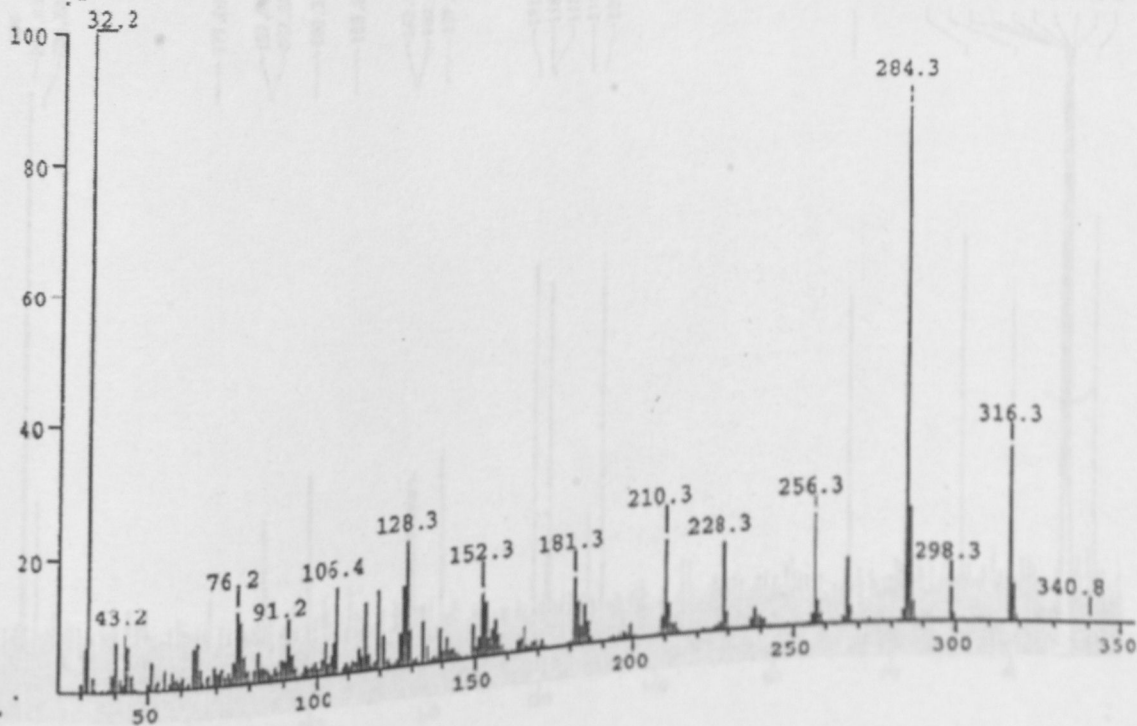
APPENDIX VII

Proton NMR (300 MHz)

SPECTRA FOR COMPOUND 10



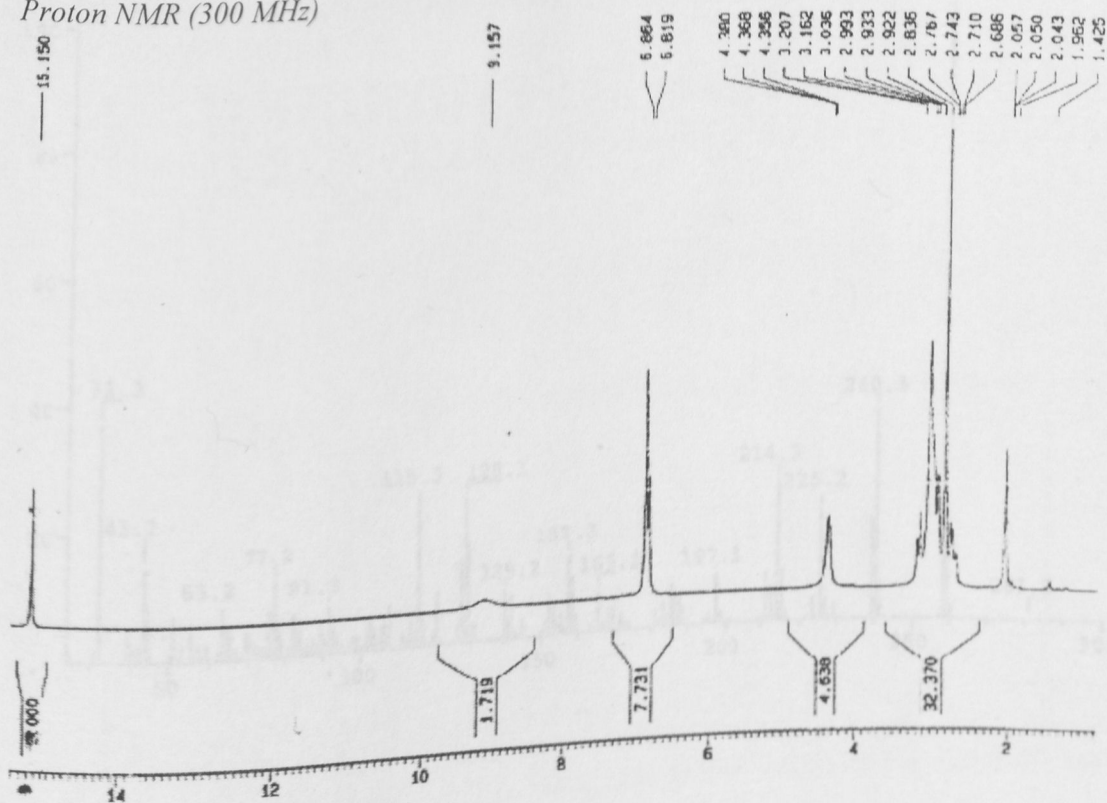
Mass spectrum



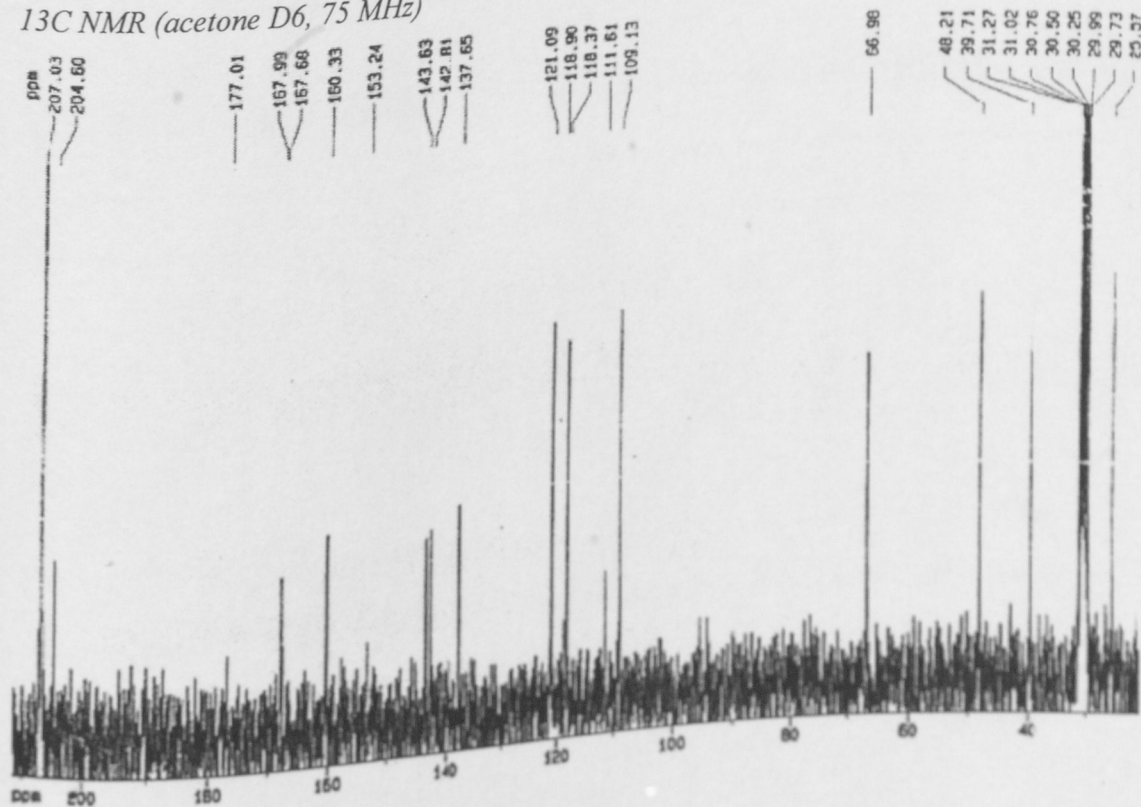
APPENDIX VIII

SPECTA FOR COMPOUND 11

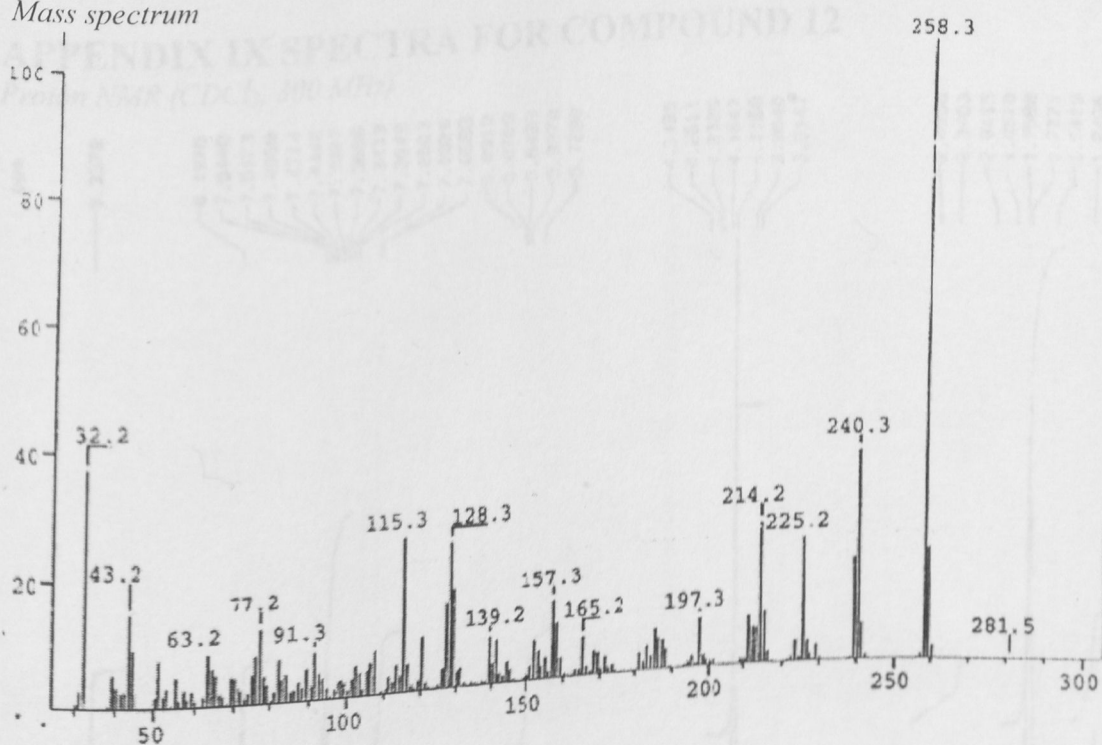
Proton NMR (300 MHz)



^{13}C NMR (acetone D₆, 75 MHz)

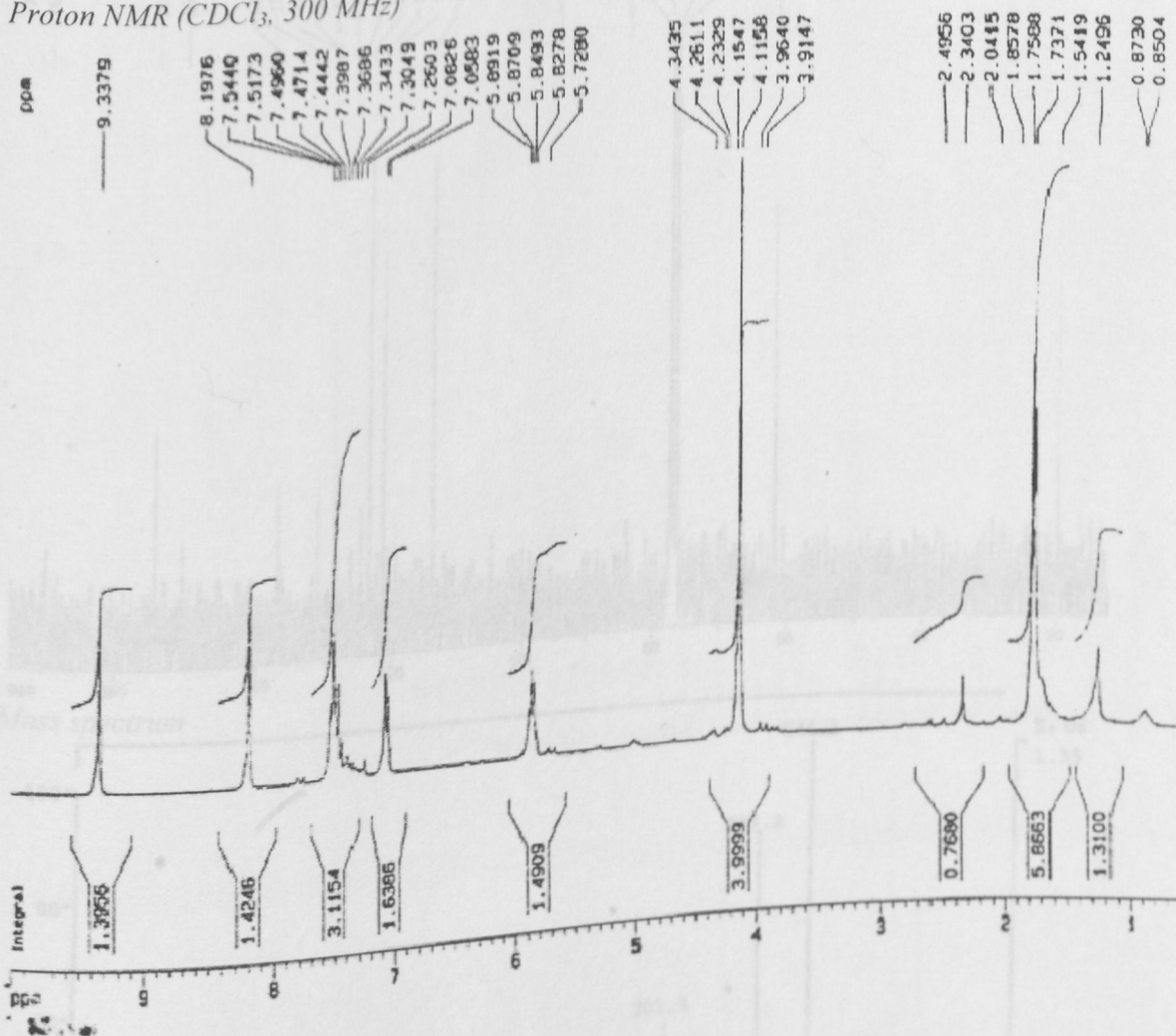


Mass spectrum

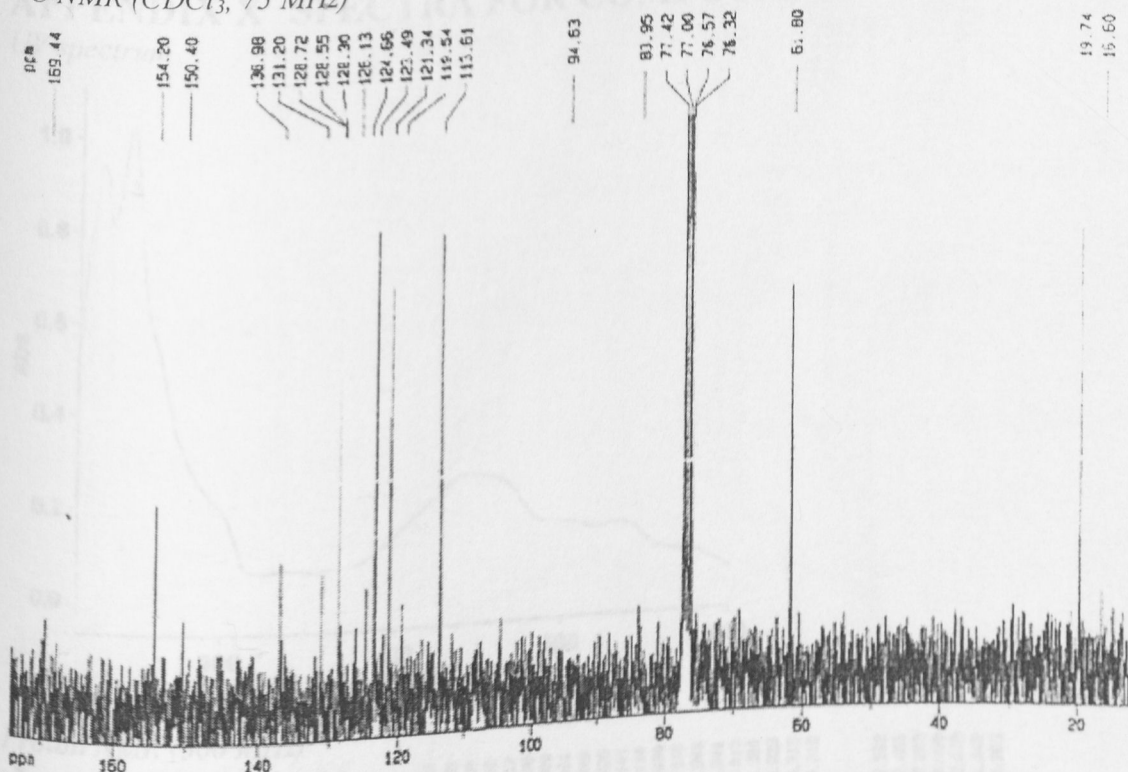


APPENDIX IX SPECTRA FOR COMPOUND 12

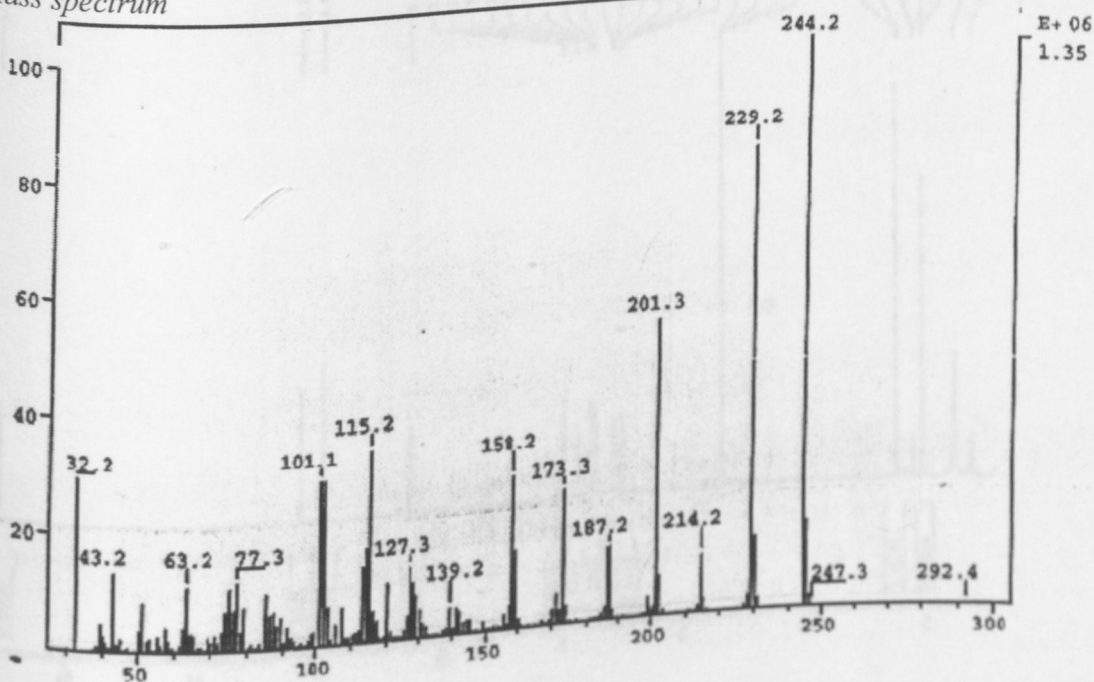
Proton NMR (CDCl_3 , 300 MHz)



^{13}C NMR (CDCl_3 , 75 MHz)

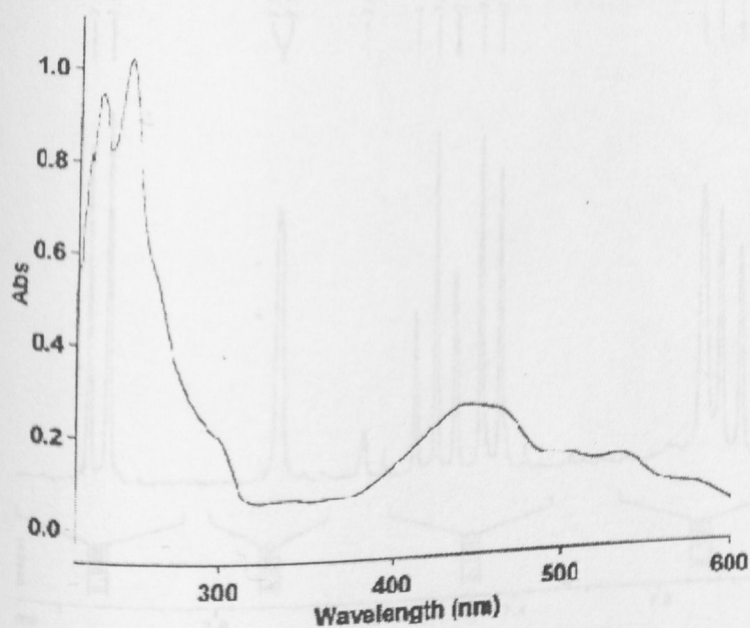


Mass spectrum

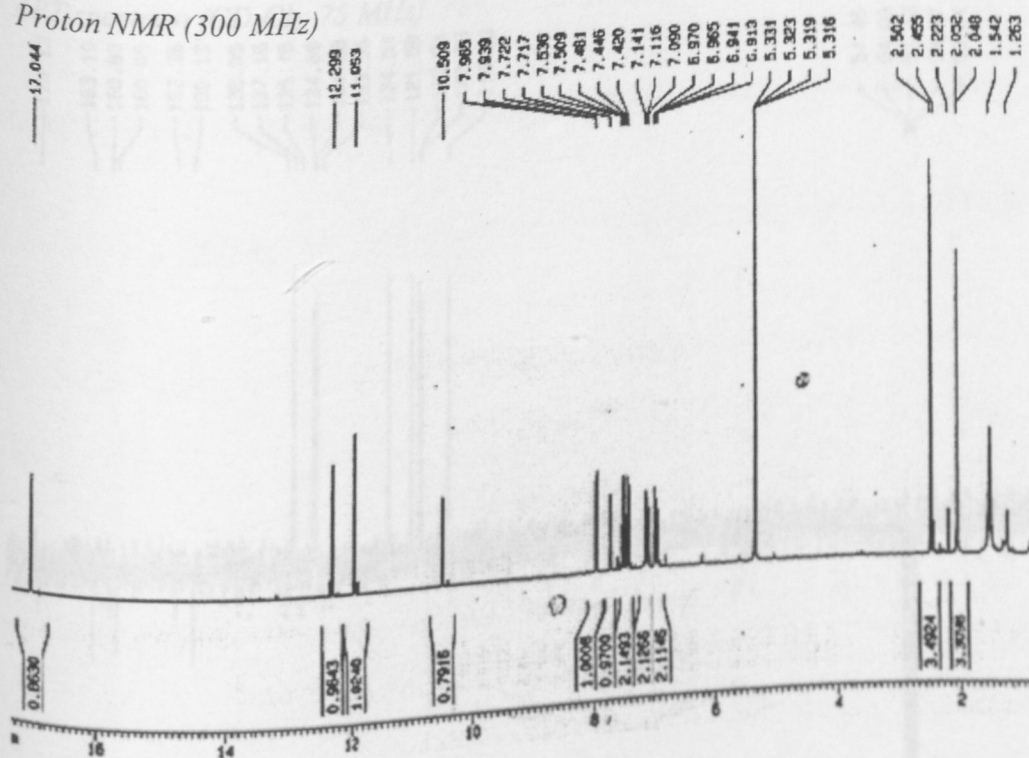


APPENDIX X SPECTRA FOR COMPOUND 13

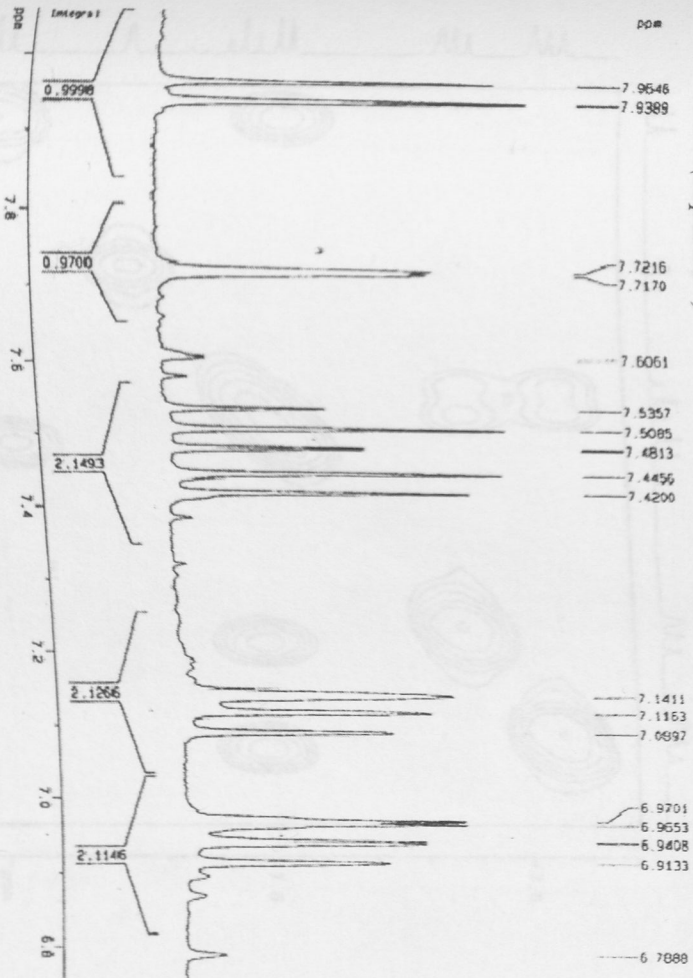
UV spectrum



Proton NMR (300 MHz)



Proton NMR (expansion)



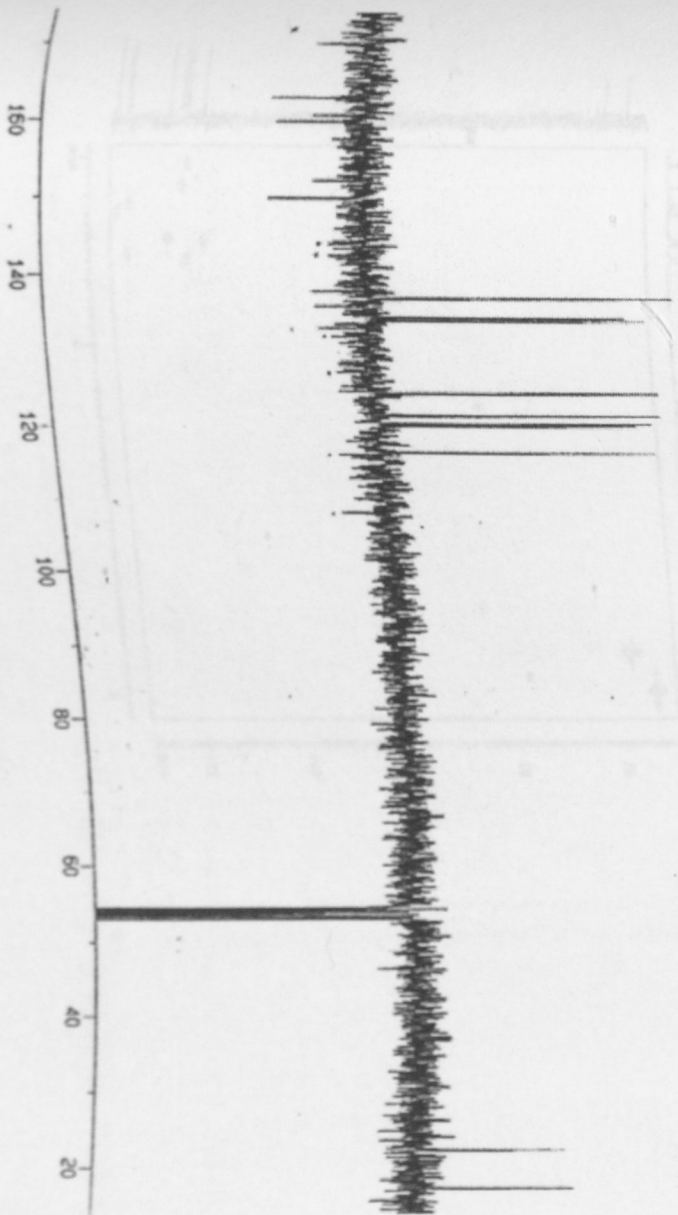
APT spectrum (CD_2Cl_2 , 75 MHz)

Chemical shifts (ppm):

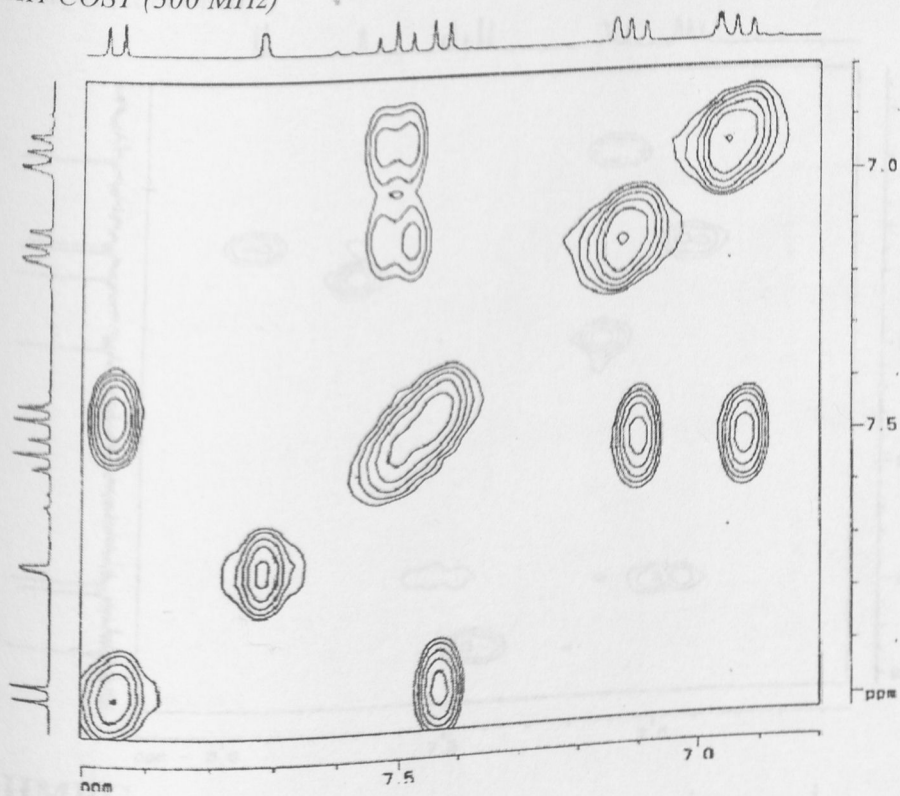
- 170.13
- 163.10
- 160.83
- 160.05
- 152.35
- 150.13
- 138.05
- 137.16
- 136.15
- 134.69
- 134.28
- 133.36
- 124.54
- 121.59
- 120.51
- 120.23
- 116.63

Chemical shifts (ppm):

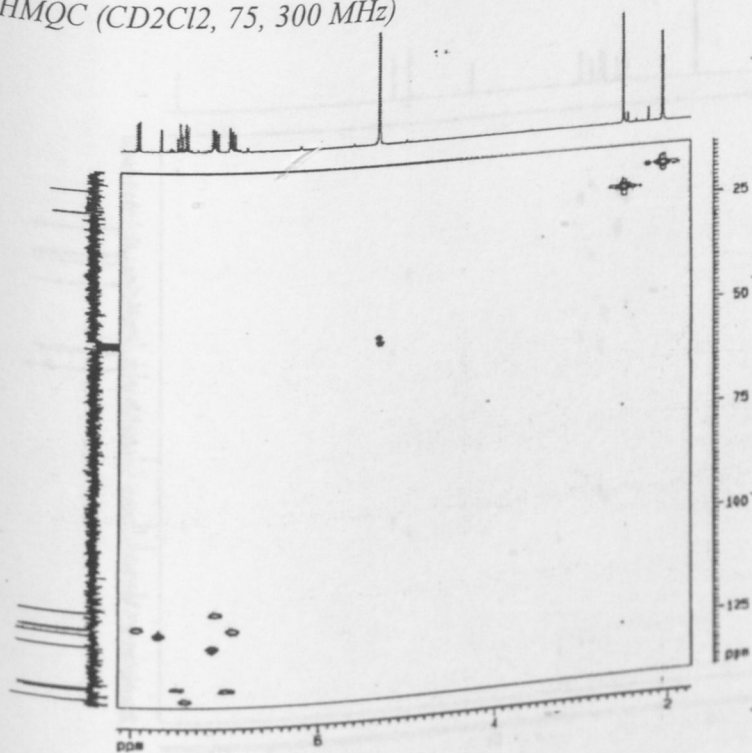
- 24.07
- 22.30
- 17.20



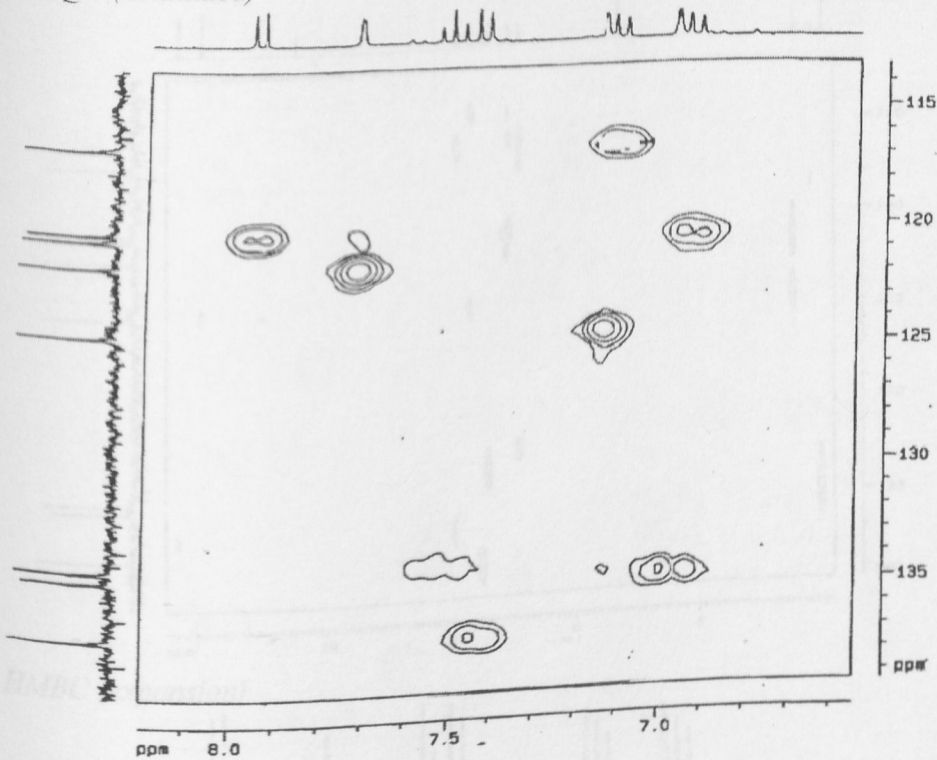
HH-COSY (300 MHz)



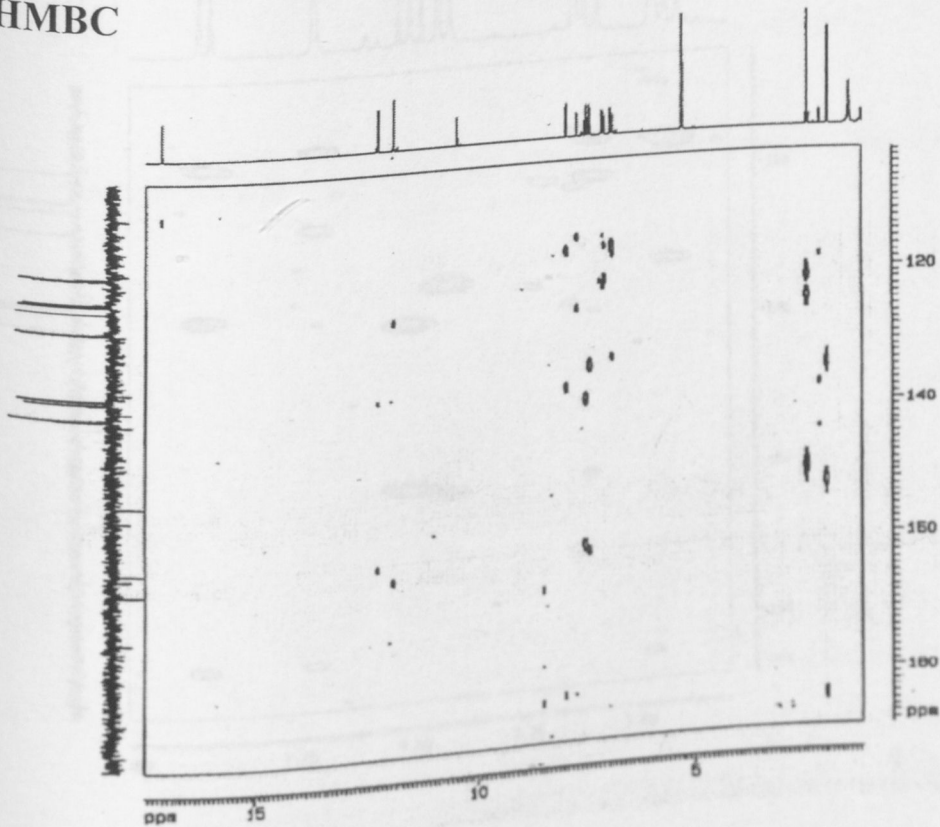
HMQC (CD_2Cl_2 , 75, 300 MHz)



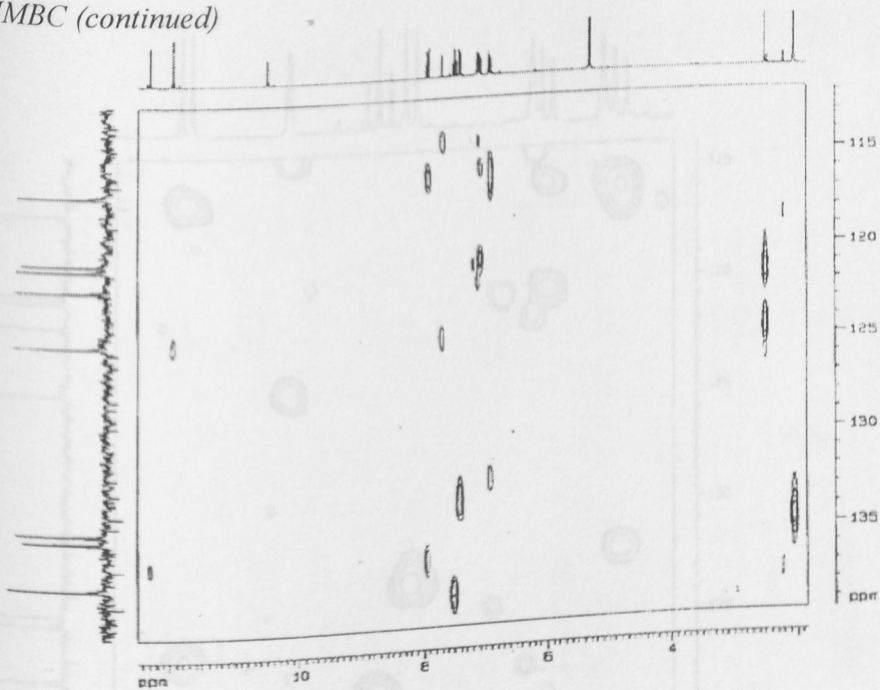
HMQC (continued)



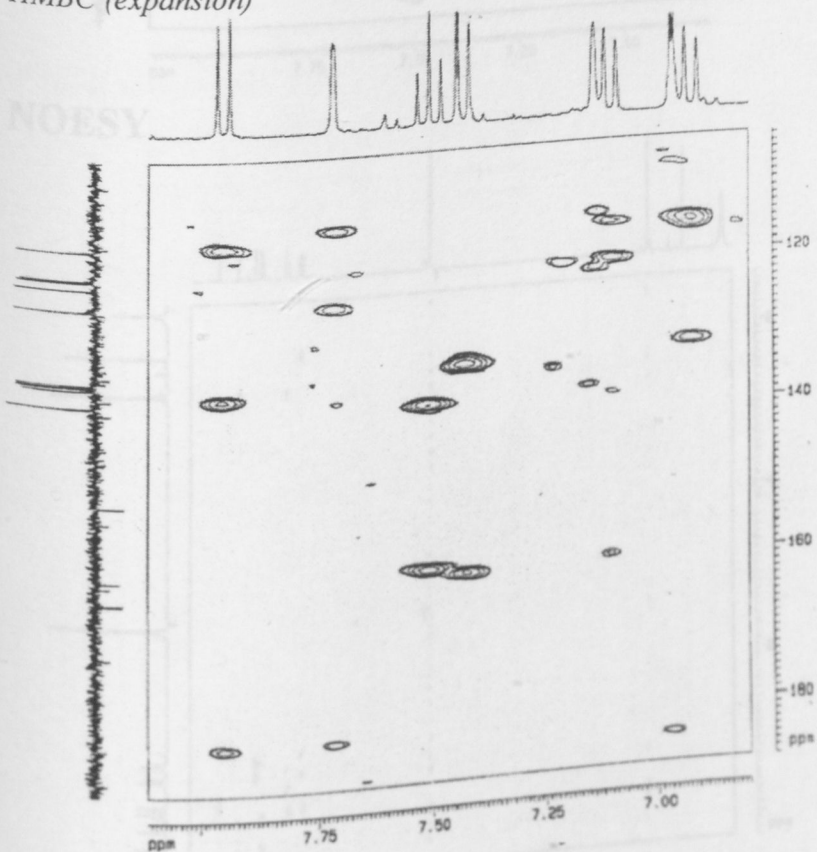
HMBC



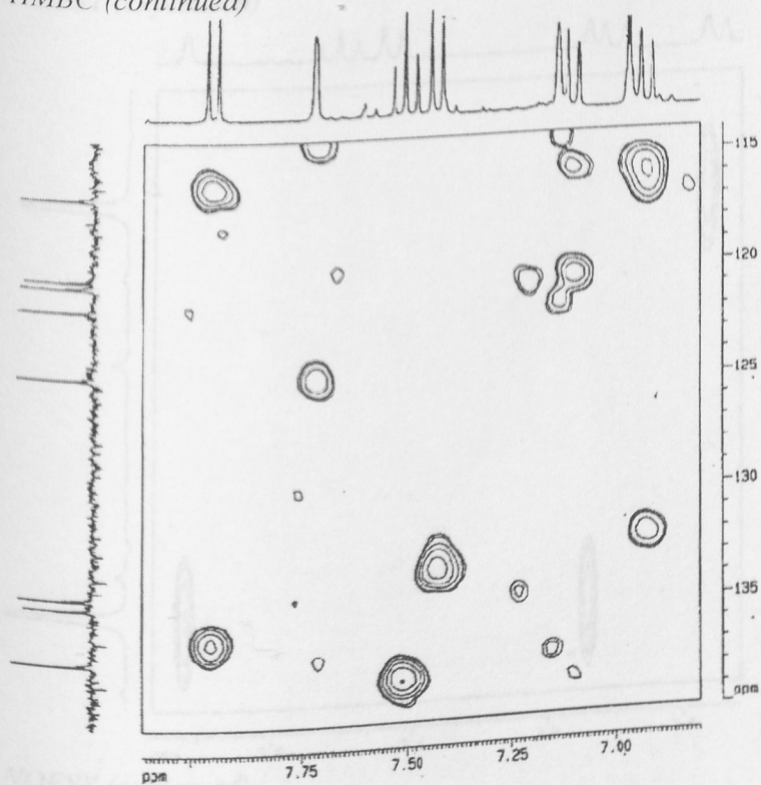
HMBC (continued)



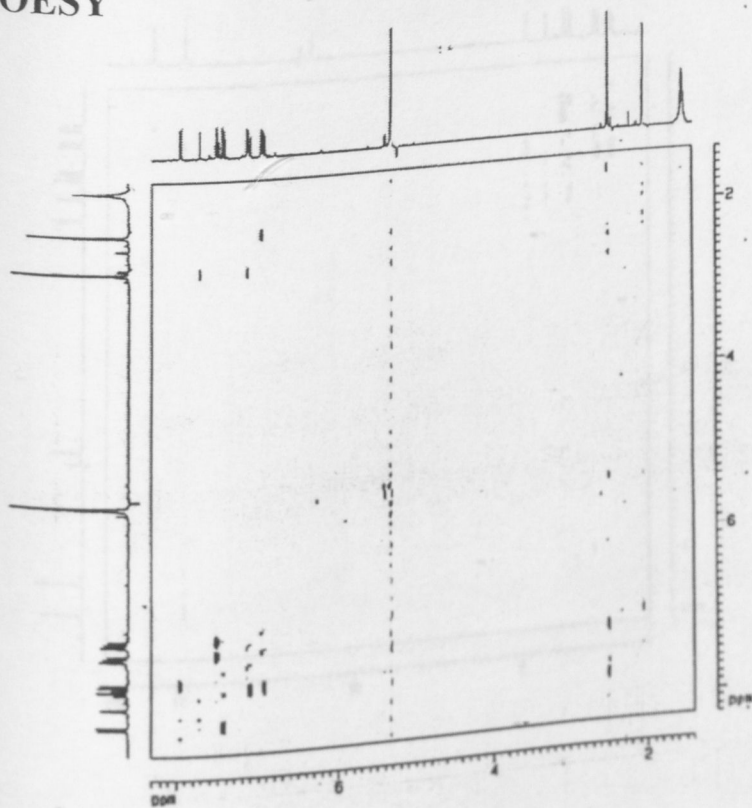
HMBC (expansion)



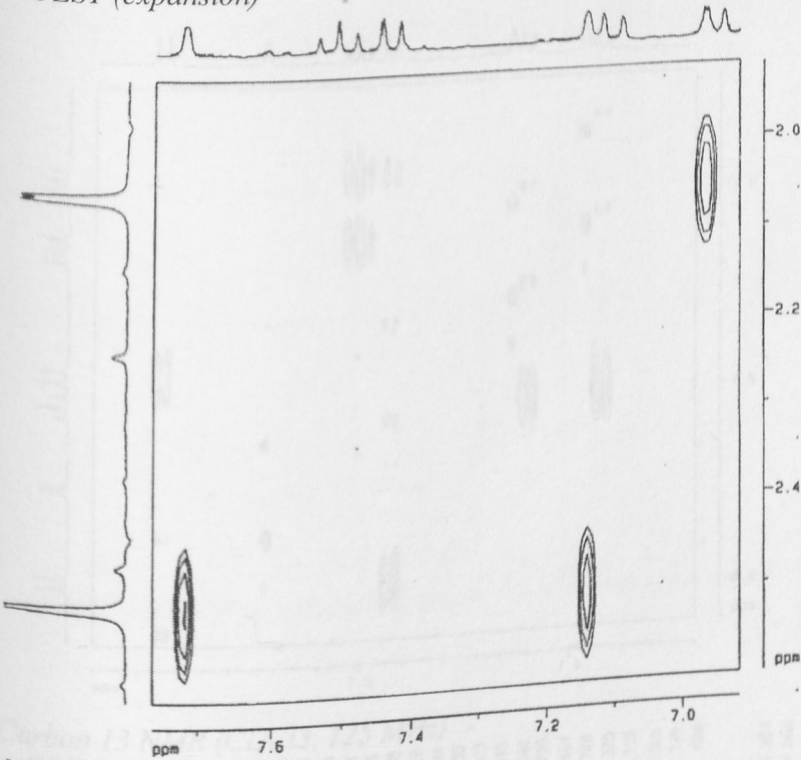
HMBC (continued)



