ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

PHYTOCHEMICAL INVESTIGATIONS ON THE LEAVES OF *ANTIDESMA LACINIATUM*

BY

AYELE TESHOME

JULY, 2005

PHYTOCHEMICAL INVESTIGATIONS ON THE LEAVES OF *ANTIDESMA LACINIATUM*

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ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

PHYTOCHEMICAL INVESTIGATION ON THE FLOWERS OF SENNA DIDYMOBOTRYA

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DECLARATION

I declare that this thesis is my original work and has not been presented for any degree in this or other university and that all sources of materials used for this thesis have been duly acknowledged.

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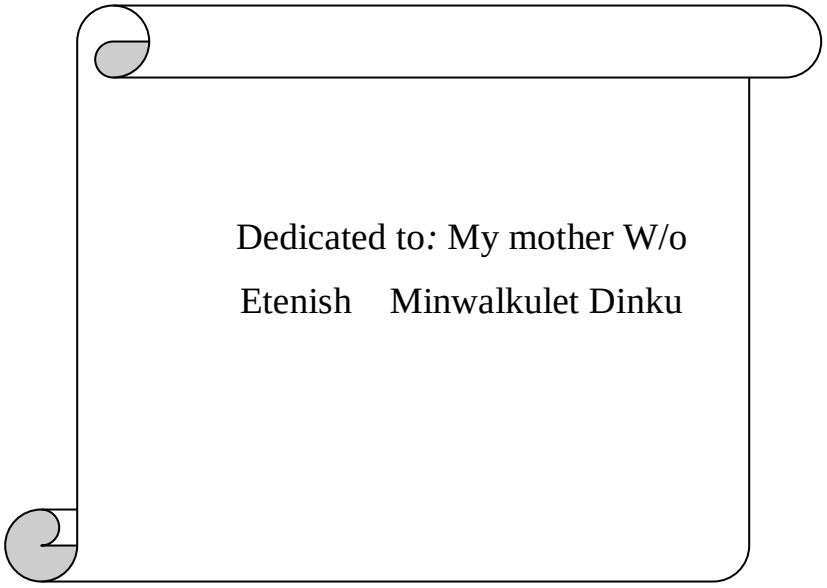
Dedicated to: My mother W/o
Etenish Minwalkulet Dinku

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ABSTRACT

Four known Compounds were identified from the leaves of *Antidesma laciniatum* namely, squalene (**37**), phyt-en-2-ol (**38**), β-sitosterol (**32)** and amentoflavone (**39**). The structures of these compounds were determined on the basis of spectroscopic data. Occurrence of squalene, phyt-en-2-ol and amentoflavone from the genus *Antidesma* is reported here for the first time.

1.0 INTRODUCTION

1.1 The Euphorbiaceae family

The Euphorbiaceae with 300 genera and 5000 species is the sixth largest family among the flowering plants (after the Orchidaceae, Compositae, Leguminosae, Gramineae and Rubiaceae). In East Africa the family comprises 77 genera and some 510 species (Gilbert, 1995; Smith, 1988; Susan & Smith, 1988).

1.2 The Genus *Antidesma*

Antidesma is one of the genera, which belongs to the Euphorbiaceae family and includes about 170 species. The word *Antidesma* was derived from the Greek *anti* (against) and the Burmese term *desma* (poison); an oblique reference to its supposed use as anti-venom. It is a relatively homogeneous genus of dioecious shrubs and trees in the tropics of the old world (Susan & Smith, 1988; Gilbert, 1995). Within the subfamily Phyllanthoideae of the Euphorbiace it belongs to the tribe Antidesmeae, subtribe Antidesminae (Buske *et al*. 2002). The subtribe Antidesminae also includes the genera *Hyeronima* with about twenty species from Americas, *Thecacoris* with about twenty species from Africa and Madagascar, *Leptonema* with two species from Madagascar, *Phyllanoa* and *Celianella* both monotypic from South America. (Buske *et al*. 2002).

1.3 African *Antidesma* species

Out of 170 *Antidesma* species in the world, less than ten species are found in Africa. Some of them are *A. venosum*, *A. laciniatum*, *A. chevalieri*, *A. membranaceum*, *A. madagascariensis* and *A. vogelianum* (Gilbert, 1995; Smith, 1988; Susan & Smith, 1988).

A. laciniatum Muell. Arg. is a small forest tree, which grows in Ivory Coast, Nigeria, Cameroon, Equatorial Guinea and Congo. The powdered bark is used as

an aphrodisiac. The essential oil of A. *laciniatum* has been found to be active against strain W2 of *Plasmodium falciparum* in culture (Boyom *et al.*, 2003; Susan and Smith, 1988). The essential oil obtained from leaves of *A. laciniatum* var. *laciniatum* collected in Cameroon was analyzed by GC and GC-MS and was found to contain terpenoids (72%), with a relatively high amount of ester derivatives (40.9%). The two major constituents were found to be esters: benzyl benzoate (19.1%), which is responsible for the sweet balsamic odor of the oil, and geranyl acetate (14.9%) (Website Reference 1).

A. venosum is a shrub or small evergreen tree, sometimes straggling, 1-9(-15) m tall. It has many or single-stemmed and sometimes with drooping branches. The bark is fibrous, fairly smooth to lightly fissured and gray-brown. Its wood is hard and has a white or pale brown coloration. The leaves are 3-7(10) mm long. It is distributed in Africa from the Gambia eastwards to Ethiopia and south to Namibia and South Africa (Susan and Smith, 1988; Gilbert, 1995; Website Reference 2).

Female flower **Leaf**

Figure 1: Flowers and leaves of *A. venosum*. (Website Reference 3).

A. membranaceum is a shrub or small tree occurring in the rain forests of tropical Africa including Senegal eastwards to the Sudan and south to Mozambique, Zimbabwe and South Africa. It is very similar to *A. venosum*, but differing in

having the leaves more markedly and acutely acuminate, with rather less dense indumentum, and in having smaller fruits (3-5 mm long when dry) (Susan & Smith, 1988; Buske *et al.*, 2001).

1.4 Use of *Antidesma* species in traditional medicine

Some *Antidesma* species were used since a long time in traditional medicine. *A. bunius*; native in Southeast Asia, contains alkaloids in the bark and leaves which is medicinal, but also reported to be poisonous. The bark is astringent and styptics for wounds, the leaves are acidic. Leaves and fruits treat hypertension, heartache, anemia, gonorrhea and syphilis. In Europe leaves of *A. bunius* were used as diaphoretic drug (Website Reference 4).

In Africa up to 80% of the population depend on traditional medicine for their primary health care needs. For example in Madagascar *Antidesma madagascariensis* is used as drug active against *Pseudomonas aeruginosa*, and *Salmonella typhimerium*. These data correlate well with traditional use as it is used to wash boils, and appears to accelerate the healing process (Website Reference 5).

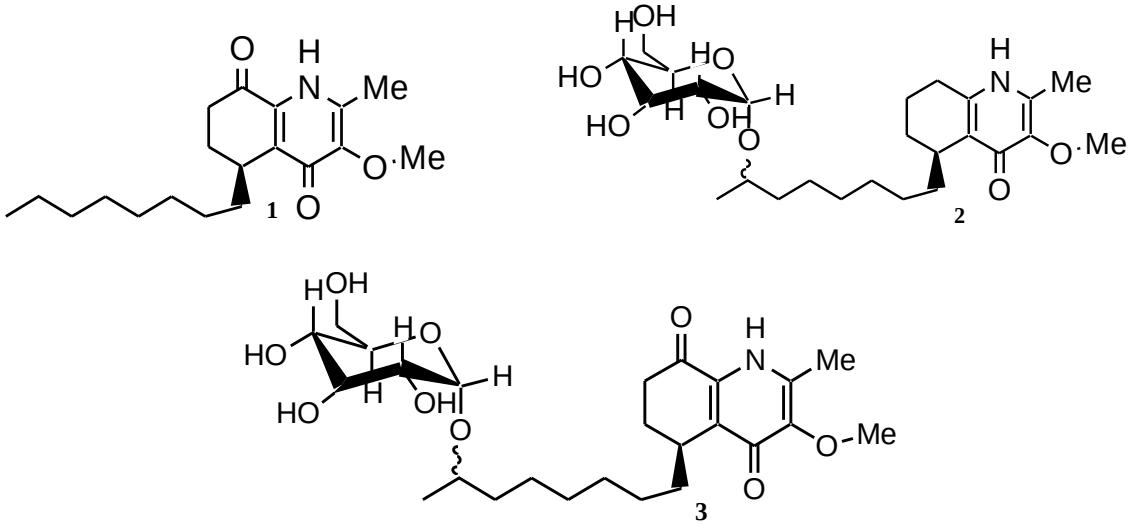
All parts of *A. venosum* are used to treat rheumarthritis. The wood is used for firewood, tool handles and knife sheaths. The leaves and roots are chewed as a remedy for snake bites, stomach-ache and hook worm. Seeds are steeped in water and drunk for liver complaints. Roots are also known to be very bitter, emetic, poisonous and used as a fish poison. Fruits are sometimes eaten (Website Reference 2).

The wood of *A. membranaceum* is used for tool handles, wooden spoons, firewood and knife sheaths. Roots are used as medicine for pneumonia and as a tonic for

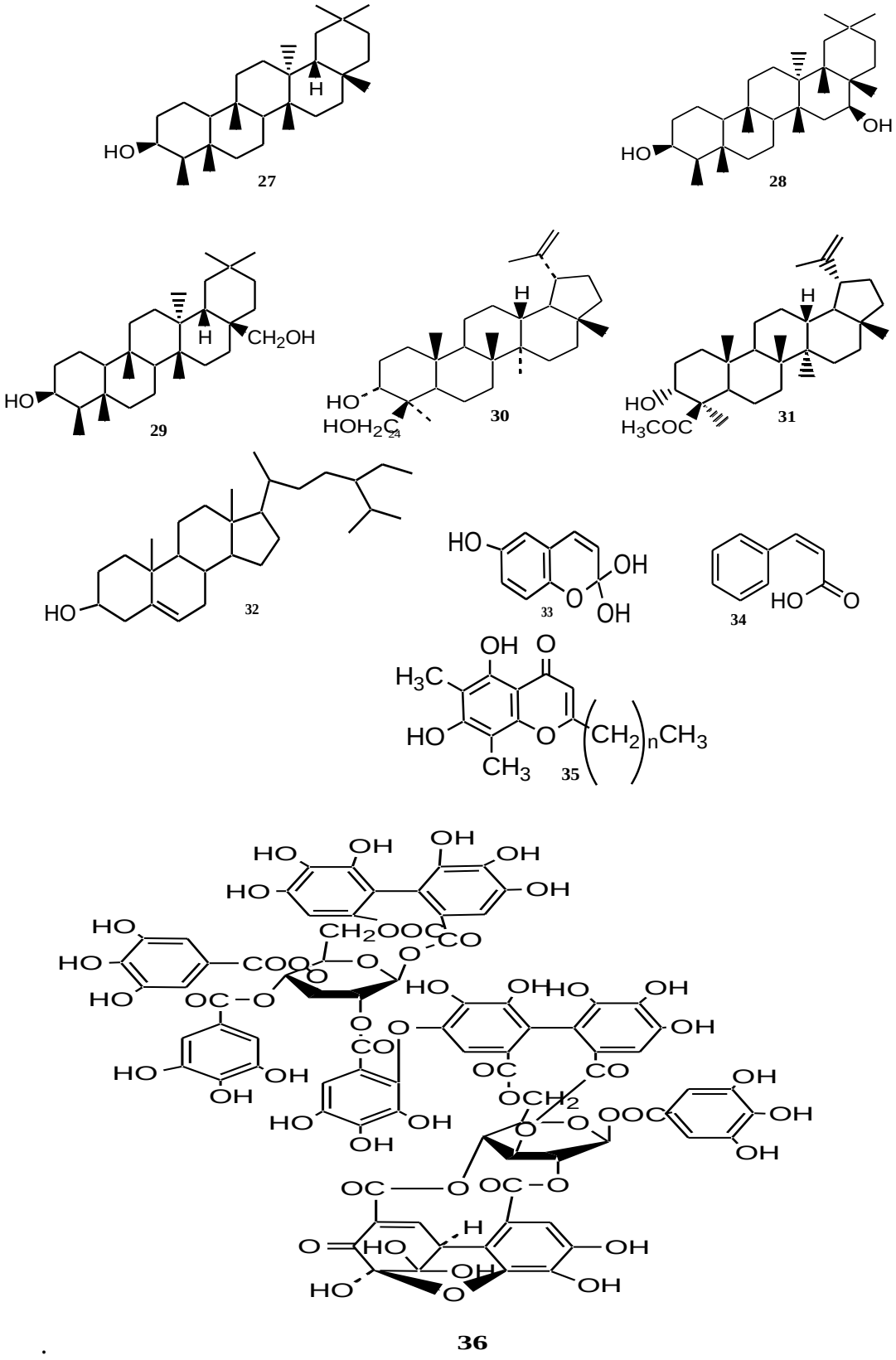
kwashiorkor. Roots are scraped and used to treat mouth ulcers in children. Leaves are used as medicine for stomachache and snakebites. The tree is used for shade (Website Reference 2).

1.5 Review of the chemistry of *Antidesma* species

Various investigations on the leaves and barks of *Antidesma* species resulted in the isolation and identification of different compounds. In the natural products isolation program by Buske *et al*. (1997), new substances such as antidesmone (**1**), 17β-D-glucopyranosyloxy-antidesmone (**2**) and 8-deoxo-17β-D-glucopyranosyloxy-antidesmone (**3**) were isolated from African *Antidesma* plants. In addition to the three substances, twenty-six compounds (Table 2) were isolated and structurally elucidated using different HPLC-MS and HPLC-NMR techniques (Buske *et al.,* 2002). Besides antidesmone and its derivatives, three lignan glucosides, four megastigmane glucosides, nine triterpenes, five phytosterols, seven flavonoid glucosides, and one biflavonoid, some of which were new compounds were also isolated. Some of the interesting compounds from *Antidesma* species are listed in Table 1 and 2.



Scheme-1: Previously isolated compounds from *Antidesma* species (for compounds **4**-**26,** refer Table 2 page 8).



. **36**

Scheme-1: Previously isolated compounds from *Antidesma* species.

Table 1: Previous Phytochemical investigations on *Antidesma* Species

Species	Compound Name	Compound No	References
A. montanum (Synoym: *A. acuminatum*, *A. pentandrum* and *A. menasu*)	◦ antidesmanol (3-keto-16α-hydroxy-friedelan) ◦ 3-Friedelanol ◦ canophyllol	**27** **28** **29**	Buske . *et al*.(2002)
A. geraniin and *A. carpusin*	◦ lupeolacetone ◦ two cyclopeptide alkaloids	**31** ---	Buske . *et al*. (2002)
A. tetrandrum	◦ two cyclopeptide alkaloids	---	Buske. *et al*. (2002)
A. diandrum	◦ Sitosterol	**32**	Buske. *et al*. (2002)
A. membranaceum	◦ antidesmone ◦ 2,2,6-Trihydroxy-2H-1-benzopyran ◦ 2-alkyl-5, 7-dihydroxychronomes ◦ 2-alkyl-2,5,7-trihydroxychromanones	**1** **33** **34** ---	Buske *et al*. (1999)
A. ghaesembilla	◦ cyanogenic substances ◦ no alkaloids	--- ---	Buske *et al*. (2002)
A. acuminatum	◦ derivatives of benzoic acid ◦ cinnamic acid	--- **35**	Buske. *et al*. (2002)
A. pentandrum. var. barbatum	◦ antidesmin A, ◦ aristolochic acid and ◦ derivatives of aristolochic acid	**36** --- ---	Buske. (2000)
A. pentandrum	◦ lupeolacetone	**31**	Buske (2000)
A. montanum	◦ cyclopeptide alkaloids	-	Buske (2000)
A. petandrum	◦ cyclopeptide alkaloids	-	Buske (2000)

1.5.1 Use of Antidesmone and its Derivatives

Antidesmone is a novel type of tetrahydroquinoline alkaloid and was isolated from *A. membranaceum* by Buske *et al*. (1999). Its structure was revised by Bringmann *et al* (2000b) from (5S)-1-hydroxy-4-methoxy-3-methyl-5-octyl-5,6,7,8-tetrahydroisoquinolin-8-one to (S)-4,8-dioxo-3-methoxy-2-methyl-5-n-octyl-1,4,5,6,7,8-hexahydroquinoline (**1**).

Antidesmone [**1**] and its derivatives (**4**-**26**) are highly potent against *Leishmania donovani* and *Trypanosoma cruzi*. Some derivatives show an up to ~1000- fold higher activity as the standard drug benznidazol used against *T. cruzi* and ~125-fold higher activity as the standard drug pentostam (*L. donovani*) (Buske *et al*., 2002).

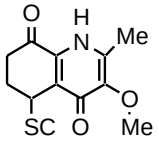
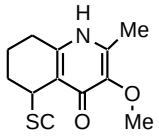
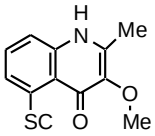
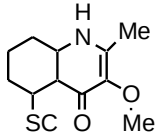
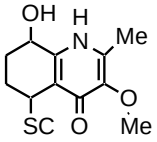
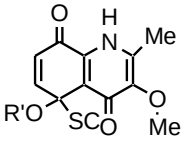
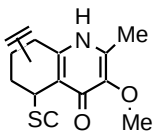
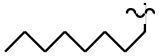
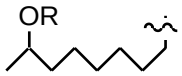
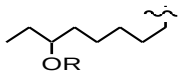
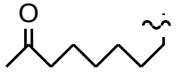
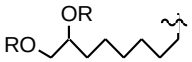
Furthermore, the compounds exhibit antihelmintic activity against the parasitic hookworms and roundworms *Nippostrongylus brasiliensis* and *Trichonelle spiralis*. The compounds are simple in structure and somewhat related to quinoid and quinoline type inhibitors of parasitic disulfide reductases. A cheap access appears possible also by synthesis. The biosynthesis of antidesmone has been studied in cell cultures by Bringmann *et al.* (2000a).

yields and distribution of antidesmone and its derivatives from herbarium material were recently published (Buske *et al*., 2002). The level of antidesmone in *Antidesma* species varies between 2 mg/kg and 65 mg/kg. These relatively low levels of antidesmone depend on a number of biotic and abiotic factors, i.e. the age of the organism or tissue, season or possible pathogenic infection. Furthermore, secondary metabolites can be degraded in time and sometimes herbarium specimens are soaked in alcohol upon collection to assist drying (Buske *et al*., 2002).

1.6 Objective of this work

The main objective of this project is to undertake phytochemical study on the leaves of *A. laciniatum*. Except for a report on the essential oil constituents of this species there is no prior report on the chemistry of the plant.

Table 1.2: Antidesmone and its derivatives from *Antidesma* species

SC=							
	**1**: antidesmone	**23**	**28**	**29**	**22**	**4**: R’ = H **5**: R’ = glc	
	**6**: R = H	**7**: R = H **2**: R = glc	**3**: R = glc		**8**: R= H	**11**: R = glc **10**: R= glc	**9**: R= glc R’ = H
	12: R= rhm **15**: R= glc-glc	**13**: R= glc-xyl			**14**: R= H		
	**16**: R= H **17**: R = glc						
	**26**						
		**18**: R = H		**19**: R = H			**20**: R= H, glc
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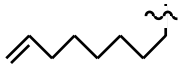
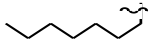
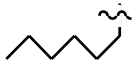
	**27**						
	**24**						
	**25**						

Sc = side chain; glc = glucose; rhm = rhamnose; xyl = xylose

2.0 RESULTS AND DISCUSSION

Six grams of the chloroform extract of *A. laciniatum* (leaves) were subjected to Flash Column Chromatography (FCC). Elution was performed with increasing polarity of the solvent starting from petrol to methanol (Table 8). The chloroform extract on TLC showed at least eleven spots upon spraying with 1% vanillin in sulfuric acid. Those fractions from the FCC that had similar retention factors were combined and further purified by Sephadex LH-20, chromatotron, Medium Liquid Pressure Chromatography (MPLC) Separo AB type apparatus, and preparative TLC. The following four compounds AL-1, AL-3, AL-3A, and AL-11 were isolated. The other expected compounds AL-2, AL-4, AL-5, AL-6, AL-7, AL-8, AL-9 and AL-10 were not isolated since they were present in small amounts. Details on the extraction and isolation of these four compounds are given in the Experimental Section.

2.1 Characterization of AL-1

AL-1 was obtained as colorless oil from Flash Column Chromatography (FCC) by petrol: chloroform (8:2) as eluting solvent. The mass spectrum (EIMS) exhibited a molecular ion peak (Appendix 1) at m/z 410.4 establishing the molecular formula as $C_{30}H_{50}$ (Scheme-2). Furthermore, the UV-Visible absorption band (Appendix 2) of AL-1 in chloroform was blank and its IR ($CHCl_3$) spectrum exhibited absorptions indicating the presence of double bond stretching (1670 cm^{-1}) and both bond stretching and bending of methyl and methylene carbon groups at 2967, 2925, and 2856 cm^{-1}.

The ^1H NMR spectrum (Appendix 3) of AL-1 in $CDCl_3$ showed a broad multiplet at δ 5.12 ppm integrating for three overlapping ethylenic protons, ten methine protons showing a broad multiplet at δ 2.0 ppm, sharp singlets at δ 1.6 and 1.5 integrating for three and nine protons respectively indicated the presence of four methyl groups. A comparison of the ^1H NMR chemical shift values of AL-1 with the previously

reported data of squalene [**37**] (Aldrich Library, 1992) (Table 3) showed almost the same value.

Table 3: ^{1}H NMR spectral data of compounds AL-1 compared with lit. data for squalene

AL-1			Squalene (Aldrich Library, 1992)		
δ_H	No of protons	Multiplicity	δ_H	No of protons	Multiplicity
5.12	3 overlaping ethylenic protons	multiplet	5.04	3 overlaping ethylenic	multiplet
2.01	10 CH_2 protons	multiplet	1.97	10 overlapping CH_2 protons	multiplet
1.69	3 methyl protons	singlet	1.60,	3 methyl protons	singlet
1.60	9 overlapping CH_3 protons	Singlet	1.52	9 overlapping CH_3 protons	singlet

The ^{13}C NMR data (Appendix 4) and DEPT-135 (Appendix 5) of AL-1 showed signals for four methyl carbons at δ 16.39, 16.43, 18.05 & 26.06 ppm, five methylene carbons at δ 27.08, 27.19, 28.68, 40.14 & 40.16 ppm, three vinylic methine carbons at δ 124.83, 124.73 & 124.70 ppm and three vinylic quaternary carbons at δ 131.56, 135.25, & 135.47 ppm). In the Heteronuclear Multiple Bond Correlations (HMBC) spectrum (Appendix 8) a strong correlation was observed between the three overlapping vinylic proton and the methyl and methylene carbons (Fig. 2). The HSQC spectrum (Appendix 7) showed these three overlapping vinylic protons (at δ 5.04 ppm) to be bonded with the three vinylic carbon atoms at δ 124.8, 124.7 and 124.7 ppm.

Its ^{13}C NMR data are virtually identical with those reported for squalene [**37**] (Aldrich Library, 1992). From the reported data of squalene and the current ^{1}H and ^{13}C spectroscopic data of AL-1 (Table 3 & Table 4) respectively, it was obvious that AL-1 is squalene. The HMBC and HSQC data of AL-1 confirm the proposed structure.

Squalene is widely found in nature and its biological source includes olives, palm oil and wheatgerm oil. However the largest concentration of squalene is from deep sea shark liver oil, from which it was first isolated [Torssell, G., 1997]. It is beneficial

for: **s**trengthening and supporting the immune system, as an anti-oxidant, improving skin complexion, to treat skin problem such as psoriasis and dermatitis and also as a general health tonic (Website Reference 6).

