DEPARMENT OF CHEMISTRY

GRADUATE PROJECT (Chem.774)

PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF *LAGGERA TOMENTOSA* (Petroleum Ether Extract)

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A Graduate project Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemistry

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By DIBABA AMENU

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ABSTRACT

Chemical investigation on the leaves of *Laggera tomentosa*

By

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Advisor: Dr. Nigist Asfaw

In this project, a phytochemical study of laggera tomentossa has been under taken and chemical investigation on the solvent extract of the plant has been conducted.

The pet-ether extract of the leaves of L. tomentossa led to isolation of two Cuauthemone type sesquiterpene compounds. The compounds were identified to be 4-O-Acetylcuauthemone 3-O-angelate (**LTP-1**) and 4-O-Acetylcuauthemone3-O-(2'-methyl-2'-hydroxy-3-acetoxy butyrate) (**LTP-3**)**.** Structural determination was accomplished by means of spectroscopic methods(UV,IR,1D,and 2DNMR). **LTP-3** was identified as cuauthemone sesquiterpenoides previously from *Bluma alata*

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1. Introduction

Phytochemical studies of plants especially of medicinal plants are of great importance in developing drugs. They are useful in the study of chemotaxonomy and plant biodiversity as well as in documenting knowledge. Enhancing the knowledge of biological and pharmacological effects of plant constituents and determination of the structures of the active principles may help in sustaining the use of these products.[1]

1.1 Natural product

Natural products are secondary metabolites of an organism. In most instances they appear to be non-essential to the plant, insect or micro organism producing them in marked contrast to the other organic compounds in nature (sugars, amino acids, nucleotides and the polymers derived from them) which are both essential and ubiquitous.[2, 3]

Natural products became a necessity to mankind. They have been immensely utilized for various purposes, such as, foodstuff, as weapons, treatment of diseases, in their crude form[3]. The contribution of Natural products to the development of medicine could be demonstrated by the amount of plant derived drugs being used. In general 40% of modern drugs are said to be of natural origin.[1, 4]

1.2 Terpenes

The terpenes are among the most widespread and chemically diverse groups of Natural products. Fortunately, despite their structural diversity, they 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 isopentane (2-methyl butane) units.[5]

1.2.1 Classification of Terpenes

Terpenes are 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} (very rare), Triterpenes C_{30}, Tertaterpenes C_{40}). Like all natural products, within this simple classification lies an enormous amount of structural diversity which leads to a wide variety of terpene like (terpenoid) compounds.[5]

To day, Monoterpenes (C_{10}) and sesquiterpenes (C_{15}) are the basis of many of our finished goods; perfumes for cosmetics, soaps and detergents; for flavoring certain processed meats and tinned goods: in confectionery; and as active ingredients of oral preparations (e.g. tooth pest and mouth wash.[6]

1.3 Biogenesis of Terpenoids

The terpenes are of a similar biogenetic origin, in which isopentenyl pyrophosphate and diethyl allyl pyrophosphate combine to yield Geranyl pyrophosphate leading to monoterpenes. Similarly, compounds derived from farnesyl pyrophosphate lead to sesquiterpenes, and triterpenes are formed from two equivalents of farnesyl pyrophosphate. These various combinations and oxidations give rise to large variety of terpenes.[7, 8, 9] (fig.1)

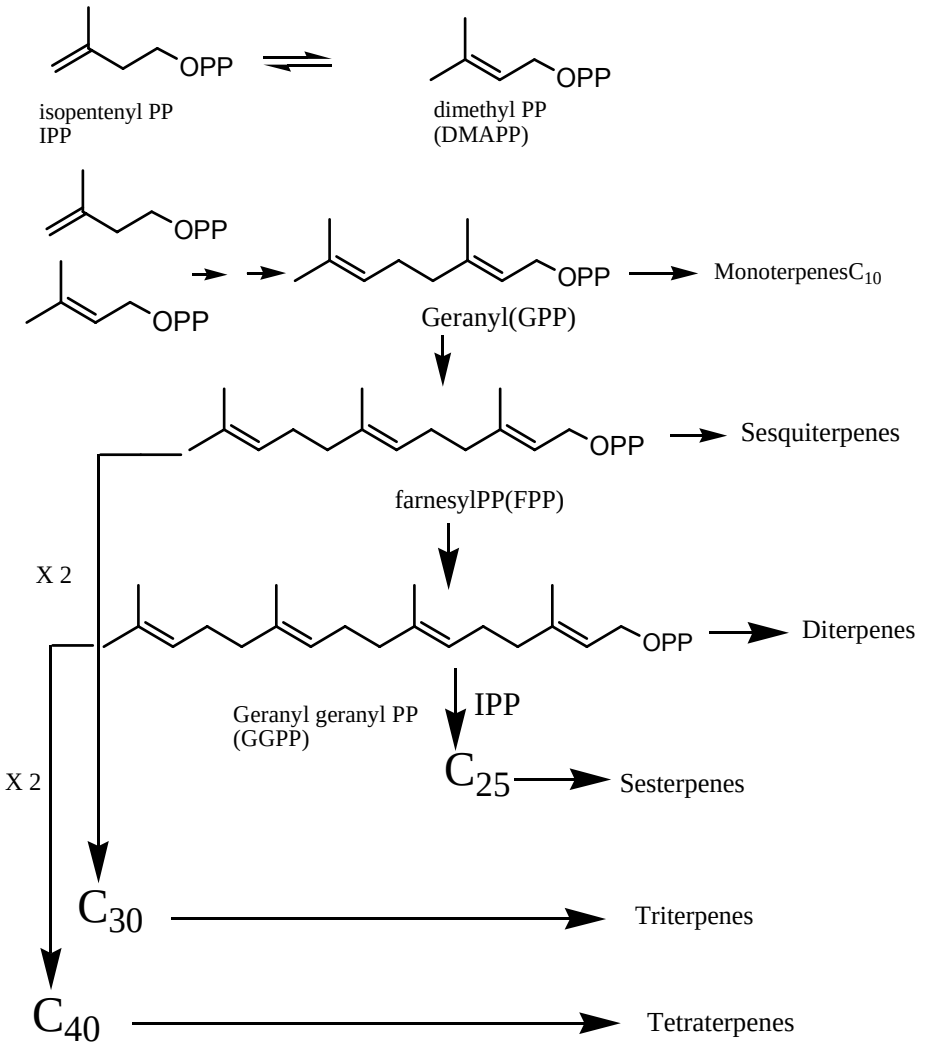
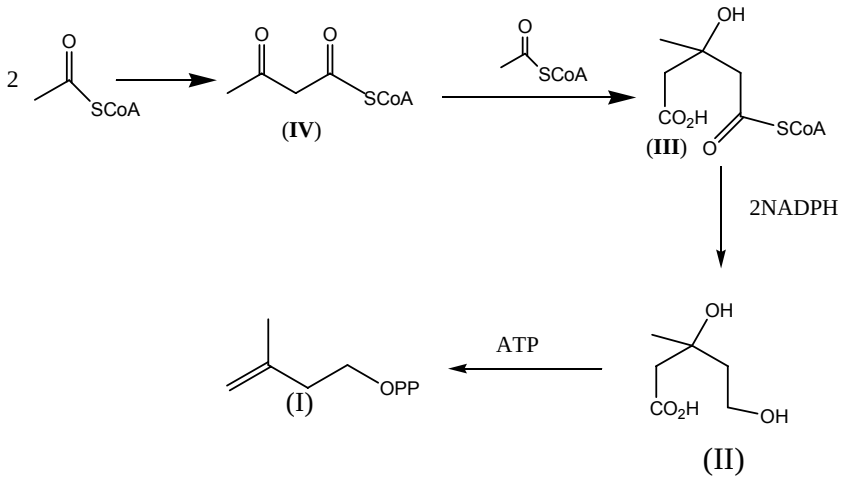


Fig 1: Biosynthesis of terpenoids.

1.3.1 Biogenesis of Isopentenyl Pyrophosphate

All terpenoids can be derived from an isoprene unit in the form of isopentenyl pyrophosphate which serves as a nucleus for the condensation of further 5-carbon

units.[7] The key building block, isopentenyl pyrophosphate(I) arises from mevalonic acid(II) via hydroxyl methyl glutarate (III)[10, 11](scheme 1).

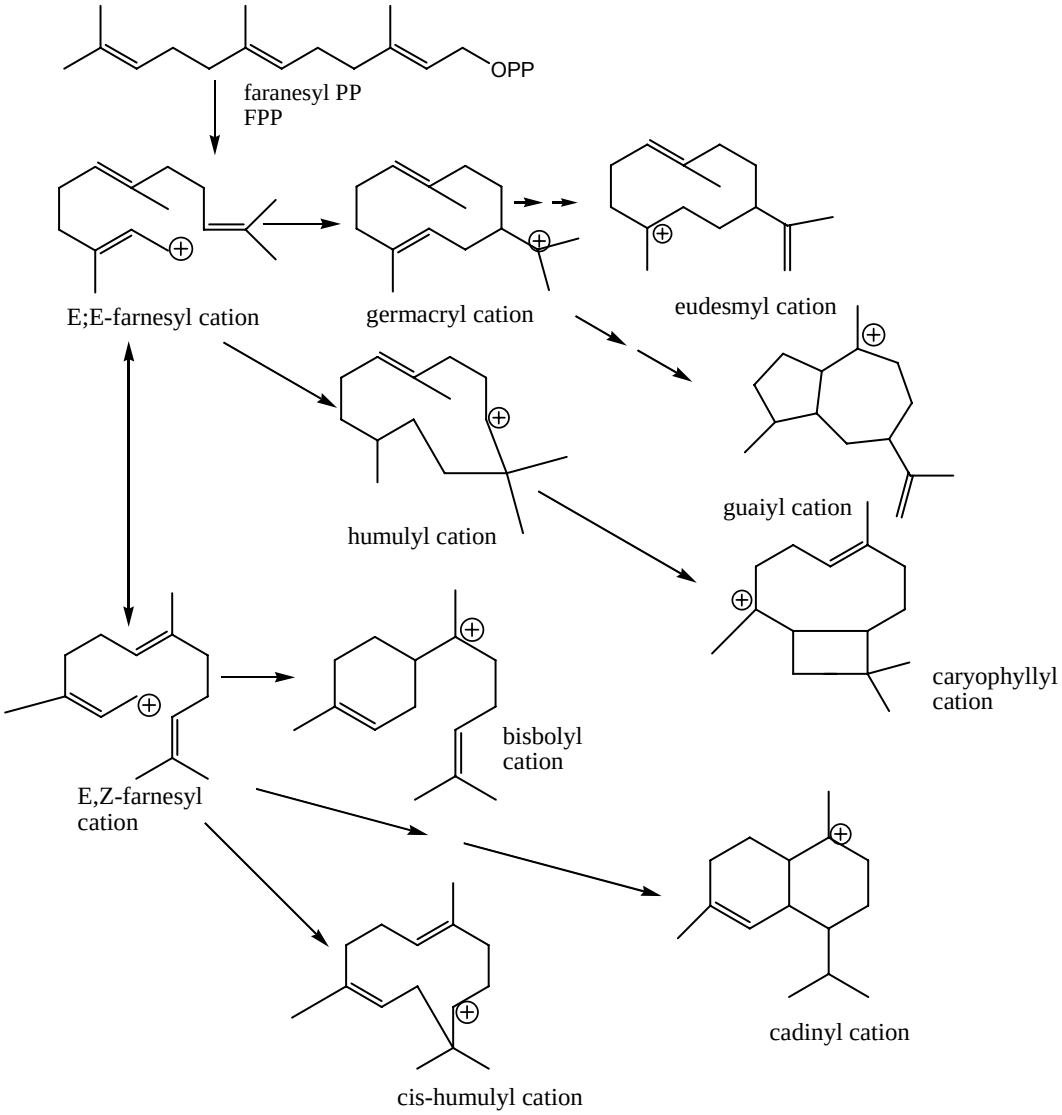


Scheme 1: Biogenesis of isopentenyl pyrophosphate.

The starting point of this metabolic path way is believed to be condensation of two molecules of acetic acid to form acetoacetyl coenzymeA (IV). This follows an essentially irreversible two step reduction via hydroxymethyl glutarate (III) to produce mevalonic acid (II). Mevalonic acid is then phosphorylated, and mevalonic-5 phyrophosphate is decarboxylated and dehydrated to yield isopentenyl pyrophosphate.

1.3.2 Biogenesis of Sesquiterepenes.

Sesquiterpenes are characteristic constituents of Asteraceae family. They arise from the cyclizaiton of farnesyl pyrophosphate (FPP) and subsequent rearrangement of the resulting carbonium ions.[9, 12] (Scheme2)



Scheme 2 ; Biosynthesis of Sesquiterpenes.