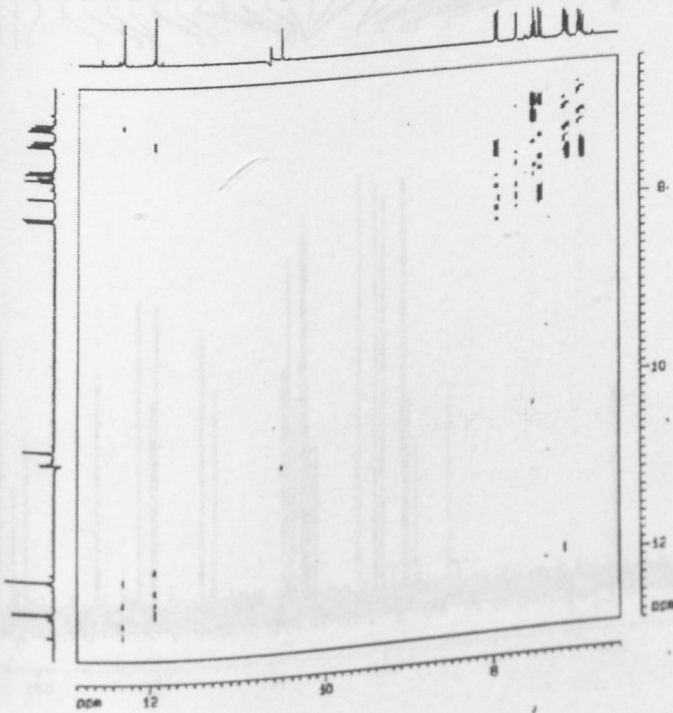
NOESY



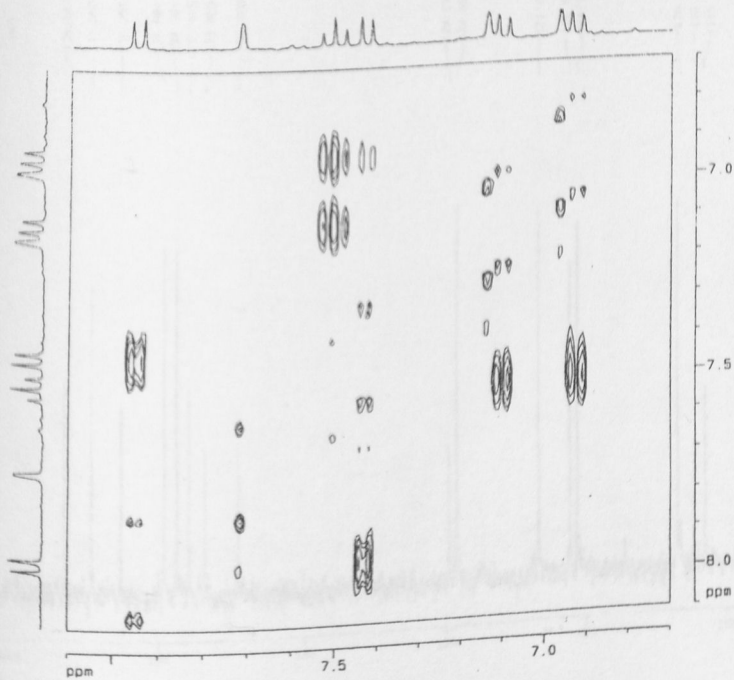
NOESY (expansion)



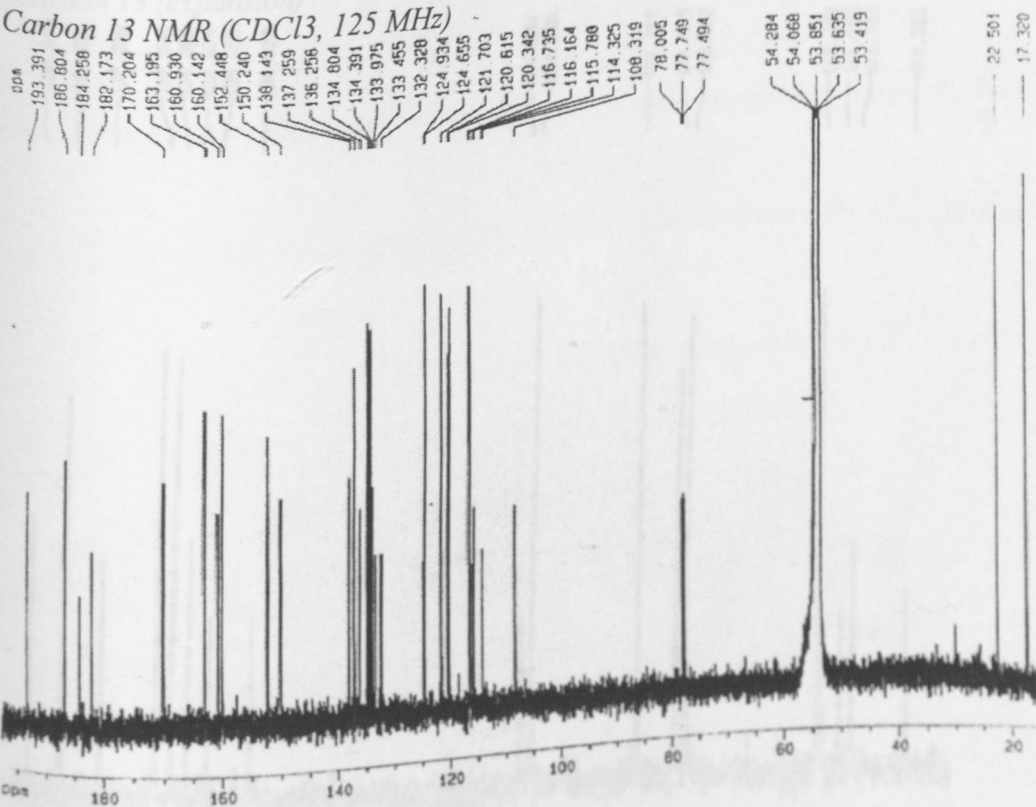
NOESY (continued)



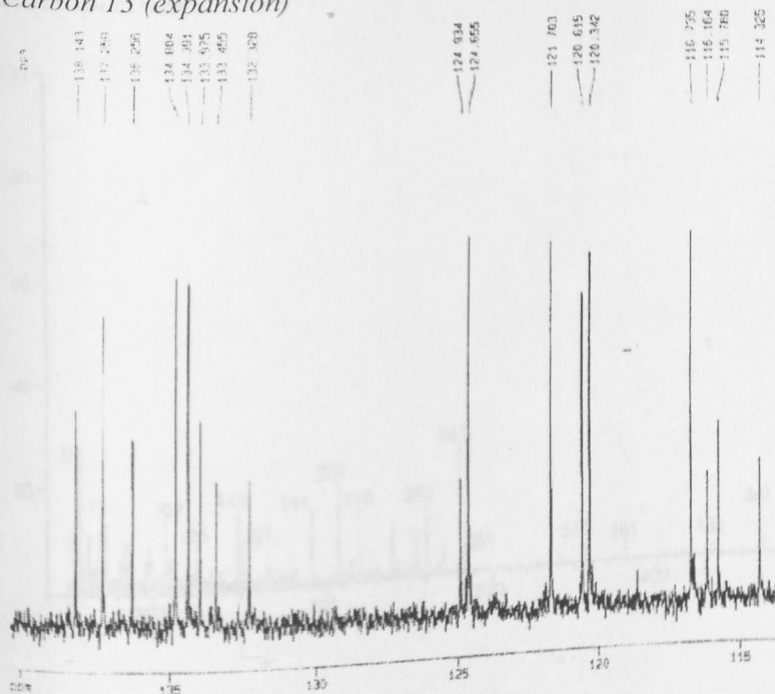
NOESY (expansion)



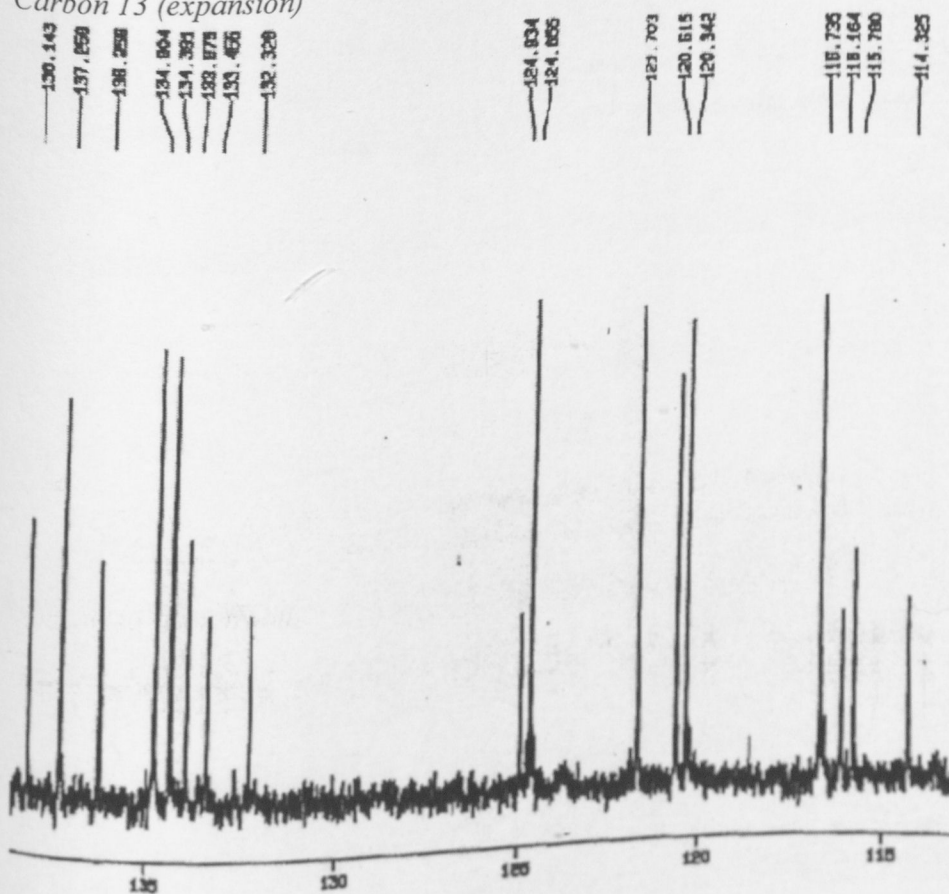
Carbon 13 NMR (CDCl3, 125 MHz)



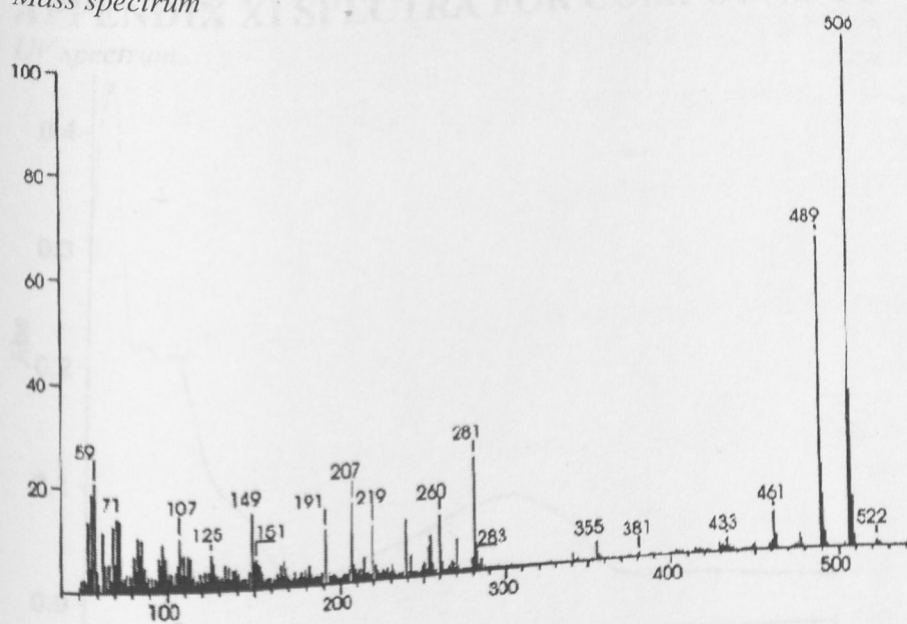
Carbon 13 (expansion)



Carbon 13 (expansion)

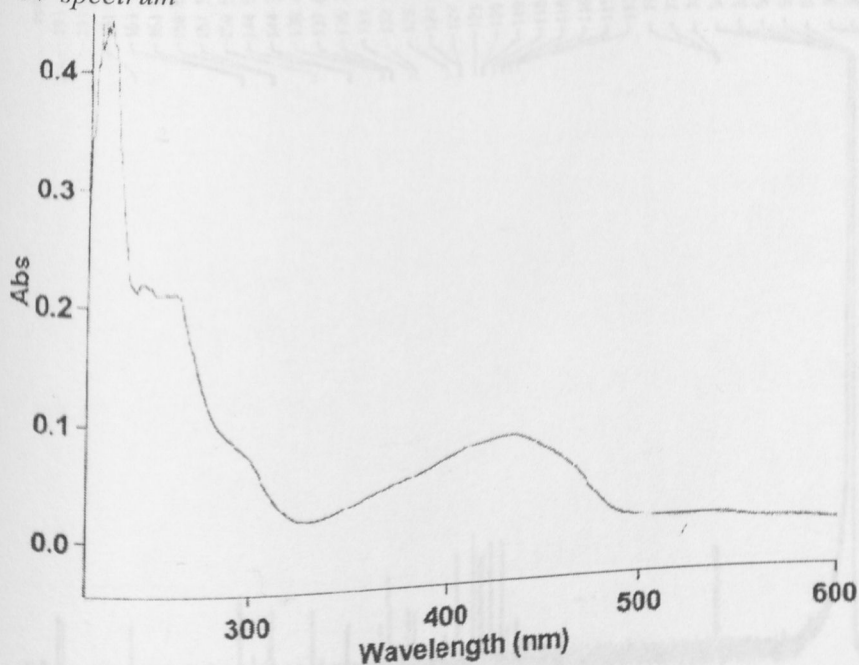


Mass spectrum

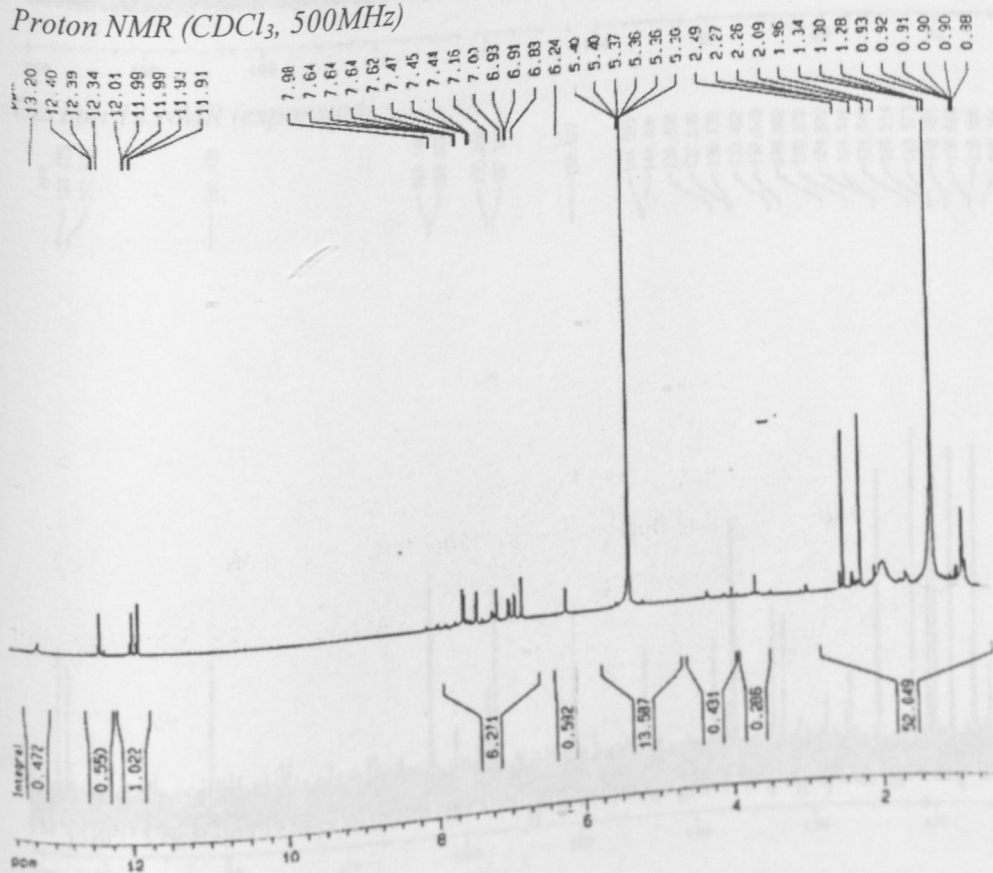


APPENDIX XI SPECTRA FOR COMPOUND 14

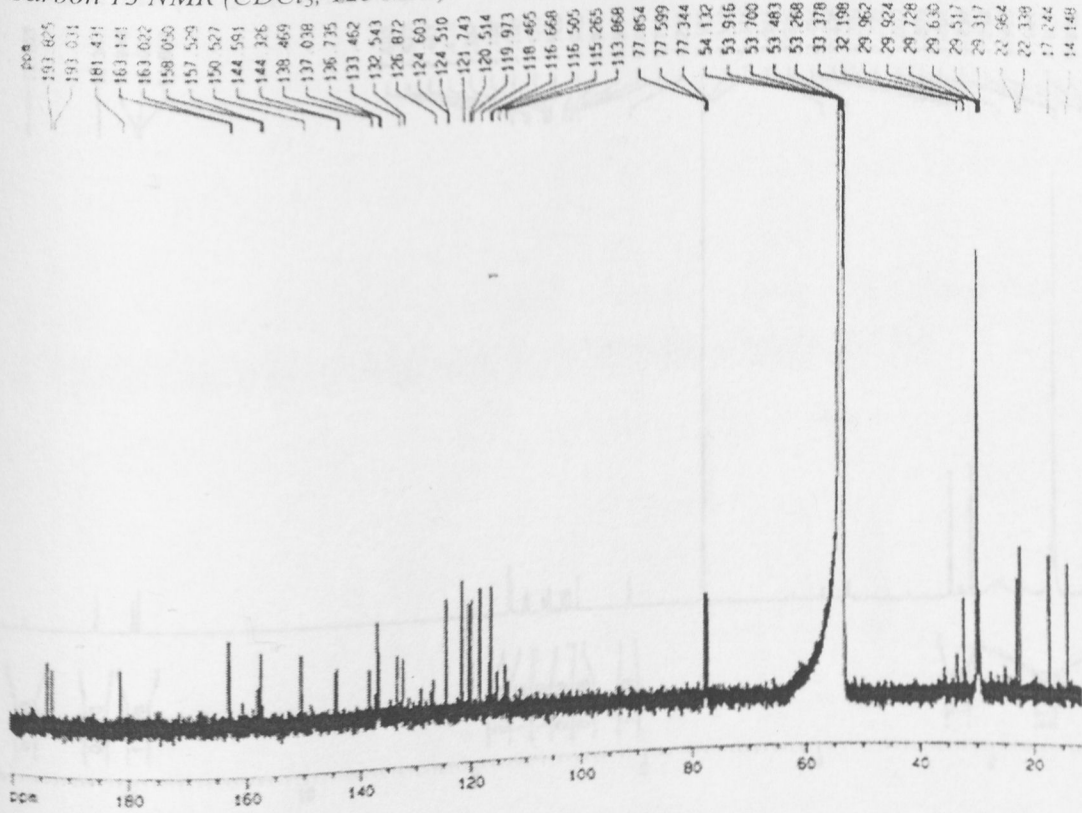
UV spectrum



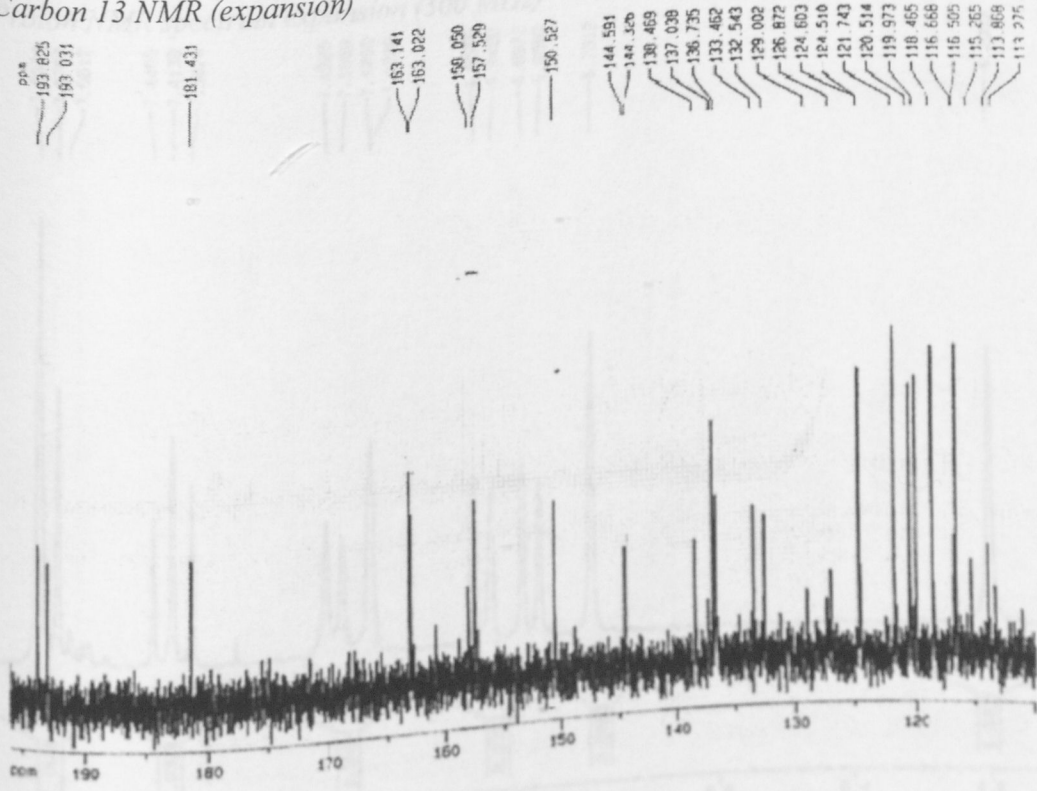
Proton NMR ($CDCl_3$, 500MHz)



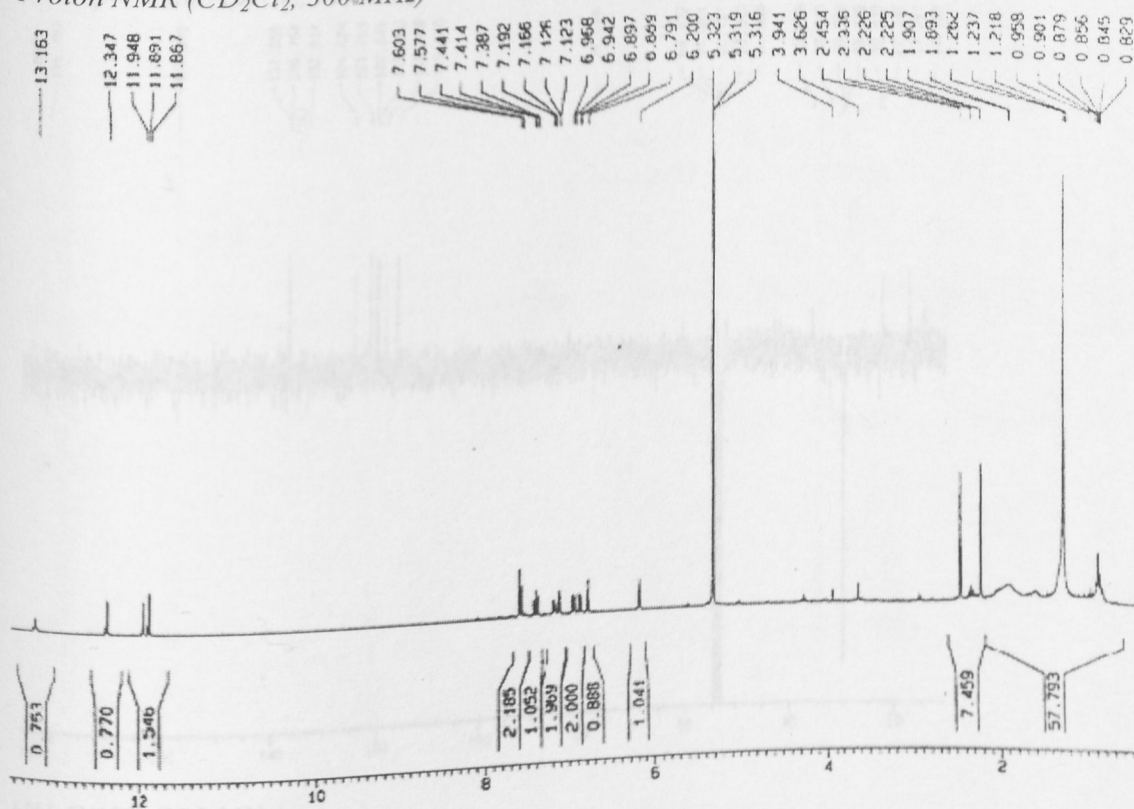
Carbon 13 NMR (CDCl₃, 125 MHz)



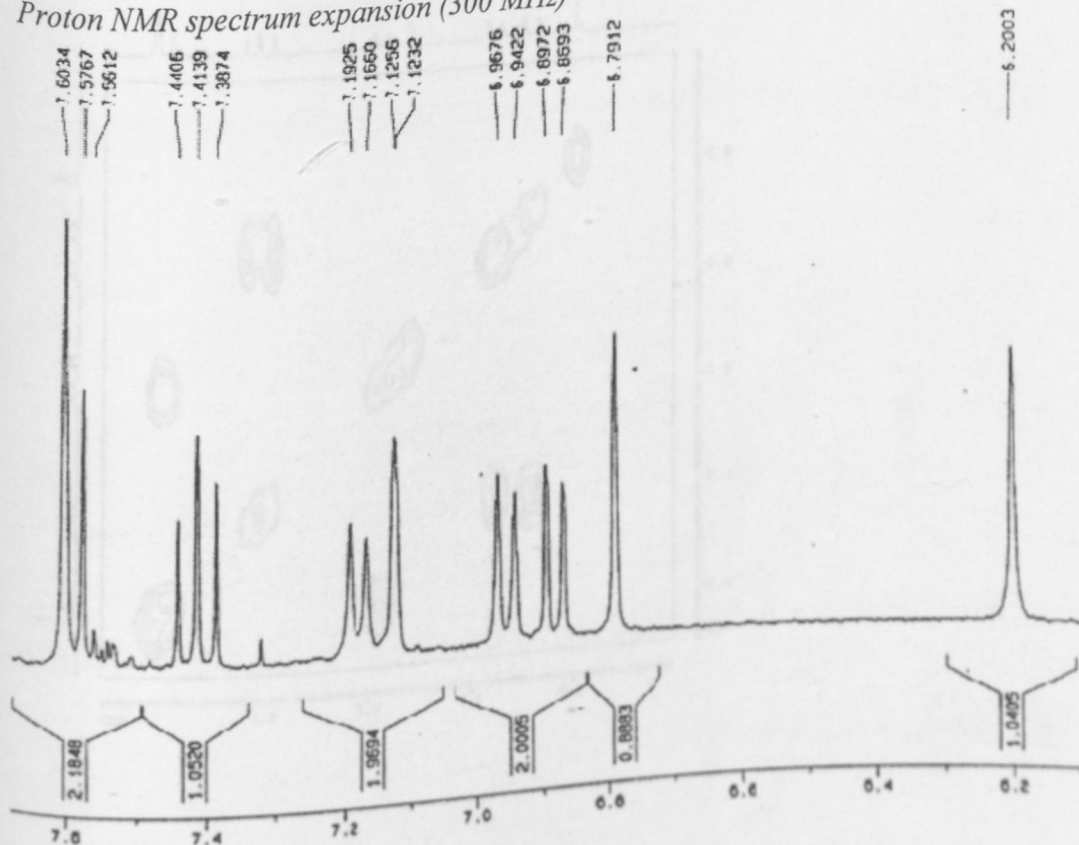
Carbon 13 NMR (expansion)



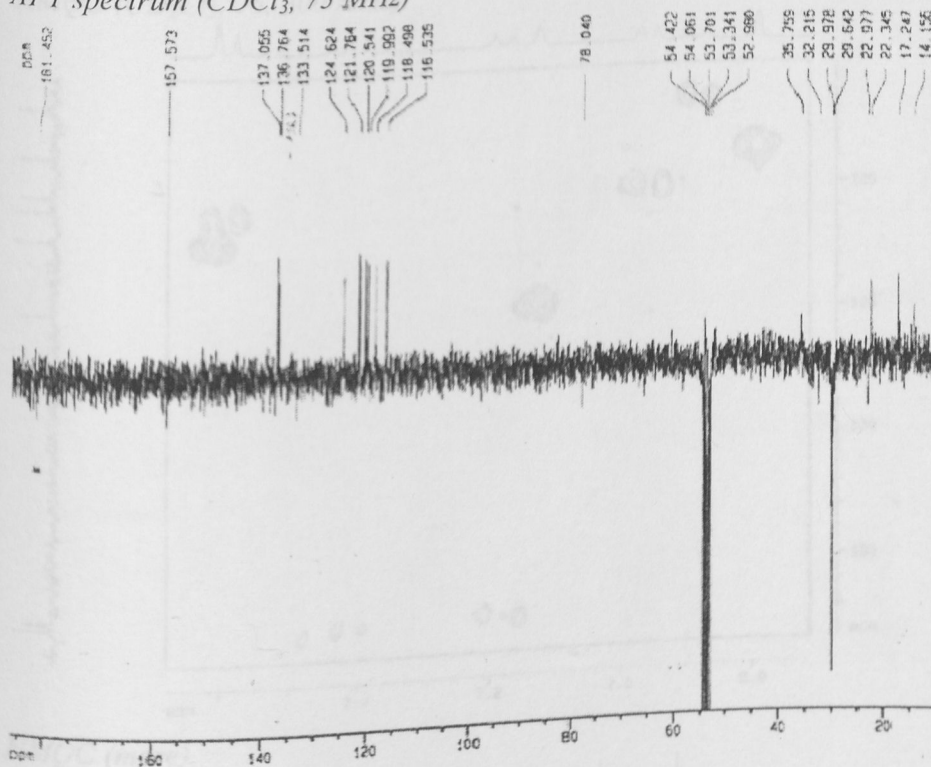
Proton NMR (CD_2Cl_2 , 300 MHz)



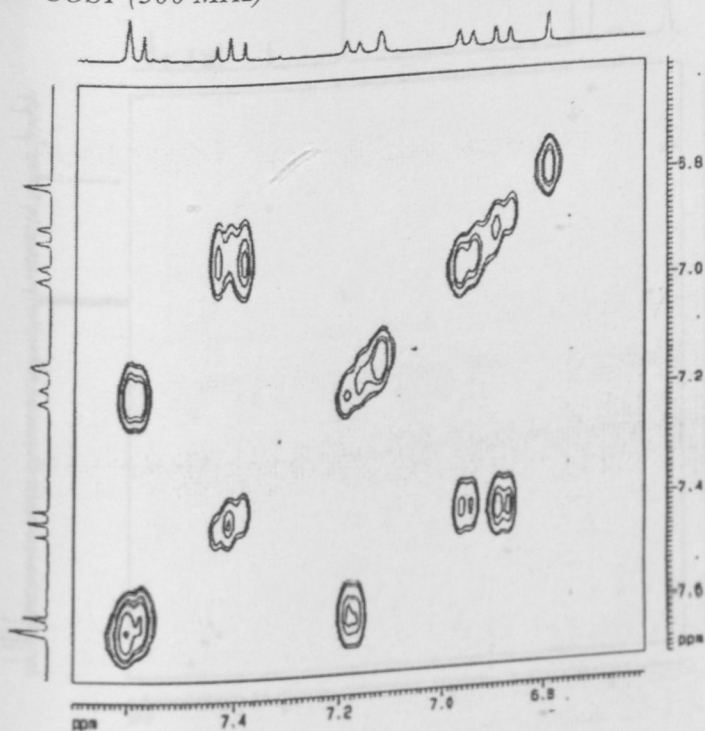
Proton NMR spectrum expansion (300 MHz)



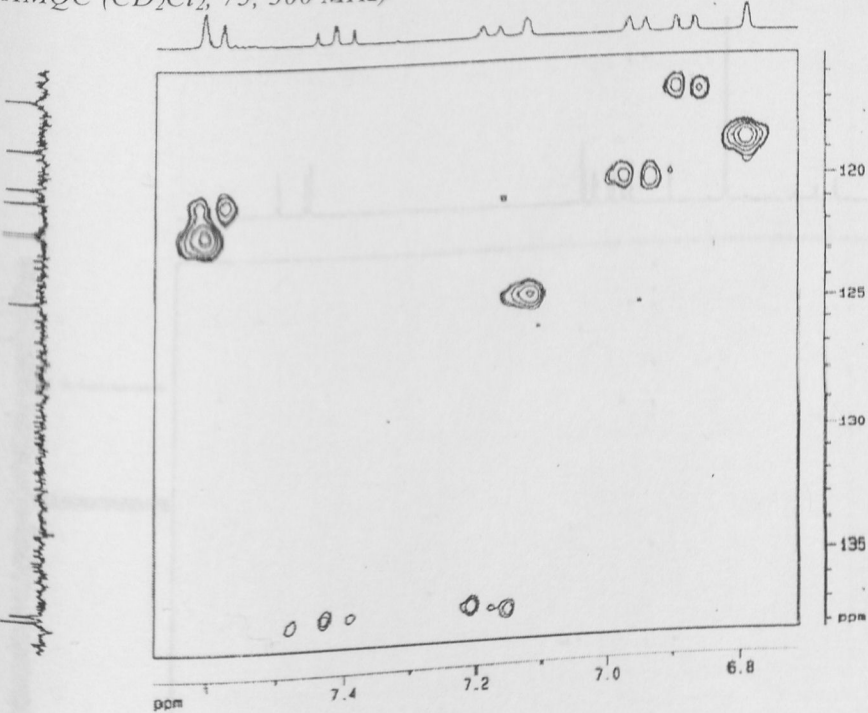
APT spectrum (CDCl₃, 75 MHz)



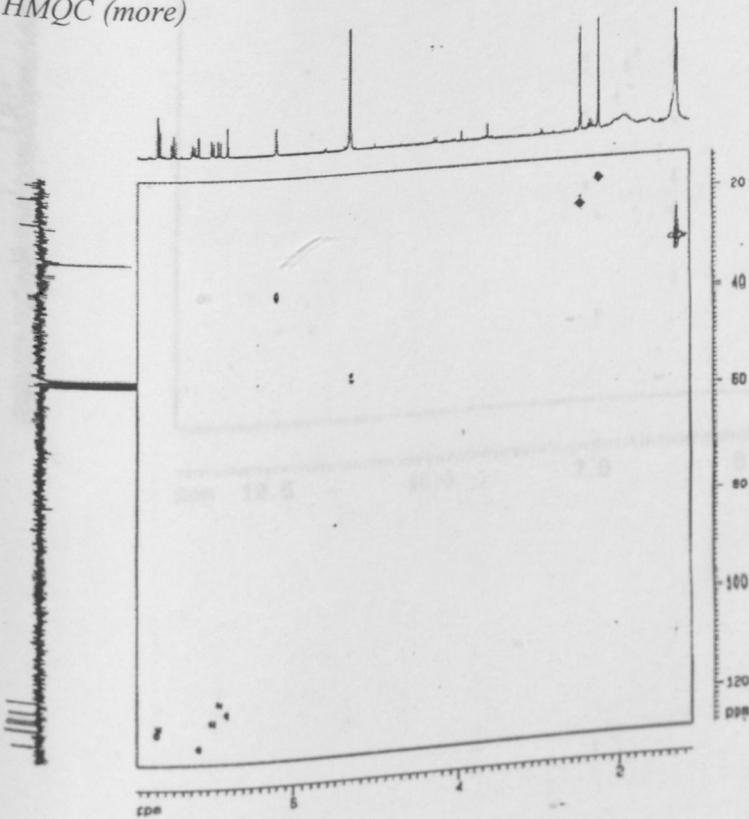
HH-COSY (300 MHz)



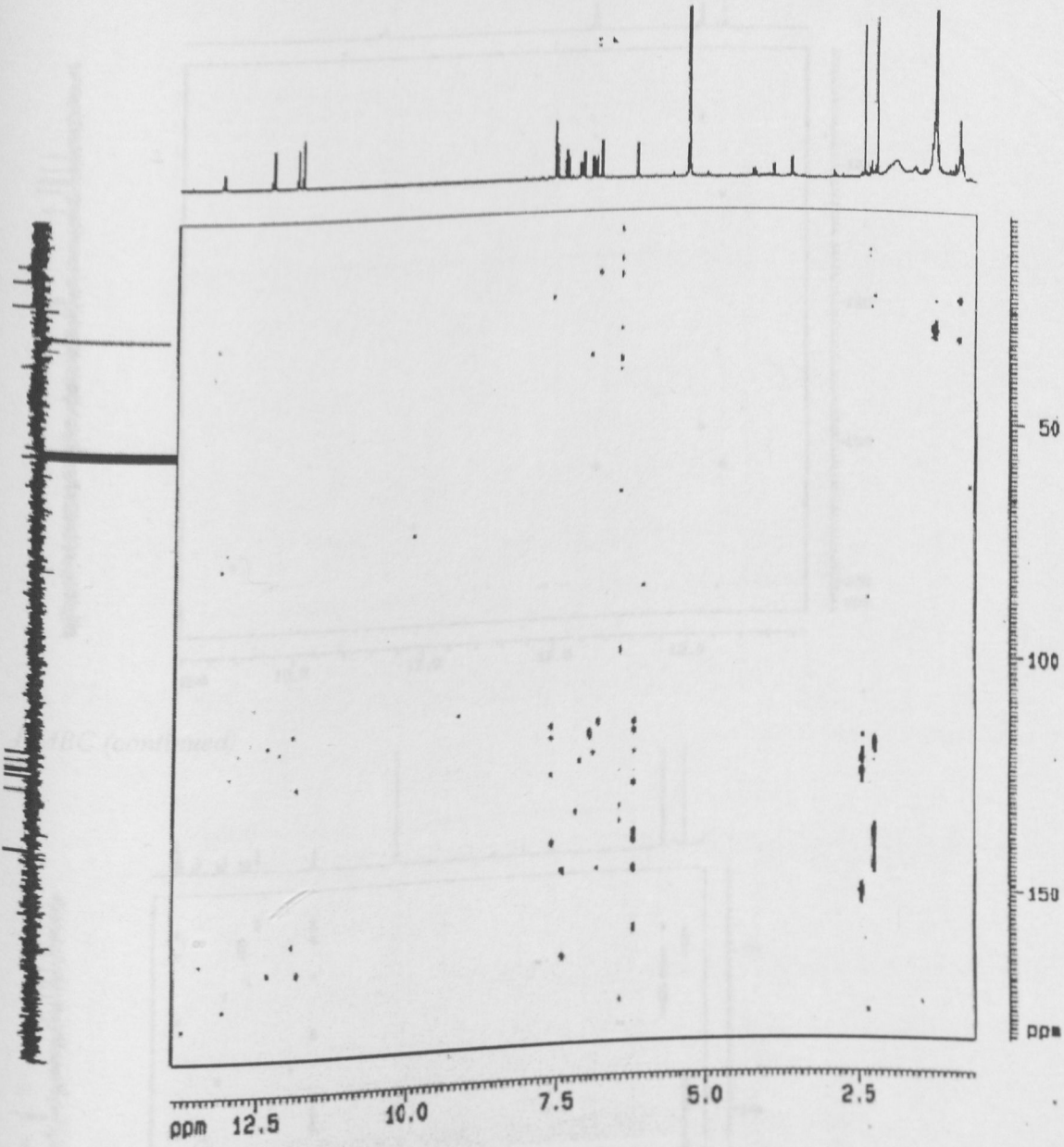
HMQC (CD₂Cl₂, 75, 300 MHz)



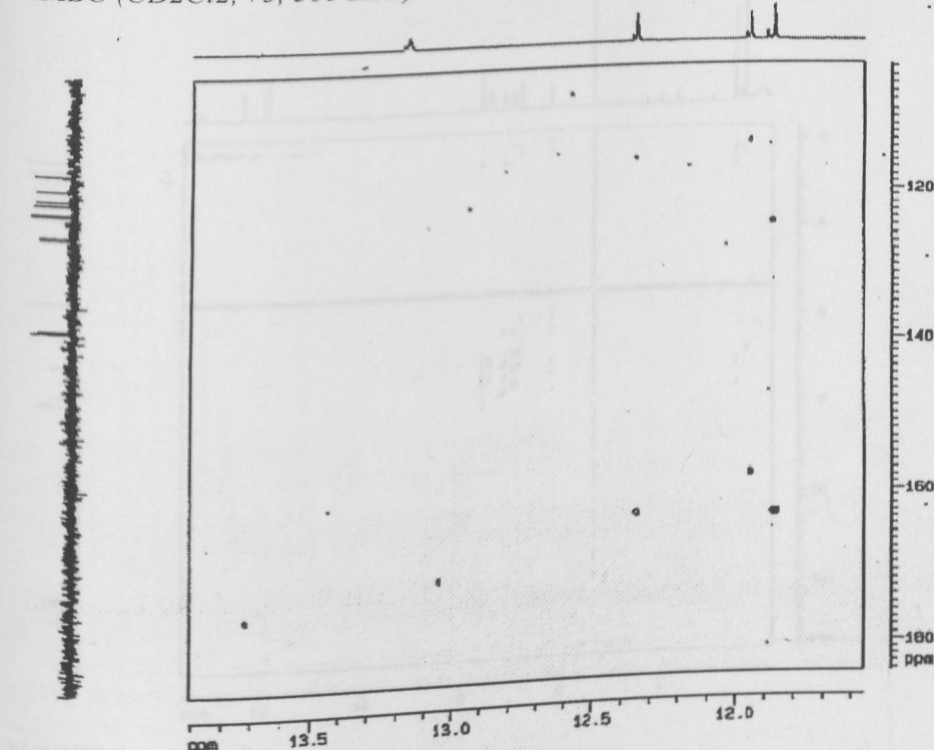
HMQC (more)



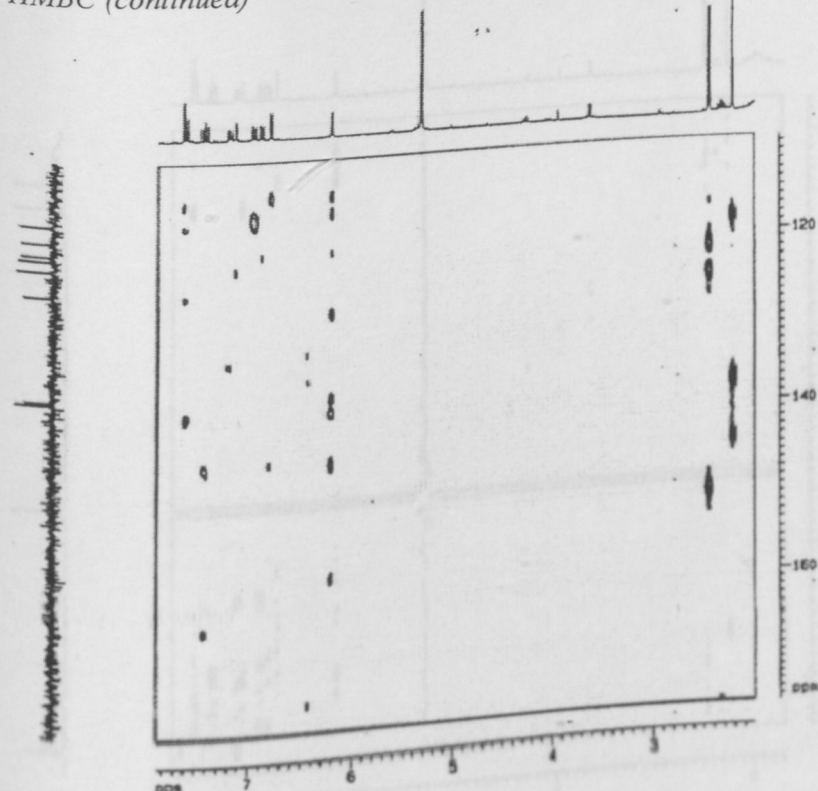
HMBC (CD₂Cl₂, 75, 300 MHz)



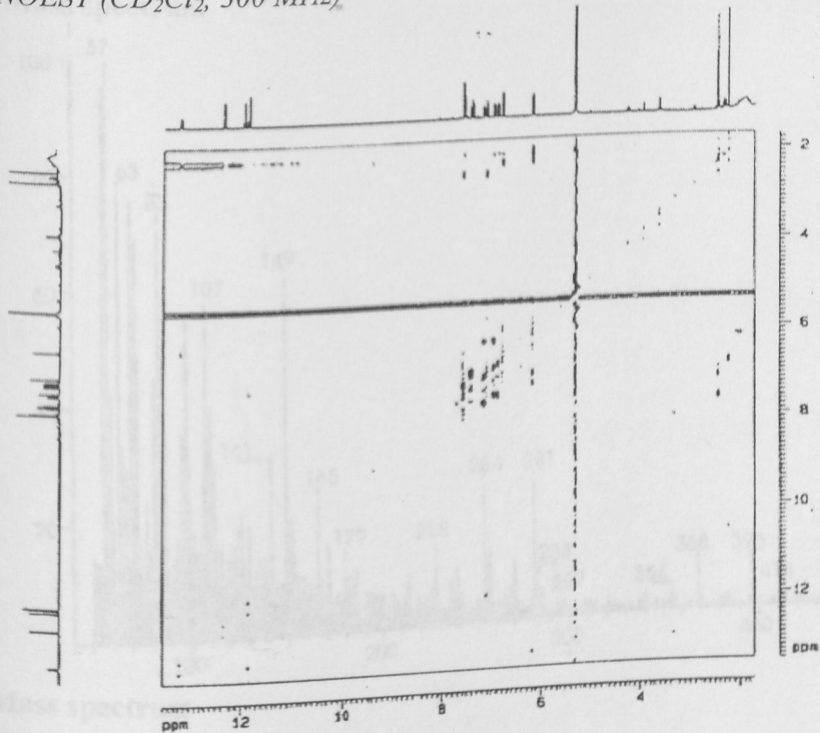
HMBC (CD₂Cl₂, 75, 300 MHz)



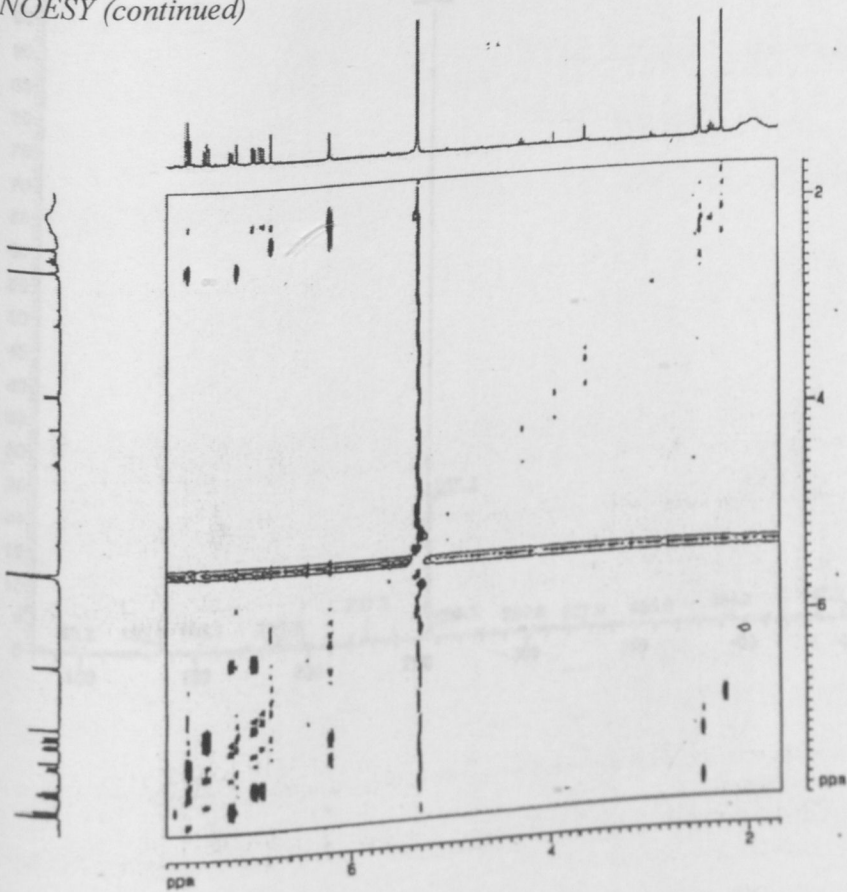
HMBC (continued)



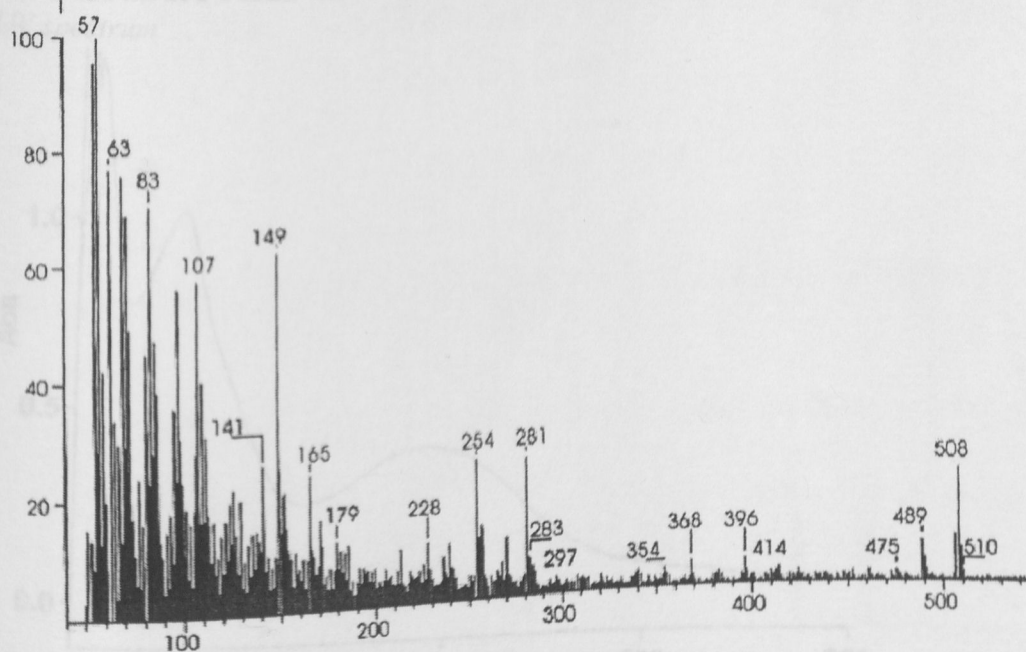
NOESY (CD₂Cl₂, 300 MHz)