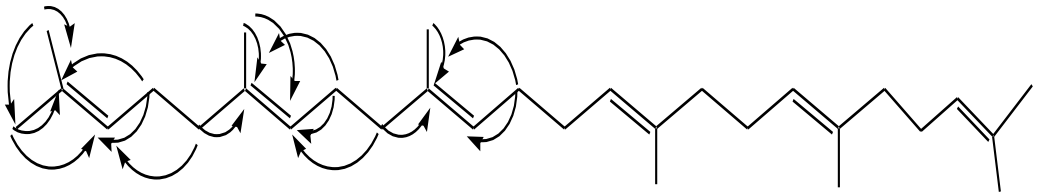
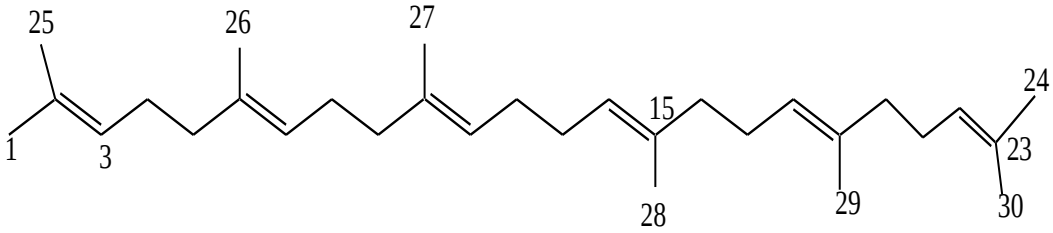


Fig. 2: Selected Heteronuclear Multiple Bond Correlations (HMBC) for compound AL-1. Arrows point from Proton to carbon.

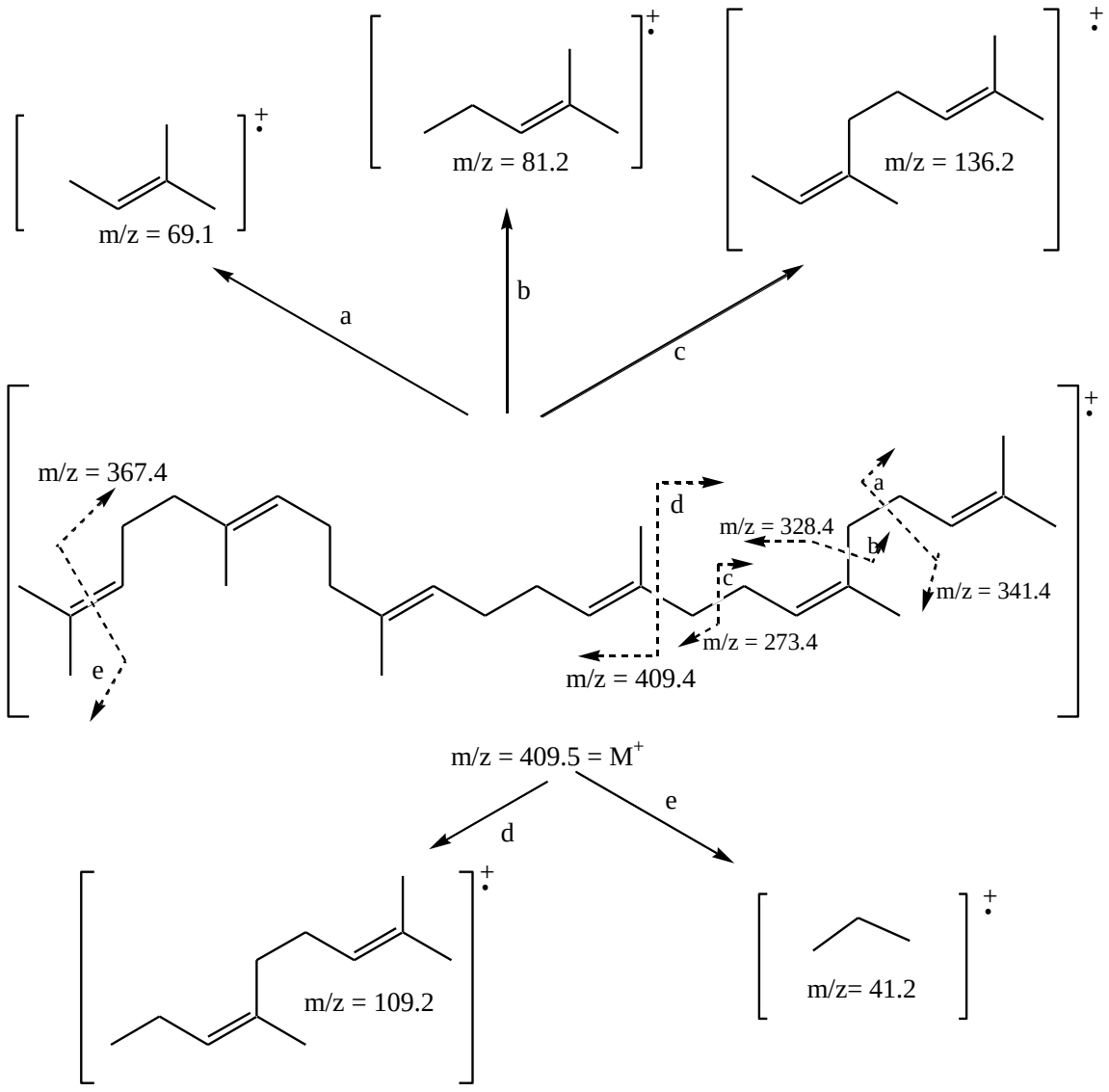


Structure of squalene (**37**)

Table 4: ^{13}C NMR spectral data of compounds AL-1 compared with lit. data for squalene

Carbon No	δ_C of AL-1 (87-191C)	δ_C of squalene (Aldrich)
1(24)	18.1	17.66
2(23)	131.6	131.12
3(22)	124.8[a]	124.39
4(21)	28.7	28.29
5(20)	27.1[e]	26.80
6(19)	135.3[c]	134.81
7(18)	124.7[a]	124.29
8(17)	40.2[d]	39.75
9(16)	27.2[e]	26.69
10(15)	135.5[c]	135.02
11(14)	124.7[a]	124.26
12(13)	40.1[d]	39.75
25(30)	26.1	25.67
26(29)	16.4[b]	16.04
27(28)	16.4[b]	16.00

a, b, c, d & e: These values may be interchanged (a with a, b with b, c with c, d with d & e with e)



Scheme-2: Major MS fragmentation patterns in compound AL-1.

2.2 Characterization of AL-3

AL-3 was obtained as colorless oil with retention factor (Rf) value of 0.6 in petrol: EtOAc (80:20) on TLC. The IR ($CHCl_3$) spectrum (Appendix 9) showed a broad absorption band at 3435 cm^{-1} indicating the presence of hydroxyl functional group. The weak absorption band at 1666 cm^{-1} is due to double bond stretching. A strong

absorption band at 1215 cm^{-1} indicated the presence of carbon oxygen bond stretching.

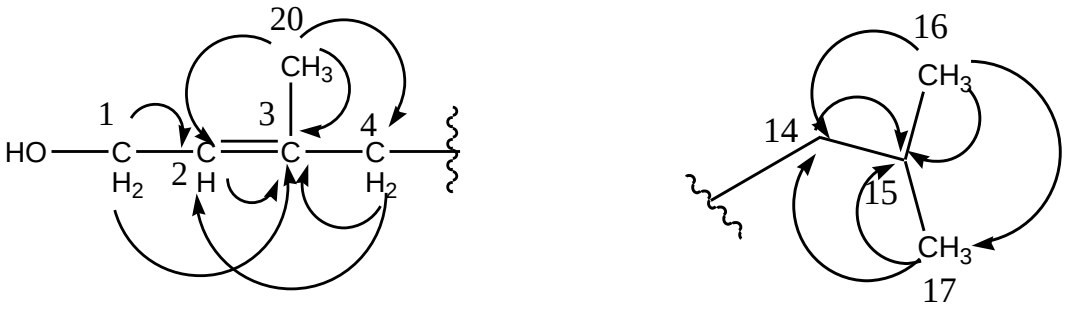
The ^{1}H NMR spectrum (Appendix 10) of AL-3 in $CDCl_3$ showed a triplet at δ 5.30 ppm integrating for one vinylic proton, two protons doublet at δ 4.04 ppm corresponding to protons attached to a carbon bearing an electronegative atom such as oxygen. Methyl proton signals at δ 0.76 and 0.74 ppm (12H, d, overlapping) and at δ 1.56 ppm (3H, s) indicate the presence of five methyl groups. The singlet peak at δ 1.56 ppm is indicative of its attachment to a vinylic quaternary carbon.

The ^{13}C NMR spectrum (Appendix 11) and the DEPT-135 spectrum (Appendix 12) in $CDCl_3$ confirmed the presence of twenty carbon atoms of which, five methyl, ten methylenes, three methines, one vinylic and one quaternary carbon atoms. The above data are consistent with two vinylic carbons and one vinylic proton, which revealed the presence of one trisubstituted double bond. Therefore the quaternary carbon is a vinylic quaternary carbon, consistent with partial skeleton I (Fig. 3).

In the HMBC spectrum (Appendix 15), pertinent correlations were observed between the vinylic carbons at δ 123.6 ppm with the methyl protons H-20, and the methylene protons H-1 and H-4, the quaternary carbon at δ 140.6 ppm with H-1, H-2, H-20 and H-4. These observations together with HSQC (Appendix 14) correlation of H-2 (triplet) at δ 5.3 ppm with C-2 at δ 123.6 ppm, the methylene protons H-1 (doublet) at δ 4.04 ppm with C-1 at δ 59.8 ppm, the methyl protons H-20 (singlet) at δ 1.56 ppm with C-20 and the methylene protons H-4 triplet at δ 1.88 ppm with C-4 at δ 40.3 ppm led to partial skeleton I.

Partial skeleton II (fig. 3) is based on the methyl protons of H-16 and H-17 and the methine proton of H-15. From the HMBC H-15 showed a strong correlation with the

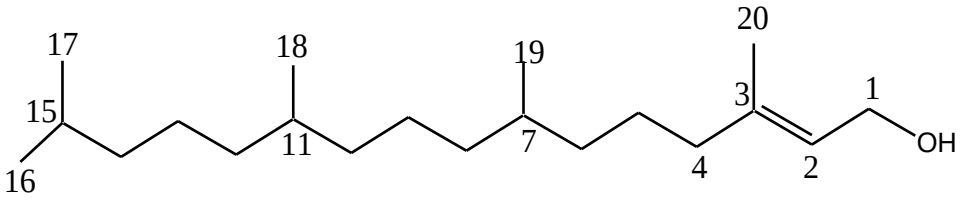
two methyl carbons C-16 and C-17 and also with the methylene carbon at δ 39.8 ppm which is C-14. The methyl proton H-16 showed a correlation with the methyl carbon at δ 23.0 ppm (C-17) also with the methylene carbon at δ 39.8ppm (C-14) and with the methine carbon at δ 28.4 ppm (C-15), this led to partial skeleton.



Partial skeleton I Partial skeleton II

Fig. 3: Selected Heteronuclear Multiple Bond Correlations (HMBC) for compound AL-3. Arrows point from Proton to carbon.

After drawing the above two partial skeletons compound AL-3 was identified as phytol (phyt-2-en-1-ol) by comparison of its ^{13}C NMR data with the ^{13}C NMR data of phytol which was previously reported in the literature (Goodman *et al.*, 1973). The remaining eleven carbon atoms are situated between the two partial skeletons. The ^{13}C chemical shift value of AL-3 and phytol are tabulated in Table 5.



Structure of phytol (phyt-2-en-1-ol) (**38**)

Phytol is an acyclic diterpene alcohol, colorless, high-boiling oil. It is used in the manufacture of synthetic vitamins E and K_1. It was first obtained by hydrolysis (decomposition by water) of chlorophyll in 1909 by the German chemist Richard Wilstätter. Its structure was determined in 1928 by the German chemist F.G. Fischer.

Phytol may be obtained in the process of separating chlorophyll from alfalfa (Website Reference 7).

Table 5: ^{13}C NMR spectral data of compounds AL-3 compared with lit. data for phytol.

No	**δ_C AL-3 (87-153A)**	**δ_C phytol (**phyt-2-en-1-ol)
1	59.8	59.4
2	123.6	123.2
3	140.6	140.2
4	40.3	39.9
5	25.6	25.2
6	37.1	36.8
7	33.2	32.8
8	37.8	37.5
9	24.9	24.5
10	37.8	37.4
11	33.1	32.7
12	37.7	37.4
13	25.2	24.9
14	39.8	39.4
15	28.4	28.0
16	23.1	22.7
17	23.0	22.8
18	20.1	19.8
19	20.1	19.8
20	16.6	16.2

2.3 Characterization of AL-3A

AL-3A was obtained as white needle crystal, soluble in chloroform with melting point 136-138 ^0C and Rf value of 0.5 in Petrol/EtOAc (80:20), not UV active (254 nm), brownish coloration with vanillin sulfuric acid reagent and recrystallized from petrol/chloroform. The UV-visible absorption band (Appendix 16) in chloroform was blank.

The MS spectrum exhibited a molecular ion peak (EIMS) at m/z 414.5, which established the molecular formula as $C_{29}H_{50}O$. The IR (KBr) spectrum (Appendix 17) exhibited absorption bands at 3434 cm^{-1} (-OH stretching), 1638 cm^{-1} (unsaturated bond stretching), 1465 cm^{-1} (-CH bending of methylene groups), 1382 cm^{-1} (-CH bending of methyl groups), and 1062 cm^{-1} (-C-O- stretching). Absorption bands at 2960 cm^{-1} and 2867 cm^{-1} are due to C-H vibrations of aliphatic CH_3 and CH_2 groups.

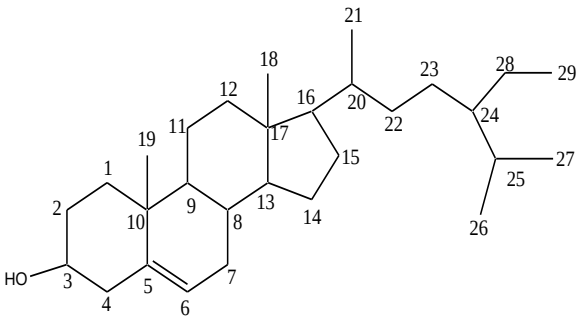
The ^{1}H NMR spectrum (Appendix 18) of AL-3A displayed signals for two quaternary methyls at δ 0.58 and 0.91 ppm (each 3H, s), one hydroxyl methine proton at δ 3.4 ppm (1H, m) and one vinylic proton at δ 5.3 ppm (1H, bs). These chemical shift values were quite similar with the data of the known compound β-sitosterol (Kojima *et al*., 1990).

The ^{13}C spectrum (Appendix 19) and DEPT-135 spectrum (Appendix 20) showed signals for two quaternary methyls at δ 12.3 and 19.5 ppm (C-18 & C-19), three secondary methyls at δ 19.2, 18.77 & 19.80 (C-21, C-26 & C-27), one primary methyl at 12.4 ppm (C-29), one hydroxyl methine carbon at δ 72.2 ppm (C-3), two trisubstituted carbons at δ 36.94 & 42.7 ppm (C-10 & C-13), six trisubstituted methine carbons at δ 32.3, 50.6, 57.2, 36.6, 46.3 & 29.6 ppm (C-8, C-9, C-14, C-21, C-24 & C-25), one vinylic methine carbon at δ 122.1 ppm (C-6), one vinylic quaternary carbon at δ 141.2 ppm (C-5) and eleven methylene carbon atoms.

From the ^{13}C NMR spectrum, the appearance of signals at δ 122.1 and 141.2 for the C-5 and C-6 carbons together with a broad singlet at δ 5.3 ppm revealed the presence of a $\Delta^{5,6}$ double bond in compound AL-3A. The proton NMR spectrum exhibited doublet of doublet at δ 3.42 ppm for the hydroxy methine proton. This doublet of doublet arose due to coupling with protons of the methylene group, which were oriented axial and equatorial. The chemical shift values of all the carbon atoms in

AL-3A are quite similar with the previously reported data of β-sitosterol [**32**] (Kojima, *et al*., 1990 and Chaurasia *et al*. 1987). Both the HMBC (Appendix 23) and HSQC (Appendix 22) spectral data of AL-3A are in good agreement with the proposed structure. The chemical shifts of the carbon atoms in AL-3A are compared with the literature data of β-sitosterol in Table 6. All the spectroscopic as well as physico-chemical results such as melting point, physical state, solubility, nature of the crystal, etc support AL-3A to be β-sitosterol.

β-sitosterol is one of several plant sterols (cholesterol is the main animal sterol) found in almost all plants. High levels are found in rice bran, wheat germ, corn oil and soybeans. Peanuts and its products, such as peanut oil, peanut butter and peanut flour, are good sources of plant sterols, particularly β–sitosterol. It is one of a group of organic compounds found in plants that, alone and in combination with similar plant sterols, reduces blood levels of cholesterol. The reduction of cholesterol levels appears to be because β-sitosterol blocks absorption of cholesterol. It has also been effective in reducing symptoms of benign prostatic hyperplasia. Although molecules quite similar to β-sitosterol inhibit cancer cells in test tubes, the relevance of this information for people remains unknown (Website Reference 8).



Structure of ß-Sitosterol (**32**)

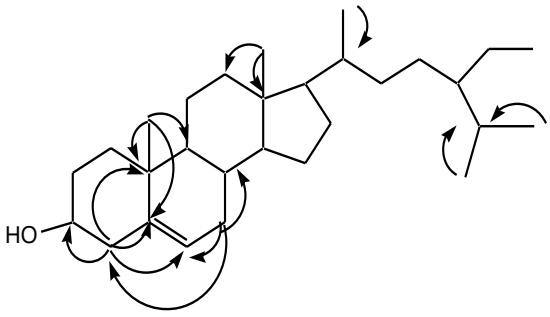


Fig. 4: Selected Heteronuclear Multiple Bond Correlations (HMBC) for compound **AL-3A**. Arrows point from Proton to carbon.

Table 6: ^{13}C NMR spectral data of compound AL-3A compared with lit. data for β-sitosterol by Kojima et al. (1990).

C	AL-3A	β-Sitosterol (Kojima et al., 1990)
1	37.7	37.2
2	32.1	31.6
3	72.2	71.8
4	42.7	42.3
5	141.2	140.7
6	122.1	121.7
7	32.3	31.9
8	32.3	31.9
9	50.6	50.1
10	36.9	36.5
11	21.5	21.1
12	40.3	39.8
13	42.7	42.3
14	57.2	56.8
15	24.7	24.3
16	28.6	28.2
17	56.5	56.0
18	12.3	11.9
19	19.5	19.4
20	36.6	36.1
21	19.2	18.8
22	34.4	33.9
23	26.5	26.0
24	46.3	45.8
25	29.6	29.1
26	20.2	19.8
27	19.8	19.0
28	23.5	23.0
29	12.4	12.0

2.4 Characterization of AL-11

AL-11 was obtained as a yellow amorphous solid, with Rf value of 0.73 in chloroform acetone (40:60), soluble in methanol, and gives yellow coloration with 1% vanillin in sulfuric acid on TLC. The MS spectrum (Appendix 24) exhibiting the molecular ion peak (HR-ESIMS) at m/z 538.08495 established the molecular formula as $C_{30}H_{18}O_{10}$ (Scheme-3). The UV-Visible absorption spectrum (Appendix 25) in methanol showed bands at the wavelength of 269 and 335 nm, which is the characteristic band for aromatic ring absorptions. In the IR (KBr) spectrum (Appendix 26) displayed absorption band at 3436 cm^{-1} showed the presence of hydroxyl functional group that is due to hydrogen bonded OH stretching vibration. Absorption at 1656 cm^{-1} is due to

conjugated-γ-pyron. A strong absorption bands at 1610, 1577, and 1501cm^{-1} are indications of aromatic ring stretching. The absorption band at 836 cm^{-1} showed the presence of p-substituted phenyl ring and the absorption bands at 1240 and 1173 cm^{-1} indicate the presence of carbon oxygen bond stretching.

The ^{1}H NMR spectrum (Appendix 27) of AL-11 in DMSO-d_6 showed signals for two chelated hydroxyl groups at δ 13.1 and 12.97 ppm, eight different kind of aromatic protons at δ 6.71(2H, d), 7.15(1H, d), 7.57(2H, d), 8.0 (2H, singlet), 6.46 (1H, d), 6.40 (1H, d), and 6.19 (1H, d) and two protons attached to sp^2 carbon atoms at δ 6.78 (1H, s) and 6.82 (1H, s).

The ^{13}C NMR spectrum (Appendix 28) and DEPT-135 spectrum (Appendix 29) displayed signals for eighteen quaternary carbon atoms of which two carbonyl carbons at δ 182.6 (C-4) and 182.9 (C-4”), ten oxygenated sp^2 quaternary carbon atoms at δ 164.5 (C-7), 162.3 (C-5), 158.3 (C-9), 164.7 (C-2), 160.6 (C-4’), 163.0 (C-7”), 161.8 (C-5”), 155.4 (C-9”), 164.9 (C-2”), and 161.4 (C-4”’), and six sp^2 quaternary carbon atoms at δ 104.6 (C-10), 121.7 (C-1’), 122.3 (C-3’), 104.9 (C-4”), 104.5 (C-10”), and 120.9 (C-1”’). The carbon spectrum also shows the presence of ten sp^2 methine carbon atoms at δ 94.9 (C-8), 99.6 (C-6), 103.8 (C-3), 117.1 (C-3’), 128.7 (C-2’), 132.0 (C-6’), 99.7 (C-6”), 103.4 (C-3”), 129.0 (C-2”’ & C-6”’) and 116.6 (C-3”’ & C-5”). Therefore from the ^{13}C and DEPT-135 NMR spectra we can conclude that there are 30 carbon atoms in compound AL-11.

From the HSQC spectrum (Appendix 31) and HMBC spectrum (Appendix 32) the following cross peak correlations between protons and carbons are observed. In the HMBC spectrum: H-8/ C-9, C-10, C-7, C-4 & C-6; H-6/ C-7, C-8, C-10, C-5, & C-4; H-3/ C-4, C-10, C-5, C-2 & C-1’, H-2’/ C-1’, C-2, C-3’, C-4’, C-5’, C-6’ & C-8”; H-5’/ C-4’, C-6’, C-1’ & C-8”; and H-6’/ C-2, C-1’, C-5’, C-2’, & C-4’. These

observations together with HSQC correlation of H-8 broad singlet at δ 6.46 with C-8 at δ 94.9, H-6 (singlet) at δ 6.4 with C-6 at 99.6, H-3 singlet at δ 6.82 with C-3 at δ 103.8, H-2’ (broad singlet) at δ 8.0 with C-2’ at δ 128.6, H-5’ doublet at δ 7.15 with C-5’ at δ 117.1 and H-6’ a broad singlet at 8.0 with C-6’ at δ 132.3 ppm led to partial skeleton I.

Similarly the correlations H-6”/ C-8”, 7”, 5”, 10”,& 4”; H-3”/ C-2”, 1”’, 4”, 10”; H-2”’/ C-1”’, 3”’, 4”’,& C-2” and H-3”’/ C-4”’, 2”’, & 1”’ were depicted. These observations together with HSQC correlation of H-6” singlet at δ 6.2 with C-6” at δ 99.7, H-3” (singlet) at δ 6.78 with C-3” at 103.4, H-2”’ (doublet) at δ 7.57 with C-2”’ at δ 129.0, and H-3”’ (doublet) at δ 6.72 with C-3”’ at δ 116.6 ppm, led to partial skeleton II.