1.4 The Genus Laggera.

The Asteraceae (also known by the older alternative name composite) is one of the largest families of vascular plants with about 1535 genera and about 23,000 species.[13] It is widely distributed with the herbaceous species found predominantly in temperate regions and the larger trees mainly at high altitudes in tropical areas.[14]

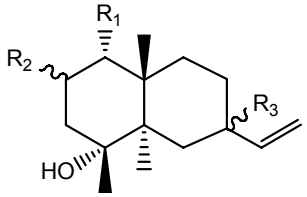
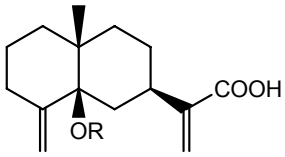
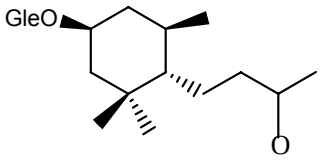
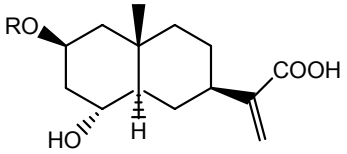
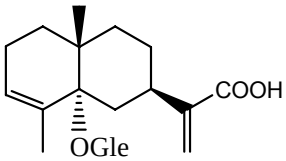
The genus Laggera, in the family Asteraceae (Astereal, Tribe, Plucheal cass) has about 20 species distributed in tropical Africa and South east Asia.[18] In Ethiopia there are six Laggera species namely, *L. Crispata* (Vahl) Hepper and wood, *L.braunii* (vatke), *L.elatior* (R.E.Fries), *L.crassifollia* (Sch. Bip exa. Rich) oliv & Hern., *L. alata* (D. Don) Oliv., *L. tomentosa*. (Sch. Bip exa. Rich) oliv & Hern.[15]

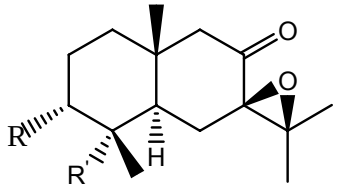
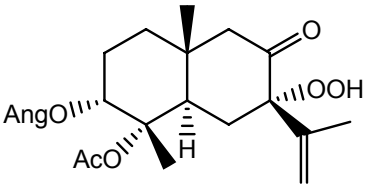
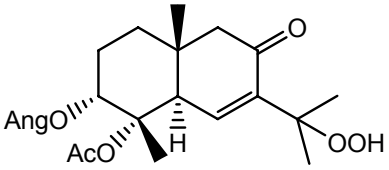
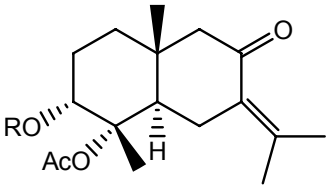
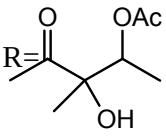
The Laggera species have been used in ethanomedical practices in Asia and Africa. For example *Laggera decurrens* (valu) Hepper and Wood is quite common in Somalia and Southern Africa and is well known for its use in traditional medicine. In Namibia, an extract of the leaves or the roots is drunk to relive stomach pains. [16]

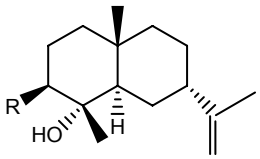
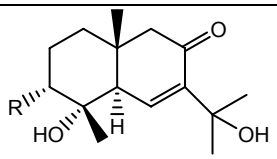
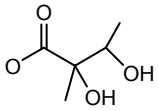
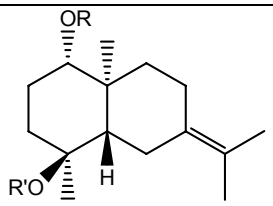
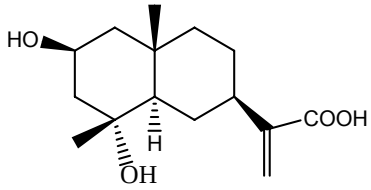
L. alata and *L. Pterodonta* are the other Laggera species employed as traditional herbal medicines in different part of the world.[14] Chinese *L. pterodonta* herb is commonly cultivated domestically and has been traditional employed as a medicine because it has been used as a cure for angina, bronchitis, influenza, and malaria. Externally applied, this plant has been of use in treating scabies, burns, bites by poisonous snakes, injuries from falls, fractures, and contusions and strains.[17] On the basis of previous biological testing both of *L. alata* and *L. Pterodonta* have been found to possess anti-leukemia, anti-inflammatory, and anti-bacterial activities.[18-22]

The chemical studies of different Laggera species have been done before. In most species, eudesman and their derivatives are the common chemical compounds. Beside to these, there are other terpenes and terpene derivatives, which are constituents of the genus (Table 1).

Table 1. Some components of crude extract of *Laggera* species

Species	Compound	Ref
L. alata	 R_1= OGle R_2 = OH R_3= H **1.** 1α - O-(β -D-glucopyranosyloxyl) -7-epi-eudesma-11-en-2β , 4 ,- diol.  . R=Gle **3**.5β - O-(β– D-glucopyranosyloxyl) -7-epi-eudesma-4(15),11(13)-dien -12-oic acid  **5 .**3β– O-(β -D-glucopyranosyloxyl) -megastigma-9-one  R=Gle **2**. 2 β O-(β -D-glucopyranosyloxyl) -7-epi-eudesma-4α - hydroxyl- 11(13)-en-12-oic acid.  **4 .** 5α - O-(β -D-glucopyranosyloxyl) -7-epi-eudesma-3, 11(13)-dien -12-oic acidl..	14

Blumea alata	 **6** **7** **8** **9** R. Ang Ang Epang Epang R' H H Ac Ac **6**. 7β .11-Epoxycuauthemone -3-O-angelate. **7**. 4-O-Acetyl-7β ,11.epoxycuathemone 3-O angelate. **8** 7β ,11-epoxycuathemone-3-O-epoxyangelate. **9** 4-O-Acetyl 7β ,11-epoxycuathemone-3-O-epoxyangelate.   **10** .4-O-Acetyl -7α -hydroperoxy-11,13-dehdro-7 ,11-diydrocuathemone -3-O-angelate. **9** .4-O-Acetyl -11 -hydroperoxy-6,7-dehdro- 7 ,11-diydrocuathemone-3-O-angelate.  10. R=Epang 11. R=  **10**. 4-O-Acetyl cuauthemone 3-O-epoxyangelte. **11**. 4-O-Acetyl cuauthemone 3-O-(2'-methyl-2'-hydroxy-3-acetoxy butyrate)	23

L.crispata	 **12**. R = OH **13**. R = OAc **12**. 3β ,4α -Dihydroxy-7-epi-eudesm-11(13) **13**. 3β -Acetoxy,4α -hydrooxy-7-epi-eudesm-11(13)  14. R =  3α (2',3'-Dihydroxy-2'-methyl butanoyl)4,11-dihydroxy-6,7-dehydroeudesman-8-one.	19
L.ptrodonta	 R R' **15** **H** **Gle** **16** **Gle** **H** **15**. 4 β-(β -D-glucopyranosyloxy) -enantio-eudasm-7(11)-en-1α - ol **16**.1α -(β -D-glucopyranosyloxy) -enantio-eudasm-7(11)-en-4 β - ol  **17** 2β– Hydroxyillicic acid	17& 22

The essential oils of some Laggera species have been also analyzed. The oil composition of L. Petrodonta (a synonym of *L. crispata*) from Cameroon was founded to contain a high percentage of oxygenated sesquiterpenes and thyme derivatives with γ-eudesmol, α- eudesmol 2.5 dimethoxy p- cymene (thymoquinol dimethyl ether) and juniper camphor as main components.[24]

L. alata oil from Madagascar contained the eduesmane type sesquiterpenes, 7-epi-γ-eudesmol, 7 epi -β eudesmol, 7- epi-α-- eudesmol, isonitermedeol, and juniper camphor as its major constituents. The oil of *L.aurita* from India has been reported to contain Laggerol, 2, 3-dimethoxy-p-cymene,δ-cadinene,α-cadinol and m-menth-6-en-8-ol.[25, 26]

1.5 Laggera tomentosa

Laggera tomentosa, called "Keskese" locally, is a perennial fragrant bushy herb (0.5-1.2 m high), endemic to Ethiopia. It grows on dry hill and mountain slopes (2345-2950m). L. tomentosa is found in Tigray, Gonder, Gojjam, Wollo, Shewa and Arsi not known else where.[15]

Locally the plant has use in traditional medicine. The juice of the crushed plant is ingested as a treatment for stomach-ache, and is also used against migraine. It can also be used as a fumigant and for cleansing milk containers.[15, 27]

Chemical investigation on the essential oil of *L. tomentossa* have been reported before [27]. However; there are no reportes on the chemical investigation of the solvent extract of the species

2. Objectives

The main objectives of this project are to isolate and characterized the constituents of the petroleum ether extract of the leaves of *L.tomentosa*. The plant was selected for the study because it is endemic to Ethiopia and its important in traditional medicine. In addition the solvent extract of the plant have not been investigated before.

3. RESULTS AND DISCUSSION

Two compounds **LTP-1** and **LTP-3** were isolated and characterized from the Pet. ether extract of *Laggera tomentosa*. Structure elucidation of the compounds was based on the spectroscopic data obtained for the compounds and in comparison with the data in the literature for similar compounds.

3.1. Characterization of LTP-1.

Characterization of **LTP-1** was determined by using spectroscopic techniques. In the IR (NaCl) spectrum (Appendix 1) the strong absorption band at 3420cm^{-1} showed the presence of a hydroxyl group. Strong absorption band at 2931cm^{-1} and medium absorption band at 1456cm^{-1} showed the presence of saturated group. Strong absorption band at 1673cm^{-1} showed the presence of α-β unsaturated ketone. A strong absorption band at 1731cm^{-1} showed the presence of ester group. The medium absorption band at 1367cm^{-1} showed the presence of H-C-H bending. The medium absorption band at 1244cm^{-1} and 1162cm^{-1} showed C-O stretching. The UV spectrum λ_{max} (in $CHCl_3$) (Appendix 2) revealed absorption band at 244nm indicating that the molecule has α-β unsaturated carbonyl chromophore.

The ^{1}H NMR (Appendix 3) showed quartate at δ 6.07 (J=12 Hz) integrated for one proton indicated the presence of olefinic proton. A doublet peak at δ 2.00 integrated for three protons indicated methyl group attached to olefinic carbon. A singlet peaks at δ 1.98, 1.79 and 1.91 integrated for three protons each indicated methyl group attached to olefinic carbons. A singlet peak at δ 1.41 integrated for three protons indicated methyl group attached to O-substituted quaternary carbon. A singlet peak at δ 0.98 integrated for three protons indicated methyl group attached to a quaternary carbon. A doublet of doublet at δ 5.94 (J=4Hz) integrated for one proton indicated proton attached to oxygen substituted tertiary carbon. A multiplet peaks at δ 1.56&2.28 integrated for two protons indicated diastereotopic protons of methylene group. A doublet of doublet peaks at δ (2.68 & 1.85) integrated for one proton each indicating diastereotopic protons. A doublet peak at δ (2.25 J=4Hz) integrated for two protons. A doublet of doublet peak at δ (3.17 J=12Hz) integrated for one proton indicating proton attached to tertiary carbon.

Table 2: ^{1}H, Proton Decoupled ^{13}C & DEPT spectra data of **LTP-1**

^{1}H δ (PPm)	^{13}C NMR of **LTP-1** δ (ppm)	DEPT
1.56(m)	37.70	CH_2
2.28 (m)	25.63	CH_2
5.94(dd)(J=4Hz, 12Hz)	73.94	CH
-	87.39	-
3.17(dd)(J=4Hz, 12Hz)	44.82	CH
1.85&2.68 (dd)	25.82	CH_2
-	129.94	-
-	201.87	-
2.25(d) (J=4Hz)	59.93	CH_2
-	36.95	-
-	144.41	-
1.98 (s)	23.33	CH_3
1.79 (s)	22.75	CH_3
0.98 (s)	19.39	CH_3
1.41 (s)	16.74	CH_3
-	166.99	-
-	128.02	-
6.07(q ,J=12Hz)	137.79	CH
2.00 (d)	15.74	CH_3
1.91 (s)	20.65	CH_3
-	170.38	-
1.97-2.00 (s)	22.52	CH_3

The proton decoupled ^{13}C NMR spectrum (appendix 4 table 2) of **LTP-1** showed well resolved resonance of the 22 carbon atoms. The multiplicity of each carbon atom was determined using DEPT-135 experiment, which revealed the presence of seven methyl groups, four methylene groups, three methine groups (one attached to carbon atom that attached with oxygen, one attached to three saturated carbons and the other is vinylic), Eight quaternary carbon (three carbonyl carbons, three vinylic carbons and two saturated carbons), indicating 32 hydrogen atoms attached to carbon atoms.

Based on ^{1}H NMR (Appendix 3) and proton decoupled ^{13}C NMR spectrum (appendix 4 table 2) data of **LTP-1** the proposed structure of the compound is

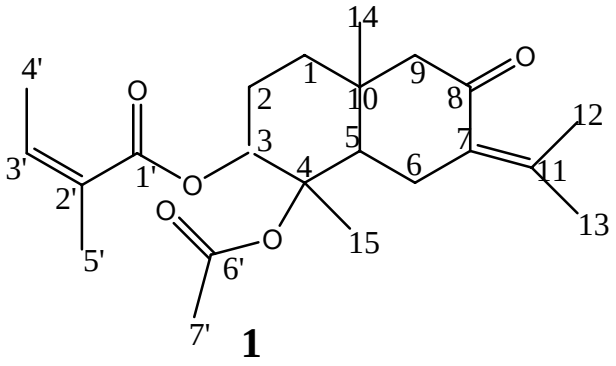


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Table 3 .Proton Decoupled ^{13}C spectra of **LTP-1** compared with literature report. (Ref.28)

No	**^{1}H δ (PPm)**	Literature value of ^{1}H NMR δ (ppm)	^{13}C NMR of **LTP-1** δ (ppm)	Literature ^{13}C NMR δ (ppm)
1	1.56(m)	-	37.70	37.7
2	2.28 (m)	-	25.63	25.8
3	5.94(dd)(J=4Hz, 12Hz)	5.93 dd	73.94	74.0
4	-		87.39	87.4
5	3.17(dd)(J=4Hz, 12Hz)	3.16 dd	44.82	44.9
6	1.85&2.68 (dd)	2.65dd& 2.27m	25.82	25.6
7	-		129.94	129.9
8	-		201.87	201.8
9	2.25(d) (J=4Hz)	2.26 & 2.20d	59.93	59.9
10	-		36.95	36.9
11	-		144.41	144.5
12	1.98 (s)	1.96d	23.33	23.3
13	1.79 (s)	1.77d	22.75	22.5
14	0.98 (s)	0.95s	19.39	19.4
15	1.41 (s)	1.38s	16.74	16.7
1’	-		166.99	167.0
2’	-		128.02	128.0
3’	6.07(q ,J=12Hz)	6.05q	137.79	137.7
4’	2.00 (d)	1.96d	15.74	15.7
5’	1.91 (s)	1.89d	20.65	20.6
6’	-		170.38	170.3
7’	1.97-2.00 (s)	1.94s	22.52	22.7

Moreover, the above prediction is also supported by using its 2D NMR spectral data as follows.

