ADDIS ABABA UNIVERSITY

DEPARTEMENT OF CHEMISTRY

Phytochemical investigation on the Leaves of Vernonia Amygdalina

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Phytochemical investigation on the leaves of vernonia amygdalina

By Salah Hamza

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Abstract

Phytochemical investigation of Vernonia amygdalina

By: Salah hamza

Project advisor: Dr. Tetemke Mehari

Methanol extract of V.amygdalina, after repeated chromatography led to the isolation of different compounds, one of these compounds is partially characterized based on the spectral data.

I. INTRODUCTION

VERNONIA

Vernonia (compositae) is a large & predominantly tropical gnus with over 500 pecies. [1]

It is found nearly in tropical and warmer parts of north and South America, tropical Africa Madagascar and SE. Asia.

So far about 49 species are recorded form Ethiopia. [2] Vernonia amygalina is a shrub or small tree of 2-5m with petiolae leaf of about 6mm diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste, [3] flowering in the dry season. [4]

The genus vernonia as reported are rich in secodary metabolites, such as sesquiterpenoid, triterpenoids & flavornoids. [5]

Phytocemical analysis of V, amygdalina revealed the presence of two major classes of bioactive compounds, sesquiterpene lactones and steroid compounds. A number of known sesquiterpene lactones and freely occurring aglycones, have been isolated. [6]

1.2. MEDICINAL USES OF V. AMYGDOLINA

V. amygdolina has been reported for its use by wild chimpanzees for the treatment of parasite related disease. [7]

A concoction made form V. amygdalina is prescribed treatment for malaria fever, Sichistosomiasis, amoebic dysentery, and several other intestinal parasites & stomach-ache. [6, 8]

It has also been reported that V. amygalina to cause a marked reduction in blood pressure. [9] Extracts from V. amygdalina have also been suggested to have cell growth inhibitory effects in prostate cancer cell line. [7]

Chloroform extract of V. amydalina was found to show a significant inhibitory activity in vitro against cell derived form human carcinoma. [10]

1.3. OTHER USE OF V. AMYGDALINA

The leaves are used for human consumption and washed before easting to get rid of the bitter taste. They are used as vegetable and simulate the digestive system. V. amygdalina has been observed to be eaten by goats in central zone of Delta state Nigeria.

However in general has there been found, that V. amygalina has an astringent taste, which affects its intake.

V. amygdalina is also used, instead of hops to make beer in Nigeria. [3] In Ethiopia the plant is used in cleaning the container (fermentation vat). [11]

1.4. OBJECTIVE OF THE PROJECT

To isolate and characterize natural compounds found in Vernonia amygdalina

2. BACKGROUND

2.1. TERPENES

Terpenes are one of the largest groups of natural products comprising numerous compounds with important role in physiological and pathological process [12] despite their structural diversity, terpenes have a simple unifying feature by which they are defined and by which they may be easily classified. Terpenes are a unique group of hydrocarbon based natural products whose structure may be derived from isoprenes. [13]

The isoprene rule states that terpenes are multiples of C_5 units linked together head-to-tail. Several modes of cyclizaiton are conceivable and lead to various skeleton. [14]

Terpenes are thus classified by the number of 5 carbon units they contain.

Hemiterpenes C_5, monoterpenes C_{10}, Sesquiterpenes C_{15}, Diterpenes C_{20},

Sesterpenes C_{25}, Triterpees C_{30}, and Tetraterpenes C_{40}

The terpenes contain a wealth of significant compounds. The perfume industry is interested in the “essential” oils. Terpentine is used for painting, and most importantly we find among the terpenes physiologically very active compounds governing the life process such as adrenal hormones (cortisone), sex hormones (oestrogen and testosterone), vitamins A, D & E etc. [14]

Most terpenoid compounds occur free in plant tissue, uncombined, with other substances, but many of them are found as glycosides, esters of organic acids and in some cases in combination with proteins.

The lower members of the class (C_{10} & C_{15} compounds) can often be obtained from the fresh or dried plant by steam distillation, while higher members (C_{20} and above) are usually isolated by extraction with solvents and separated and purified by crystallization, and chromatography.

2.2. SESQUITERPENE LACTOES

A large structurally varied, and botanically closely allied class of sesquiterpenes is a group of lactones found distributed throughout plants of the family compositae. [15]

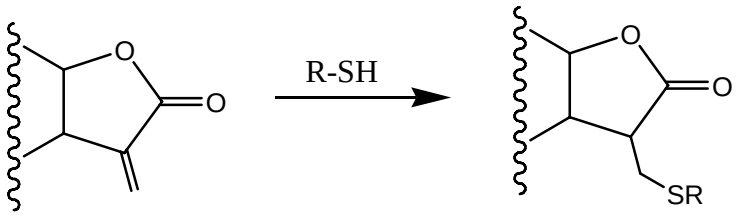
New sesquiterpen lactones are being found at a surprising rate there were 1300 sesquiterpene lactones known in 1981 & by 1987 there were 3,200. The rate of discovery has continued this place the sesquiterpene lactones among the largest classes of natural product.

Advances in the understanding of the biosynthesis of this group together with the great structural variety make these compounds of great value in the chemotoxonomy. [16]

2.3. BIOLOGICAL ACTIVITIES OF SESQUITERPENE LACTONES

Of all of the terpenoid compounds the sesquiterpene lactones possess the greatest variety of biological activities. More than 1000 of these compounds have been isolated chiefly from the plant family Asteraceae (compositae) but also form the umbellifereae & manaoliaceae.

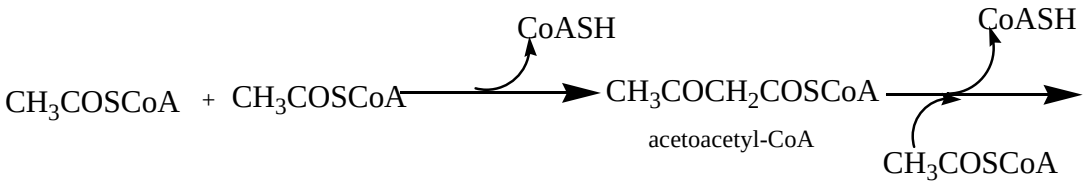
The activities these compounds possess include antineoplastic agent, insect feeding deterrents, plant growth regulators antimicrobial agents, schisosomicidal agents, vertebrae poisons and contact dermatitis in humans. Several structure-activity studies led to the conclusion that plant growth regulation requires the presence of an exocyclic α,β-unsaturated lactones moiety which can combine with sulfahydryl groups in key enzymes that control cell division. [18]

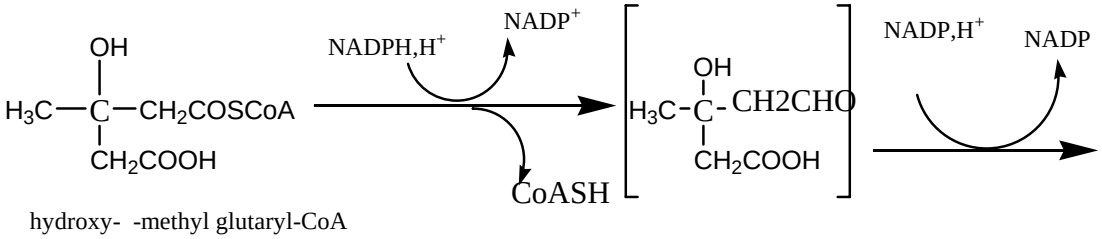


2.4. BIOSYNTHESIS OF TERPENOIDS

1. Formation of basic precursors

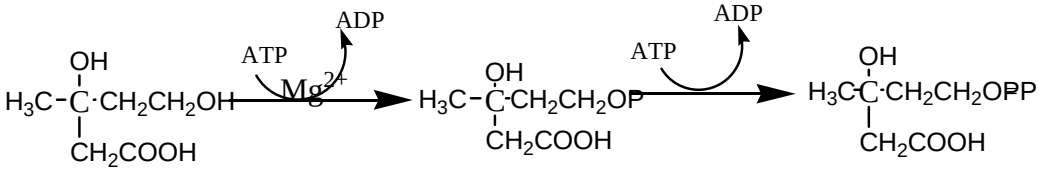
The biological isoprene unit isopentenyl pyrophosphae (IPP), from wich all terpenoids appear to be synthesized, is formed form three molecules of acetyl- coA via mevalonic acid (MVA) (Scheme-1)





hydroxy- -methyl glutaryl-CoA

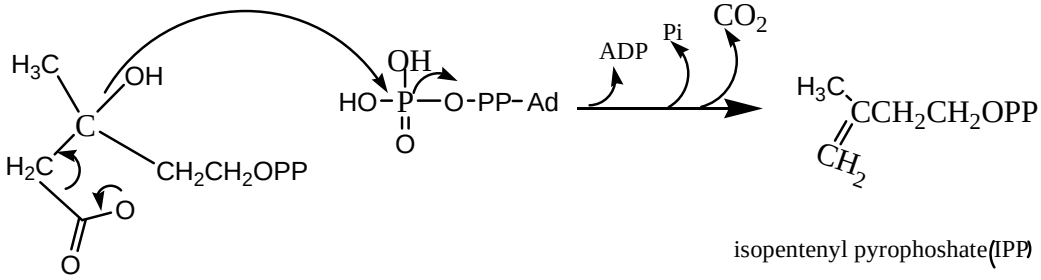
mevaldic acid
[enzyme bound]



mevalonic acid

mevalonyl-5-phosphate

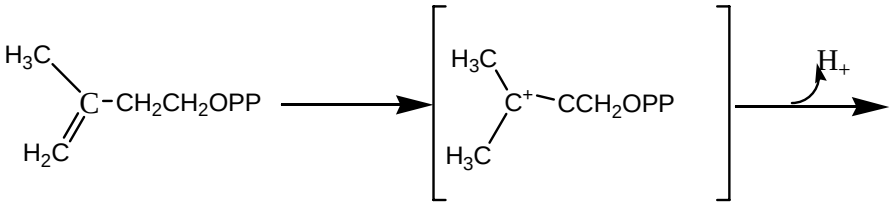
mevalonyl-5-pyrophosphate



isopentenyl pyrophoshate (IPP)

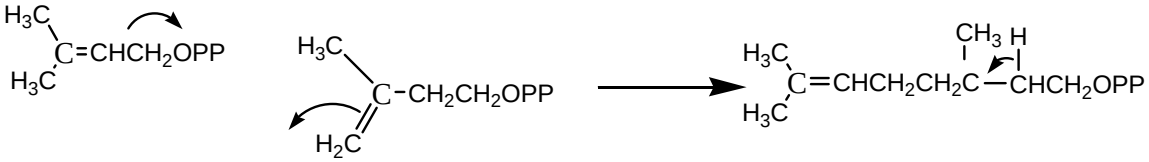
Scheme 1: formation of isopentenyl pyrophpsphate