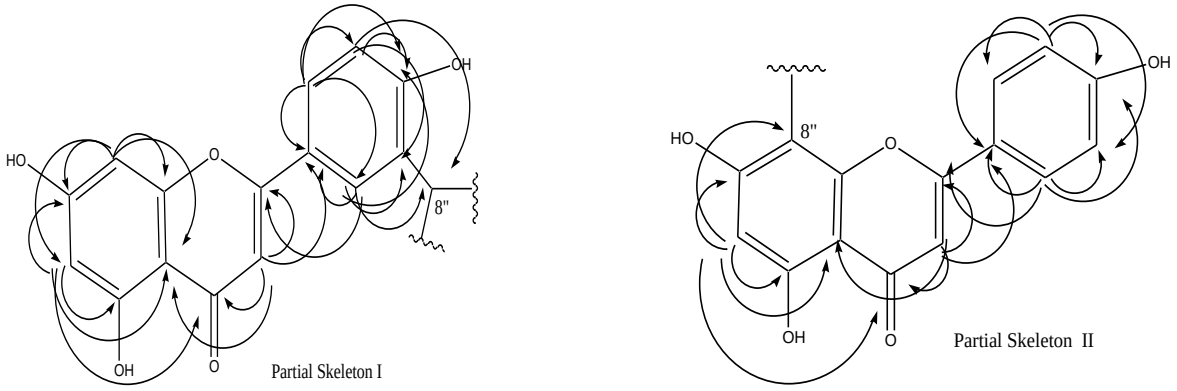
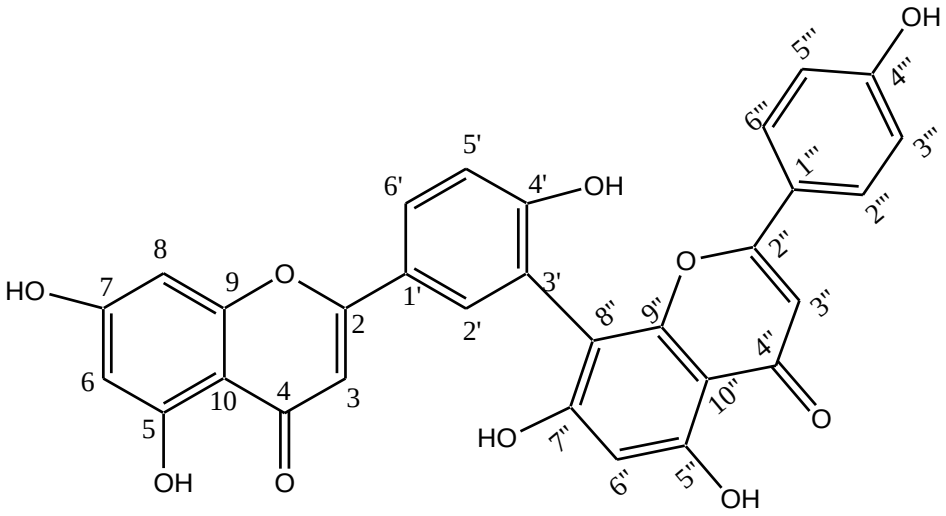


Fig. 5: Selected HMBC correlations of AL-11 in partial skeletons I and II (arrows point from proton to carbon).

From partial skeleton I H-2’, and H-5’ and from partial skeleton II H-6” showed a cross peak correlation in the HMBC spectrum with C-8” which is at δ 104.9 ppm. These cross peak correlation indicates that partial skeleton I and Partial skeleton II are linked to each other. The bond formed between the two partial structure lies on C-3’ and C-8”. When these two partial skeletons are combined through C-3’ and C-8” bond, the resulting compound is consistent with a bis-5-hydroxyflavone. The

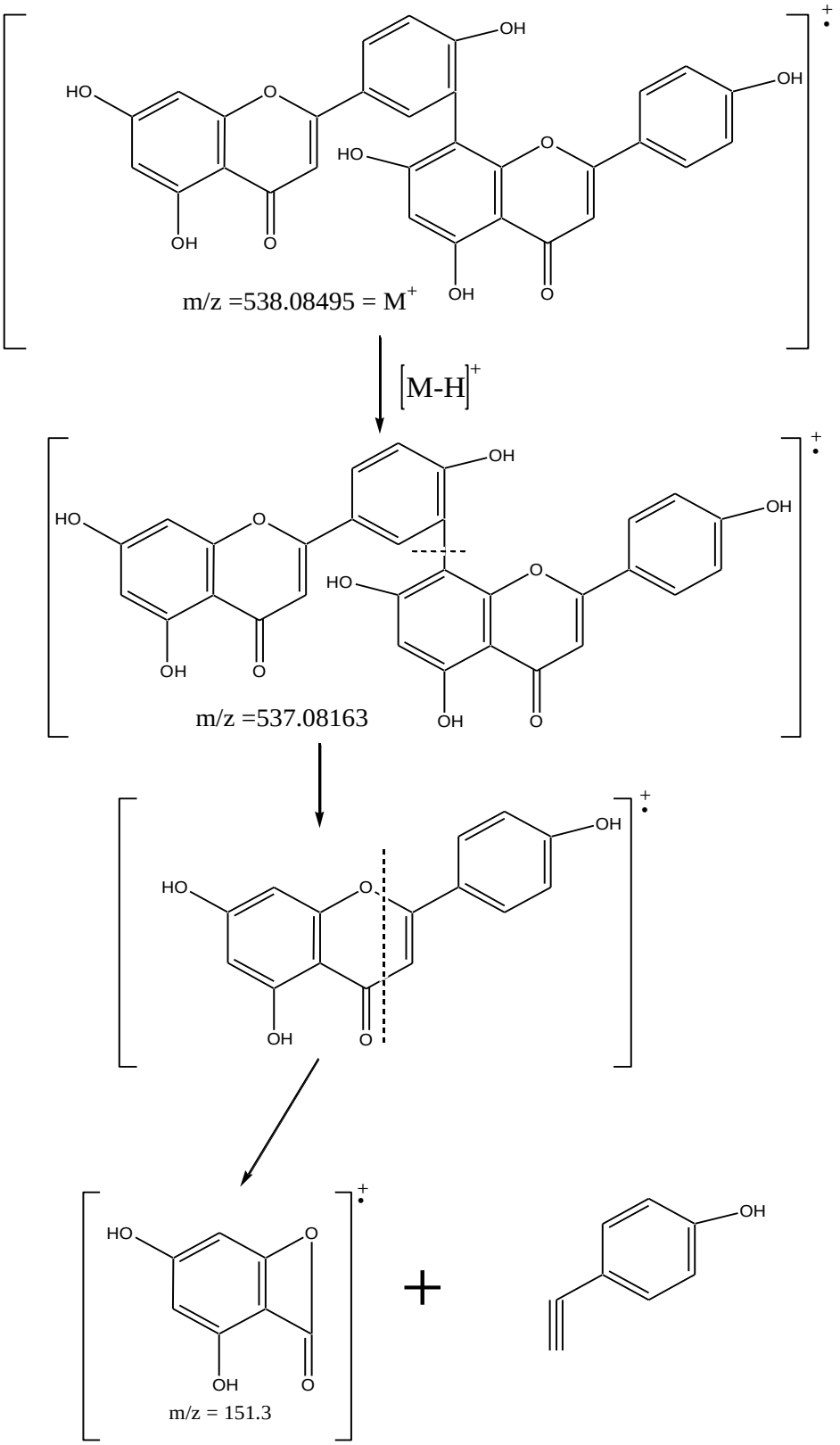
compound appears to be identical with bisflavone previously reported from *Rhussuccedanea* (Anacardiaceae) (Chari *et al*., 1977) and from the seeds of *Canariun schweinfurthii* (Burseraceae) (Helene *et a*l., 2000) and named amentoflavone. The chemical shifts of the ^{13}C NMR spectrum of this compound in DMSO-d6 were compared (Table 7) with those published by Chari *et al*. and Helen *et al*. for amentoflavone measured in the same solvent. We noticed that all the chemical shifts data of AL-11were shifted downfield by *ca* o.6 ppm from Chari *et al*. data and quite similar to the data reported by Helen *et al*. All the spectroscopic as well as physico-chemical results support AL-11 to be amentoflavone (Livira *et al*., 2002; Markham *et al*., 1987; Dora, *et al*., 1991).



Structure of amentoflavone (AL-11) (**39**)

Table 7: ^{13}C NMR assignment of amentoflavone and AL-11 in DMSO-d_6; chemical shifts are given in ppm.

C	Chari *et al*., 1977	Helene *et a*l., 2000	AL-11
2	164.0	164.7	164.7
3	103.2	103.8	103.8
4	181.8	182.6	182.6
5	161.6	162.3	162.3
6	98.9	99.5	99.6
7	163.9	164.5	164.5
8	93.9	94.9	94.9
9	157.6	158.2	158.3
10	104.0	104.6	104.6
1’	121.3	121.8	121.7
2’	127.9	128.7	128.6
3’	121.6	122.3	122.3
4’	159.6	160.4	160.4
5’	116.4	117.0	117.1
6’	131.6	132.3	132.0
2”	164.3	165.0	164.9
3”	102.8	103.5	103.4
4”	182.2	183.0	182.9
5”	160.8	161.9	161.8
6”	99.1	99.7	99.7
7”	162.0	162.7	163.0
8”	104.1	104.8	104.9
9”	154.7	155.3	155.4
10”	104.0	104.5	104.5
1”’	120.3	120.8	120.9
2”’	128.3	129.1	129.0
3”’	116.0	116.6	116.6
4”’	161.1	161.4	161.4
5”’	116.0	116.6	116.6
6”’	128.3	129.1	129.0



Scheme-3: Major MS fragmentation patterns for AL-11

3.0 EXPERIMENTAL

3.1 General

TLC was performed on a 0.25 mm thick silica gel GF_{254} (Merck). Preparative TLC was run on 0.5 and 0.75 mm thick layer silica gel coated on 20 cm X 20 cm glass plates. IR spectra were measured on a Perkin-Elmer BX spectrometer (400-4000 cm^{-1}) in KBr pellets for solid samples and in chloroform for liquid compounds. Melting points were determined on electro thermal digital melting point apparatus. Components of the *A. laciniatum* were detected by their UV fluorescence and by spraying with 1% vanillin in H_2SO_4. Flash Column Chromatography (FCC) was performed using silica gel 60 (230-400 mesh). Samples were applied on top of the column by adsorbing on silica gel. The speed of the mobile phase was increased by applying pressure from the top. UV spectra were measured on a Shimadzu UV-VIS recording spectrophotometer, UV-160, spectronic genesys spectrophotometer. NMR spectra in $CDCl_3$ and DMSO-d_6 (400 MHz) were run on a Bruker Avance spectrometer and δ values are given in ppm relative to TMS as internal standard.

3.2 Plant Material

The leaves of *Antidesma laciniatum* (Euphorbiaceae) were collected in August 2004 in Cameroon at Mount Kala (1800m), about 15 km from Yaoundé by Mr. Nana, Botanist at the National Herbarium, Yaoundé and Dr. Tchinda T. Alembert. The plant was found at around the middle of the hill. They were dried at room temperature for four weeks. The dried leaves were ground and readied for extraction.

3.3 Extraction of the plant material

The leaves powder of *A. laciniatum* obtained (2.3 kg) was macerated with MeOH/H_2O (80:20) of 7.5 L for 48 h, concentrated to 1800 mL, then treated with $CHCl_3$/MeOH/H_2O (4:4:1) (2.5 L). The organic phase was dried with $MgSO_4$,

concentrated to yield 36 g of extract. The aqueous phase was reduced to 1.5 L by evaporation of MeOH and dried at 50°C in a ventilated oven for 24 h to give 138 g of a brown-red extract.

3.4 Coding System

In the coding system **A** stands for the genus name *Antidesma*, **L** stands for species name *laciniatum* and the number attached to AL indicate the position of the compound starting from the highest R_f value to the lowest. Thus, AL-1 stands for the first compound that is the least polar and AL-11 is the last compound, which is the most polar. TLC examination of the crude extracts revealed the presence of at least 11 spots when sprayed by 1% vanillin in conc. H_2SO_4 indicating the extract to be rich in secondary metabolites.

3.5 Isolation of compounds

The chloroform extract (6 g) was chromatographed using FCC (silica gel, 140 g) by eluting successively with increasing polarity. A total of 28 fractions, 50 ml each were collected (Table 8). Purification of all the fractions were done by using Sephadex LH-20, Flash Column Chromatography (FCC), Medium Pressure liquid Chromatography (MPLC) Separo AB type, Chromatotron, Preparative Thin Layer Chromatography (PTLC) and recrystalization techniques. From eleven spots on the crude TLC the following four compounds AL-1, Al-3, AL-3A and AL-11 were isolated. Details of the isolation procedure for each case are described below (Table 8).

Table 8: Fractions collected from FCC to isolate compounds from the leaves of *A. laciniatum*

Solvent	TLC Result	Label	Remark
Petrol (100%)	Empty	-	Discarded
Petrol/$CHCl_3$ (90:10)	2-spots	87-191B	Chlorophyll
Petrol/$CHCl_3$ (80:20)	1 clear spot	87-191C	Pure compound
Petrol/$CHCl_3$ (70:30)	1 major spot	87-191D	Small amount
Petrol/$CHCl_3$ (50:50)	2 spots	87-191E	Chlorophyll
Petrol/$CHCl_3$ (30:70)	Mixture	87-191G	Chlorophyll
Petrol/$CHCl_3$ (20:80)	Mixture	87-191H	Chlorophyll
$CHCl_3$ (100%)	3 spots	87-191I	Chlorophyll
$CHCl_3$/EtOAc (90:10)	Mixture	87-191J	Chlorophyll
$CHCl_3$/EtOAc (80:20)	Mixture	87-191K	Passed through Sephadex
$CHCl_3$/EtOAc (70:30)	3 major spots	87-191L	Applied on Separo
$CHCl_3$/EtOAc (60:40)	3 major spots	87-191M	Applied on sephadex
$CHCl_3$/EtOAc (50:50)	2 major spots	87-191N	Chlorophyll
$CHCl_3$/EtOAc (40:60)	3 major spots	87-191O	Applied on sephadex
$CHCl_3$/EtOAc (20:80)	Mixture	87-191P	Applied on sephadex
EtOAc (100%)	Mixture	87-191Q	Applied on sephadex
EtOAc/Acetone(75:25)	Mixture	87-191R	Discarded
EtOAc/Acetone(50:50)	Mixture	87-191S	Discarded
Acetone (100%)	Mixture	87-191T	Discarded
Acetone/MeOH (50:50)	Mixture	87-191U	Discarded
MeOH (100%)	Mixture	87-191W	Discarded

3.5.1 Isolation of AL-1

Fraction 87-191C (73 mg) was directly obtained from the FCC with the solvent system petrol/chloroform (85:15). Upon TLC analysis with the solvent petrol: chloroform (85:15) AL-1 shows blue coloration after spraying with 1% vanillin in conc. H_2SO_4. The spot of AL-1 on TLC has Rf value of 0.24.

AL-1: colorless oil, IR ($CHCl_3$) ν_{max} 2925, 1670, 1449, 1379 cm^{-1}; UV ($CHCl_3$) λ_{max} blank; ^1H NMR (400 MHz, $CDCl_3$) (Table 3); ^{13}C NMR (400 MHz, $CDCl_3$,) (Table 4); TIC m/z (ret. time, mn.) 409.5 (24.8) $[M]^+$; EIMS m/z (rel. int.) 410.4 (4), 341.4 (7), 273 (3), 149. 2 (32), 136.2 (48), 121.2 (43), 109.2 (34), 95.2 (49), 81.2 (79), 69.2 (100), 67.2 (39), 41.2 (54).

3.5.2 Isolation of AL-3

Fraction 87-191K contains AL-3 as the dominant components. 80 mg of 87-191K was passed through sephadex to look for AL-3 that shows a blue coloration with vanillin sulfuric acid spraying reagent on TLC. From the sephadex fifteen fractions were collected and after TLC analysis regrouped as follows.

Table 9: Fractions collected from sephadex to isolate AL-3

Fractions	TLC result	Label	Remark
1-8	Chlorophyll	--------	Discarded
9	Chlorophyll + AL-3	87-193A	Small amount
10-12	One major spot	87-193B	Dominant Target cpd
13-15	1 dominant + impurities	87-193C	

Cpd = compound

87-193B was applied on PTLC by using petrol: EtOAc (8:2) as eluting solvent. On PTLC three bands were observed, of which the middle band gave AL-3 (25 mg) whereas the amount of both the bottom and top bands were not enough for further analysis. For the spectroscopic data of AL-3 refer result and discussion part.

AL-3: colourless oil; IR ($CHCl_3$) ν_{max} 3435, 2928, 1666, 1463, 1379, 1215 cm^{-1} ; UV ($CHCl_3$) λ_{max} blank; ^1H NMR (400 MHz, $CDCl_3$): 5.32 (1H,m, H-2) , 4.04(2H, d, H-1), 1.89, 1.57, 1.44, 1.20, 0.86, 0.78, 0.76, 0.74; ^{13}C NMR (400 MHz, $CDCl_3$) (Table 5).

3.5.3 Isolation of AL-3A

250 mg of 87-191L contain AL-3A as dominant component among with chlorophyll, few polar and non-polar components. It was applied on Medium Liquid Pressure Chromatography Separo AB type. From the Separo thirteen fractions were collected and after TLC analysis the fractions were regrouped as follows (Table 10).

Table 10: Fractions collected from Separo to isolate AL-3A.

Solvent	Fraction	TLC result	Label	Remark
Petrol/EtOAc (95:5)	1	2 spots	87-196A	Small amount
Petrol/EtOAc (90:10)	2	1 major spot	87-196B	Small amount
Petrol/EtOAc (85:15)	3 & 4	1 major spot	87-196C	Target cpd
EtOAc (20, 30, 50, 100%)	5-9	Major chlorophyll	87-196D	discarded
Acetone (50,100) MeOH (50)	10-13	Chlorophyll	87-196E	discarded

Cpd = compound

After collecting and regrouping all the above fractions AL-3A was recrystallized from 87-196C by petrol/$CHCl_3$ and white needle crystals was obtained. AL-3A gives violet coloration with vanillin sulfuric acid on TLC.

AL-3A: white needle crystals, mp.137-139 ^oC; IR (KBr) ν_{max} 3434, 2960, 1638, 1469, 1382, 1062 cm^{-1} ; UV ($CHCl_3$) λ_{max} blank; ^1H (400 MHz, $CDCl_3$): 5.26, 3.42, 2.19, 1.90, 1.73, 1.42, 0.91, 0.83, 0.73, 0.58; ^{13}C NMR (400 MHz, $CDCl_3$) (Table 6); HRMS m/z (ret. time in mn) 414.5 (30) $[M]^+$, 399.5 (30).

3.5.4 Isolation of AL-11

Fractions 87-191O, P, & Q were combined (120 mg) and were passed through sephadex LH-20 eluted with $CHCl_3$: MeOH (50:50) and twenty fractions (Table 11) were collected and fraction 14-20 contains the target compound AL-11, which shows

yellow coloration with vanillin sulfuric acid on TLC, soluble in methanol and obtained as a yellow amorphous crystal.

Table 11: Fractions from Sephadex LH-20 to isolate AL-11.

Fraction	TLC result	Label	Remark
1-8	Major chlorophyll	-	discarded
9-13	Mixture	-	discarded
14-20	One clear yellow spot	87-189D	The target compound AL-11

AL-11: yellow amorphous solid, IR (KBr) ν_{max} 3435-3100 (broad), 1730, 1654, 1579, 1492, 1286, 1246, 1167 cm^{-1}; UV ($CHCl_3$) λ_{max} 335, 269; ^1H (400 MHz, DMSO-d_6): at δ 13.1(1H, s, H-5), 12.97 (1H, s, H-5"), 6.71(2H, d, H-3"' & H-5"'), 7.15(1H, d, H-5'), 7.57(2H, d, H-2"' & H-6"'), 8.0 (2H, s, H-2' & H-6'), 6.46 (1H, d, H-8), 6.40 (1H, d, H-6), 6.19 (1H, d, H-6"), 6.78 (1H, s, H-3"), 6.82 (1H, s, H-3); ^{13}C NMR (400 MHz, DMSO-d_6) (Table 7); HR-ESIMS (positive mode) m/z 561.07800 ($[M+Na]^+$,calcd. 561.15668 for $C_{30}H_{18}O_{10}Na$), HR-ESIMS (negative mode) m/z 538.08495 $[M]^+$, 537. 08163 (84) $[M-H]^+$, EIMS m/z (rel. int.) 537.4 (100) $[M-H]^+$, 339.3 (15), 325.3 (20), 311.4 (12), 255.3 (14), 179.2 (8), 151.3 (72), 137 (34).

4.0 Conclusions and Recommendations

This investigation was conducted because of the importance of members of the genus *Antidesma* as plant medicine. We investigated *A. laciniatum* for the first time and isolated the triterpenes squalene and β-sitosterol, the diterpene phytol, and the flavonoid amentoflavone. We noted the absence of antidesmone, a compound found in many *Antidesma* species. Further work on the minor components of this plant and similar studies on other members of the genus is recommended.

