DEPARMENT OF CHEMISTRY

GRADUATE PROJECT (Chem.774)

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

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Advisor: Dr. NIGIST ASFAW

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

July 2006

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By KIBROM G/HEIWOT

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My heartfelt thanks are to my wife, Yordanos Brihanu, for her love, support and encouragement.

ABSTRACT

Chemical investigation on the leaves of *Laggera tomentosa*

By

Kibrom Gebrehiwot

Advisor: Dr. Nigist Asfaw

Laggera tomentosa is a perennial fragrant bushy herb endemic plant of Ethiopia. Chemical investigation on the ethanol extract of the leaves resulted in the isolation of one sesquiterpene and two flavones namely, 3-(3'- acetoxy-2'-hydroxy-2'-methyl butyryl)- cuauhtemone(**LTE-3**), 3', 5, 6-trihydroxy -3, 4', 7-Trimethoxyflavone (**LTE-5**), 3', 4', 5, 7-tetrahydroxy -3, 6- dimethoxyflavone (**LTE-6**). Of these **LTE-3** and **LTE-6** were not reported before to the best of our knowledge. The structures of the isolated compounds were established by spectroscopic techniques and chemical correlation.

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1. Introduction

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[1,2].

Natural products became a necessity to mankind. They have been immensely utilized for various purposes like for instance as foodstuff, as weapons, for treatment of diseases, in their crude form. 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 [3].

1.2 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. It is widely distributed with the herbaceous species found predominantly in temperate regions and the larger trees mainly at high attitudes in tropical areas.

The genus *Laggera*, in the family Asteraceae (Astereal, Trib, Plucheal cass) has about 17 species confined to the old world. In Ethiopia there are six *Laggera* species namely, *L. tomentosa* Sch. Bip ex Oliv and Hern., *L. crassifora* (Sch. Bip exa. Rich) oliv & Hern, *L. alata* (D. Don) Sch. Bip ex Oliv., *L. Crispata* (Vahl) Heeper and Wood, *L. braunii* Vatke, and *L. elatior* R.E. Fries [4].

A number of *Laggera* species have been widely used in traditional medicine in Asia and Africa. For example preparations have been used as analgesics, as disinfectants and to treat various diseases such as fever, pneumonia and skin tumor [5]. *L. alata and L. pterodonta* are employed as traditional herbal medicines in China for their anti-inflammatory and anti- bacterial activities [6]. In addition *L. pterodonta* was reported to posses anti-leukemia activity [7]. *Laggera decurrens* (Vahl.) Hepper and Wood, formerly known as B*lumea decurrens*, is quiet 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 of *B. decurrens* is drunk to relieve stomach pains and is also used against acne [8].

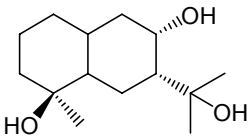
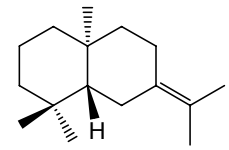
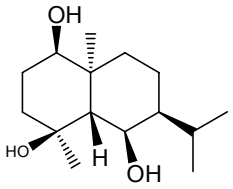
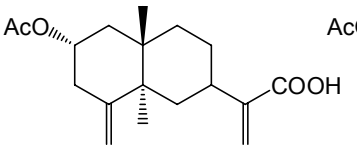
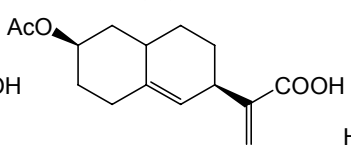
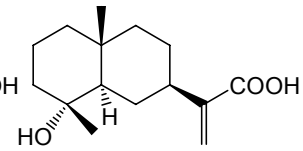
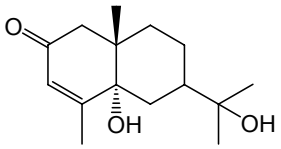
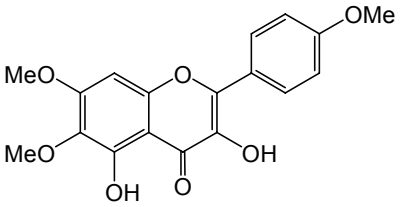
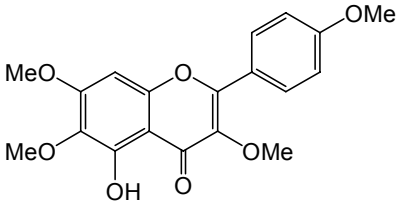
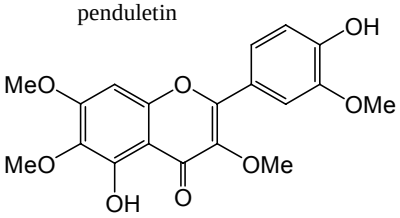
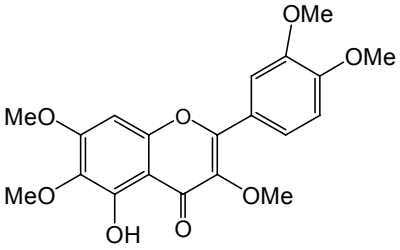
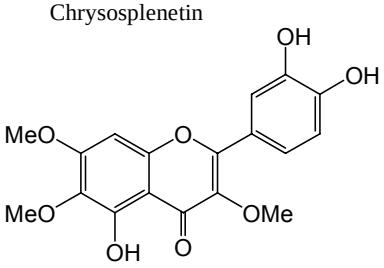
Solvent extract of some of *Laggera* species have previously analyzed. A number of compounds have been isolated from the species, the most characteristic of which are the eudesmane sesquiterpenes and flavones [6, 12-15] (Table 1).

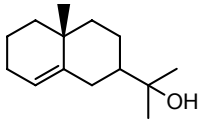
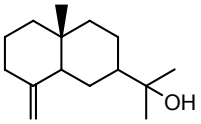
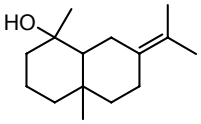
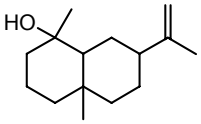
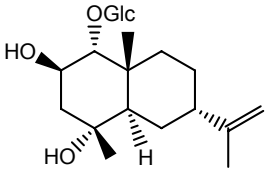
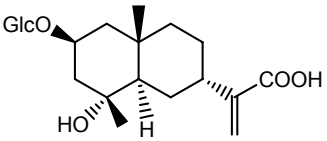
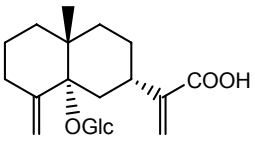
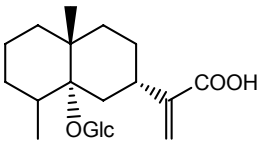
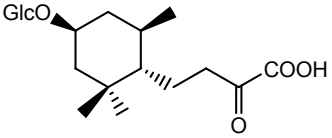
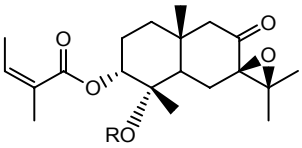
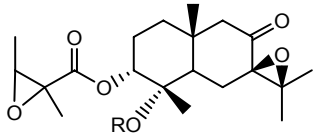
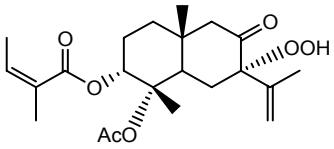
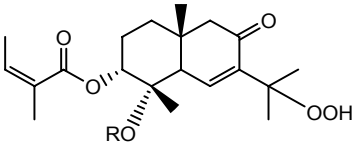
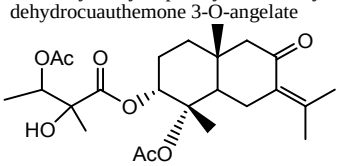
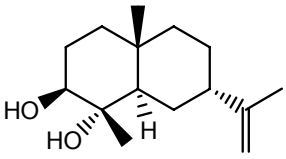
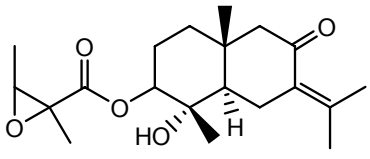
There is a close relationship between the genera *Laggera* and *Blumea* both belonging to the tribe Inuleae. They can be distinguished by the anther bases, being tailed in *Blumea* and obtuse in *Laggera*. Some authorities include *Laggera* species in the genus *Blumea*, while others exclude them; both genera kept apart in the monograph on *Blumea* [9]. Some of *Laggera* species have *Blumea* as synonym.

1.3 *Laggera tomentosa*

L. tomentosa Sch. Bip ex Oliv and Hern (Asteraceae) known locally as “keskese”, is a perennial fragment bushy herb (0.5-1.2 m high)endemic to Ethiopia. It is found in Tigray, Gonder, Gojjam, Wollo, Shewa and Arsi on dry hill and mountain slopes at an altitude of 2345-2950m [4]. 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 [4]. Phytochemical study on the essential oil of *L.tomentosa* has been reported before [10, 11]. However; there are no reportes on the chemical investigation of the solvent extract of the species.

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

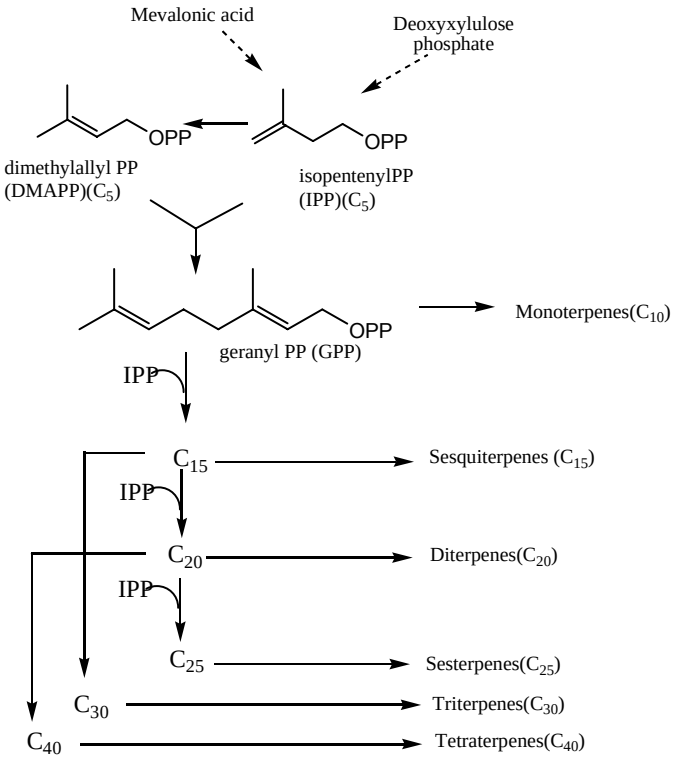
Species	Compound	Ref.
L. pterodonta	 Pterodontriol C  Ent-7 (11)-selinen-4-ol [enantio-7(11)-eudesmen-4-ol]  Pterodontriol D  2-Acetoxy costoate  2-Acetoxy pterodotric acid  Illicic acid  5,11-Dihydroxy-3-eudesmane-2-one  penduletin  5-Hydroxy-3,4',6,7-tetramethoxy flavone  Chrysosplenetin  Artemitin  3',4',5- trihydoxy-3,6,7-trimethoxyflavone	12

L. alata	 7-epi-γ-eudesmol  7-epi-β-eudesmol  Juniper camphor  Isointermedeol  Alatoside A  Alatoside B  Alatoside C  Alatoside D  Alatoside E	6, 13
Blumea alata	 **R=H:** 7,11-Epoxycuauthemone 3-O-angelate **R=Ac:** 4-O-Acetyl-7,11-Epoxycuauthemone 3-O-angelate  **R=H:** 7,11-Epoxycuauthemone 3-O-epoxyangelate **R=Ac:** 4-O-Acetyl-7,11-Epoxycuauthemone 3-O-epoxyangelate  4-O-Actyl-7-hydroperoxy-11,13-dehydro-7,11-dehydrocuauthemone 3-O-angelate  4-O-Actyl-11-hydroperoxy-6,7-dehydro-7,11-dehydrocuauthemone 3-O-angelate  4-O- acetylcuauthemone 3-O-[2-mehtyl-2hydroxy-3-acetoxybutytate]	14
L. crispata	 3,4- Dihydroxy-7-eudesm-11-ene  3-(2'3'-epoxy-2'- methylbutanoyl)cuauhtemone	15

1.4. Biosynthesis of Terpenoids

The terpenoids from a large and structurally divers family of natural products derived from C_5 isoprene unit joined in a head-to-tail fashion. Typical structure contain carbon skeletons represented by $(C_5)_n$, and classified as hemiterpenes (C_5), monotepenes(C_{10}), sesquiterpens (C_{15}), diterpenes (C_{20}), triterpenes(C_{30}).

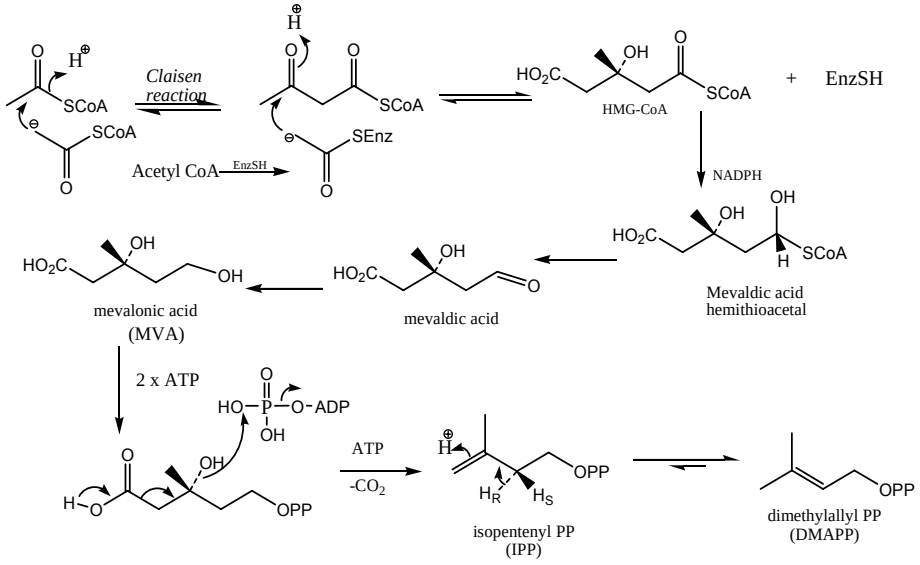
Isoprene itself had been characterized as a decomposition product from various natural cyclic hydrocarbons, and was suggested as the fundamental building block for these compounds. Isoprene is produced naturally but is not involved in the formation of these compounds, and the biologically active isoprene unit were identified as the diphosphate (pyrophosphate) esters **dimethylallyl diphosphate** (DMAPP) and **isopentenyl diphosphate**(IPP). In the biosynthesis of terpenoids IPP are condensed to DMAPP (head- tail addition) by various prenyltransferases to finally form prenyl diphosphates of different chain lengths (Scheme 1) [3].