In order to start the polymerization reactions which produce the higher terpenes IPP is converted into granyl pyroposphate which is considered to be the precursor of all monoterpenes (*scheme 2*)



IPP

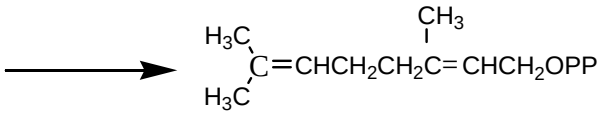
intermediate carbonium ion



dimethyl allyl pyrophosphate

IPP

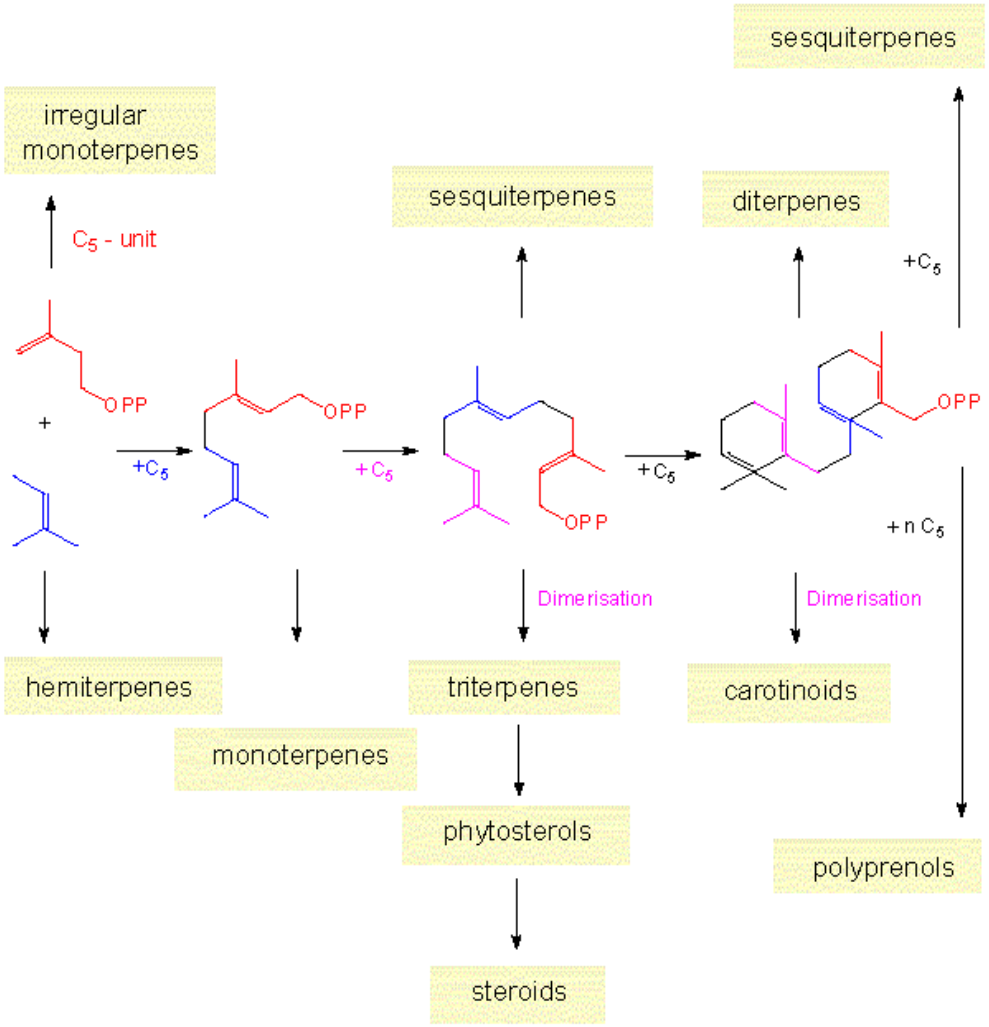
intermediate carbonium ion



Gernanyl pyrophosphate

Scheme 2: formation of geranyl pyrophosphate

This isoprene polymerizes subsequently to give all the different groups of compounds. [21] (*Scheme 3)*



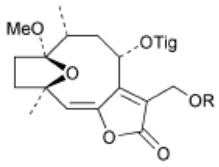
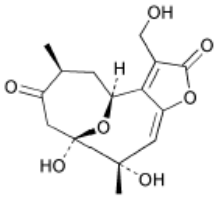
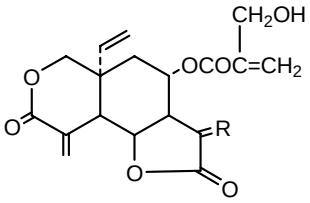
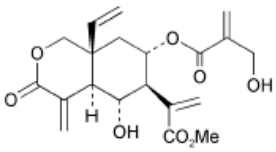
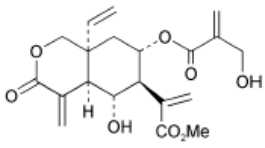
Scheme 3: formation of the different groups of terpenes

2.5. SESQUITERPENE LACTONES FROM VERNONIA SPECIES

A number of known sesquiterpene lactones have been isolated from vernonia species

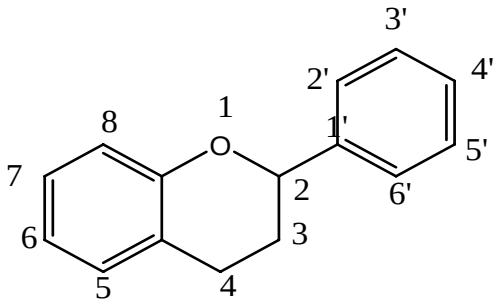
Some of the sesquiterpene lactones isolated from vernonia species are given in the table bellow

Table-1 some sesquiterpene lactones from V. species

compound	source	ref.
 1 R=Ac 2 R=H	V. cinera	19
	V. potamophila	19
 1 R=CH_2 2 R=CH_2CH_3	V.amygdalina	20
	V.lasiopulide	19
	V. lasiopus	19

3 FLAVONOID

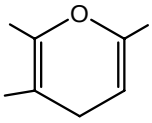
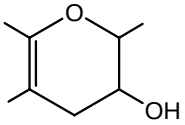
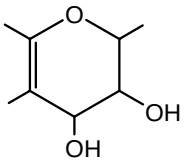
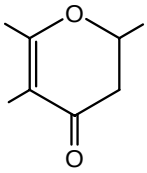
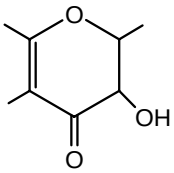
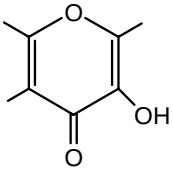
Flavonoids represent one of the largest classes of naturally occurring phenolic compounds. Chemically flavonoids are phenolic glycosides, and their aglycones consists of two aromatic rings joined by a 3- C unit in other words they are phenyl propane derivatives well over a hundred aglycones have been found in plants but these fall into a much smaller number of groups which are classified according to the oxidation level of the 3-C unit of the flavone nucleus. (1)

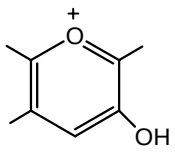
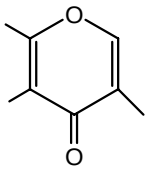
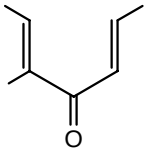
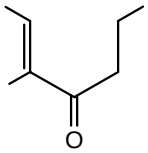
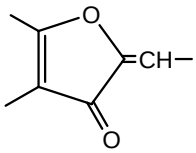


(1)

Classification of flavonoids according to the oxidation state of the 3-carbon Residue of the phenyl propane unit. [22]

Table 2 - classification of flavonoids

type	structure	example
flavone		apigenin (5,7,4')
flavonol		catechin (5,7,3',4')
flavandiol		leucocyanidin (5,7,3',4')
flavanone		butin (7,3',4')
dihydroflavonol		taxifolin (5,7,3',4')
flavonol		myricetin (5,7,3',4',5')

anthocyanidin		pelargonidin (5,7,4')
isoflavone		genistein (5,7,4')
chalcones		butein (3,4,2',4')
dihydrochalcones		phoretin (4,2',4',6')
aurones		sulphuretin (6,3',4')

the number in bracket indicate the location of additional hydroxyl grops; except for aurones and chalcones

The flavonoids include many of the most pigments and occur throughout the entire plant kingdom form the fungi to the angiosperms

About 150 aglycones are known in higher plants they are found both in vegetative parts and in flowers, as flower pigments, they have a well known role in attracting pollinating birds and insects.

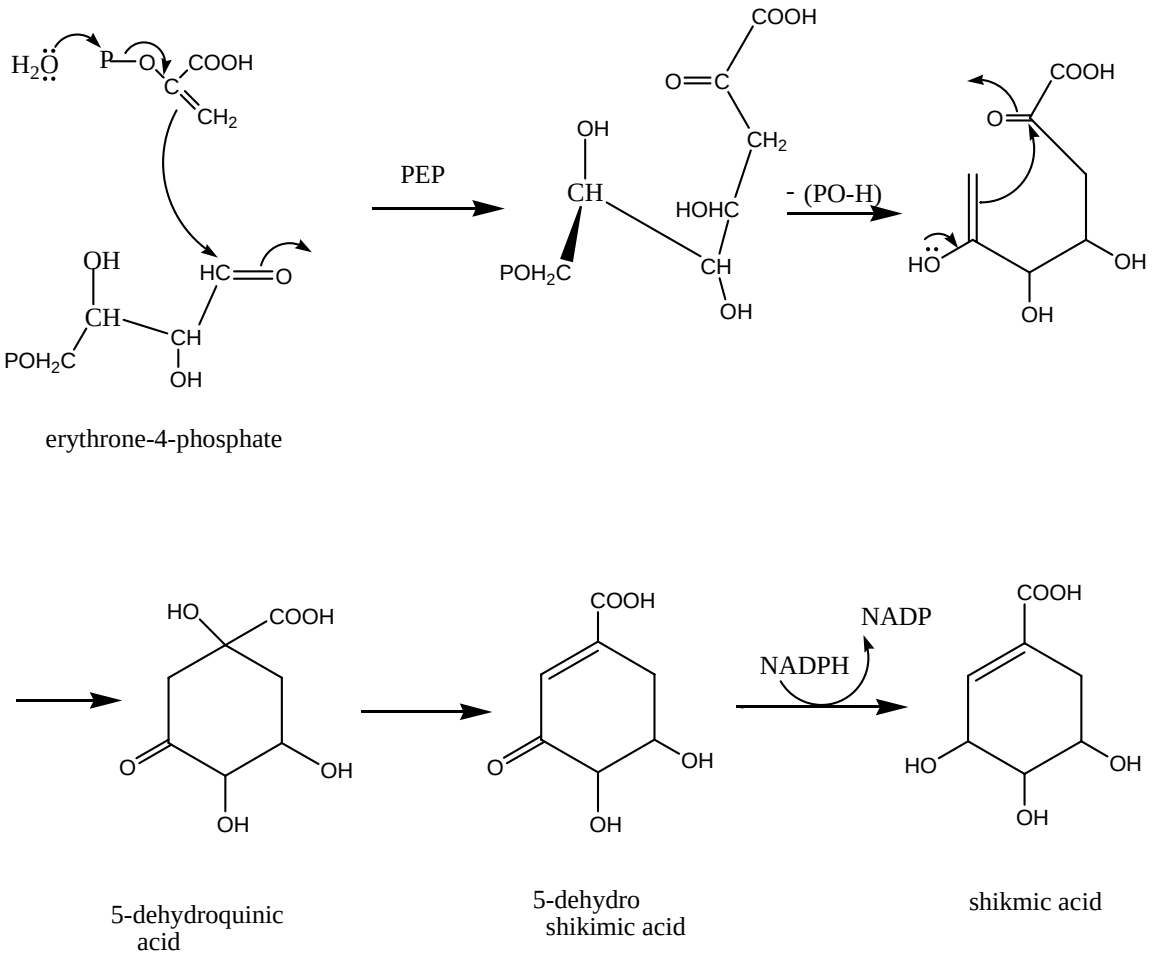
Flavonoids can also be powerful inhibitor of oxidative phosphorylatio