5.0 References

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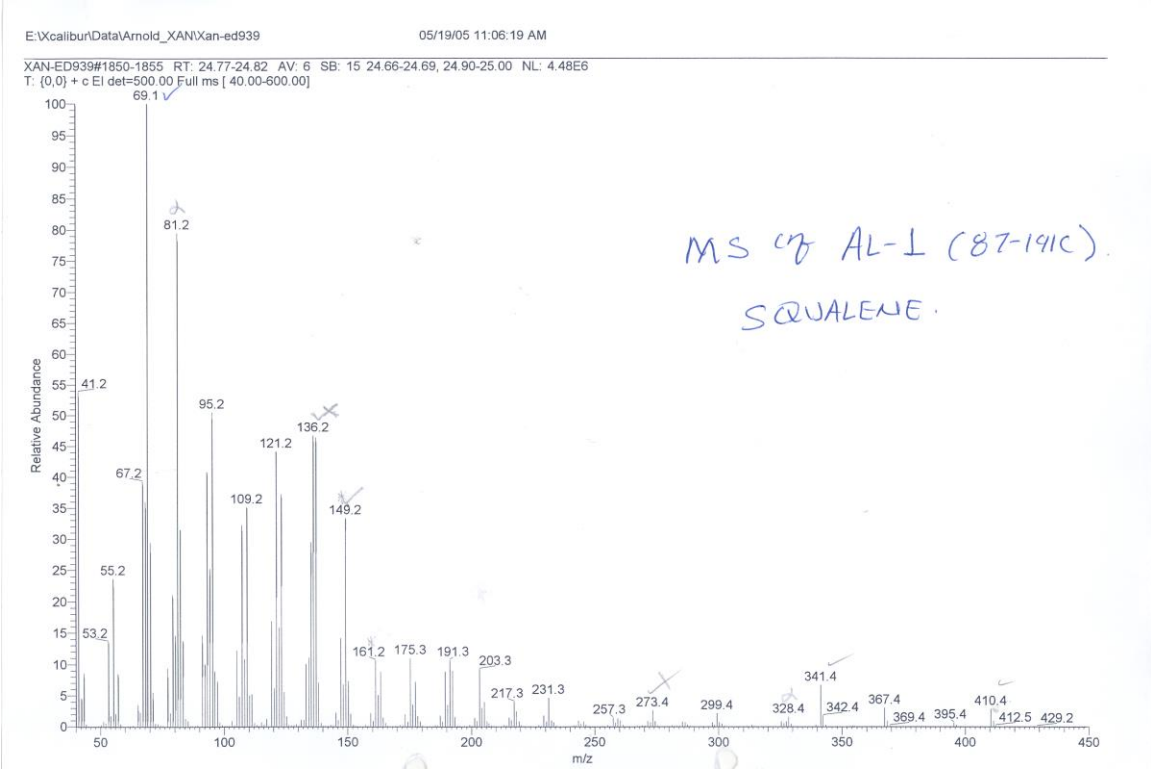
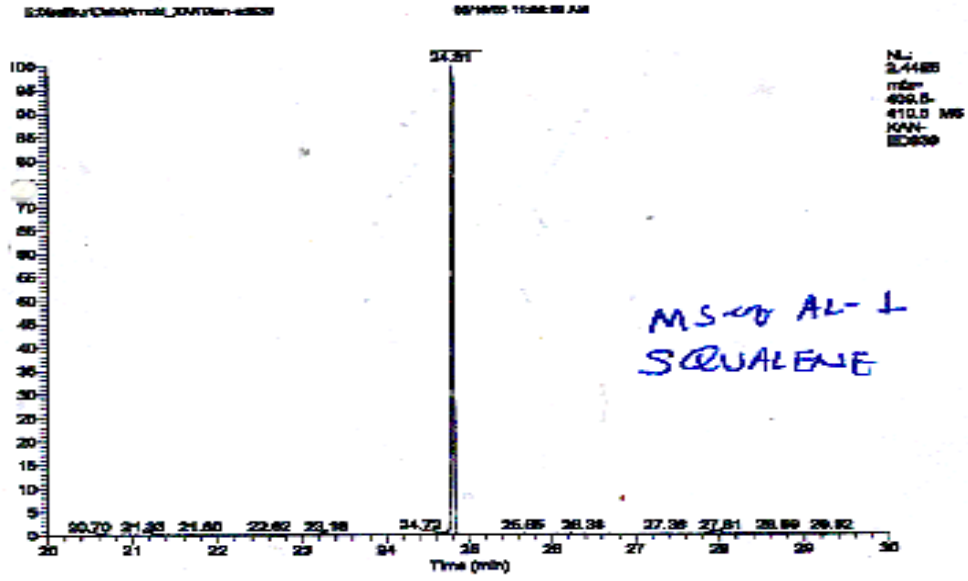
Website Reference 7:
http://www.britannica.com/eb/article?tocId=9059875 Website Reference 8: Beta-Sitosterol
http://www.vitacost.com/science/hn/Supp/Beta_Sitosterol.htm

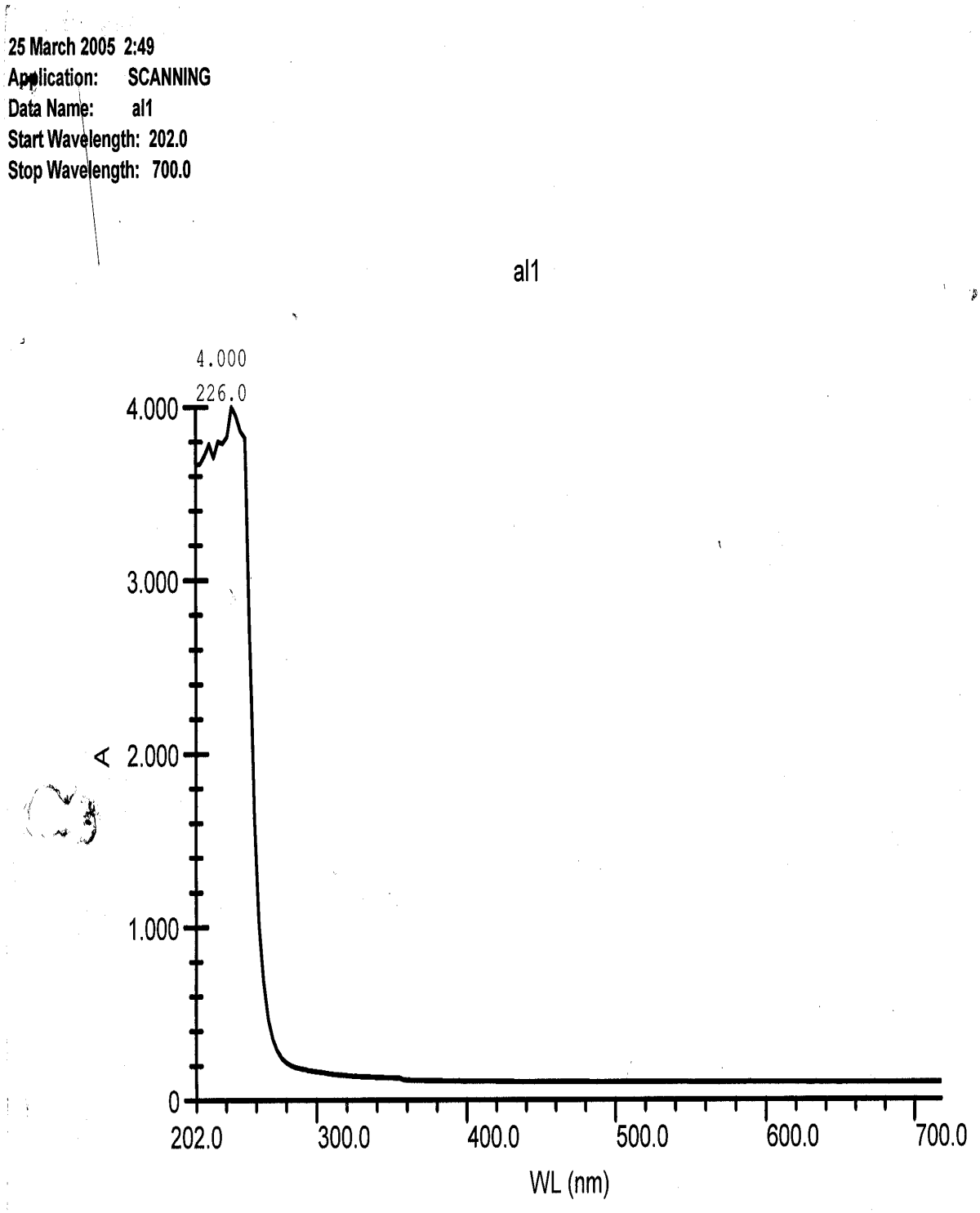
6. Appendices

Appendix 1: MS spectrum of AL-1

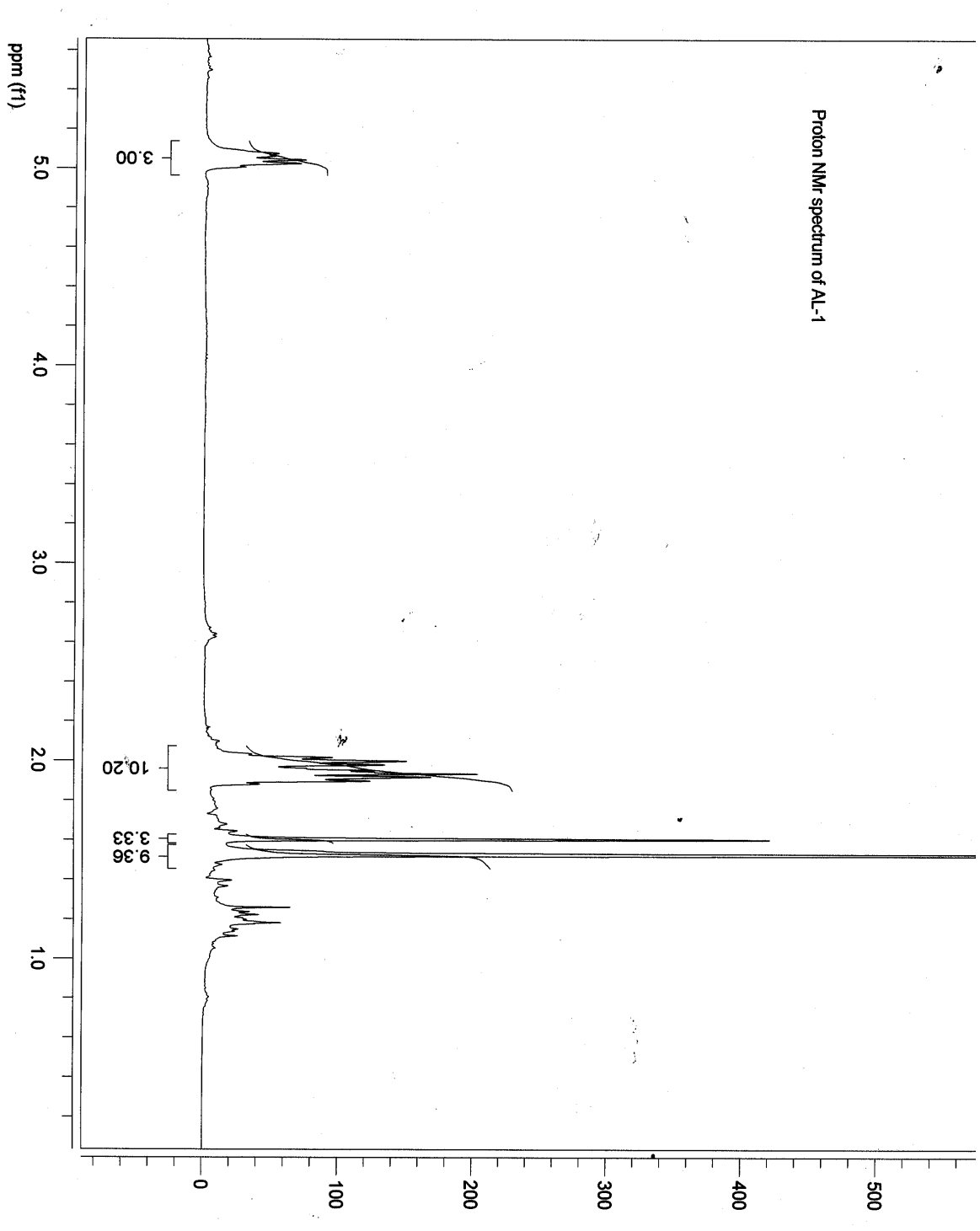




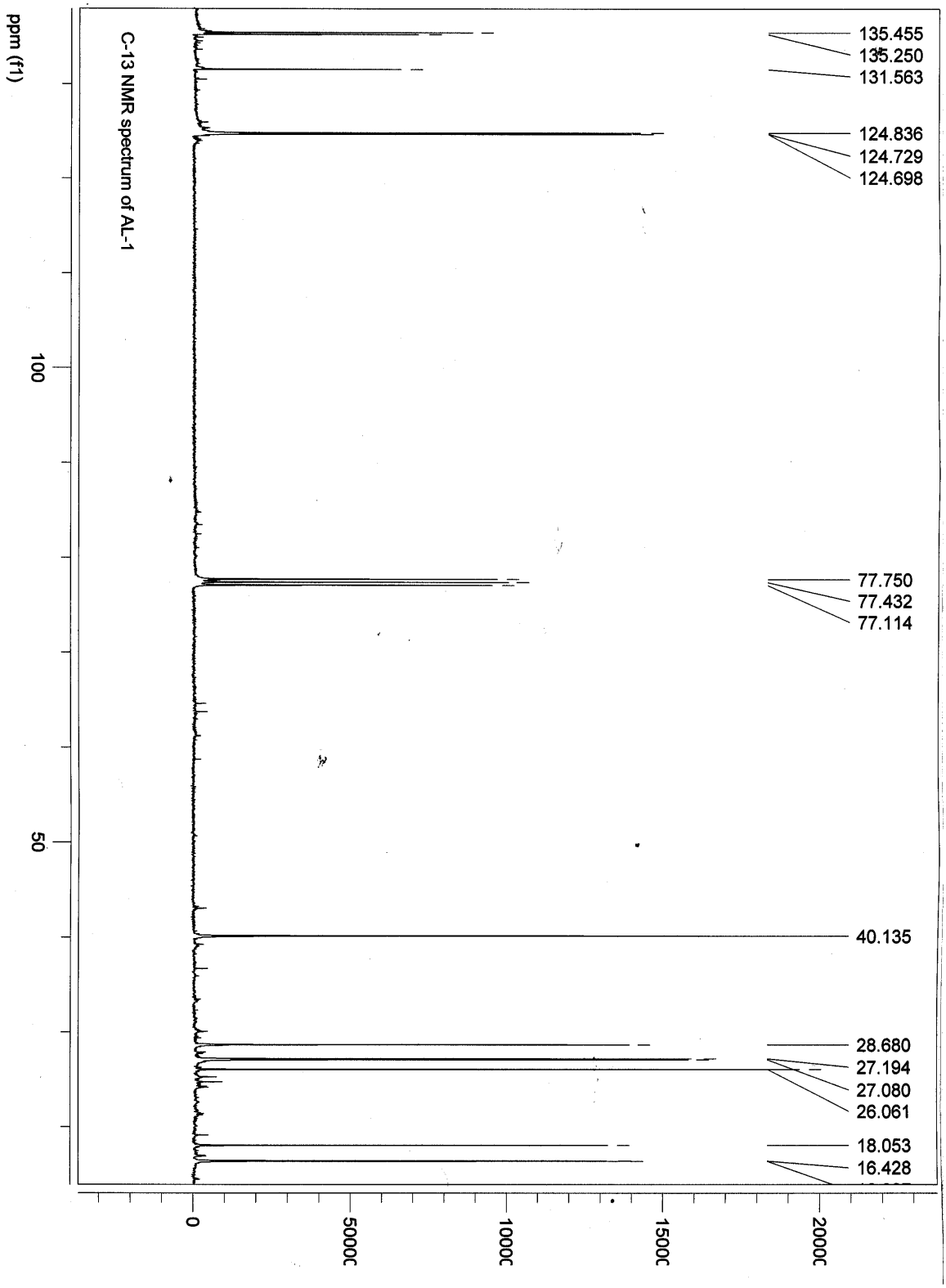
Appendix 2: UV-Visible absorption band spectrum of AL-1



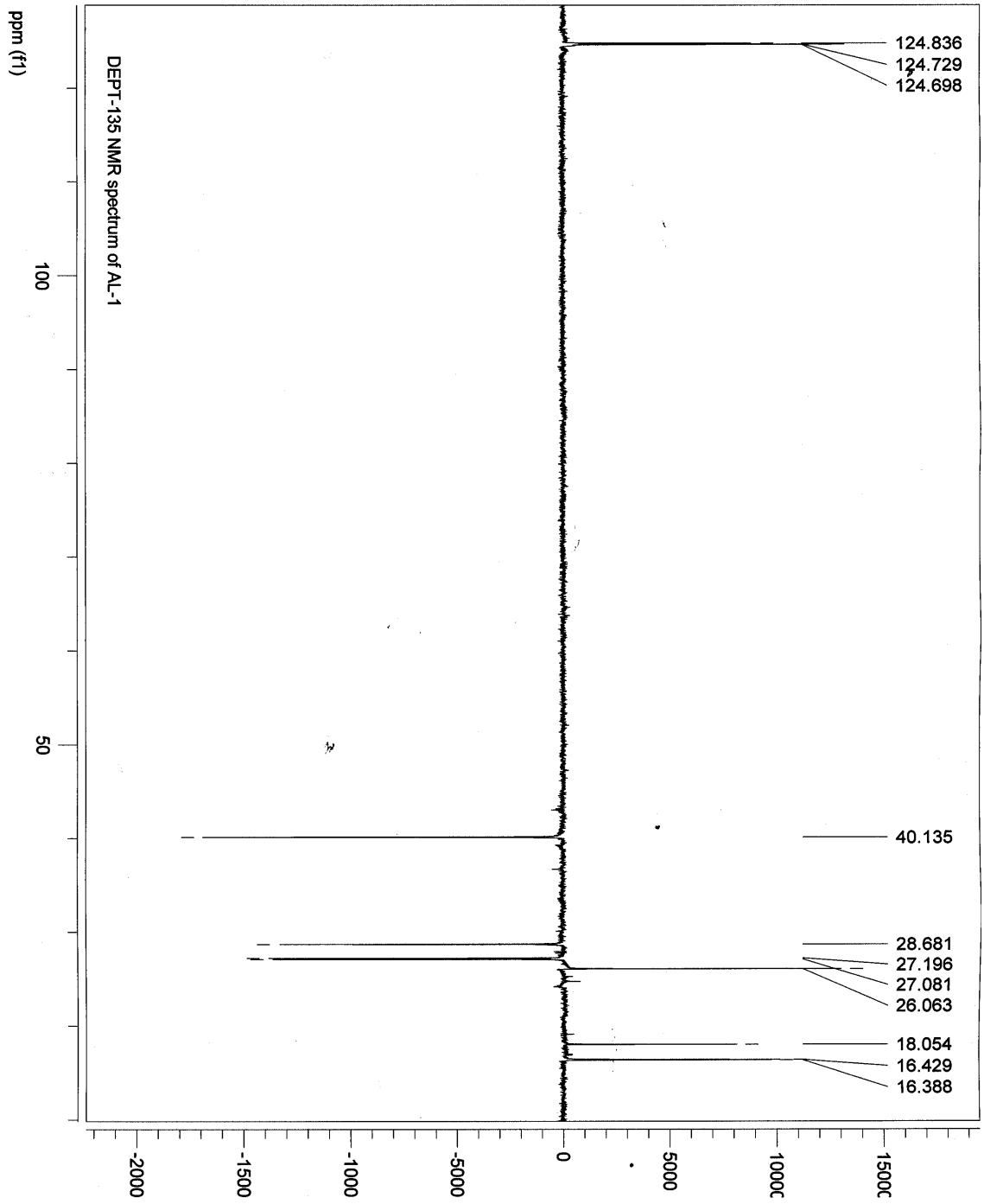
Appendix 3: ^{1}H NMR spectrum of AL-1



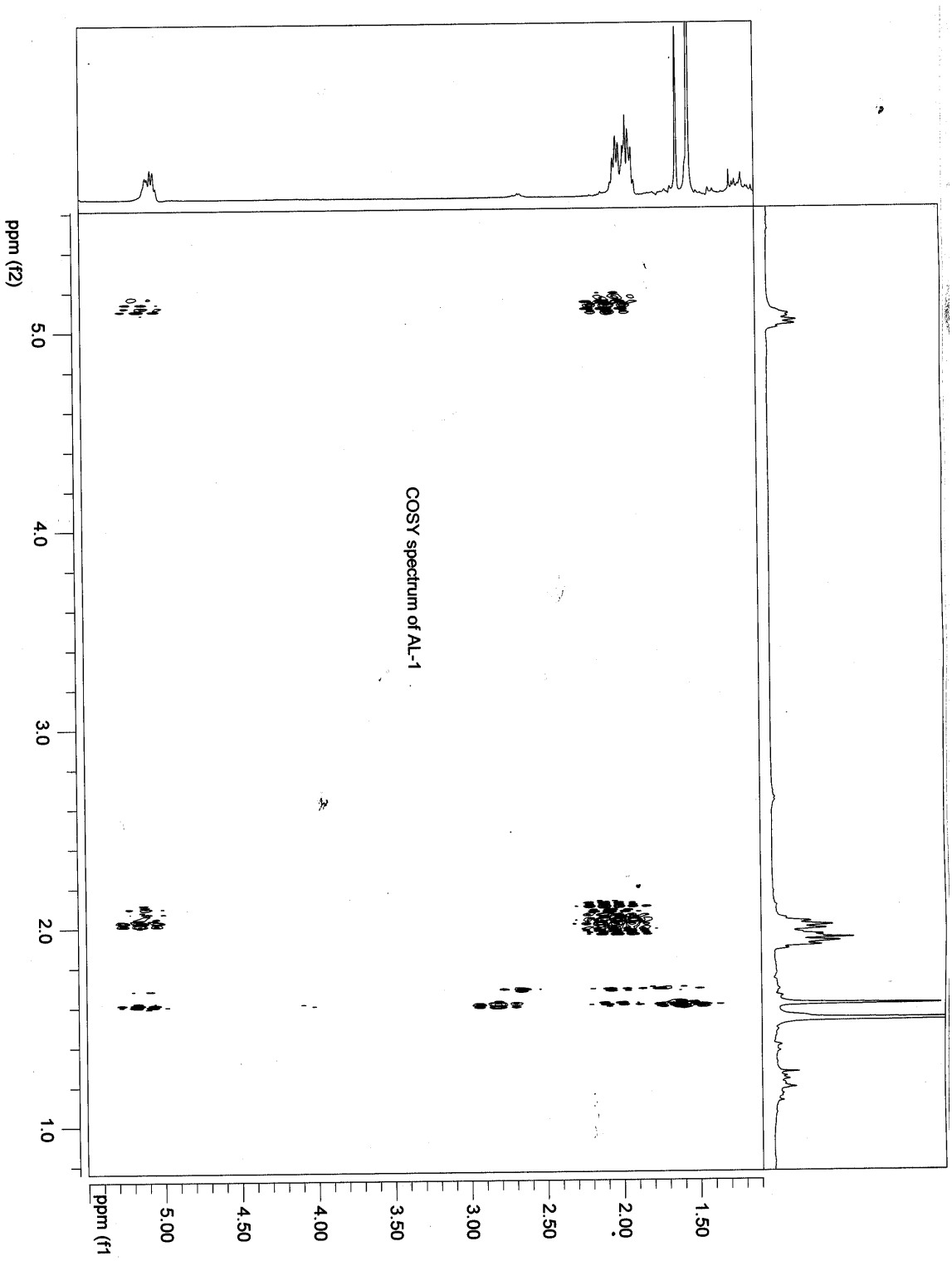
Appendix 4: ^{13}C NMR spectrum of AL-1



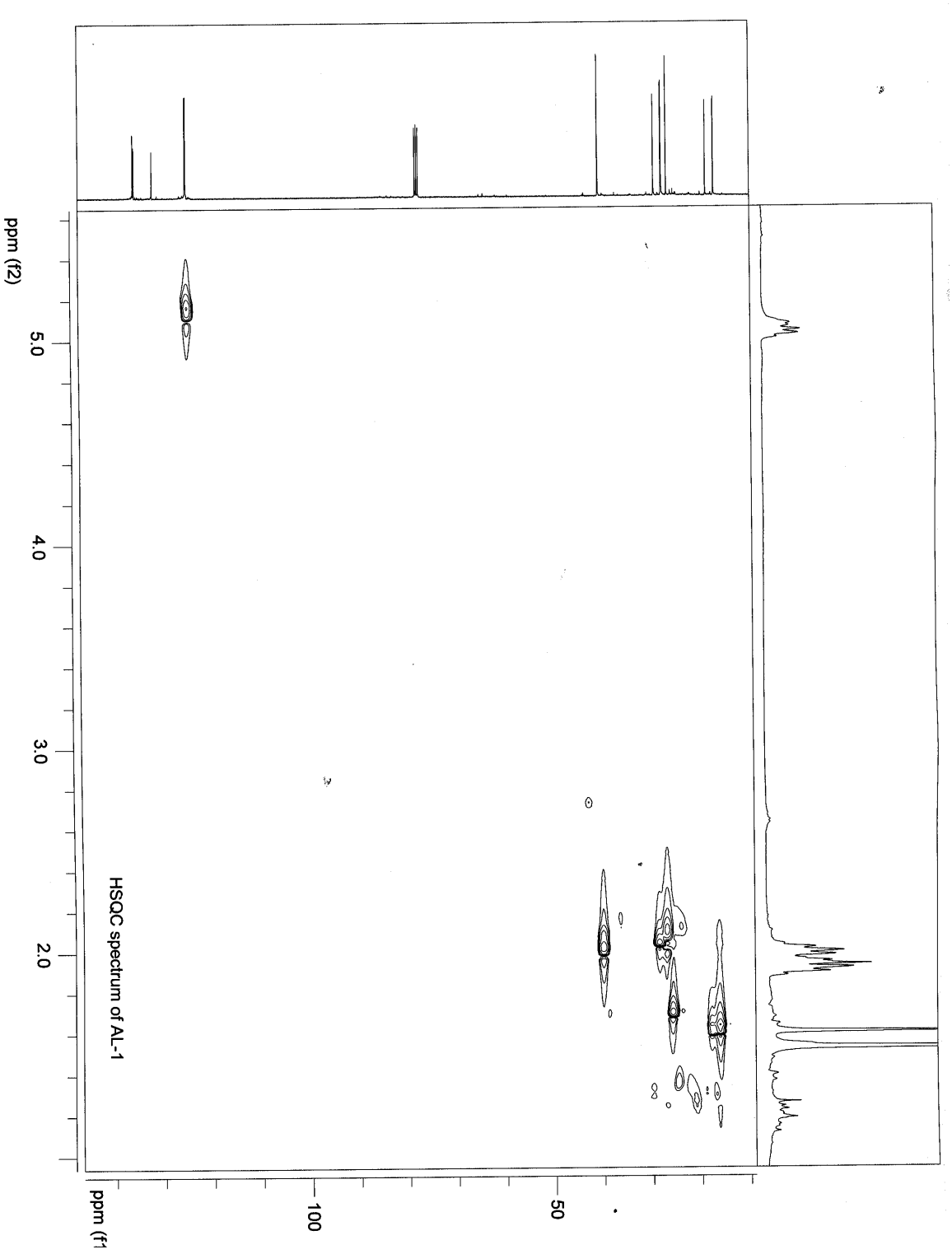
Appendix 5: DEPT-135 NMR spectrum of AL-1