^{1}H-^{1}H correlation spectroscopy (COSY) (Appendix 5,table 4) showed strong correlation between H-3 δ 5.94 and H-2 δ 2.28 indicating methylene protons at C-2 are diastereotopic and the oxygen containing carbon is chiral. Similarly, strong coupling observed between H-5 δ 3.17 and H-6 δ 2.68 indicating methylene protons at C-6 are diastereotopic and the tertiary at C-5 is also chiral. Coupling between H-9 δ 2.25 and H-14 δ 0.98 showing the two methylene protons at C-9 are diastereotopic. There are also coupling between H-3' δ 6.07 and H-4' δ 2.00.

Table 4: ^{1}H and ^{1}H-^{1}H COSY spectra data of compound **LTP-1**

carbon No.	**^{1}H δ (PPm)**	**COSY**
1	1.56(m)	
2	2.28 (m)	
3	5.94 (dd) (J=4Hz, 12Hz)	H^{3}-H^{2}
5	3.17 (dd) (J=4Hz, 12Hz)	H^{5}-H^{6}
6	1.85 (dd), 2.68 (dd)	H^{6}-H^{5}
9	2.25 (d) (J=4Hz)	H^{9}-H^{14}
12	1.98 (s)	
13	1.79 (s)	
14	0.98 (s)	H^{14}-H^{9}
15	1.41 (s)	
3'	6.07 (q ,J=12Hz)	$H^{3'}$-$H^{4'}$
4'	2.00 (d)	$H^{4'}$-$H^{3'}$
5'	1.91 (s)	
7'	1.97-2.00 (s)	

Hetronuclear Single Quantum Correlation (HSQC) experiment correlates the chemical shift of proton with the chemical shift of directly bonded carbon. In the HSQC spectral data (Appendix 6) showed three protons at δ 0.98 (s) connected with C-14 δ19.39, three protons at δ1.41 (s) connected with C-15 δ16.74 three protons at δ1.79 (s) connected with C-13 δ 22.75, three protons at δ 1.91 (s) connected with C-5' δ 20.65, nine protons at 1.98-2.00 connected with C-4' δ 15.74, C-12 δ 23.33 and C-7' δ 22.52, two protons at

δ 1.56 (m) connected with C-1 δ 37.70, two protons at δ 2.28 (m) connected with C-2 δ 25.63, one proton at δ 2.68 (dd) and one proton at δ 1.85 (dd) attached to C-6 δ 25.82, two protons at 2 δ.25 (d) connected with C-9 δ 59.93, one proton at δ 5.94 (dd) attached to C-3 δ 73.94, one proton at δ 3.17 (dd) connected with C-5 δ 44.82 and one proton at δ 6.07 (q) connected with C-3' δ 137.79.

Table 5: ^{13}C & HMBC spectra data of compound **LTP-1**

Carbon NO	**^{13}C δ (ppm)**	**DEPT**	**HMBC**
1	37.70	CH_2	H-1 ↔C-3, , C-9, C-14
2	25.63	CH_2	H-2 ↔C-4
3	73.94	CH	H-3 ↔ C-4, C-1'
4	87.39	-	
5	44.82	CH	H-5 ↔C-3, C-4, C-6, C-9, C-10,C-14, C-15
6	25.82	CH_2	H-6 ↔C-5,C-7,C-8,C-10,C-11
7	129.94	-	
8	201.87	-	
9	59.93	CH_2	H-9 ↔C-5, C-7, C-8, C-10, C-14
10	36.95	-	
11	144.41	-	
12	23.33	CH_3	H-12 ↔C-13
13	22.75	CH_3	H-13 ↔C-12
14	19.39	CH_3	H-14↔C-1, C-5, C-9, C-10
15	16.74	CH_3	H-15↔C-3, C-4, C-5,C-6'
1'	166.99	-	
2'	128.02	-	
3'	137.79	CH	H-3' ↔C-1', C-5', C-4'
4'	15.74	CH_3	H-4' ↔C-3'
5'	20.65	CH_3	H-5'↔C-3', C-2'
6'	170.38	-	
7'	22.52	CH_3	H-6'↔C-6'

Hetroneuclar Multiple Bond Correlation (HMBC) experiment gave information about coupling of hydrogen's and carbons that are two or three bonds away. In the HMBC (Appendix 7), the methyl protons at δ 1.91 (s) (H-5') correlated with δ 137.79 C-3';δ 128.03 C-2'. Methylene proton at δ 1.56 (H-1) correlated with δ 73.94 c-3; δ 59.93 C-9 and δ 19.39 C-14. The methylene protons at δ 2.28 (m) (H-2) correlated with δ 87.39 C-4. Methine proton at δ 5.94 (dd) H-3 correlated with the δ 25.63 C-1; δ 87.39 C-4 and δ166.99 C-1'. Another methine proton at δ 3.17 (dd)H-5 correlated with δ 733.94 C-3; δ 87.39 C-4; δ25.82 C-6; δ 59.93 C-9; δ 36.95 C-10; δ 19.39 C-14; δ 16.74 C-15.The methylene proton at δ 2.68 (dd) H-6 correlated with δ 44.82 C-5; δ 129.94 C-7; δ 201.87 C-8; δ 36.95 C-10; δ 144.41 C-11. The methylene protons at δ 2.25 (d) H-9 correlated with δ 44.82 C-5; δ129.94 C-7; δ 201.87 C-8; δ36.95 C-10; δ19.39 C-14. The methyl protons at δ 1.98 (s) (H-12) correlated with δ 22.73 C-13 and methyl proton at δ 1.79 (s) (H-13) correlated with δ 23.33 C-12.

The methyl protons at δ 0.98 (s) (H-14) correlated with C-1 δ 37.70; C-5 δ 44.82; C-9 δ 59.93 and C-10 δ 36.93. The methyl protons at δ 1.41 (s) (H-15) correlated with C-3 δ 73.94; C-4 δ 87.39; C-5 δ 44.83 and δ 170.38 C-6'. Methine proton at δ 6.07 (q) (H-3') correlated with δ 20.65 C-5' and δ 166.99 C-1'.The vinylic proton at δ 6.07 (q) (H-3') correlated with δ 20.65 C-5'; δ 166.99 C-1' and δ 15.743 C-4'. The methyl proton at δ 1.9-2.00 (s) (H-7') correlated with δ 170.38 C-6'.

Based on COSY& HMBC correlations the following partial structures (**I** and **II**) can be drawn.

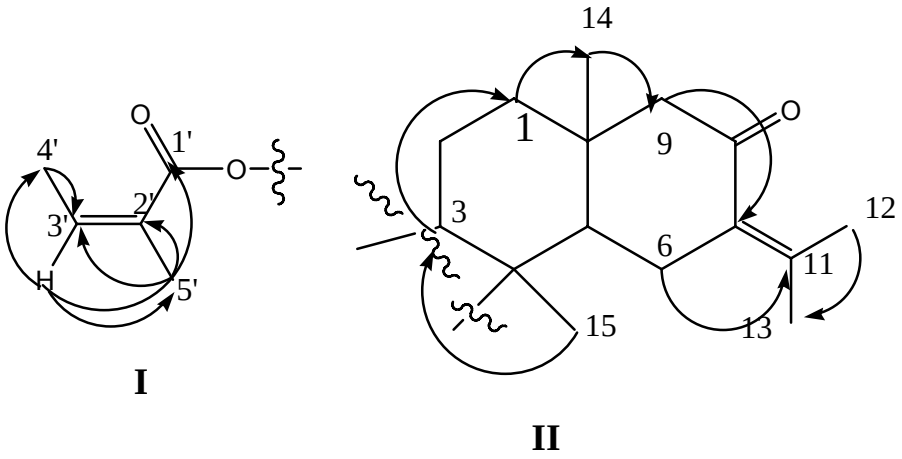
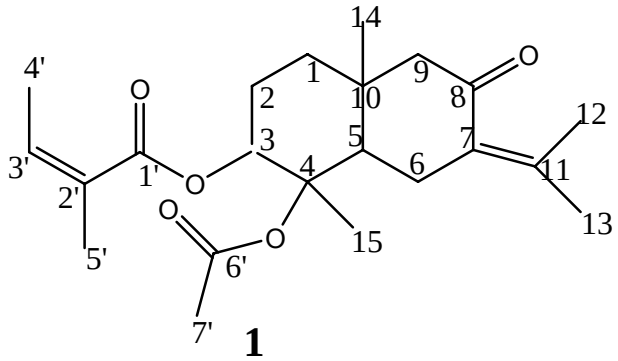


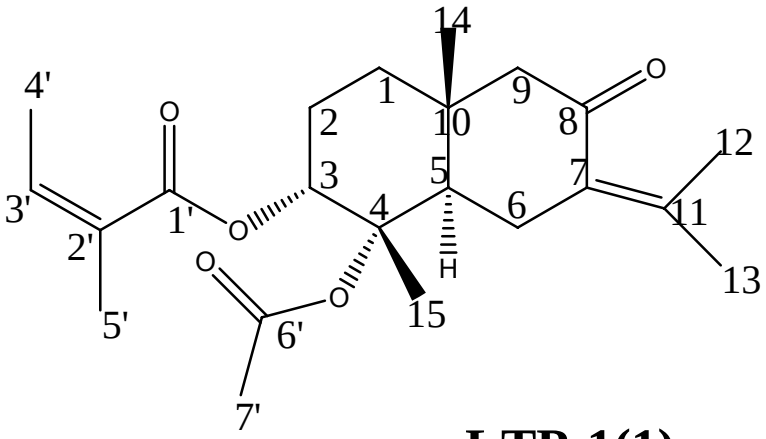
Figure-2.The proposed fragments of **LTP-1**. (**1**)

Combination of fragment **I** and **II** (Fig-) gave the proposed structure **1** for compound **LTP-1.**



4-Acetoxy-3 -angeloyloxy-7, 11-dehydroeudesman-8-one

From the literature reported before (Ref. 28) the configuration of LTP-1 is β-substituted at C-3 and C-4.The coupling constant of H-5 and H-6 also confirm the configuration.



LTP-1(1)

4β-Acetoxy-3β -angeloyloxy -7, 11-dehydroeudesman-8-one

3.2. Characterization of LTP-3.

Practical characterization of LTP-3 was determined by using spectroscopic techniques. In the IR (NaCl) spectrum (Appendix 8) the absorption bands at 3467cm^{-1} showed the presence of a hydroxyl group. Strong absorption band at 2935.74cm^{-1} and medium absorption band at 1447cm^{-1} showed the presence of saturated group. Strong absorption band at 1671cm^{-1} showed the presence of α-β unsaturated ketone. A strong absorption band at 1738cm^{-1} showed the presence of ester group. The medium absorption band at 1371cm^{-1} showed the presence of H-C-H bending. The medium absorption band at 1246cm^{-1} and 1145cm^{-1} showed C-O stretching. The UV spectrum at λ_{max} (in $CHCl_3$) (Appendix 9) revealed absorption band at 250nm indicated that the molecule has α-β unsaturated carbonyl chromophore.

The ^1H NMR (Appendix 10) showed multiplet peak at δ 1.39 &1.22 integrated for two protons indicating the presence of methylene group. Signals appearing at δ 1.94 (1H, m) and δ 1.79 (1H, m) showed diastereotopic proton of methylene group. A broad peak at δ 5.89(1H, bdt) revealed methine proton attached with oxygen substituted tertiary carbon. A complex peak at δ 2.21-2.26 integrated for five protons, showed two methylene groups and one methylene proton. A singlet peaks at δ 2.06, 1.86, 0.99.1.59, 1.35, 1.97 and 1.98 each integrated for three protons indicated methyl protons. A quartet peak at δ 5.05 integrated for one proton indicated methine proton. A broad singlet peak at δ 3.37 integrated for one proton showed hydroxyl proton.

Table 6:^{1}H NMR , Proton Decoupled ^{13}C & DEPT spectra data of **LTP-3**

^{1}H δ (ppm)	13C δ (ppm)	DEPT δ (ppm)
1.39)&1.22(m)	32.51	CH2
1.94&1.79 (m)	23.03	CH2
5.89(bdt) (J=4Hz)	74.69	CH
-	83.04	Quaternary carbon
2.21-2.26 (complex)	45.26	CH
2.89&2.18 (complex)	25.83	CH2
-	130.24	Quaternary carbon
-	201.46	Carbonyl carbon
2.22(d,J=4Hz)	59.99	CH2
-	36.01	Quaternary carbon
-	145.22	Quaternary carbon
2.06 (s)	23.54	CH3
1.86 (s)	22.76	CH3
0.99 (s)	19.00	CH3
1.59 (s)	17.98	CH3
-	169.41	Carbonyl carbon
-	75.86	Quaternary carbon
5.05(q, J = 8Hz)	74.34	CH
1.30 (d J=4Hz)	13.27	CH3
1.97(s)	22.20	CH3
-	173.81	Carbonyl carbon
.35 (s)	22.26	CH3
-	170.00	Carbonyl carbon
1.98(s)	21.06	CH3

The proton decoupled ^{13}C NMR spectrum (appendix 11, table 6) of LTP-3 showed 24 carbon atoms. The multiplicity of each carbon atom was determined using DEPT-135 experiment, which revealed the presence of seven methyl groups, four methylene groups three methine groups and ten quaternary carbons indicating 32 hydrogen atoms attached to carbon atoms.