3.2. BIOSYNTHESIS OF FLAVOOIDS

The C_5 Skeleton of flavonoids is derived from two separate pathways.

Ring A arises by head-to-tail condensation of two malonyl CoA units & acetyl C_OA. [24]

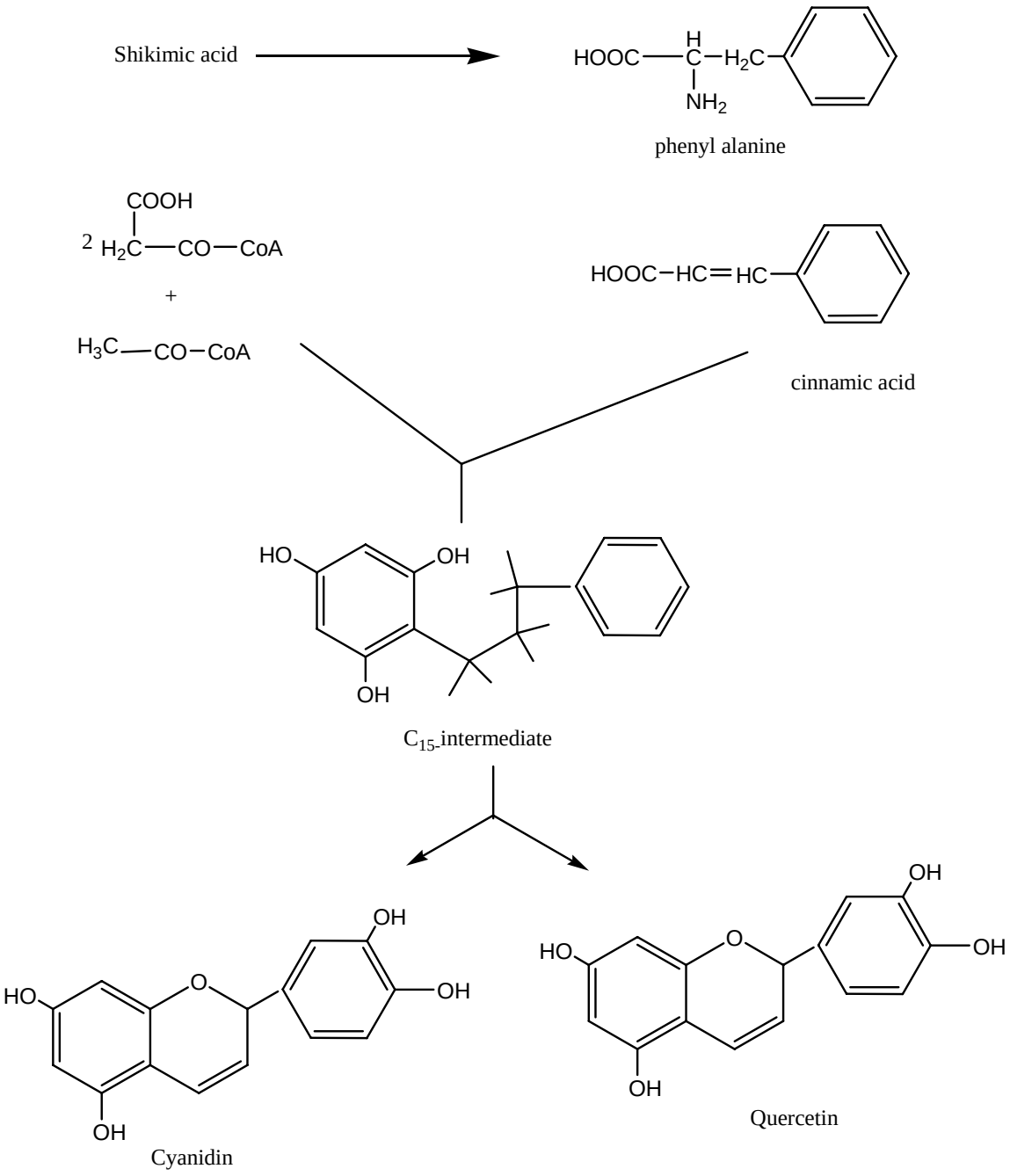
The B-ring & the side chain is derived form p-hydroxy cinnamic acid via shikimic acid. The formation of shikimic acid proceeds from the three- & four-carbon atom precursor phosphoenol pyruvic acid and eryhrose-4-phosphate through a series of steps. [25] (*Scheme 4*)



Scheme 4: formation of shikmic acid

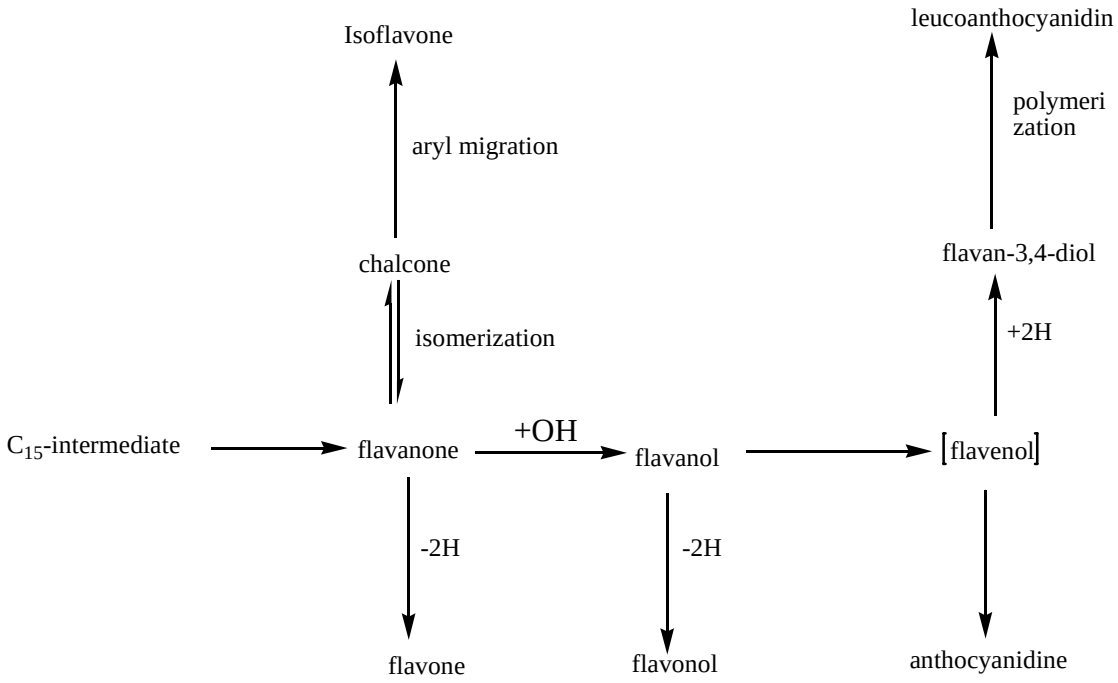
Chalcon synthase (CH) is the first enzyme in the biosynthesis of all classes of flavonoids in plants. It catalyzes the stepwise condensation of three acetate residues. [25]

Both cinnamic acid and phenylalanine are excellent flavonoid precursor and the enzyme for the one-step deamination of phenylalanine to cinnamic acid has been found in a number of plants. [24] *(Scheme 5)*



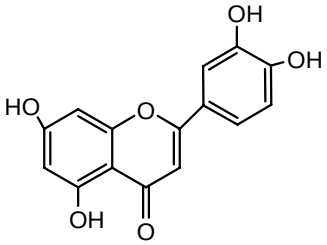
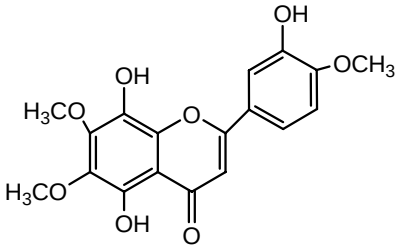
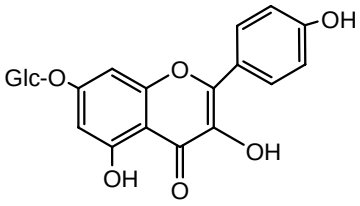
Scheme 5: formation C_{15}- intermediate

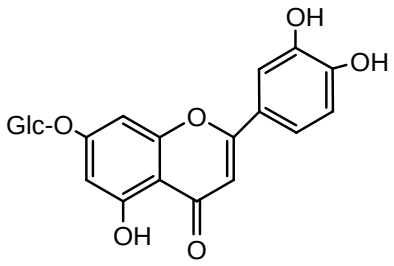
Once the C_{15}-intermediae is formed, it is modified in a variety of ways but in a limited number of steps, to yield the range of different flavonoid classes encountered in nature. [24] *(Scheme 6)*



Scheme 6: formation of the different classes of flavonoids

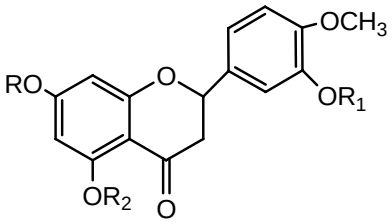
Table-3 some flavonoids from vernonia species

compound	source	ref
 luteolin	vernonia amygdalina	28
 8,3'-dihydroxy-5,6,7,4' tetra methoxyflavone	vernonia saligna	27
 kampetferol- 7-O- -glucoside	vernonia saligna	27



luteolinl- 7-O-glucoside

vernonia amygdalina 30



vernonia diffusa 29

$R=R_1=R_2=H$ (hesperetin)

$R=R_2=H$ $R_1=Me$ (homoesperetin)

4. RESULTS AND DISCUSSION

4.1. PARTIAL CHARACTERIZATION OF VAM-1

The UV spectrum of VAM-1 (appendix-1) shows absorbance peaks at 223 and 274nm which indicate easter substituted aromatic ring.