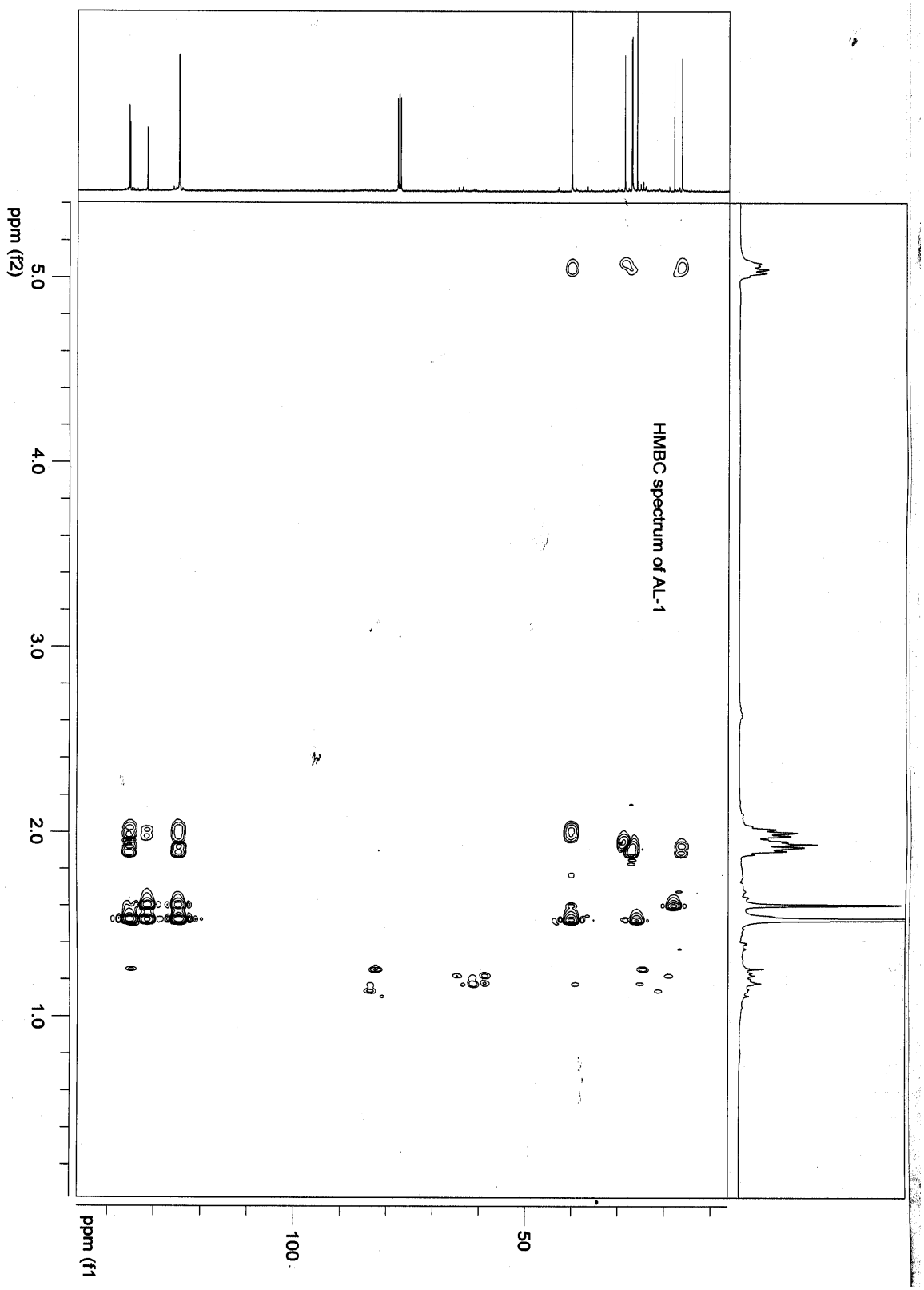
Appendix 6: COSY spectrum of AL-1



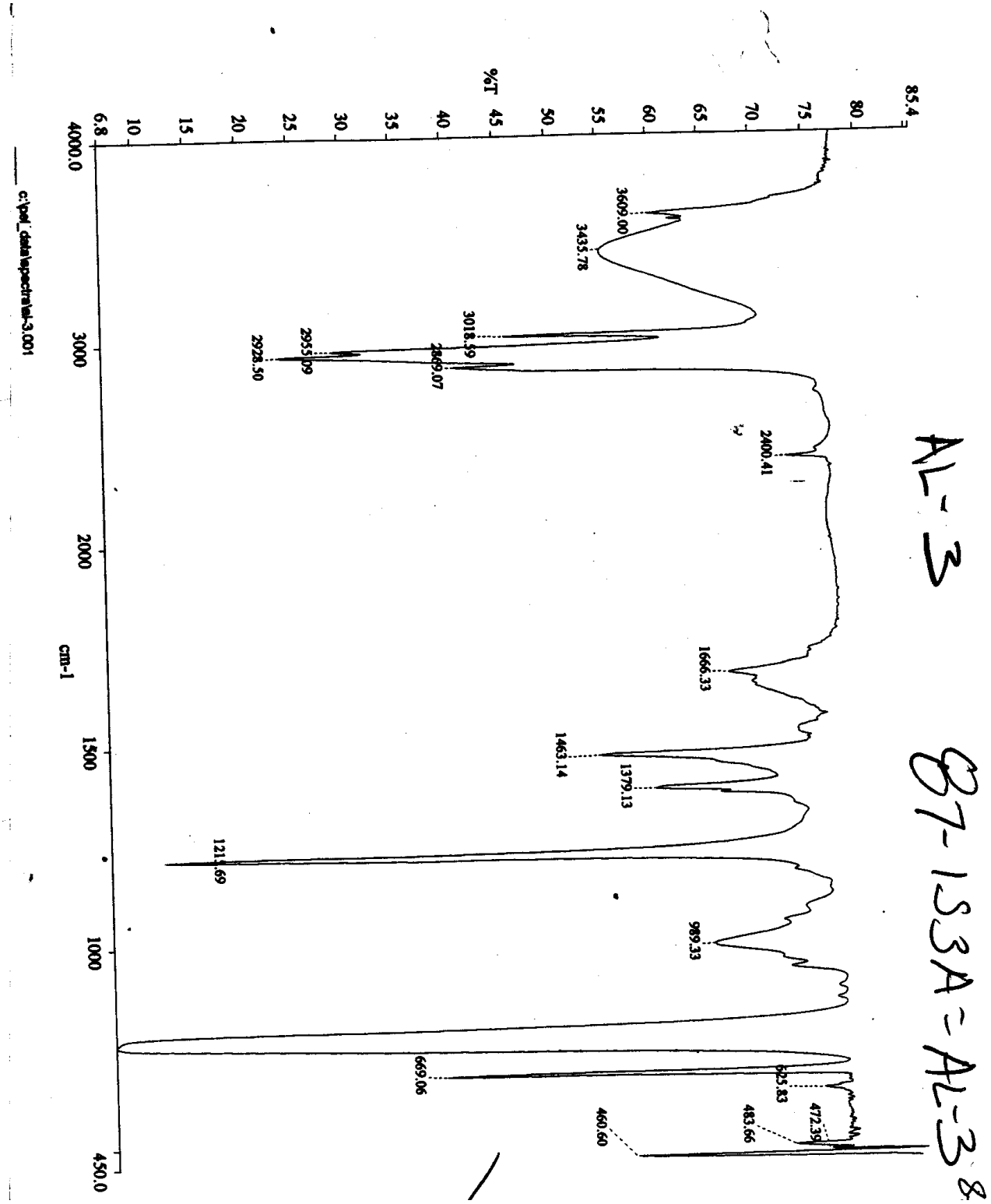
Appendix 7: HSQC spectrum of AL-1



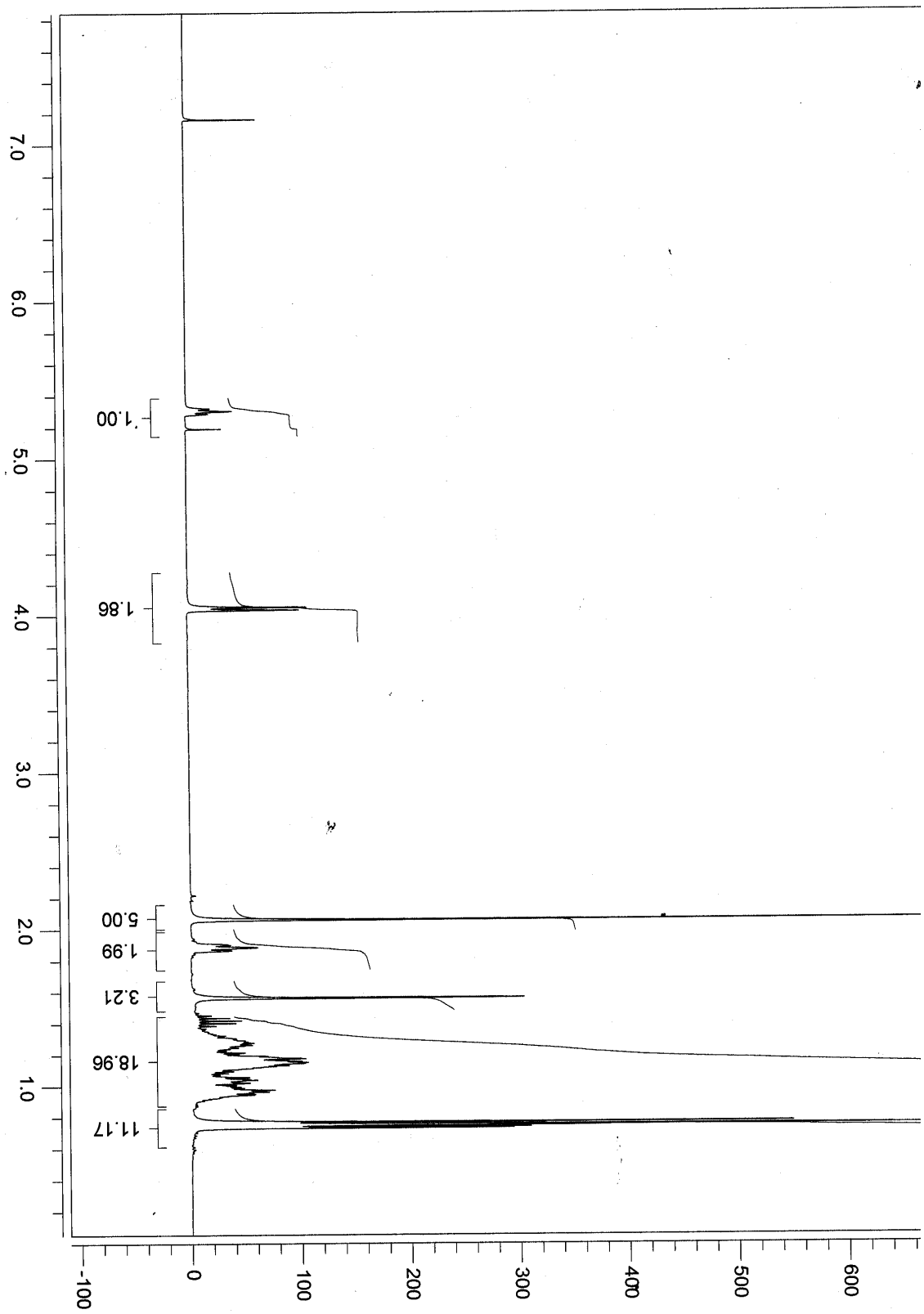
Appendix 8: HMBC spectrum of AL-1



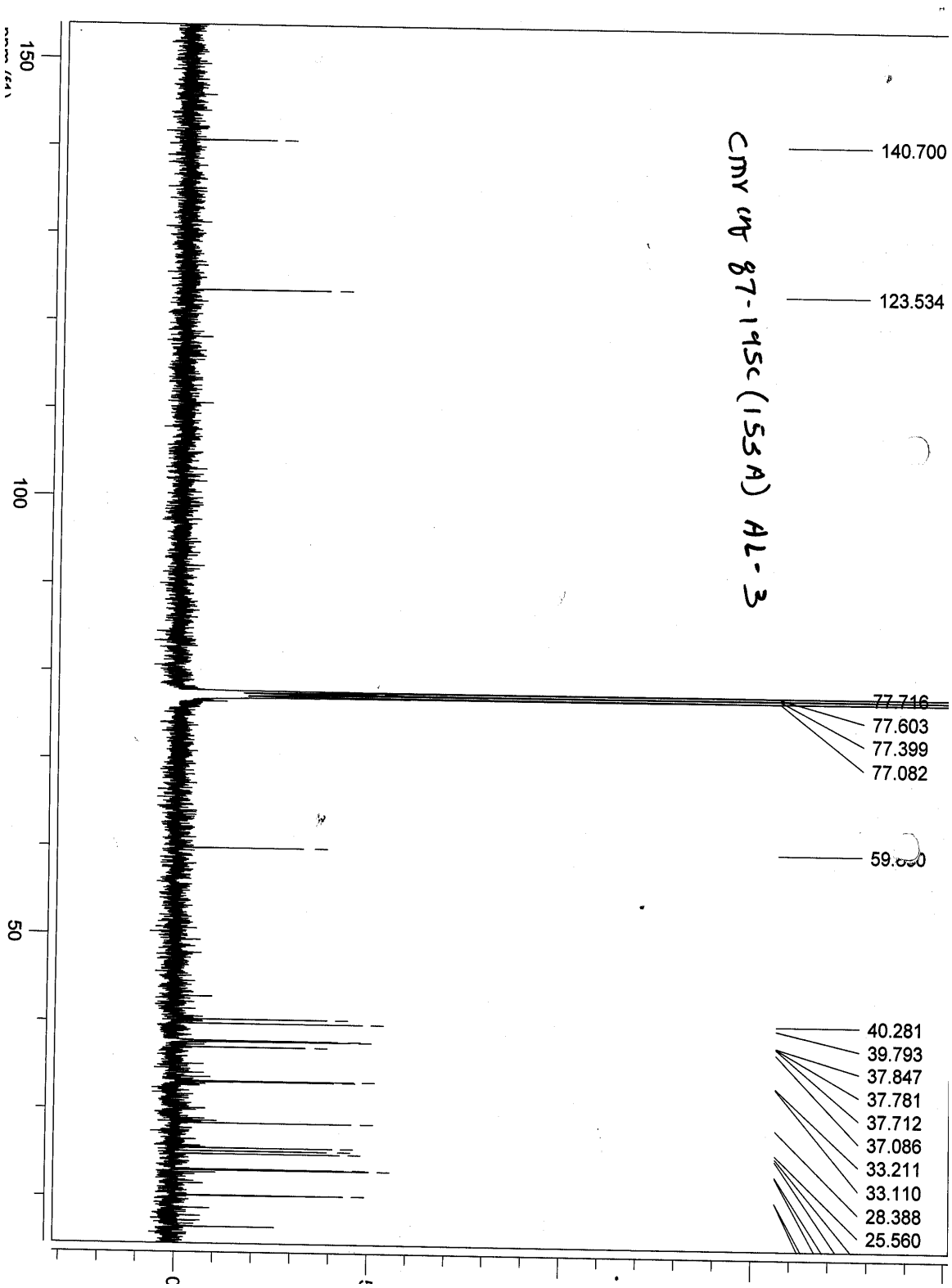
Appendix 9: IR (CHCL3) spectrum of AL-3



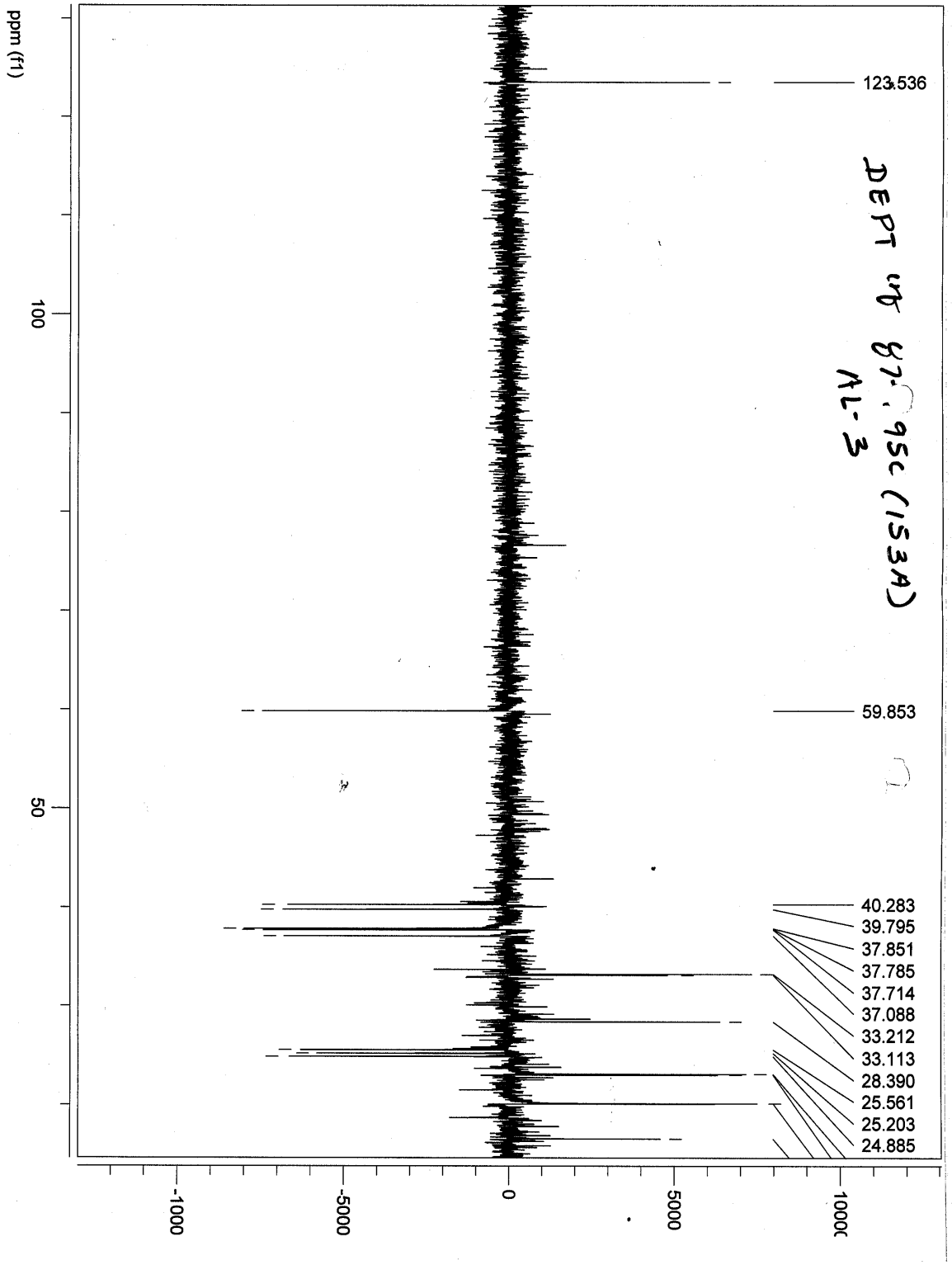
Appendix 10: ^{1}H NMR spectrum of AL-3



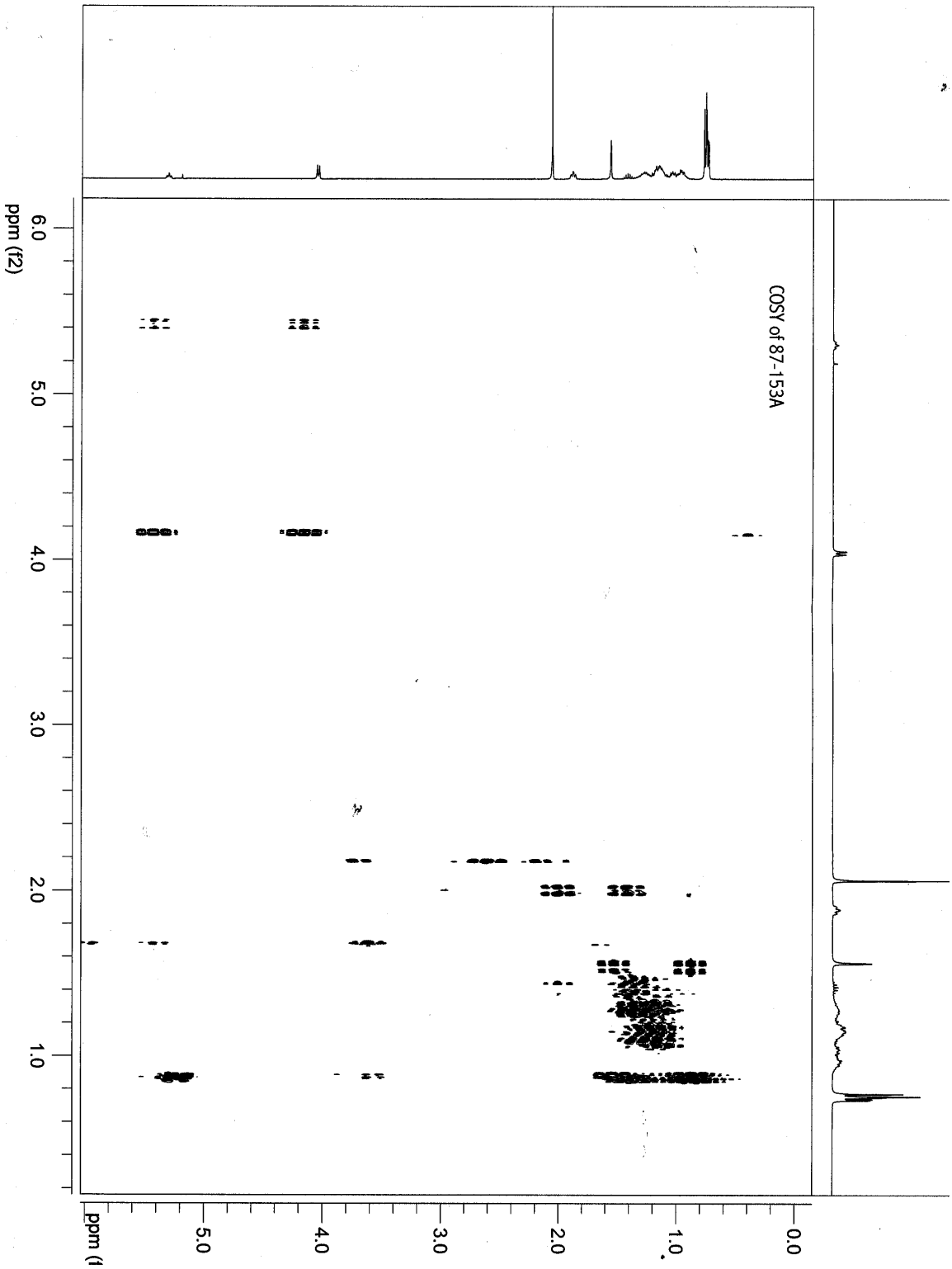
Appendix 11: ^{13}C NMR spectrum of AL-3