From ^{1}H NMR , Proton Decoupled ^{13}C & DEPT spectra data of **LTP-3**(appendix9 & 10, table 6), the proposed structure of compound **LTP-3** is,

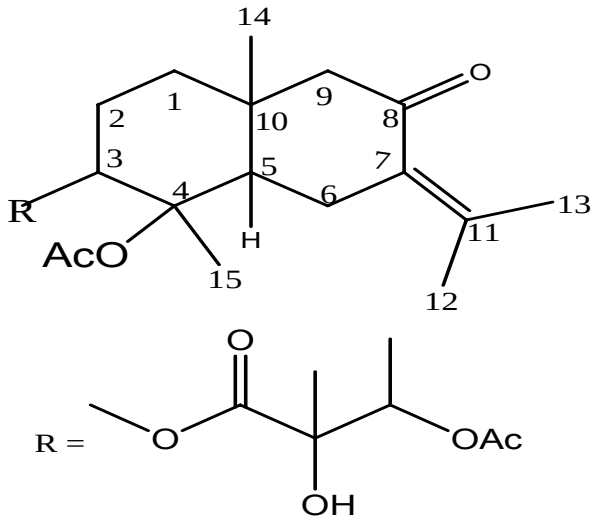


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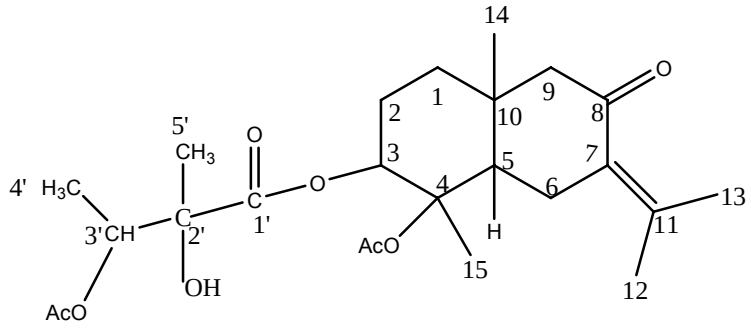
Table 7: ^{13}C spectra of **LTP-3** compared with literature report.

No	^{1}H δ (PPm)	^{13}C NMR of **LTP-3(3)** δ (ppm)	Literature value(Ref.29)
1	1.39)&1.22(m)	32.51	32.9
2	1.94&1.79 (m)	23.03	23
3	5.89(bdt) (J=4Hz)	74.69	73.7
4	-	83.04	82.9
5	2.21-2.26 (complex)	45.26	45.1
6	2.89&2.18 (complex)	25.83	25.8
7	-	130.24	130.1
8	-	201.46	201.4
9	2.22(d,J=4Hz)	59.99	60.1
10	-	36.01	35.9
11	-	145.22	145.5
12	2.06 (s)	23.54	23.6
13	1.86 (s)	22.76	22.8
14	0.99 (s)	19.00	19.1
15	1.59 (s)	17.98	18.0
1’	-	169.41	-
2’	-	75.86	-
3’	5.05(q, J = 8Hz)	74.34	-
4’	1.30 (d J=4Hz)	13.27	-
5’	1.97 (s)	22.20	-
6’	-	173.81	-
7’	1.35 (s)	22.26	-
8’	-	170.00	169.3
9’	1.98(s)	21.06	22.2

From ^{1}H NMR & ^{13}C spectra (Appendix 10&11) of **LTP-3** the poroposed structures of the molecule is,



3

The above prediction is also supported by using its 2D NMR spectral data as follows

Table 8: ^{1}H and ^{1}H-^{1}H COSY spectra data of compound **LTP-3**

Hydrogen on carbon No.	^{1}H δ (PPm)	COSY
1	1.39)& 1.22 (m)	^{1}H-^{2}H
2	1.94(m), &1.79 (m)	^{2}H-^{1}H,^{3}H
3	5.89 (bdt) (J=4Hz)	H^{3}-H^{2}
5	2.21-2.26 (complex)	H^{5}-H^{6}
6	2.89 &2.18 (complex)	H^{6}-H^{5}
9	2.22(d) (J=4Hz)	H^{9}-H^{14}
12	2.06 (s)	-
13	1.86 (s)	-
14	0.99 (s)	H^{14}-H^{9}
15	1.59 (s)	-
3’	5.05 (q, J=8Hz)	-
4’	1.30 (d J=4Hz)	-
5’	1.97 (s)	-
7’	1.35 (s)	-
9’	1.98(s)	-

^{1}H-^{1}H correlation spectroscopy (COSY) (Appendix 12 Table 8) showed strong correlation between H-3 δ 5.89 and H-2 δ 1.94;H-1δ 1.39 and H-2; H-3 and H-1 indicted C-1, C-2 and C-3 exist in the same region. There are also coupling between H-9 δ 2.22 and H-14 δ .99. Also, there are other correlations. (table 8)

In the HSQC spectral data (Appendix 13) showed two protons at δ 1.39(m) and1.22(m) connected with C-1 δ 32.51, two protons at δ1.98(m) and 1.79(m) connected with C-2 δ23.03 ,one protons at δ5.89 (bdt) connected with C-3 δ 74.69, one protons at δ 2.21-2.26 (complex) connected with C-5 δ 45.26, two protons at 2.89(complex) and-2.18(complex) connected with C-4 δ 25.83, two protons at δ 2.22 (d) connected with C-9 δ 59.99, three protons at δ 2.06 (s) attached to C-12 δ 23.54, three proton at δ 1.86 (s) connected with C-13 δ 22.76, three protons at δ 0.99 (s) attached to C-14 δ 19.00, three protons at 1.59(s) attached to C-15 δ.17.98, one proton at δ 5.05 (q) attached to C-3' δ 74.34, three proton at δ 1.30 (d) connected with C-4' δ 13.27, three proton at δ 1.97 (s) connected with C-5' δ 22.20, three proton at δ 1.35 (d) connected with C-7' δ 22,26 and three protons at δ 1.98 (s) connected with C-9' δ 21,06.

Table 9 ^{13}C &HMBC spectra data of compound LTP-3

Carbon NO	^{13}C δ (ppm)	DEPT	HMBC
1	32.51	CH_2	
2	23.03	CH_2	
3	74.69	CH	H-3 ↔ C-1, C-4
4	83.04	-	
5	45.26	CH	H-5 ↔, C-4, C-7, C-9, C-10
6	25.83	CH_2	H-6 ↔ C-4, C-5, C-7, C-10, C-11
7	130.24	-	
8	201.46	-	
9	59.99	CH_2	H-9 ↔ C-7, C-10, C-14
10	36.01	-	
11	145.22	-	
12	23.54	CH_3	H-12 ↔ C-7, C-11, C-13
13	22.76	CH_3	H-13 ↔C-7,C-8, C-12
14	19.00	CH_3	H-14↔C-1, C-5, C-9, C-10
15	17.98	CH_3	H-15↔C-3, C-4, C-5
1’	169.41	-	
2’	75.86	-	
3’	74.34	CH	H-3’ ↔ C-1’,C-4’
4’	13.27	CH_3	H-4’ ↔C-3’
5’	22.20	CH_3	H-5’↔ C-1’
6’	173.81	-	
7’	22.26	CH_3	H-7’↔C-6’,C-3’
8’	170.00	-	
9’	21.06	CH_3	H-9’↔C-8’

In the HMBC (Appendix 14), the methine proton at δ 5.89 (bdt) (H-3) correlated with δ 32.51 C-1 and δ 83.04 C-4,another methine proton at δ 2.21-2.26(complex)(H-5) correlated with δ 83.04 C-4 ; δ 130.24 C-7; δ 59.99 C-9 and δ 36.01 C-10, methylene proton at δ 2.89(complex)(H-6) correlated with δ 83.04 C-4; δ 45.26 C-5 ; δ 130.24 C-7;

δ 36.01 C-10 and δ 145.22 C-11,anther methylene proton at δ 2.22(d)(H-9) correlated with δ 130.24 C-7; δ 201.46 C-8; δ 36.01 C-10;and δ 19.00 C-14 , methyl proton at δ 2.06(s) (H-12) showed correlated with δ 22.76 C-13, the methyl protons at δ 0.99 (s) (H-14) showed correlation with δ 32.51 C-1; δ 45.26 C-5; δ 59.99 C-9; δ 36.01 C-10; another methyl protons at δ 1.59 (s) (H-15) correlated with δ 74.69 C-3; δ 83.04 and δ 45.26 C-5 , methine proton at δ 5.05 (q)(H-3') displayed correlation with δ 13.27 C-4' and δ 169.41 C-1', methyl proton at δ 1.30(d)(H-4') correlated with δ 74.34 C-3', methyl proton at δ 1.97(s)(H-5') correlated with δ 169.41 C-1', methyl proton at δ 1.35(s)(H-7') correlated with δ 74.34 C-3'and δ.81 C-6'and anther methyl proton at δ 1.98(s)(H-9') correlated with δ 170.00 C-8'.

Based on COSY& HMBC correlations the following fragments (**I** and **II**) can be drawn.

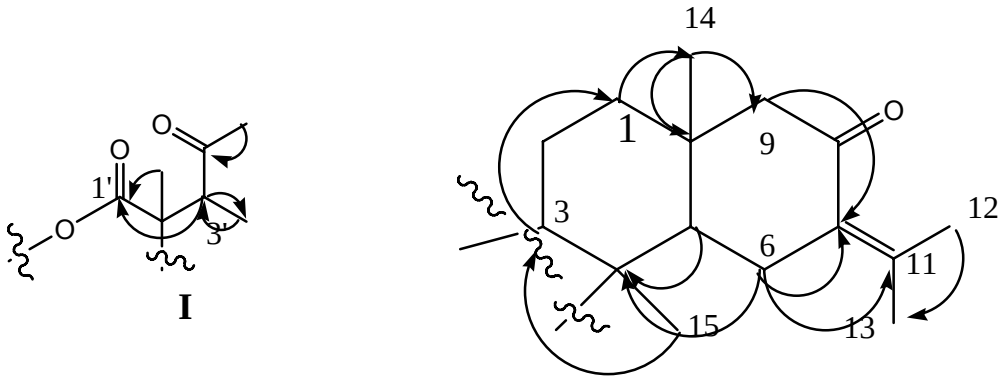
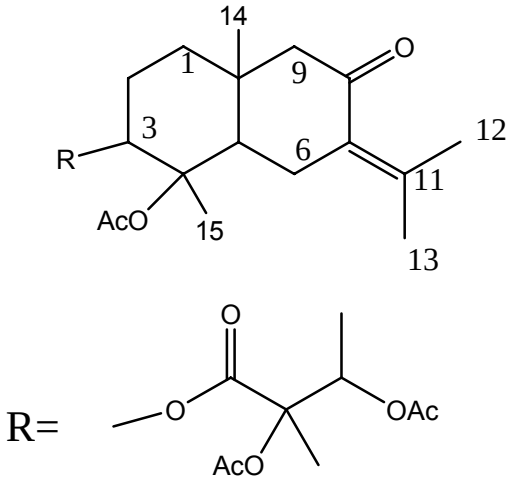


Figure-3.The proposed fragments of **LTP-3**.

Combination of fragment **I** and **II** (Fig-3) give the following proposed structure **3** for compound **LTP-3.**



3

4-O-Acetyl - 3-O-(2'-methyl-2'-hydroxy butyrate) -7, 11-dehydroeudesman-8-one

4. Experimental

4.1 General

UV spectrum was measured with GENEY'S spectrometer (200-400) in $CHCl_3$ at room temperature.

IR spectrum was obtained as NaCL pellets on Perkin-Elmer BX Infrared spectrum in the range 4000-400cm^{-1}

^{1}H NMR, ^{13}C, and 2D NMR spectra were recorded on a Bruker Advance 400 MHZ spectrometer with TMS as internal standard.

The purity of compounds was monitored on silica gel GF_{254}. Analytical thin layer chromatograph were run on silica gel(Merck)coated on aluminum foil, 0.2mm thickness. The spots were detected by their UV fluorescence and by spraying with 0.5% viniline in sulpheric acid solution.

Silica gel with fluorescent indicator 254 nm on aluminum cards with layer thickness 0.2mm used for TLC.

Silica gel 60(Merck), particle size 0.063-0.200(70-230 mesh ASTM) used for column chromatography.

4.2 Coding system

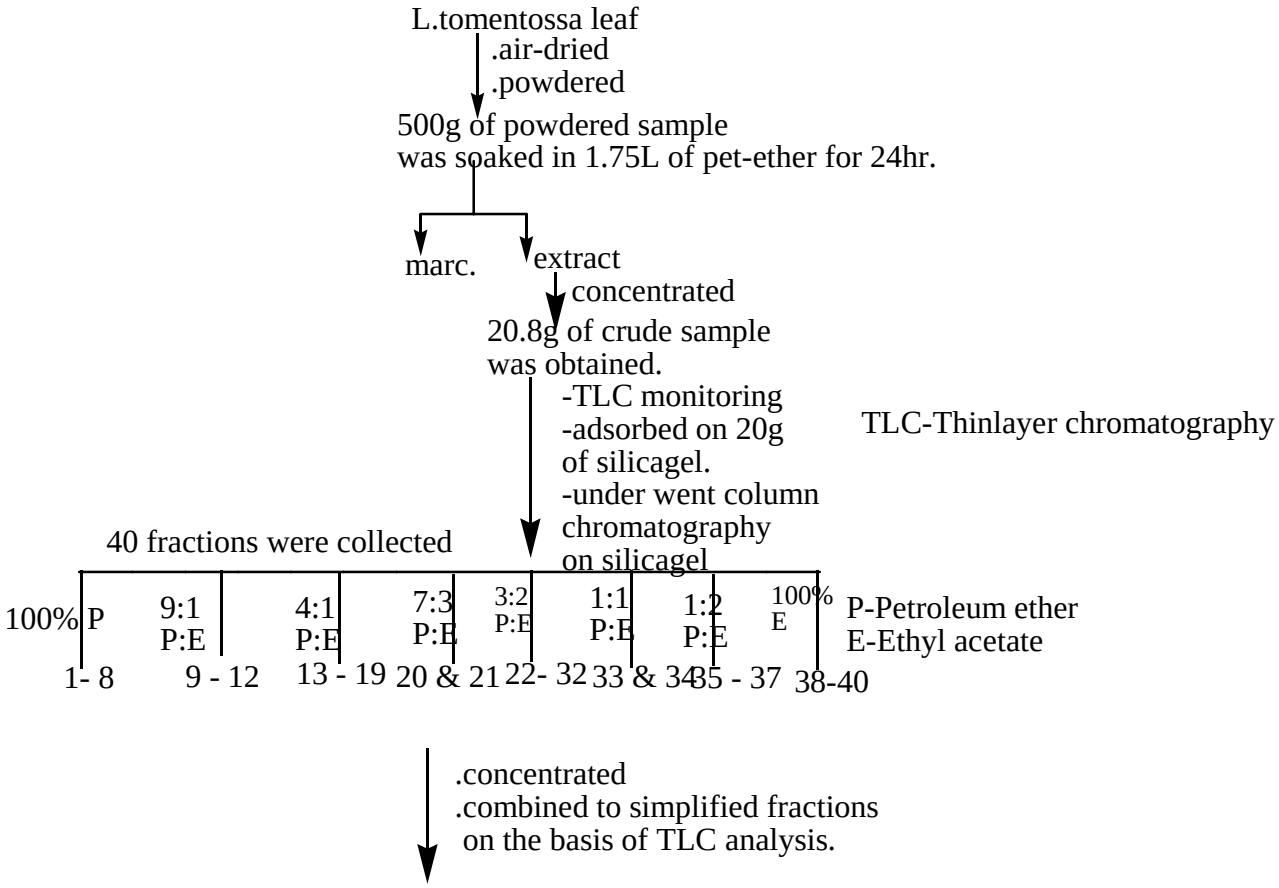
L stands for the genus name Laggera, **T** stands for species name tomentosa, **p** stands for petroleum ether extract and the number behind LTP indicates the pure compounds isolated.