Scheme 1. Biosynthesis of terpenoids

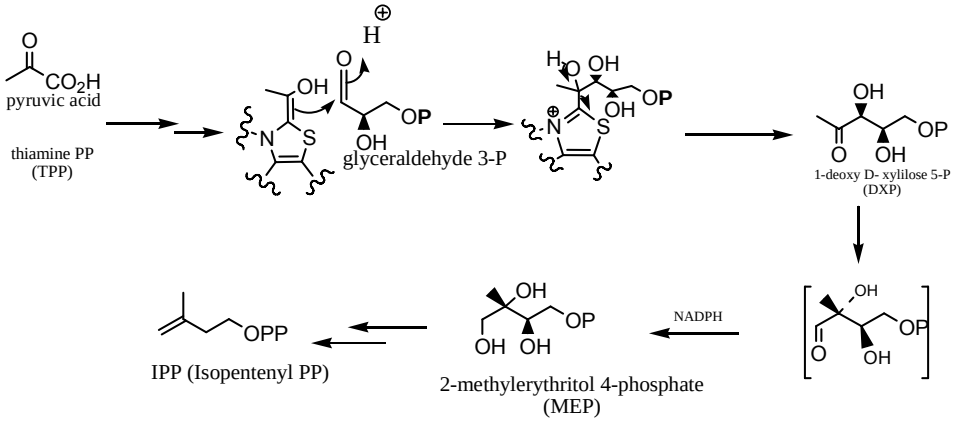
The biochemical isoprene unit, IPP, may be derived by two pathways , by way of intermediates mevalonic acid (MVA)(Scheme 2) or 1-deoxy D-xylose 5-phosphate (deoxyxylose phosphate:DXP) (Scheme 3)

In the mevalonate pathway three molecules of acetyl CoA are used to form mevalonic acid. Two molecules combine initially in a Claisen condensation to give acetoacetyl CoA, and a third is incorporated via a stereospecific aldol addition giving the branched-chain ester β-hydroxy-β-methylglutaryl-CoA (HMG-Co A). Mevalonate is then transformed to IPP by phosphylation twice at C_5 followed by a decarboxylation step.



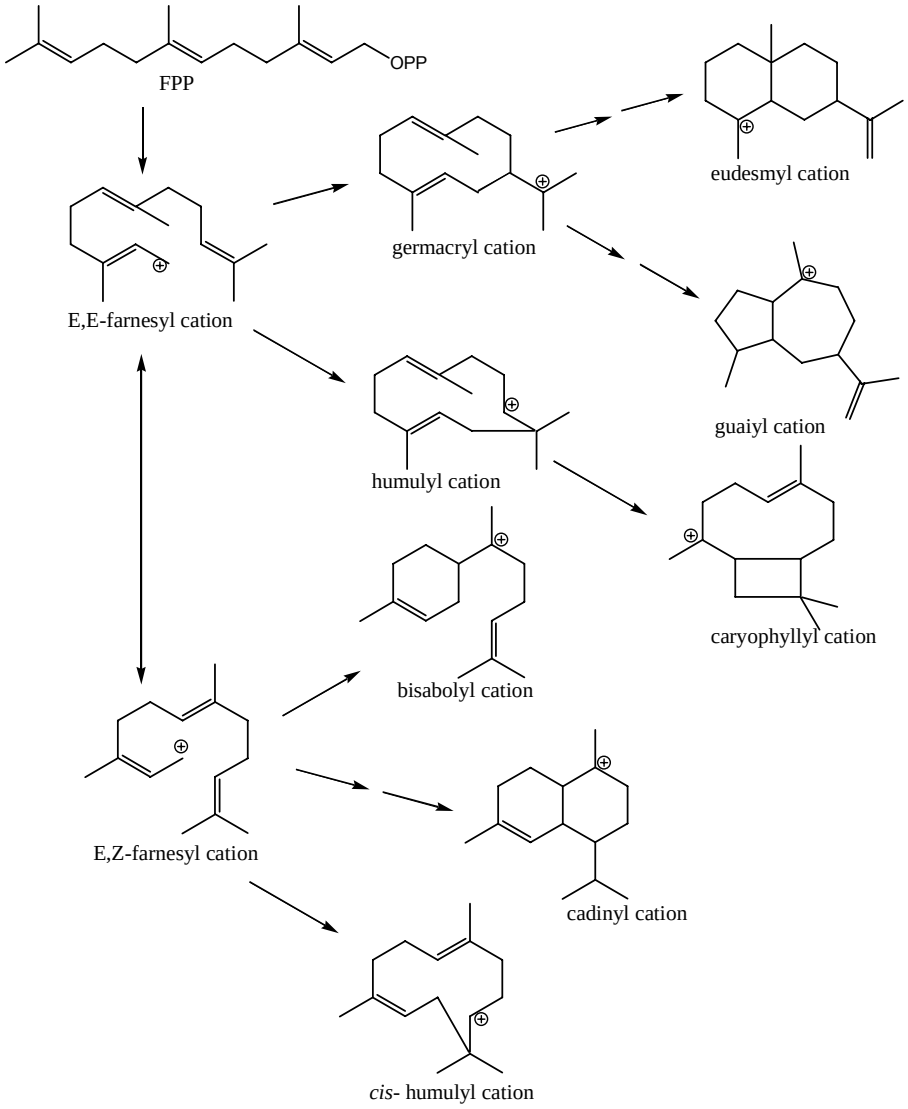
Sheme 2. Mevalonic acid pathway for the biosynthesis of isoprenoid

In the alternative path way 1-Deoxy-D-xylose 5-phosphate is formed from the glycolytic pathway intermediates pyruvic acid and glyceraldehydes 3-phosphate with the loss of the pyruvate carbonyl. Thiamine diphosphate-mediated decarboxylation of pyruvate produces an acetaldehyde equivalent bound in the form of an enamine, which reacts as a nucleophile in an addition reaction with the glyceraldehydes 3- phosphate. Subsequent release from the TPP carrier generates 1-decarboxyxylose 5-phosphate which is transformed in several steps to IPP [3].



Sheme 3. deoxyxylose phosphate pathway for the biosynthesis isoprenoid

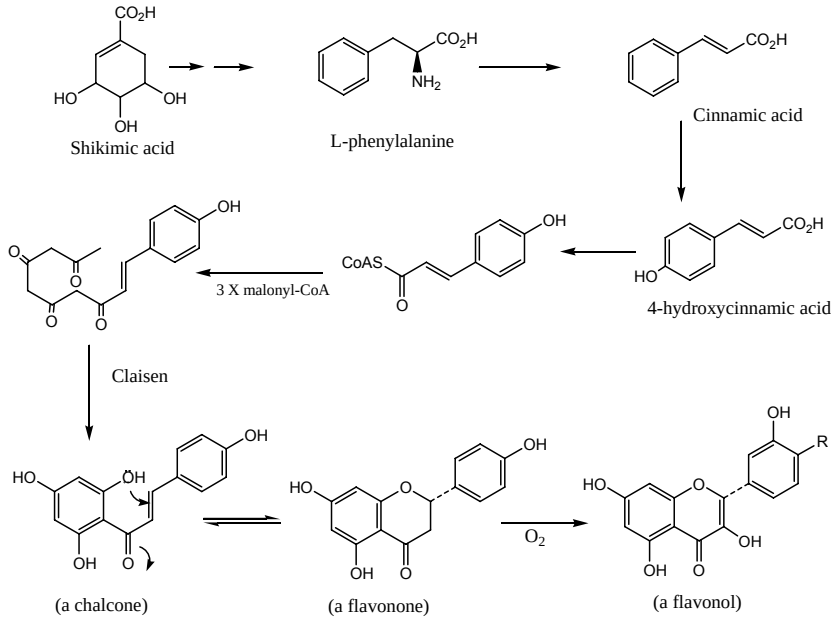
Addition of a further C_5 IPP unit to geranyldiphosphate in an extension of the prenyl transferase reaction leads to the fundamental sesquiterpene precursor, farnesyl diphosphate(FPP). FPP can then give rise to linear and cyclic sesquiterpenes(Scheme 4) [3].



Scheme 4. Biosynthesis of sesquiterpenes

1.5 Biosynthesis of Flavones

Flavonoids are products from a cinamoyl-CoA starter unit, which is produced from phenylalanine via the Shikimic acid pathway, with chain extension using three molecules of malonyl-CoA. The resulting intermediate is then transformed to a flavone by Claisen like reaction [3].



Scheme 5. Biosynthesis of flavonoids

1.6 Objectives of the Project

The main objective of the project was isolation and structural elucidation of the constituents of the ethanol extract of *L. tomentosa*. The plant was selected for this study because it endemic to Ethiopia and important in traditional medicine. Furthermore, there are no previous reports on the solvent extract of the plant.

2. Result and discussion

Three compounds LTE-3, LTE-5 and LTE-6 were isolated and characterized from the ethanol 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.

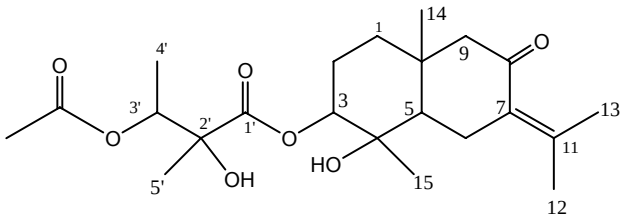
2.1 Characterization of LTE-3(1)

LTE-3 is a colorless solid with melting point of 138°C, and R_f=0.4. In the IR spectrum (Appendix 6), sharp absorption band at 3510cm^{-1} showed the presence of a free OH group. Absorption band at 1733 and 1667cm^{-1} indicated the presence of ester carbonyl and α,β-unsaturated ketone carbonyl groups, respectively. The UV spectrum displayed an absorption band at λ_{max} (in $CHCl_3$) 256nm indicating the presence of α,β-unsaturated carbonyl chromophore.

The ^{1}H spectrum (Appendix 1, Table 2) indicated the presence of an acetate group (δ 1.98, 3H, *s*), quaternary methyl groups (δ 0.94 and 1.26, 3H each, *s*) and two olefinic methyl groups (δ 1.82, and 2.03 3H each, s). Two other methyl groups in the spectrum (δ 1.28 and 1.40) were assigned to groups in an ester side chain. A downfield quartet integrated for one proton at δ 5.10 (J=8Hz) coupled to the methyl doublet at δ 1.28 (J=8Hz) was assigned to proton in the ester side chain. A downfield broadened triplet at δ 4.89, which showed a δ shift to 3.67 after hydrolysis, was assigned to methine proton on the carbon atom attached to oxygen(C-3). The other doublet of doublet methine proton at δ 1. 29 (J= 4, 16Hz) was attached to C-5. Four methylene proton appeared at δ 2.22(2H, s), 2.91, 2.12(2H, dd), 1.45, 1.27(2H, td) and 1.79. 1.86 (m). The OH signals appeared at δ 3.55.

^{13}C NMR and DEPT-135 (Appendix 2, Table 2) indicated that **LTE-3** has 22 carbon atoms: 8 quaternary, 3 methine, 4 methtylene and 7 methyl carbons, of which four resonated in the region corresponding to oxygenated carbons (δ 72.16, 74.35, 76.37, 78.88 ppm). It showed one ketone carbonyl at δ 202.08, two ester carbonyls at δ 196.84 and 174.74, two olefinic carbons at δ 130.48 and 145.89.

Structure **1** was proposed for compound **LTE-3** from the spectroscopic data obtained for the compound and in comparison with published NMR data for compounds in this series [15-17]. The 2D NMR spectra of **LTE-3** further supported the proposed structure.

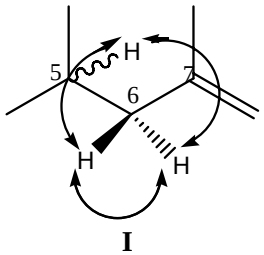


1
(**LTE-3**)

Table 2: ^{1}H, ^{13}C, and COSY ($^{1}H \leftrightarrow {}^{1}H$) NMR data of compound **1** (in $CDCl_3$, δ in ppm)

C no.	^{13}C	^{1}H	COSY
1	33.36	1.45, 1.27(td)	$H^1 \leftrightarrow H^2$
2	23.86	1.79. 1.86 (m)	$H^2 \leftrightarrow H^1$
3	78.88	4.89(bd t)	-
4	72.16	-	-
5	46.60	1.92(dd)	$H^5 \leftrightarrow H^9$, $H^5 \leftrightarrow H^{6a}$, $H^5 \leftrightarrow H^{6b}$
6	25.47	2.91, 2.12(dd)	$H^{6a} \leftrightarrow H^{6b}$, $^{6} \leftrightarrow H^5$, $H^6 \leftrightarrow H^9$
7	130.48	-	-
8	202.08	-	-
9	59.74	2.22(s)	$H^9 \leftrightarrow H^6$, $H^9 \leftrightarrow H^{14}$
10	35.78	-	-
11	145.89	-	-
12	23.61	2.03(s)	$H^{12} \leftrightarrow H^{13}$
13	22.92	1.82(s)	$H^{13} \leftrightarrow H^{12}$
14	18.65	0.94(s)	$H^{14} \leftrightarrow H^9$
15	21.45	1.26(s)	-
1’	174.74	-	-
2’	76.37	-	-
3’	74.35	5.10(q)	$H^{3'} \leftrightarrow H^{4'}$
4’	13.31	1.28(d)	$H^{4'} \leftrightarrow H^{3'}$
5’	22.36	1.40(s)	-
-OAc	169.84 21.01	- 1.98(s)	- -

The position of hydrogens on carbon was deduced from the HSQC correlation spectrum (Appendix 4). The 2D ^{1}H-^{1}H COSY spectrum (Appendix 3) showed strong correlation between the two protons on C-6 indicating that methylene protons on C-6 are diasteriotopic and C-5 is chiral. In addition the proton on C-5 showed strong correlations with the two protons on C-6 (See I).



I

In HMBC spectrum (Appendix 5, Table 3) methyl carbon signal appearing at δ 18.65 showed correlation with C-10, C-9, C-1 and C-5 indicating the position of C-14 to be on C-10. Protons on C-13 and C-12 correlated with the two olefinic carbons (C-7 and C-11) and carbonyl carbon C-8. This observation suggests the presence of α,β-unsaturated carbonyl group. Correlation of proton of C-3 with carbonyl carbon C-1' indicated that the position of the side chain to be C-3(Fig. 1).