- In the IR (KCl) spectrum (appendix-2), the absorption bands at 3459.84 cm^{-1} is due to solvent
- The absorption band at 3019.19 cm^{-1} indicate aromatic C= C-H stretch
- The absorption band at 2928.58cm^{-1} indicate –C-H stretch or methyl group
- Absorption band at 1723.82 cm^{-1} indicate ester group
- Absorption band at 1601.72 cm^{-1} indicate –C=C- stretching

The HNMR spectrum (appendix-3) show speaks at δ 0.92 and 0.94 indicate the two methyl group

- The peaks form δ 1.28 – 1.35 indicate three methylene groups.
- The peak at δ 1.43 multiplet indicate one methylene group
- The peak at δ 1.75 multiplet indicate methine group
- The peaks at δ 4.24 triplets indicate one methylene group attached to oxygen.
- The peaks at δ 7.5 & 7.71 indicate protons of aromatic ring

^{13}C NMR ($CDCl_3$)

The ^{13}C NMR & DEPT spectra in ppm (appendix 4 & 5, table-4) indicate VAM-1 has 13 carbon atoms, two equivalent carbons.

The spectra show two-methyl carbon at δ 10.95 and 14.2

Four-methylene carbon at δ 22.98, 23.79, 28.95 and 30.4 4

One methylene carbon that is attached with oxygen at δ 68.18

Four methine carbon at δ 38.78, 128.84, 128.80, and 130.5 4 &

Two quaternary carbons at δ 167.73 and 132.5

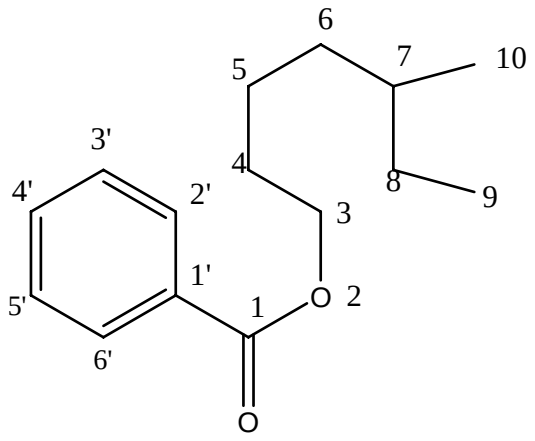
Table-4

^{13}CNMR & DEPT Spectra of VAM-1

Carbon No.	^{13}C (PPM)	DEPT	Remark
1	167.73	-	Quaternary carbon
2	-	-	
3	68.18	CH_2	
4	23.79	CH_2	
5	22.98	CH_2	
6	30.4	CH_2	
7	38.78	CH	
8	28.95	CH_2	
9	10.96	CH_3	
10	14.02	CH_3	
1’	1325	-	Quaternary carbon
2’	128.84	CH	
3’,5’	128.80	CH	
4’,6’	130.5	CH	

The 13C NMR Spectrum (appendix-4, table-4) of VAM-1 shows well resolved resonance of 13 carbon atoms, two carbon atoms signals at δ128.84 and 128.80 overlapped.

The DEPT shows the presence of five-methylene group, one is bonded to oxygen, four methine, tow quaternary carbon atoms & two methyl groups. The ^{13}C NMR spectrum of VAM-1, indicate the presence of aromatic ring.



The proposed structure of VAM-1.

The spectra of VAM-5 are the same to the spectra of VAM-1 and represent the same compound (appendices -6, 7, 8, & 9)

5. EXPERIMENTAL

5.1. PLANT MALARIAL

The leaves of V. amygdalina were collected form Addis Ababa in April 2006. After collection the plant material was air-dried & then extracted.

5.2. MATERIAL

UV spectrum was measured with GENESY'S spectrometer (200-400) in CH_3OH at room temperature

IR spectrum was recorded as KCl pellets on Perk-Elmer BX Infrared

Spectrometer in the range 4000-400 cm^{-1}

HNMR, ^{13}C NMR spectra were recorded on a Bruker advance 400 MHz spectrometer with TMS as internal standard.

Analytical TLC was done on 0.2 mm thick layer silica gel on aluminium card detection was done using vanillin/sulphuric acid for terpennoids and KOH for flavonoids

Column chromatography was carried using silica gel 60(Merck)

5.3. EXTRACTIONS AND ISOLATION

The powder of air dried leaves of vernonia amygadlina (500 gms.) were extracted with petroleum ether followed by extraction with chloroform (13 gms.) & then the marc after chloroform extraction was extracted with methanol (44 gms.) at room temperature.

The solvents were evaporated using roatary vapor

20 gms. of the methanol extract was subjected to CC gradient elution with hexane/ chloroform & chloroform/ methanol to give 28 fractions

Fraction 23 (chloroform/ methanol) (10:90) was subjected to repeated CC elution with chloroform methanol (60:40) afford compound VA-5 (table 5)

Table- 5

Fraction	Solvent	Ratio	Code
1	$CHCl_3$	100%	
2	,,	”	
3	$CHCl_3/CH_3OH$	9:1	
4	”	8.5:1.5	
5	”	8:2	
6	”	7.5:2.5	
7	”	7:3	
8	”	6:4	VA-5
9	”	5:5	

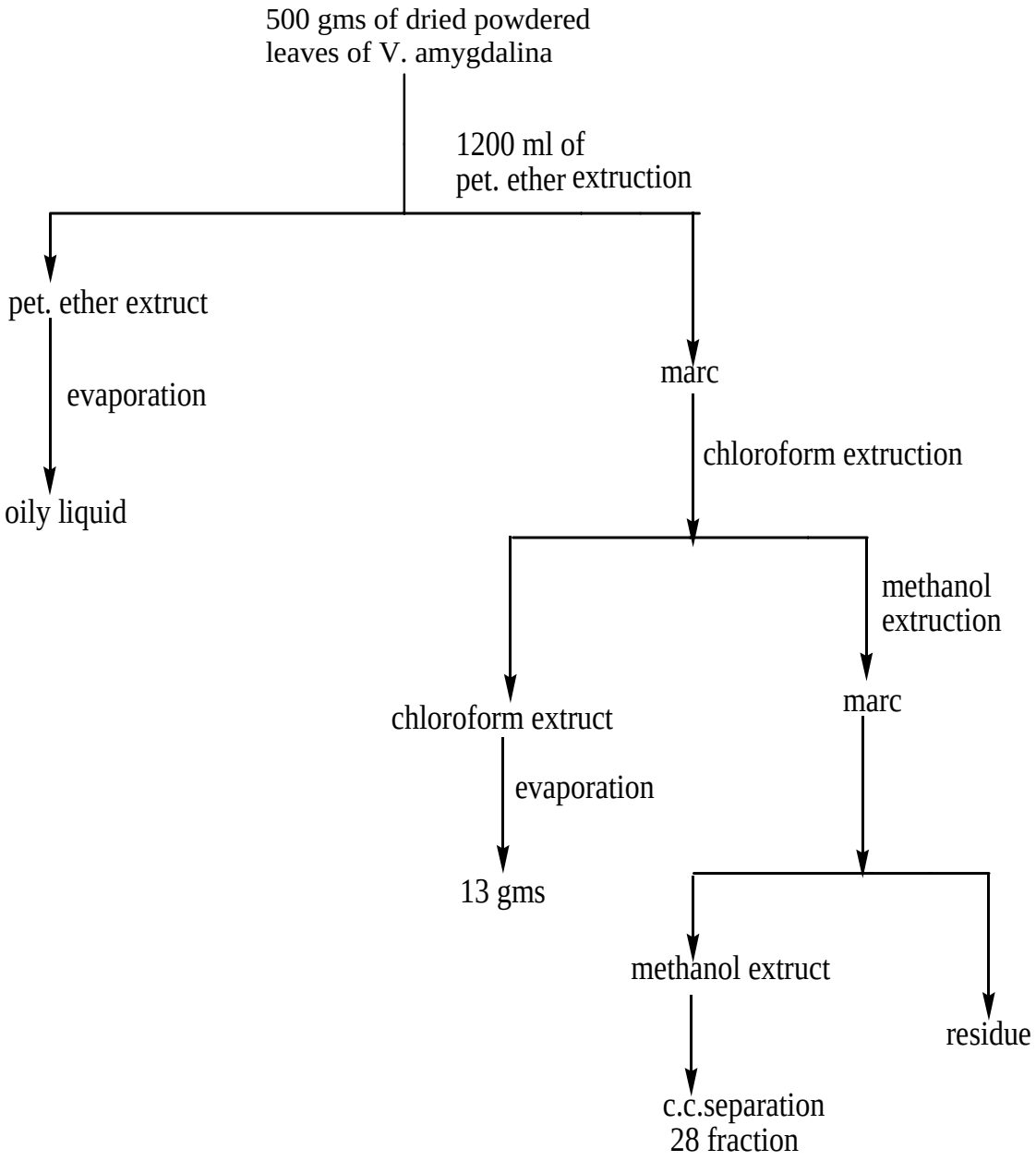
Fraction 5 ($CHCL_3/CH_3OH$) (8:2) was further eluted with dichloromethane/methanol (9.5:0.5) & (8.5:1.5) affords compounds VAM-1 and VAM-3 respectively table 6

Table- 6

Fraction	Solvent	Ratio	Code
1	CH_2Cl_2	100%	
2	”	”	
3	CH_2Cl_2/CH_3OH	9.5: 0.5	VAM-1
4	”	9:1	
5	”	8.5:1.5	VAM-3
6	”	8:2	
7	”	7.5:2.5	

Fraction 25 (100% methanol) was also chromatographed using a solvent system dichloromethane/methanol and a total of 10 fraction was collected, the spectra of the fraction collected with a solvent dichloromethane/ methanol (7:3) was not pure to characterize (appendices 10 and 11)

5.4. Out line of the extraction



6. CONCLUSION

The TLC test of the fraction of 100% methanol extract indicate the presence of flavonoid but due to lack of preparative tlc isolation of pure sample couldn’t be achieved.