4.3 Sample collection.

Laggera tomentosa was collected from Daletti, Western Shewa of Ethiopoia (26 Km far from Addis Ababa near Alemgena) in November 2005. A voucher specimen (SD 6487) is deposited at the National Herbarium (ETH), Department of Biology, Addis Ababa University.

4.4 Extraction

The leaf part of the plant was dried with air and powdered; 500g of powdered *L.tomentossa* was soaked with 1.75lit. of petroleum ether (54-930c) for 24hrs. The extract , on removal of the solvent by using rota vaporator afforded 22.8g of black Gummy material and labeled as **LTP**.



4.5 Isolation

20.0g of LTP was adsorbed on 20.0g of silica gel, by dissolving the sample with chloroform. After drying the solvent, the dry sample was applied on a column packed with 200g of silica gel. Isolation was carried out using the solvents pet-ether, ethyl acetate by increasing polarity. A total of 40 fractions were collected. Fractions that showed the same R_f value and the same characteristic color on TLC were combined. (Table10)

Table10: Petroleum ether extract fractions.

+

Solvent system	Ratio	Fraction	Volume (ml)	Fraction Combined	Code
Pet-ether	10:0	1-8	150	1 and 2 3-5 6 and7	Fr-1 Fr-2 Fr-3
Pet-ether/EtOAc	9:1	9-12	150	8-10 11 and 12	Fr-4 Fr-5
Pet-ether/EtOAc	4:1	13-19	150	13-15 16-19	Fr-6 Fr-7
Pet-ether/EtOAc	7:3	20 and 21	150	20 and 21	Fr-8
Pet-ether/EtOAc	3:2	22-32	100	22 23 24 25-27 28 29-33	Fr-9 Fr-10 Fr-11 Fr-12 Fr-13 Fr-14
Pet-ether/EtOAc	1:1	33 and 34	100	34	Fr-15
Pet-ether/EtOAc	1:2	35-37	100	35-37	Fr-16
Pet-ether/EtOAc	0:10	38-40	100	38-40	Fr-17

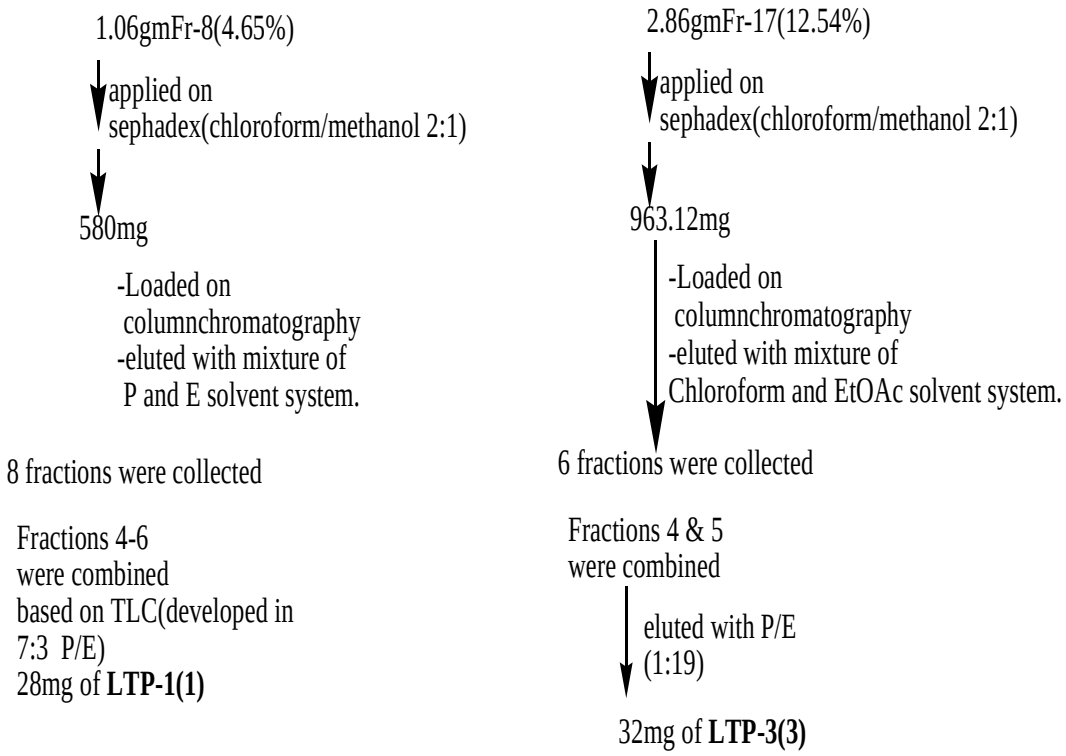
Fractions collected with 100% pet-ether and pet-ether-EtOAc (9:1) ratios were discarded because their TLC result did not show spots.

4.5.1 Isolation of LT P-1

Fr-8 was concentrated under reduced pressure (on a rotaevaporator) to yield 1.06gm(4.56%) and was applied on a sephadex column using 2:1 ratio of chloroform and methanol solvent mixture as eluant. The chlorophyll free part of the fraction (580mg) was applied on a column packed with 20g of silicagel and fractionated further in to 8 fractions each 40 ml. Fractions (4-6) (**LTP-1**) were combined based on TLC analysis (developed in 7:3 ratio of pet-ether/EtOAc)that showed R_f value of 0.46 in this solvent system. Concentrating this fraction under reduced pressure yielded 28mg of pure **LTP-1**

LTP-1 is white Gummy substance. R_f 0.46 (4:1pet-ether/EtOAc) IR Vmax (cm^{-1}) 2931 1456 1673, 1732 1367, 1244, 1162, UV λ_{max} (in $CHCl_3$) 250nm ^{1}H NMR (400MHZ, $CDCl_3$) 1.56(2H, m, H-1); 2.28 (2H, m,H-2) 5.94(1H, dd, H-3); 3.17(1H,dd,H-5):1.85(1H,dd,H-6);2.68(1H,dd,H-6);2.25(2H,d,H-9):1.98(3H,s,H-12):1.79(3H, s, H-13):

0.98(3H, s, H-14):1.41(3H, s, H-15):6.07(1H, q, H-3'):2.00(3H, s, H-4'):1.9(3H, s, H-5')1.97-2.00(3H, s, H-7')



Scheme 3: Isolation of **LTP-1** & **LTP-3**

4.5.2 Isolation of LTP-3

2.86gm (12.54%) of Fr-17 was applied on a sephadex column using 2:1 ratio of chloroform and methanol solvent mixture. The chlorophyll free part of the fraction (963.12mg) was applied on a silicagel column and eluted with mixture of chloroform and EtOAc solvents of increasing polarity. A total of six fractions each 150ml were collected. The fractions were combined according to their R_f value and characteristic color. Fraction 4 and 5 were combined and concentrated under reduced pressure (on rotavaporator) .Eluting this fraction with pet-ether/EtOAc mixture (1:19) yielded 32mg of **LTP-3**.

LTP-3 is brown Gummy substance. . R_f 0.62 (1:4 $CHCl_3$/EtOAc) IR Vmax (cm^{-1}) 3467,2936, 14571671, 1738, 1371., 1245.99, 1144.90 UV λ_{max} (in $CHCl_3$) 250nm, ^1H NMR (400MHZ, $CDCl_3$) 1.39(1H, m, H-1); 1.22(1H, m, H-1); 1.94 (1H, m,H-2),1.79(1H, m,H-2) 5.89(1H, bdt, H-3); 2.21-2.26(1H,complex,H-5):2.89(1H,complex,H-6), 2.18(1H,complex,H-6);2.22(2H, d, H-9):2.06(3H, s, H-12):1.86(3H, s, H-13): 0.99(3H, s, H-14);1.59(3H, s, H-15):5.05(1H, q, H-'): 1.30(3H, d, H-4'):1.97(3H, s, H-')1.35 (3H, s, H-7'); 1.98(3H, s, H-9').

5. Conclusions

The presence of oily components made separation difficult. However, with repeated use of column chromatography two compounds were isolated.

LTP-1(1) was identified previously from *Teucrium carolipaui and Pluchea quitic*

LTP-3(3) from *Bluma alata.*

The base skeleton of **LTP-1** & **LTP-3** are the same, except the configuration on C-3 and C-4 (β in **LTP-1** and α in **LTP-3**).

6. References

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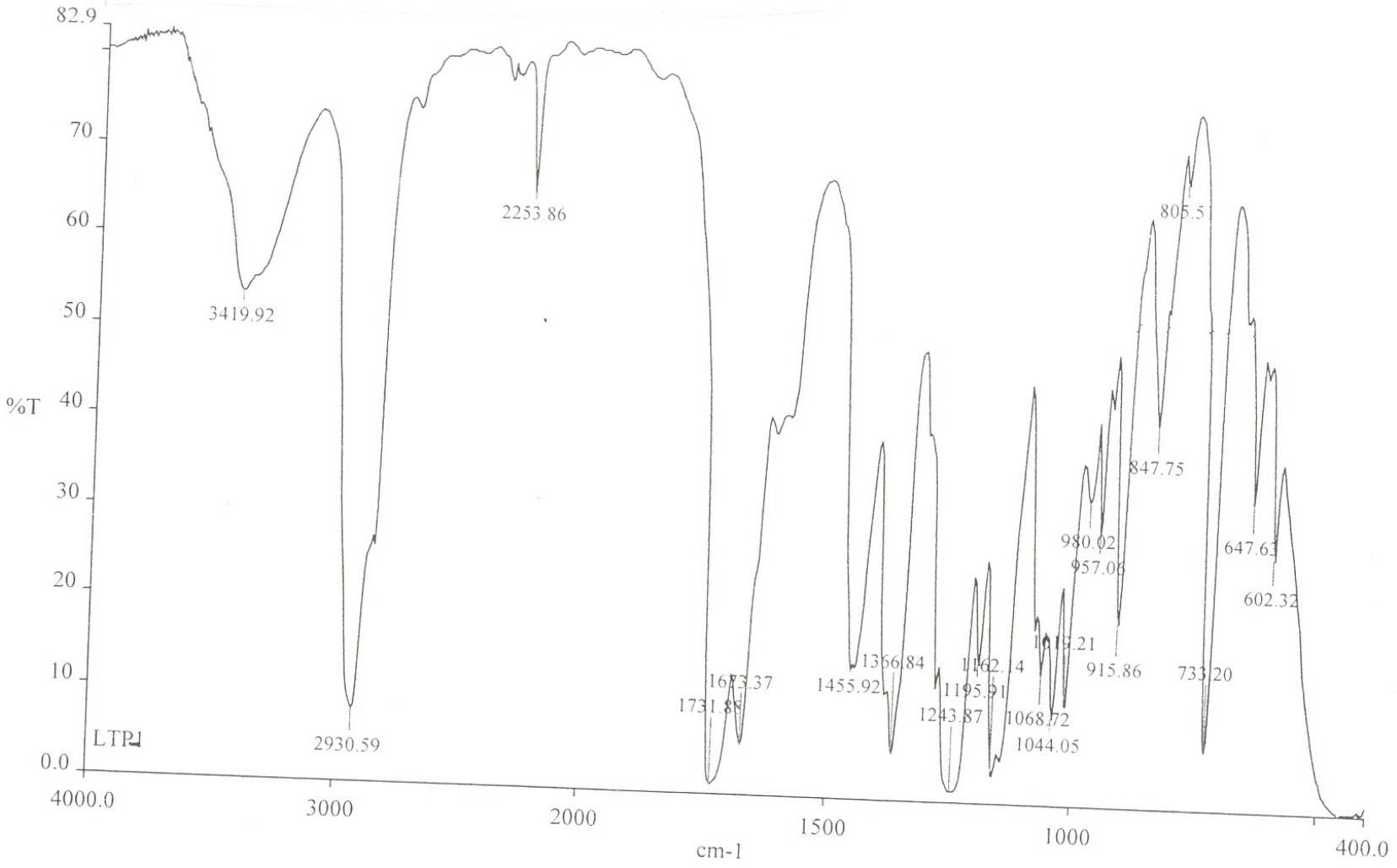
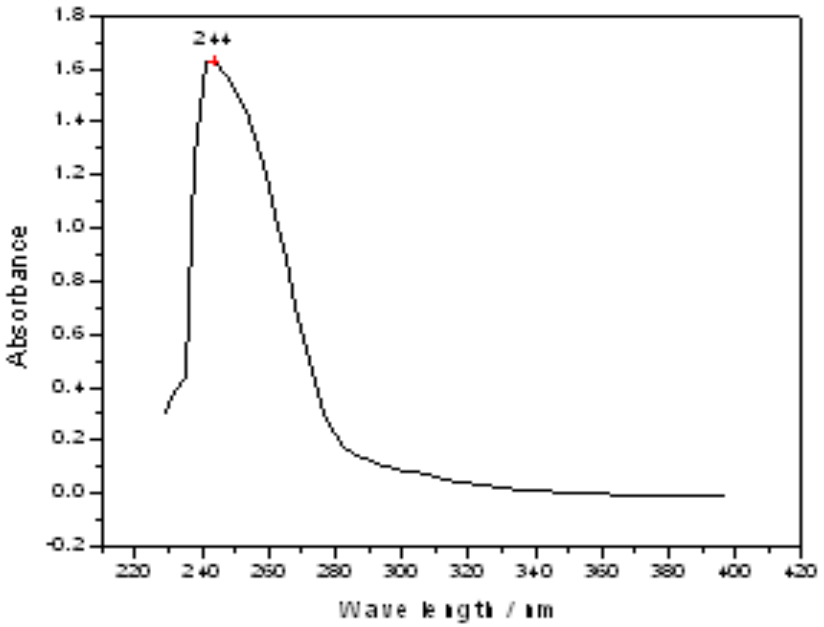
APPENDIX