Table 3: HMBC ($^{1}H\rightarrow^{13}C$) NMR correlation data of compound **1**

C no.	**^{13}C**	**HMBC** ($^{1}H\rightarrow^{13}C$)
2	23.86	$H^{2}\rightarrow C^{10}$
3	78.88	$H^{3}\rightarrow C^{1}$, C^{4}, C^{5}, C^{15}, $C^{1'}$
5	46.60	$H^{5}\rightarrow C^{6}$, C^{7}, C^{9}, C^{14}, C^{15}
6	25.47	$H^{6}\rightarrow C^{5}$, C^{7}, C^{8}, C^{11}
9	59.74	$H^{9}\rightarrow C^{1}$, C^{5}, C^{7}, C^{8}, C^{14}
12	23.61	$H^{12}\rightarrow C^{7}$, C^{8}, C^{11}, C^{13}
13	22.92	$H^{13}\rightarrow C^{7}$, C^{8}, C^{12}
14	18.65	$H^{14}\rightarrow C^{1}$, C^{5}, C^{9}, C^{10}
15	21.45	$H^{15}\rightarrow C^{5}$
3'	74.35	$H^{3'}\rightarrow C^{1'}$, $C^{4'}$, $C^{5'}$, $\underline{C}OCH_3$
5'	22.36	$H^{5'}\rightarrow C^{1'}$, $C^{3'}$
-OCOC$\underline{H}_3$	21.01	CO$C\underline{H}_3\rightarrow C^{3'}$, $\underline{C}OCH_3$

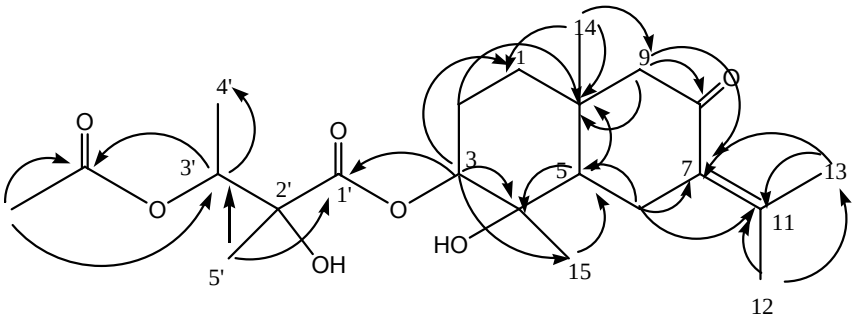
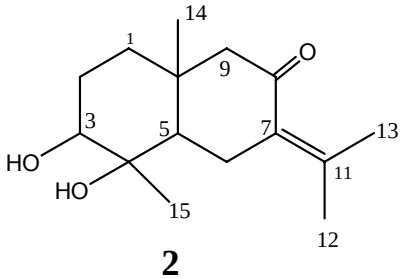


Fig. 1: Selected Proton- Carbon ($\rightarrow$) HMBC correlation of **LTE-3**.

Hydrolysis of LTE-3

Mild hydrolysis of the isolate afforded compound **2** (see experimental part). The ^{13}C of the hydrolyzed product revealed the absence of the signals for the side chain of **LTE-3,** δ 174.74, 169.84, 76.37, 74.35, 22.36, 21.01, and 13.31(Appendix 9). This confirms the presence of ester side chain in **LTE-3**. The ^{1}H spectrum (Appendix 8) showed a δ shift from 4.89 to 3.67 for H-3 indicating that the position of the side chain to be at C-3.



2

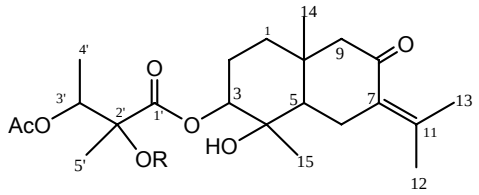
The ^{1}H and ^{13}C NMR data of **2** found to be consistent with those reported for the compound in the literature [16, 17] (Table 4)

Table 4 Comparison of ^{13}C and ^{1}H NMR of **2** with the literature.

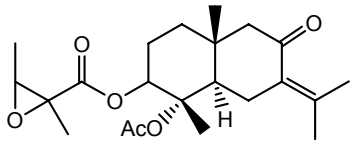
C no.	2		Literature value	
	^{13}C	^{1}H	^{13}C	^{1}H
1	32.92	1.25, (m)	32.9	1.20, 1.80
2	25.71	1.79. (m)	25.7	1.8
3	74.43	3.67(bd t)	74.3	3.65
4	73.12	-	73.1	-
5	45.60	2.06(dd)	45.6	2.0
6	25.75	2.95, 2.01(m)	25.7	2.94, 2.0
7	131.29	-	131.2	-
8	202.78	-	202.0	-
9	60.17	2.23(m)	60.2	2.21
10	36.33	-	36.3	-
11	144.09	-	144.1	-
12	23.38	2.02(s)	23.4	2.03
13	22.66	1.83(s)	22.7	1.84
14	18.58	0.93(s)	18.6	0.94
15	21.45	1.22(s)	21.4	1.22

Literature and NMR data confirms compound **2** to be Cuauhtemone.

The ^{1}H and ^{13}C NMR data obtained for **LTE-3** is comparable with the data for compounds **3** [16] and **4** [15, 17] (Table 5).



1: R=H
3: R= Ac



4

Table 5: Comparison of the ^{13}C and ^{1}H NMR of **LTE-3** (**1**) with compounds **3** & **4**.

C no.	^{13}C(ppm)		^{1}H(ppm)	
	1	**4**	**1**	**3**
1	33.36	32.9	1.45, 1.27(dt)	1.33(dt), 1.48(ddd)
2	23.86	23.0	1.79, 1.86 (m)	1.81(m), 1.84(m)
3	78.88	73.7	4.89(t)	5.02(t)
4	72.16	82.9	-	
5	46.60	45.1	1.92(dd)	1.92(dd)
6	25.47	25.8	2.91, 2.12(dd)	3.01(dd),2.17(dd)
7	130.48	130.1	-	
8	202.08	201.4	-	
9	59.74	60.1	2.22(s)	2.17(d), 2.25 (d)
10	35.78	35.9	-	
11	145.89	145.5	-	
12	23.61	23.6	2.03(s)	2.10(s)
13	22.92	22.8	1.82(s)	1.86(s)
14	18.65	18.0	0.94(s)	0.98(s)
15	21.45	19.1	1.26(s)	1.28(s)
1’	174.74	168..2	-	
2’	76.37	59.2	-	
3’	74.35	59.6	5.10(q)	5.24(q)
4’	13.31	13.9	1.28(d)	1.26(d)
5’	22.36	19.4	1.40(s)	1.66(s)
-OAc	169.84 21.01	169.3 22.2	- 1.98(s)	- 2.09(s)

*The ^{13}C NMR data for Compound **3** was not reported[16].

Based on spectroscopic data and literature survey, the compound **LTE-3** is characterized to be 3-(3’- acetoxy-2’-hydroxy-2’-methyl butyryl)- cuauhtemone.

So far **LTE-3** was not isolated from other plant, however, its acetate derivative, 3-(2’3’-diacetoxy-2’-Methyl Butyryl)-Cuauhtemone(**3**) was isolated from *Pluchea Indica* [16].

2.2 Characterization of LTE-5 (5)

LTE-5 is a yellowish solid with melting point 208°C and R_f=0.4. A strong IR absorption band at 3493cm^{-1} (Appendix 13) showed the presence of a free OH group and broad absorption band at 3324 cm^{-1} indicating presence of a hydrogen bonded OH group. Absorption band 1677cm^{-1} indicated the presence of α,β-unsaturated carbonyl groups.

Flavonoid consists of two absorption maxima in the UV Spectrum: Band I, 300-550nm, for B-ring cinnamoyl system and band II, 240-285nm, for A ring benzoyl system.

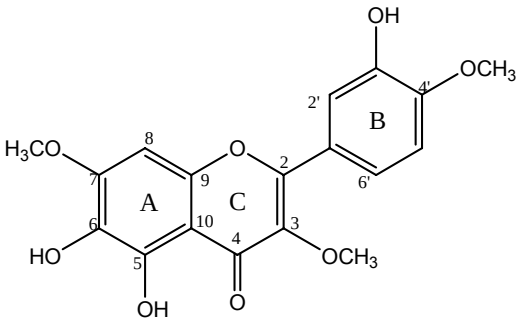
Absorption at λ_{max} (in MeOH) 349nm and at λ_{max} 280 nm a are observed for band I and band II respectively.

The ^{1}H NMR spectrum (Appendix 10) displayed four aromatic protons at δ 7.74 (1H, d, J=1.6Hz), 7.66 (1H, dd, J=1.6, 8.4 Hz), 7.04 (1H, d, J=4Hz), 6.55(1H, s). A proton singlet at δ 12.41 confirming the presence of a hydrogen bonded OH group.

The ^{13}C NMR (Appendix 11) revealed 18 carbons, which were identified as three methoxy, four methines, and eleven quaternary carbon atoms from the DEPT experiment. The ^{13}C shift assignment is based on the use of flavone as a model and additive substituent parameter for benzene[18]. All fifteen signals due to the flavonoes nucleus resonate in the region 90-200 ppm. The chemical shift of the carbons of C-ring usually distinct for flavones: C-2 (155- 165), C-3 (136-139), C-4 (176-184)[19]. For the isolated compound the three carbons are found in the expected range. This confirms **LTE-3** to be a flavone.

Table 6: ^{1}H and ^{13}C NMR data of compound **5** (in $CDCl_3$, δ in ppm from TMS)

C no.	^{13}C	^{1}H
2	156.19	-
3	138.69	-
4	178.73	-
5	145.33	-
6	129.26	-
7	152.91	-
8	90.19	6.55 (s)
9	149.74	-
10	106.39	-
1’	122.60	-
2’	111.01	7.74 (d)
3’	146.40	-
4’	148.37	-
5’	114.61	7.04 (d)
6’	122.63	7.66 (dd)
3-OCH_3	60.16	3.86 (s)
7- OCH_3	56.48	4.00(s)
4’- OCH_3	56.15	3.99(s)



5
(LTE-5)

The position of methoxy groups was determined from the HMBC spectra. The methoxy at δ 56.48 correlated with carbon at δ 152.9 9 indicating its position to be C-8. Similarly methoxy at 60.16 correlated with C-3 and methoxy at 56.15 with C-4’ (Fig. 2). The presence of a C-3 methoxy was further supported by the ^{13}C in which the methoxy signal appeared down field shift at δ 60.5. Methoxy carbons usually resonate at δ 55- 56.5. However, a down field shift to the range δ 59.5-60.30 is observed when the methoxy group is di-ortho substitution by a bulky substituent like –OH, -OMe or a ring junction.

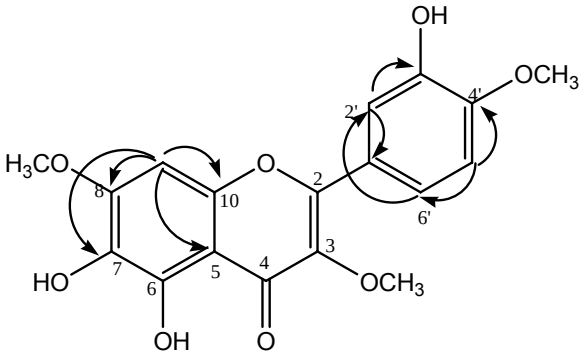
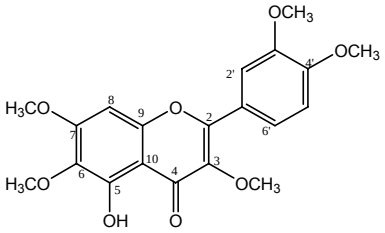


Fig 2: Propton-Carbon($\rightarrow$) conectivities in HMBC of **LTE-5**

Its ^{13}C NMR data shows comparable value with literature [18, 19].

Table 7: Comparison of the ^{13}C NMR of **LTE-5(5)** with compound **6** and **7**.

C no.	^{13}C (ppm)		
	5	**6** [18]	**7**[19]
2	156.19	151.4	155.3
3	138.69	138.0	138.2
4	178.73	180.0	178.1
5	145.33	151.9	161.0
6	129.26	131.5	98.8
7	152.91	158.0	164.4
8	90.19	89.8	93.8
9	149.74	155.0	151.8
10	106.39	105.7	104.5
1’	122.60	122.1	122.6
2’	111.01	110.7	111.8
3’	146.40	148.0	146.6
4’	148.37	150.8	150.3
5’	114.61	110.3	115.2
6’	122.63	121.5	120.5



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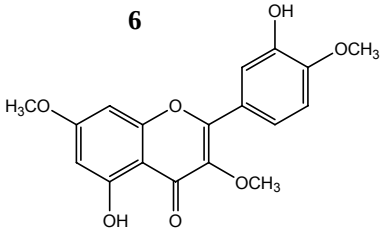


7

Based on spectroscopic data and literature survey, the compound **LTE-5** is 3’, 5, 6-trihydroxy -3, 4’, 7- Trimethoxyflavone. Dictionary of natural product on CD ROM indicated that this compound was isolated from *Pulicaria dysenterica,* and *Ambrosia cordifolia* [20].

2.3 Characterization of LTE-6 (8)

LTE-6 is a yellowish solid with melting point 202°C and R_f=0.2. A strong IR absorption band at 3403cm^{-1} (Appendix 18) showed the presence of a free OH group and broad absorption band at 3152 cm^{-1} indicated presence of a hydrogen bonded OH group. Absorption band 1654cm^{-1} indicated the presence of α,β-unsaturated carbonyl groups.

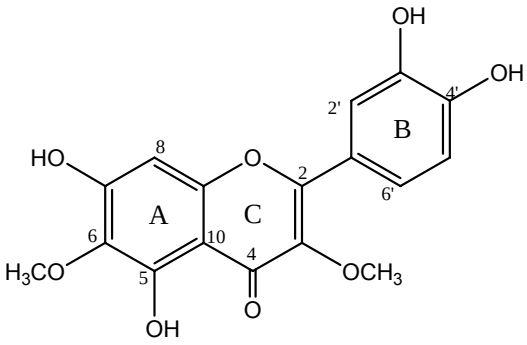
In the UV spectrum λ_{max} (in MeOH) 352nm and 256 nm are due to band I and band II of the flavone nucleus

The ^{1}H NMR spectrum (Appendix 15) displayed four aromatic protons at δ 7.65 (1H, s), 7.56 (1H, d, J= 8.4 Hz), 6.93 (1H, d, J= 8.4Hz), 6.51(1H, s). A proton singlet at δ 12.71 confirming the presence of a hydrogen bonded OH group.

The ^{13}C NMR (Appendix 16 revealed 17 carbons, which were identified as two methoxy, four methines, eleven quaternary carbon atoms from the DEPT experiment. The ^{13}C shift assignment is based on the same principle as that of **LTE-5.** The three carbons, C-2, C-3, C-4, have chemical shift in the expected range for flavone, 156.82, 138.36, and 179.21 respectively.