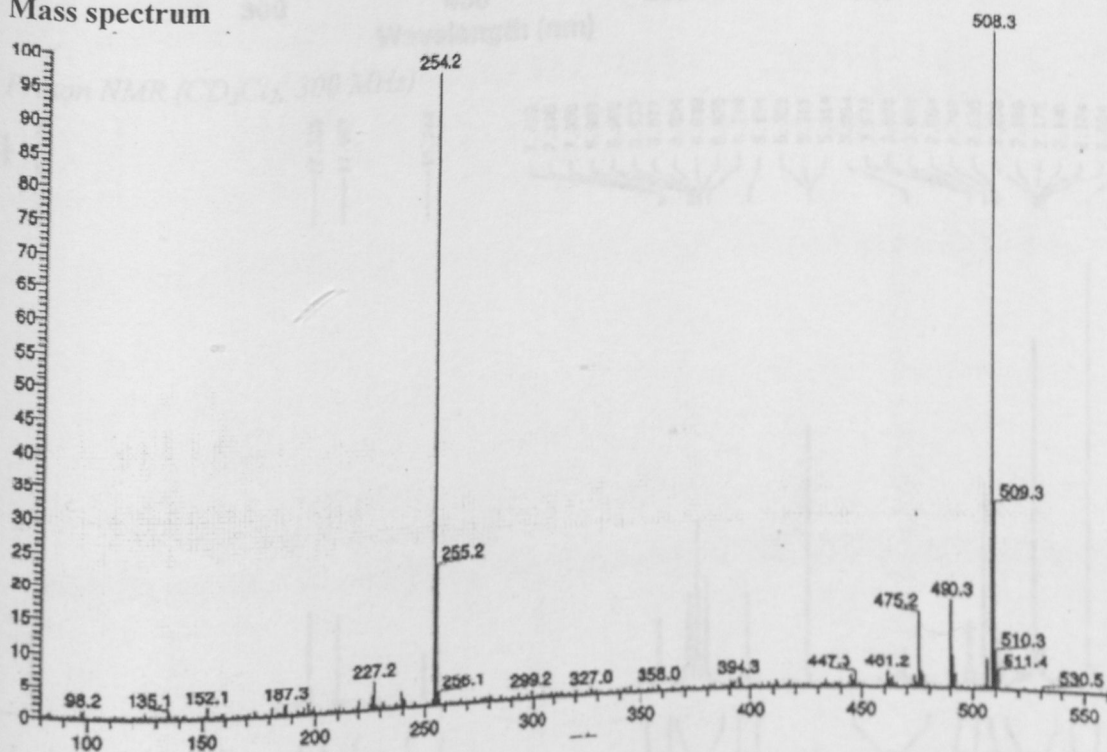
NOESY (continued)



Mass spectrum

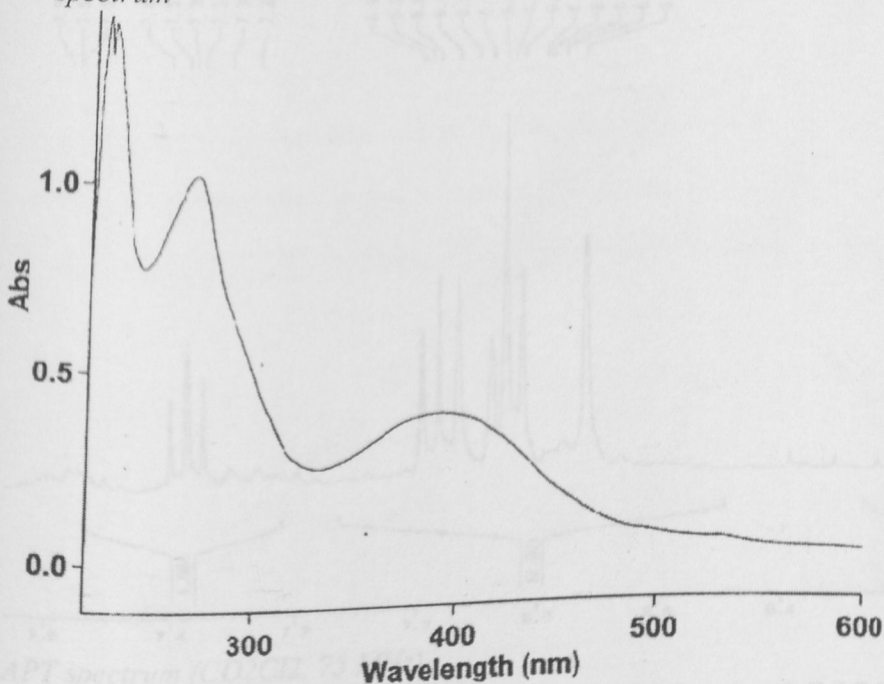


Mass spectrum

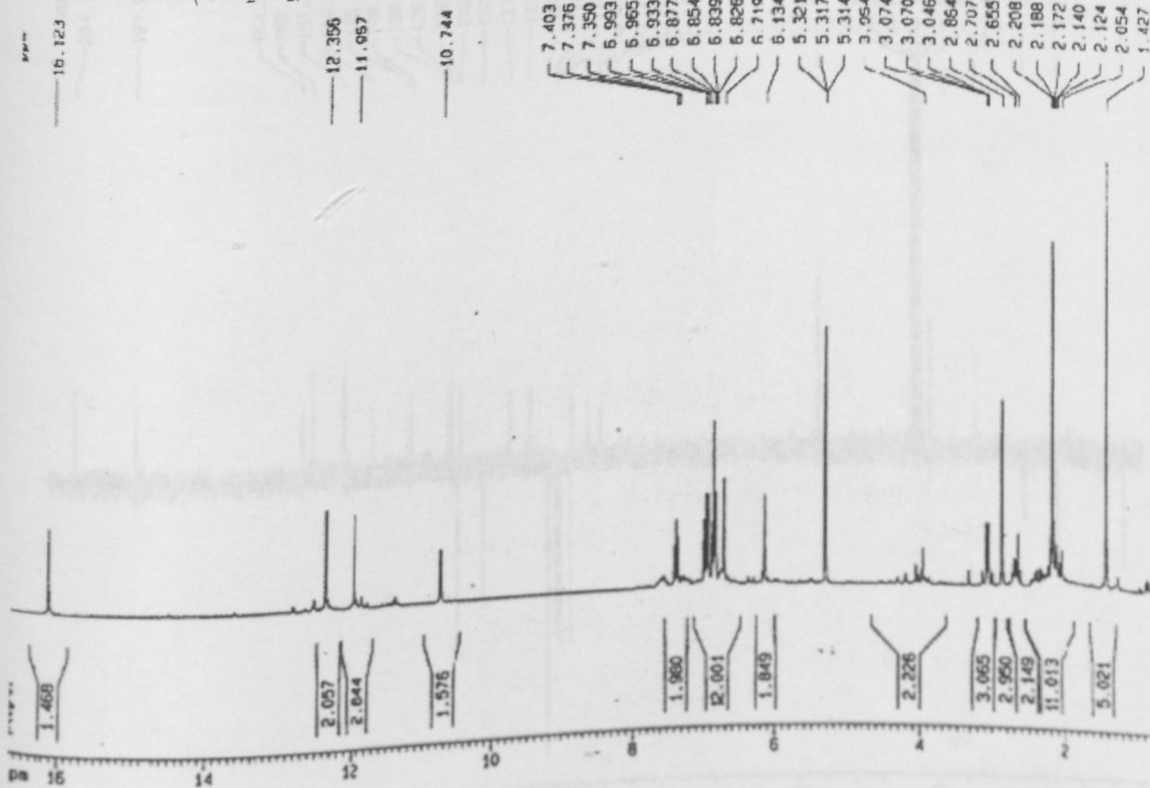


APPENDIX XIII SPECTRA FOR COMPOUND 15

UV spectrum



Proton NMR (CD_2Cl_2 , 300 MHz)

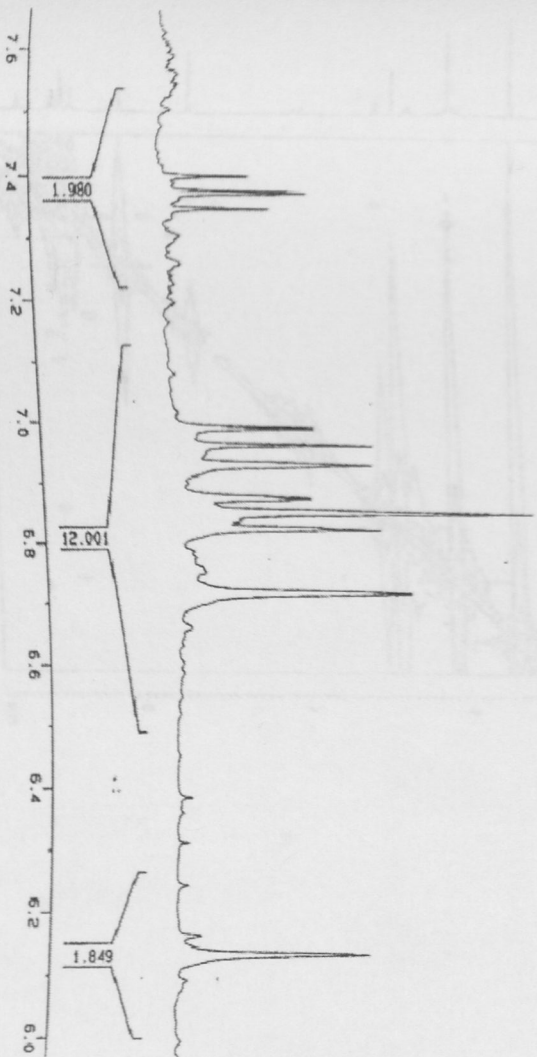


Proton NMR (expansion)

7.5633
7.5473
7.5421
7.4026
7.3758
7.3539
7.3496
7.3059
7.2612

6.9935
6.9789
6.9648
6.9333
6.9015
6.8774
6.8537
6.8393
6.8259
6.7887
6.7794
6.7732
6.7551
6.7193

6.1626
6.1339

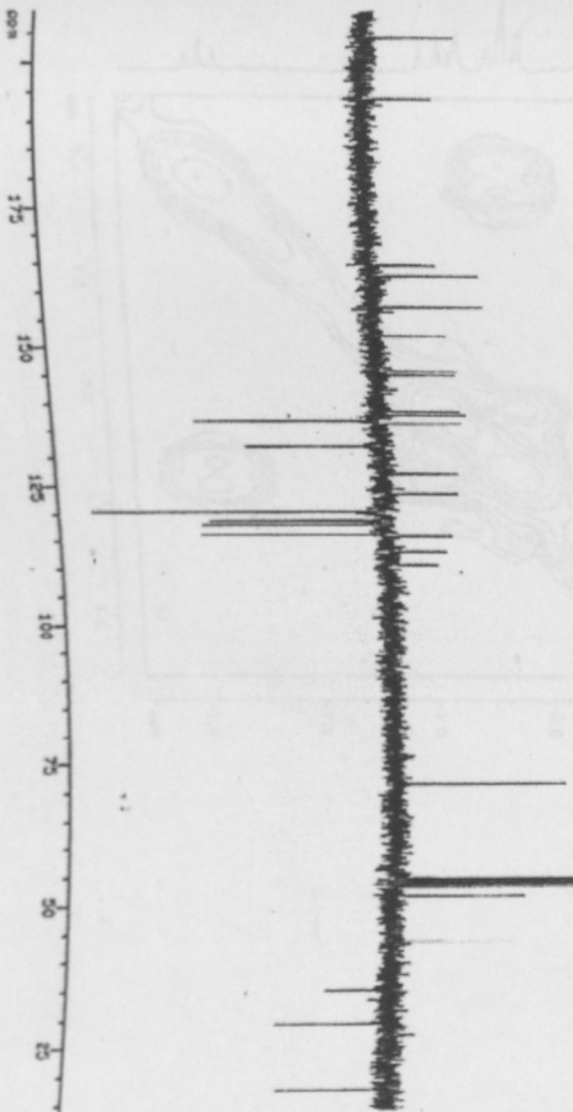


APT spectrum (CD₂Cl₂, 75 MHz)

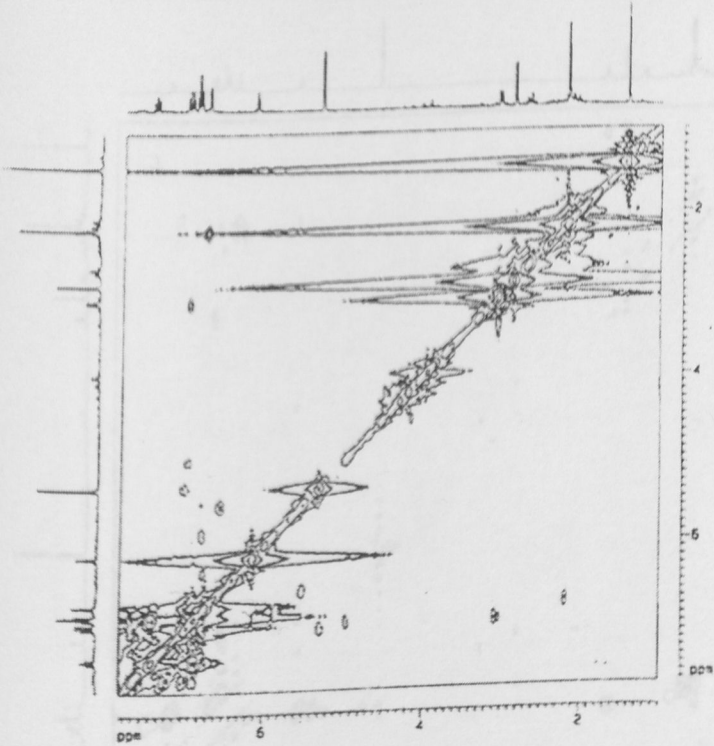
ppm
204.433
194.069

164.805
162.902
157.484
152.135
145.871
145.148
138.652
138.083
136.999
136.471
132.035
127.125
123.563
120.464
116.904
118.218
116.138
115.733
113.011
112.929
110.606

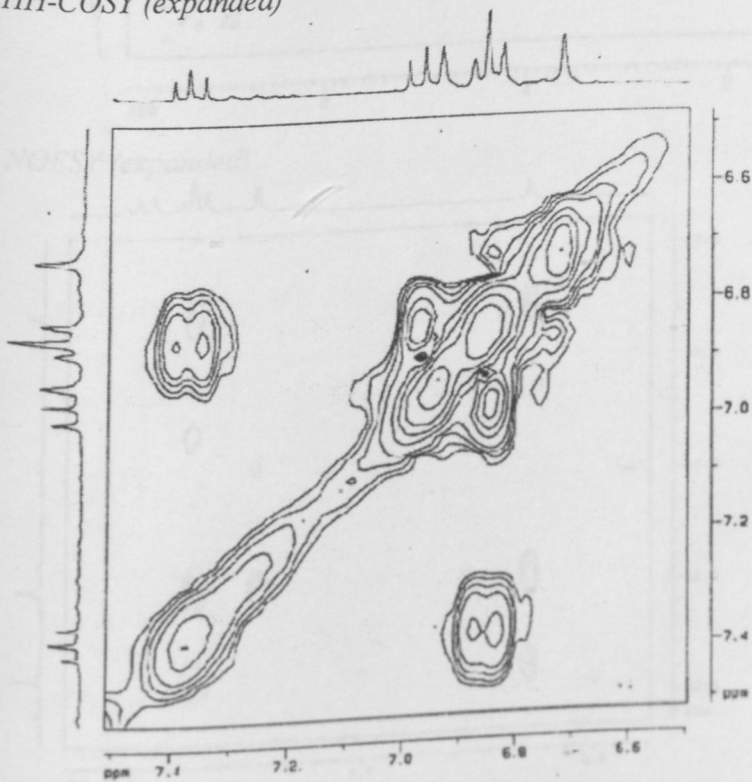
71.371
54.565
54.305
53.844
53.464
53.124
51.589
43.463
35.035
29.348
17.539



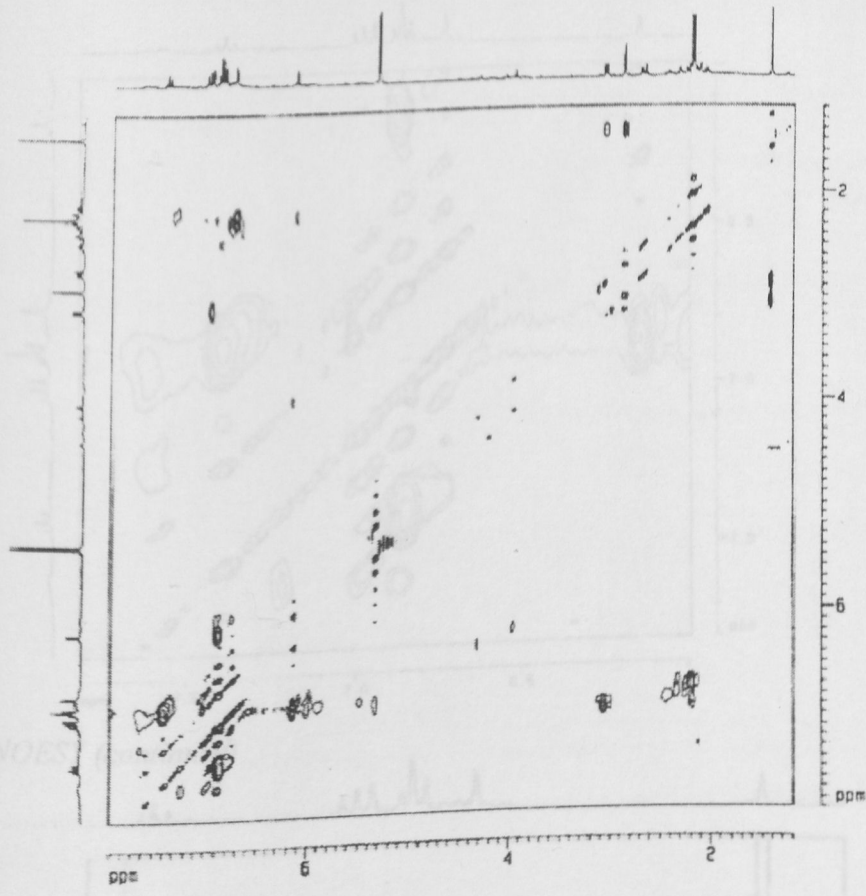
HH-COSY (CD₂Cl₂, 300 MHz)



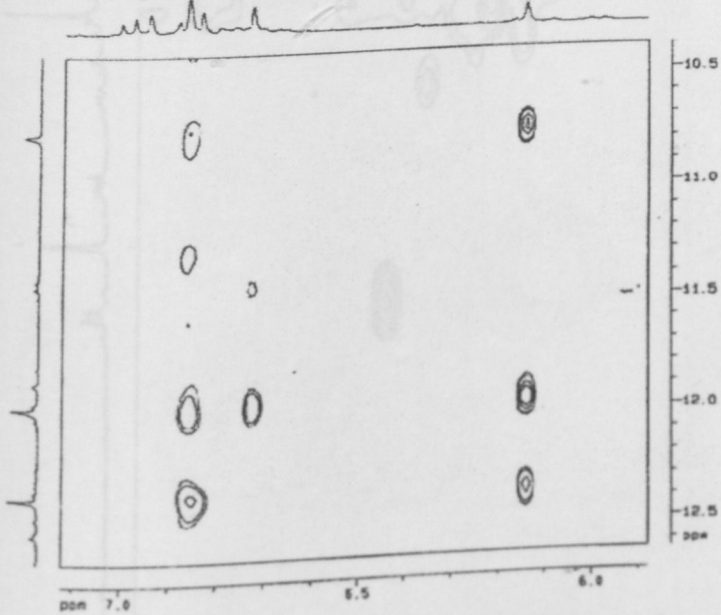
HH-COSY (expanded)



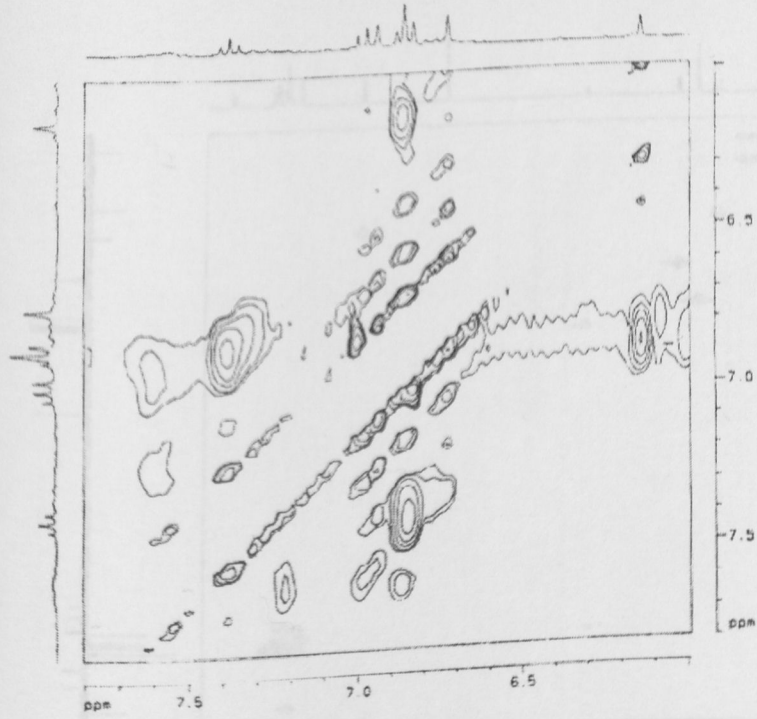
NOESY (CD₂Cl₂, 300 MHz)



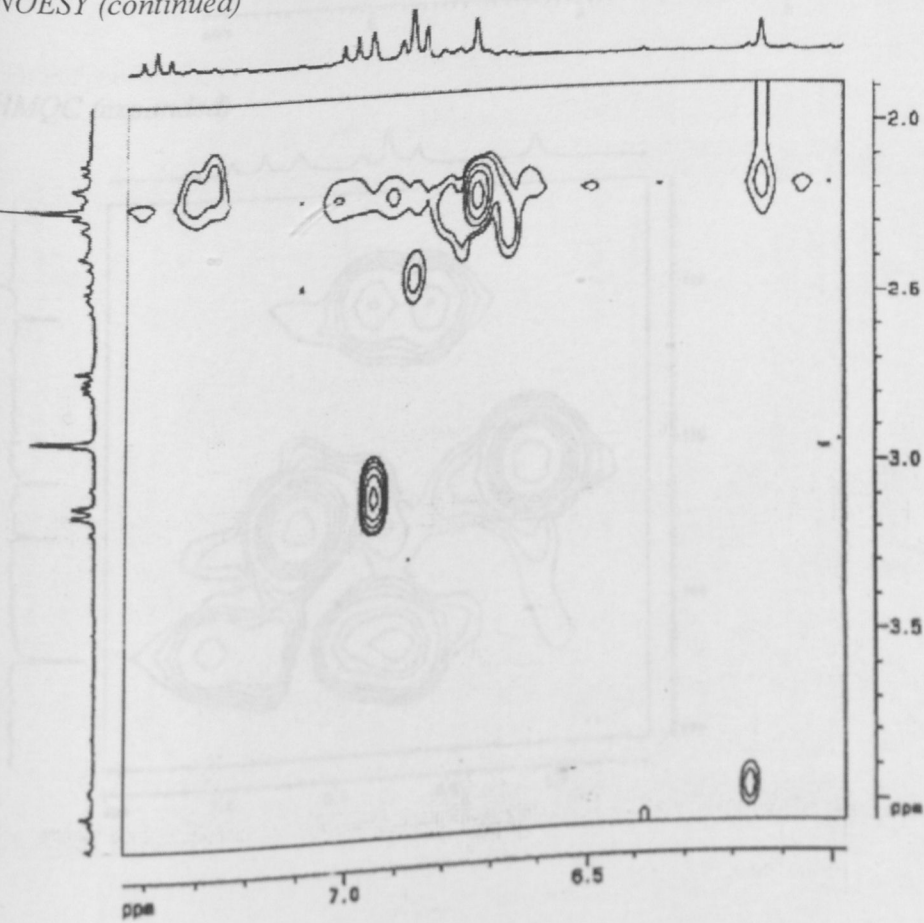
NOESY (expanded)



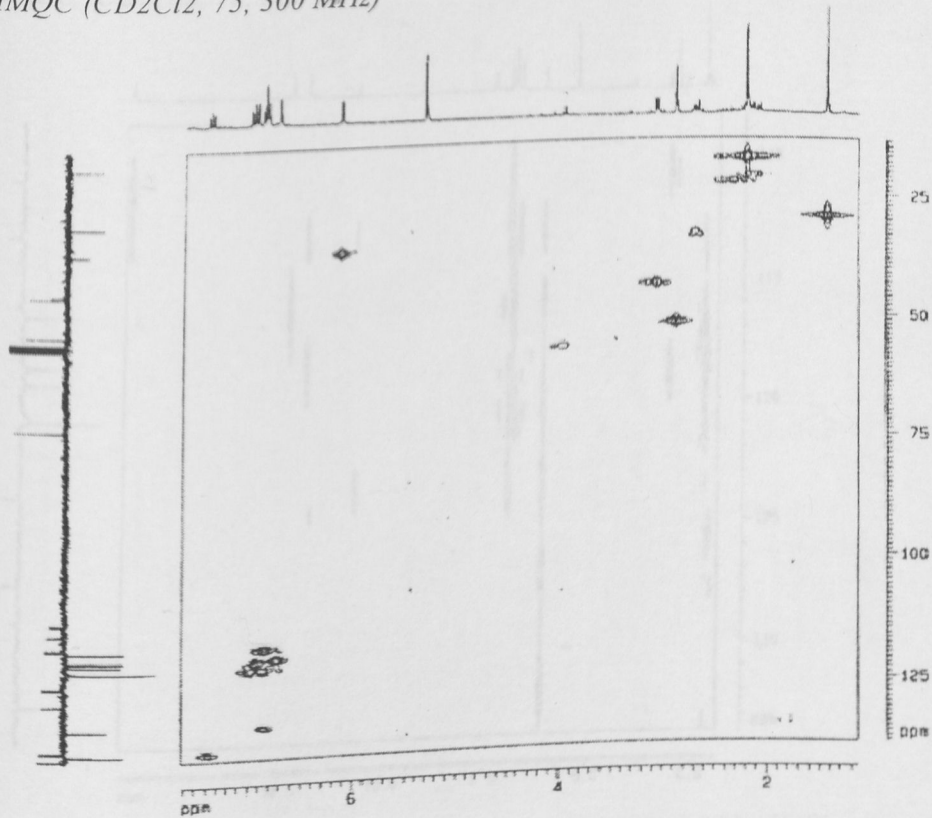
NOESY (continued)



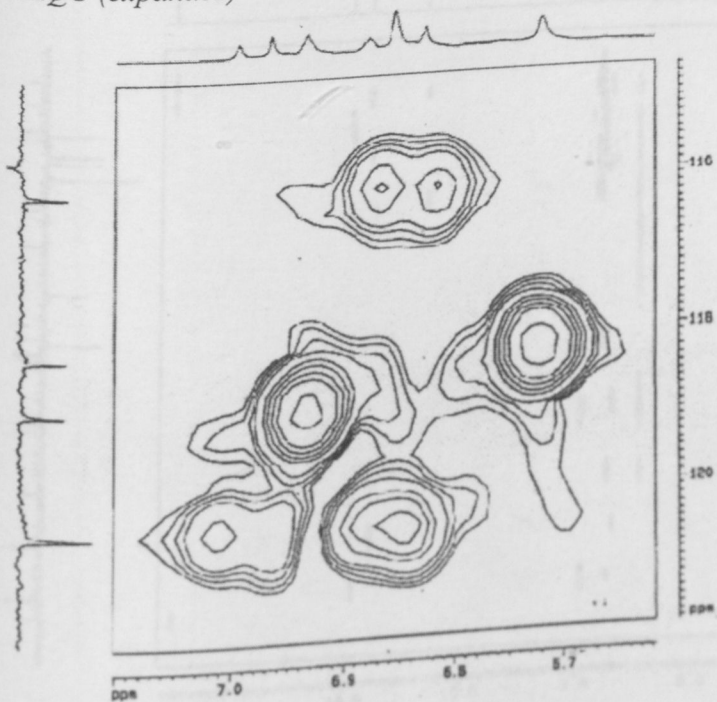
NOESY (continued)



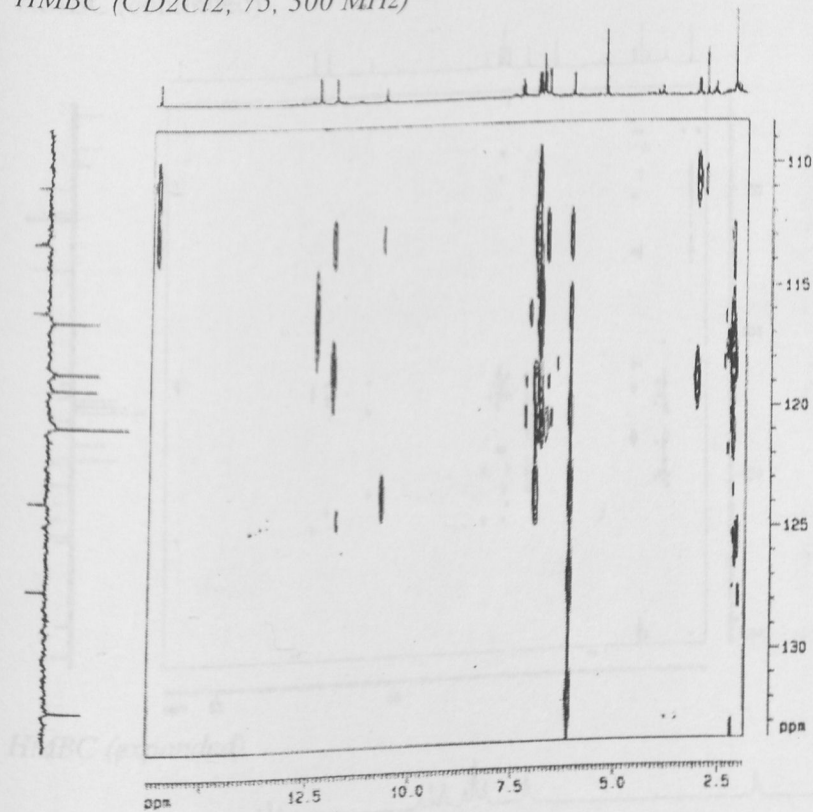
HMOC (CD₂Cl₂, 75, 300 MHz)



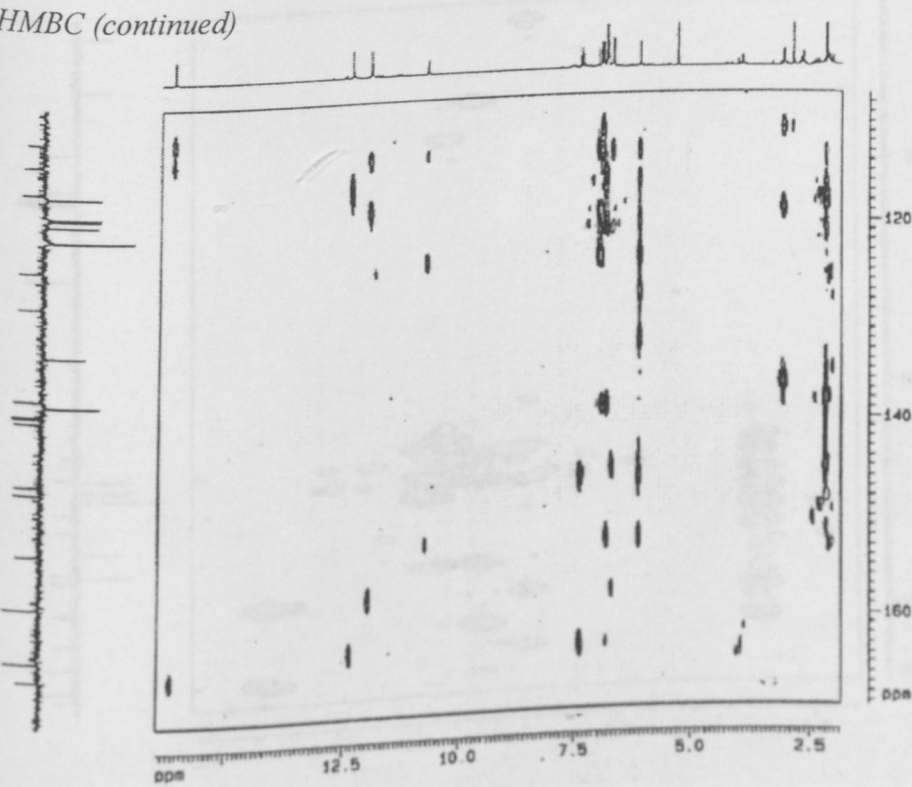
HMOC (expanded)



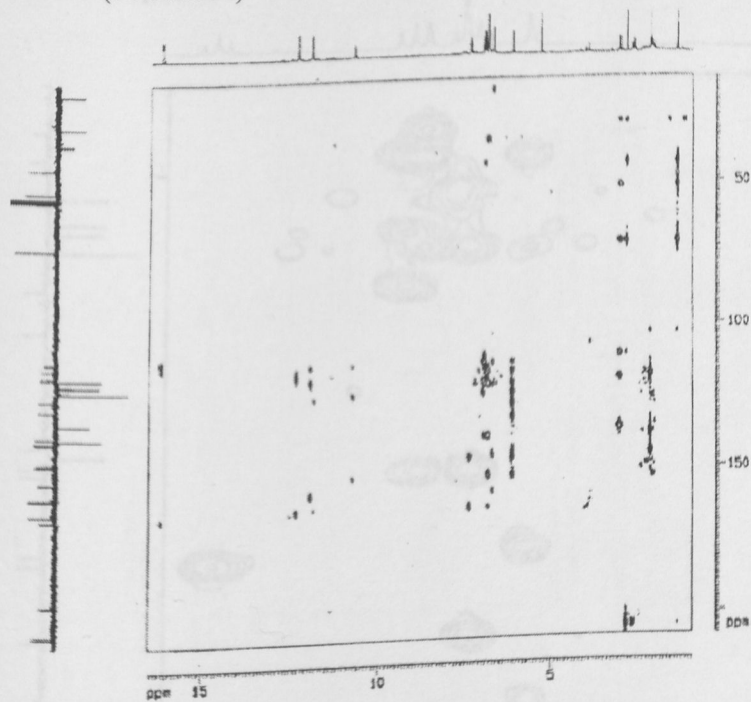
HMBC (CD₂Cl₂, 75, 300 MHz)



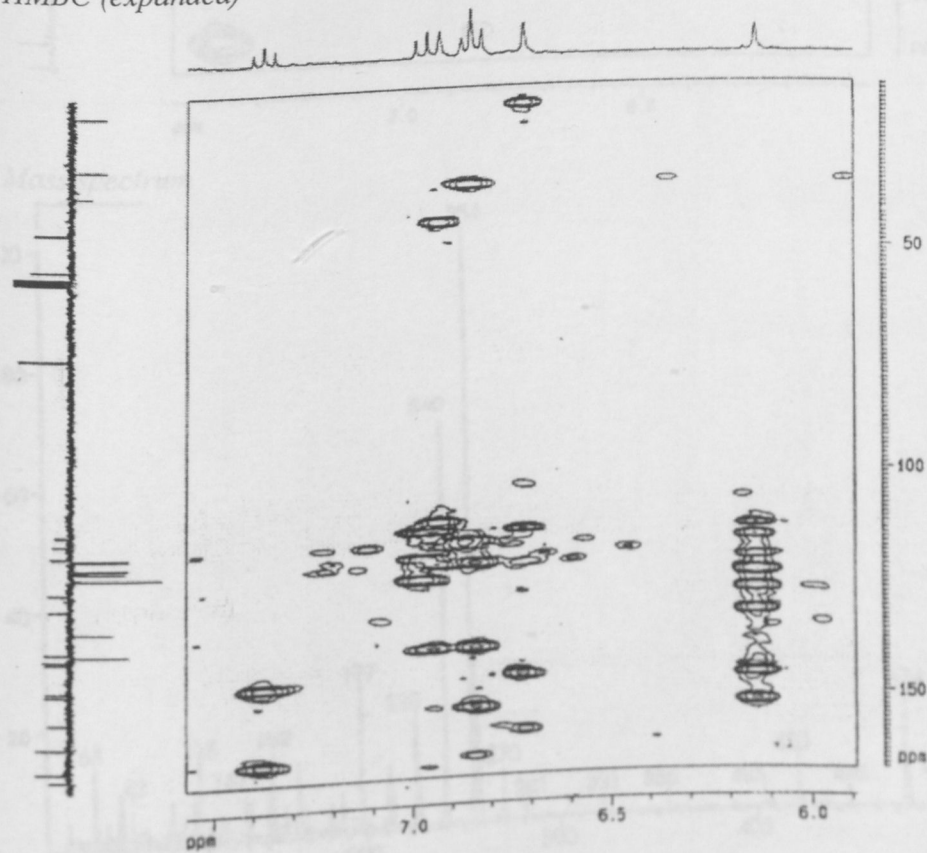
HMBC (continued)



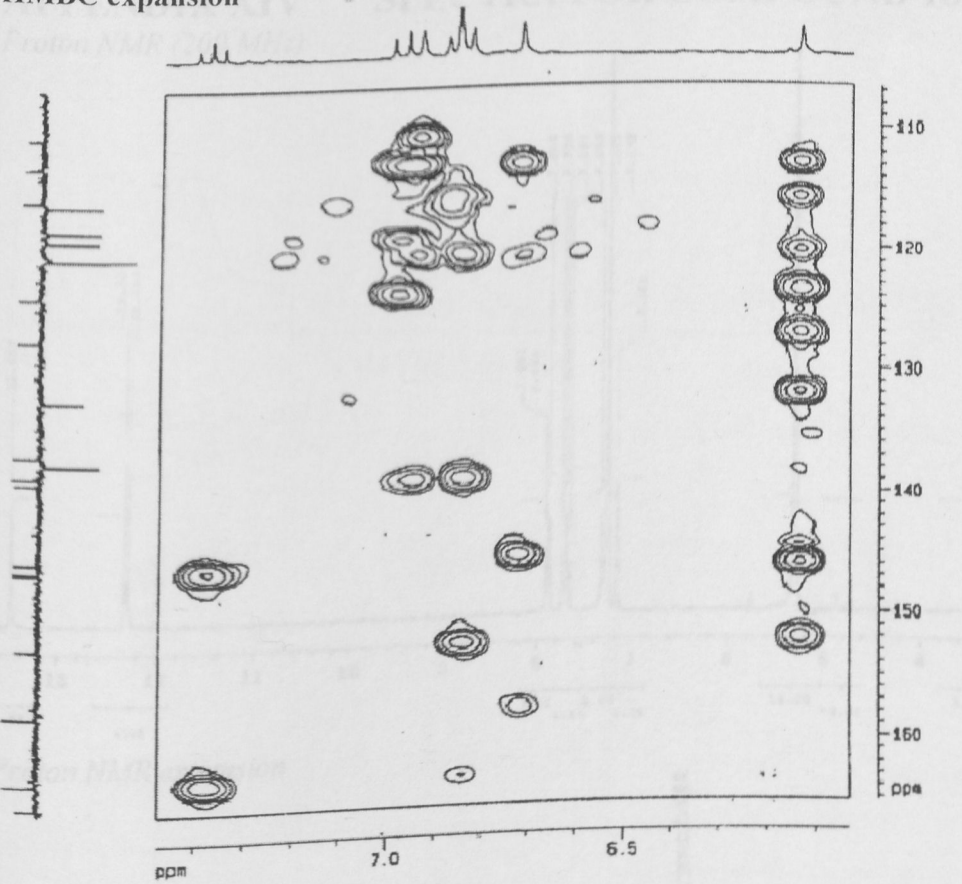
HMBC (continued)



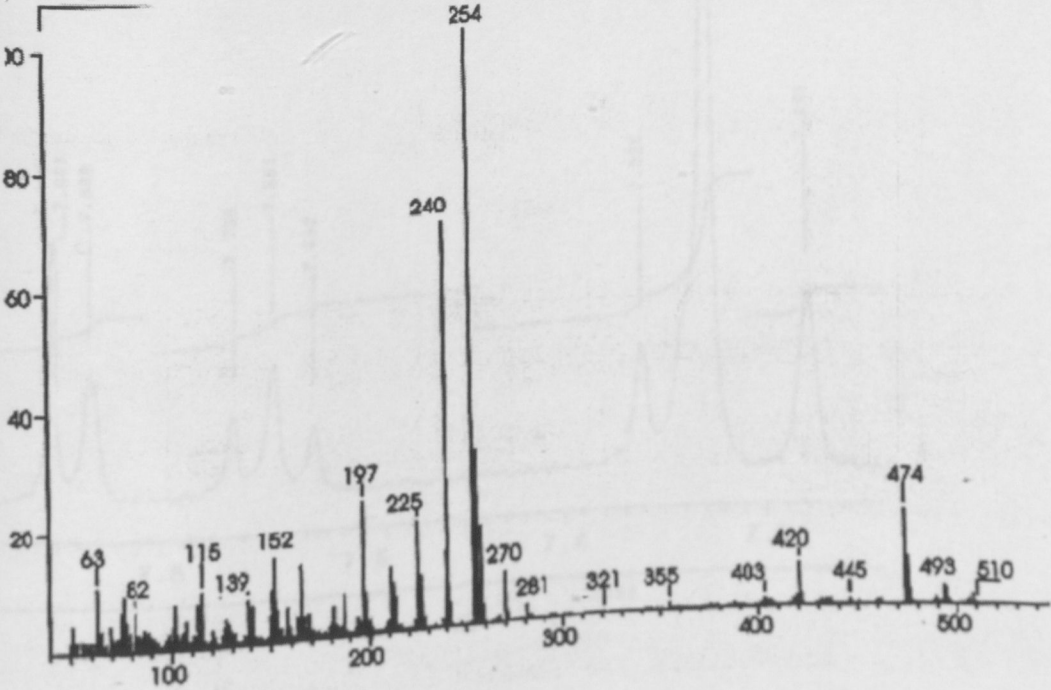
HMBC (expanded)



HMBC expansion

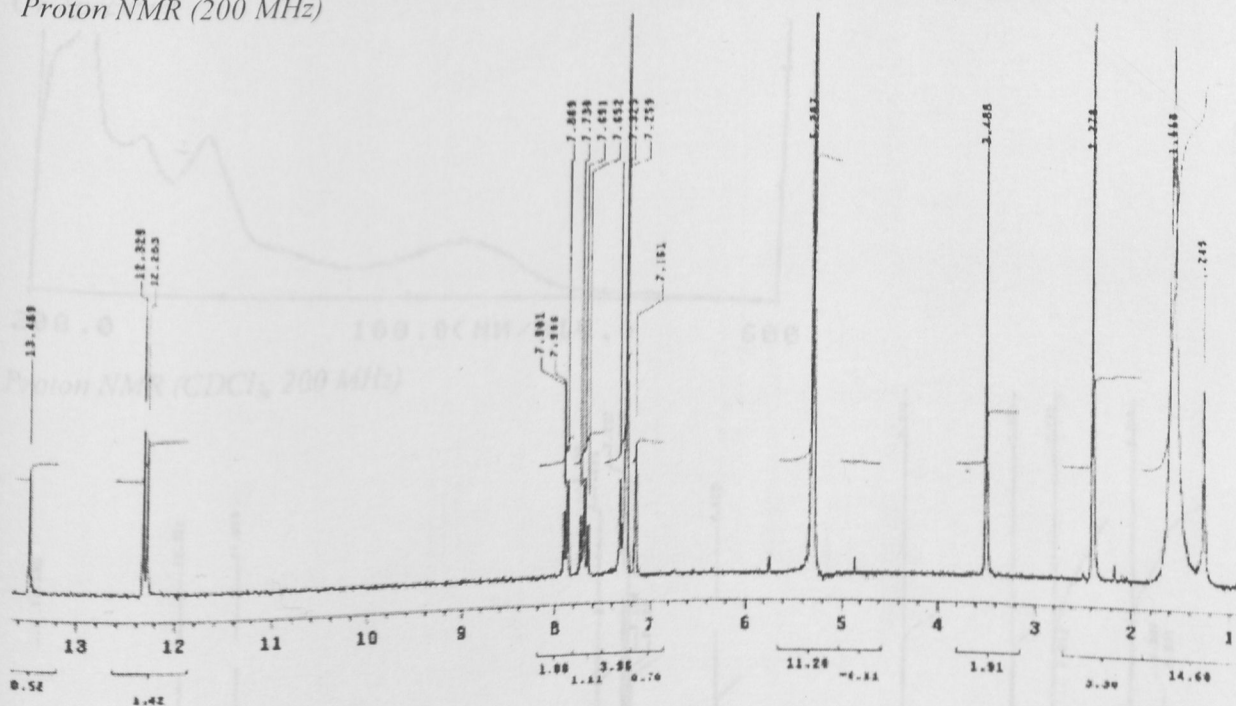


Mass spectrum

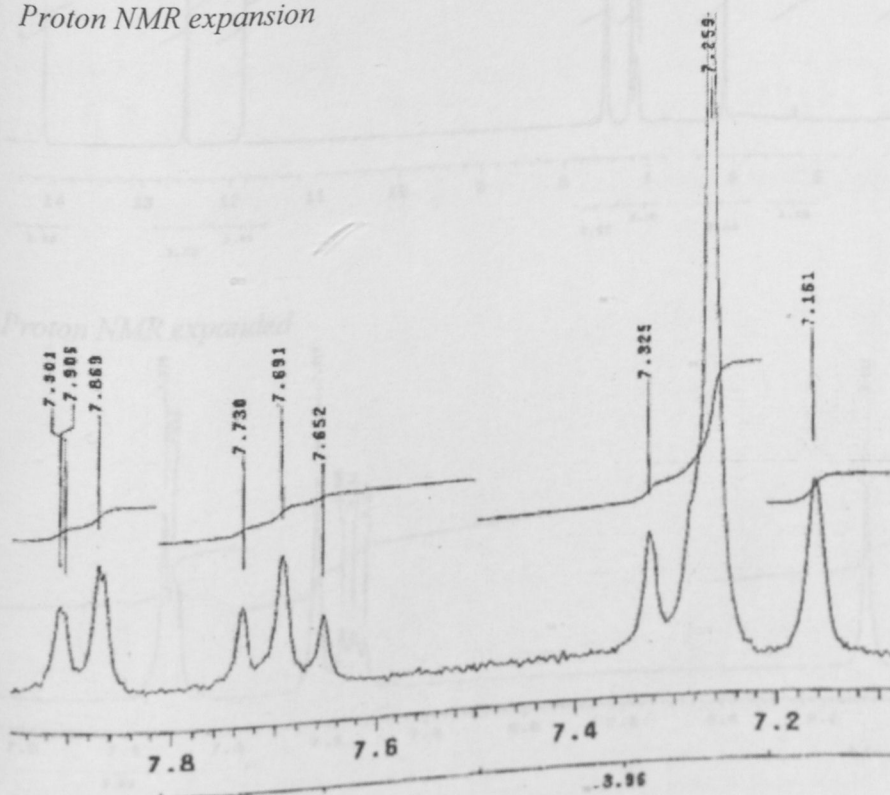


APPENDIX XIV SPECTRA FOR COMPOUND 16

Proton NMR (200 MHz)