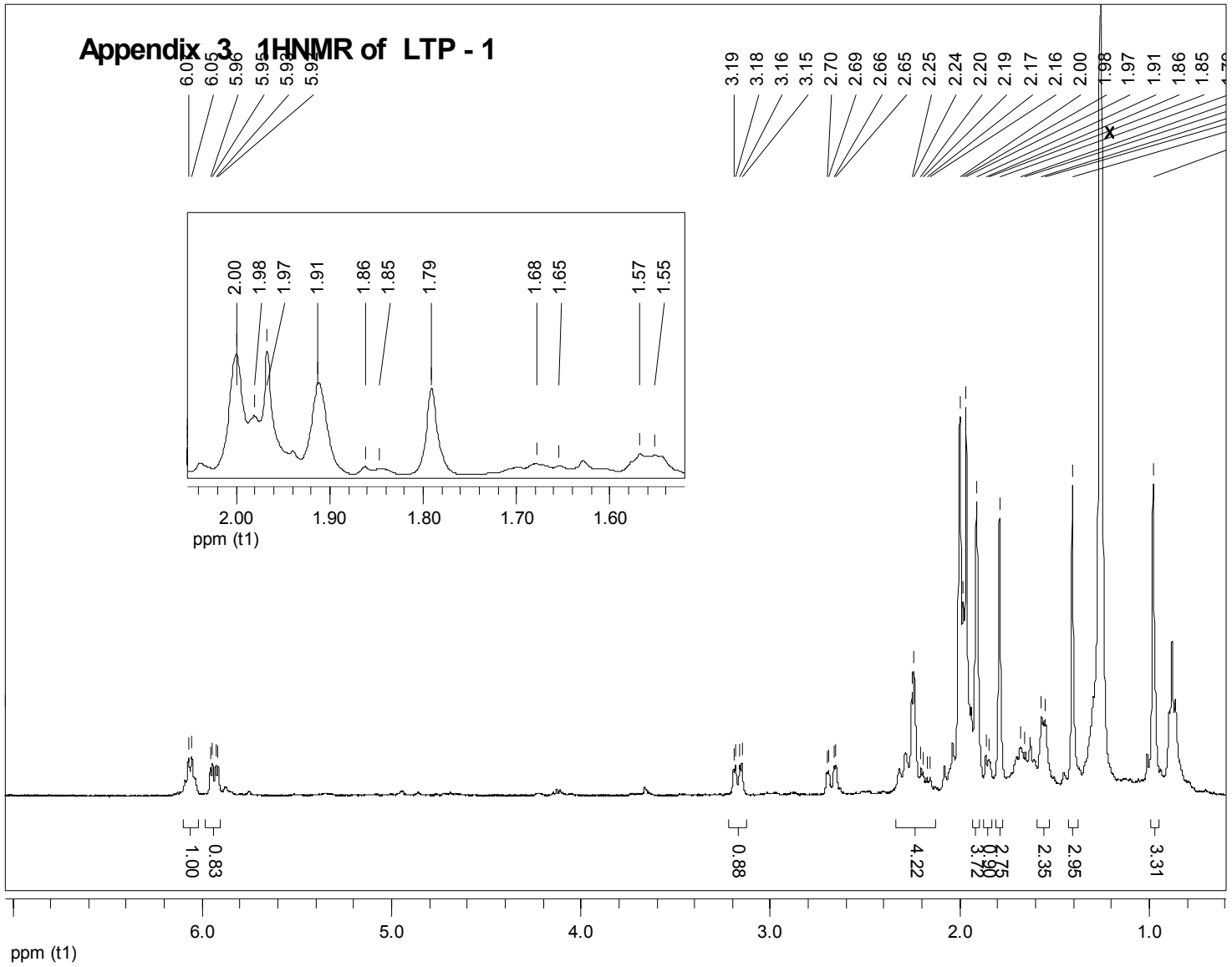
Appendix 1: IR Spectrum of LTP-1



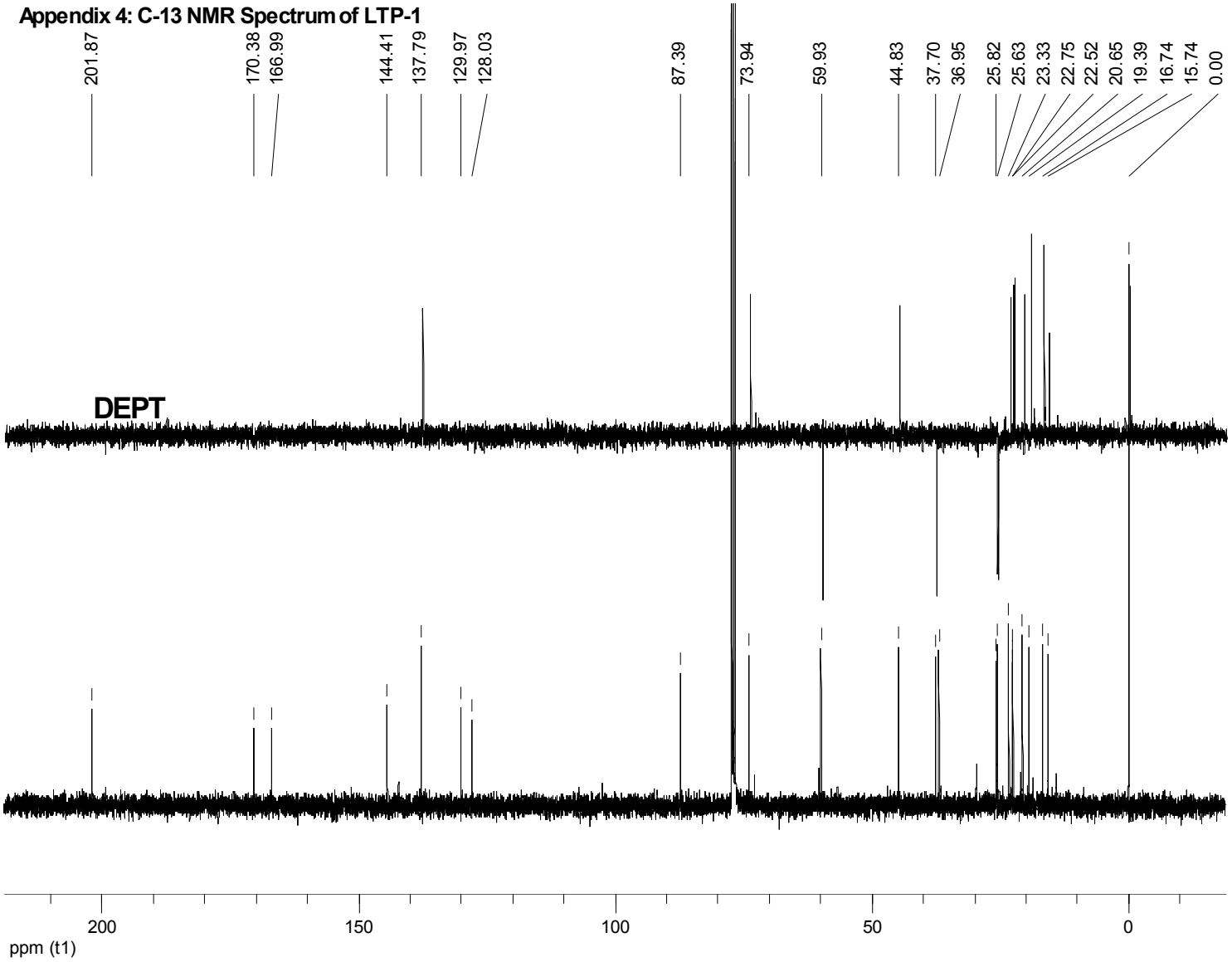
Appendix 2: UV spectrum of LTP-1

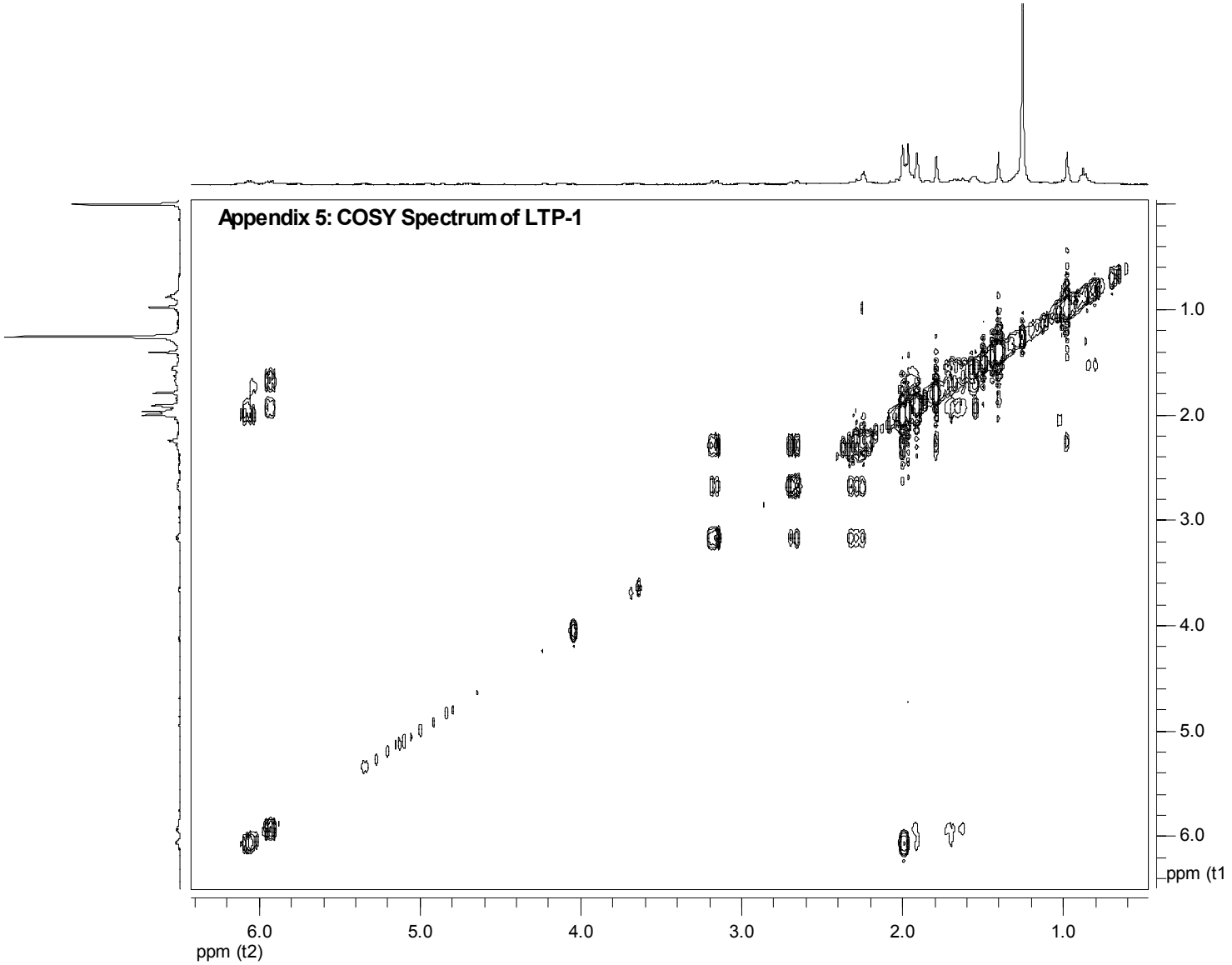


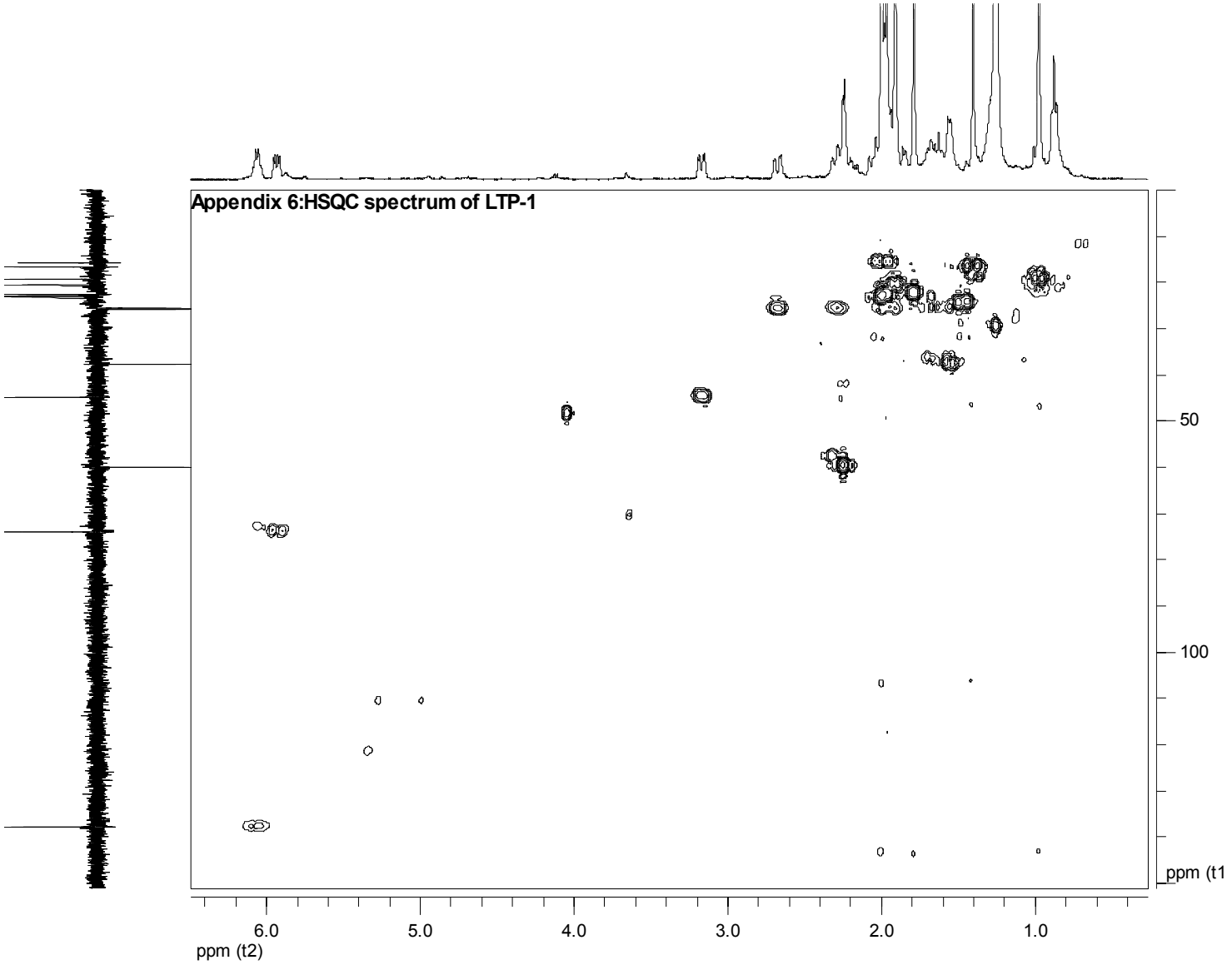
Appendix 3 1HNMR of LTP - 1

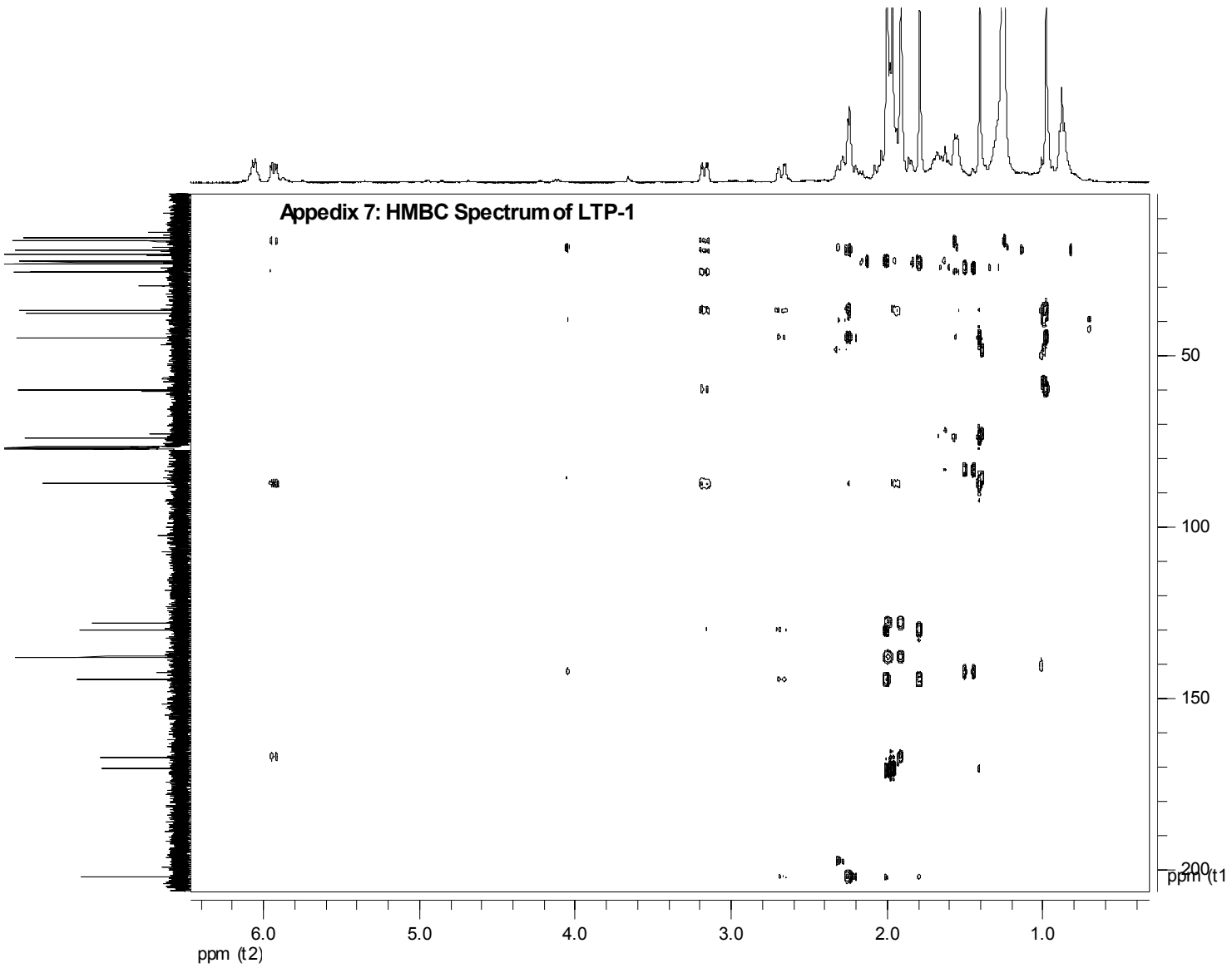


Appendix 4: C-13 NMR Spectrum of LTP-1

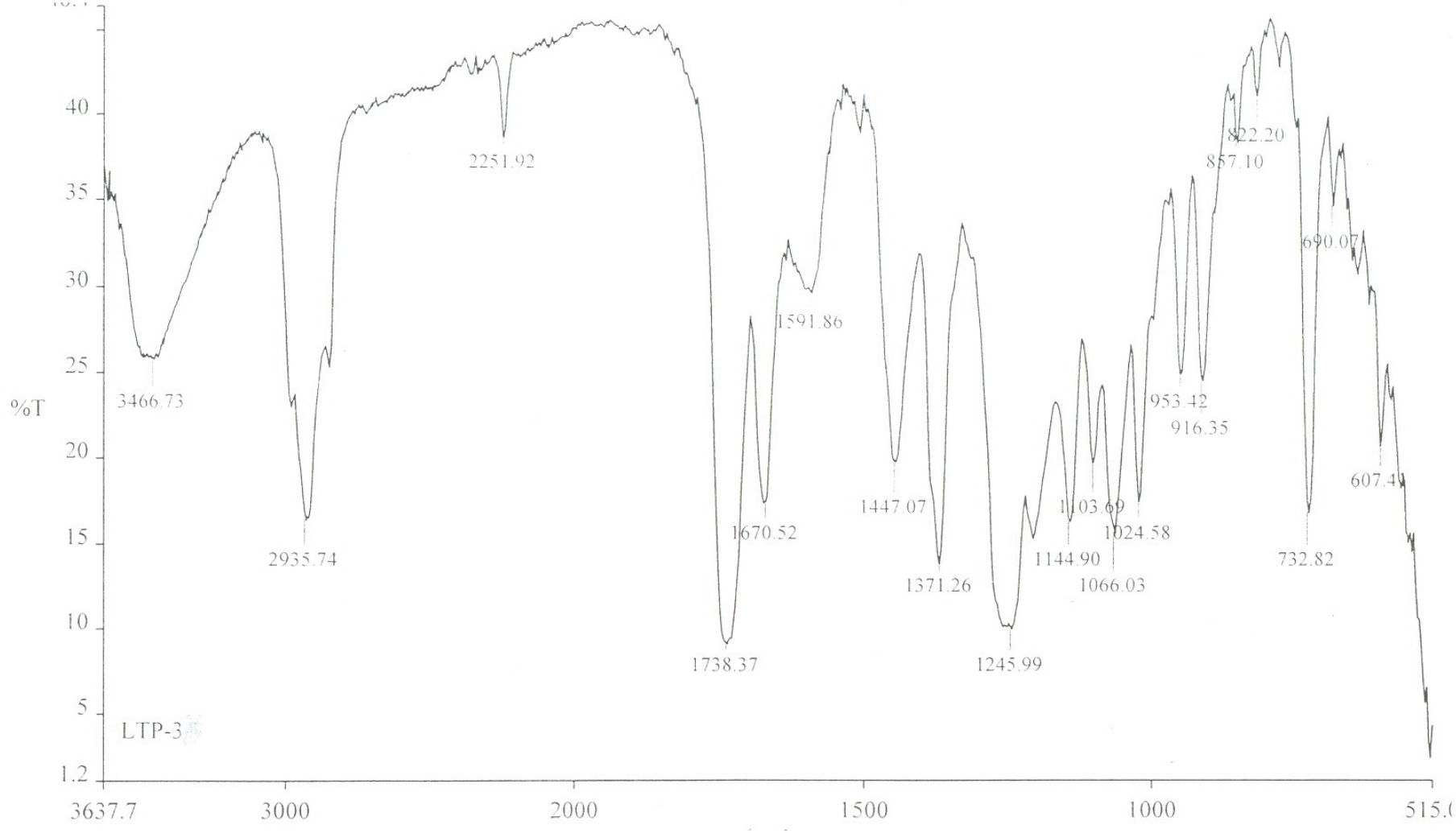