Table 8: ^{1}Hand ^{13}C NMR data of compound **8** (in $CDCl_3$ + Methanol-d_4, δ in ppm)

C no.	^{13}C	^{1}H
2	156.82	-
3	138.36	-
4	179.21	-
5	152.57	-
6	131.10	-
7	156.62	-
8	94.05	6.51
9	152.28	-
10	105.81	-
1’	122.21	-
2’	115.40	7.65
3’	144.77	-
4’	148.14	-
5’	115.47	6.93
6’	121.65	7.56
3-OCH_3	60.18	3.79
6- OCH_3	60.81	3.94



8
(LTE-6)

The position of methoxy groups was determined from the HMBC spectra. The methoxy at δ 60.18 correlated with carbon at δ 138.36 indicating its position to be C-3. Similarly methoxy at 60.81 correlated with C-6. Both methoxy shos a down field δ grater than 60. this showed that they are either di- orthosubstituted by -OH or on ring junction.

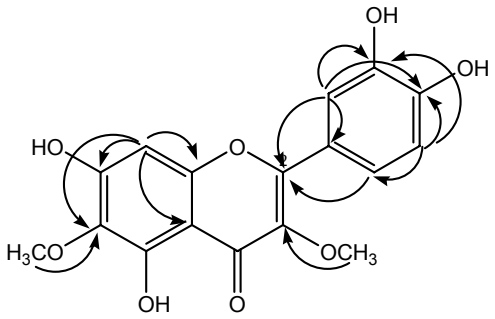
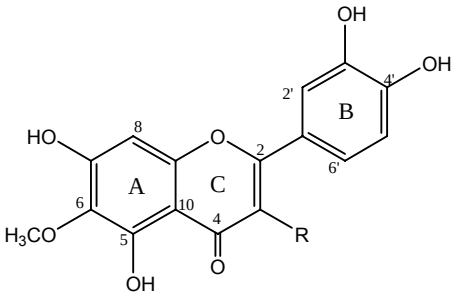


Fig 3: Propton-Carbon($\rightarrow$) conectivities in HMBC of **LTE-6**

Its ^{13}C NMR data shows comparable value with literature [19].

Table 9: Comparison of the ^{13}C NMR of **LTE-6 (8)** with the compound **9**.

	^{13}C (ppm)	
C no.	**8**	**9**
2	156.82	147.8
3	138.36	135.5
4	179.21	176.1
5	152.57	151.8
6	131.10	130.9
7	156.62	157.2
8	94.05	93.7
9	152.28	151.8
10	105.81	103.5
1’	122.21	122.1
2’	115.40	115.2
3’	144.77	145.1
4’	148.14	147.1
5’	115.47	115.7
6’	121.65	120.1



8 R=OME
9 R=OH

Based on spectroscopic data and literature survey, the compound **LTE-6** is 3’, 4’, 5, 7-tetrahydroxy -3, 6- dimethoxyflavone. To the best of our knowledge, the compound has not been reported before.

3. Conclusions

To the best of our knowledge, the isolated sesquiterpene, 3-(3'-acetoxy-2'-hydroxy-2'-methyl butyryl)-cuauhtemone(**1**), was reported for the first time from a natural source. The occurrence of other molecules having eduesmane skeleton (cuauhtemone derivative) has been reported for *L. alata* grown in South Africa [17] and *L. Crispata* grown in Ethiopia [15]. Eudesmane sesquiterpenes in the cuauhtemone derivative have not been isolated from other species of the genus. This indicated that African *Laggera* species are reach in Eudesmane sesquiterpenes in the cuauhtemone series.

Of the two isolated flavone 3', 5, 6-trihydroxy -3, 4', 7- Trimethoxyflavone, 3', 4', 5, 7-tetrahydroxy -3, 6- dimethoxyflavone, the latter to the best of our knoledge is reported for the first time.

4. Experimental

4.1. *General*

^{1}H, ^{13}C, and 2D NMR spectra were recorded on a Bruker advance 400MHz spectrometer with TMS as internal started. The ultraviolet and visible (UV-Vis) spectra were taken on GENESY'S 2PC UV-Vis scanning spectrometer in the range 200-1000 cm^{-1}. Infrared (IR) spectra were obtained on Perkin-Elmer BX Infrared spectrometer using KBr in the range 4000-400 cm^{-1}. Melting points were recorded using Thomas HOOVER Capillary melting point apparatus.

TLC analyses were carried out on TLC plates 0.2 mm thick layer of Merck silica gel 60 F_{254} coated on aluminum foil. Compounds on TLC were detected using UV light and spraying with 1% vanillin in sulfuric acid and 5% methanolic KOH solution.

4.2 Coding system

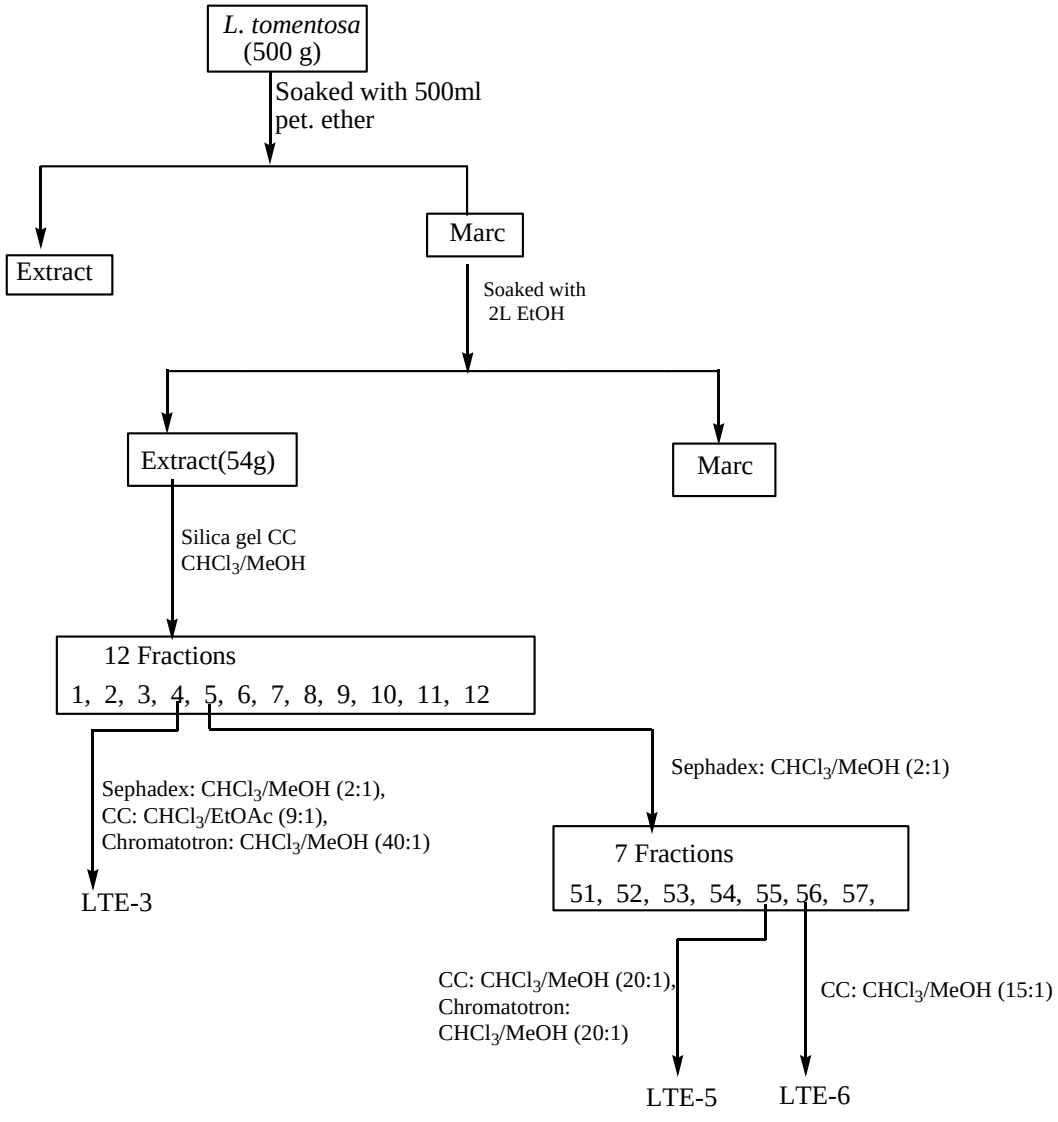
L stands for the genus name Laggera, **T** stands for species name tomentosa, **E** stands for ethanol extract and the number behind LTE indicates the location of the compound starting from the highest R_f value to the lowest.

4.3 Plant material:

Laggera tomentosa was collected from Daletti, Western Shoa 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 Isolation and Analysis

500g of powdered *L. tomentosa* was first extracted with petroleum ether (1.5L). The marc was then soaked with ethanol twice (1L ethanol each time) for 24 hrs. The filtrates were combined and evaporated under reduced pressure using Rotavapor to yield 54g solid. About 20g of crude extract was applied to a silicagel (150g) CC and eluted with $CHCl_3$/MeOH in increasing proportion. A total of 29 fractions were collected and combined in to 12 fractions on the bases of similar TLC profile (Scheme 6). Those fractions from flash column chromatography (FCC) that had similar retention factor were combined. The isolated compounds were analyzed using spectroscopic technique.



CC= column chromatography

Scheme 6: Method of isolation

4.4.1 Isolation of LTE-3

Fraction 4 from column chromatography was passed through Sephadex LH-20 using chloroform/methanol (2:1) as eluent and four fractions were collected. Fraction 2 was chromatographed on a 10g silica gel column, using chloroform/ethyl acetate (9:1) as eluent. Further purification using chromatotron ($CHCl_3$/MeOH- 40:1) afforded 163mg **LTE-3**. It's TLC (R_f=0.4) run with $CHCl_3$/MeOH at 4.5:5drops showed brown color after spraying with 1% vanillin in H_2SO_4.The compound obtained is a colorless solid with melting point of 138^oC.

IR**:** (KBr) ν_{max}: 3510, 2954, 2942, 2884, 1732, 1667, 1584, 1449, 1380, 1267, 1204, 1148, 1105, 1080. 1065, 1019, 966cm^{-1} (Appendix 6);

UV spectrum λ_{max} ($CHCl_3$) 256nm (Appendix 7)

^1H NMR (400 MHz, $CDCl_3$) δ 0.94(3H, s, 14-H_3), 1.26(3H, s, 15-H_3), 1.28(3H, d, J=8Hz, 4'-H_3), 1.40(3H, s, 5'-H_3), 1.45, 1.27(2H, dt, J=4, 12Hz, 1-H_2), 1.79. 1.86 (2H, m, 2-H_2) 1.82(3H, s, 13-H_3), 1.92(1H, dd, J=4, 16 Hz, 5-H), 1.98(3H, s, OAc), 2.03(3H, s, 12-H_2), 2.22(2H, s, 9-H_2), 2.91, 2.12(2H, dd, J=4, 16 Hz, 6-H_2), 4.89(1H, br t, 3-H), 5.10 (1H, q, J= 4, 8Hz, 3'-H) (Appendix 1)

^{13}C NMR (100MHz, $CDCl_3$) δ 202.08 (C-8), 174.74 (C-1'), 169.84 (-O<u>C</u>OCH_3), 145.89 (C-11), 130.48 (C-7), 78.88 (C-3), 76.37 (C-2'), 74.35 (C-3'), 72.16 (C-4), 59.74 (C-9), 46.60 (C-5), 35.78 (C-10), 33.36 (C-1), 25.47 (C-6), 23.86 (C-2), 23.61 (C-12), 22.92 (C-13), 22.36 (C-5'), 21.45 (C-15), 21.01 (-OCO<u>C</u>H_3),18.65 (C-14), 13.31(C-4') (Appendix 2).

4.4.2 Hydrolysis of LTE-3

63 mg of LTE-3 was dissolved in 20ml of methanol (99%) and a small quantity of $NaCO_3$ was added. After 2hrs at room temperature, the solvent was removed using Rota vapor, cold water (10ml) was added and the mixture extracted with $CHCl_3$(50ml). The extract was dried over Na_2SO_4 and evaporated in Rota vapor to afford **2** (5mg).

^1H NMR (400 MHz, $CDCl_3$) δ 0.93(3H, s, 14-H_3), 1.22(3H, s, 15-H_3), 1.25(2H, m, 1-H_2), 1.79 (2H, m, 2-H_2) 1.83(3H, s, 13-H_3), 2.02 (3H, s, 12-H_2), 2.06 (1H, dd, 5-H), 2.23 (2H, s, 9-H_2), 2.95, 2.01 (2H, m, 6-H_2), 3.67(1H, bd t, 3-H) (Appendix 8).

^{13}C NMR (100MHz, $CDCl_3$) δ 202.78 (C-8), 144.09 (C-11), 131.29 (C-7), 74.43 (C-3), 73.12 (C-4), 60.17 (C-9), 46.60 (C-5), 36.33 (C-10), 32.92 (C-1), 25.75 (C-6), 25.71 (C-2), 23.38 (C-12), 22.66 (C-13), 21.45 (C-15), 18.58 (C-14) (Appendix 9).

4.4.3 Isolation of LTE-5

Fraction 5 from column chromatography was passed through Sephadex LH-20 using chloroform/methanol (2:1) as eluent and 7 fractions were collected. Fractions 5 (55) was applied to a 10g silica gel column and using chloroform/methanol (20:1) as eluant. Further purification using chromatotron ($CHCl_3$/MeOH- 20:1) afforded **LTE-5** (38mg). Its TLC run with $CHCl_3$/MeOH at 4.5:10drops showed yellow color after spraying with methanolic KOH solution. The compound obtained is a yellow solid with melting point of 208^oC.

IR**:** (KBr) ν_{max}: 3493, 3323, 2942, 1677, 1603, 1577, 1514, 1485, 1458, 1355, 1275, 1252, 1213, 1166, 1136, 1091, 1061, 1032, 988, 819.cm^{-1} (Appendix 13)

UV λ_{max} (MeOH) 349, 280, 214nm (Appendix 14)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (1H, d, J=1.6Hz, 2'-H), 7.66 (1H, dd, J=1.6, 8.4 Hz, 6'-H), 7.04 (1H, d, J=4Hz, 5'-H), 6.55(1H, s, 8-H), 4.00(3H, s, 7- OCH3), 3.99 (3H, s, 4'-OCH_3), 3.86 (3H, s, 3-OCH_3), 12.41 (1H, s, 6-OH) (Appendix 10)

^{13}C NMR (100MHz, $CDCl_3$) δ 178.73 (C-4), 156.19(C-2), 152.91 (C-7), 149.74 (C-9), 148.37(C-3'), 146.40 (C-4'), 145.33 (C-5), 138.69(C-3), 129.26 (C-6), 122.63 (C-6'), 122.60 (C-1'), 114.61 (C-5'), 111.01 (C-2'), 106.39 (C-10), 90.19 (C-8), 60.16 (3-OCH_3), 56.48(7- OCH_3), 56.15 (4'- OCH_3). (Appendix 11)

4.4.4 Isolation of LTE-6

Fraction 5 was passed through Sephadex LH-20 using chloroform/methanol (2:1) as eluent and 7 fractions were collected. Fraction 6 (56) from Sephadex was applied to a 10g silica gel column, and eluting with chloroform/methanol (15:1) afforded **LTE-6** (43mg). It's TLC(R_f=0.2) run with $CHCl_3$/MeOH at 4.5:15drops showed yellow color after spraying with methanolic KOH solution. The compound obtained is a yellow solid with melting point of 202^oC.