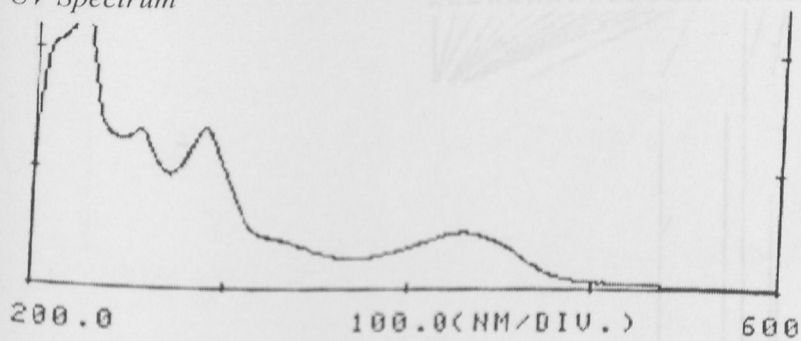


Proton NMR expansion

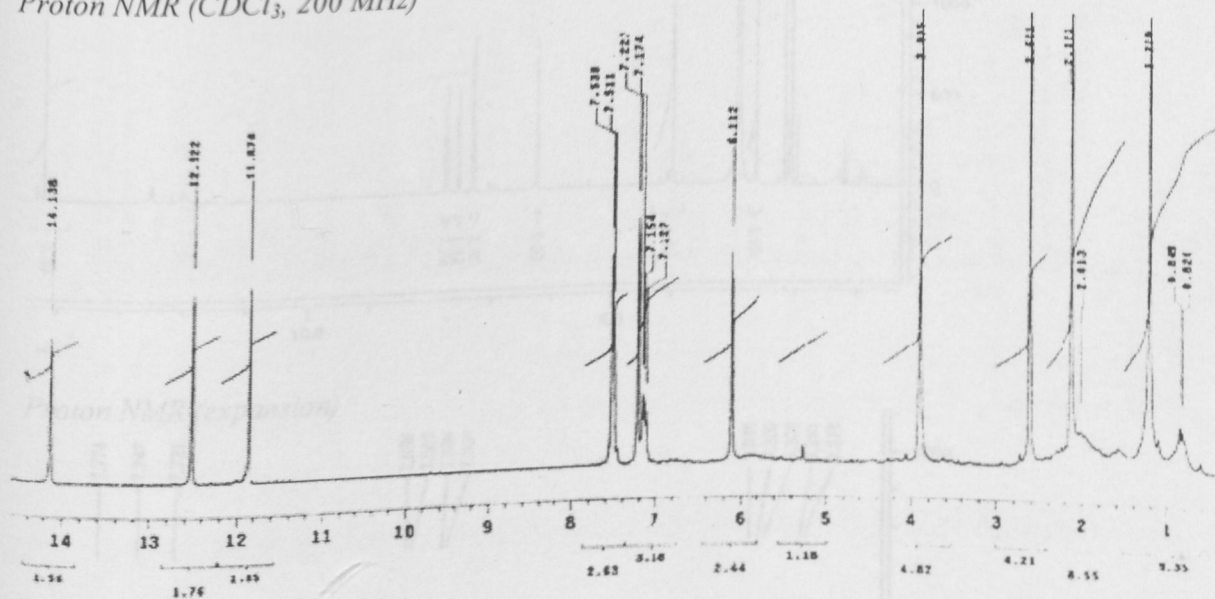


APPENDIX XV SPECTRA FOR COMPOUND 17

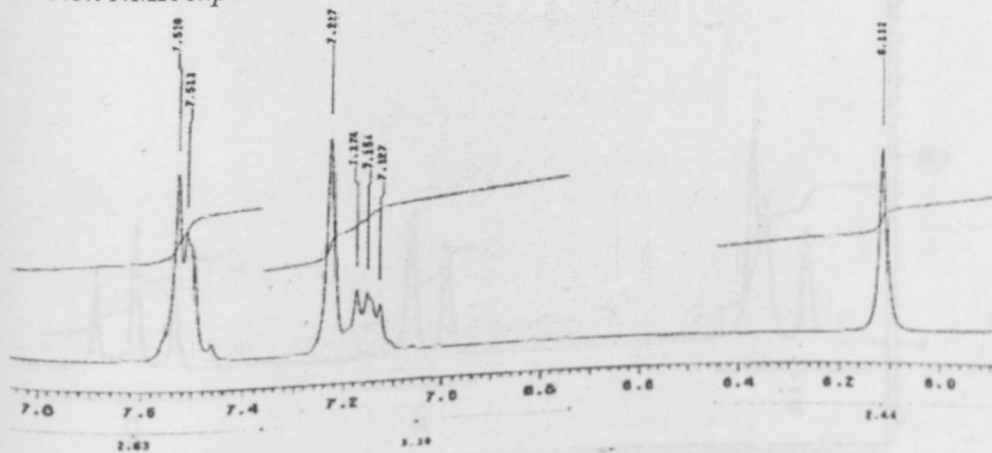
UV Spectrum



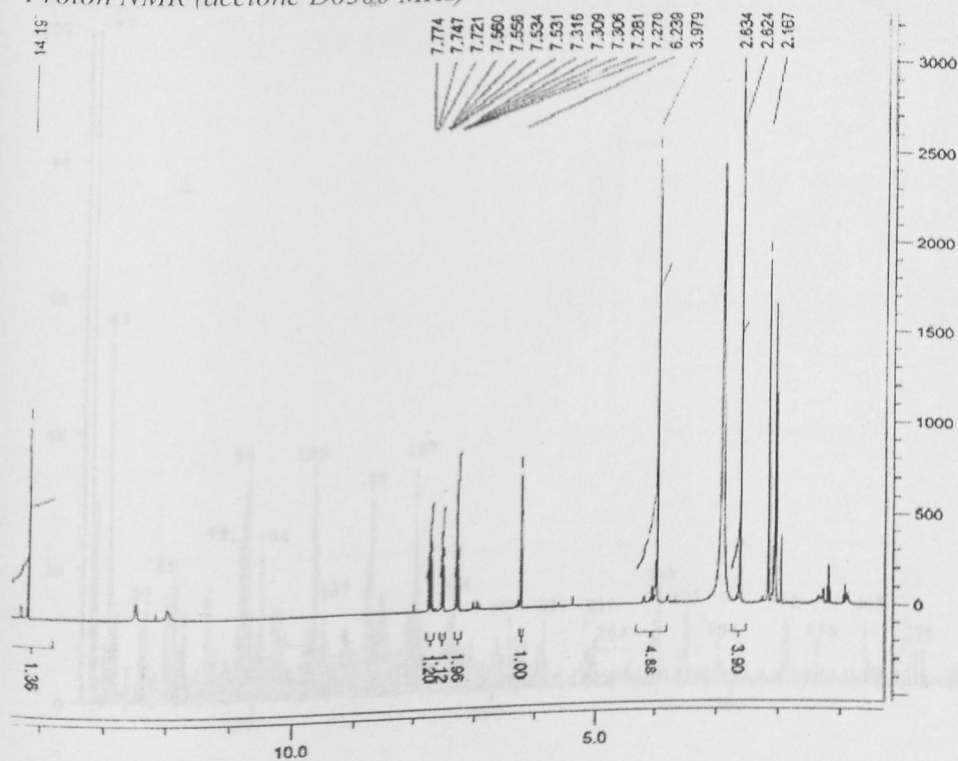
Proton NMR (CDCl_3 , 200 MHz)



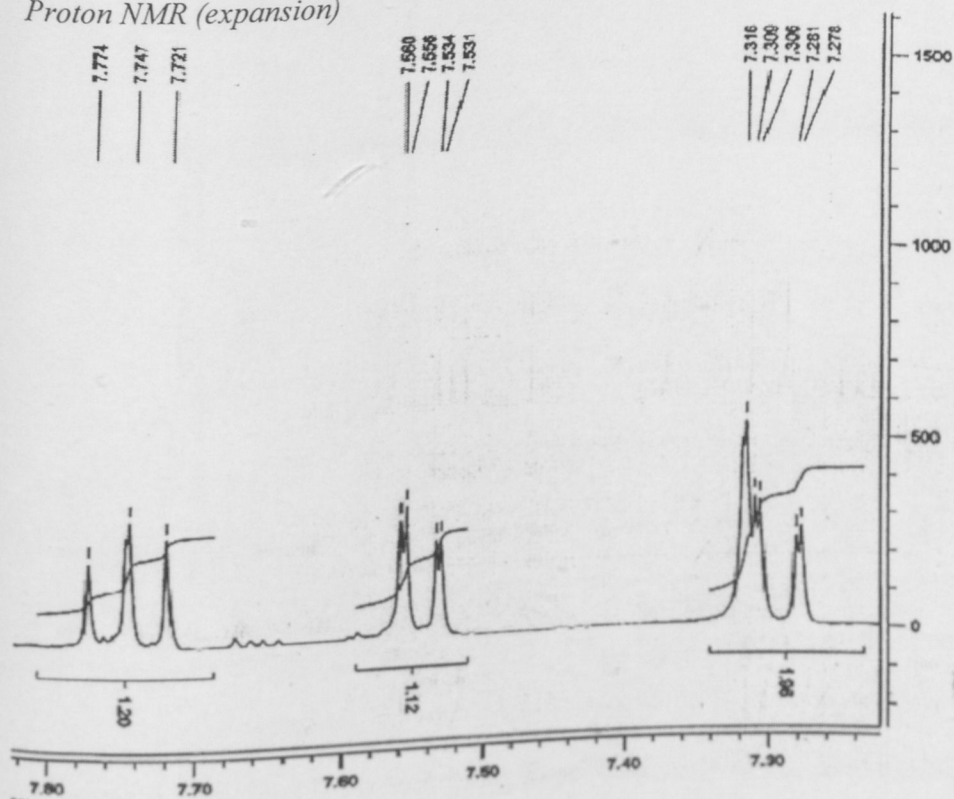
Proton NMR expanded



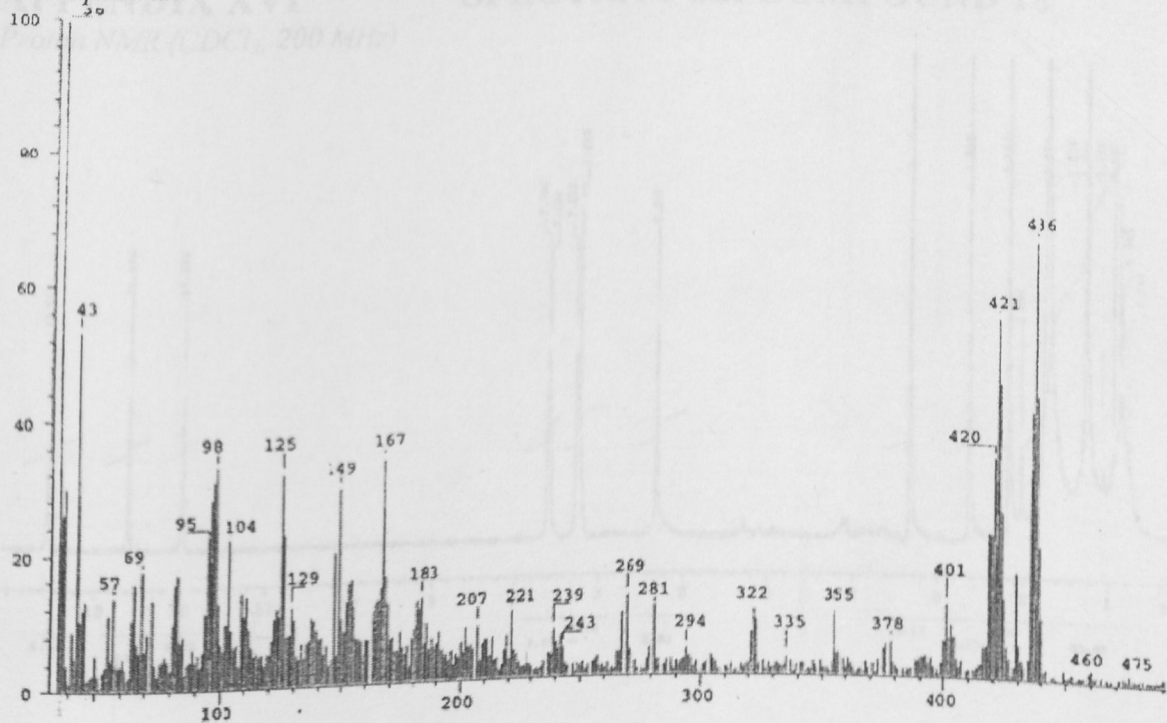
Proton NMR (acetone D6300 MHz)



Proton NMR (expansion)



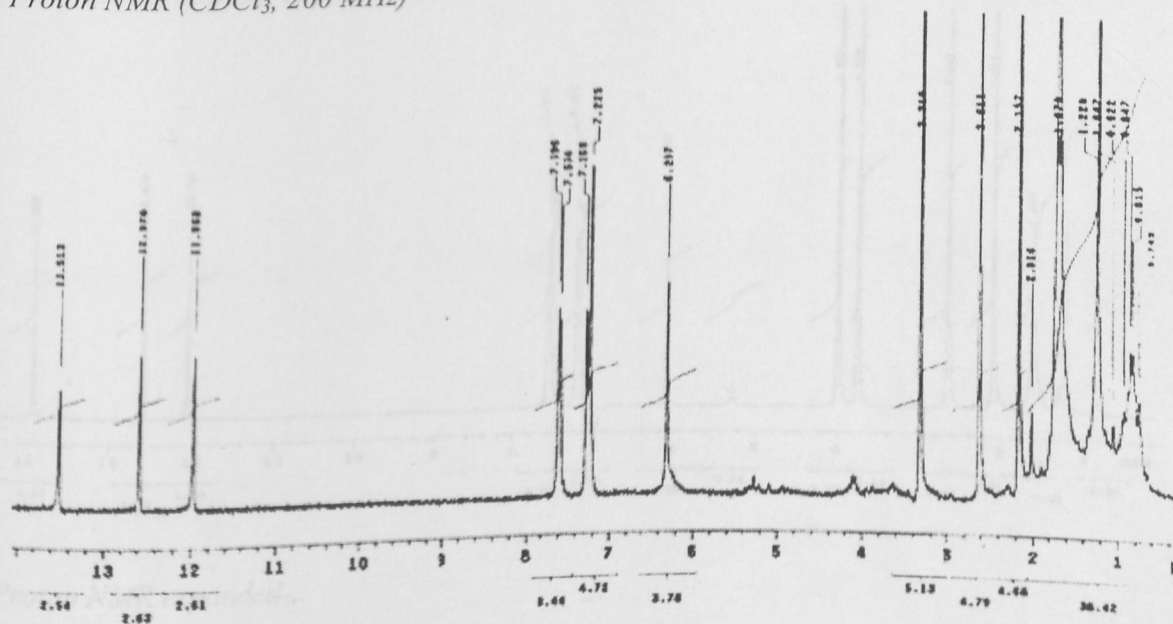
Mass spectrum



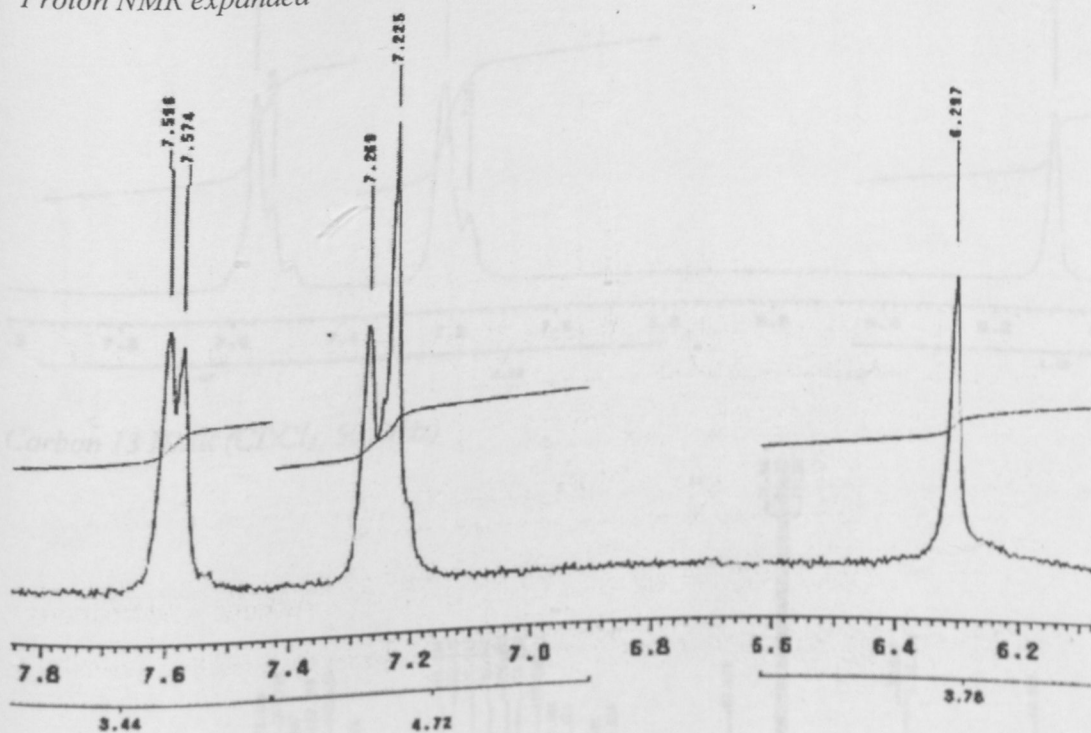
APPENDIX XVI

SPECTRA FOR COMPOUND 18

Proton NMR (CDCl_3 , 200 MHz)

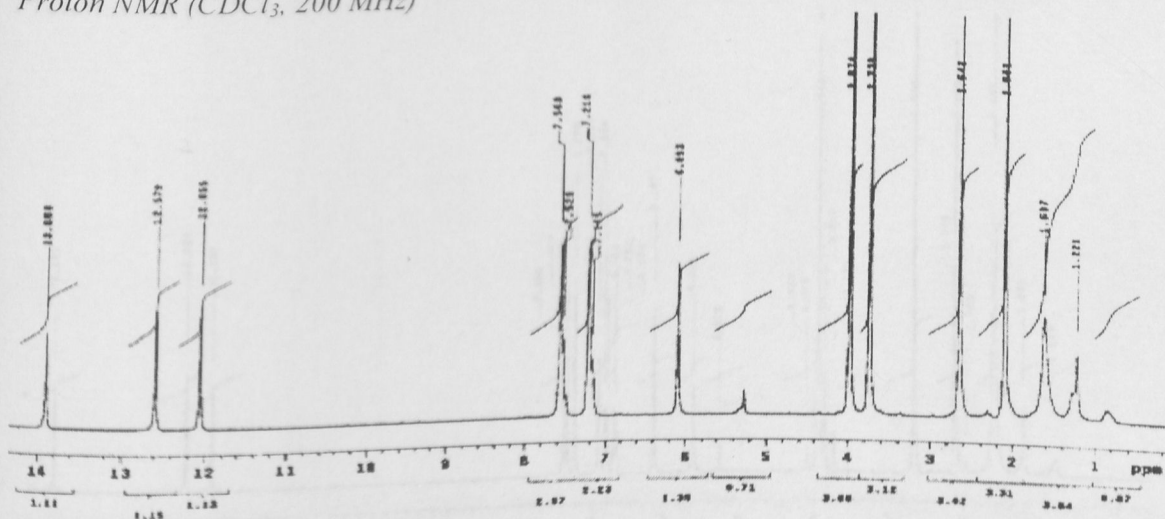


Proton NMR expanded

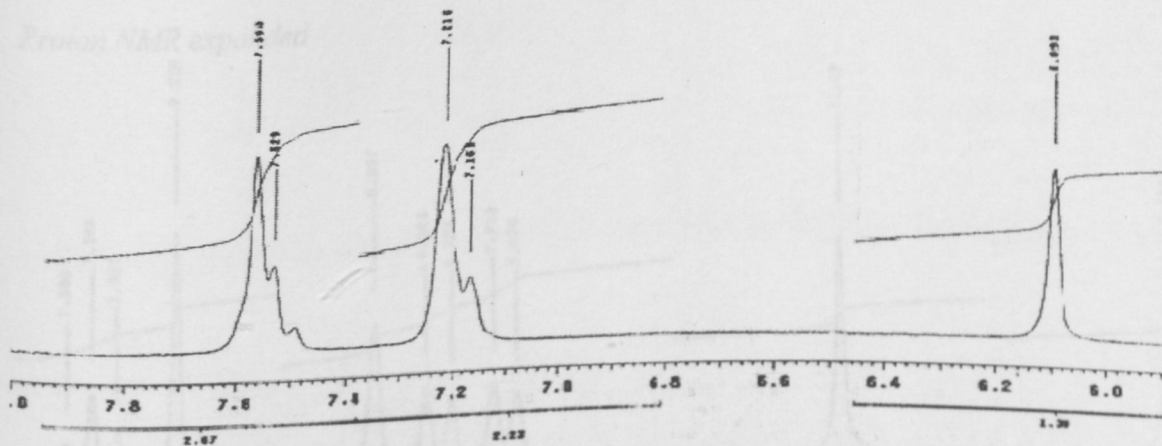


APPENDIX XVII SPECTRA FOR COMPOUND 19

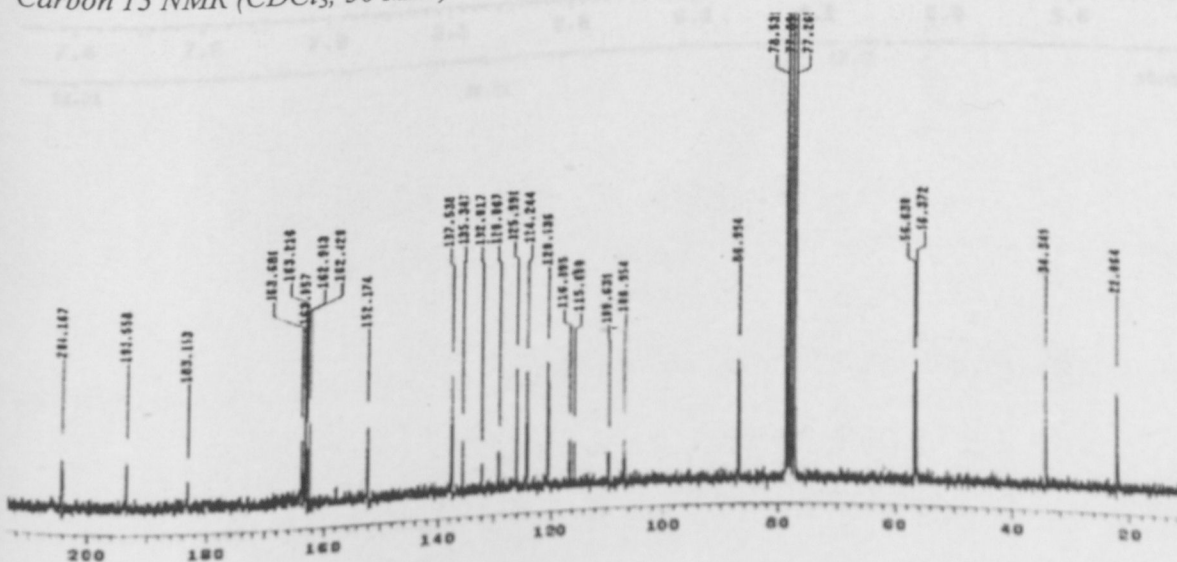
Proton NMR (CDCl_3 , 200 MHz)



Proton NMR expanded

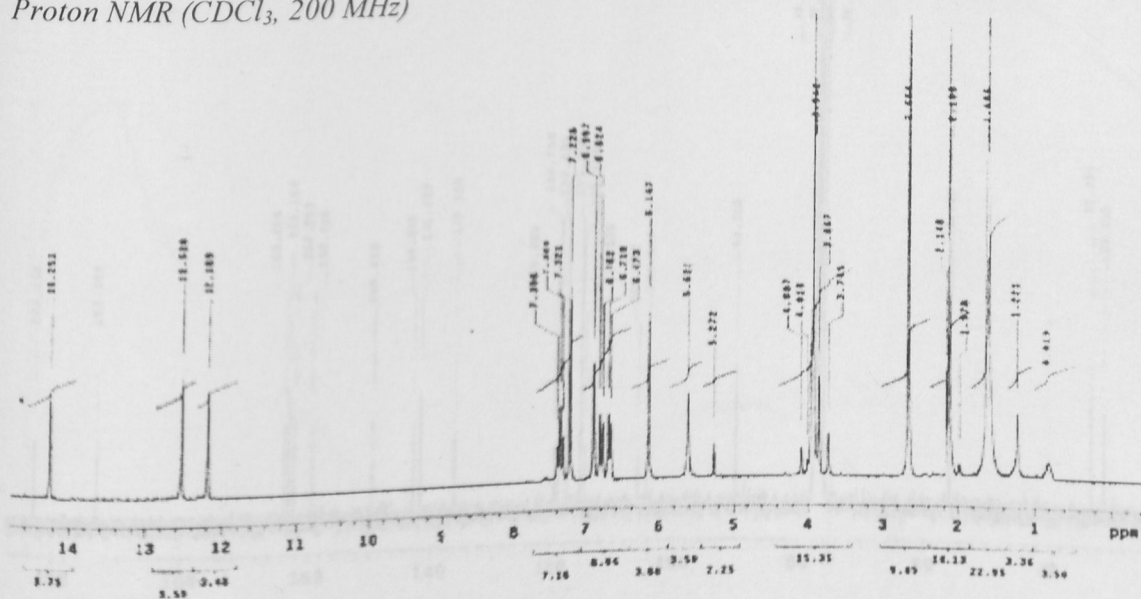


Carbon 13 NMR (CDCl_3 , 50 MHz)

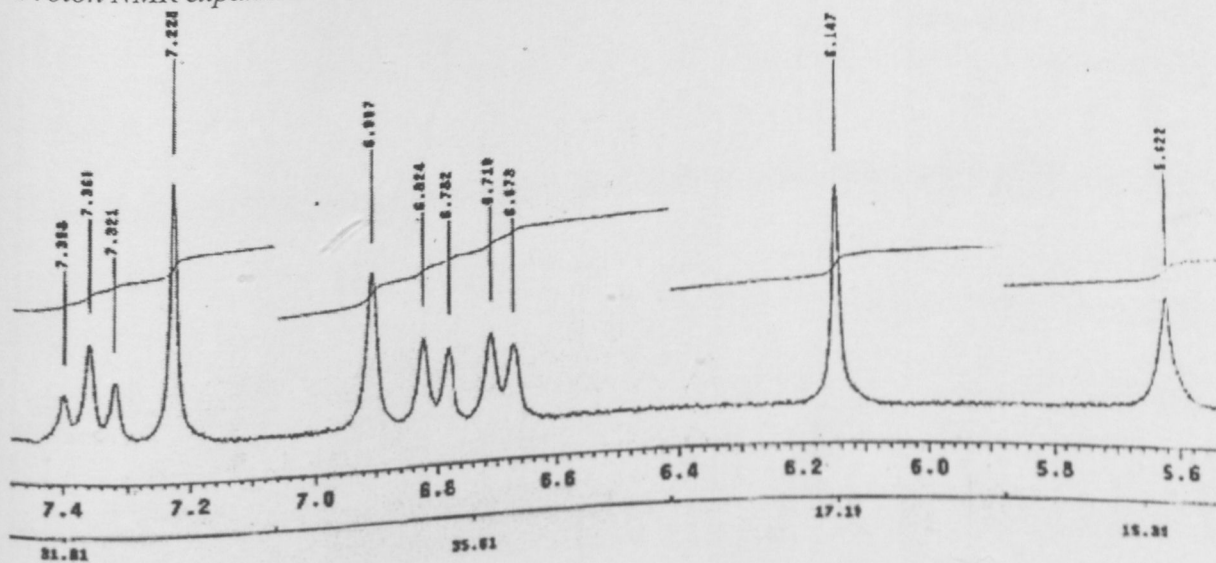


APPENDIX XVIII SPECTRA FOR COMPOUND 20

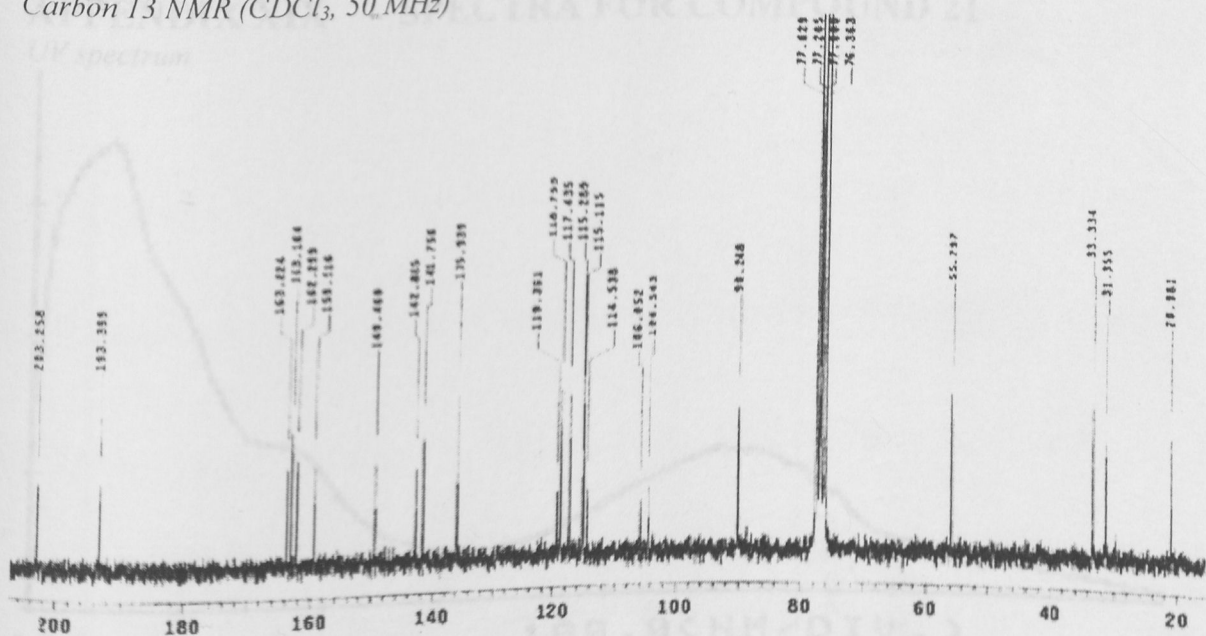
Proton NMR (CDCl₃, 200 MHz)



Proton NMR expanded



Carbon 13 NMR (CDCl₃, 50 MHz)

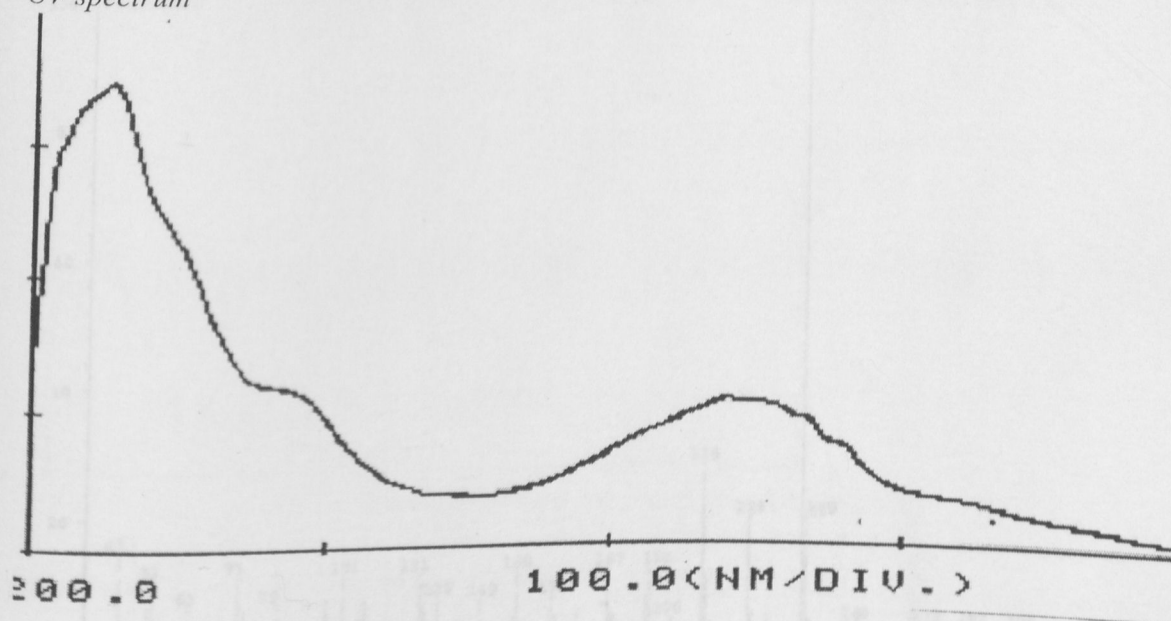


Proton NMR spectrum (CDCl₃, 500 MHz)

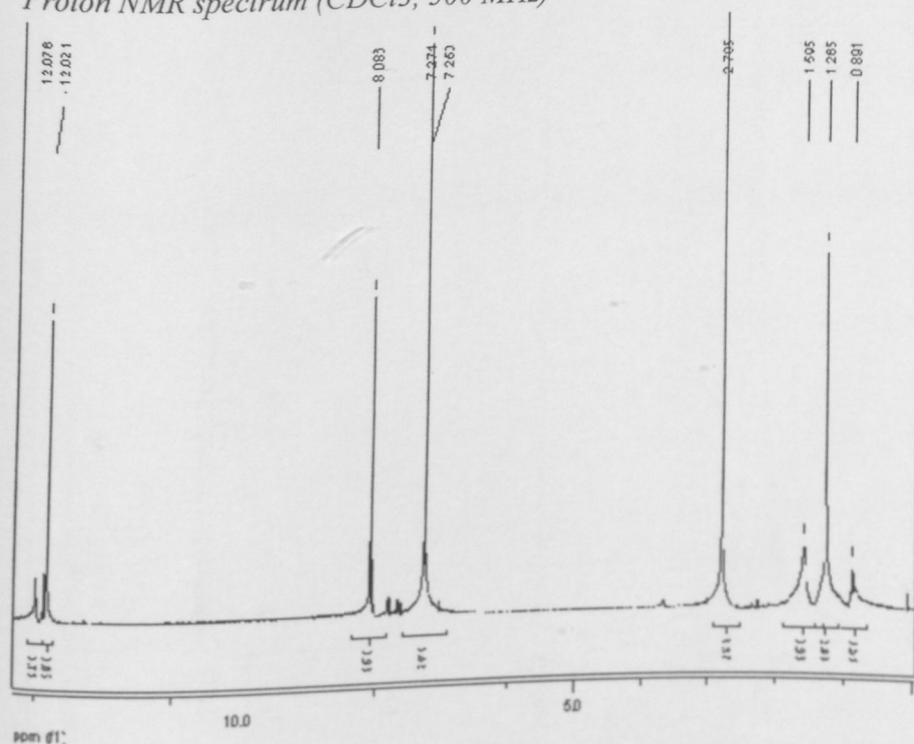


APPENDIX XIX SPECTRA FOR COMPOUND 21

UV spectrum

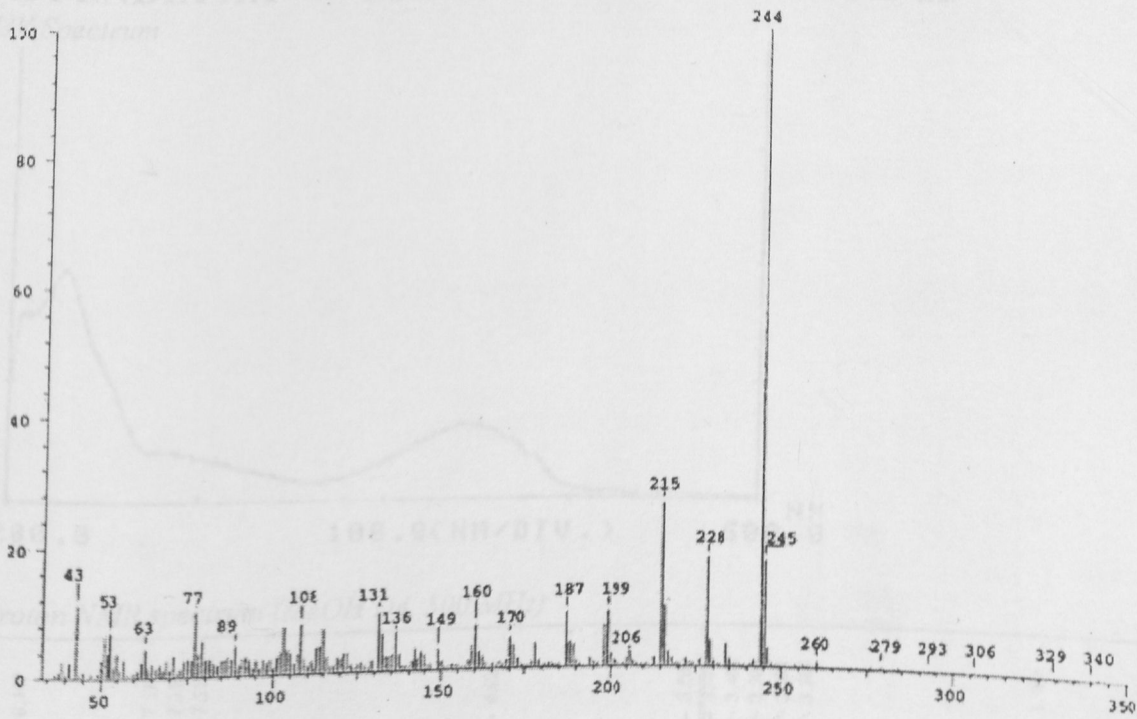


Proton NMR spectrum (CDCl₃, 500 MHz)



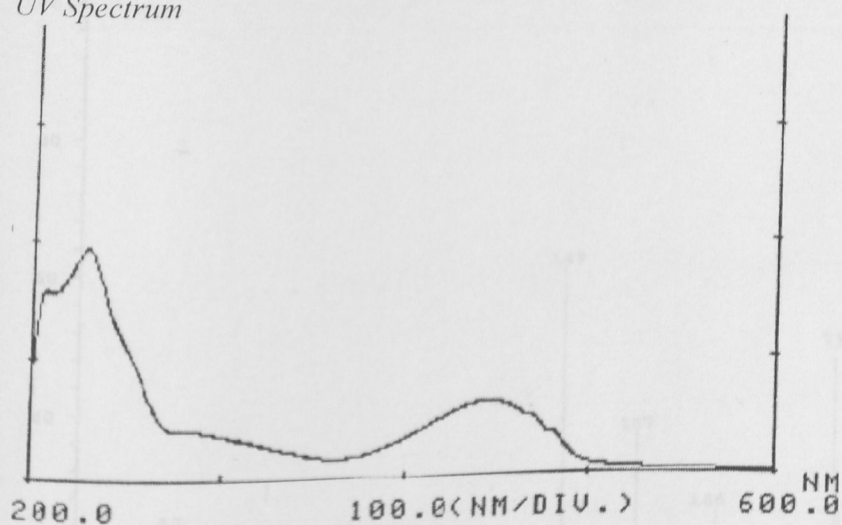
Mass spectrum

SPECTRA FOR COMPOUND 22

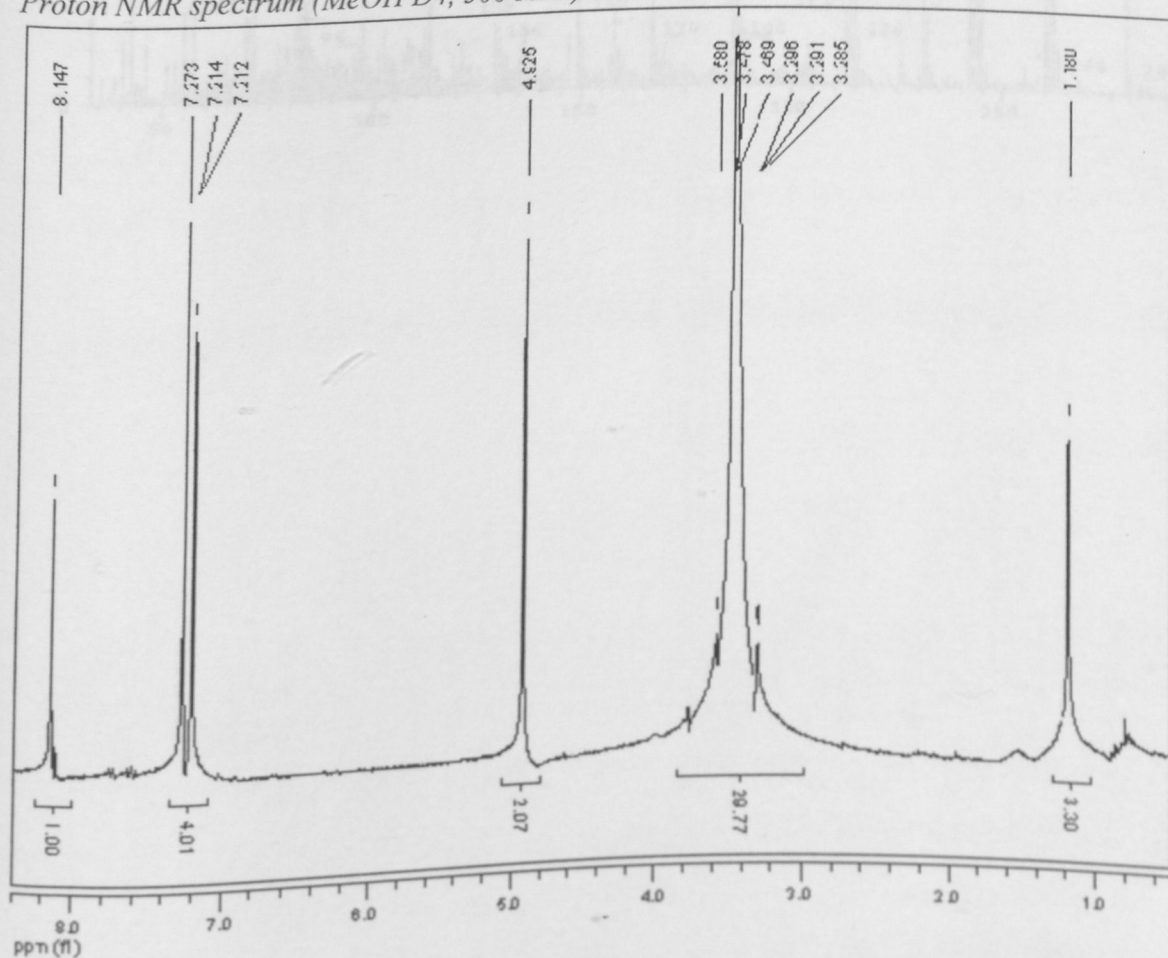


APPENDIX XX SPECTRA FOR COMPOUND 22

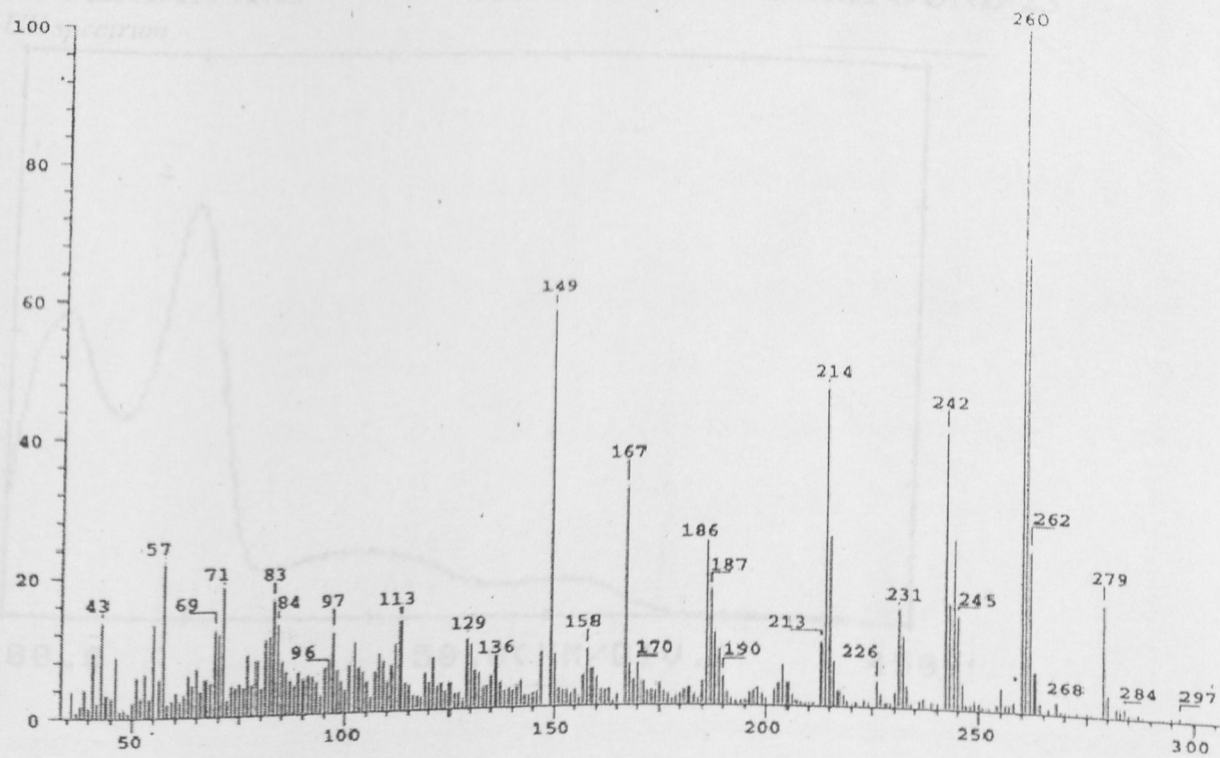
UV Spectrum



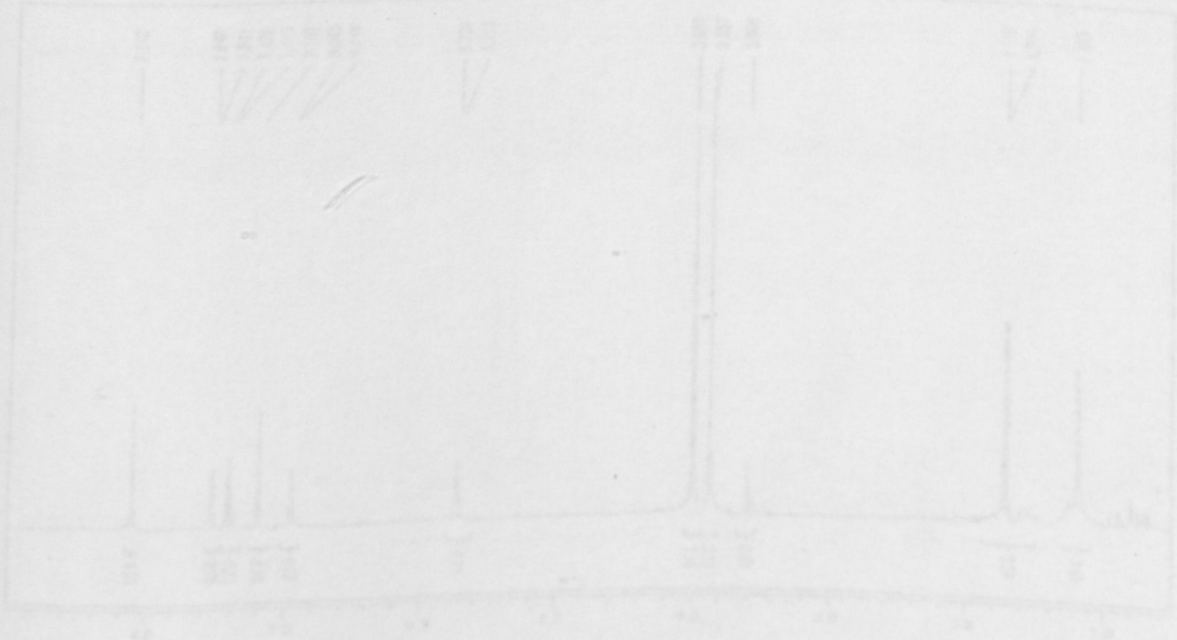
Proton NMR spectrum (MeOH D4, 500 MHz)



Mass Spectrum for compound 22



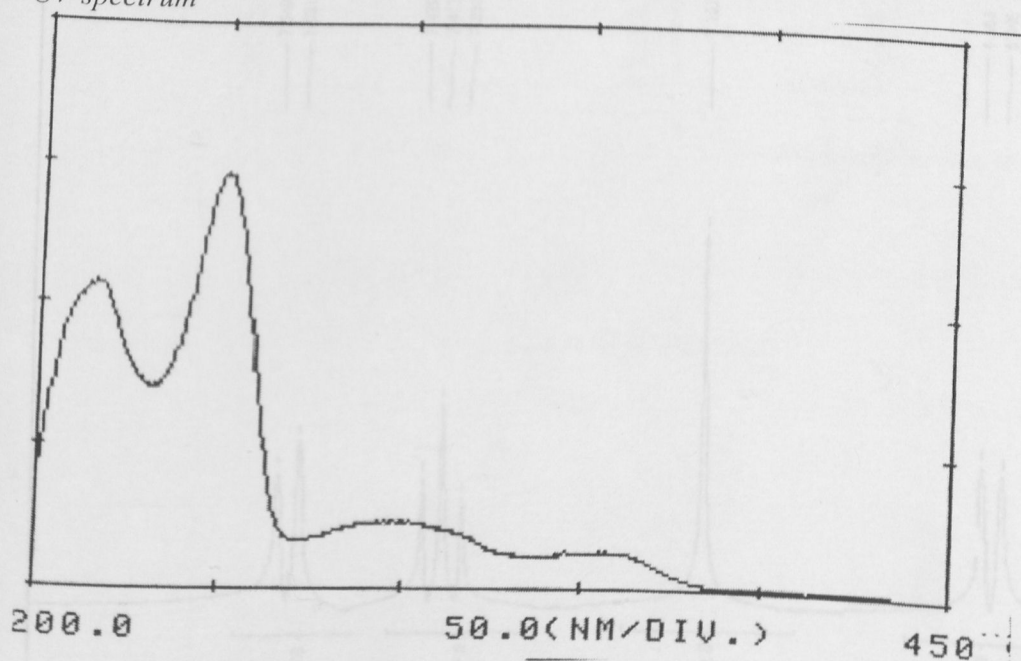
Proton NMR (500 MHz)