Appendix 5: COSY Spectrum of LTP-1
1.0
2.0
3.0
4.0
5.0
6.0
ppm (t1
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t2)

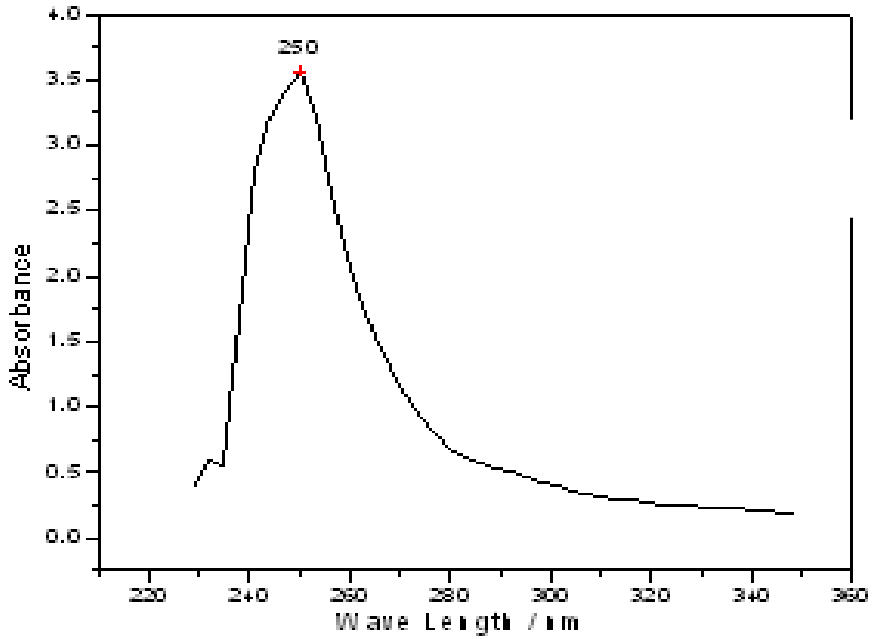

Appendix 6:HSQC spectrum of LTP-1
50
100
ppm (t1
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t2)


Appedix 7: HMBC Spectrum of LTP-1
50
100
150
200
ppm (t1
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t2)