IR**:** (KBr) ν_{max}: 3403, 3152, 2935, 2369, 1654, 1600, 1555, 1466, 1220, 1216, 1172, 990cm^{-1} (Appendix 18). UV λ_{max} (MeOH) 352, 256, 214nm (Appendix 19).

^{1}H NMR (400 MHz, $CDCl_3$ + Methanol-d_4) δ 7.65 (1H, s, 2'-H), 7.56 (1H, d, J= 8.4 Hz, 6'-H), 6.93 (1H, d, J= 8.4Hz, 5'-H), 6.51(1H, s, 9-H), 3.94 (3H, s, 7- OCH_3), 3.79 (3H, s, 3-OCH_3), 12.72 (6-OH) (Appendix 15).

^{13}C NMR (100MHz, $CDCl_3$ + Methanol-d_4) δ 179.21 (C-4), 152.57 (C-5), 156.62 (C-7), 152.28 (C-9), 144.77 (C-3'), 148.14 (C-4'), 156.82 (C-2), 138.36 (C-3), 131.10 (C-6), 121.65 (C-6'), 122.21 (C-1'), 115.47 (C-5'), 115.40 (C-2'), 105.81 (C-10), 94.05 (C-8), 60.18 (3-OCH_3), 60.81 (6- OCH_3) (Appendix 16).

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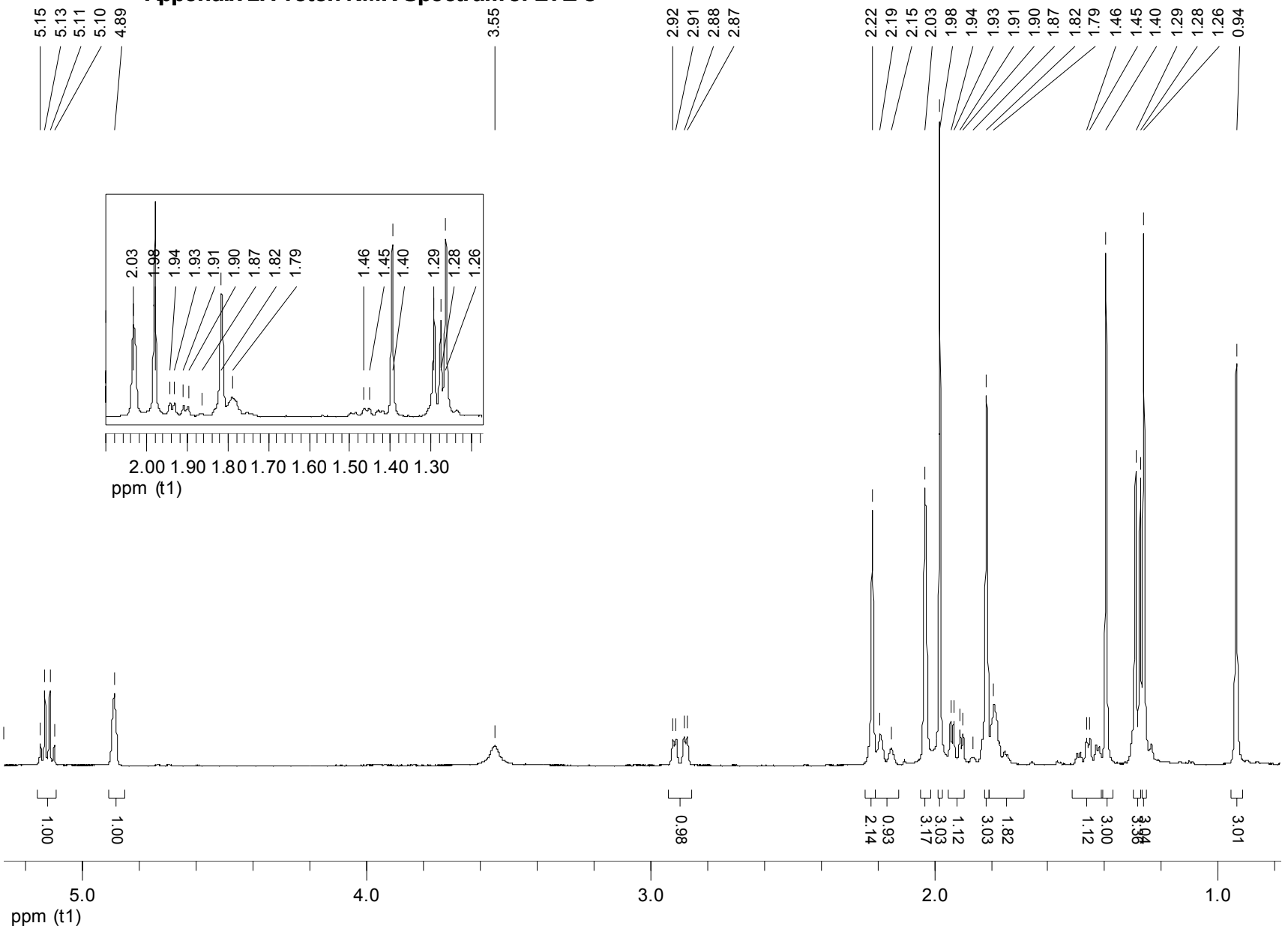
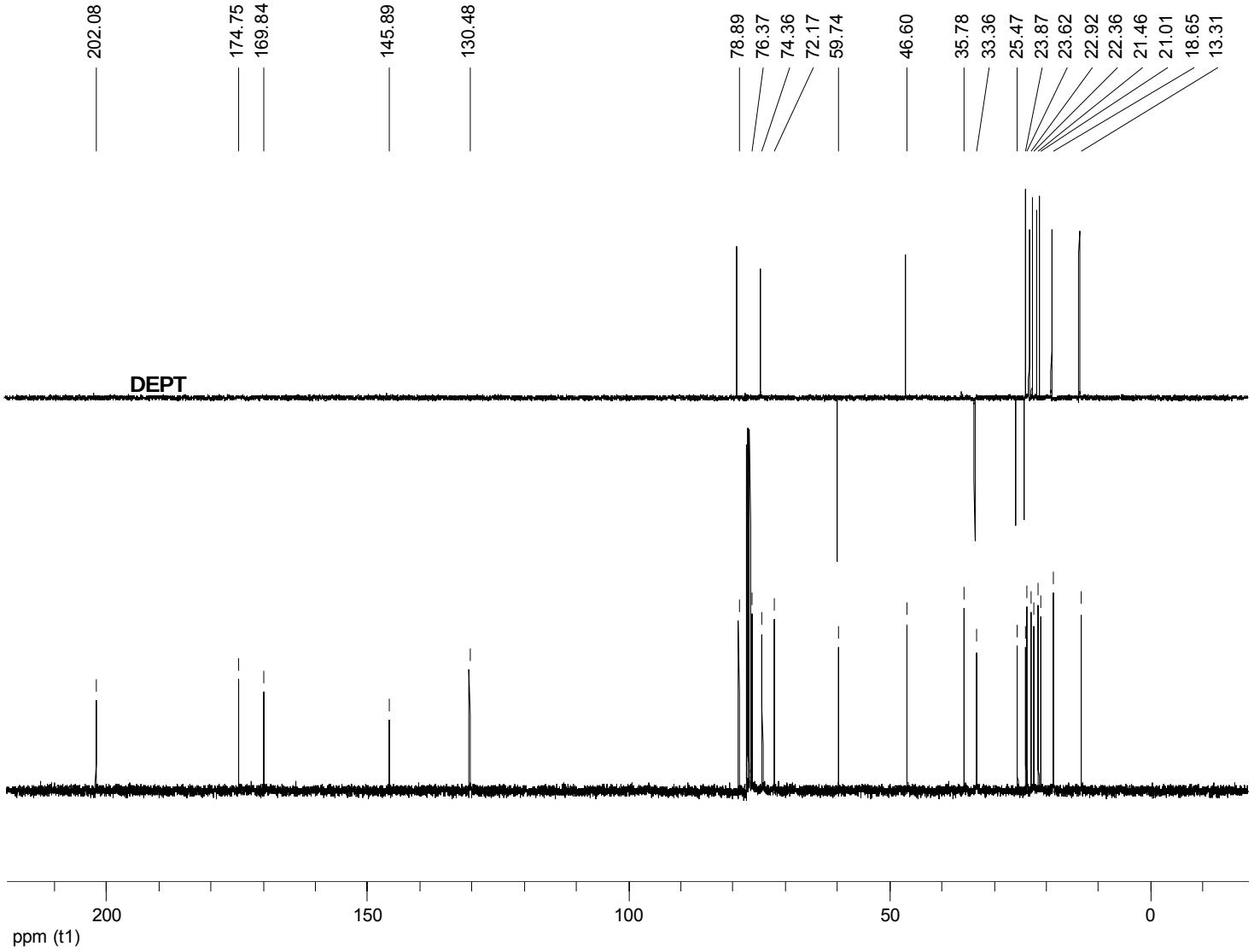
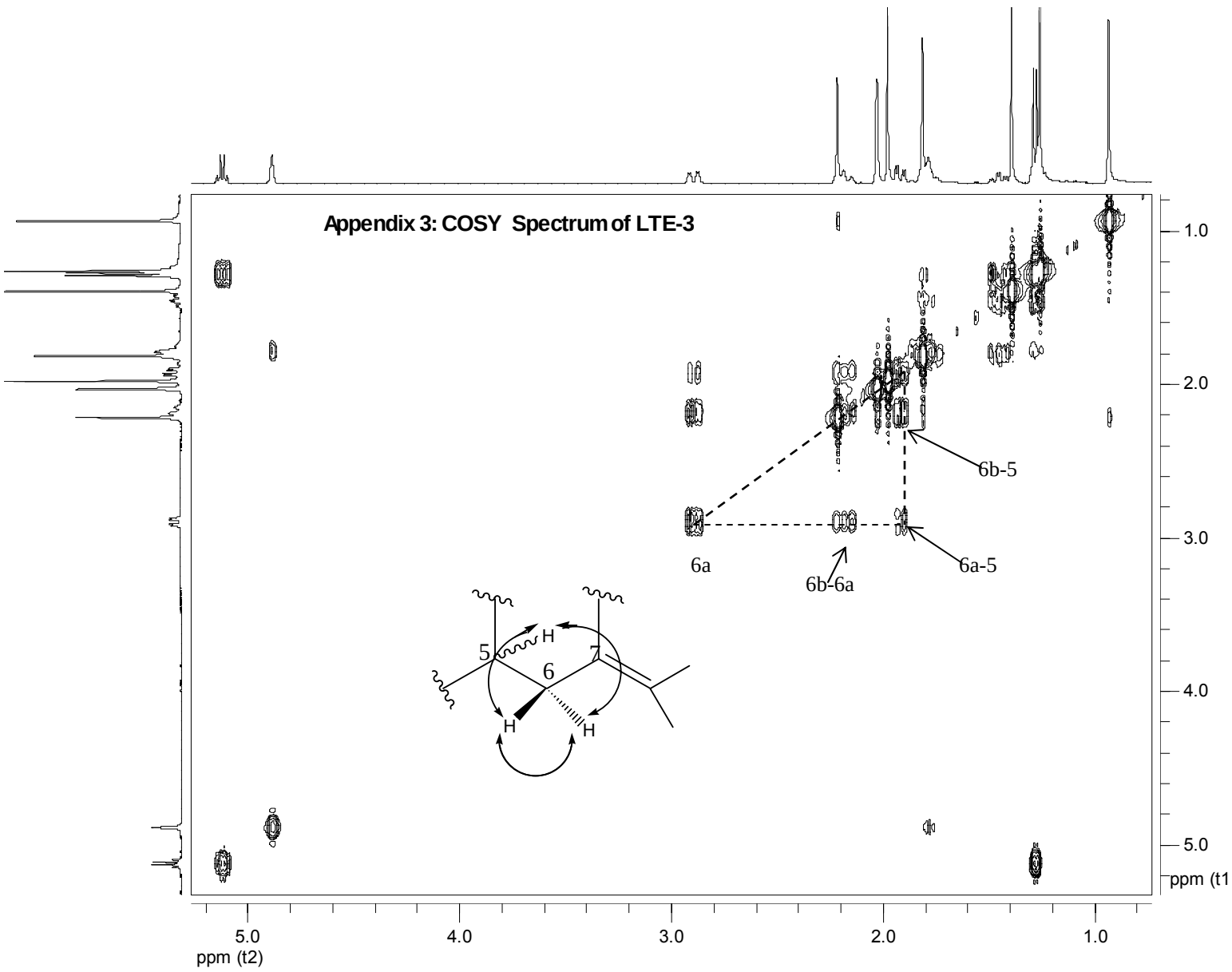
Appendixes