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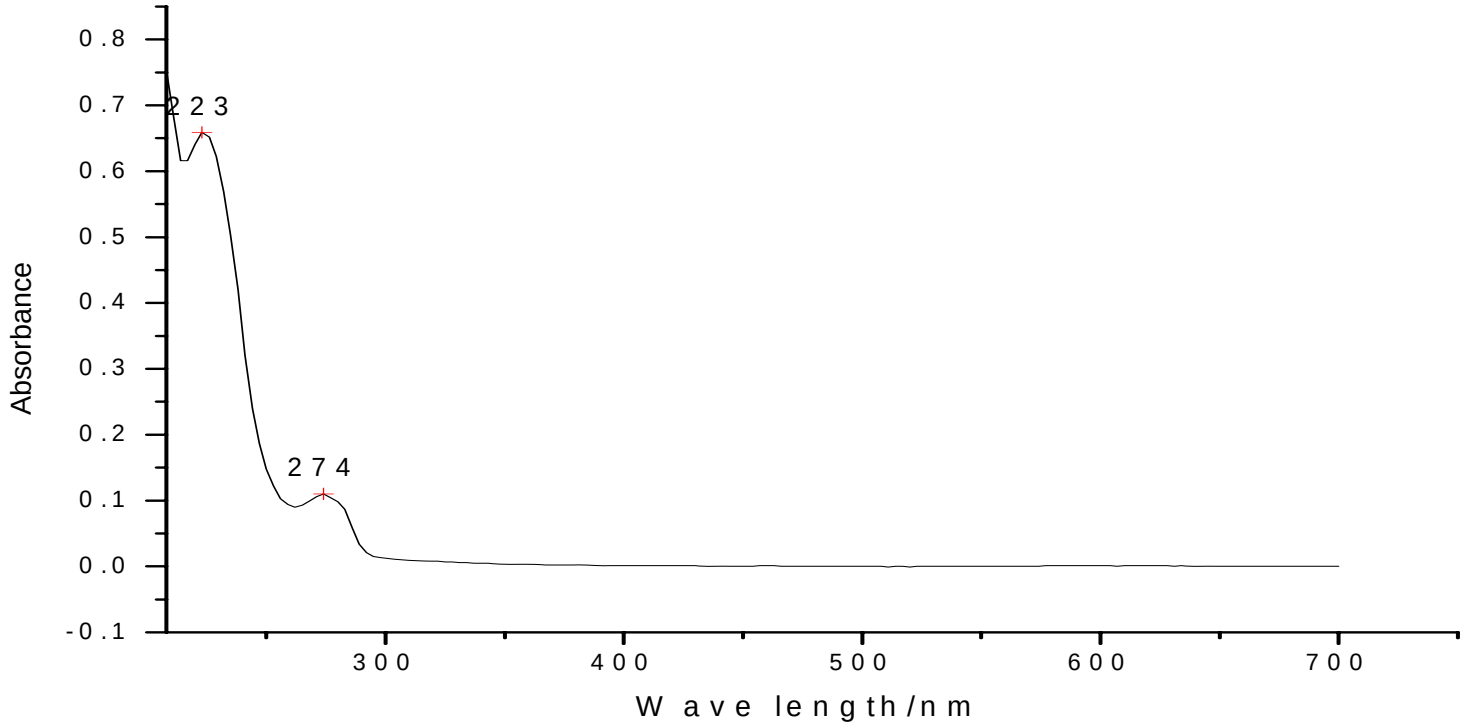
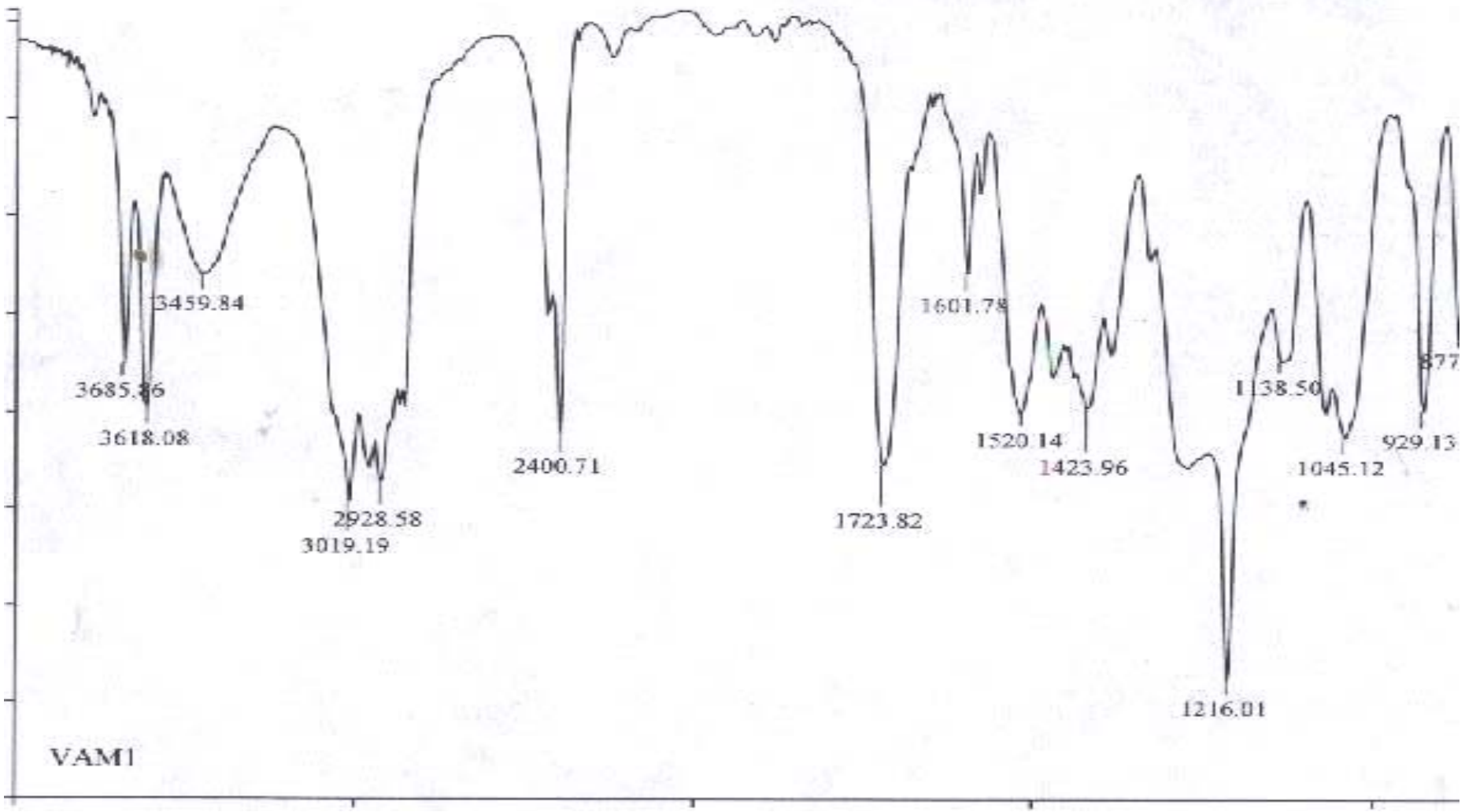
Appendices