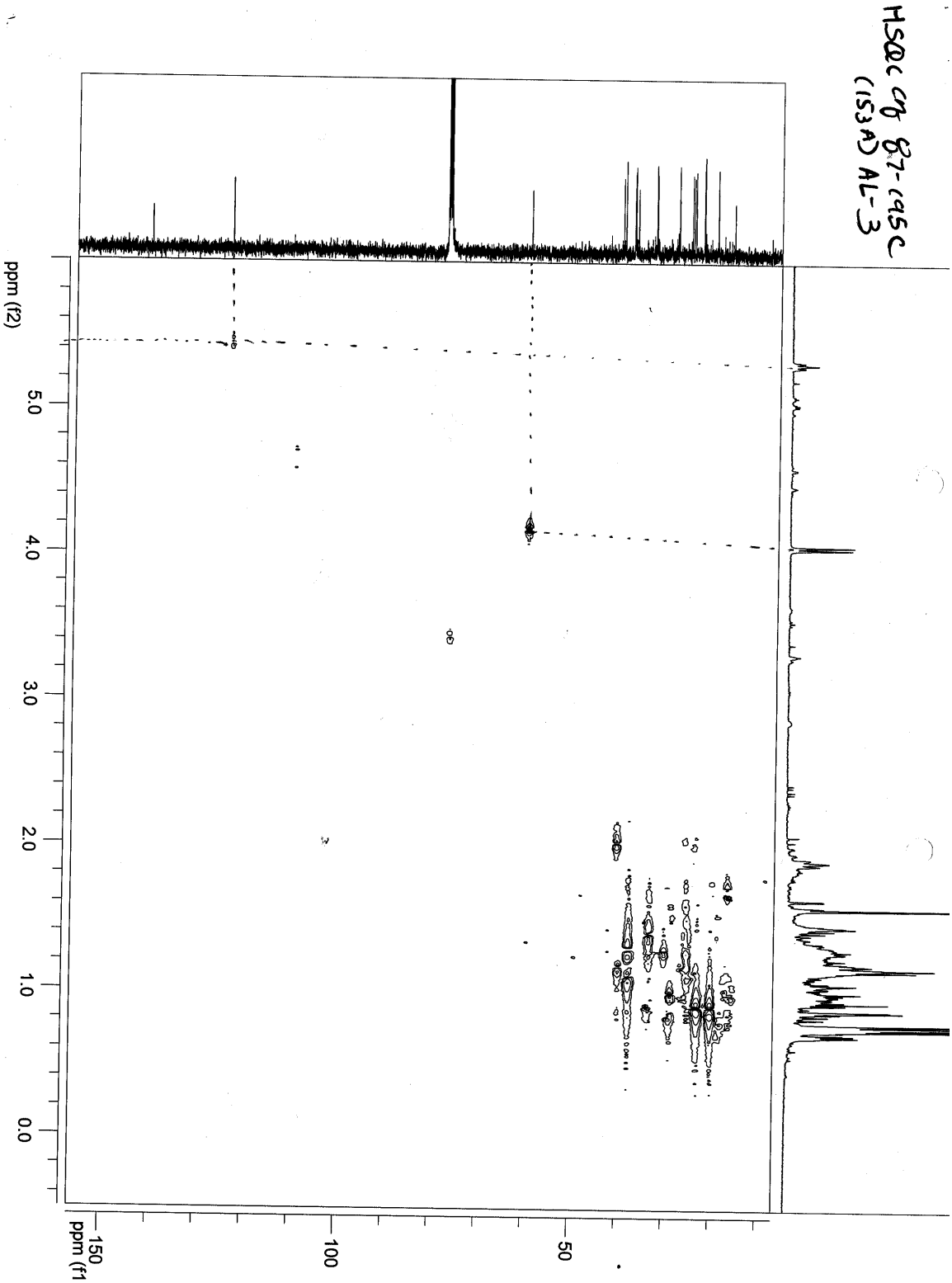
Appendix 12: DEPT-135 NMR spectrum of AL-3



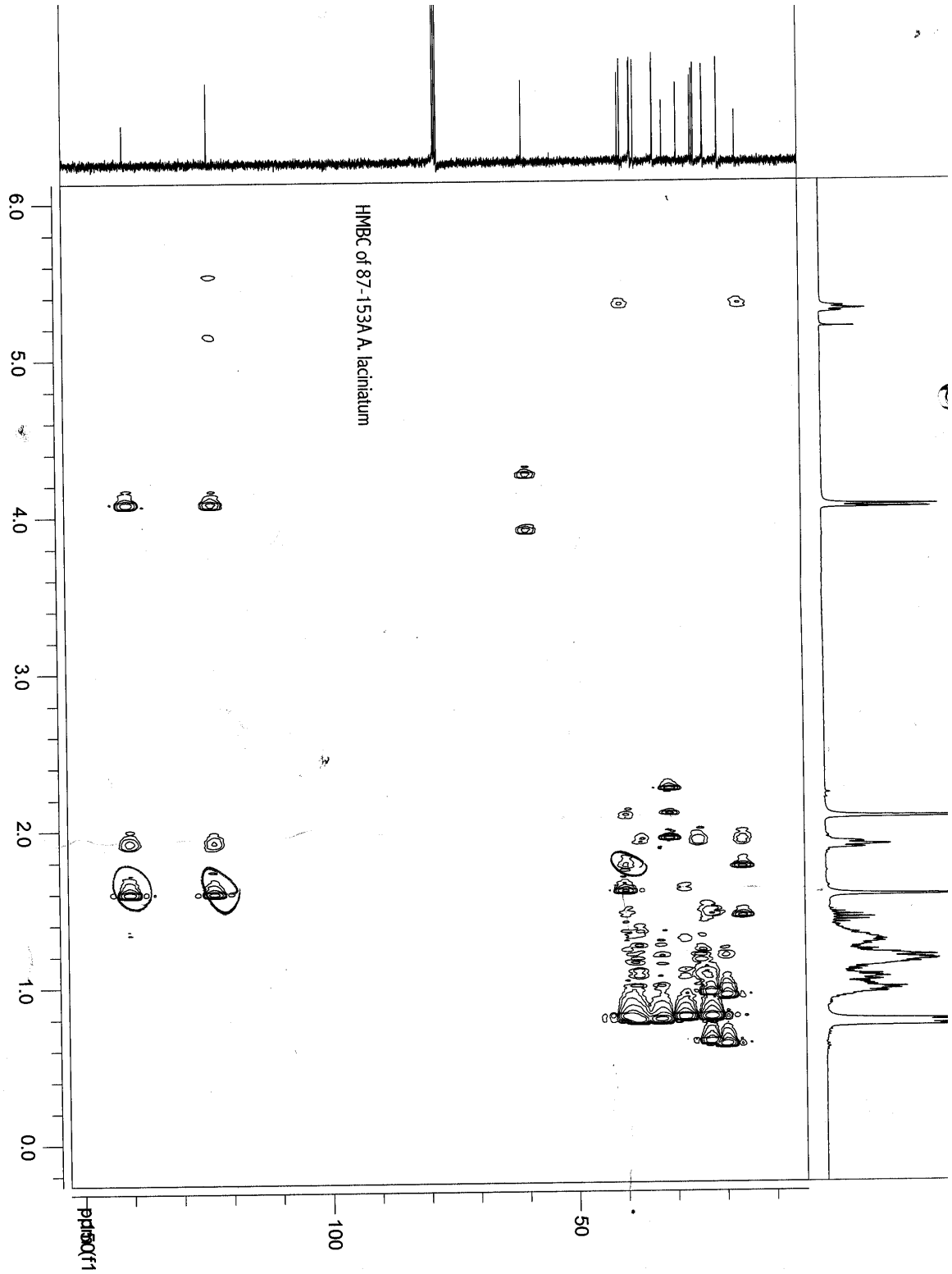
Appendix 13: COSY spectrum of AL-11



Appendix 14: HSQC spectrum of AL-3



Appendix 15: HMBC spectrum of AL-3



Appendix 16: UV-Visible absorption band spectrum of AL-3A

25 March 2005 2:50
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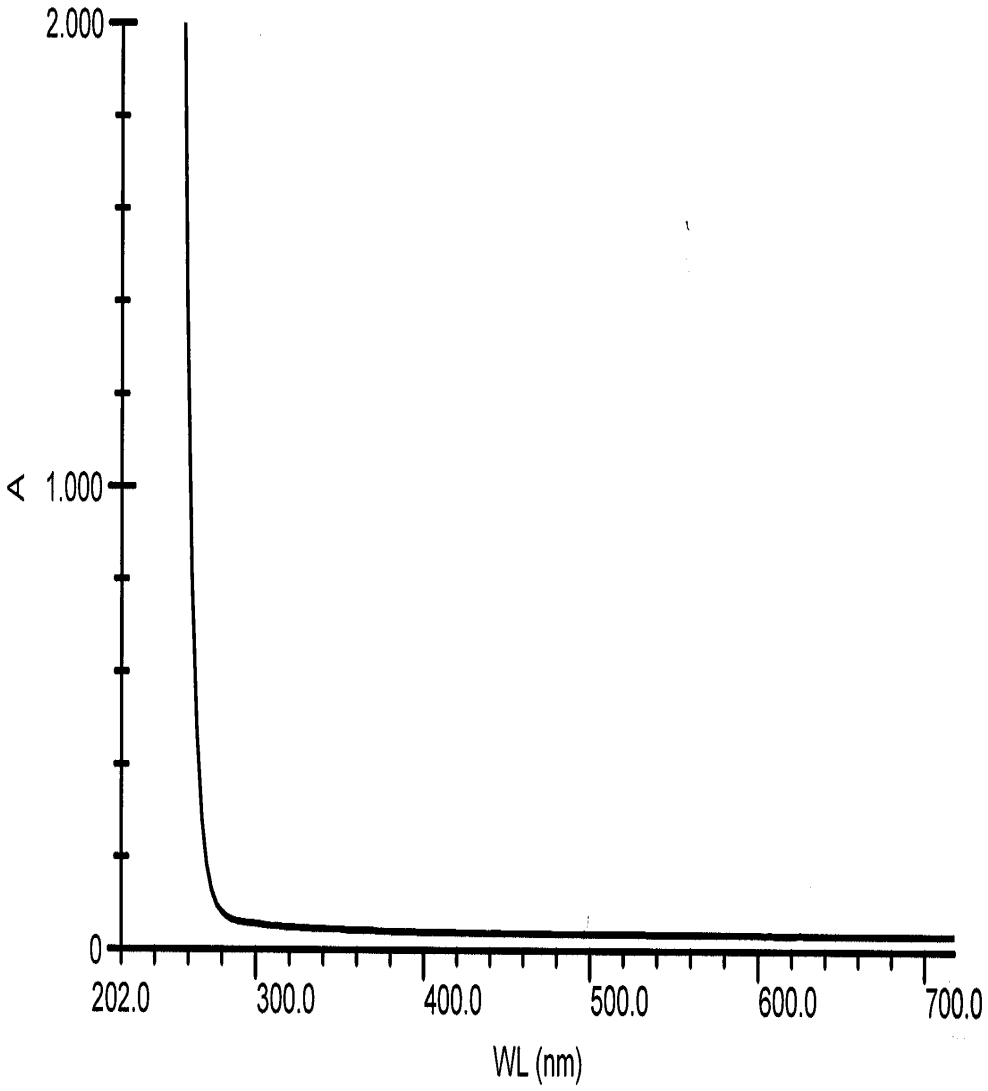
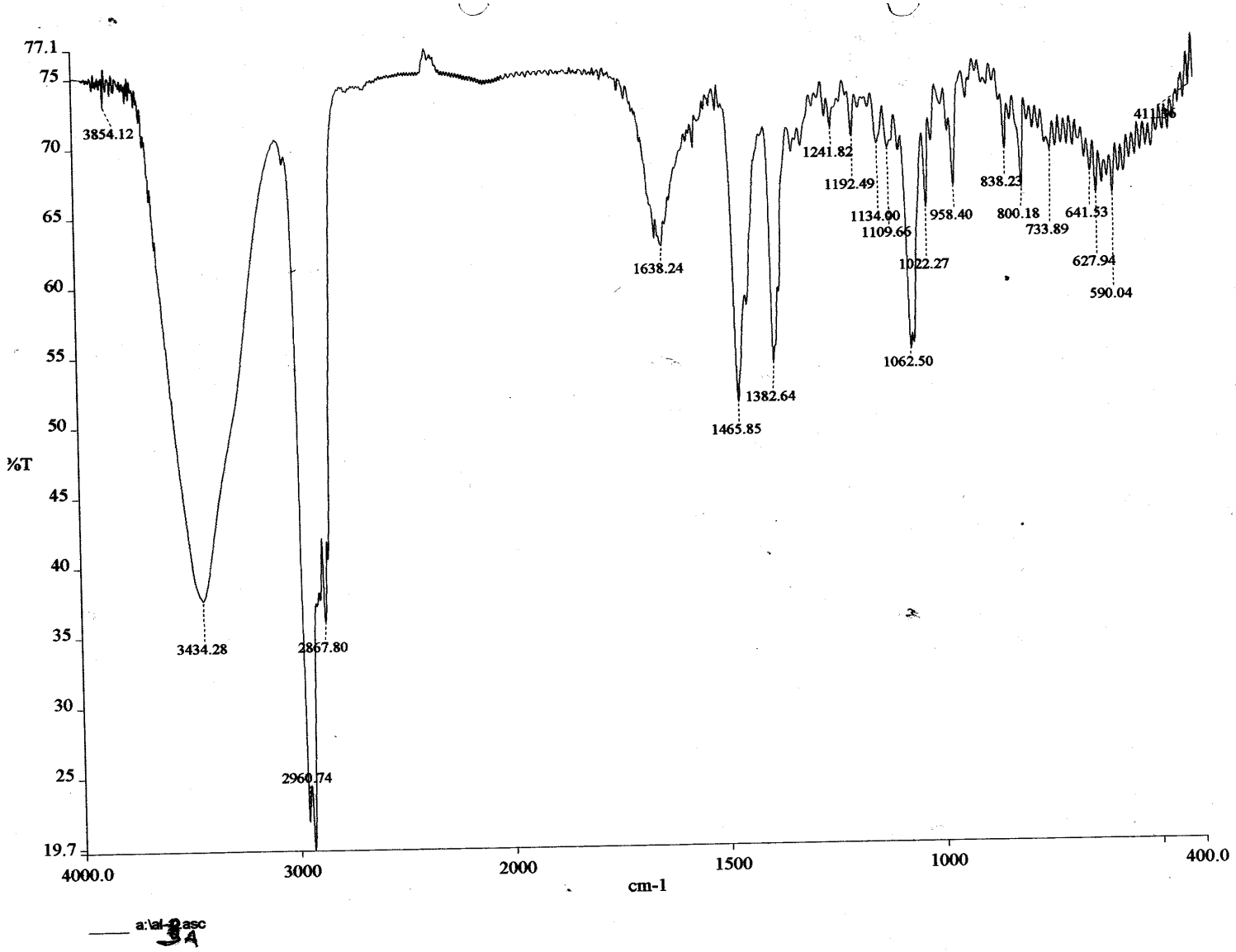
Start Wavelength: 202.0
Stop Wavelength: 700.0

Al8-3A



Appendix 17: IR (KBr) spectrum of AL-3A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50
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2960.74
1638.24
1465.85
1382.64
1241.82
1192.49
1134.00
1109.65
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1022.27
958.40
838.23
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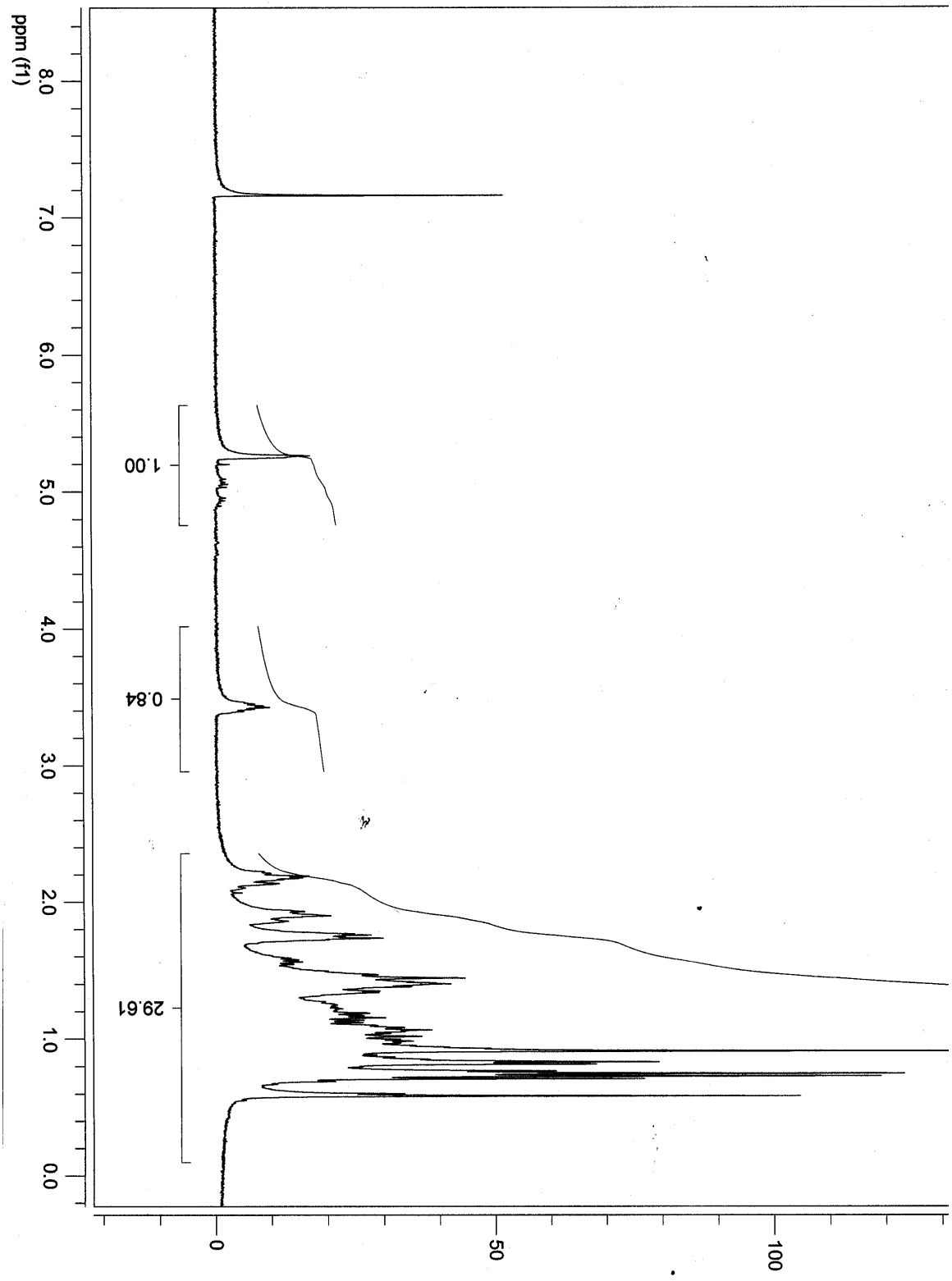
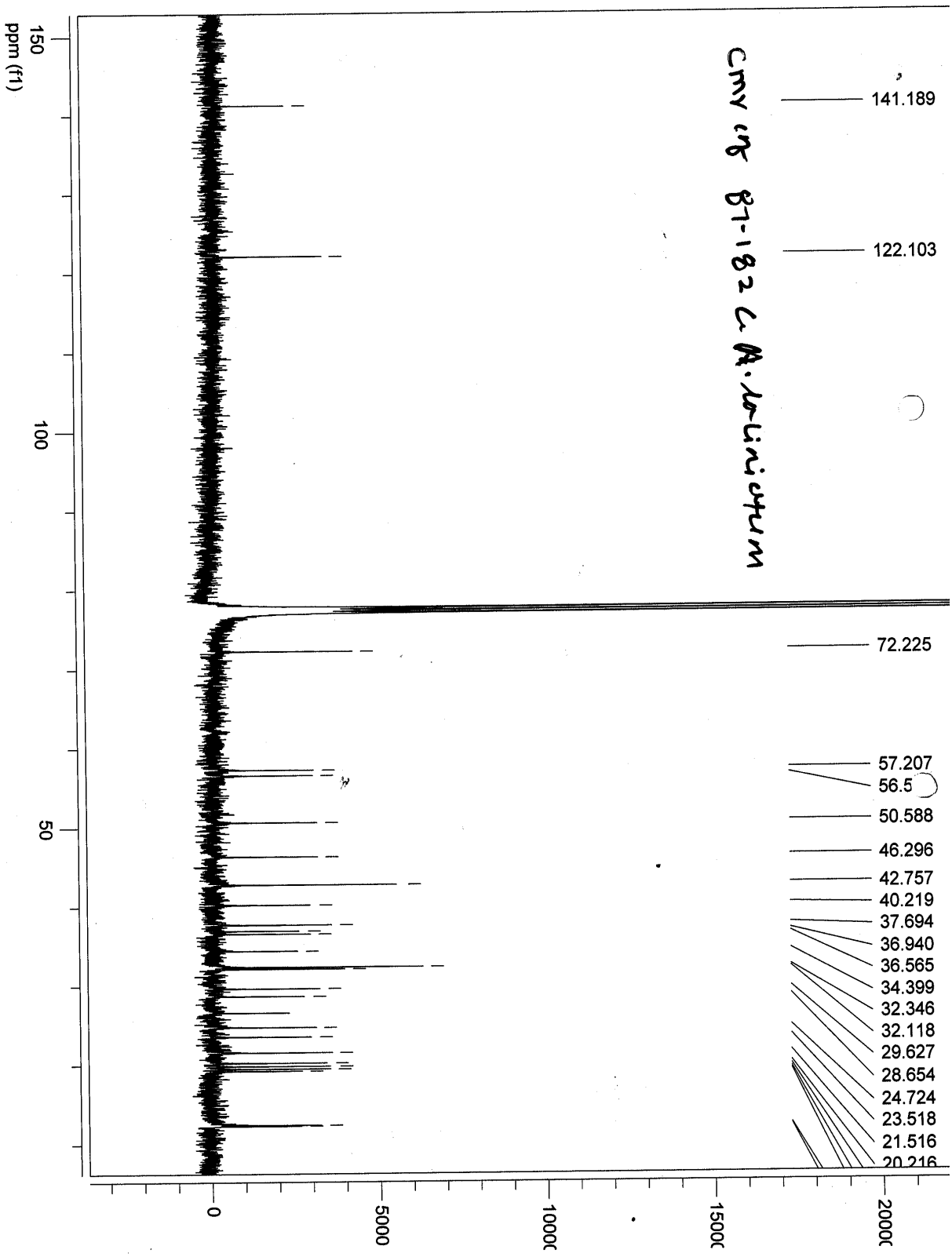
cm-1
a:\al-2.asc