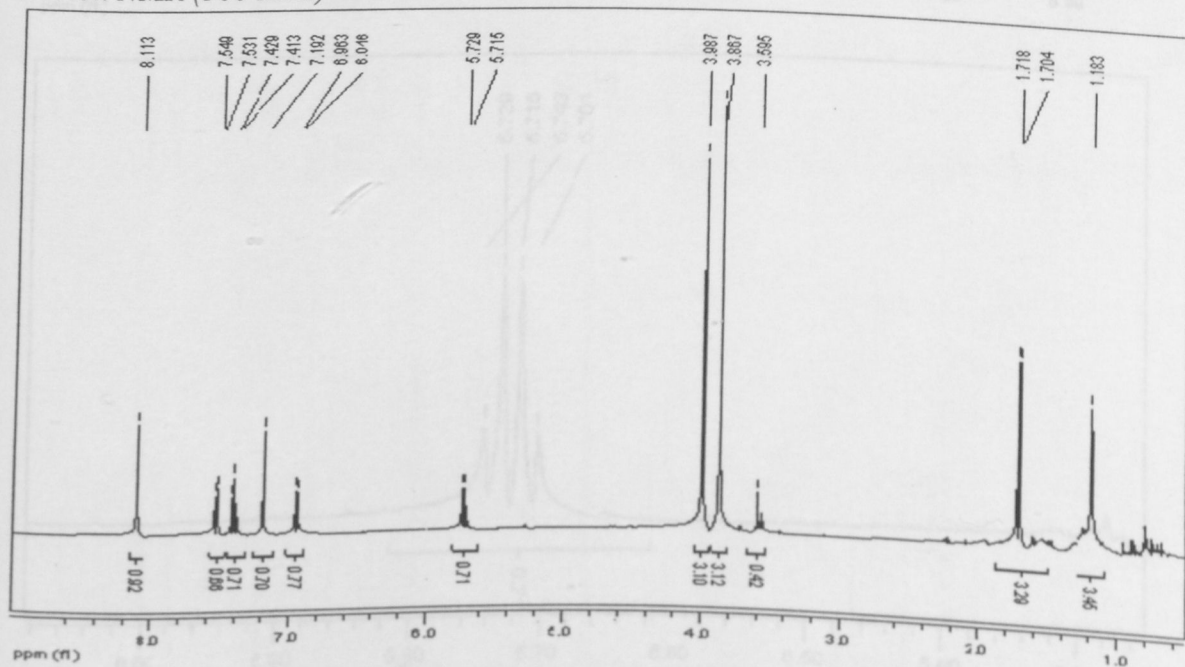
APPENDIX XXI

SPECTRA FOR COMPOUND 23

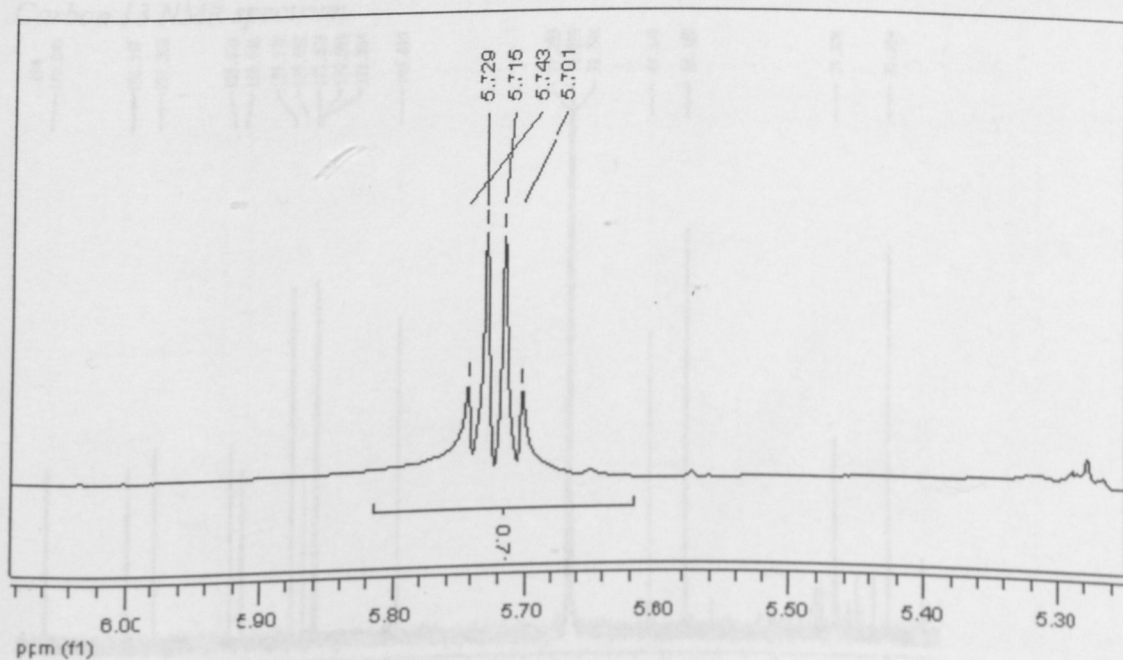
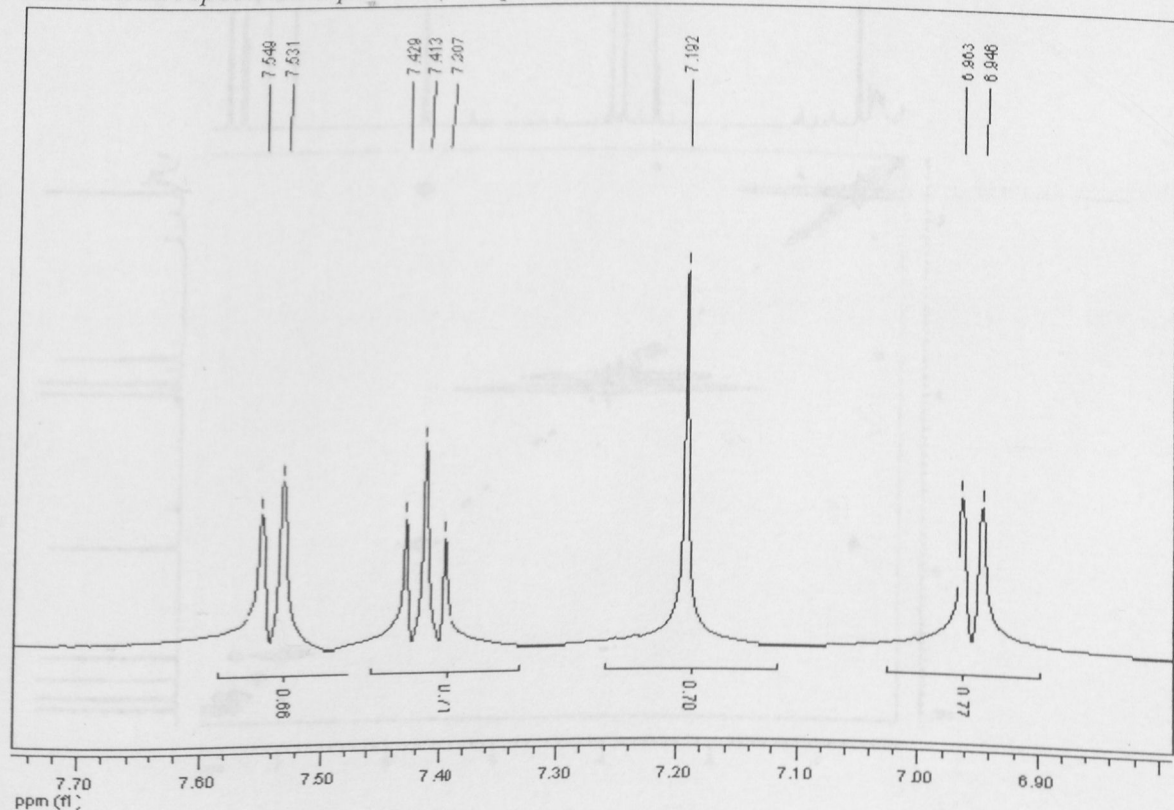
UV spectrum



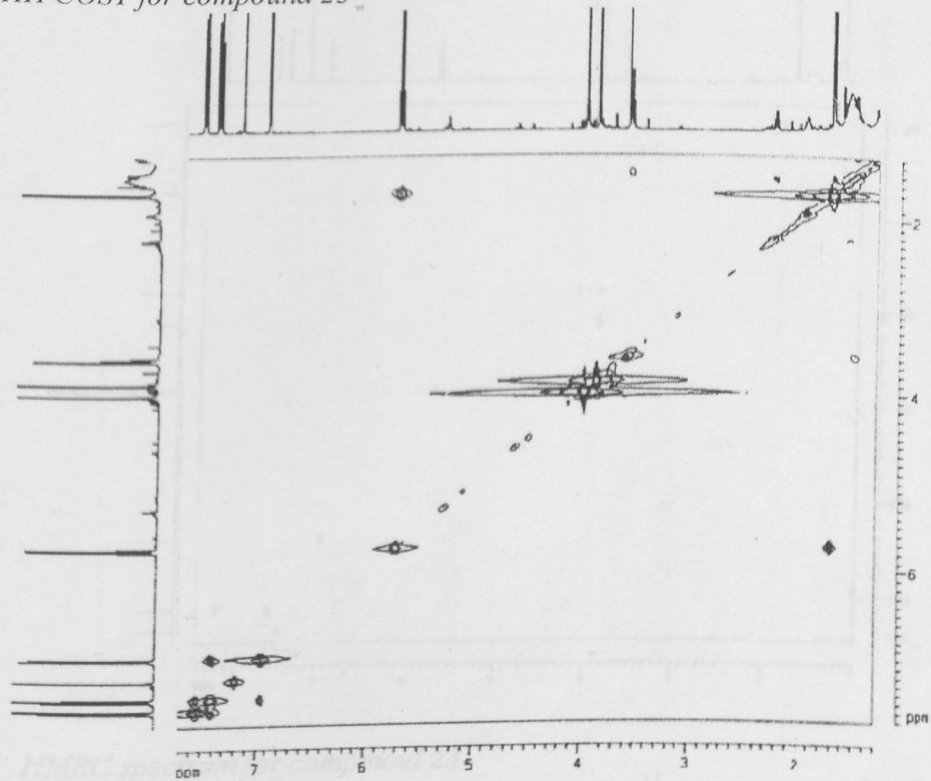
Proton NMR (500 MHz)



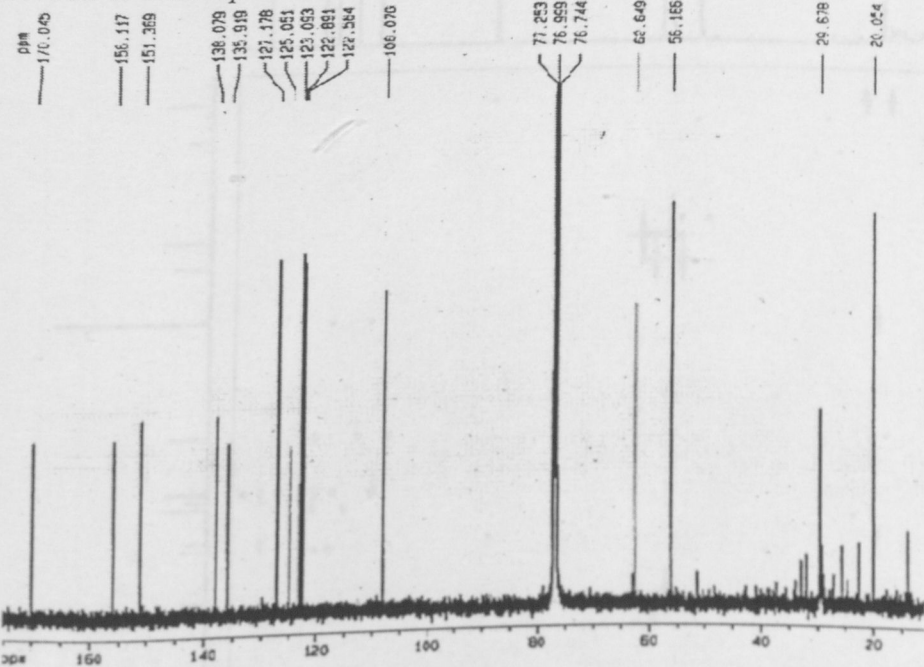
Proton NMR spectrum expanded (Compound 23)



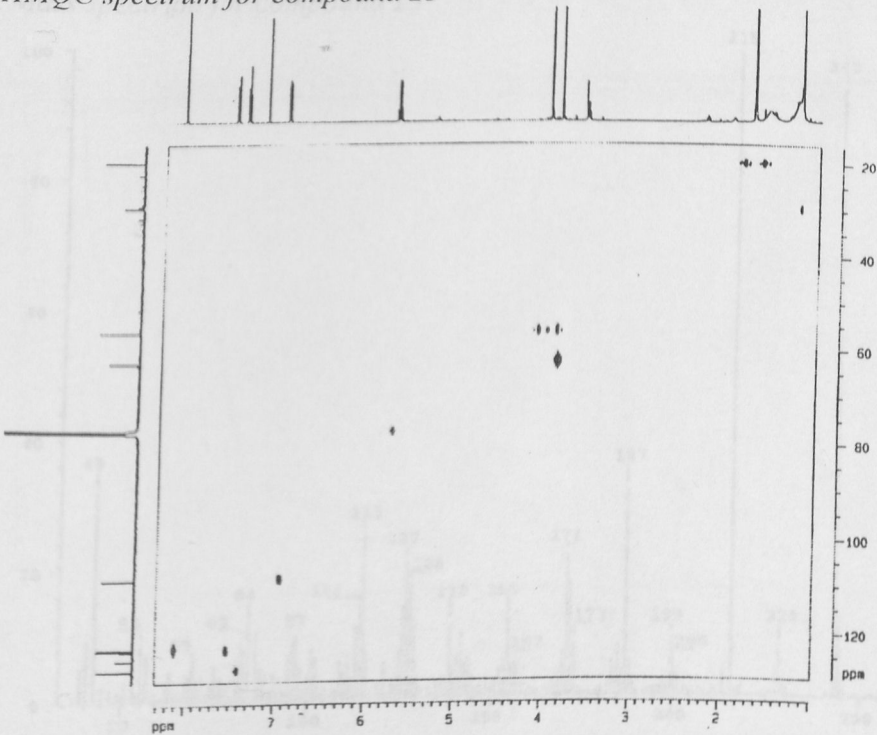
HH COSY for compound 23



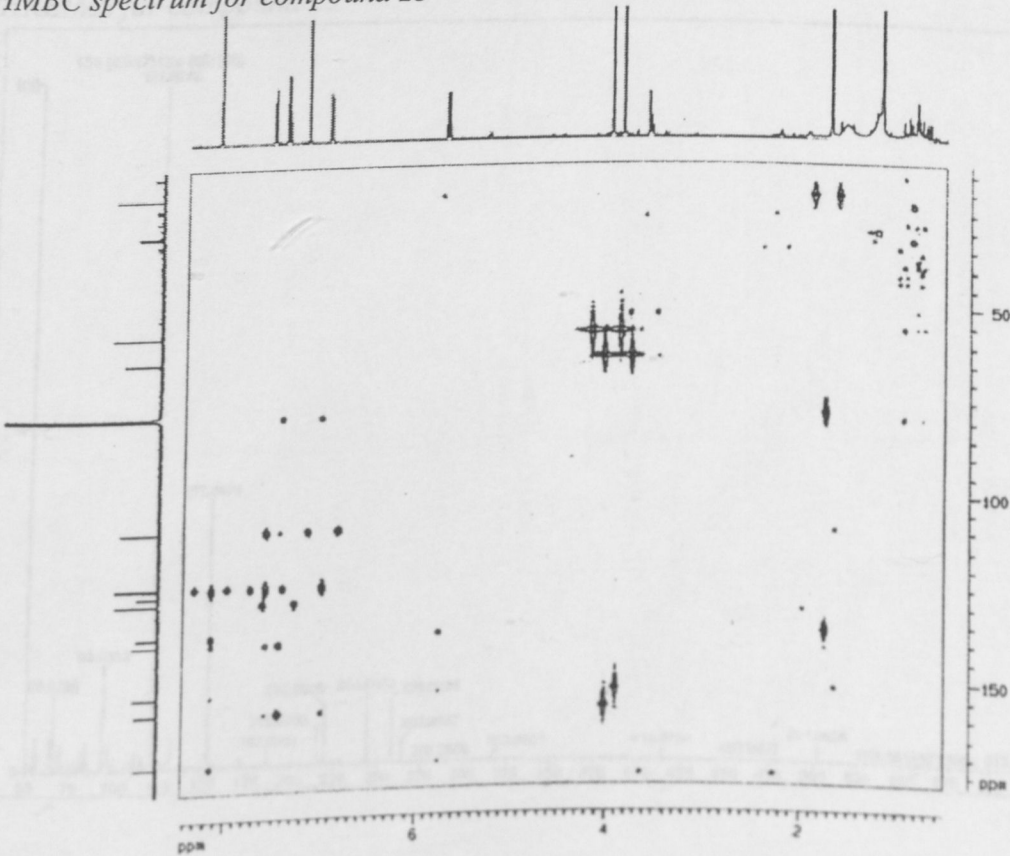
Carbon 13 NMR spectrum



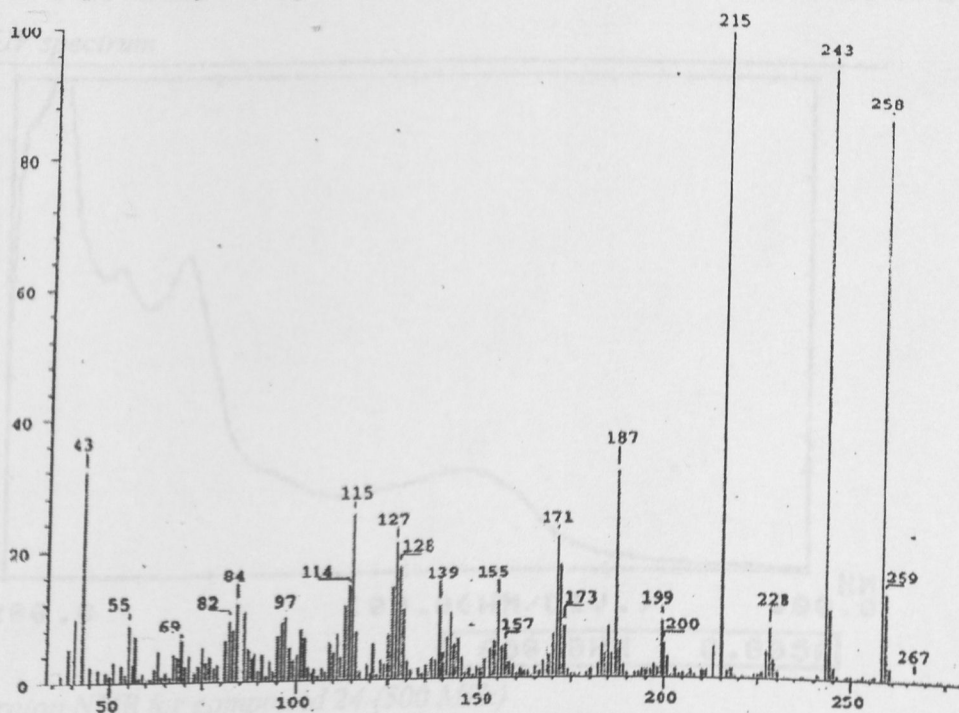
HMQC spectrum for compound 23



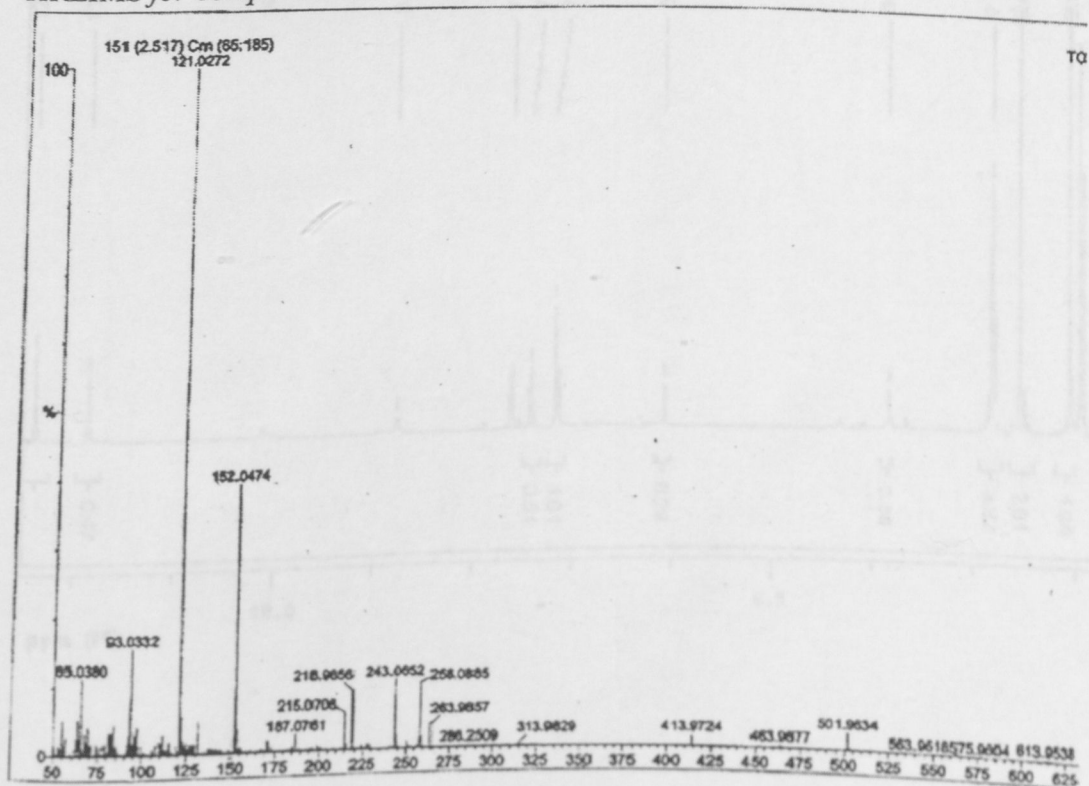
HMBC spectrum for compound 23



Mass spectrum for compound 23

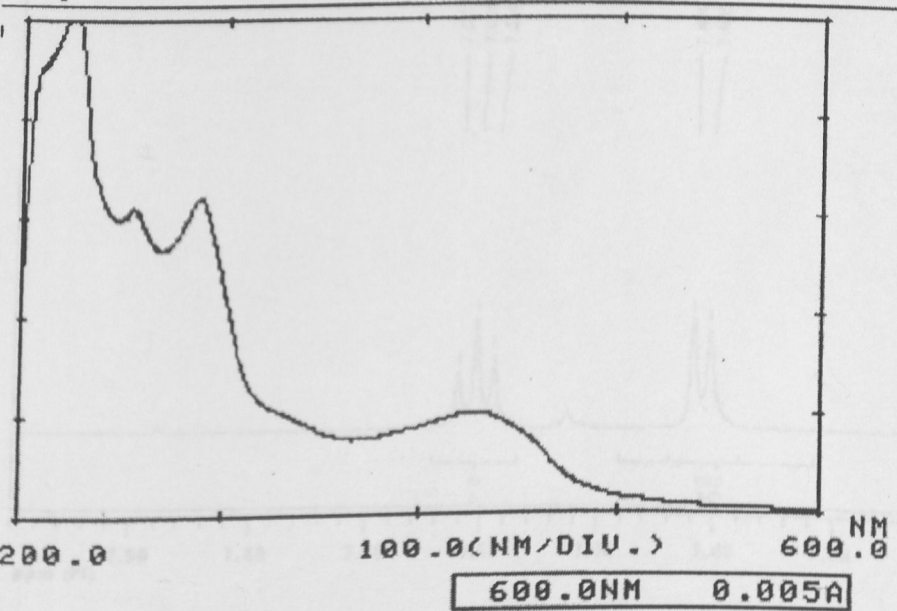


HREIMS for compound 23

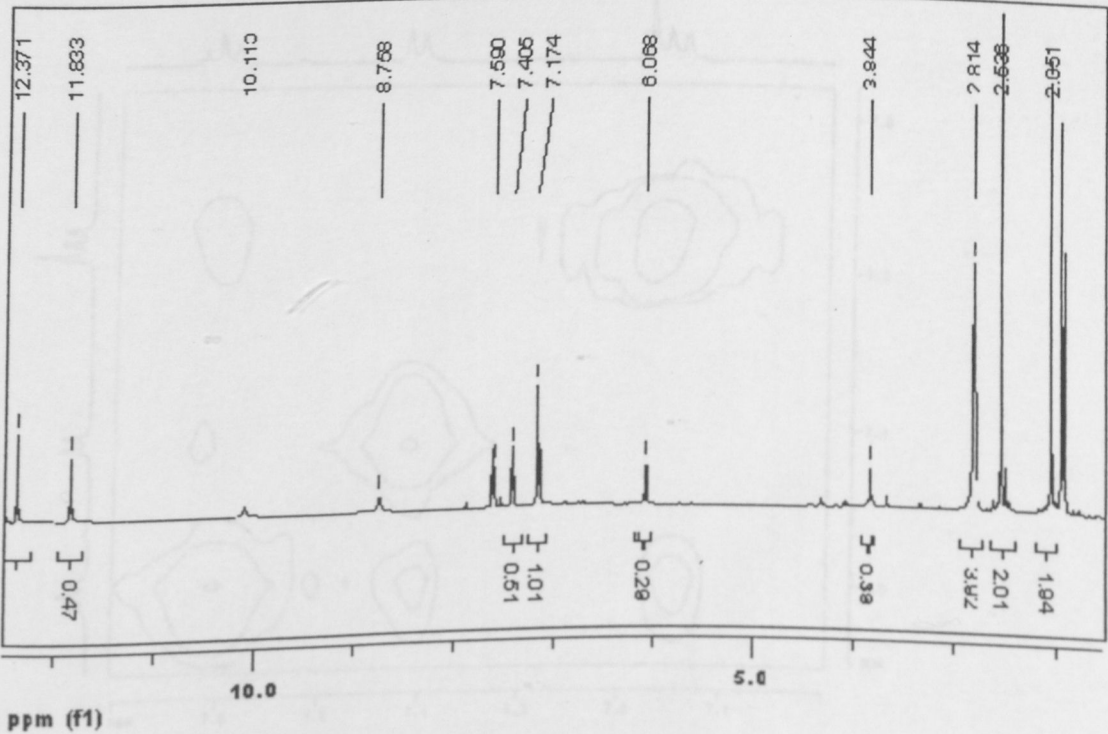


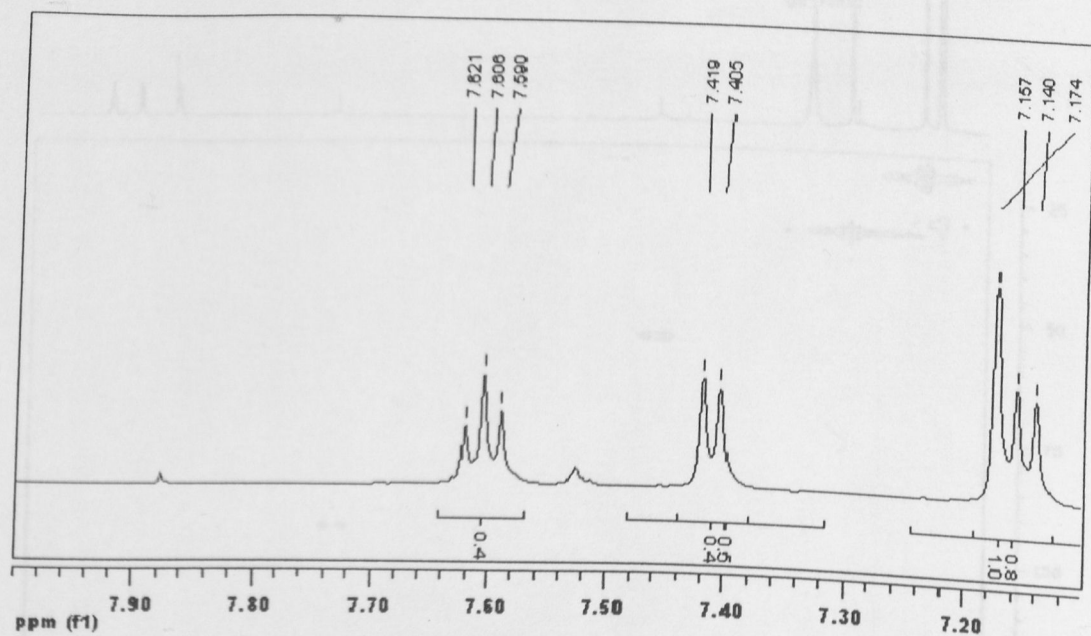
APPENDIX XXII SPECTRA FOR COMPOUND 24

UV spectrum

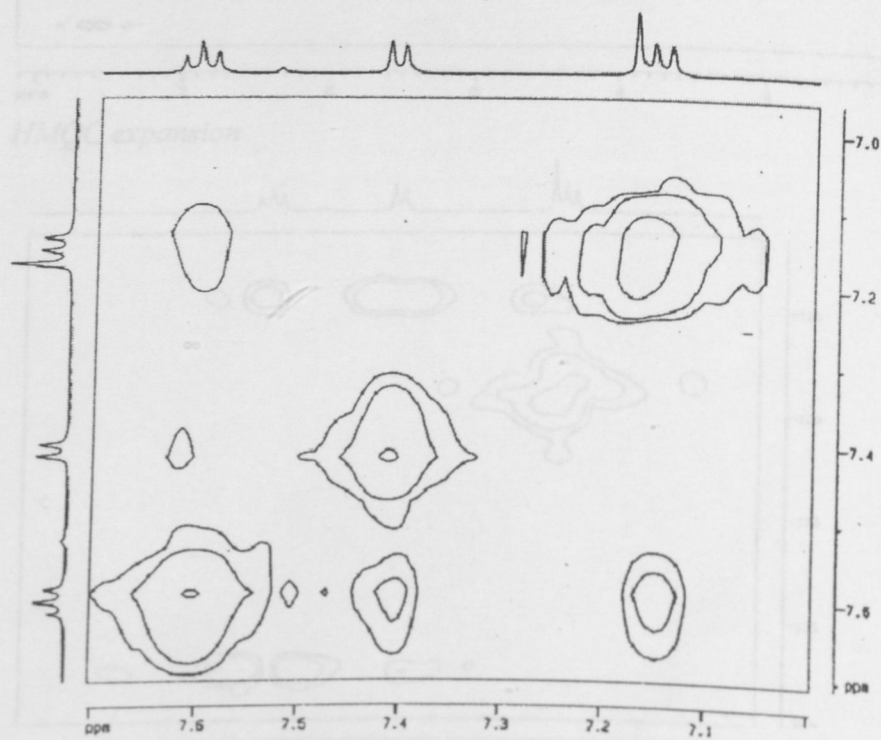


Proton NMR for compound 24 (500 MHz)

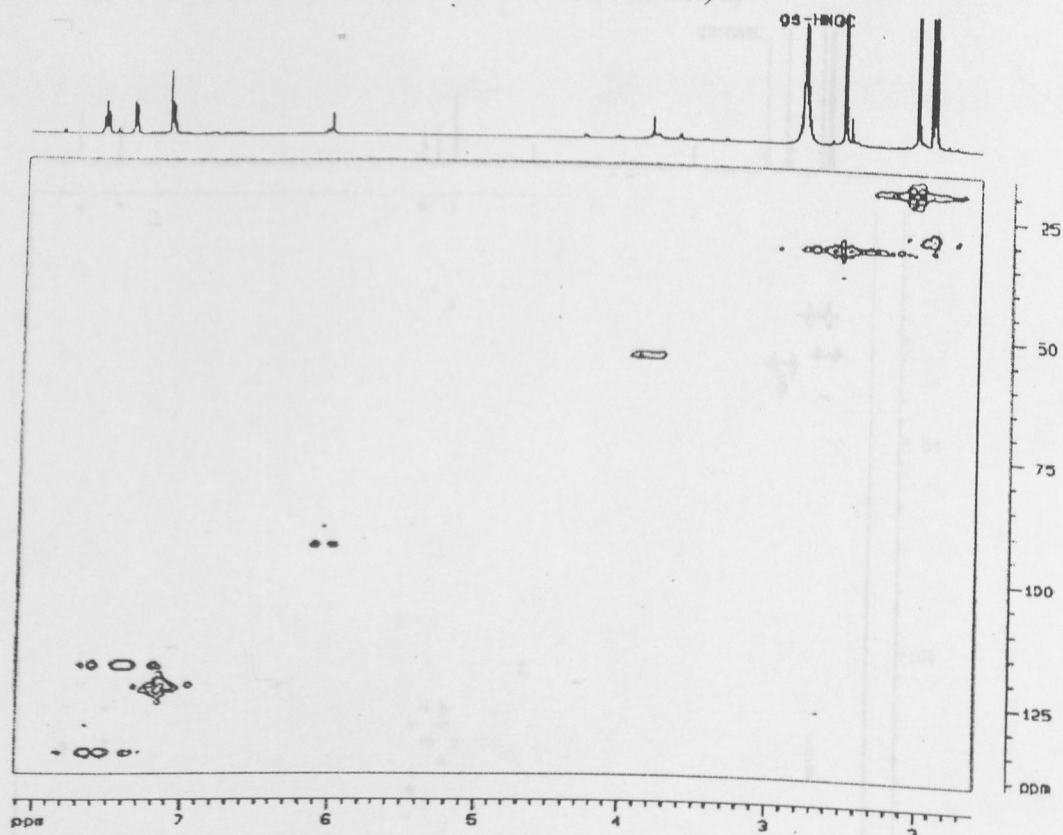




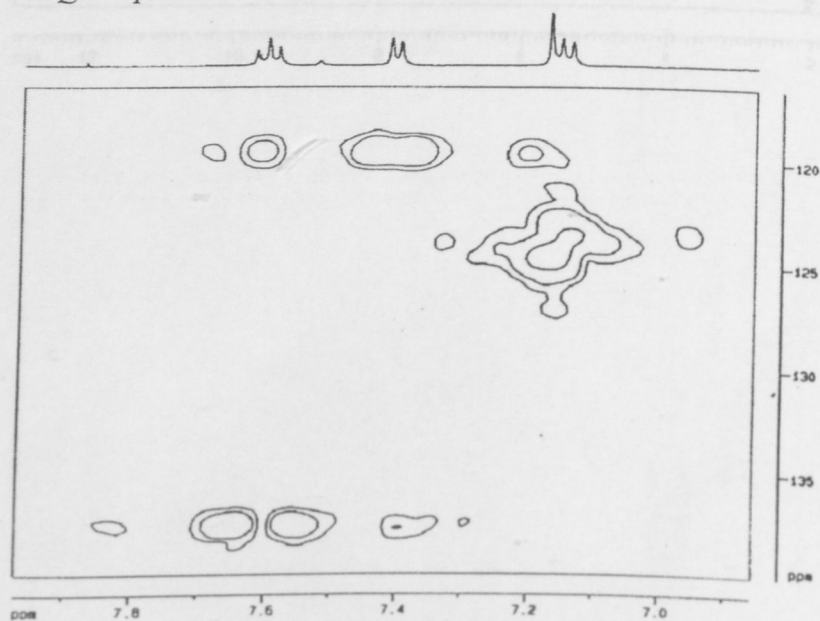
HH COSY for compound 24 (500 MHz)



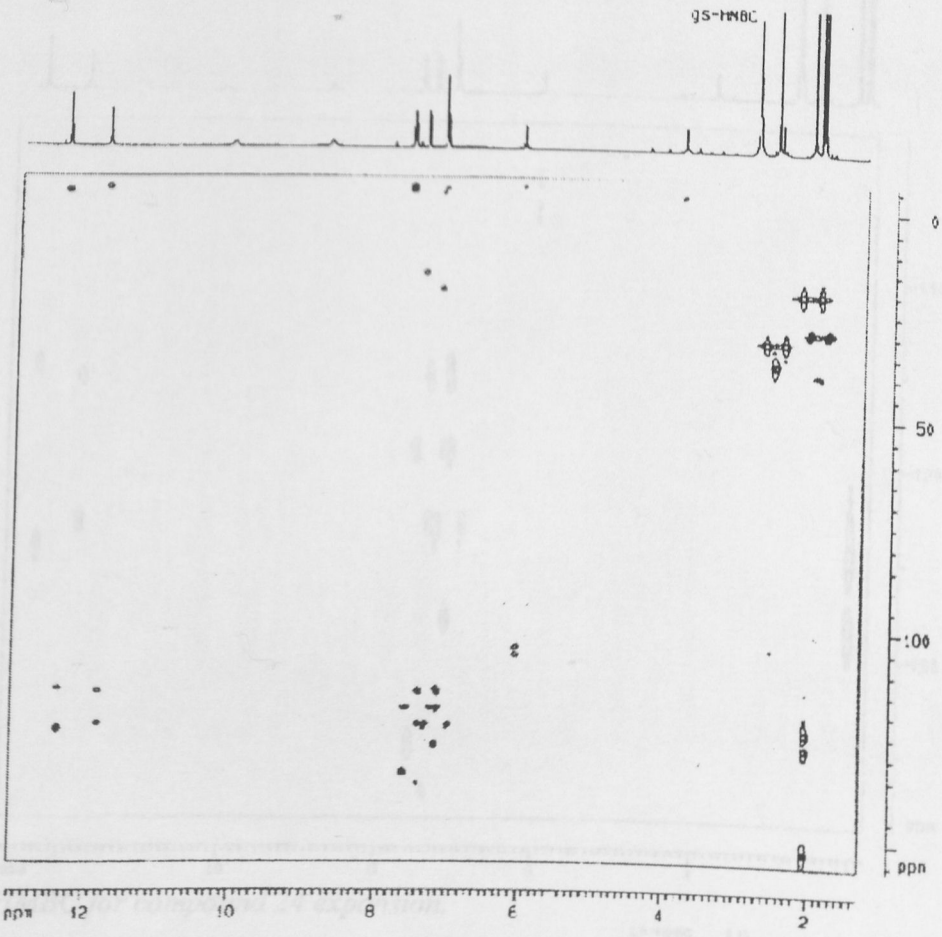
HMQC for compound 24 (Acetone D6, 125, 500 MHz)

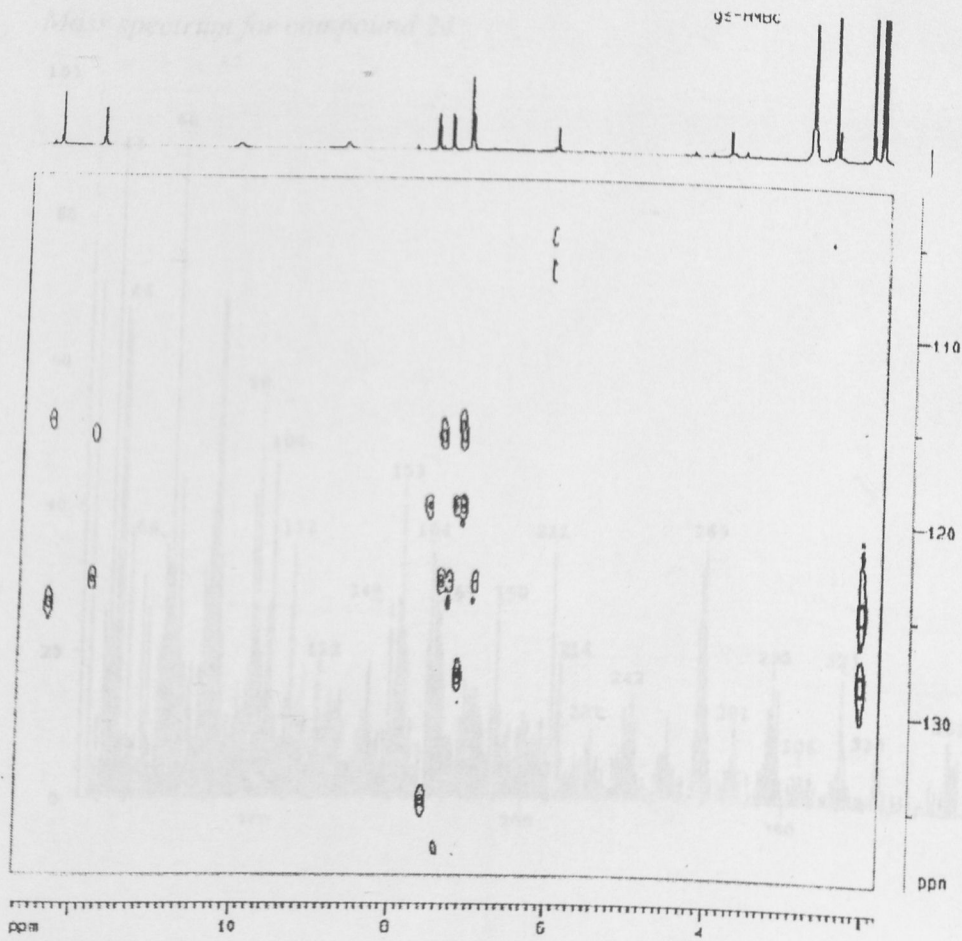


HMQC expansion

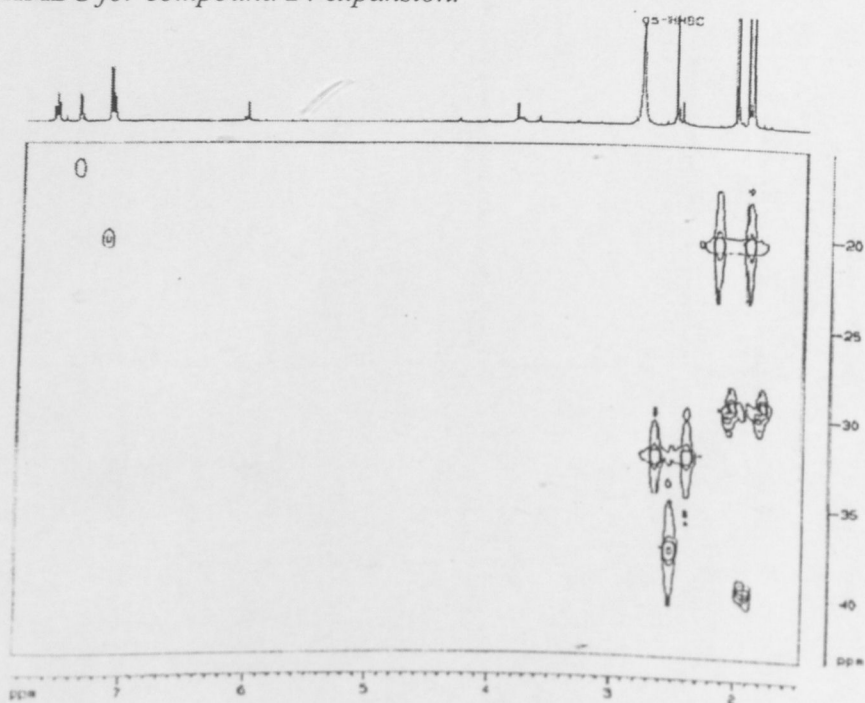


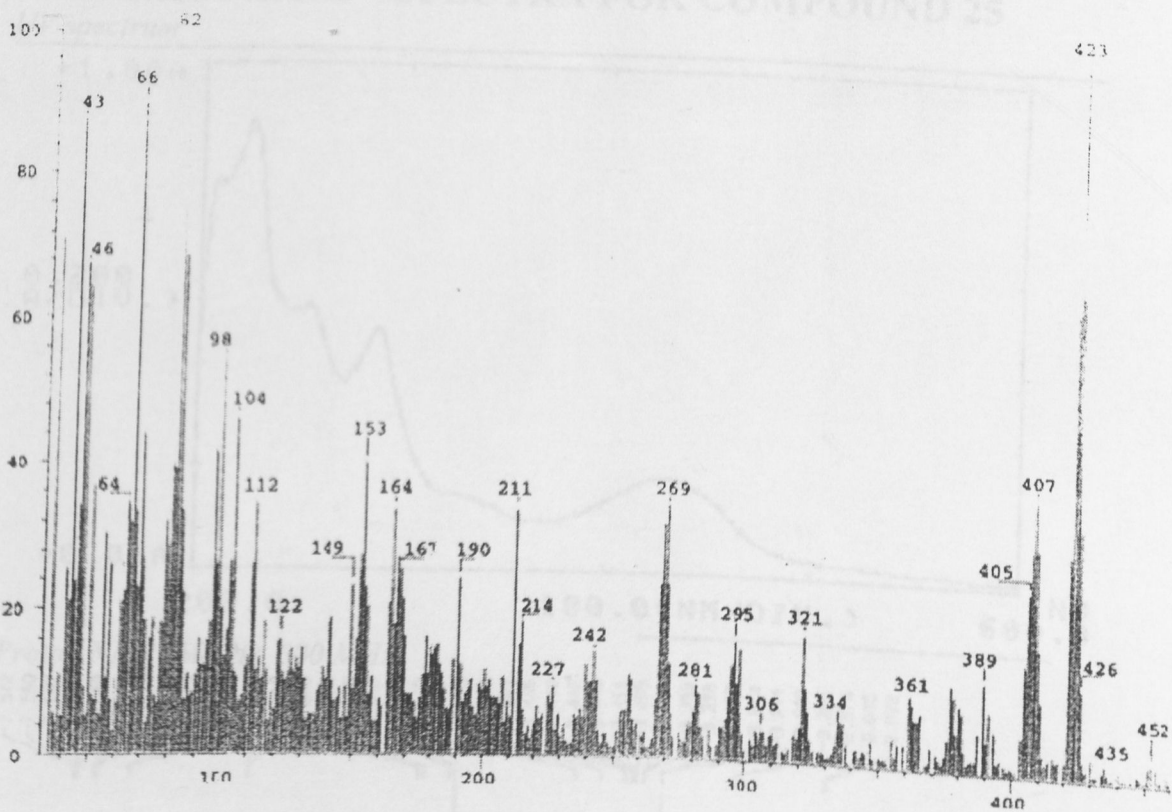
HMBC for compound 24 (acetone D6, 125, 500 MHz)





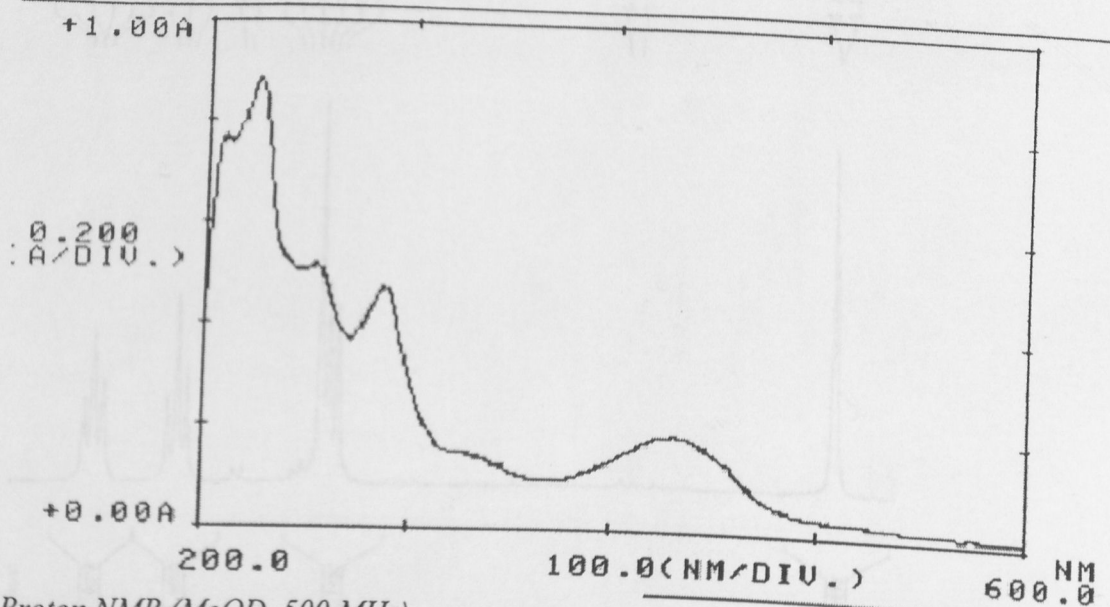
HMBC for compound 24 expansion.