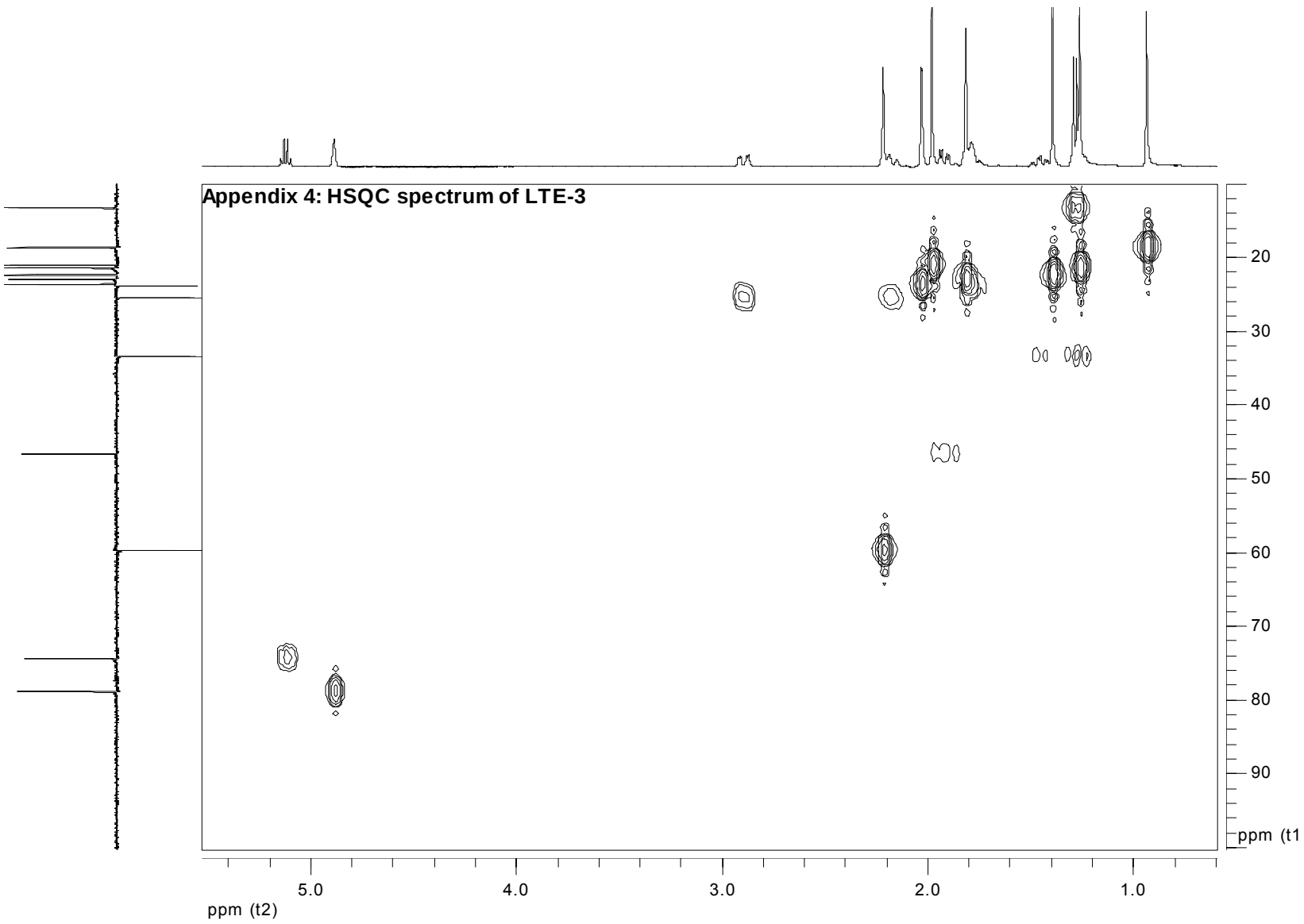
Appendix 1: Proton NMR Spectrum of LTE-3

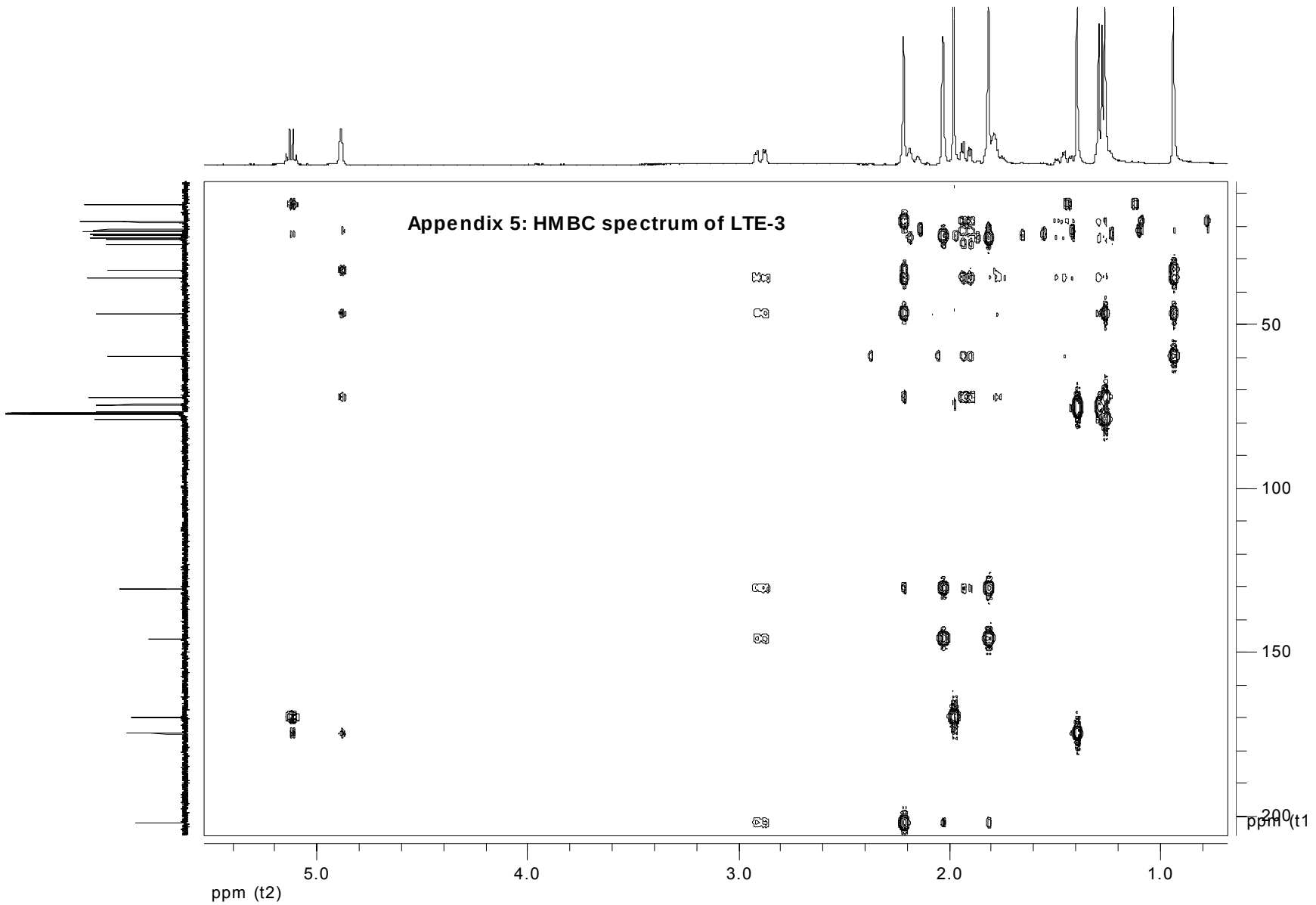


Appendix 2: C-13 and DEPT Spectrum of LTE-3

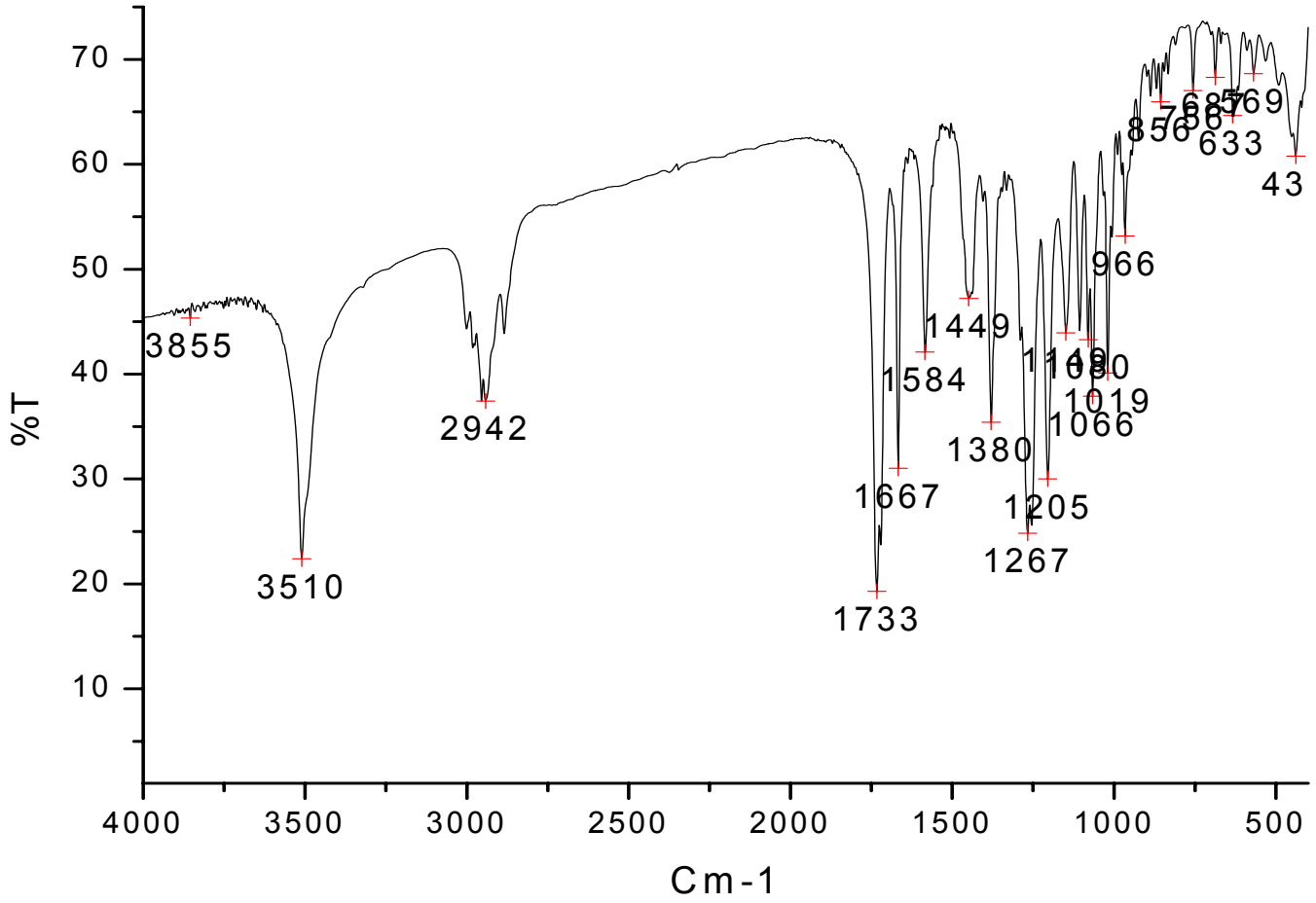



Appendix 3: COSY Spectrum of LTE-3
6b-5
6a
6b-6a
6a-5
5
6
7
H
H
H
1.0
2.0
3.0
4.0
5.0
ppm (t1
5.0
4.0
3.0
2.0
1.0
ppm (t2)

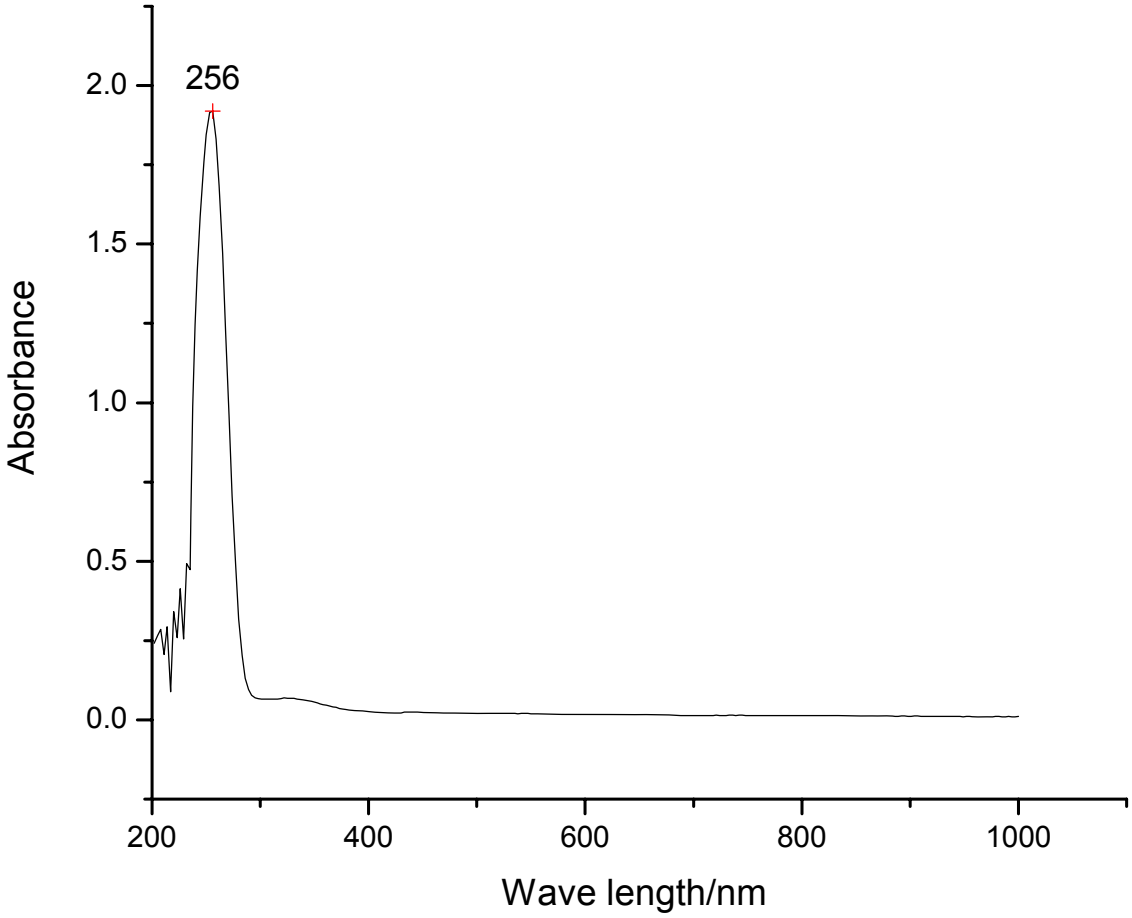

Appendix 4: HSQC spectrum of LTE-3
20
30
40
50
60
70
80
90
ppm (t1
5.0
4.0
3.0
2.0
1.0
ppm (t2)


Appendix 5: HMBC spectrum of LTE-3
50
100
150
200
ppm (t1
5.0
4.0
3.0
2.0
1.0
ppm (t2)