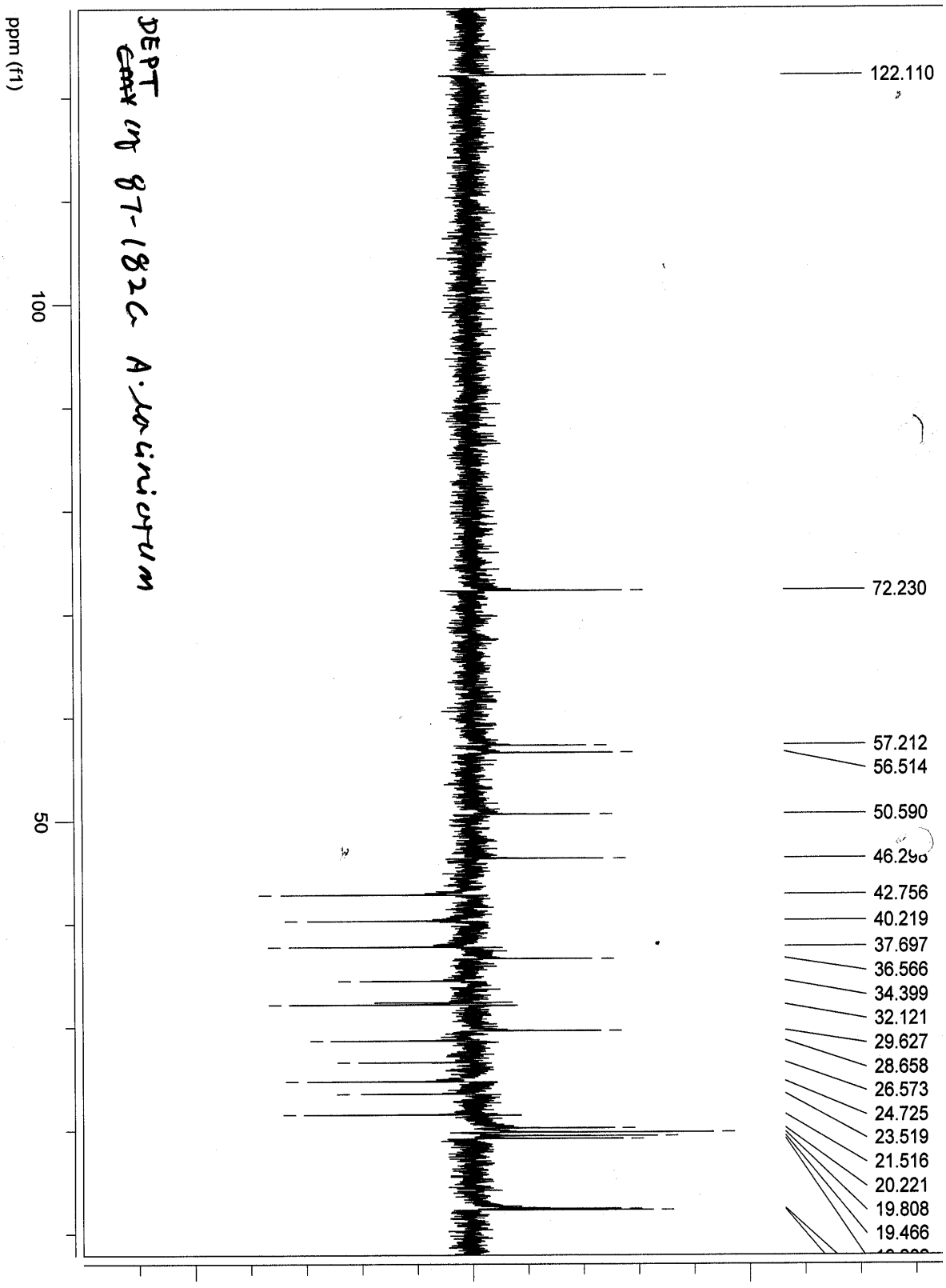
Appendix 18: ^{1}H NMR spectrum of AL-3A



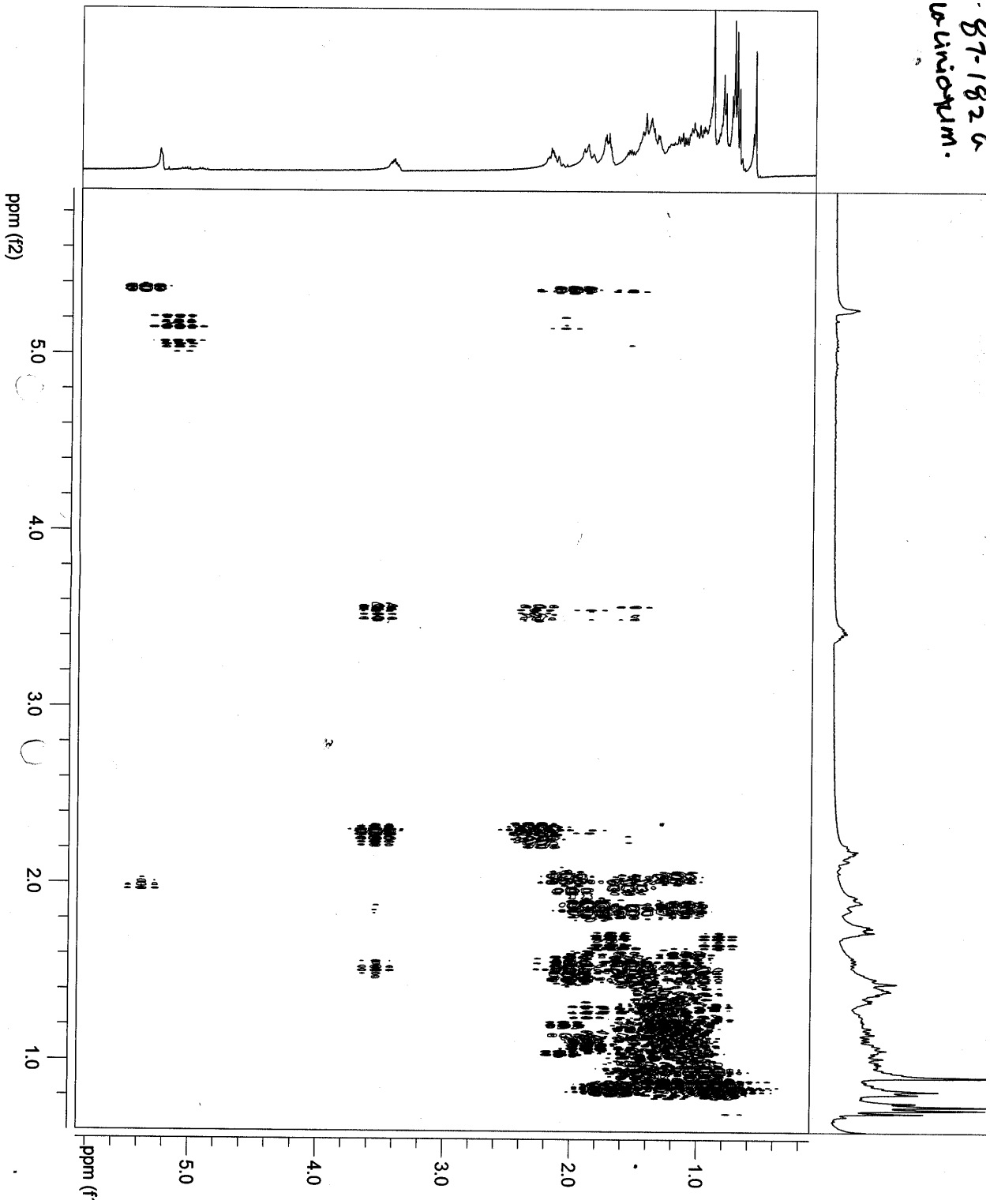
Appendix 19: ^{13}C NMR spectrum of AL-3A



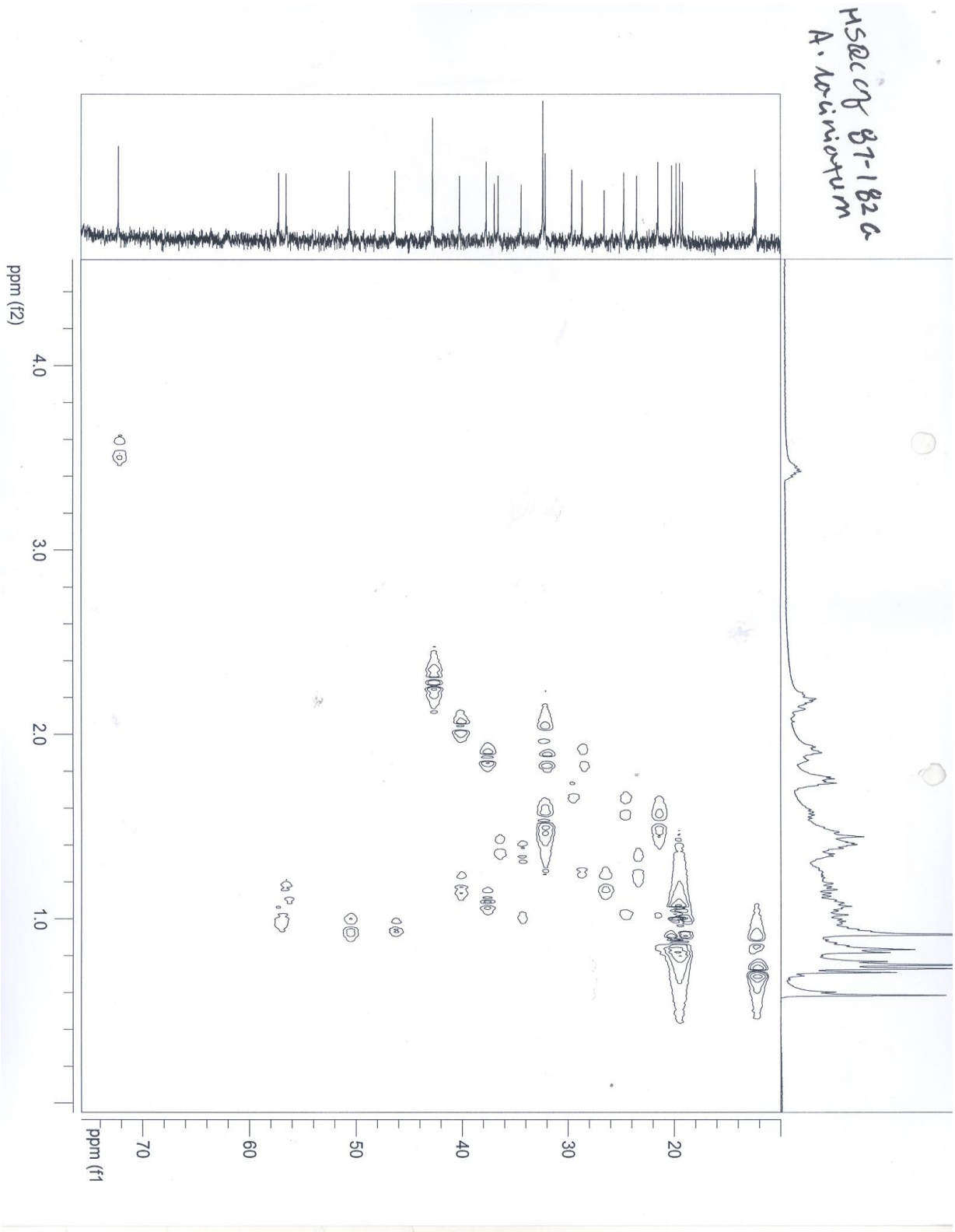
Appendix 20: DEPT-135 NMR spectrum of AL-3A