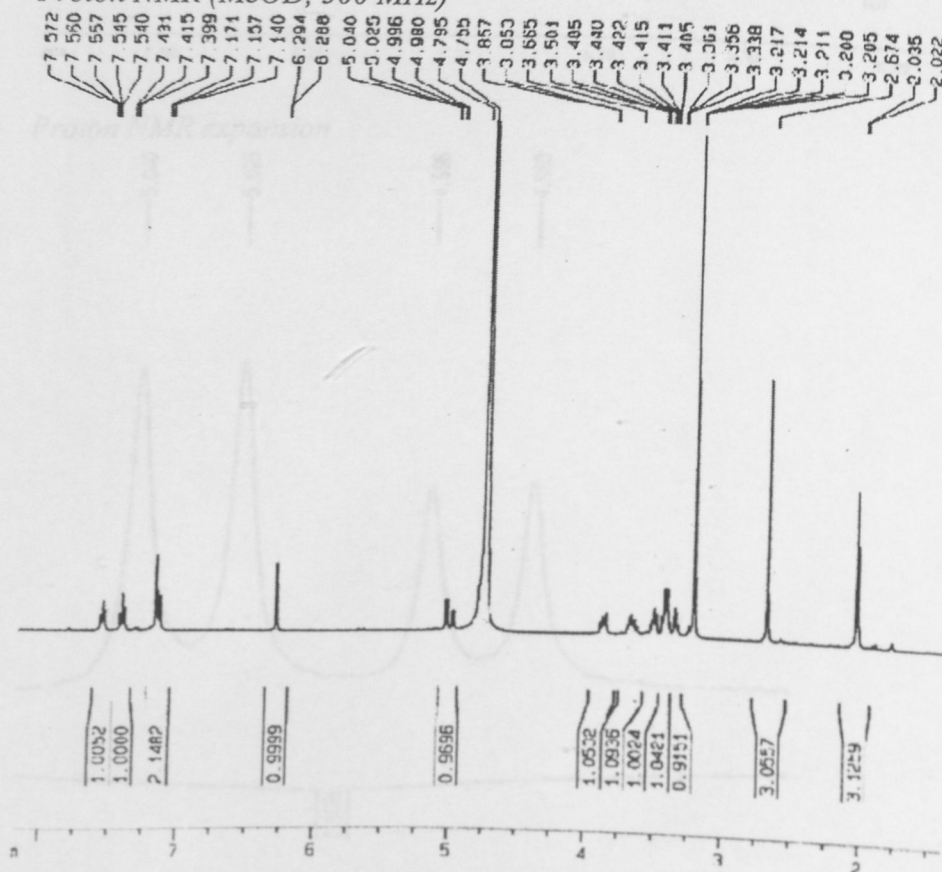


APPENDIX XXIII SPECTRA FOR COMPOUND 25

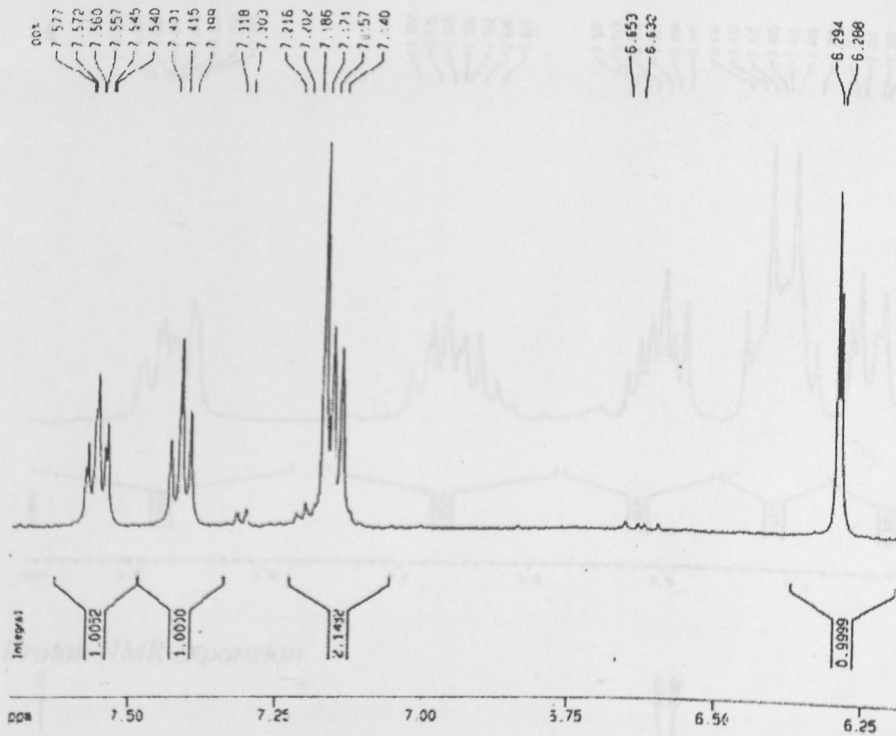
UV spectrum



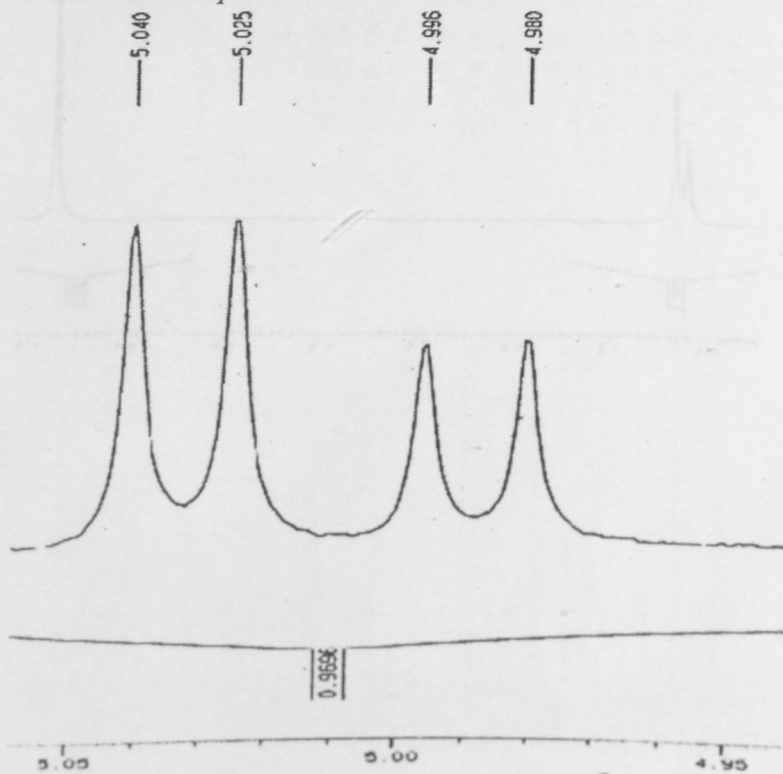
Proton NMR (MeOD, 500 MHz)



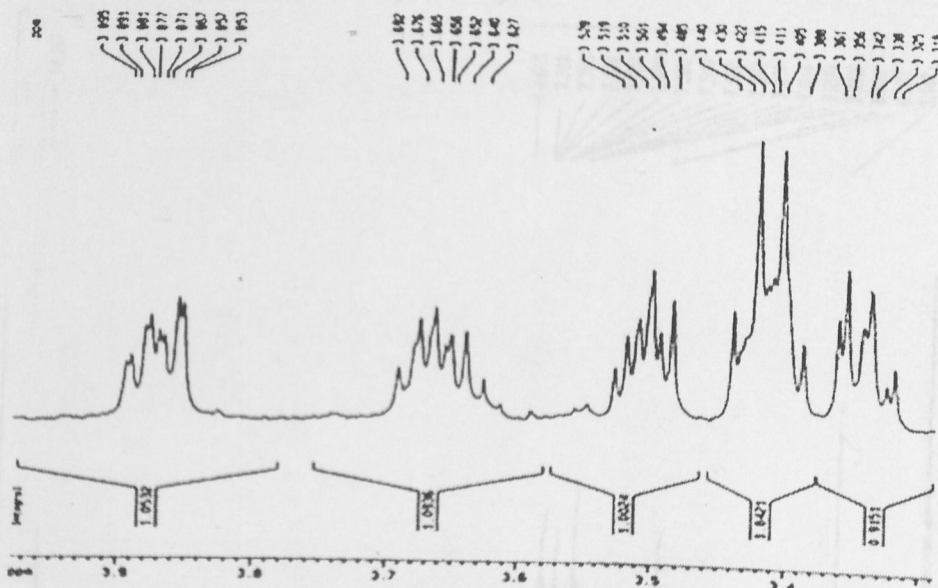
Proton NMR expansion



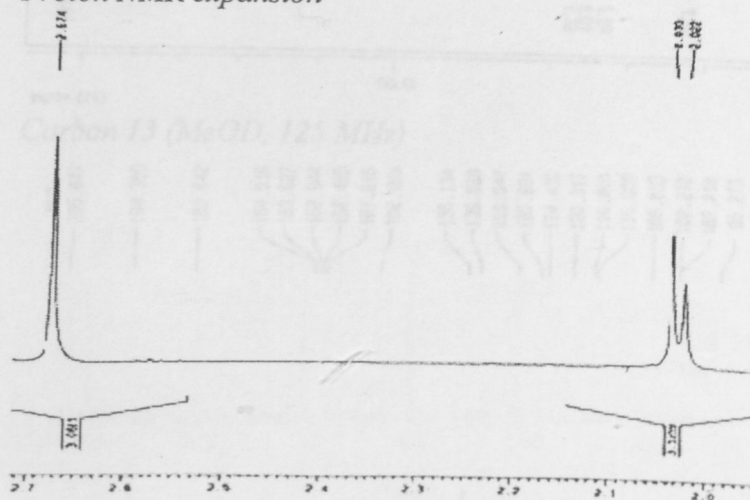
Proton NMR expansion



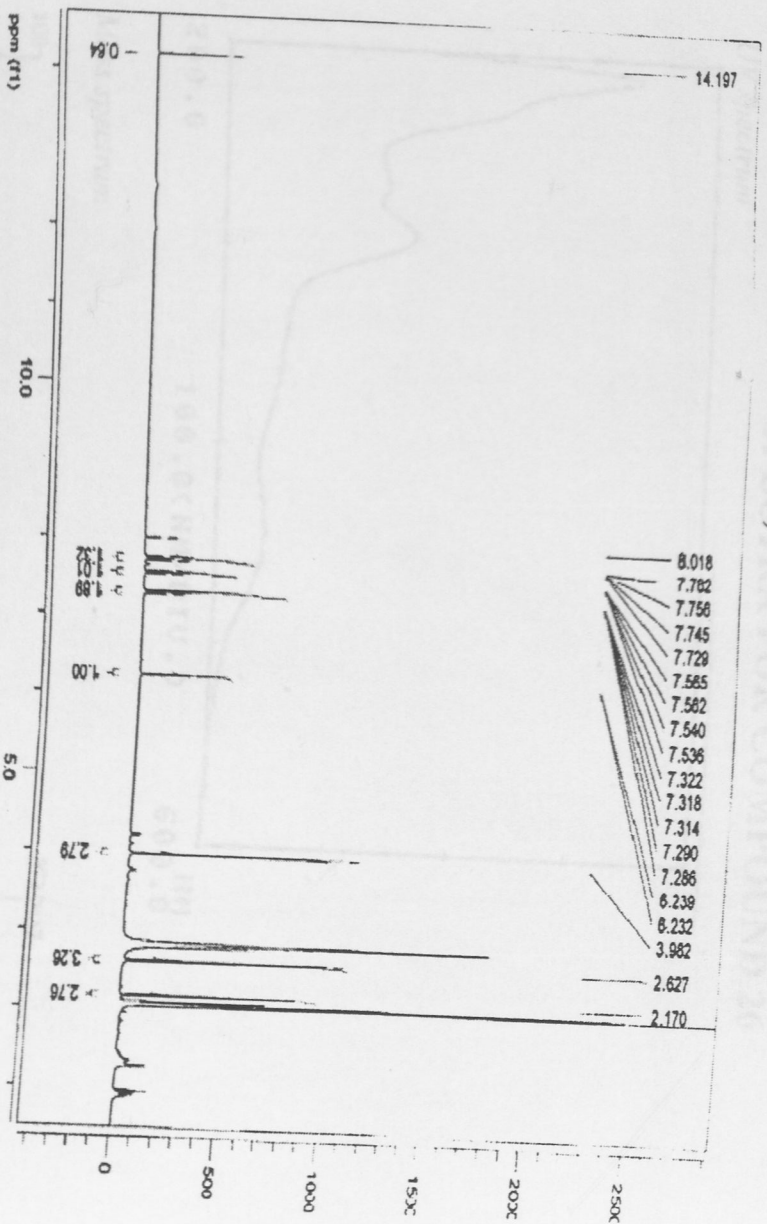
Proton NMR expansion



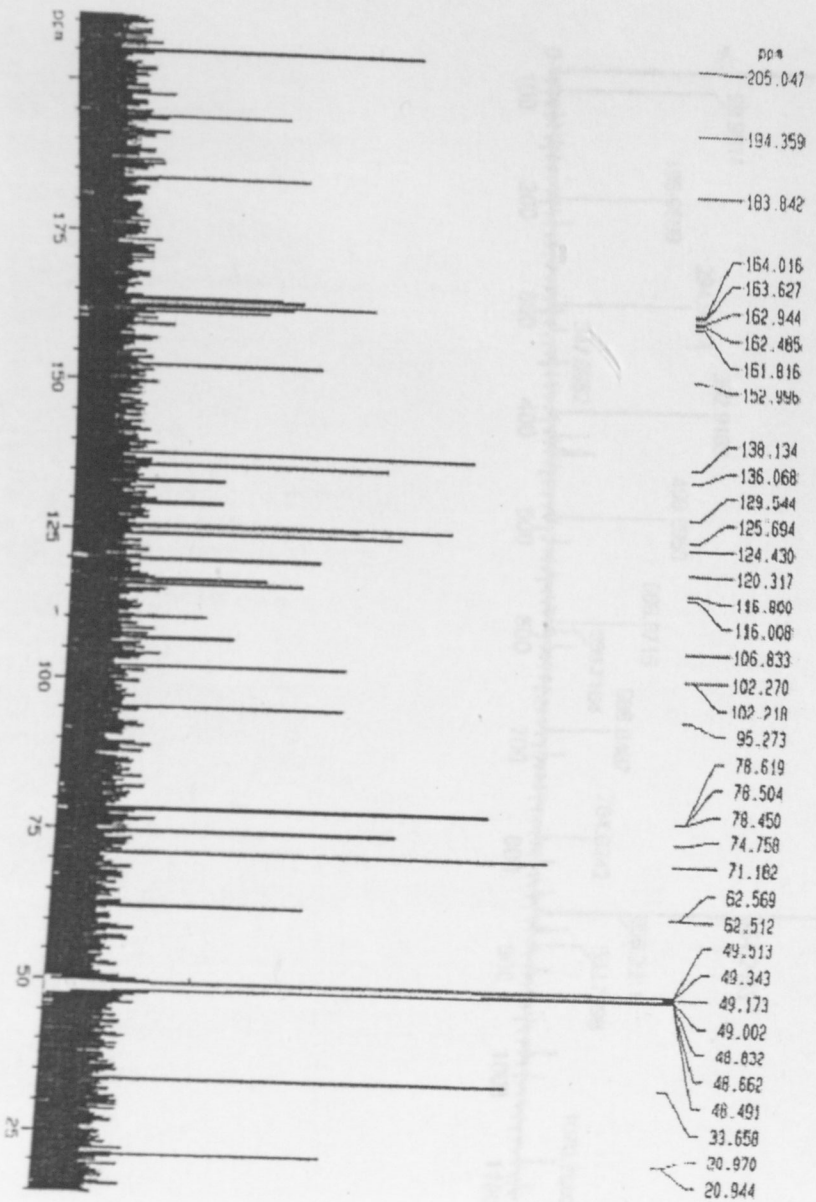
Proton NMR expansion



Proton NMR (acetone D6, 300 MHz)

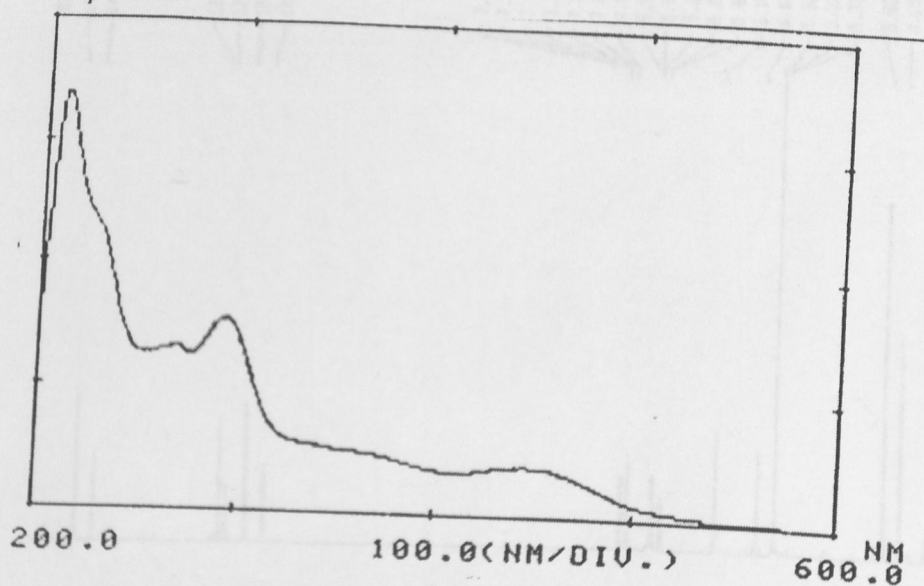


Carbon 13 (MeOD, 125 MHz)

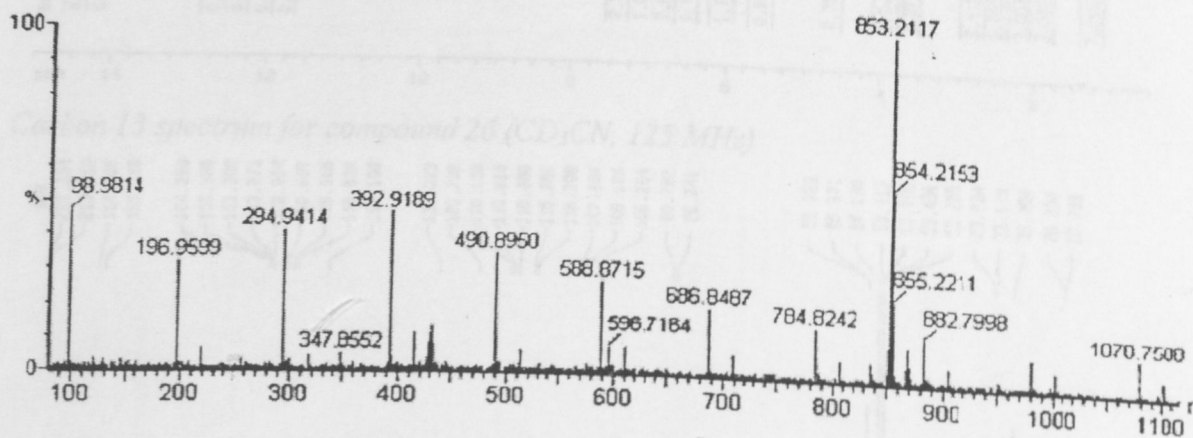


APPENDIX XXIV SPECTRA FOR COMPOUND 26

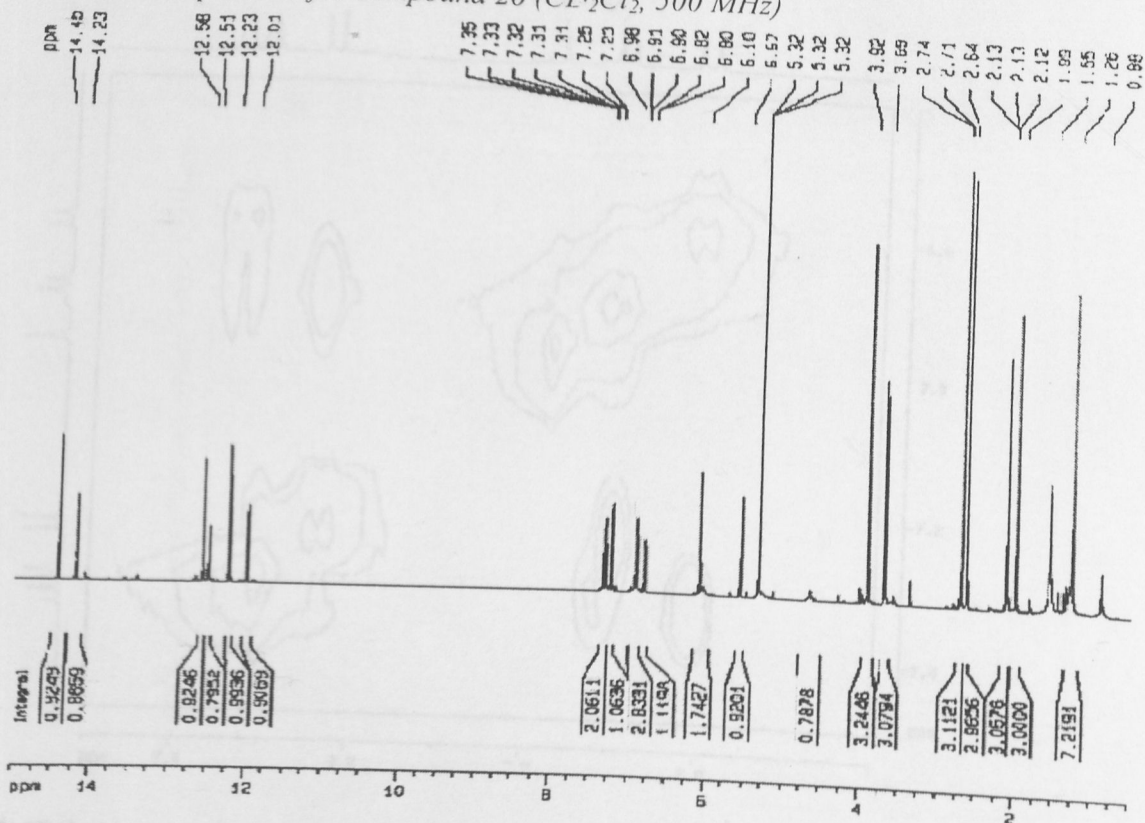
UV spectrum



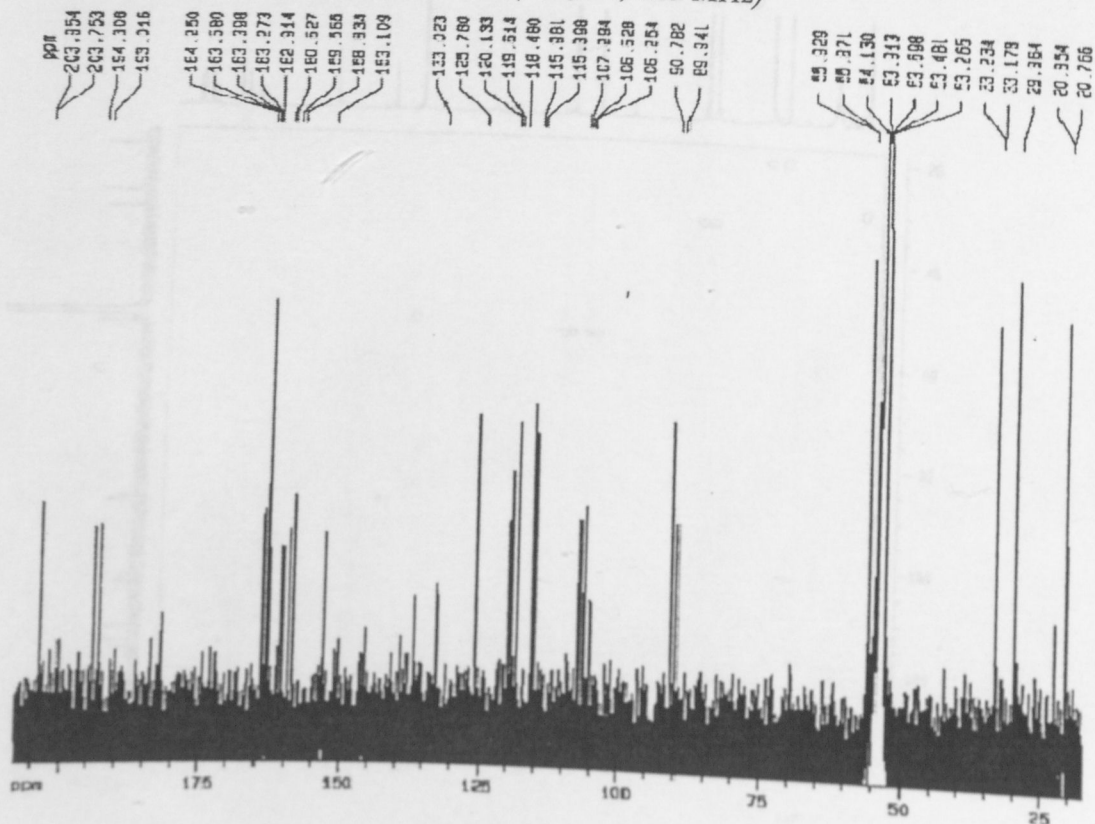
Mass spectrum



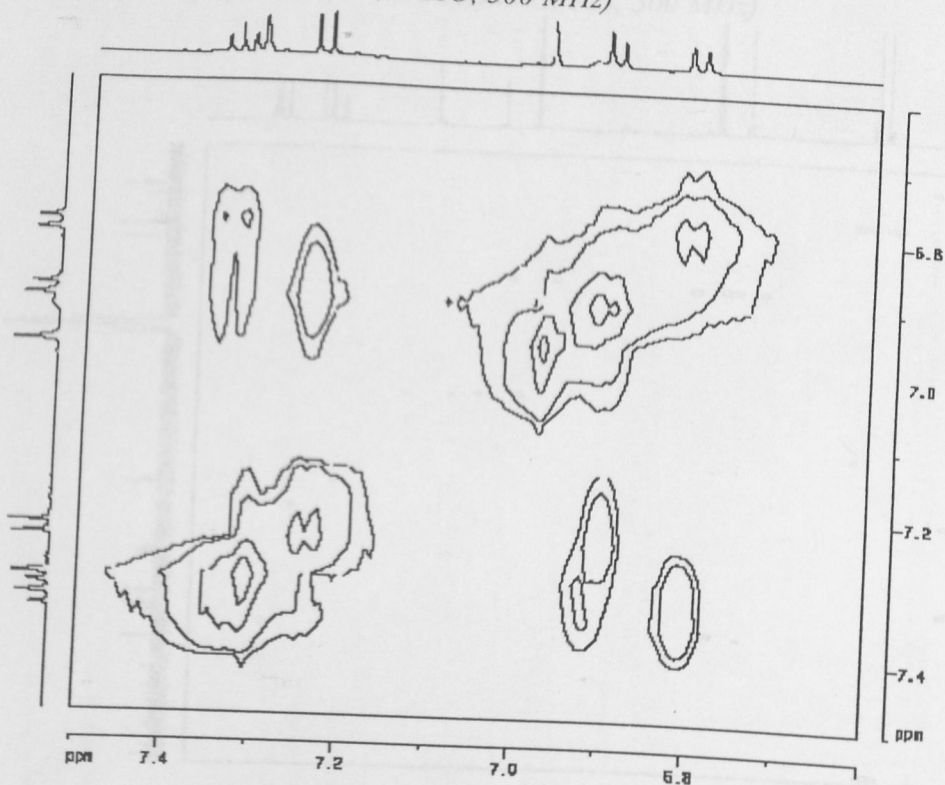
Proton NMR spectrum for compound 26 (CD_2Cl_2 , 500 MHz)



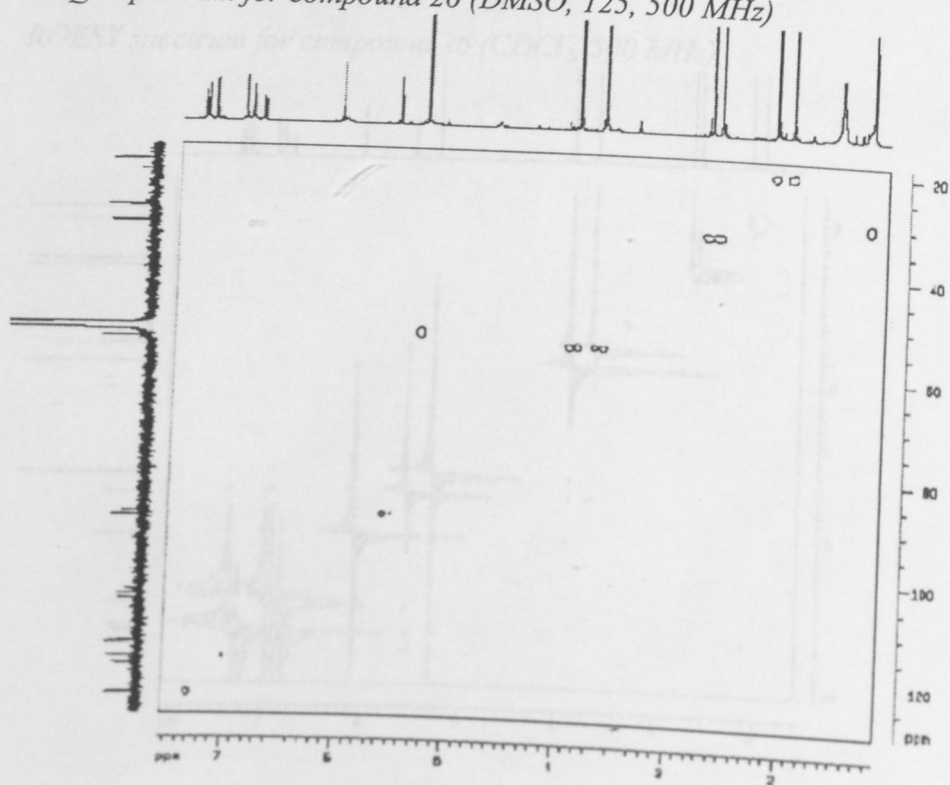
Carbon 13 spectrum for compound 26 (CD_3CN , 125 MHz)



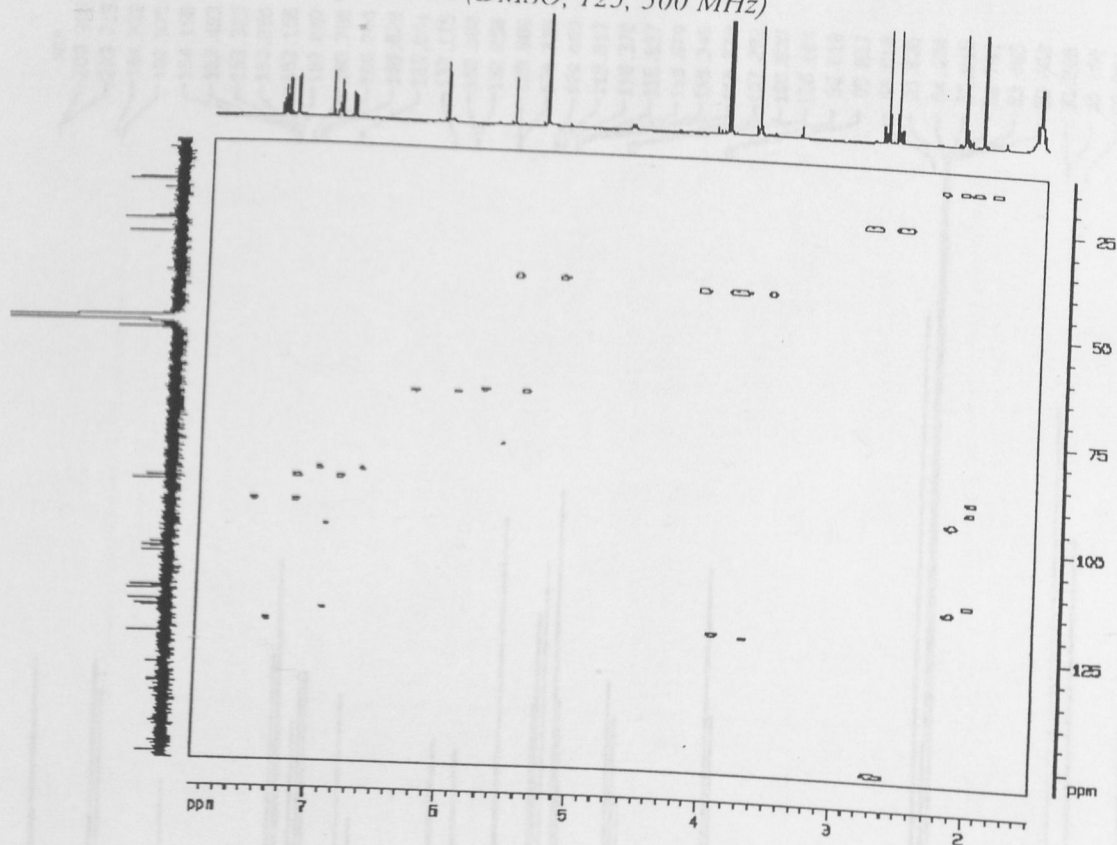
HH-COSY for compound 26 (DMSO, 500 MHz)



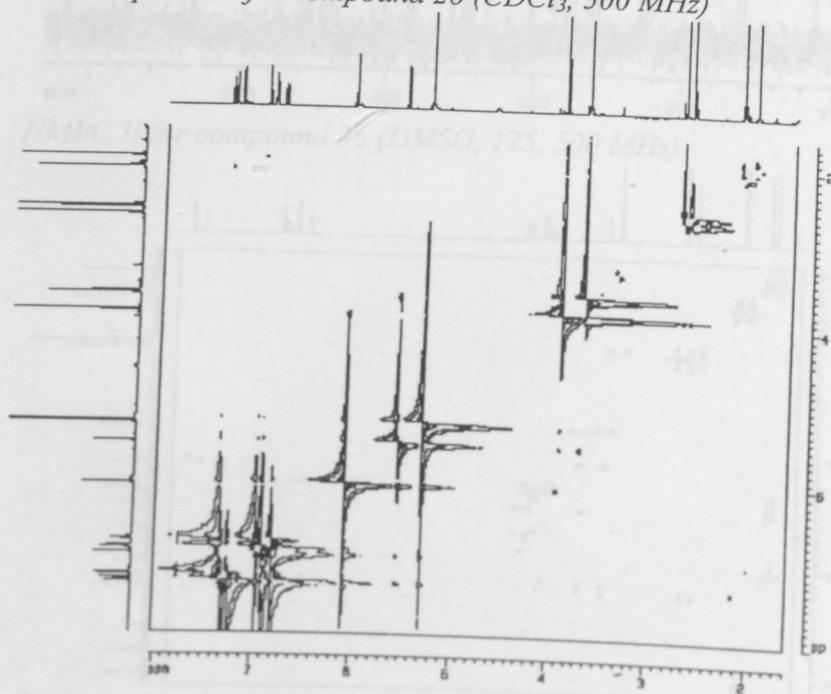
HMQC spectrum for compound 26 (DMSO, 125, 500 MHz)



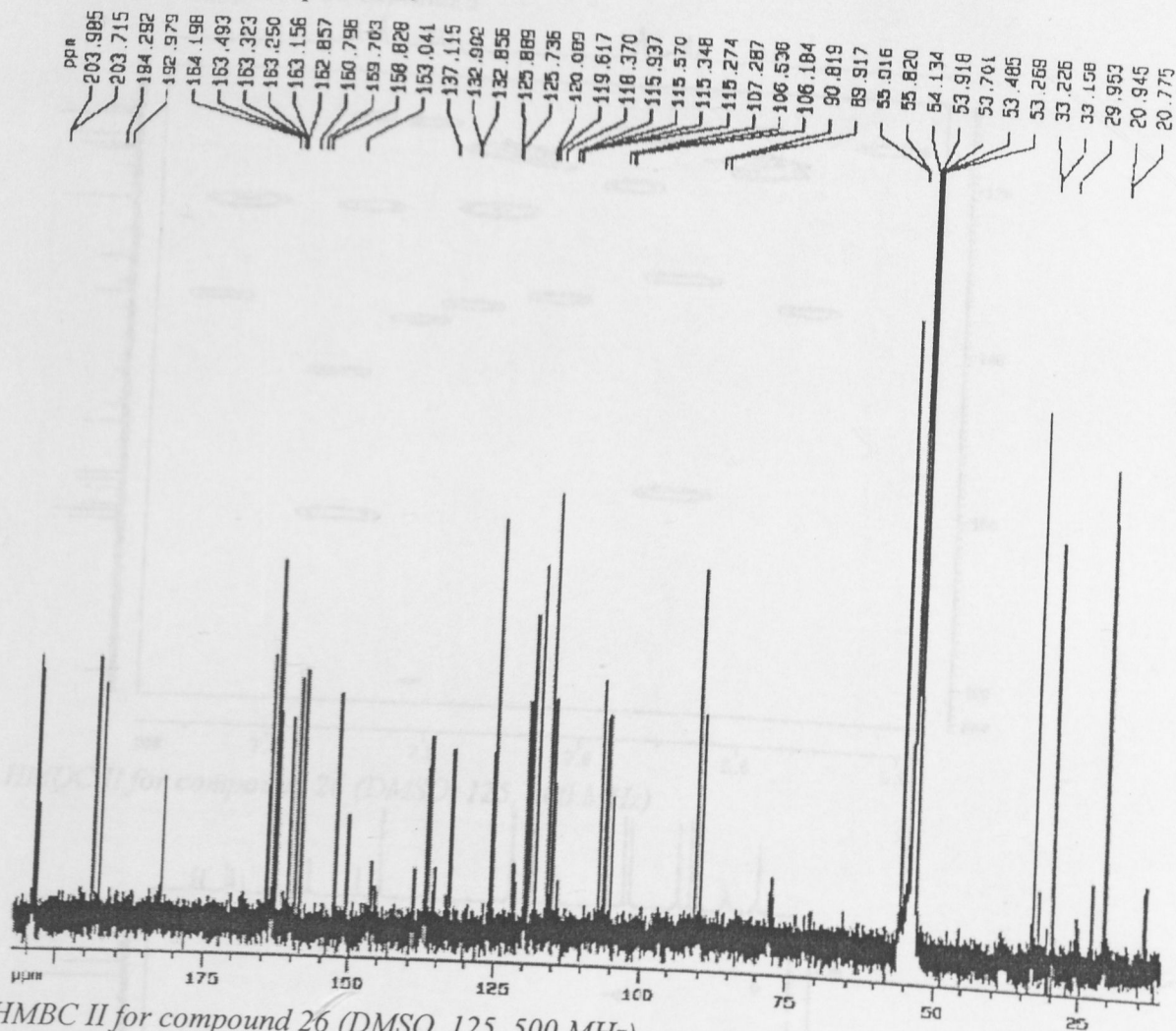
HMBC spectrum for compound 26 (DMSO, 125, 500 MHz)



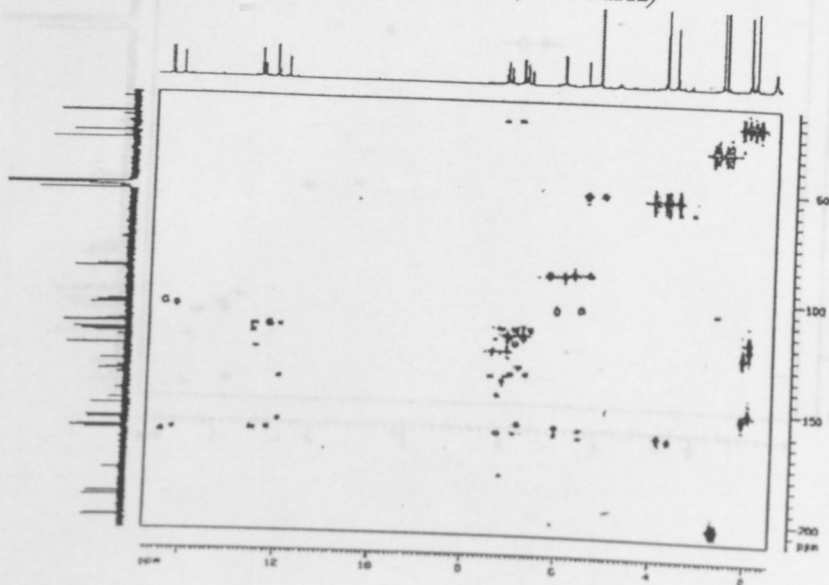
ROESY spectrum for compound 26 (CDCl_3 , 500 MHz)



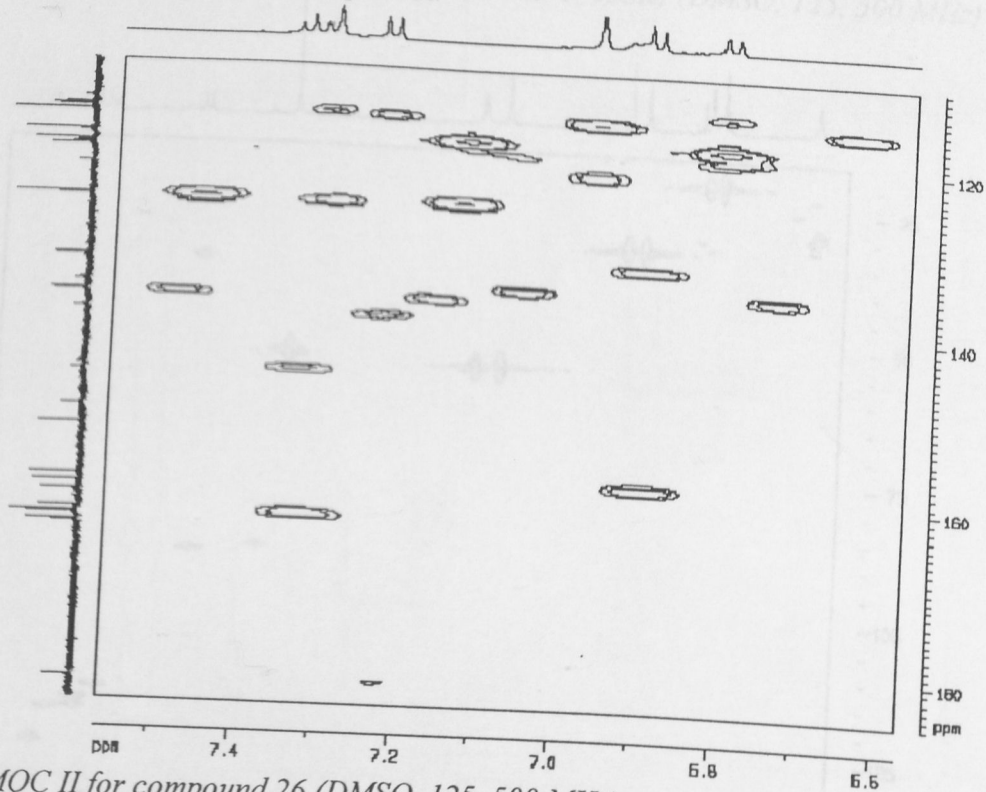
Carbon 13 II for compound 26



HMBC II for compound 26 (DMSO, 125, 500 MHz)



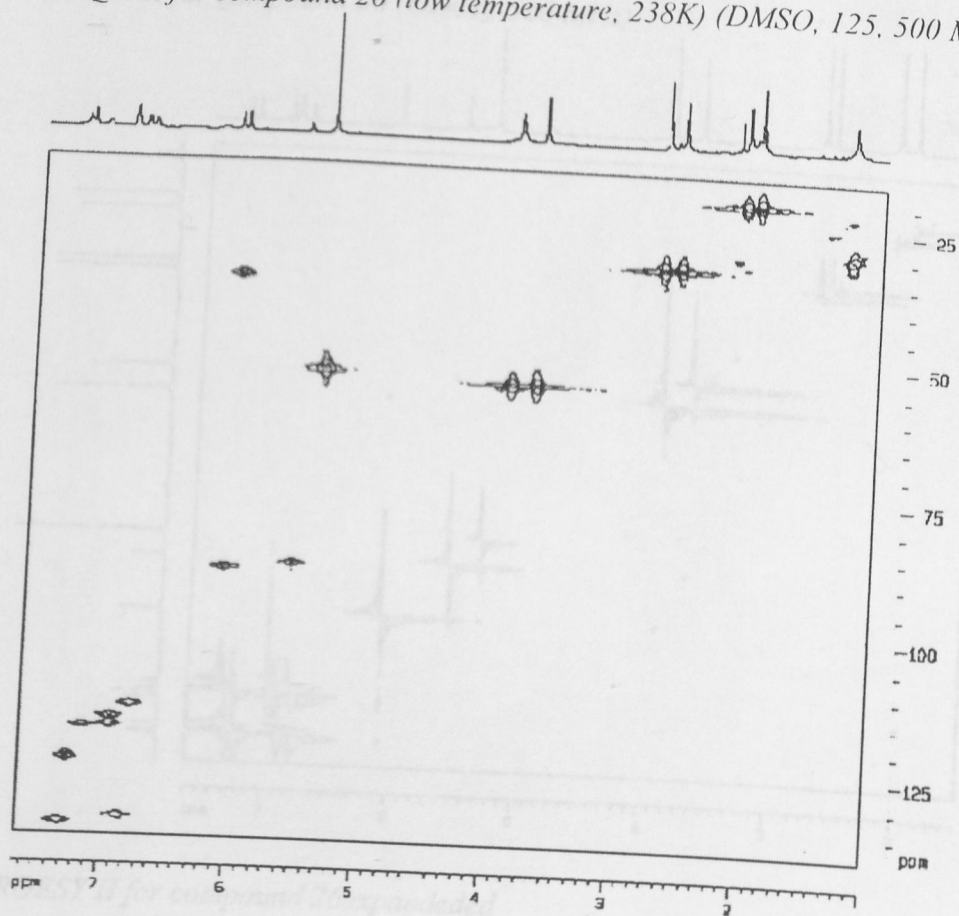
HMBC for compound 26 expanded



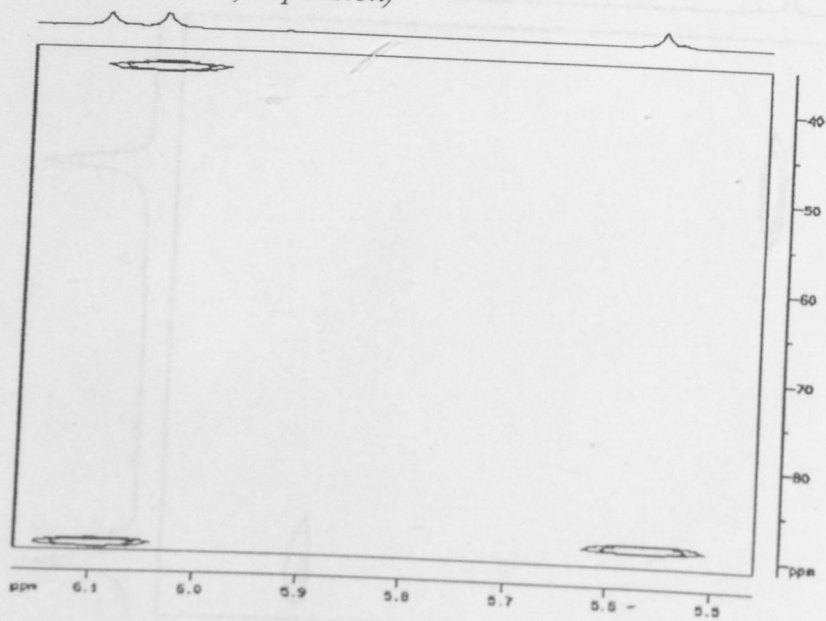
HMQC II for compound 26 (DMSO, 125, 500 MHz)



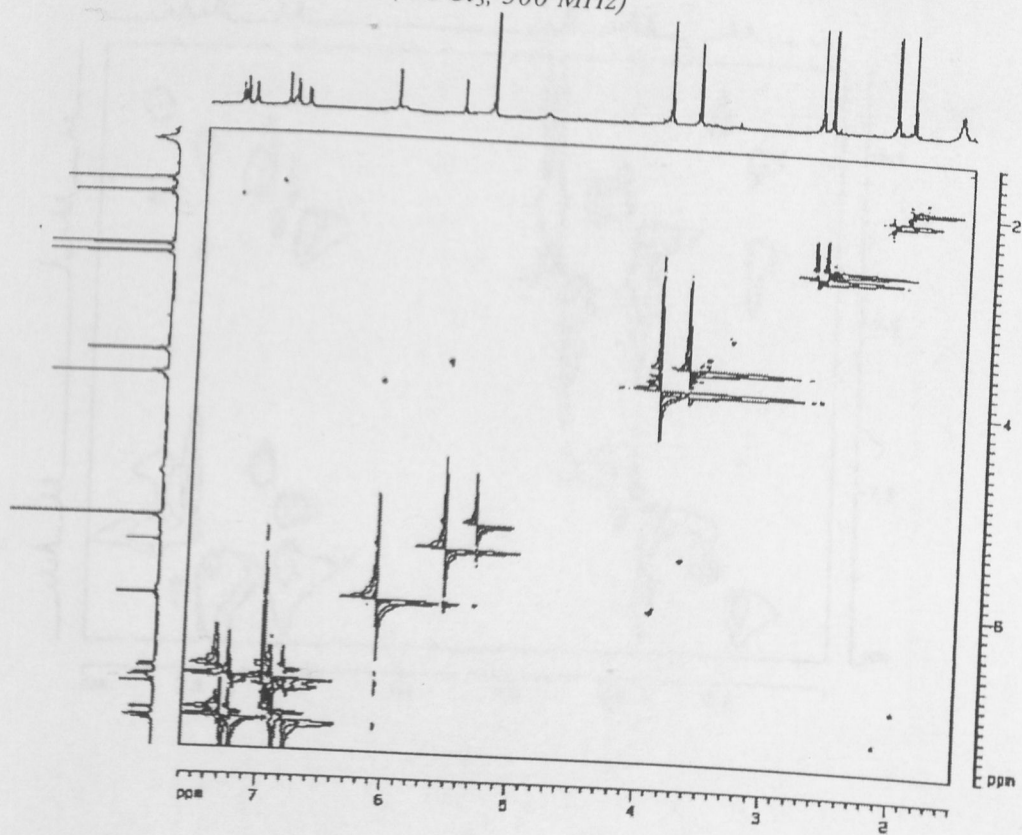
HMQC II for compound 26 (low temperature, 238K) (DMSO, 125, 500 MHz)