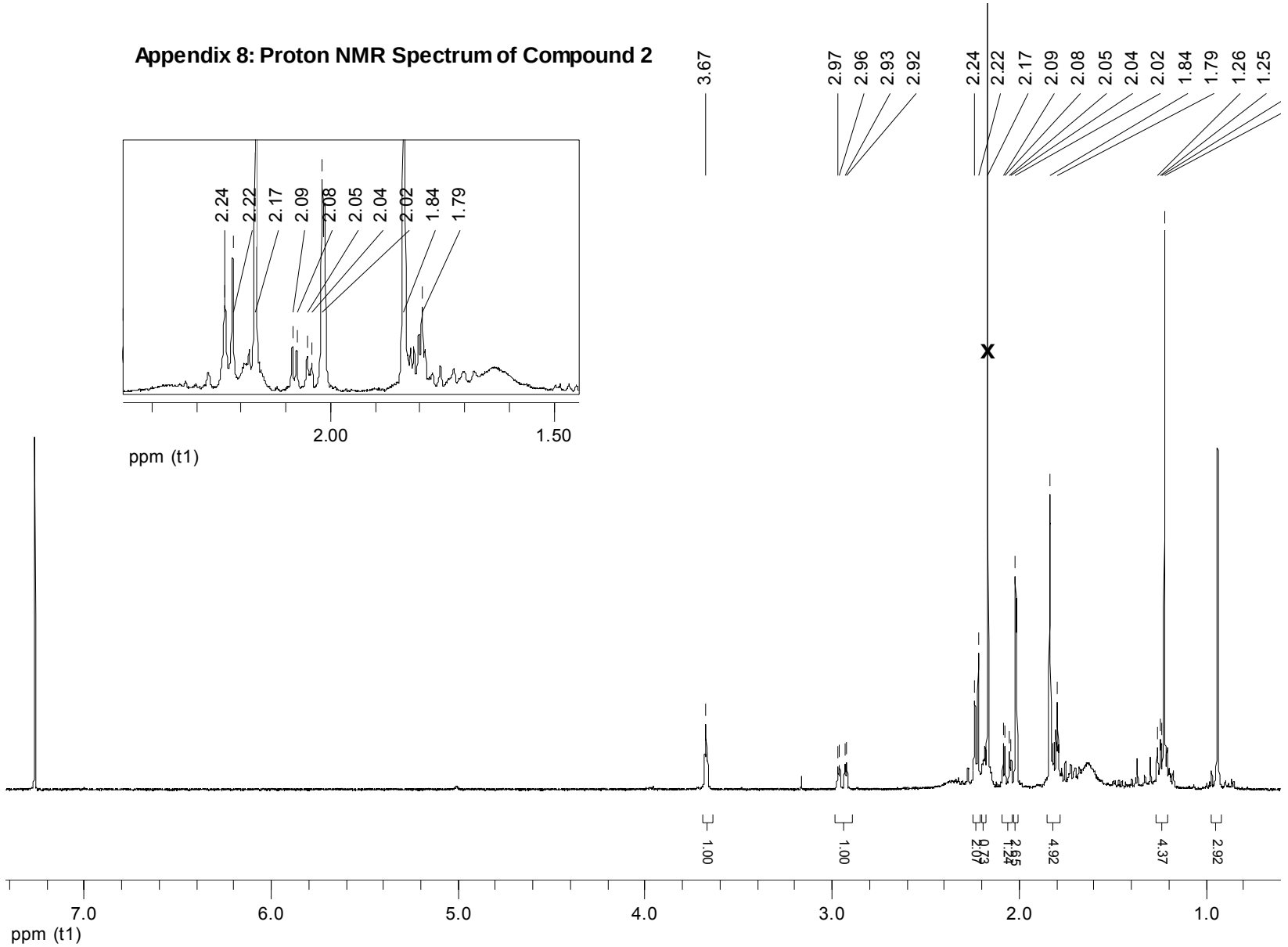
Appendix 6: IR Spectrum of LTE-3



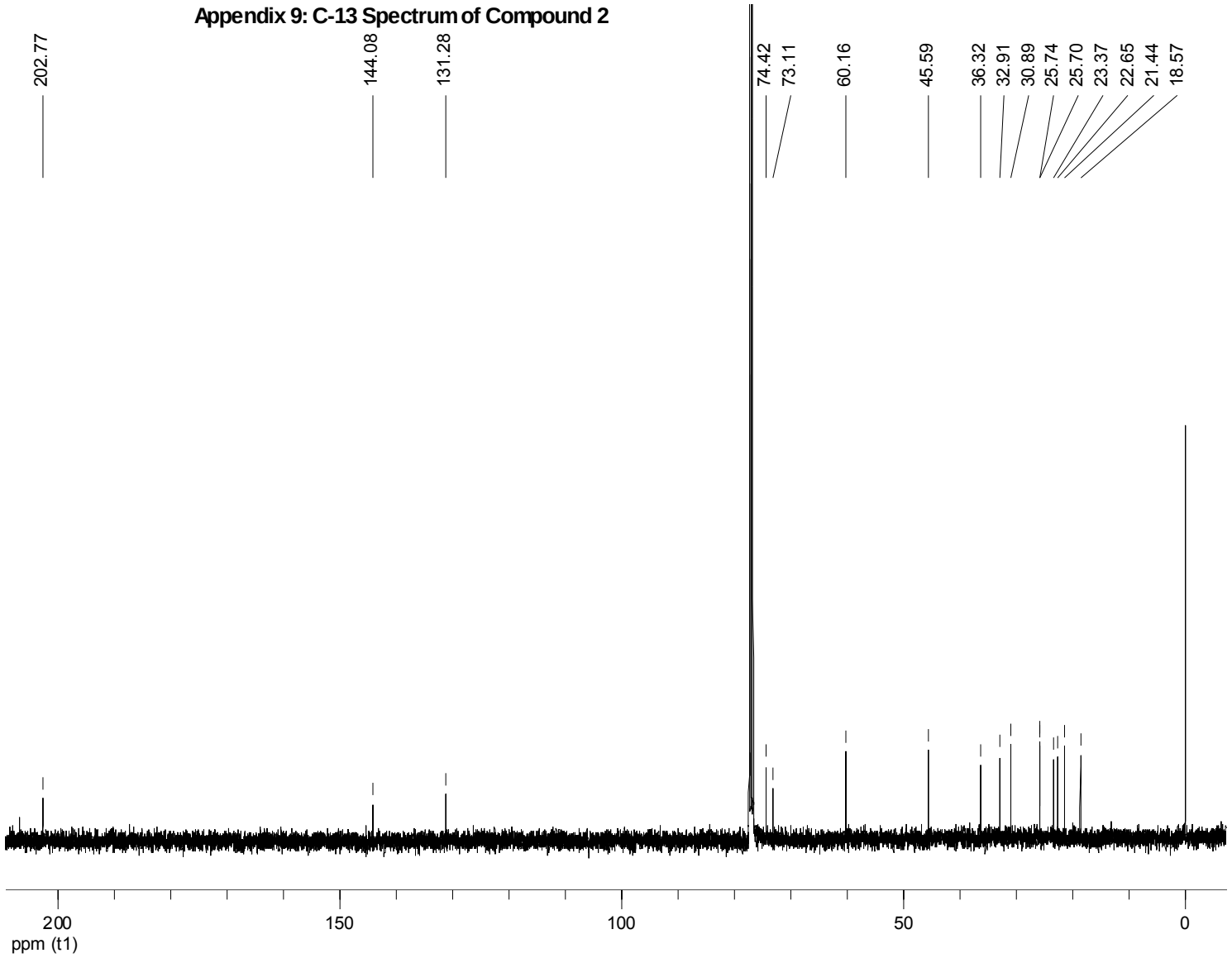
Appendix 7: UV Spectrum of LTE-3



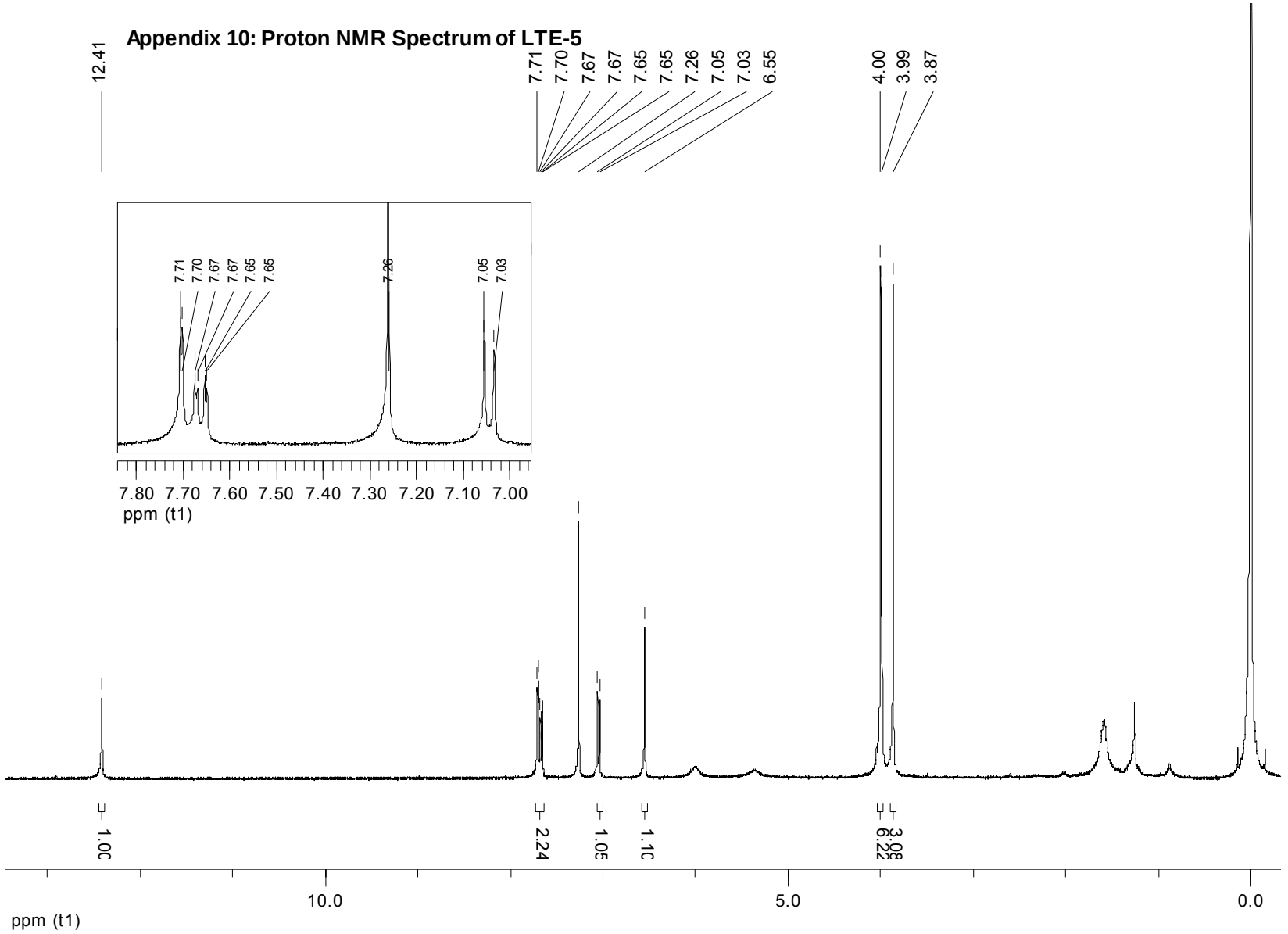
Appendix 8: Proton NMR Spectrum of Compound 2