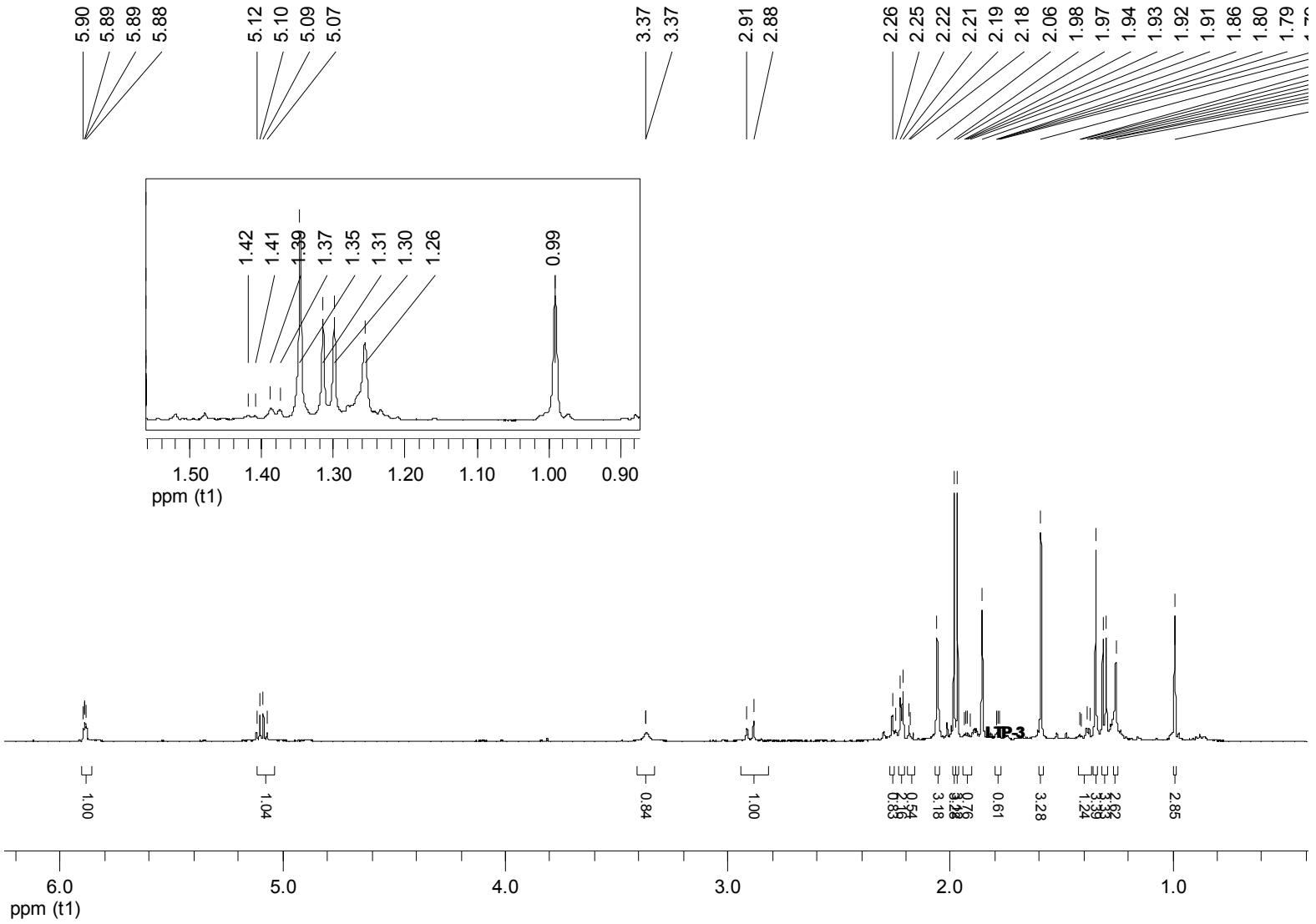
Appendix 8: IR Spectrum of LTP-3



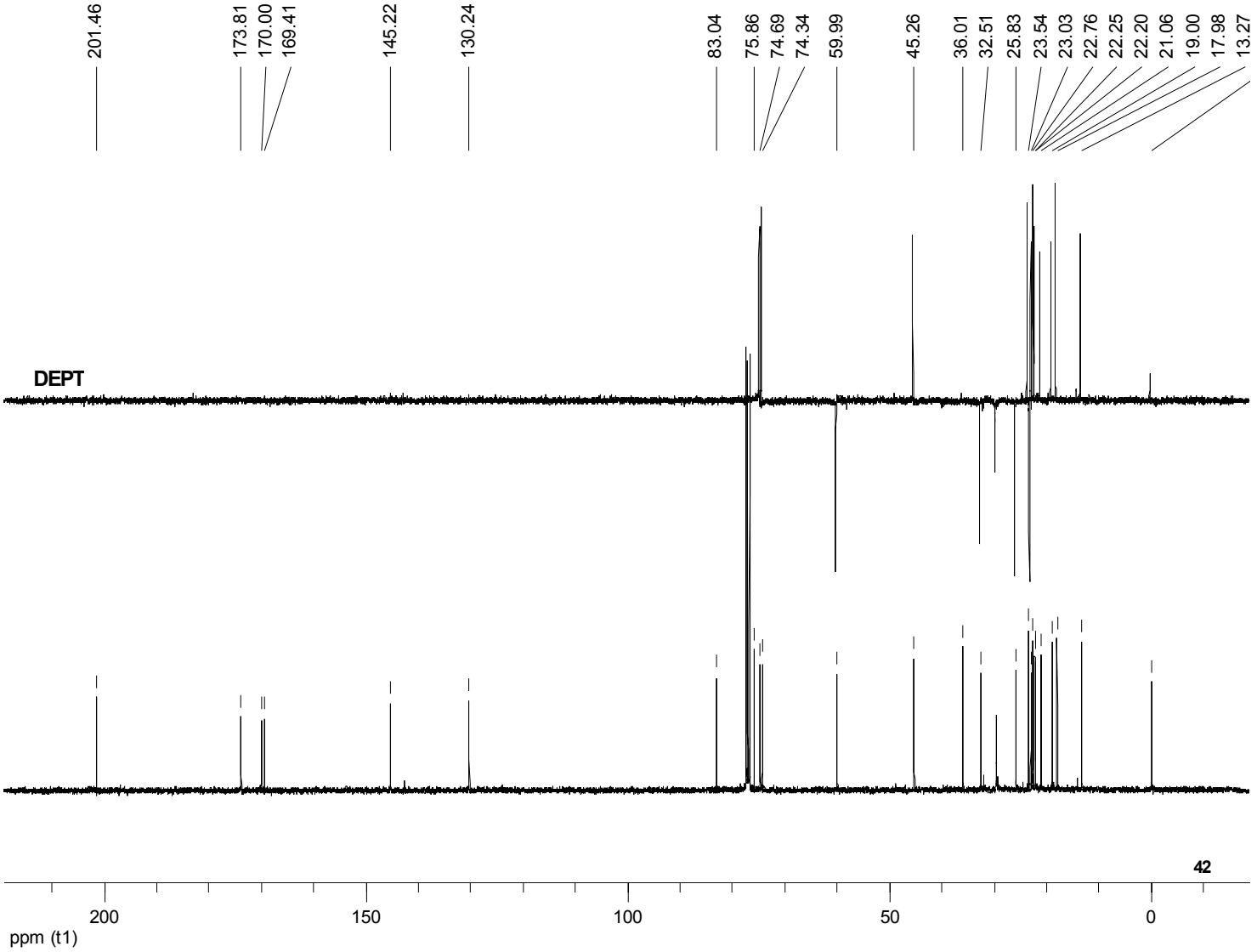
Appendix 9: UV Spectrum of LTP-3

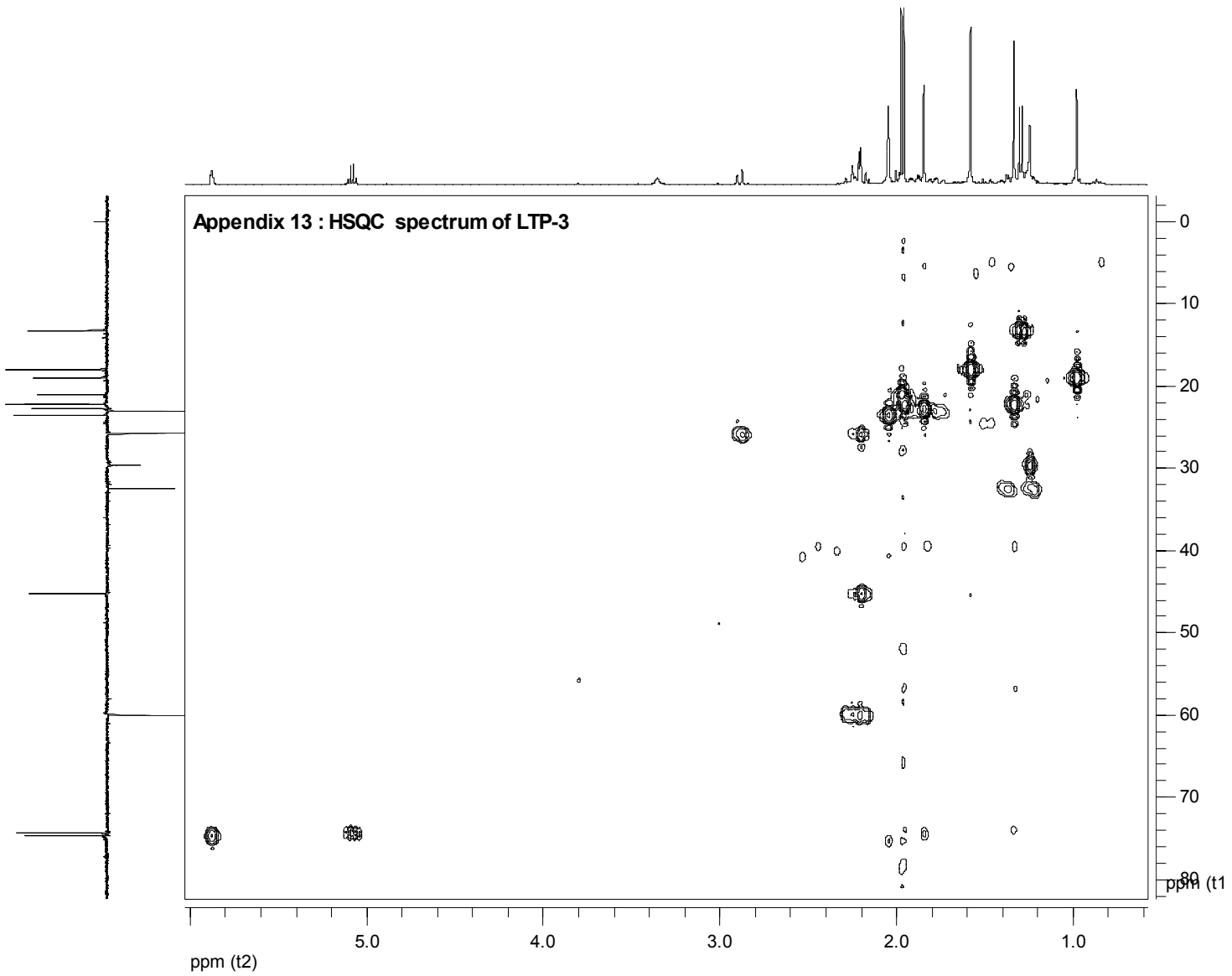


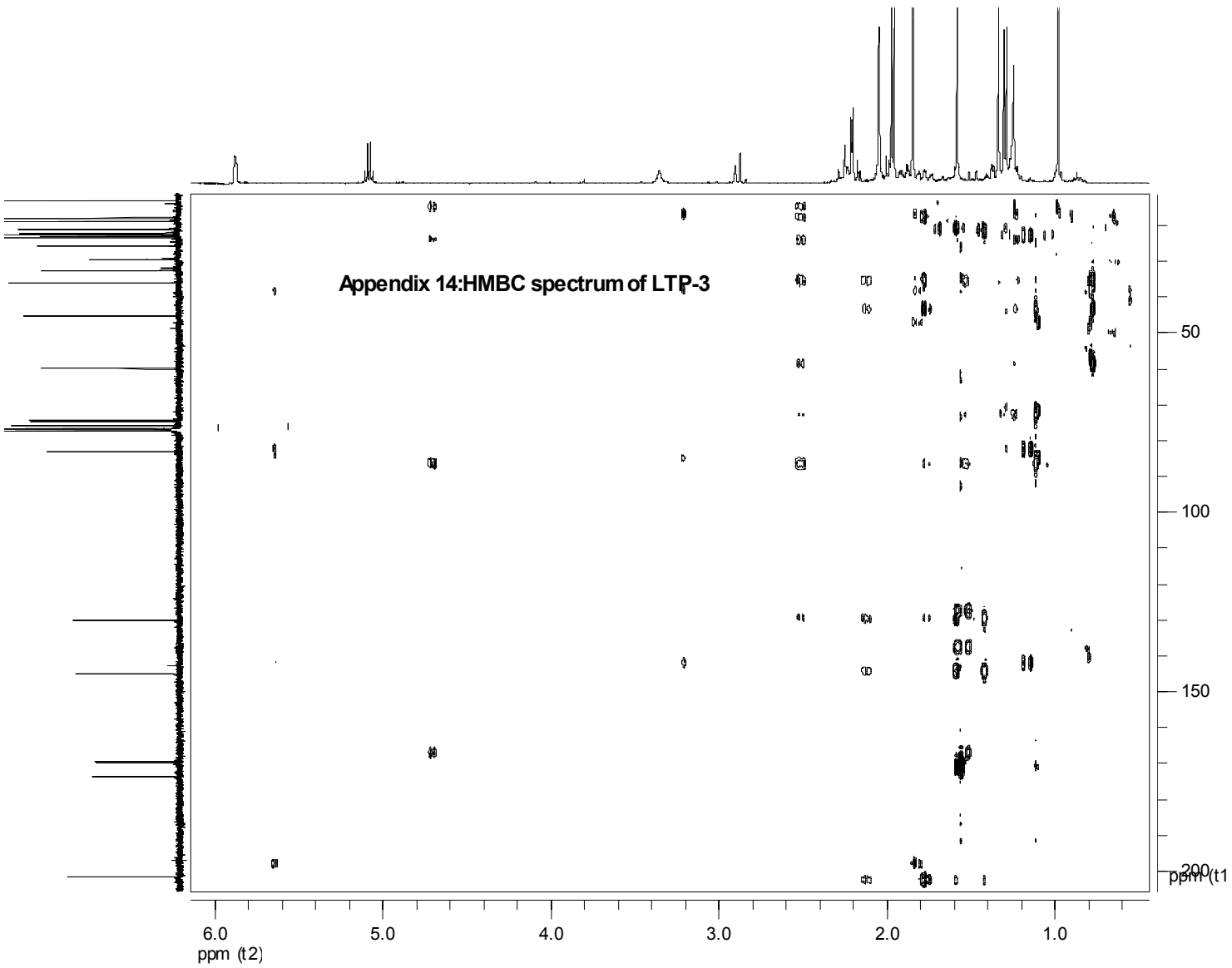
Appendix10: Proton NMR spectra of LTP-3



Appendix 12: 13-C NMR spectrum of LTP-3




Appendix 13 : HSQC spectrum of LTP-3
0
10
20
30
40
50
60
70
80
ppm (t1
5.0
4.0
3.0
2.0
1.0
ppm (t2)


Appendix 14:HMBC spectrum of LTP-3
50
100
150
200
ppm (t1
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t2)

DECLARATION

I, undersigned, declare that this project is my original work and has not been presented for a degree in any other university and that all the sources of materials used for this project has been duly acknowledged.

Name: Dibaba Amenu

Signature: _____

This project has been submitted for examination with my approval as university advisor.

Name: Dr. Nigist Asfaw

Signature: _____

Place and date of submission: Department of Chemistry

Addis Ababa University

July 2006