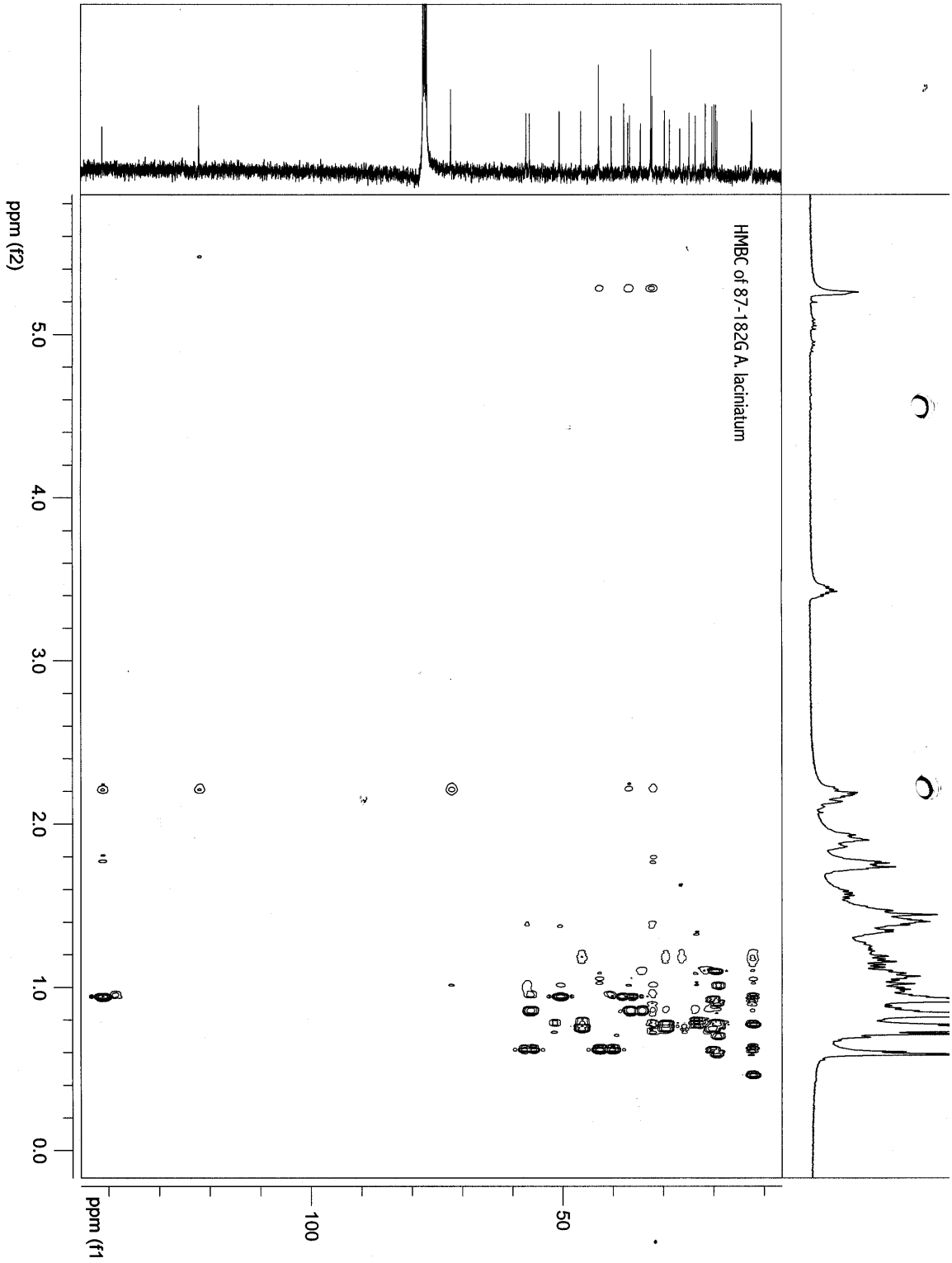
Appendix 21: COSY spectrum of AL-3A



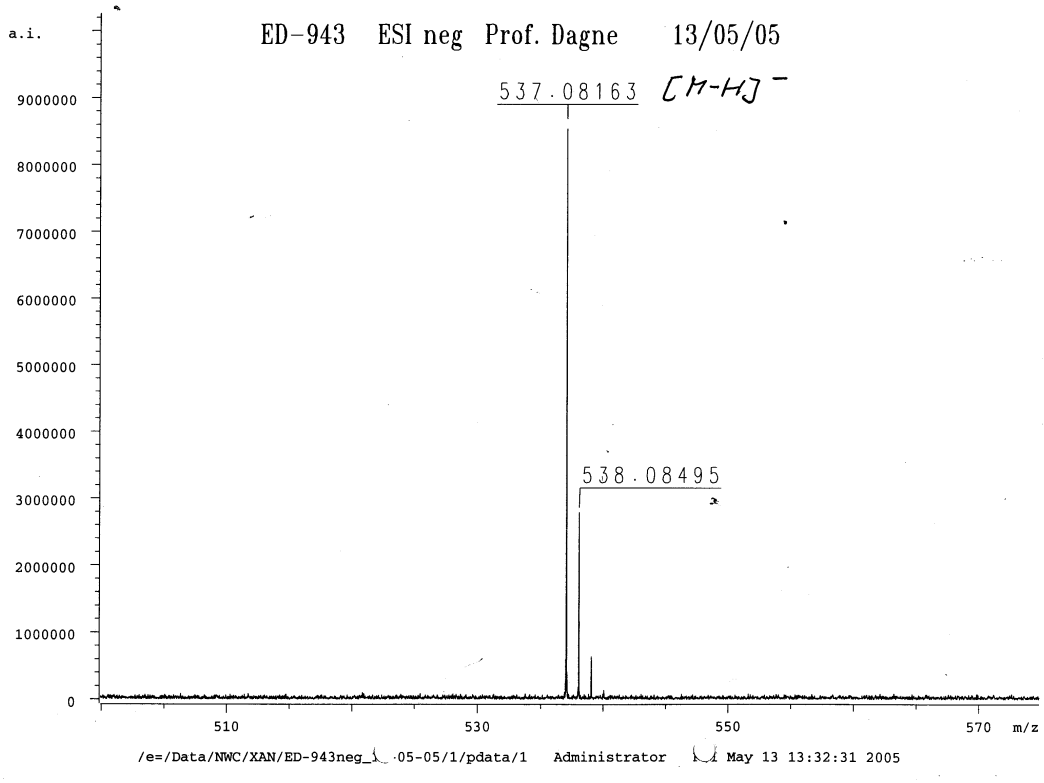
Appendix 22: HSQC spectrum of AL-3A

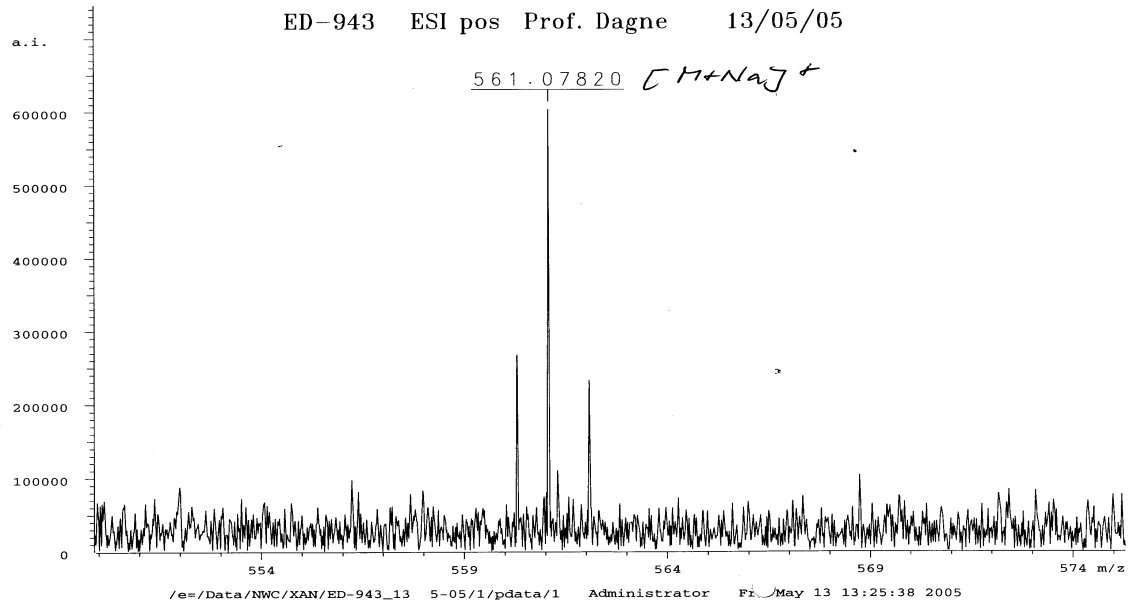


Appendix 23: HMBC spectrum of AL-3A

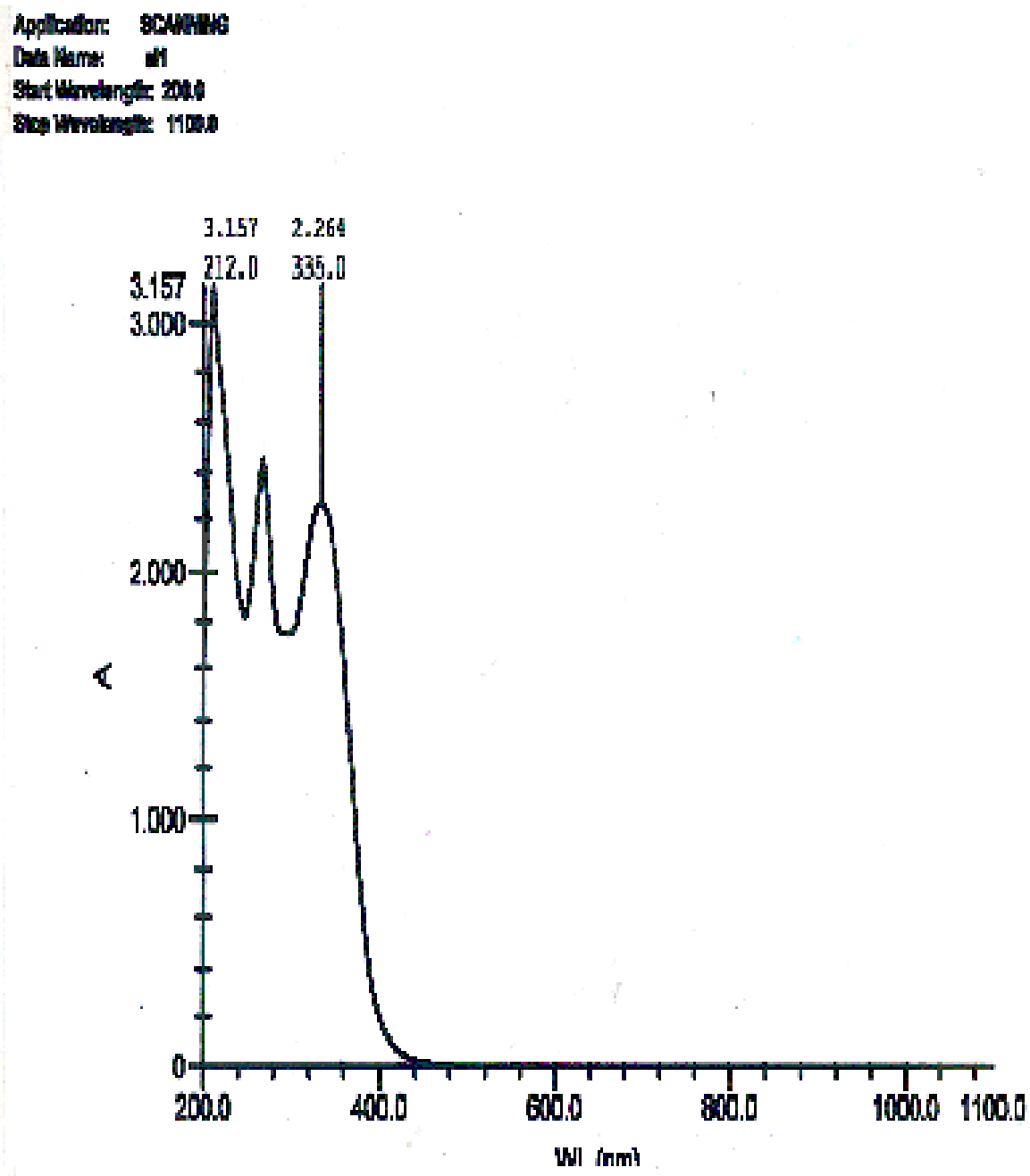


Appendix 24: MS spectrum of AL-11

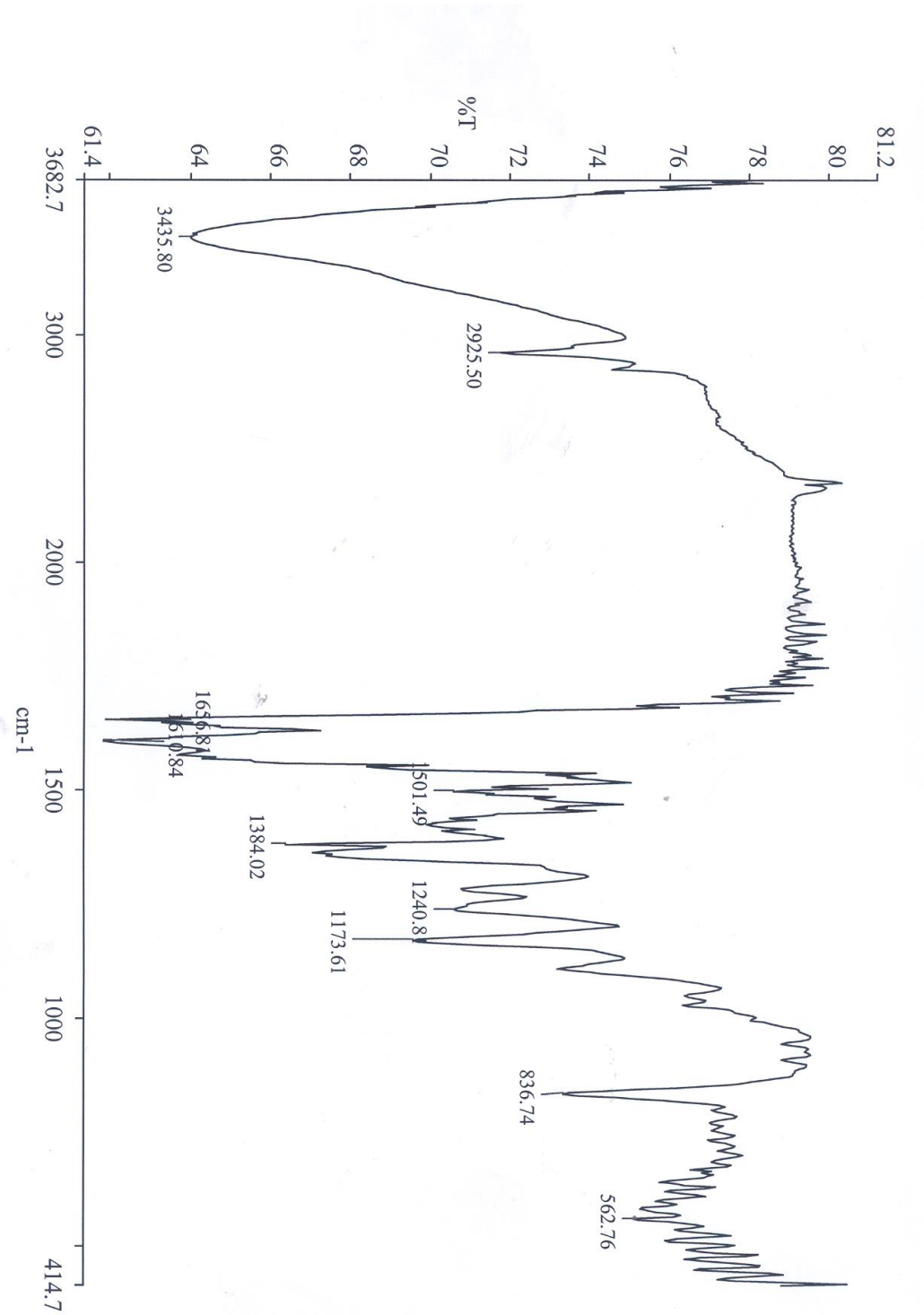

ED-943 ESI neg Prof. Dagne 13/05/05
537.08163 [M-H]-
538.08495
a.i.
9000000
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
510
530
550
570 m/z
/e=/Data/NWC/XAN/ED-943neg_1 05-05/1/pdata/1 Administrator May 13 13:32:31 2005


ED-943 ESI pos Prof. Dagne 13/05/05
561.07820 [M+Na]+
a.i.
600000
500000
400000
300000
200000
100000
0
554
559
564
569
574 m/z
/e=/Data/NWC/XAN/ED-943_13 5-05/1/pdata/1 Administrator Fr May 13 13:25:38 2005

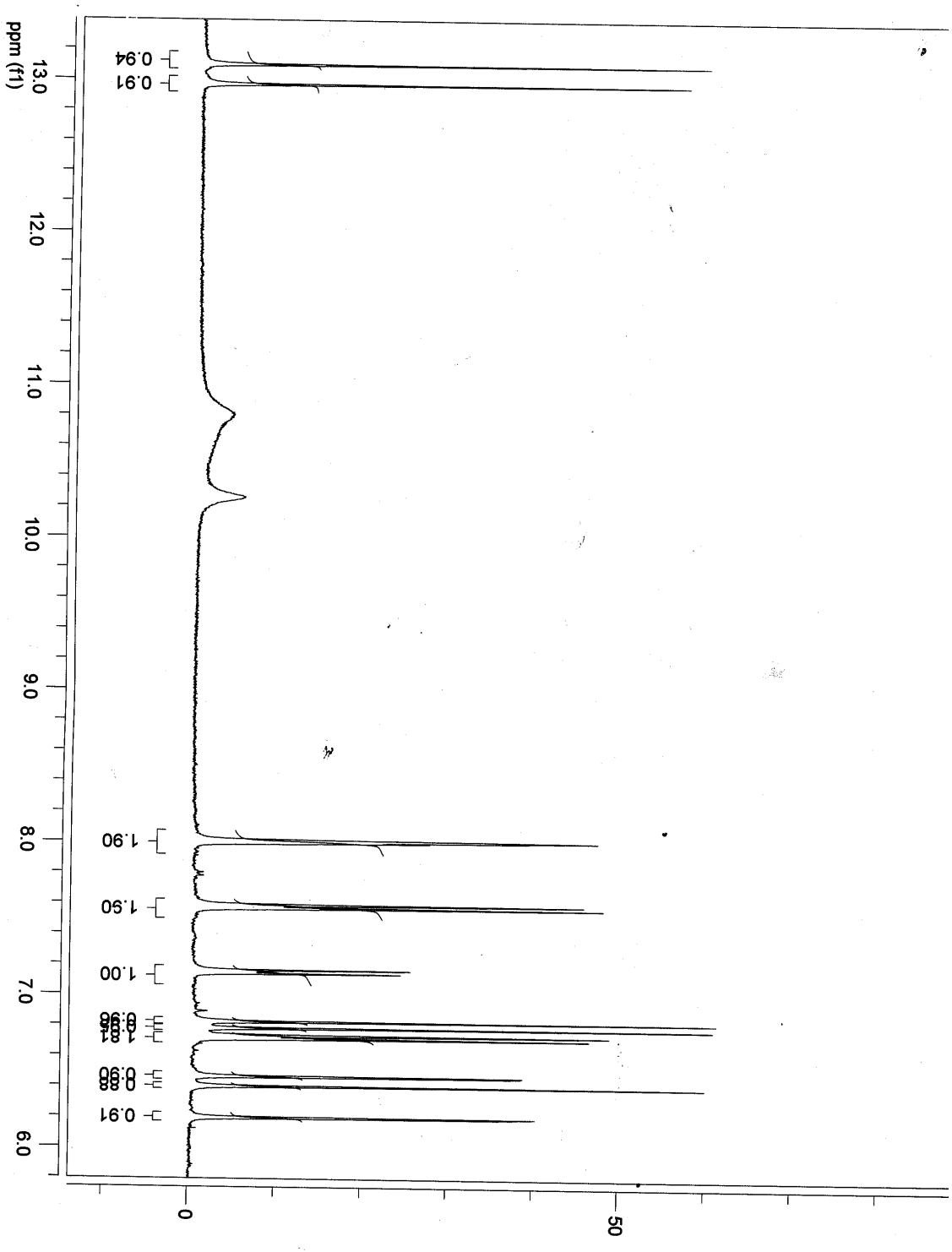
Appendix 25: UV-Visible absorption band spectrum of AL-11



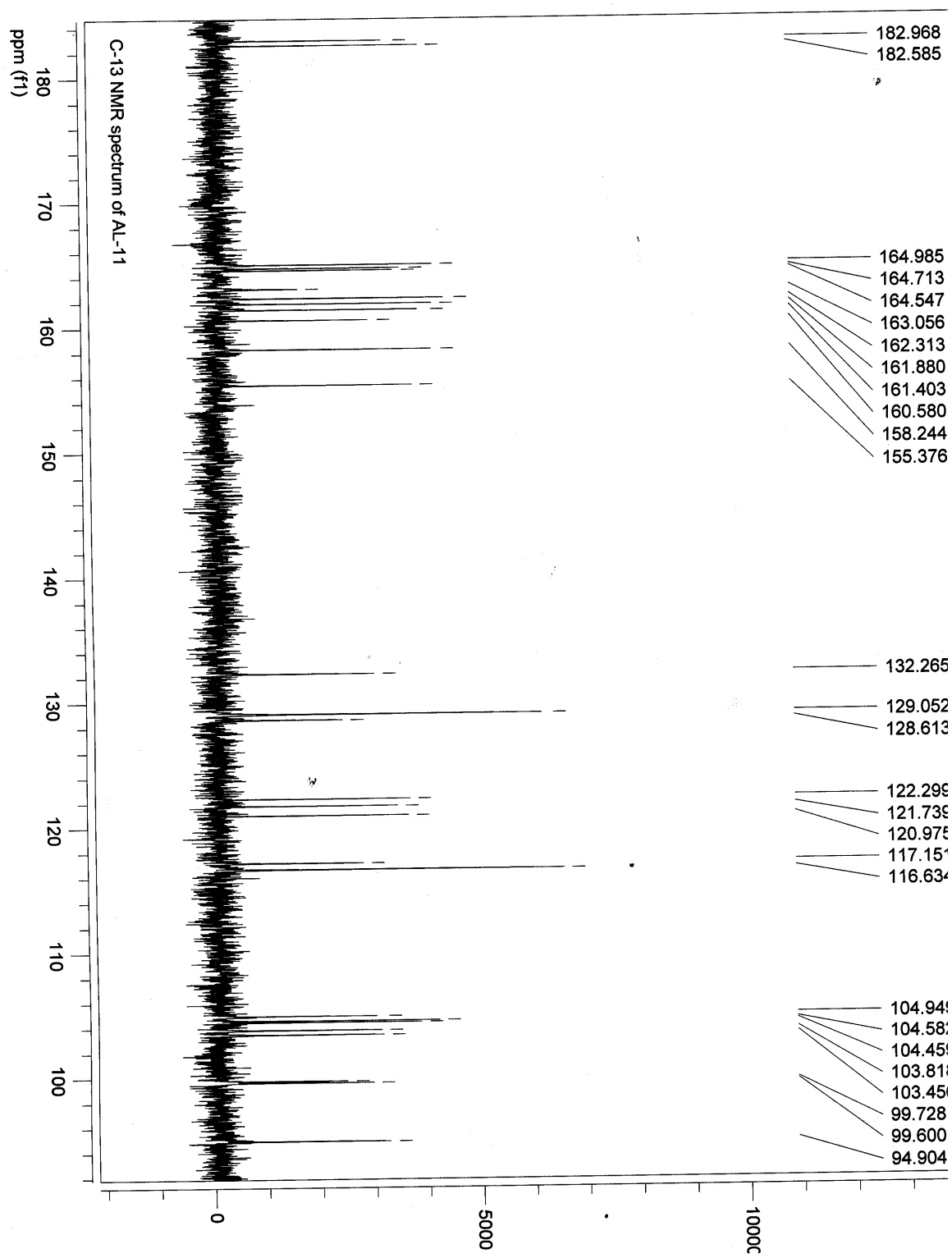
Appendix 26: IR (KBr) spectrum of AL-11



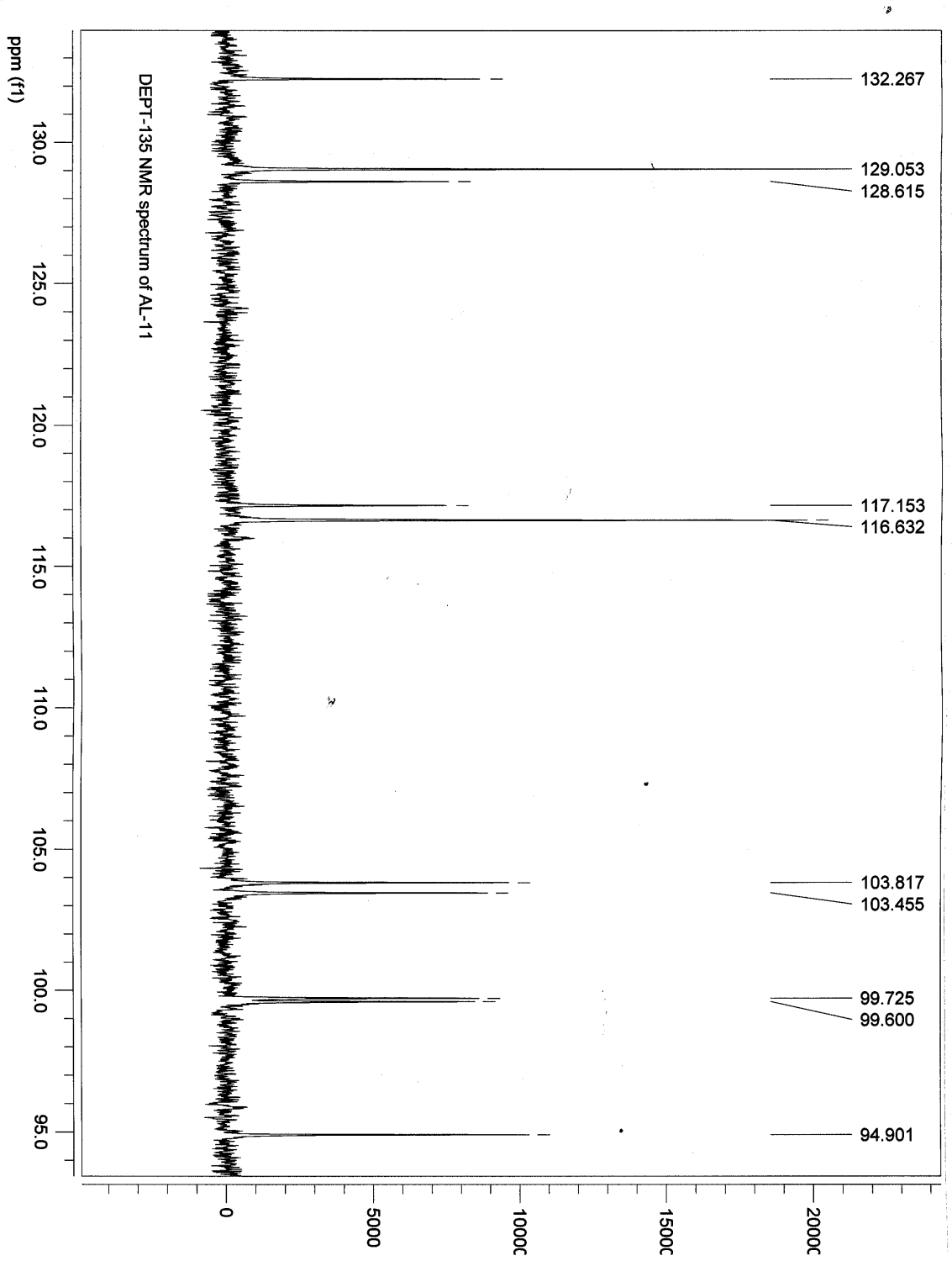
Appendix 27: ^{1}H NMR spectrum of AL-11



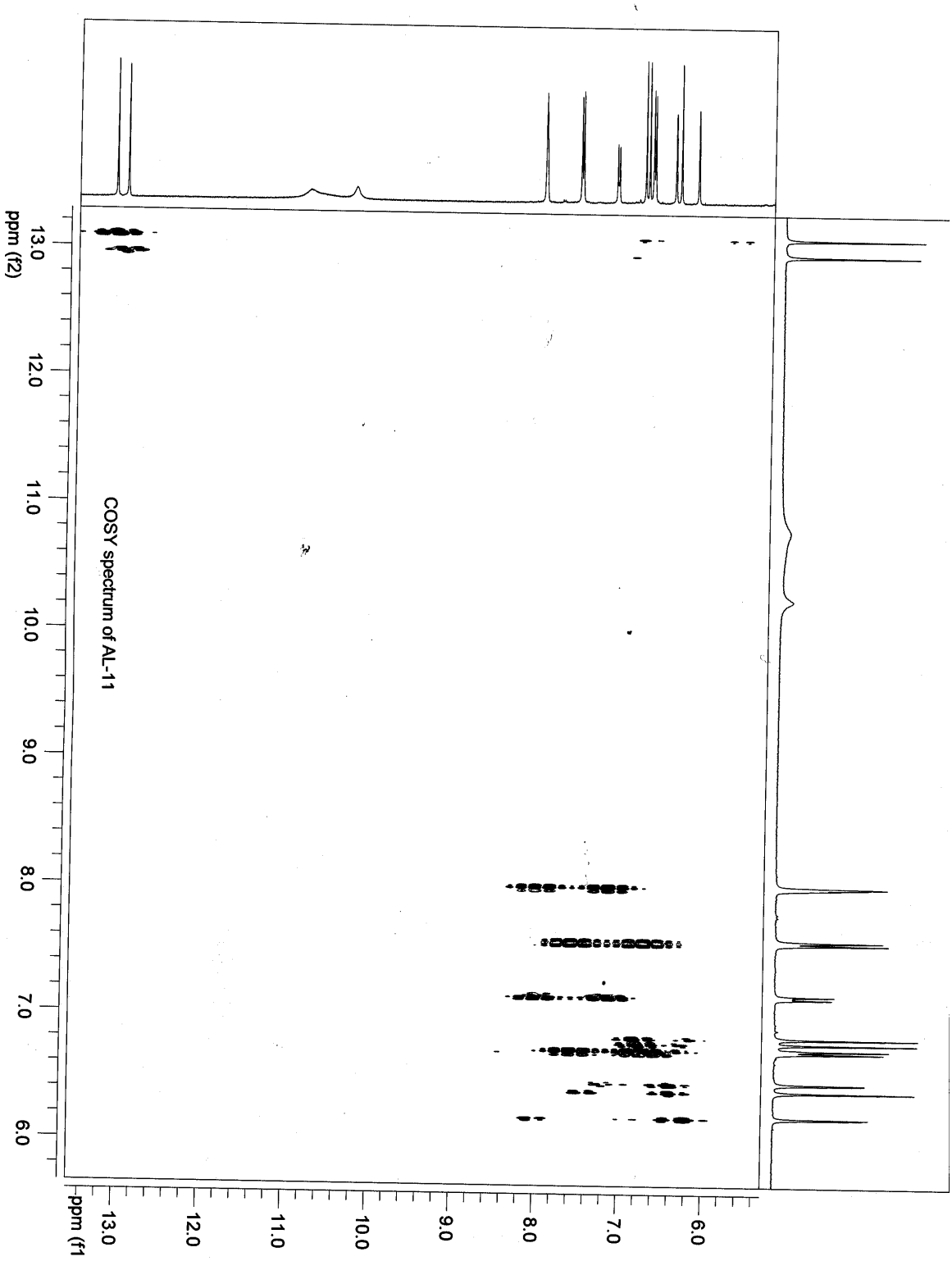
Appendix 28: ^{13}C NMR spectrum of AL-11



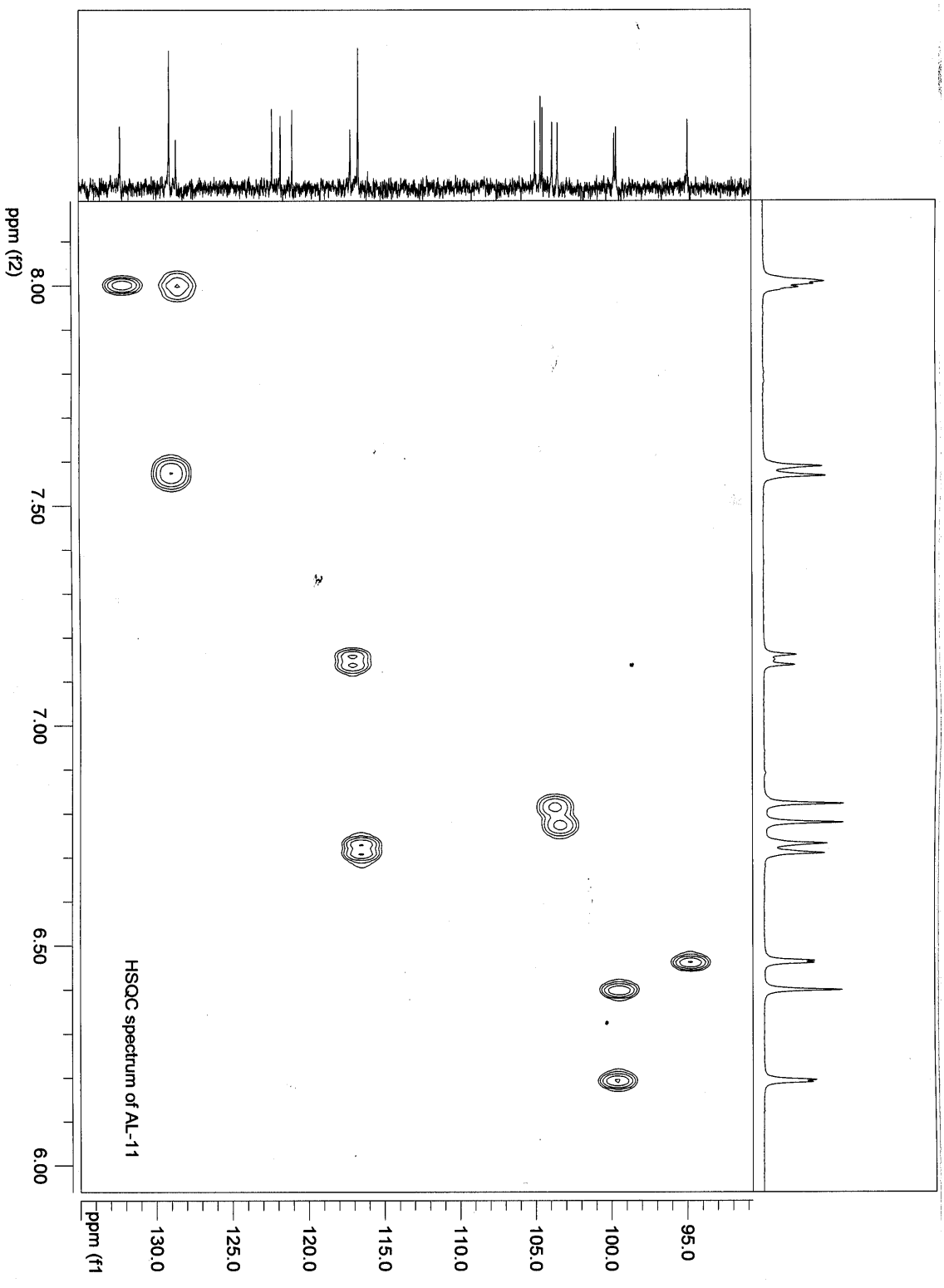
Appendix 29: DEPT-135 NMR spectrum of AL-11



Appendix 30: COSY spectrum of AL-11



Appendix 31: HSQC spectrum of AL-11



Appendix 32: HMBC spectrum of AL-11