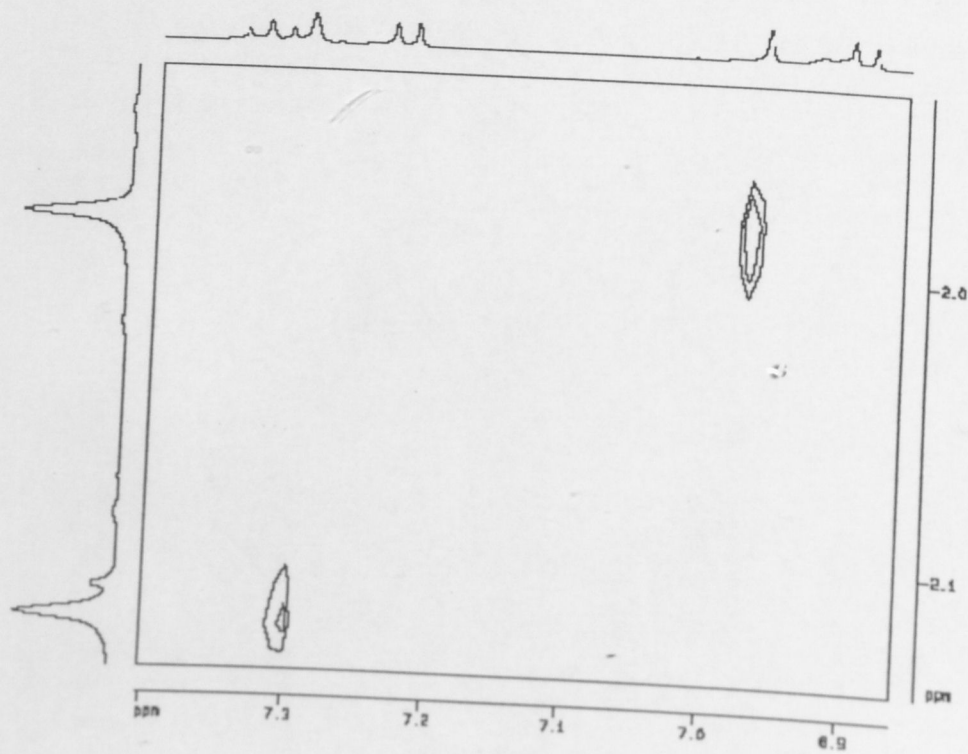
HMQC II (238K) expansion)



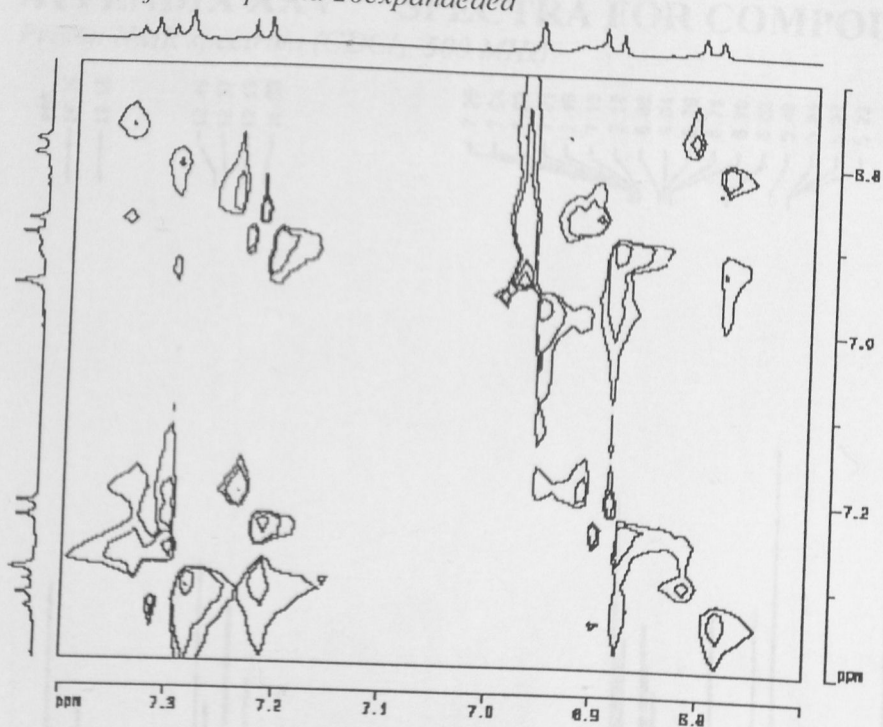
ROESY II for compound 26 (CDCl₃, 500 MHz)



ROESY II for compound 26 expanded



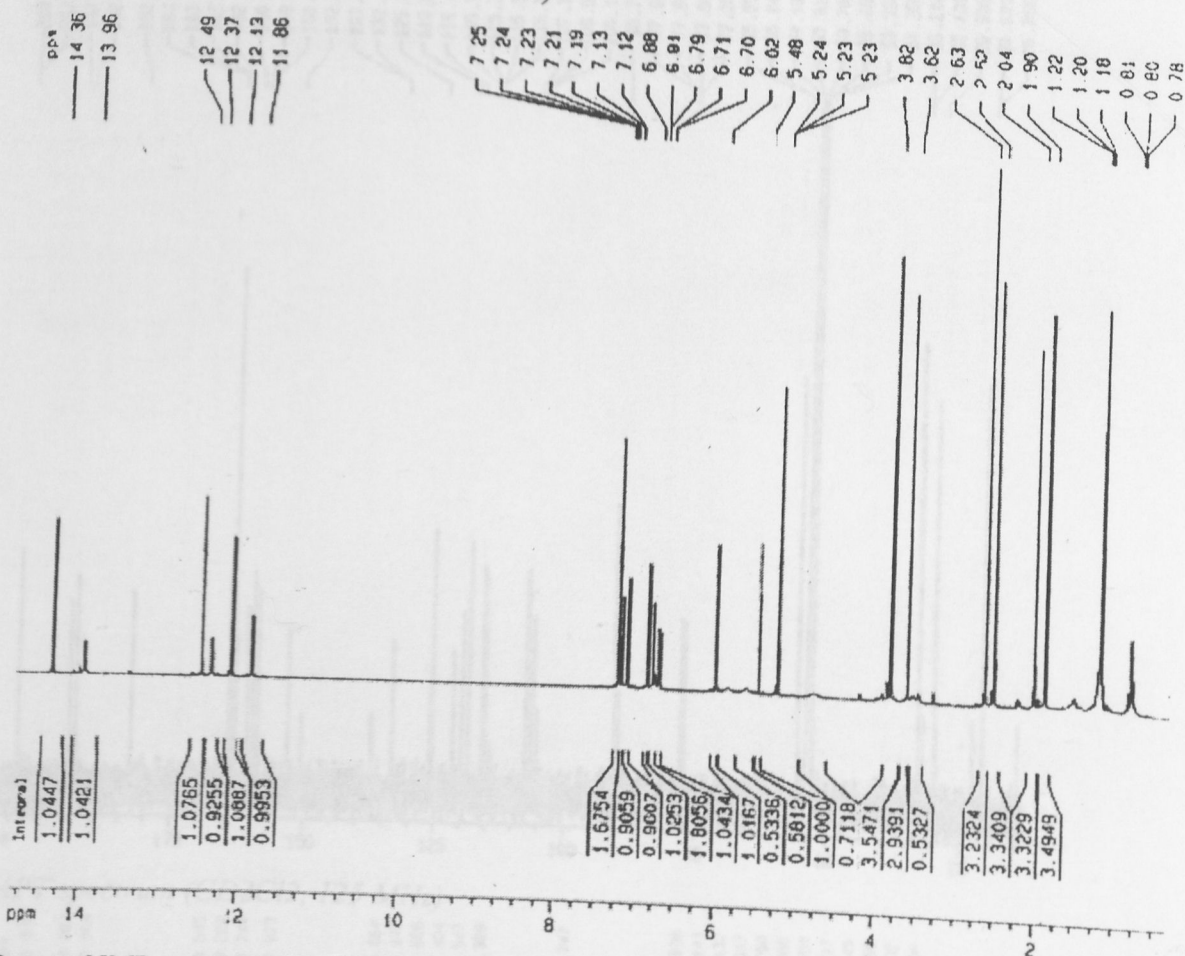
ROESY II for compound 26expanded



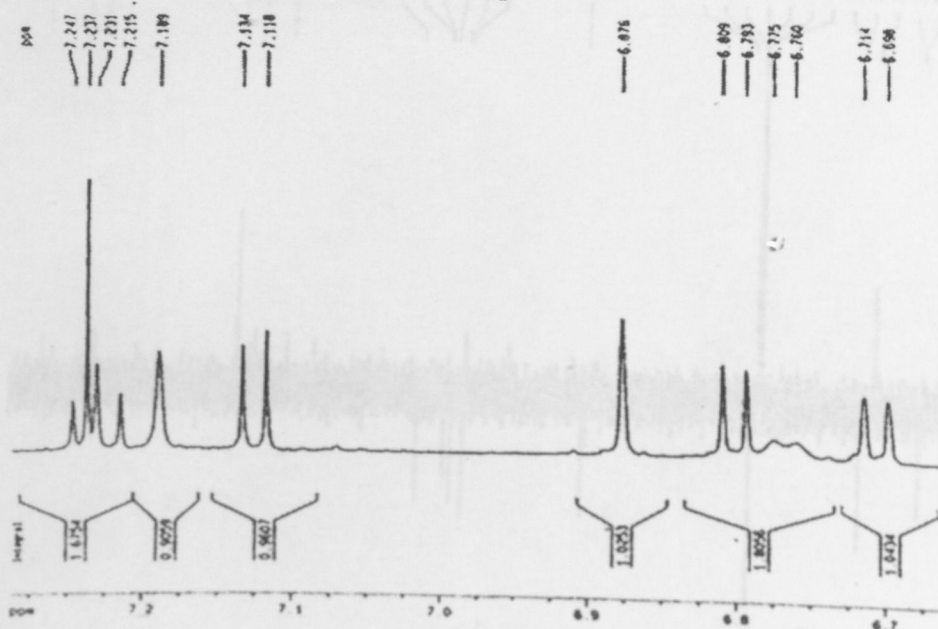
From 1D 1H NMR spectrum of compound 27

APPENDIX XXV SPECTRA FOR COMPOUND 27

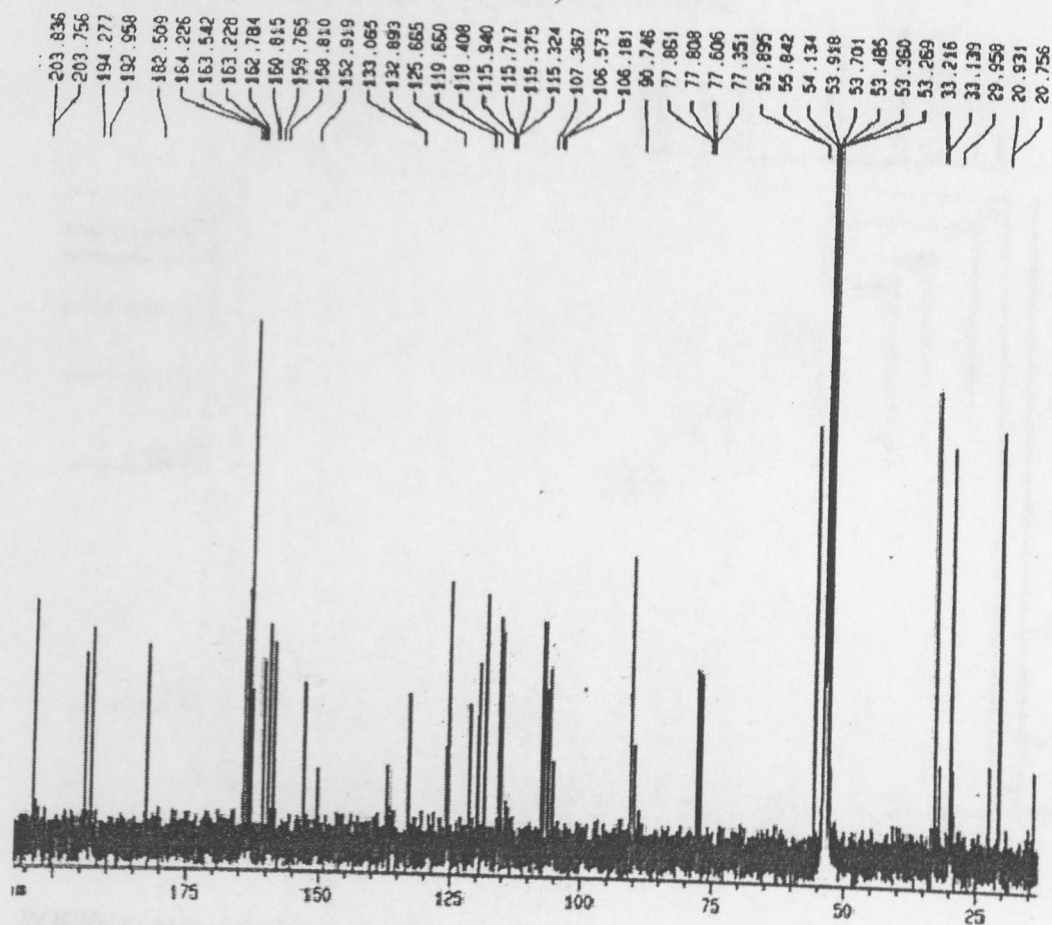
Proton NMR spectrum (CDCl₃, 500 MHz)



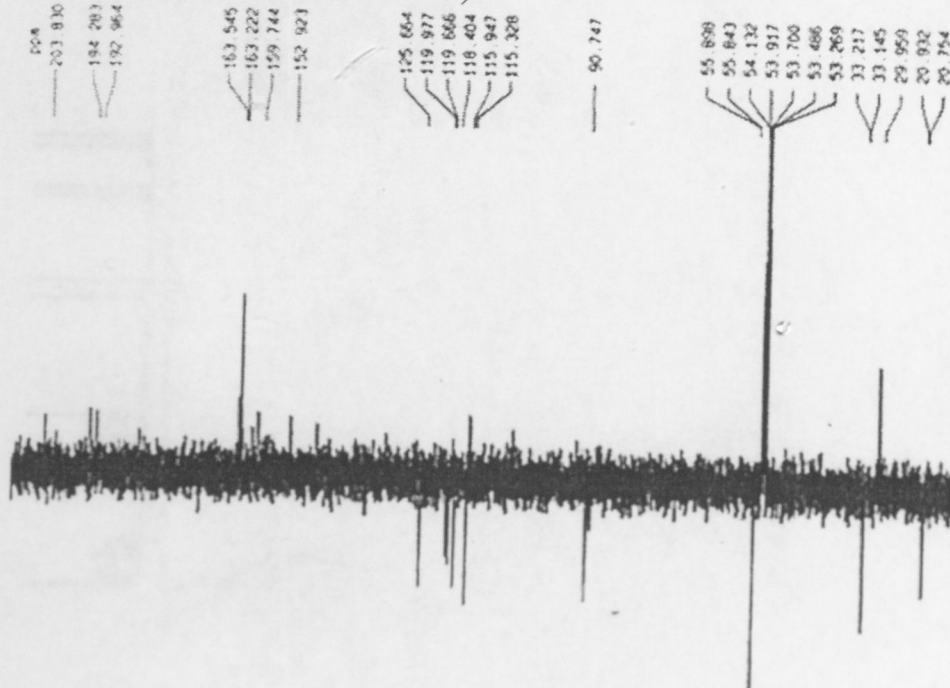
Proton NMR spectrum expansion compound 27



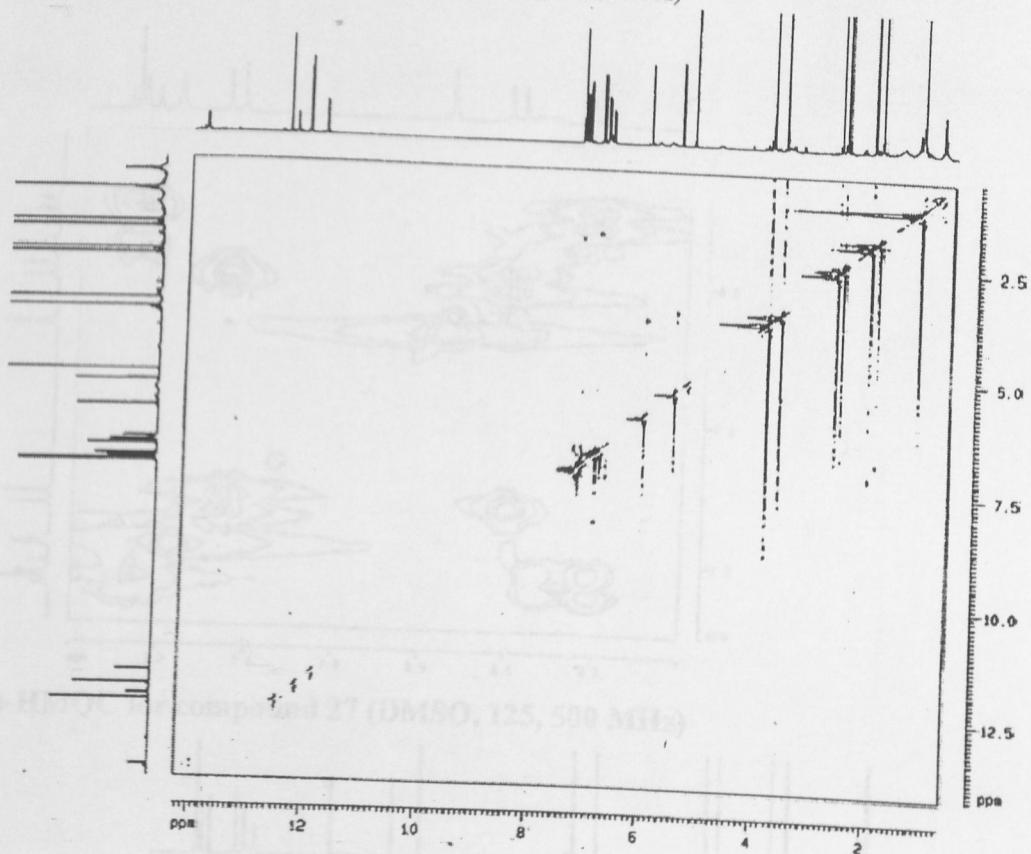
Carbon 13 spectrum (CD₂Cl₂, 125 MHz)



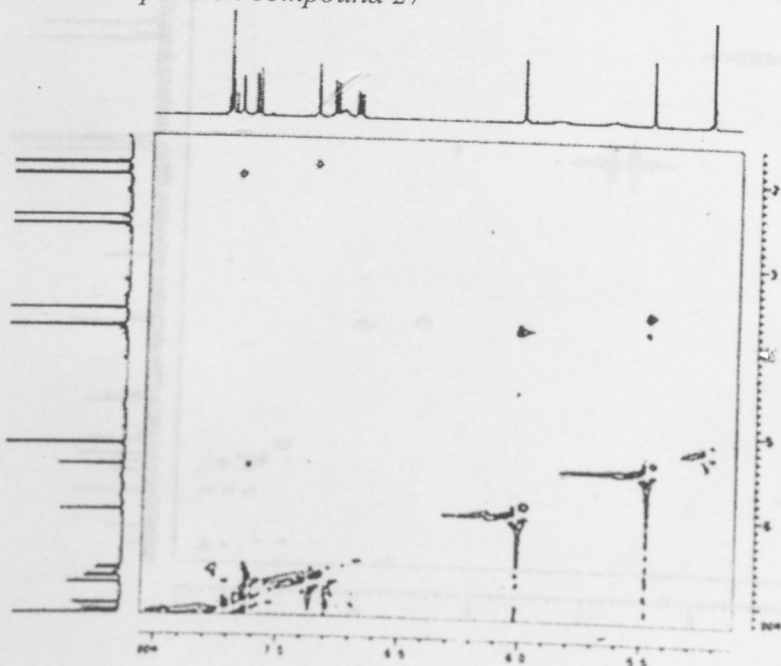
APT spectrum (CD₂Cl₂, 125 MHz)



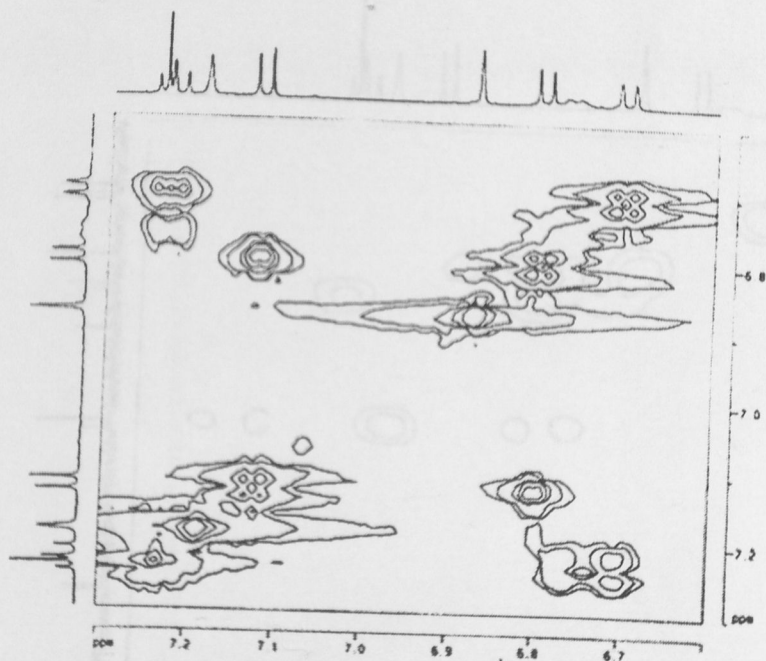
ROESY spectrum for compound 27 (CDCl₃, 500 MHz)



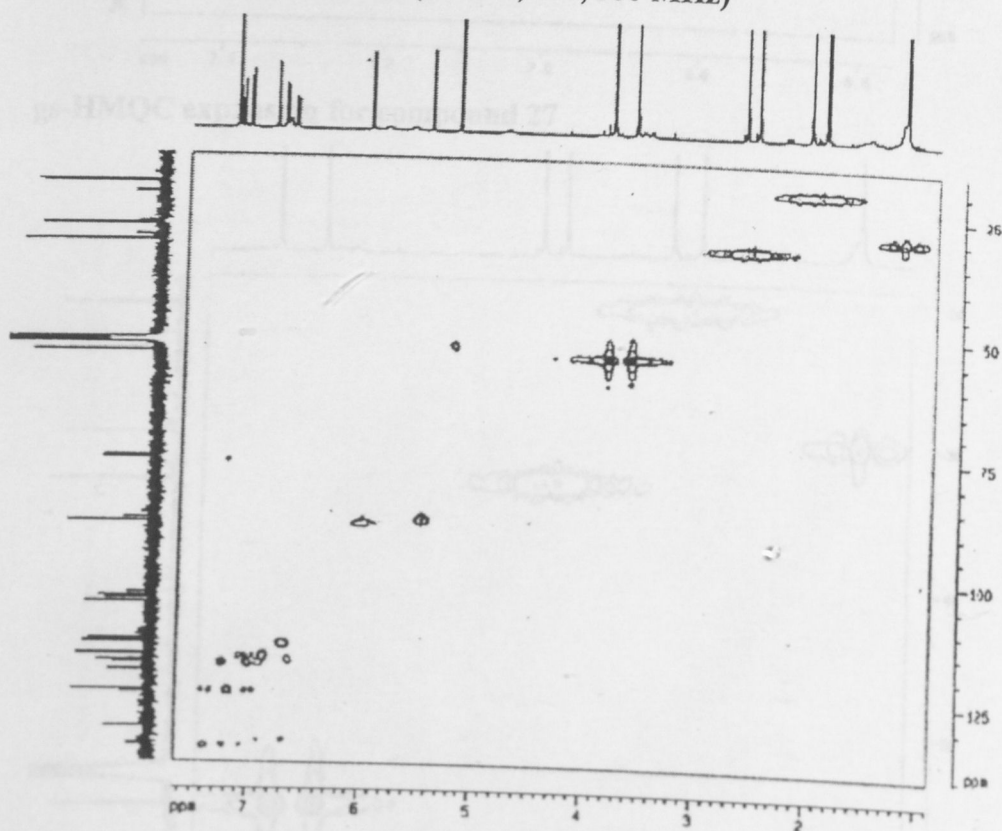
ROESY expansion compound 27



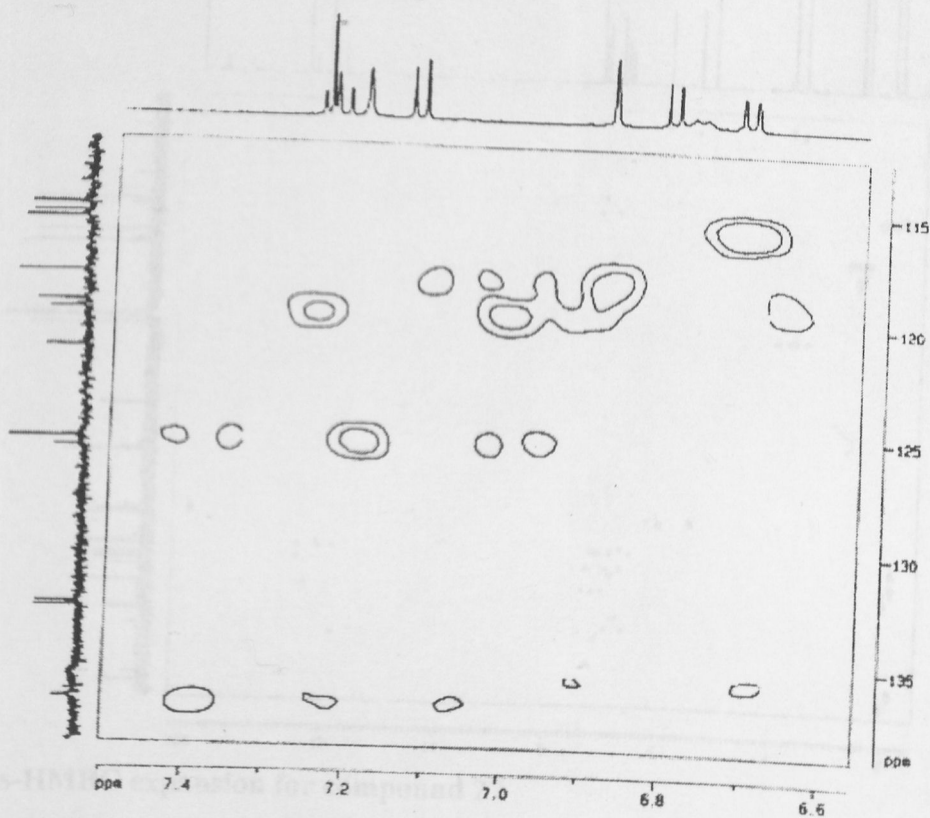
HH-COSY for compound 27 (CDCl₃, 500 MHz)



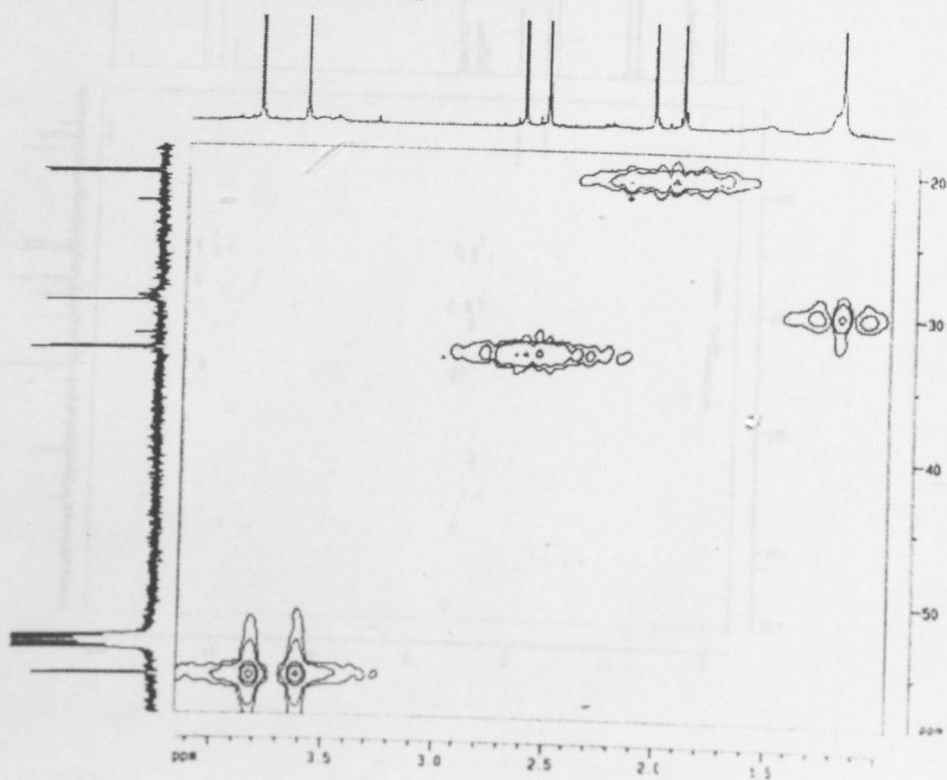
gs-HMQC for compound 27 (DMSO, 125, 500 MHz)



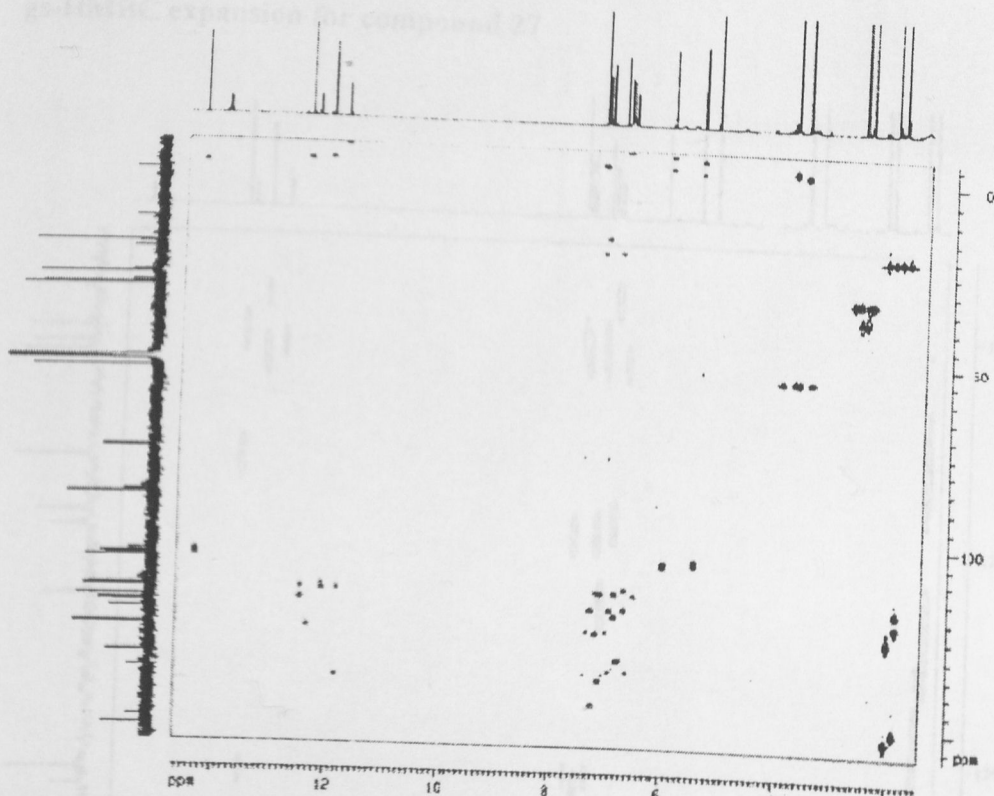
gs-HMQC expansion for compound 27



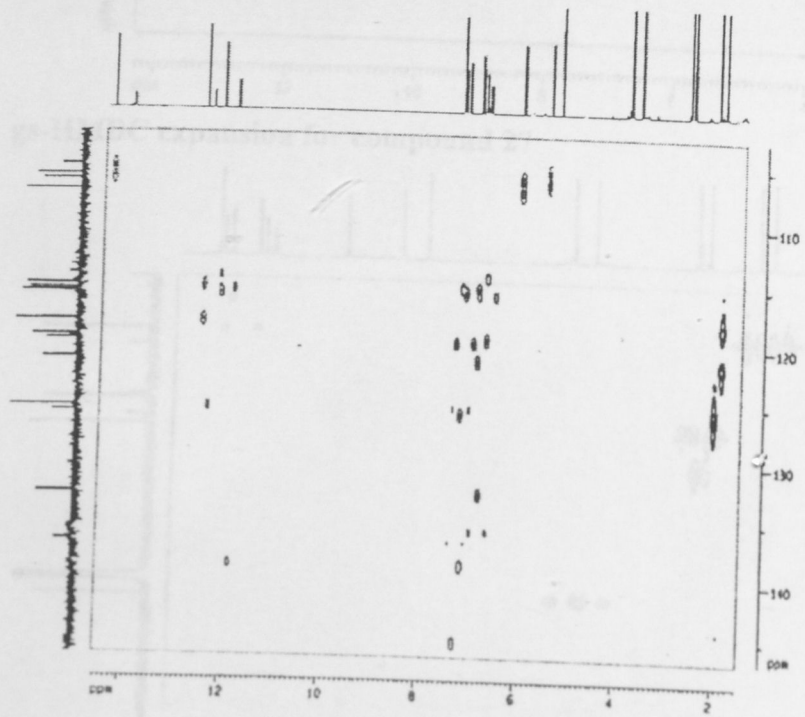
gs-HMQC expansion for compound 27



gs-HMBC for compound 27 (DMSO, 125, 500 MHz)

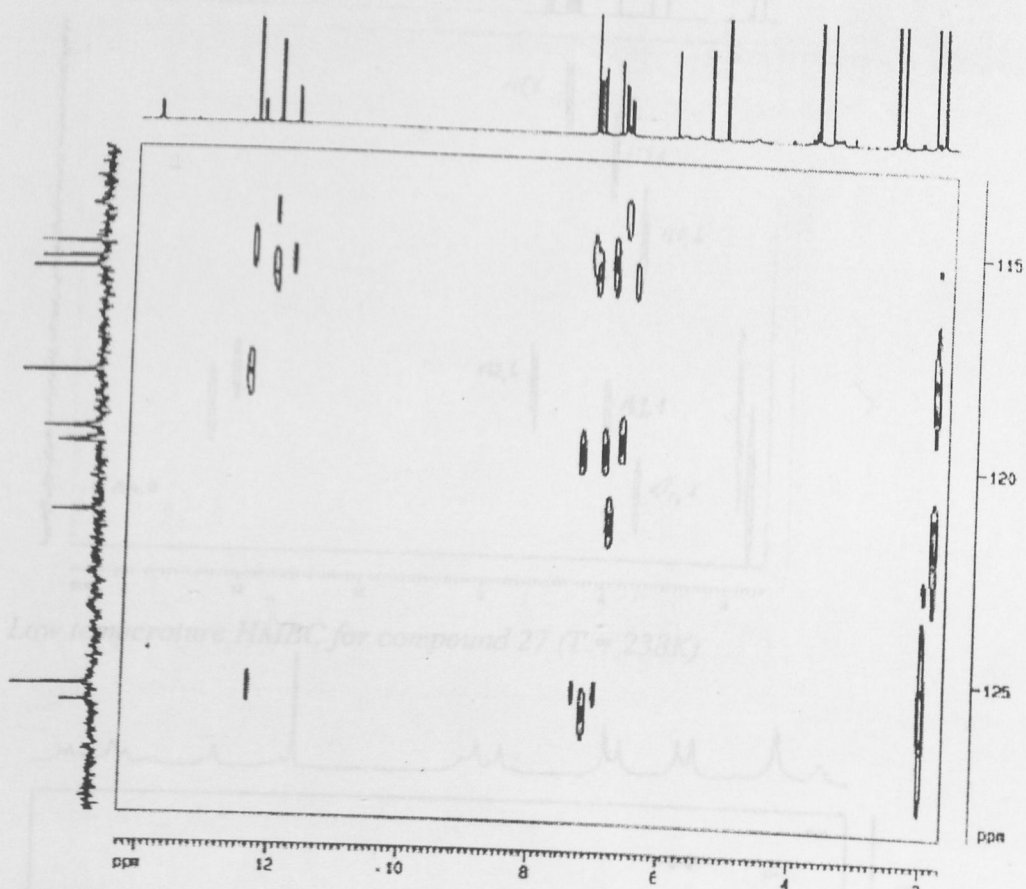


gs-HMBC expansion for compound 27

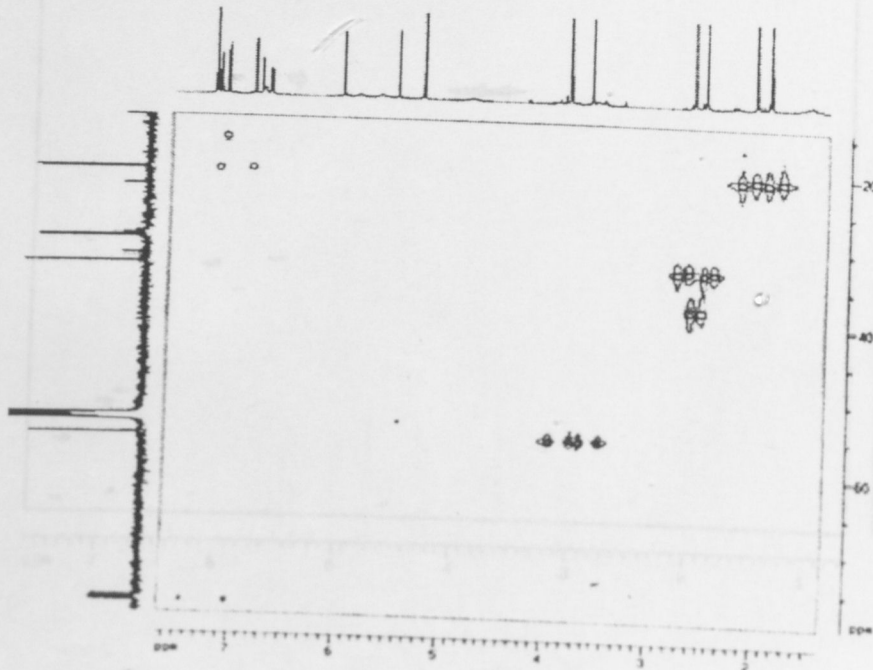


gs-HMBC expansion for compound 27

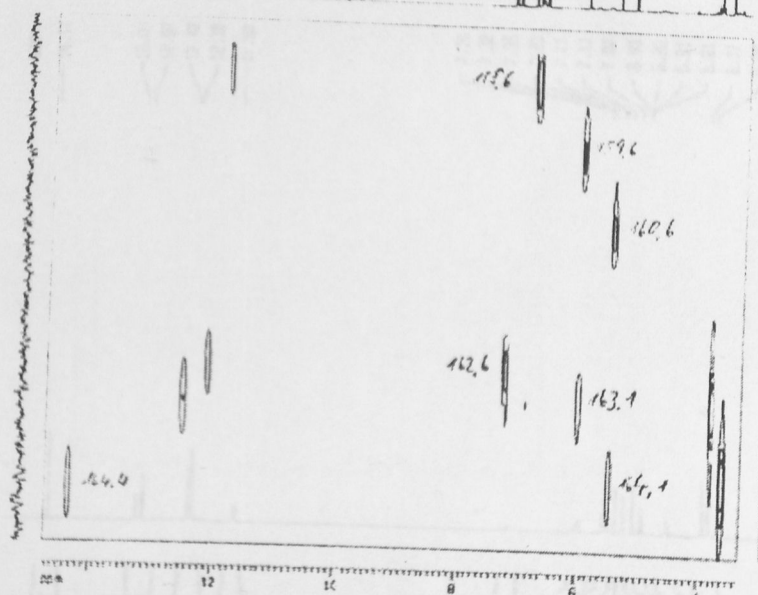
gs-HMBC expansion for compound 27



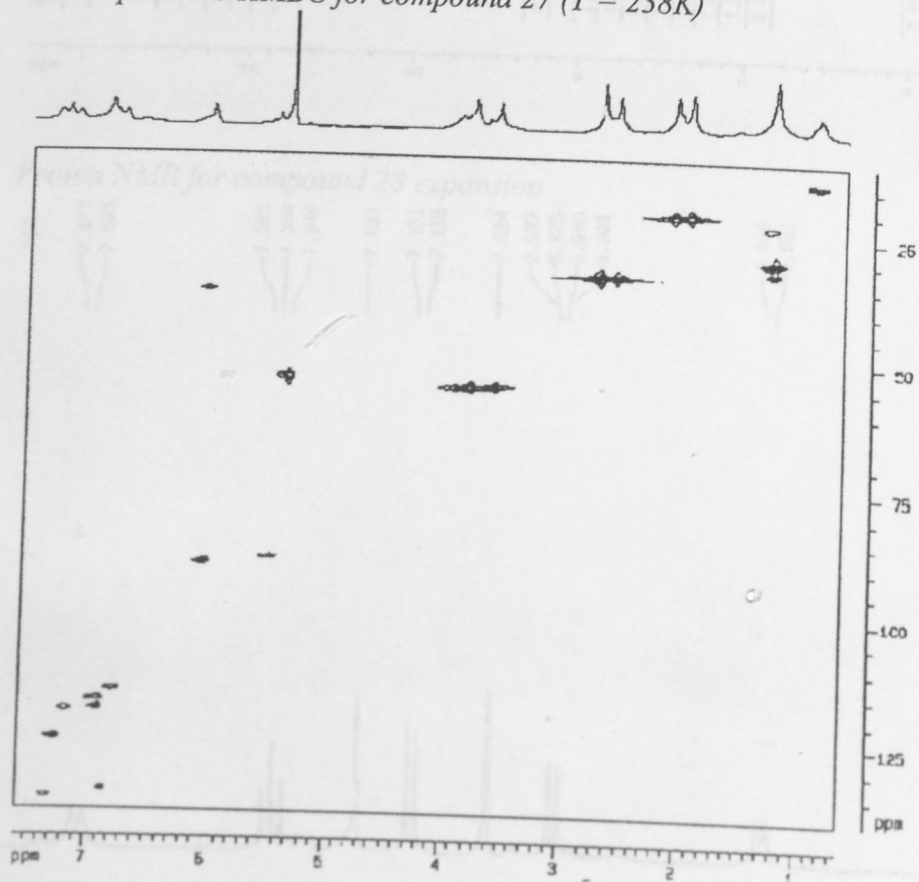
gs-HMBC expansion for compound 27



gs-HMBC expansion for compound 27

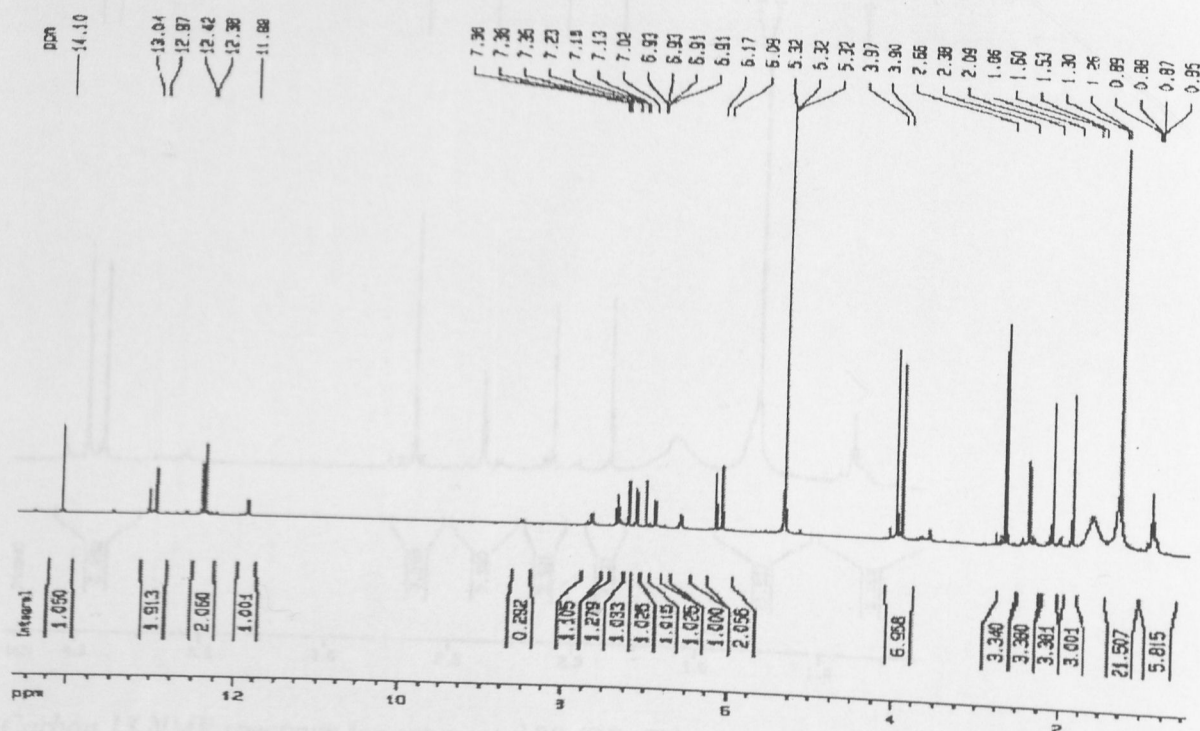


Low temperature HMBC for compound 27 ($T = 238\text{K}$)

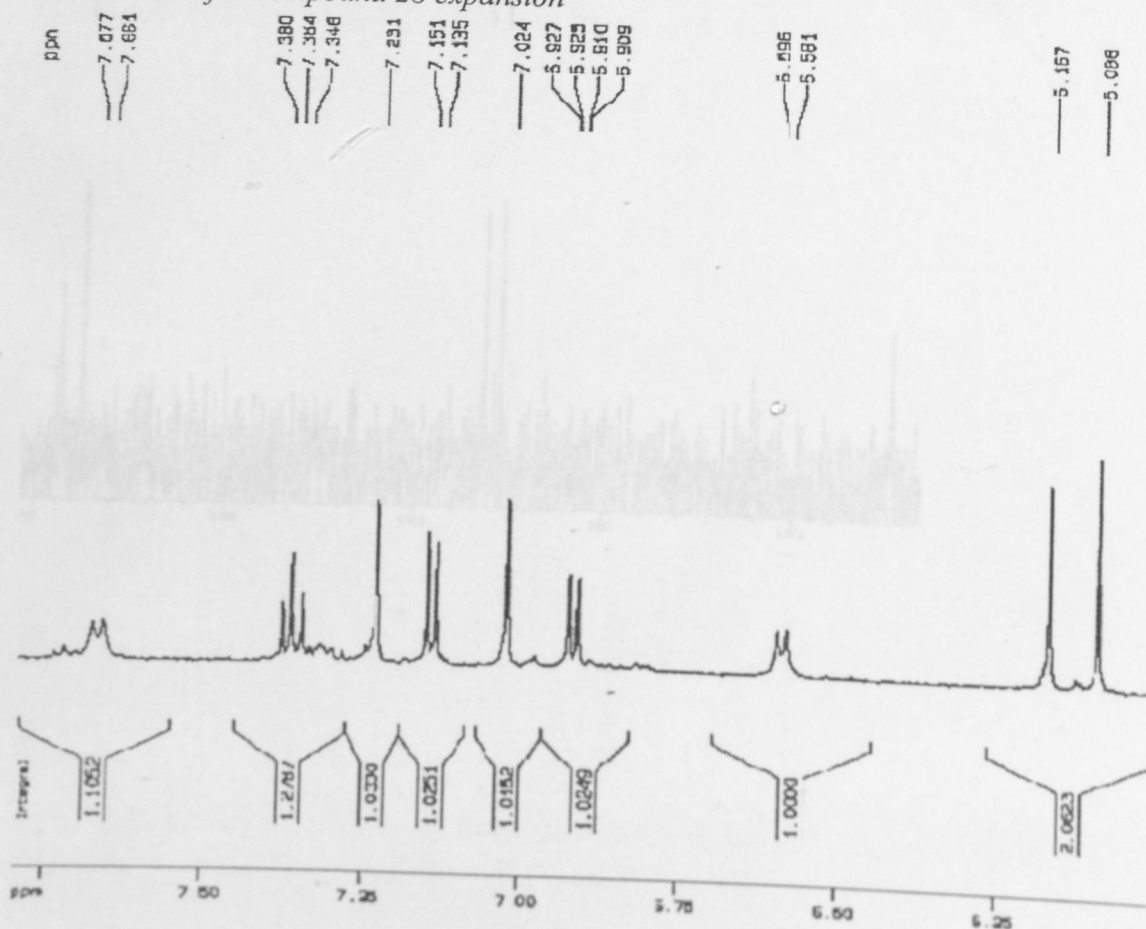


APPENDIX XXVI SPECTRA FOR COMPOUND 28

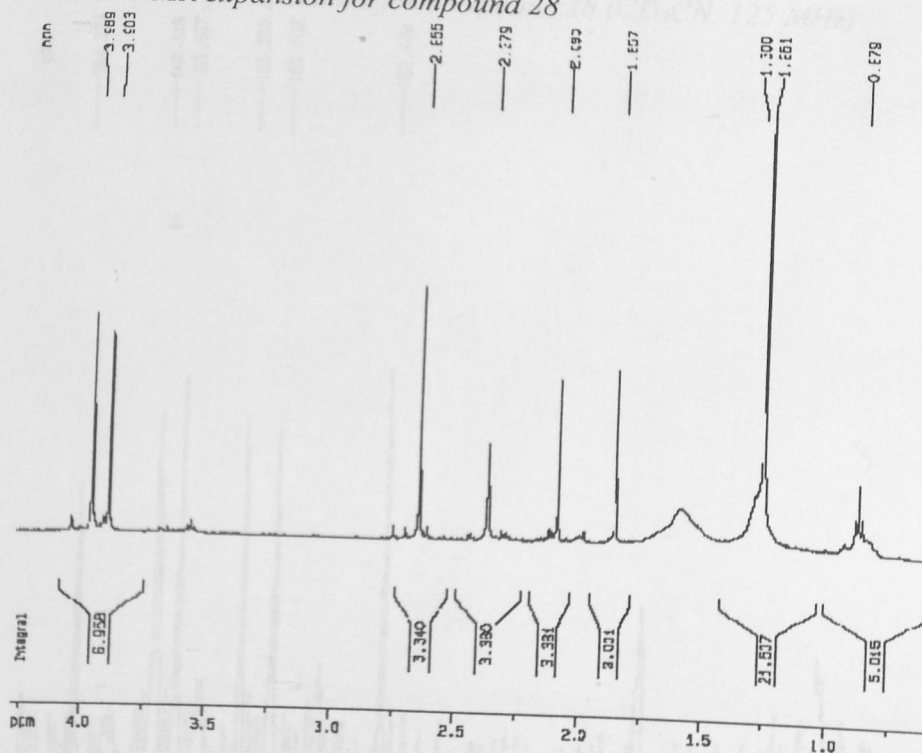
Proton NMR spectrum (CDCl₃, 500 MHz)