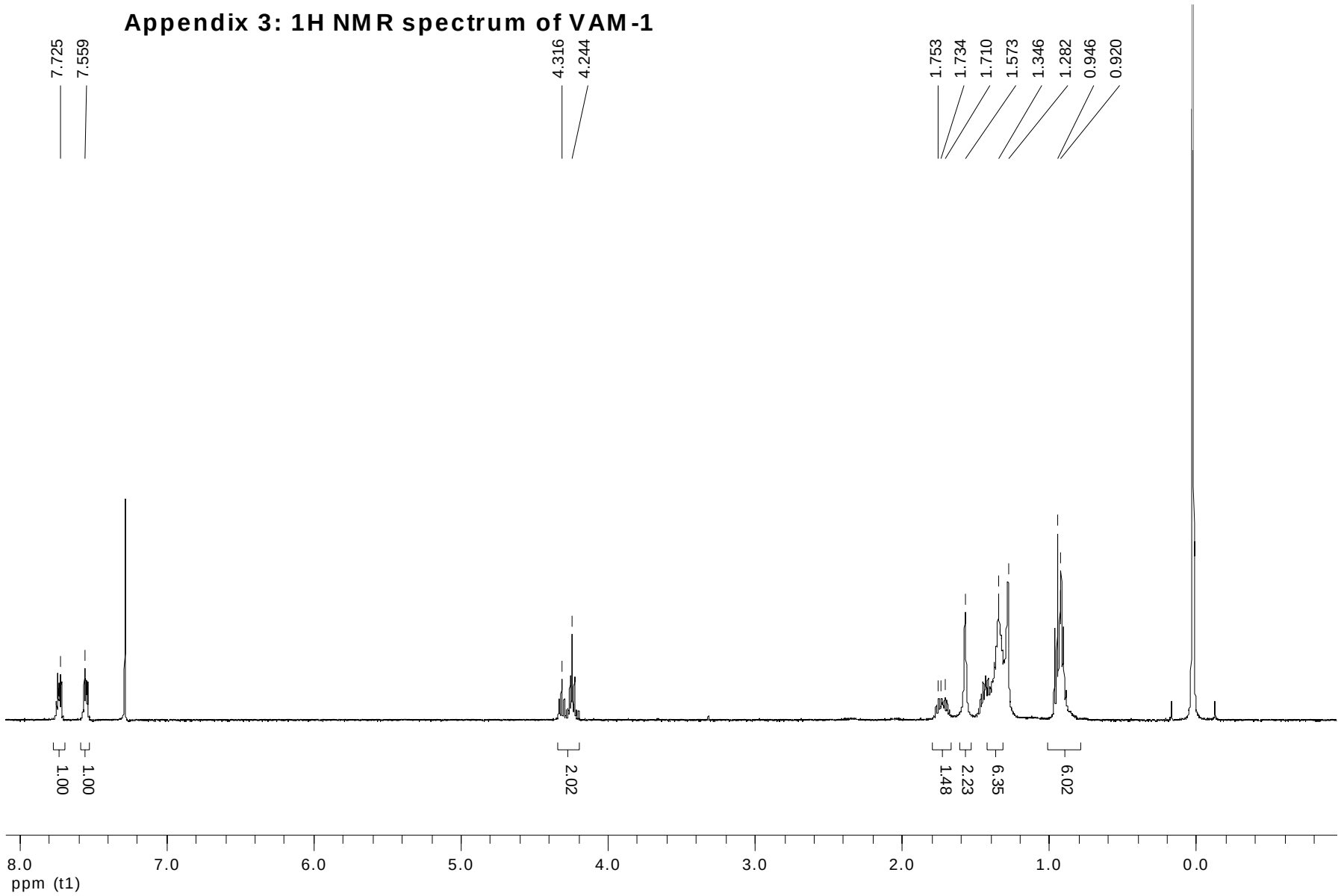
Appendix 1 VAM-1



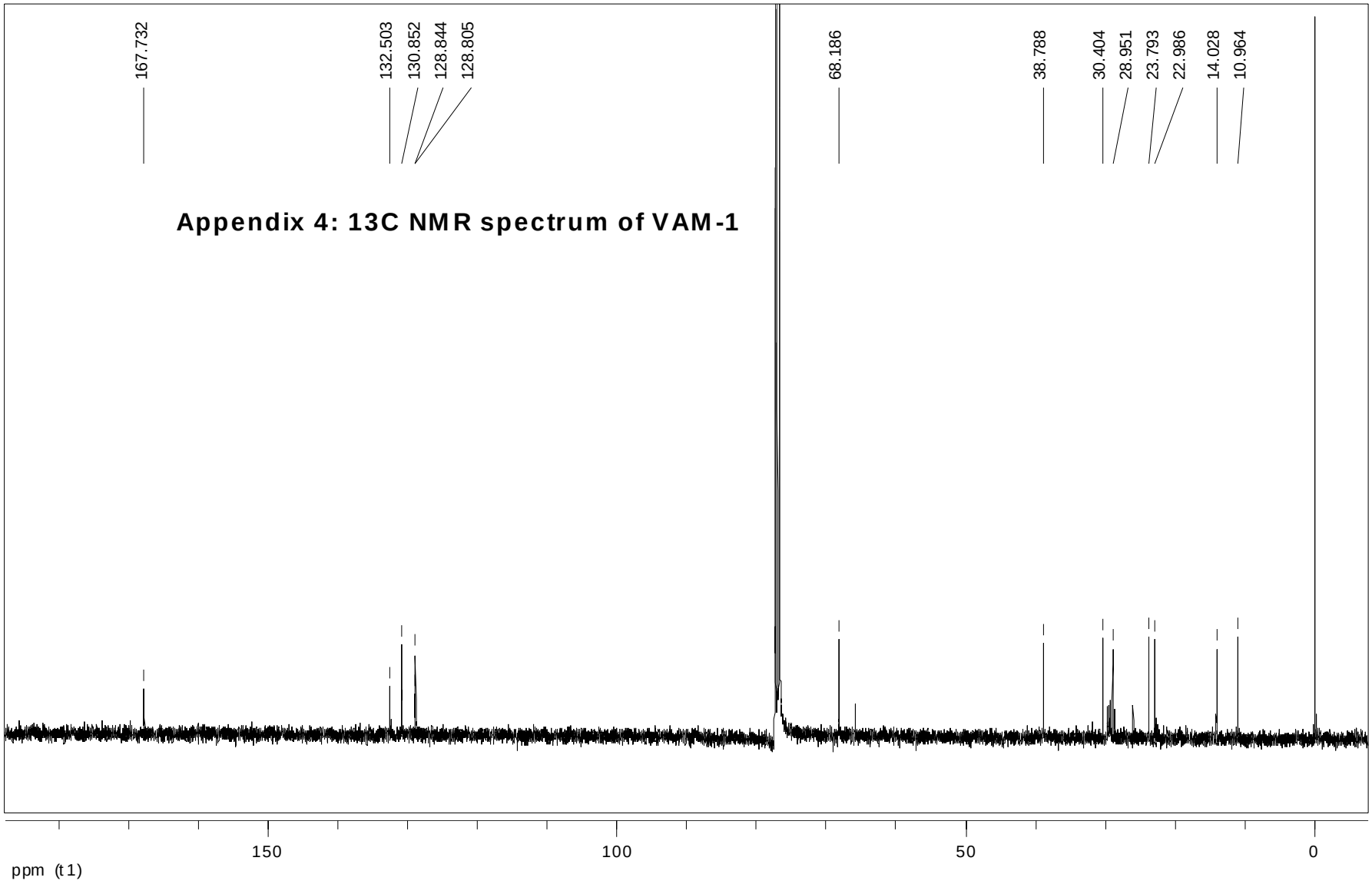
Appendix 2: IR spectrum of VAM-1

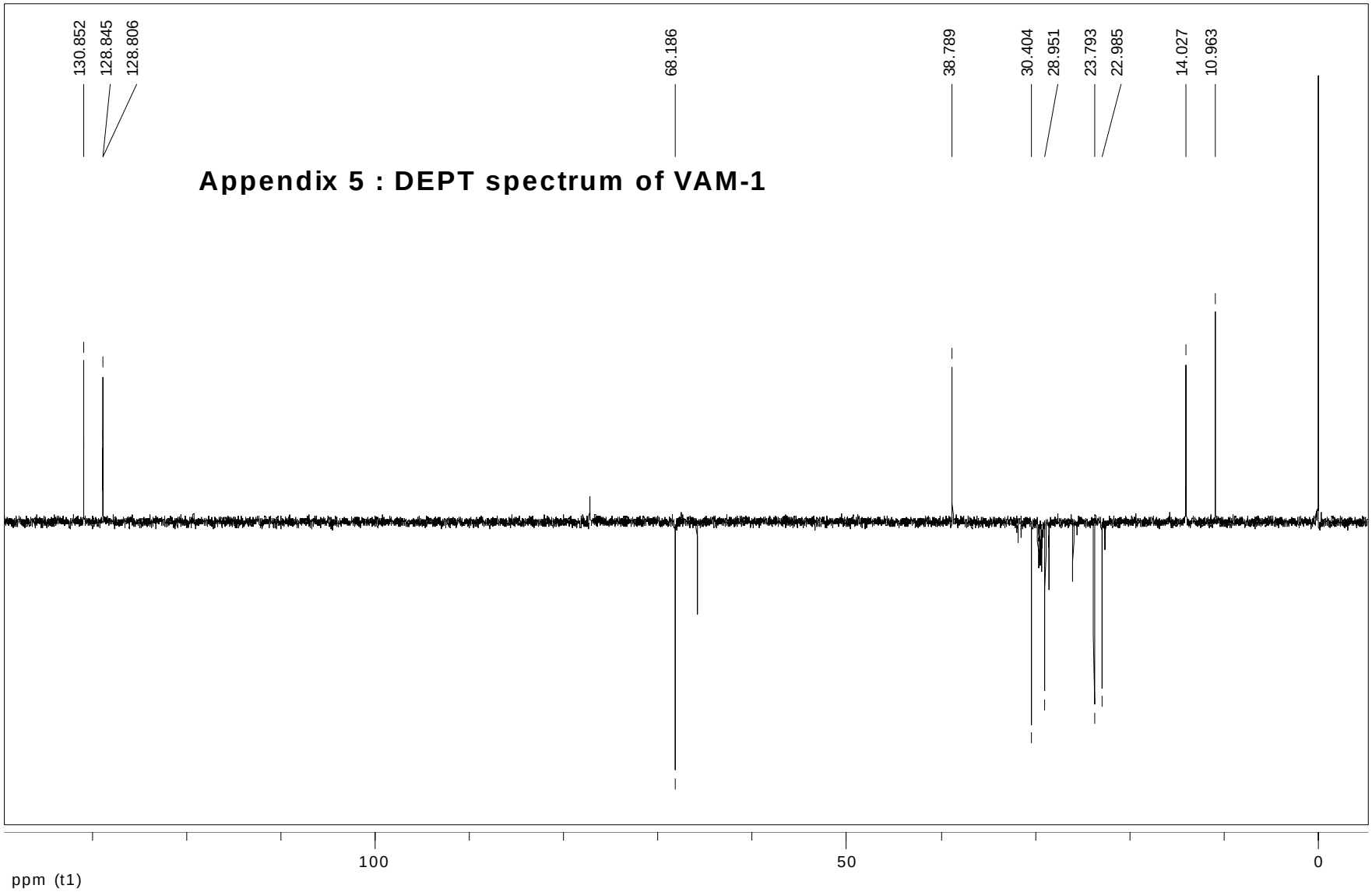


Appendix 3: 1H NMR spectrum of VAM-1



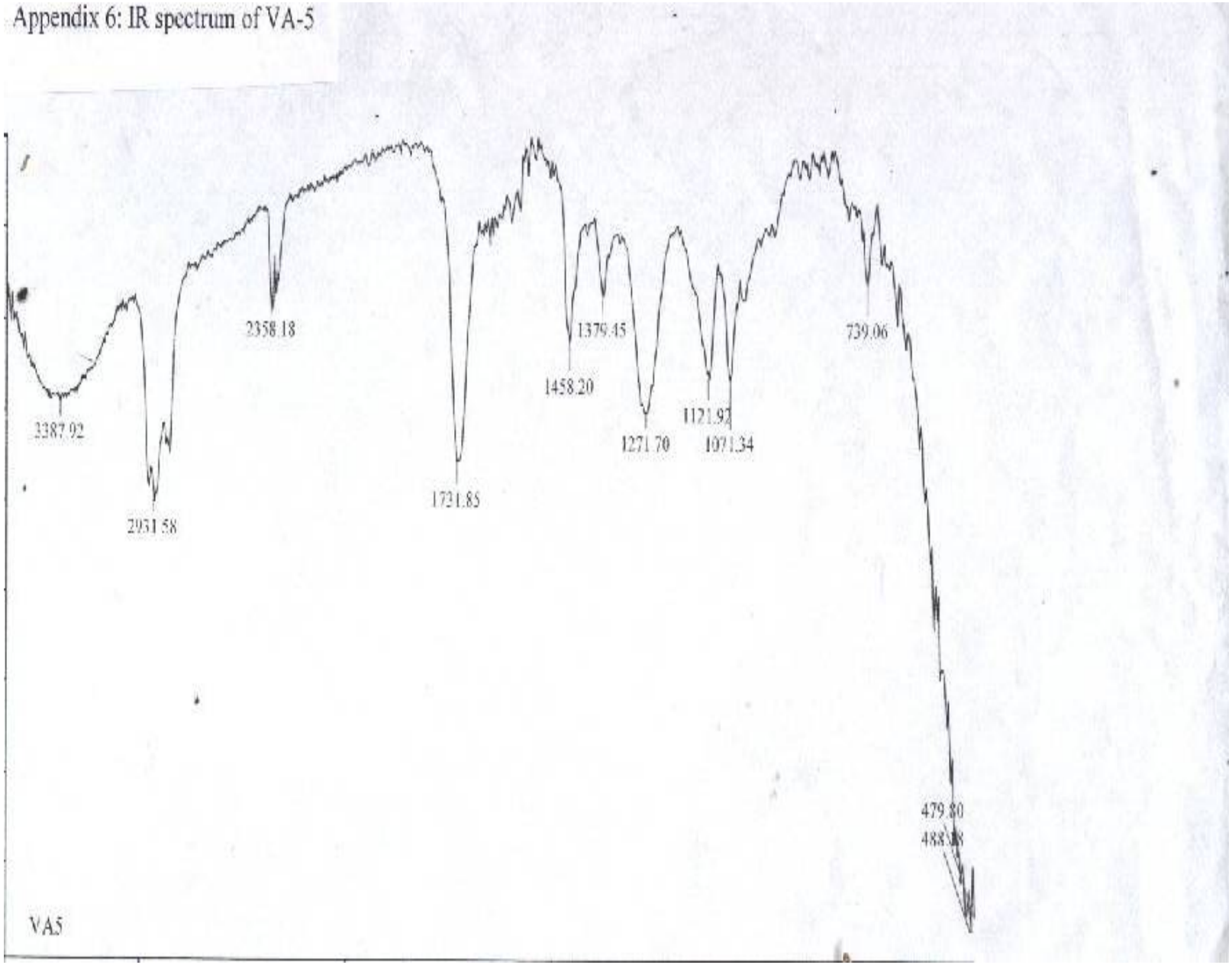
Appendix 4: 13C NMR spectrum of VAM-1

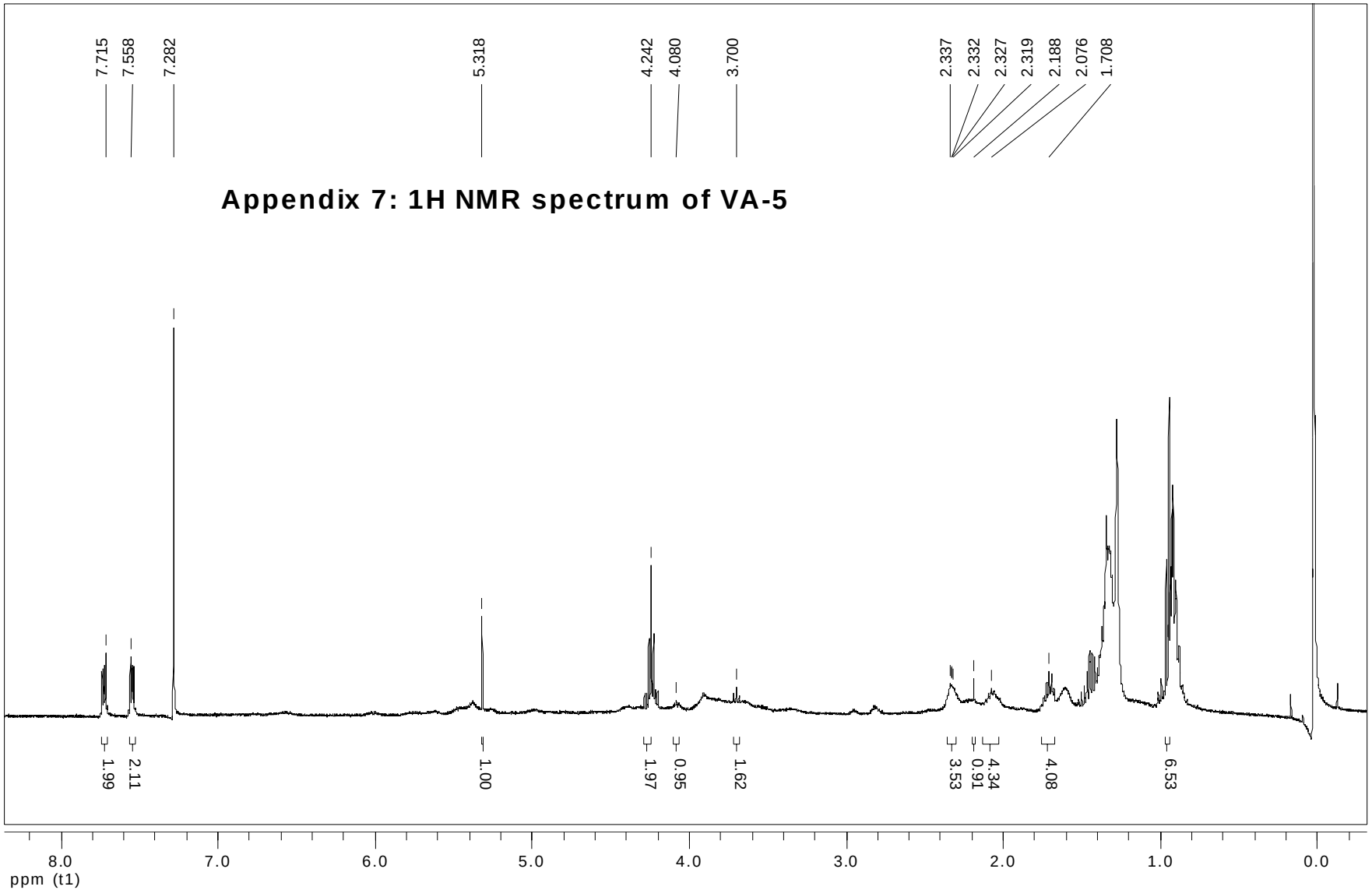




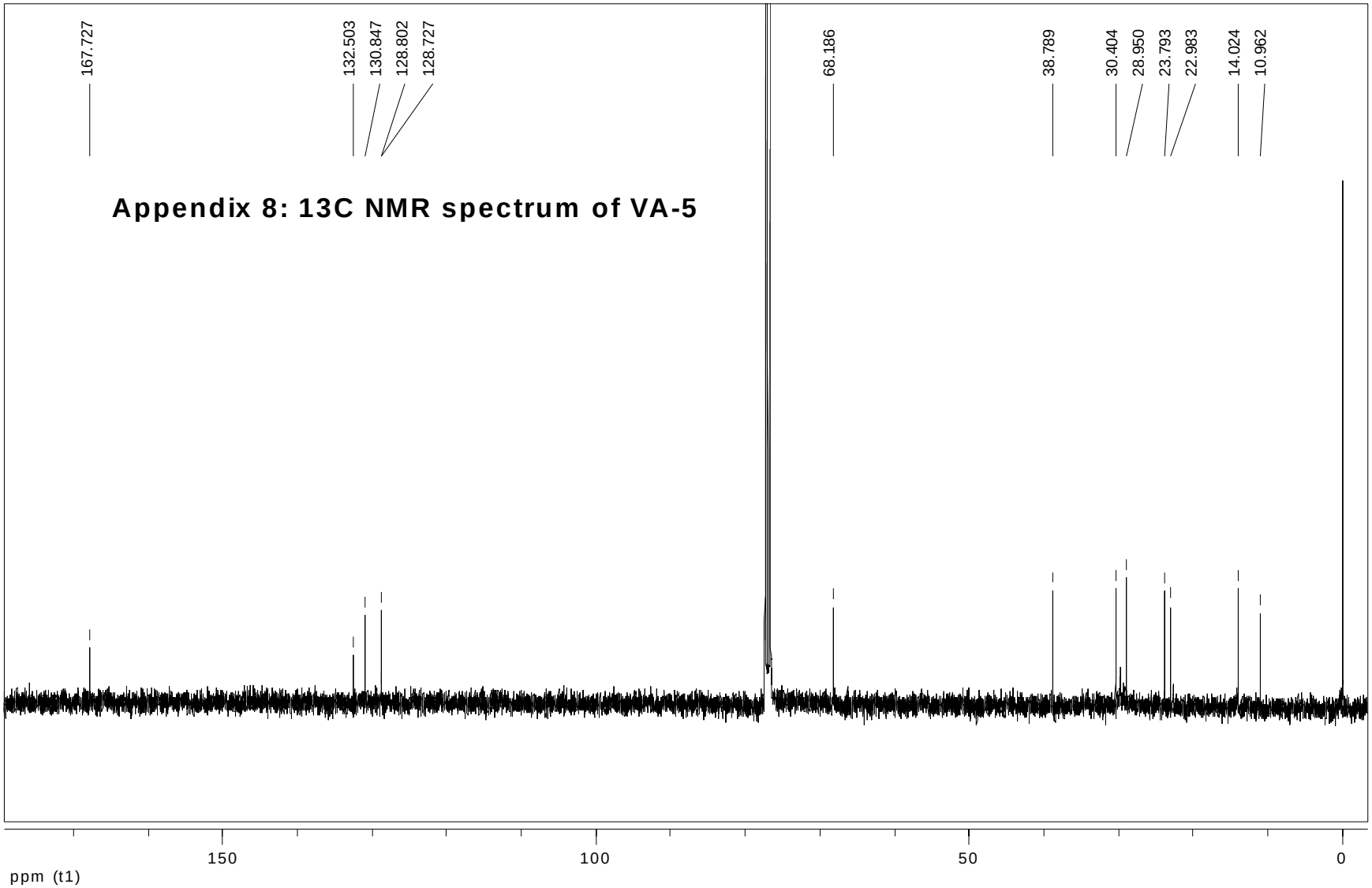