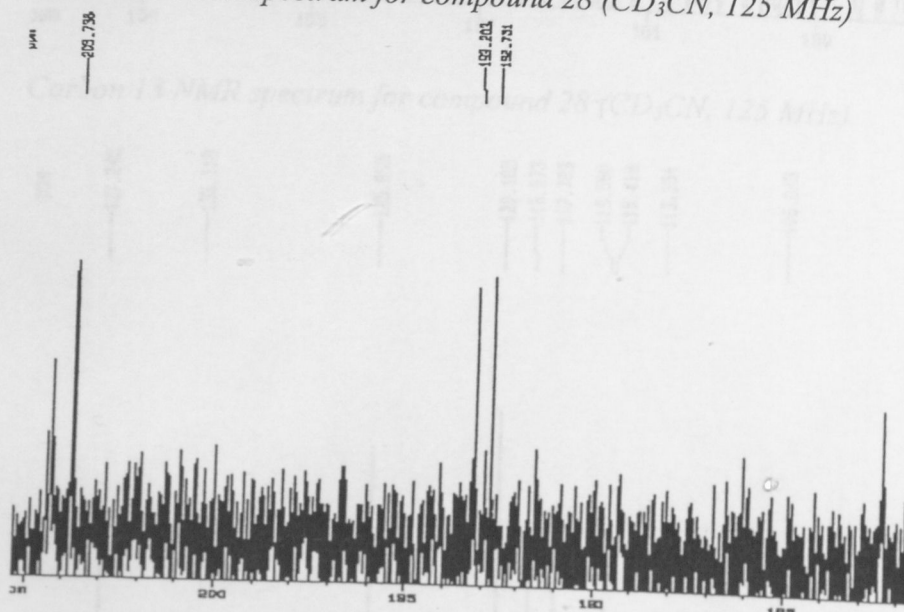
Proton NMR for compound 28 expansion



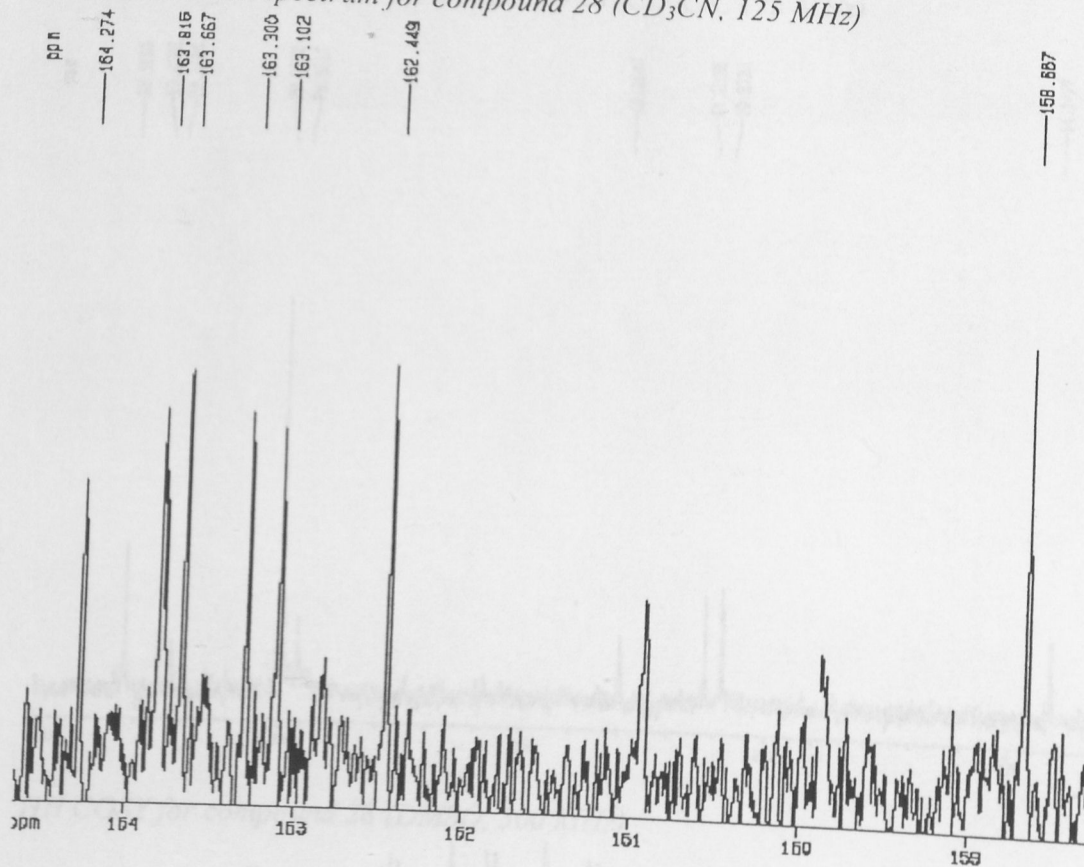
Proton NMR expansion for compound 28



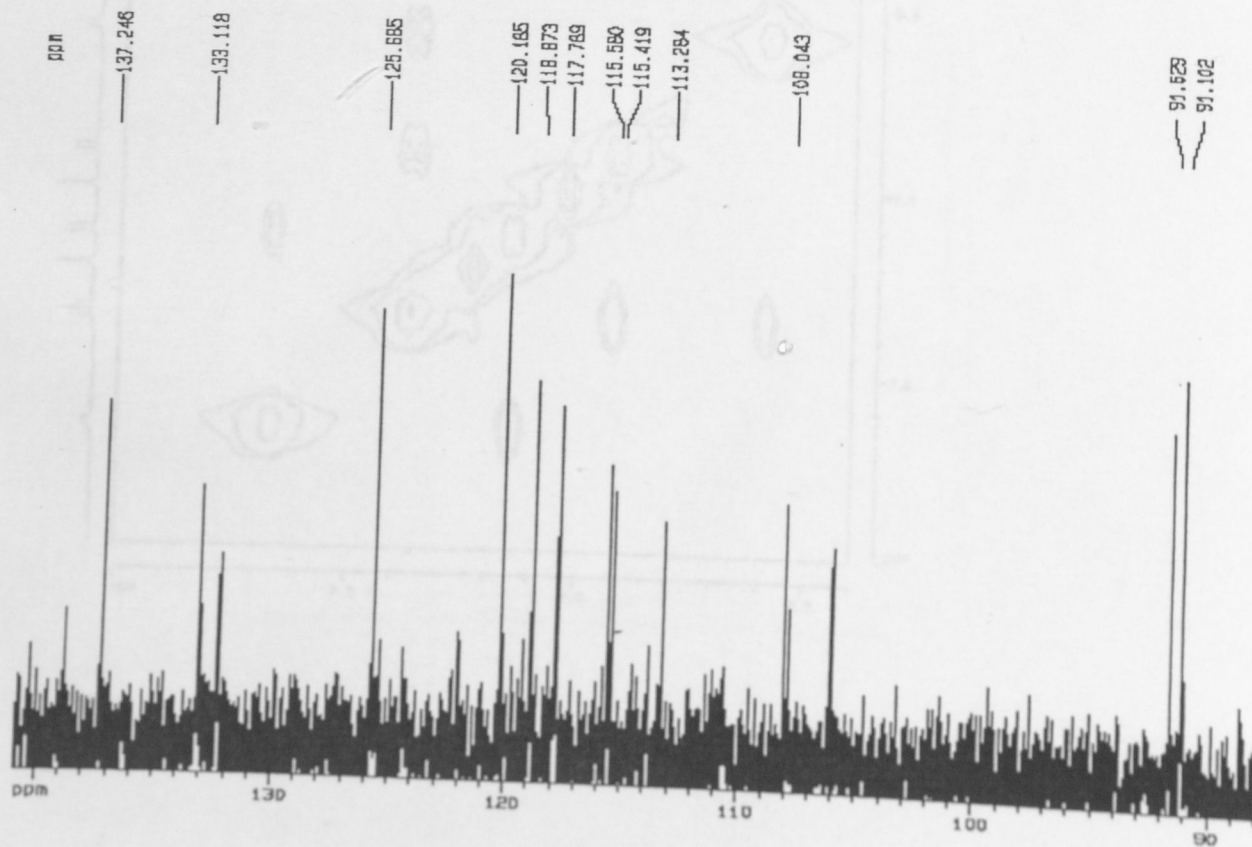
Carbon 13 NMR spectrum for compound 28 (CD_3CN , 125 MHz)



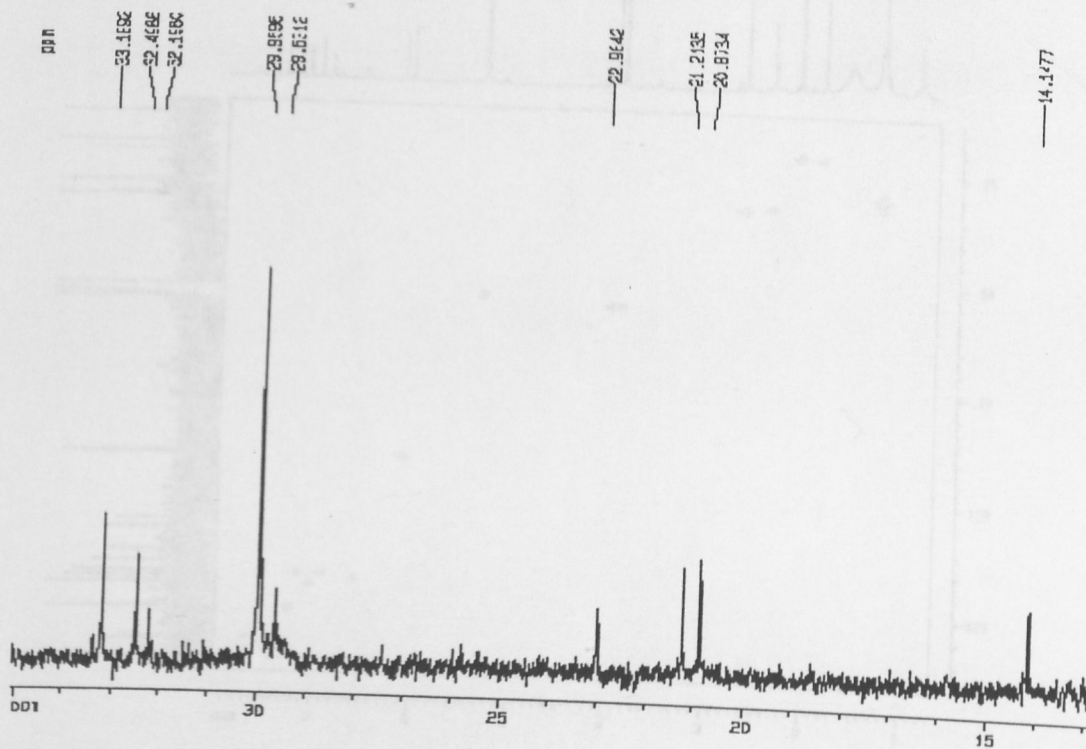
Carbon 13 NMR spectrum for compound 28 (CD₃CN, 125 MHz)



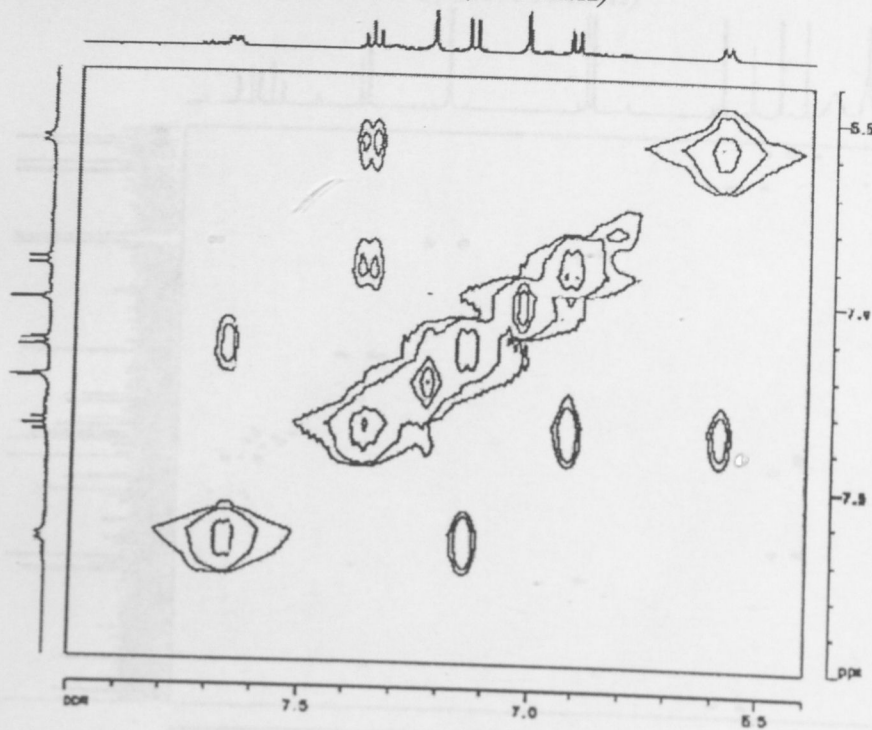
Carbon 13 NMR spectrum for compound 28 (CD₃CN, 125 MHz)



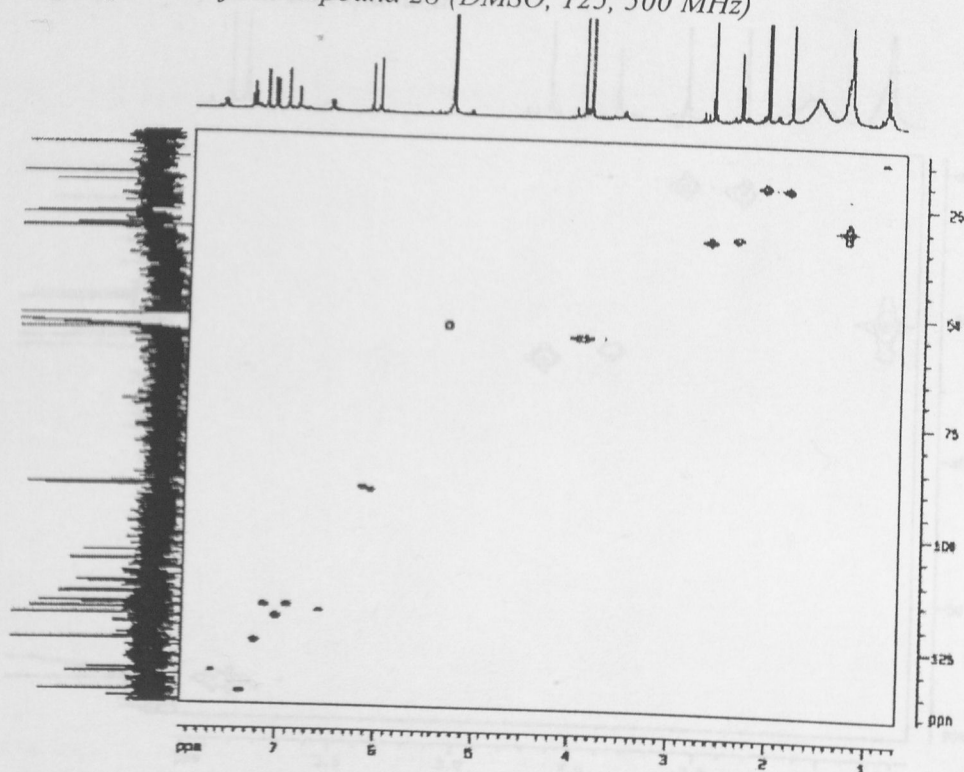
Carbon 13 NMR spectrum for compound 28 (CD₃CN, 125 MHz)



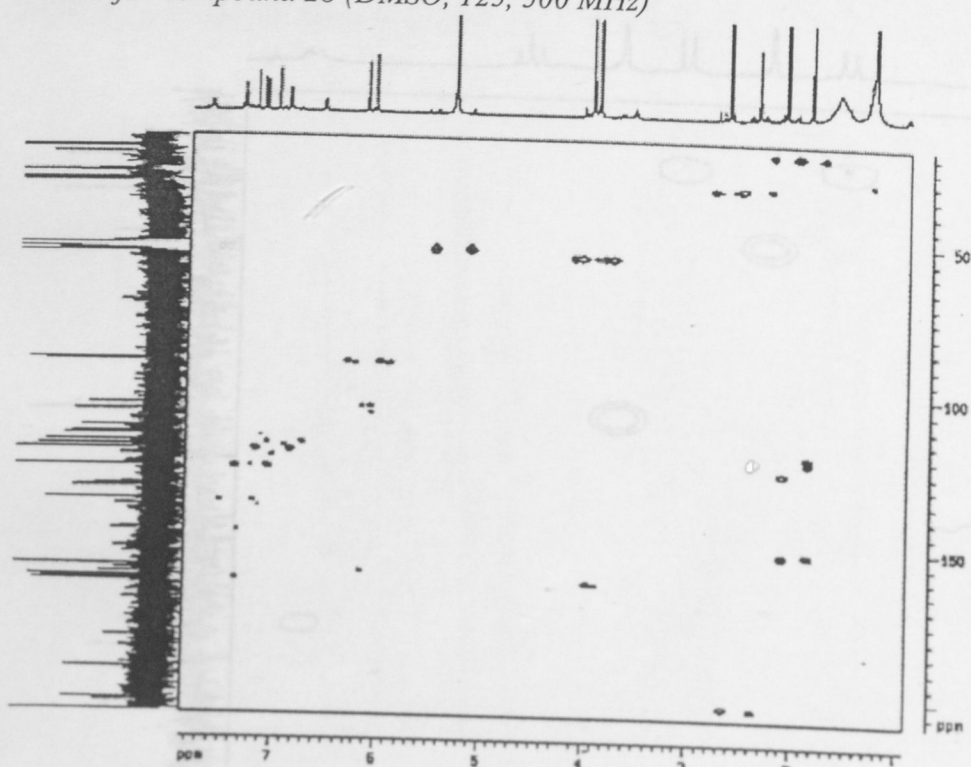
HH COSY for compound 28 (DMSO, 500 MHz)



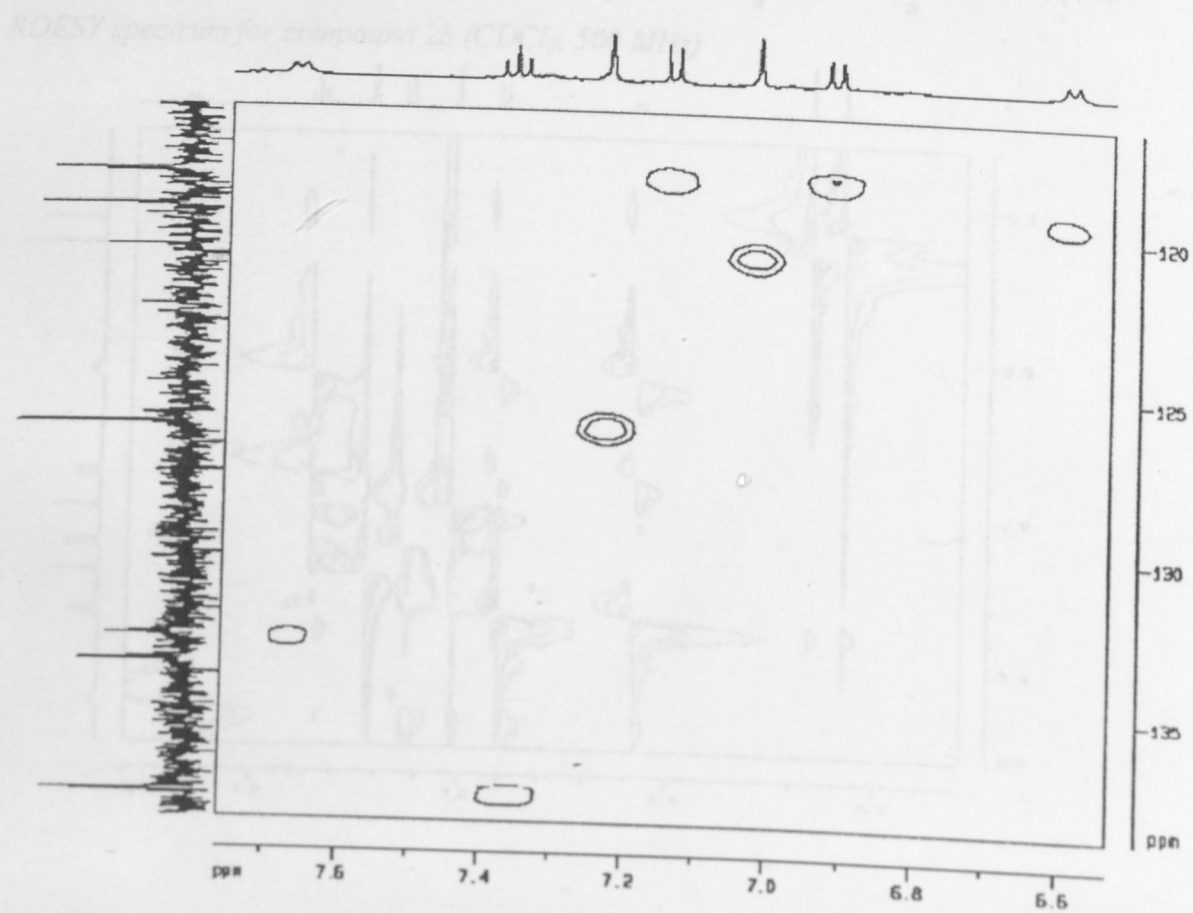
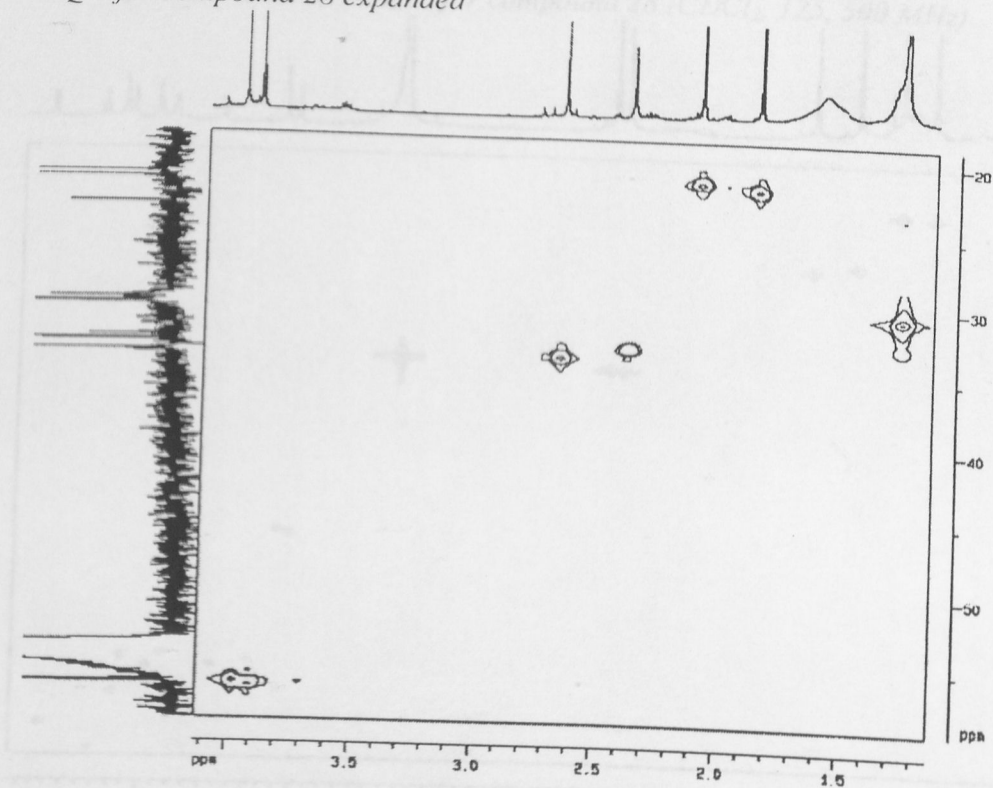
HMQC spectrum for compound 28 (DMSO, 125, 500 MHz)



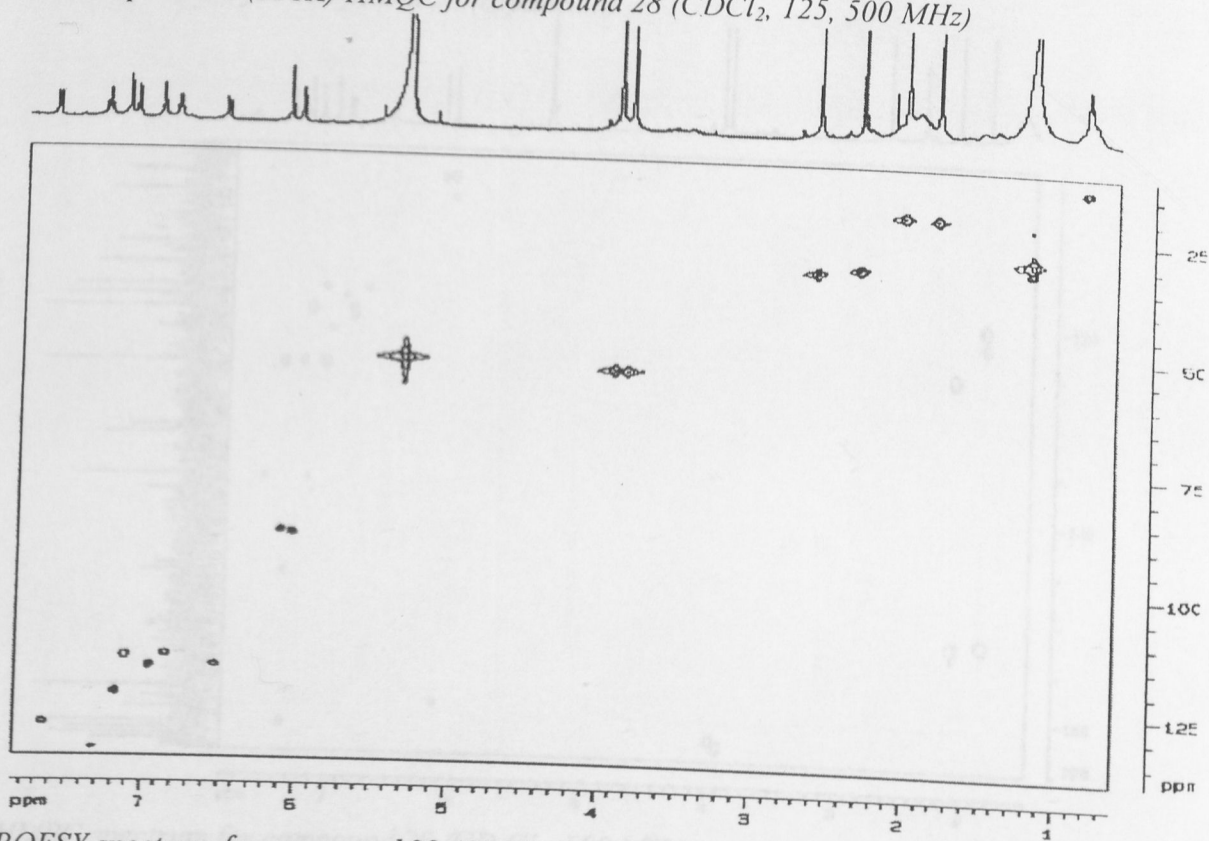
HMBC for compound 28 (DMSO, 125, 500 MHz)



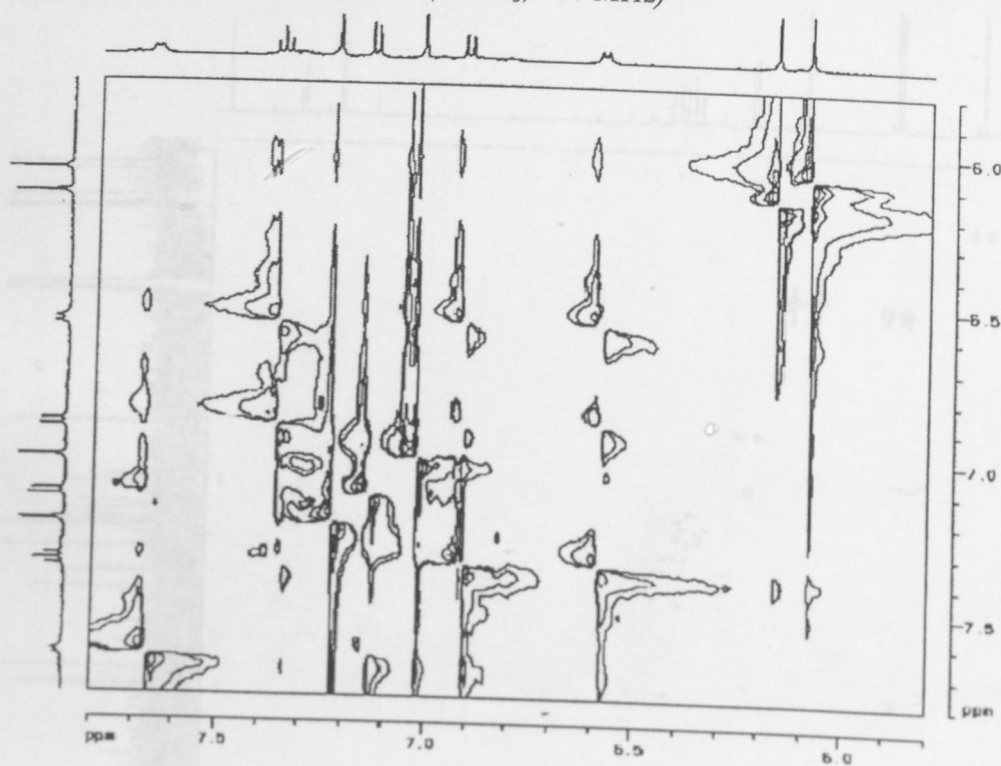
HMQC for compound 28 expanded



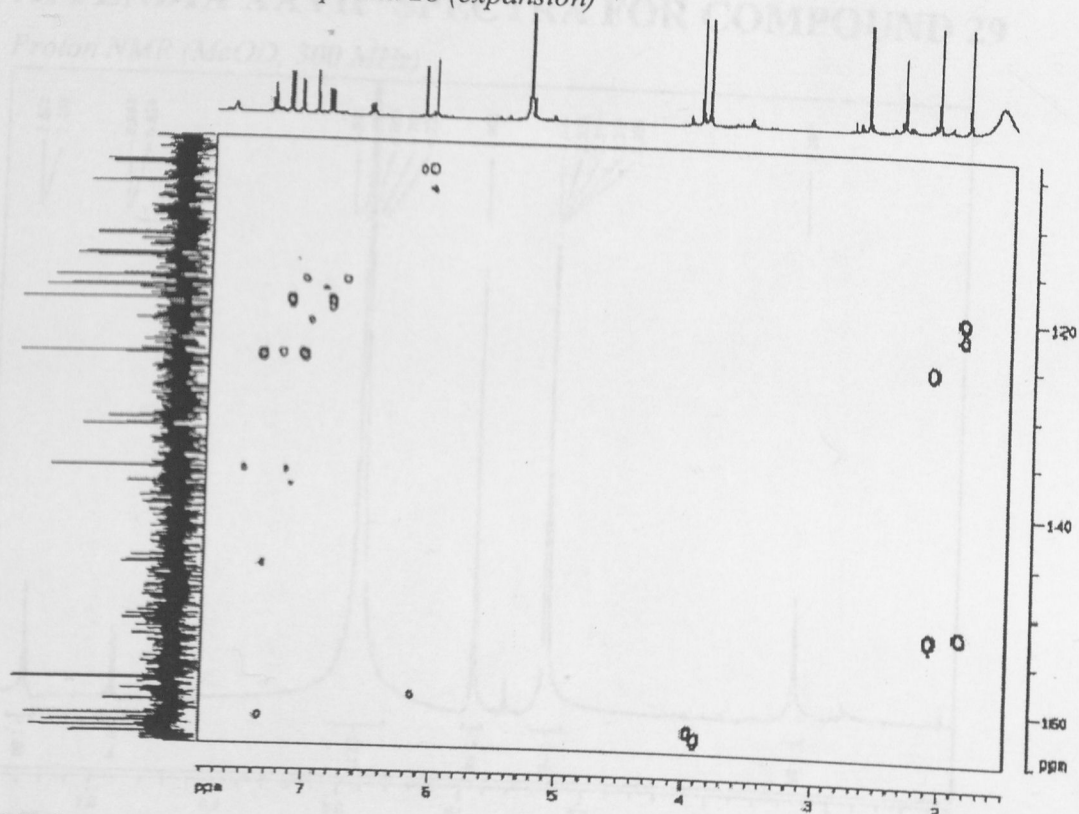
Low temperature (238K) HMQC for compound 28 (CDCl_2 , 125, 500 MHz)



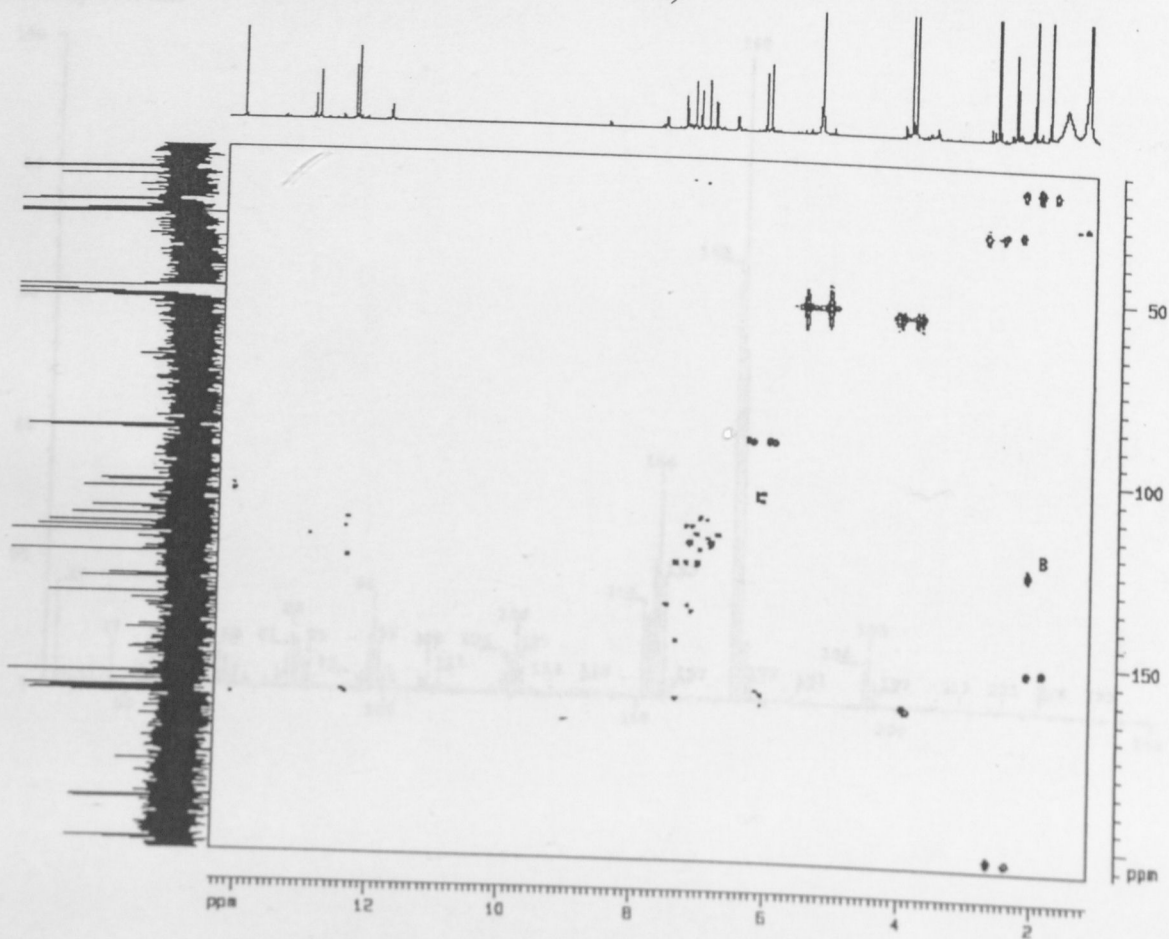
ROESY spectrum for compound 28 (CDCl_3 , 500 MHz)



HMBC spectrum for compound 28 (expansion)

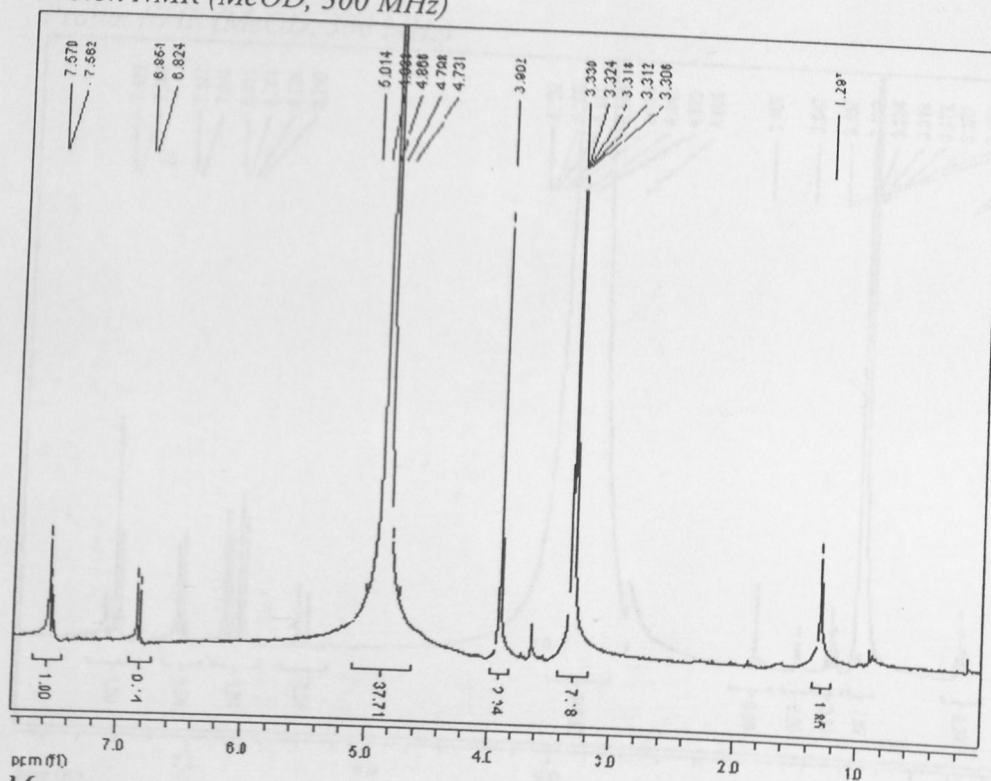


HMBC spectrum for compound 28 (CD₂Cl₂, 500 MHz)

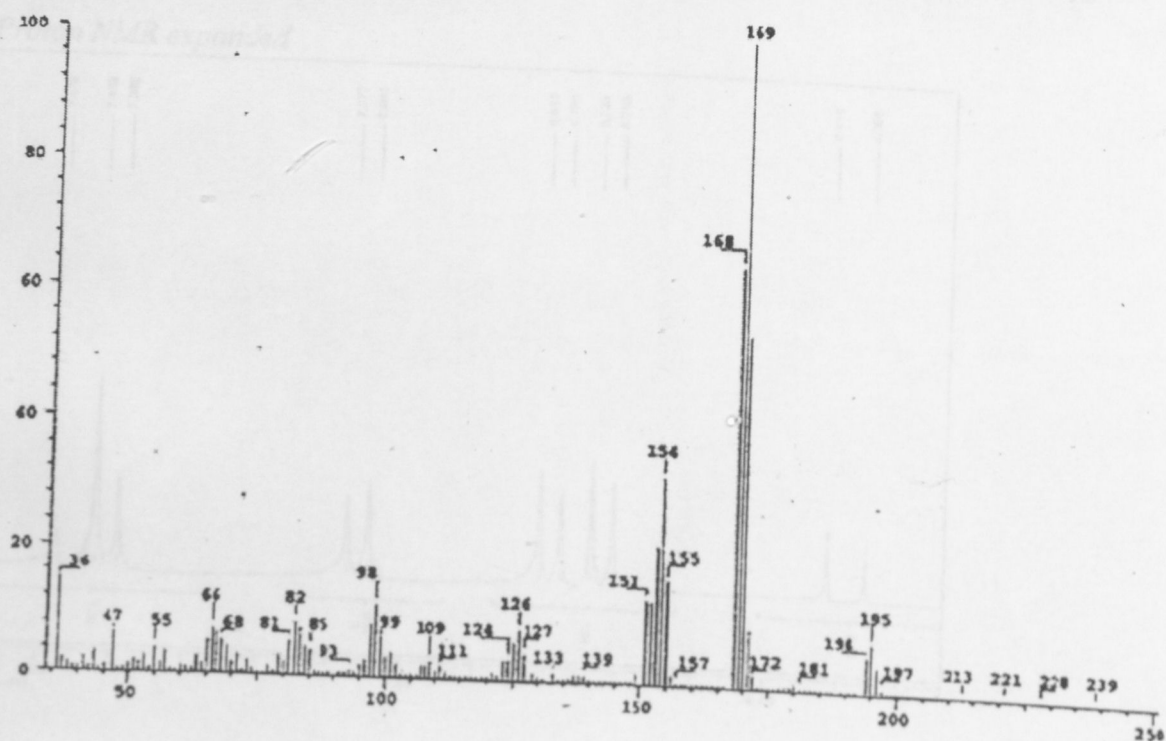


APPENDIX XXVII SPECTRA FOR COMPOUND 29

Proton NMR (MeOD, 300 MHz)

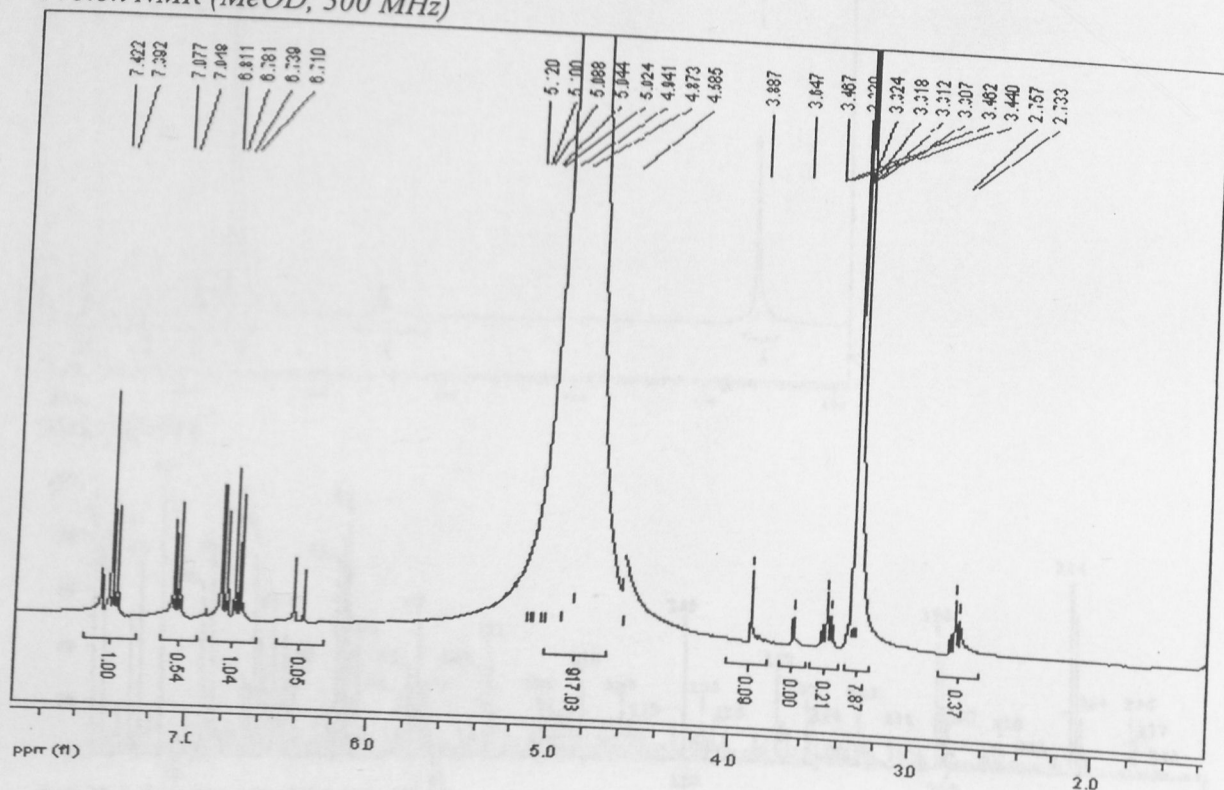


Mass spectrum

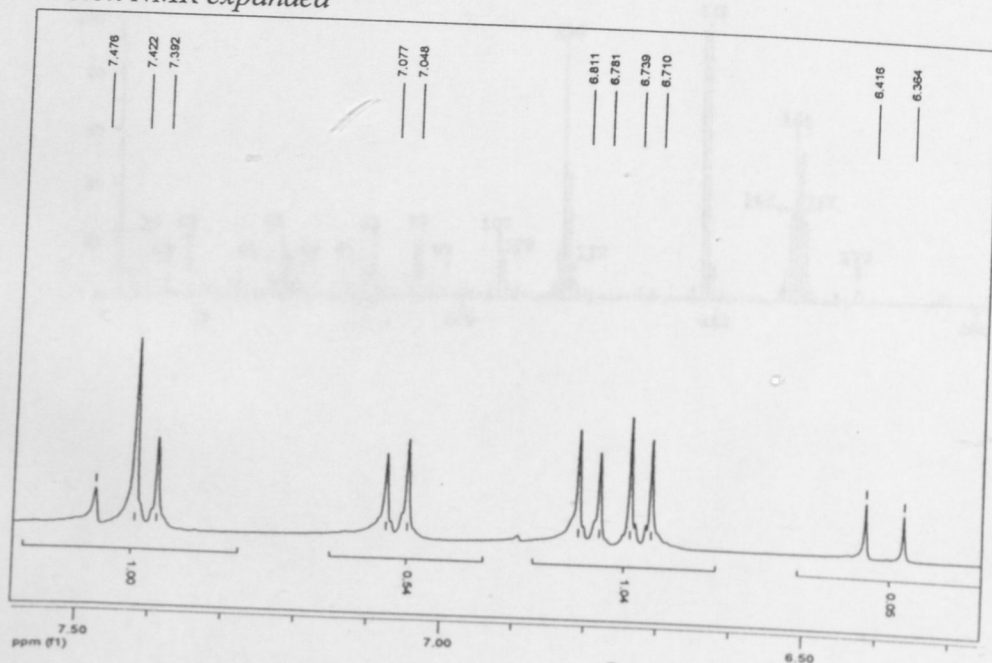


APPENDIX XXVIII SPECTRA FOR COMPOUNDS 30 AND 31

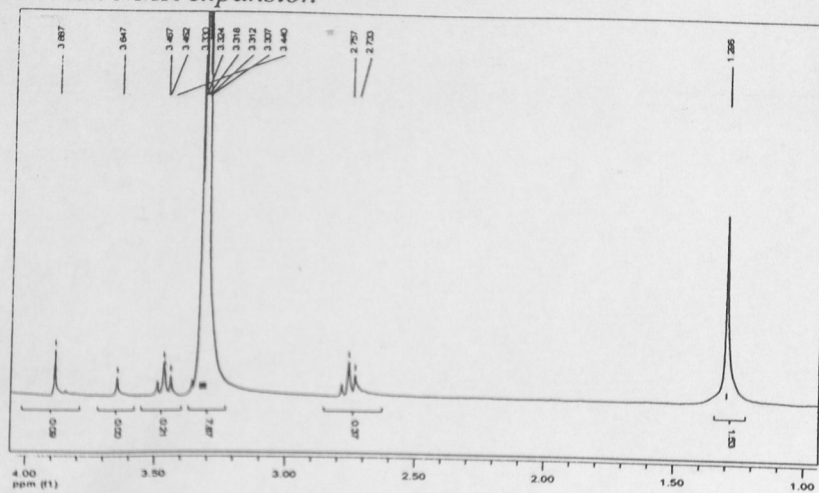
Proton NMR (MeOD, 300 MHz)



Proton NMR expanded



Proton NMR expansion



Mass spectra