Appendix 5 : DEPT spectrum of VAM-1

Appendix 6: IR spectrum of VA-5

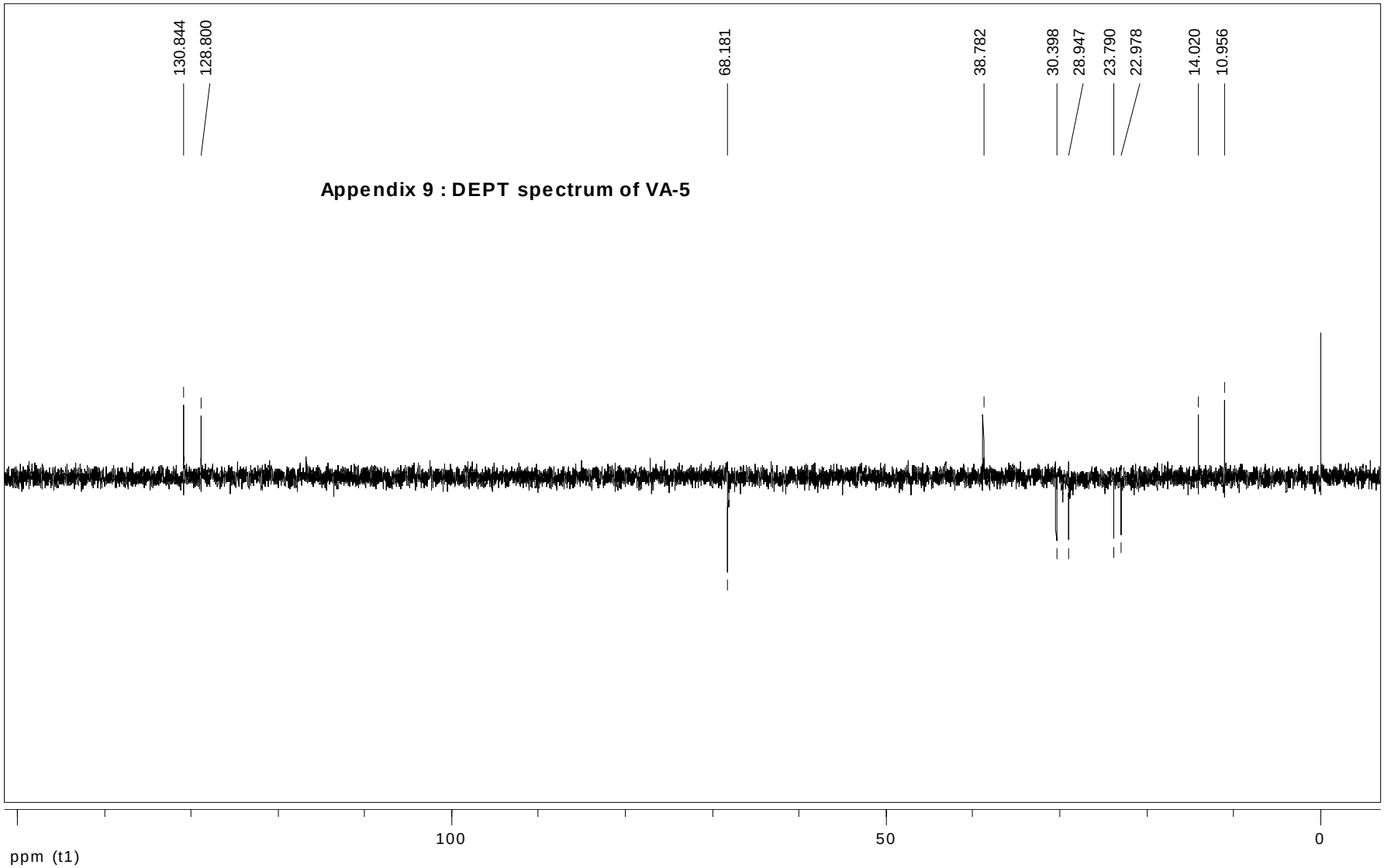




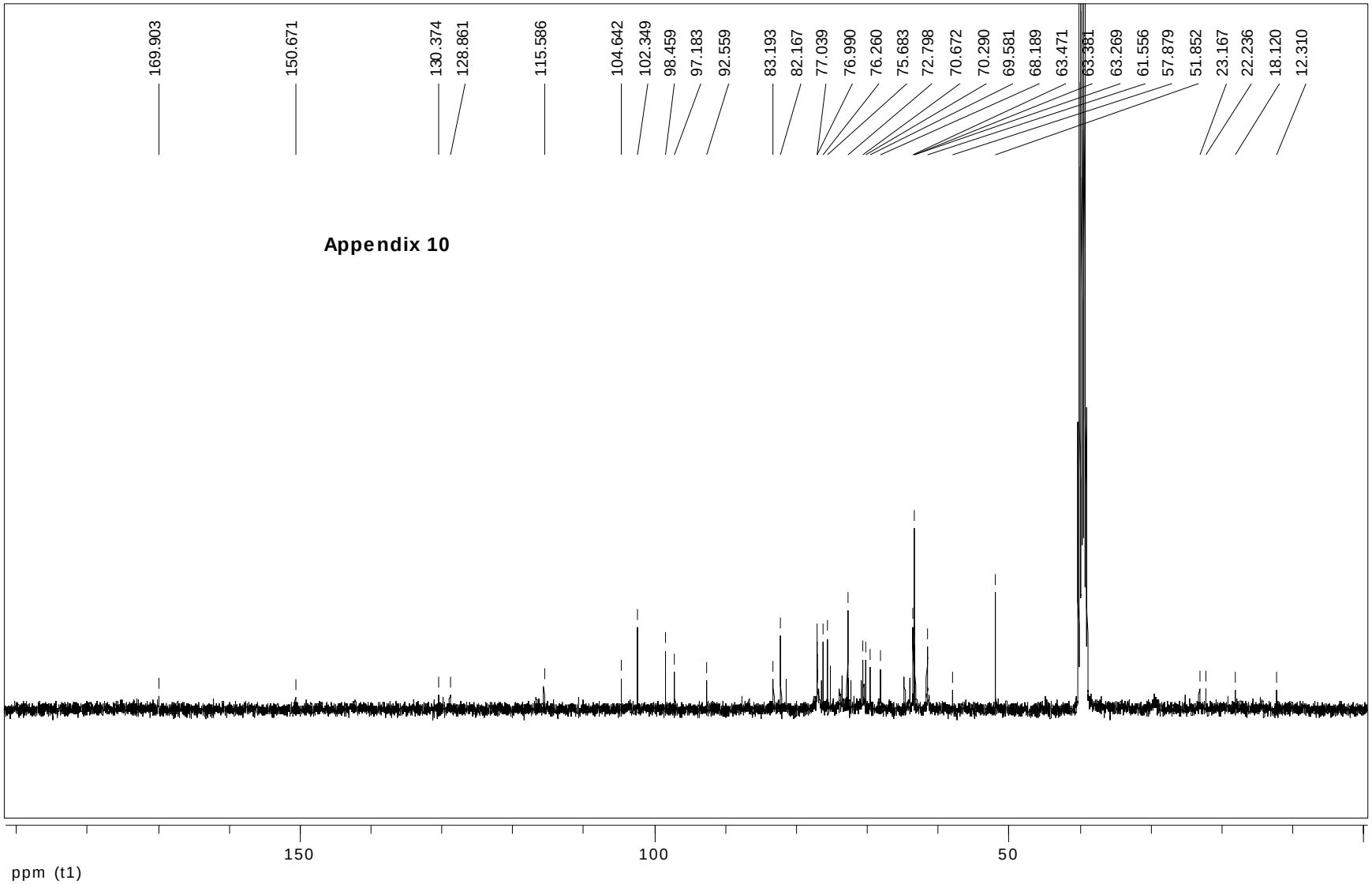
Appendix 7: 1H NMR spectrum of VA-5

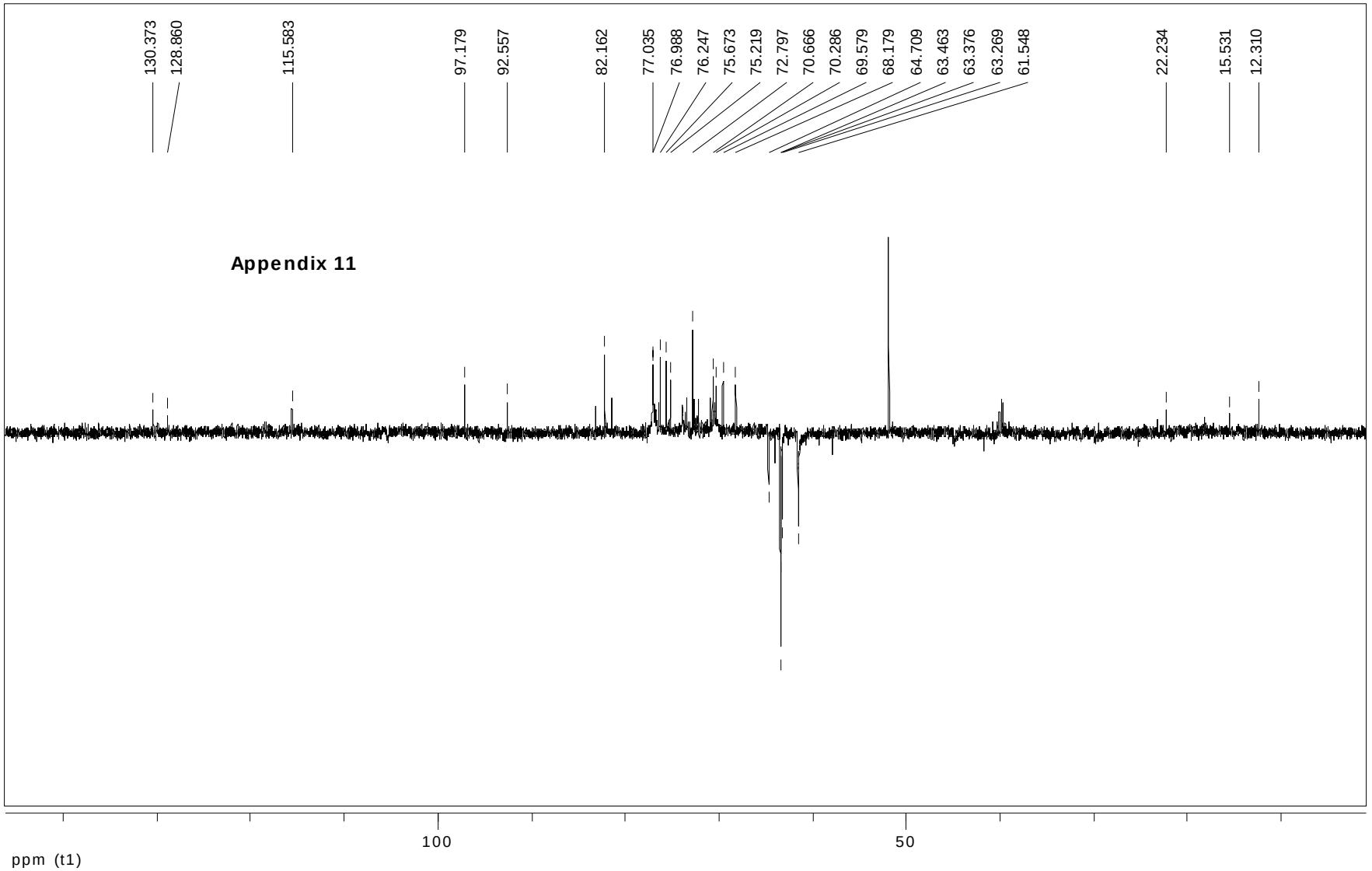


Appendix 8: 13C NMR spectrum of VA-5



Appendix 9 : DEPT spectrum of VA-5


169.903
150.671
130.374
128.861
115.586
104.642
102.349
98.459
97.183
92.559
83.193
82.167
77.039
76.990
76.260
75.683
72.798
70.672
70.290
69.581
68.189
63.471
63.381
63.269
61.556
57.879
51.852
23.167
22.236
18.120
12.310
Appendix 10
150
100
50
ppm (t1)


130.373
128.860
115.583
97.179
92.557
82.162
77.035
76.988
76.247
75.673
75.219
72.797
70.666
70.286
69.579
68.179
64.709
63.463
63.376
63.269
61.548
22.234
15.531
12.310
Appendix 11
100
50
ppm (t1)