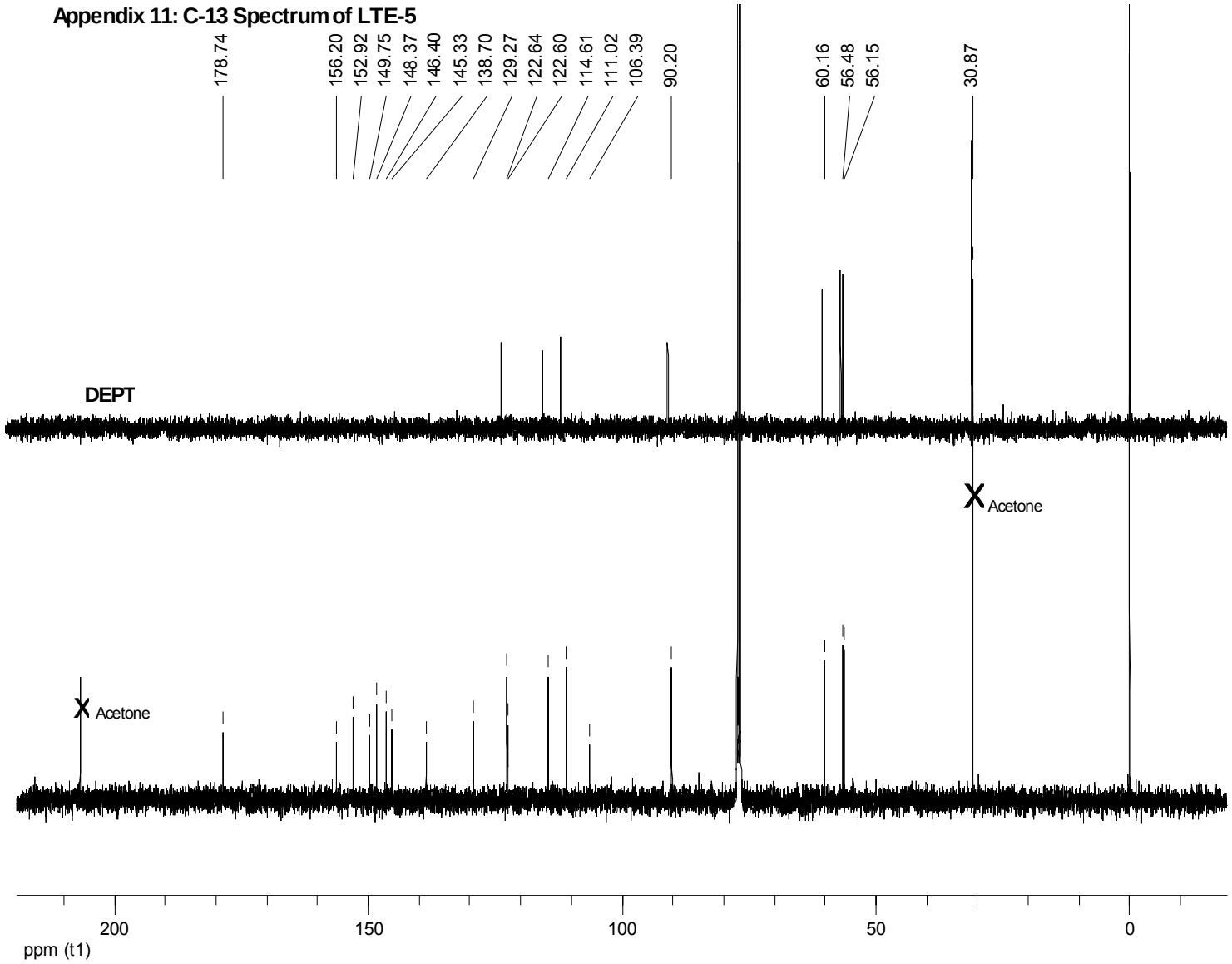
Appendix 9: C-13 Spectrum of Compound 2



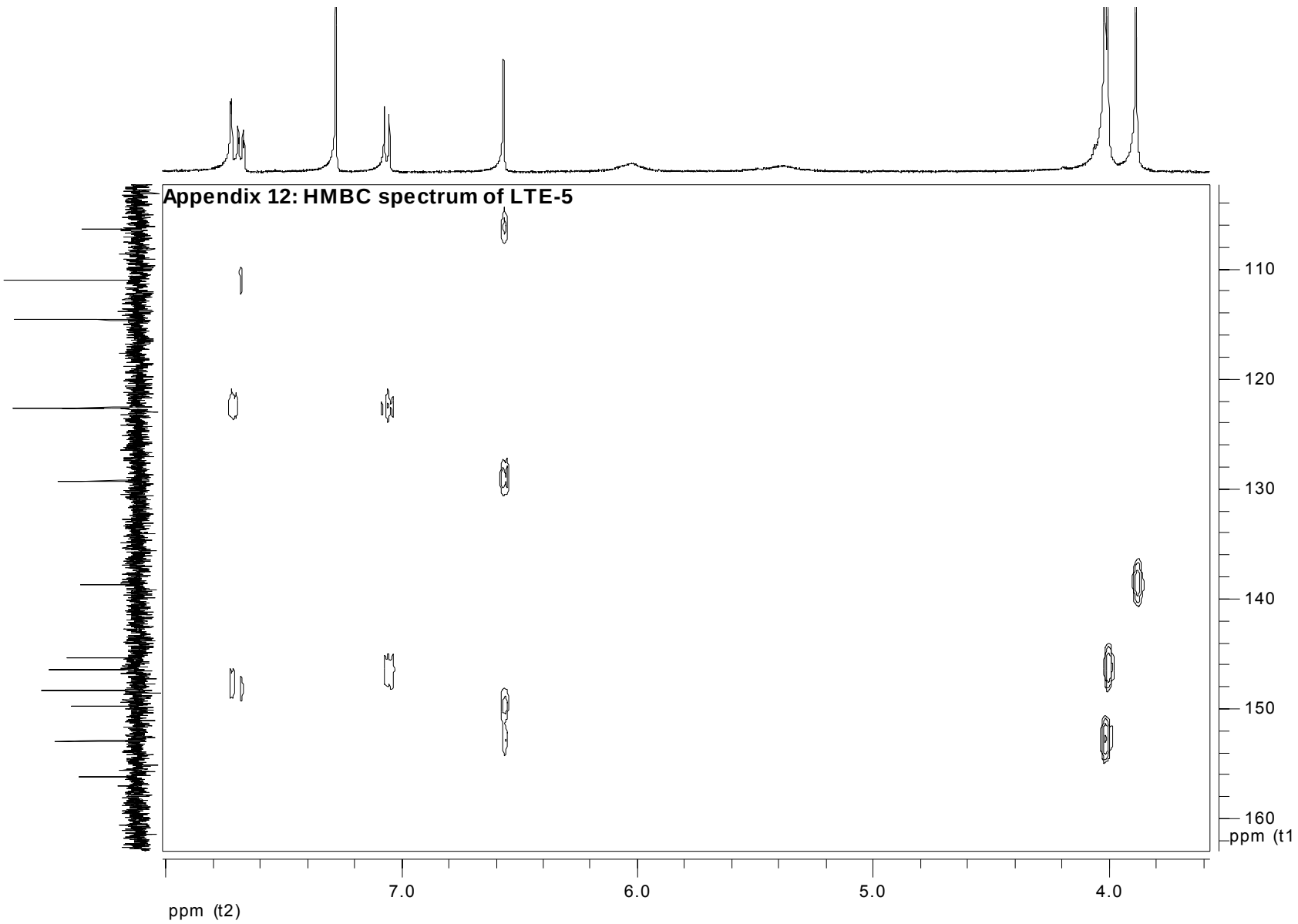
Appendix 10: Proton NMR Spectrum of LTE-5



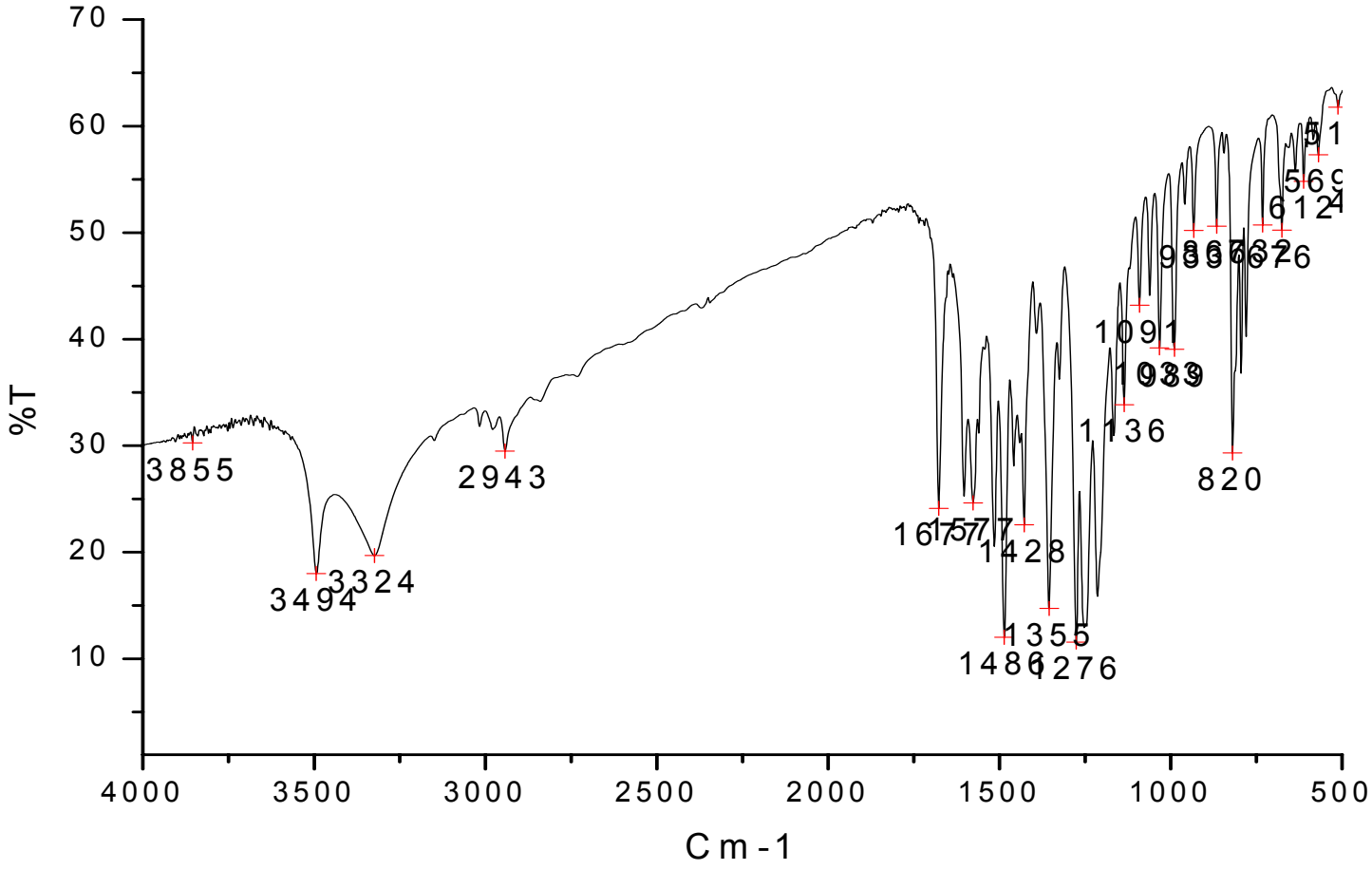
Appendix 11: C-13 Spectrum of LTE-5



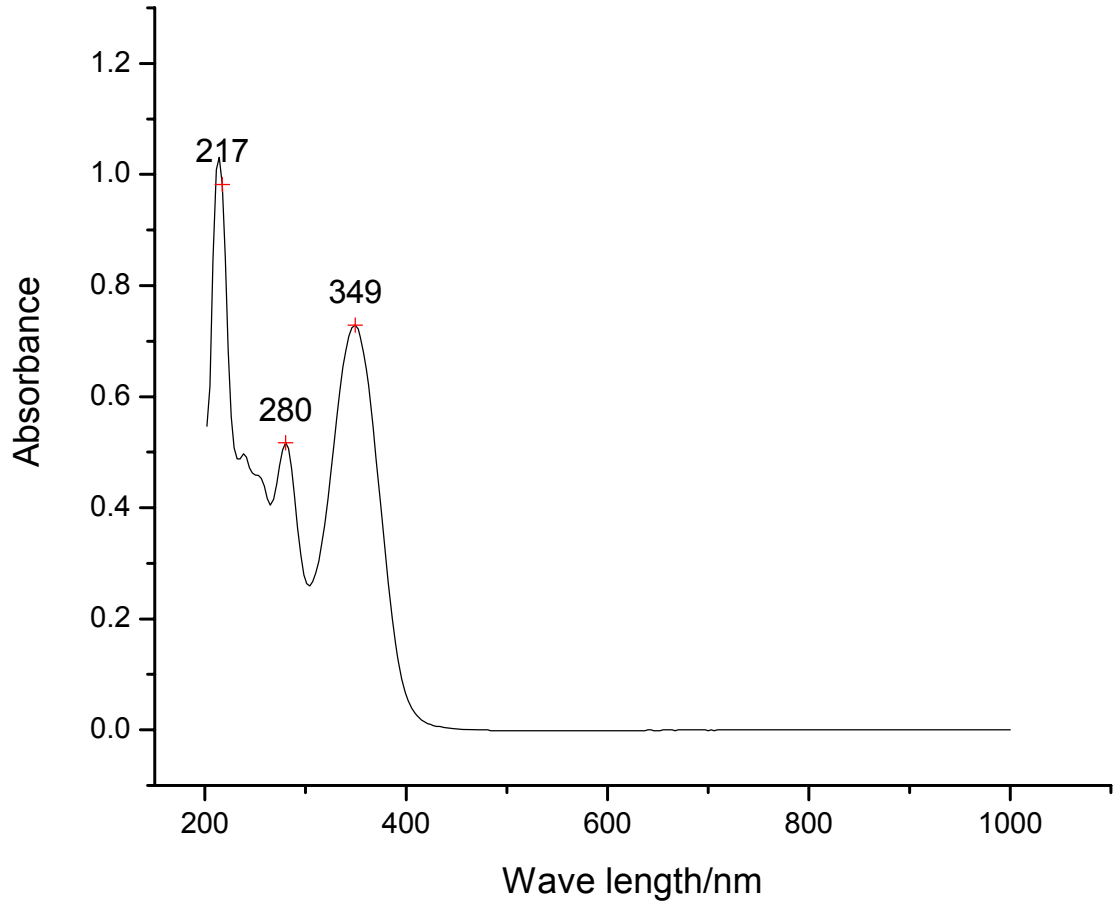
Appendix 12: HMBC spectrum of LTE-5



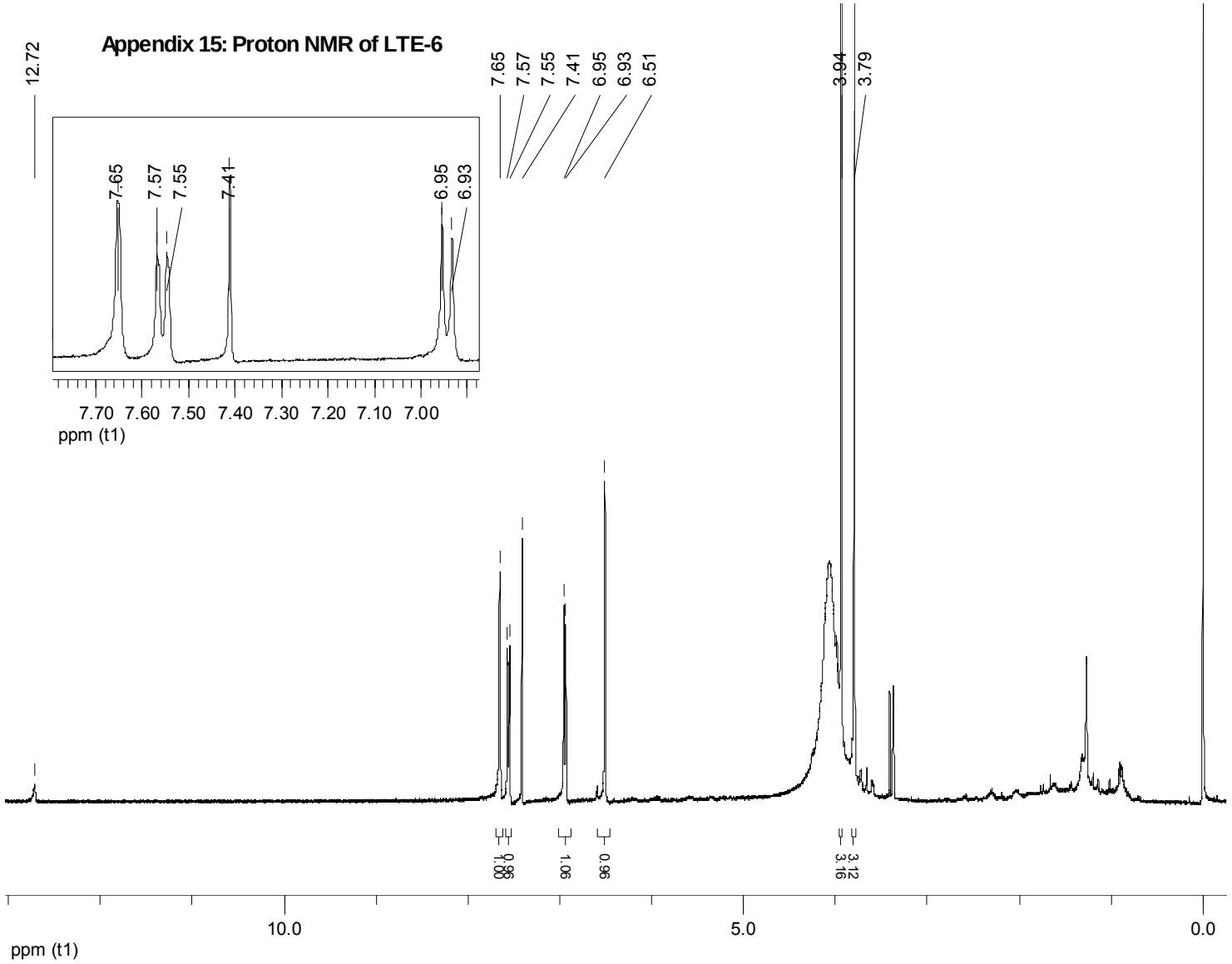
Appendix 13: IR Spectrum of LTE-5



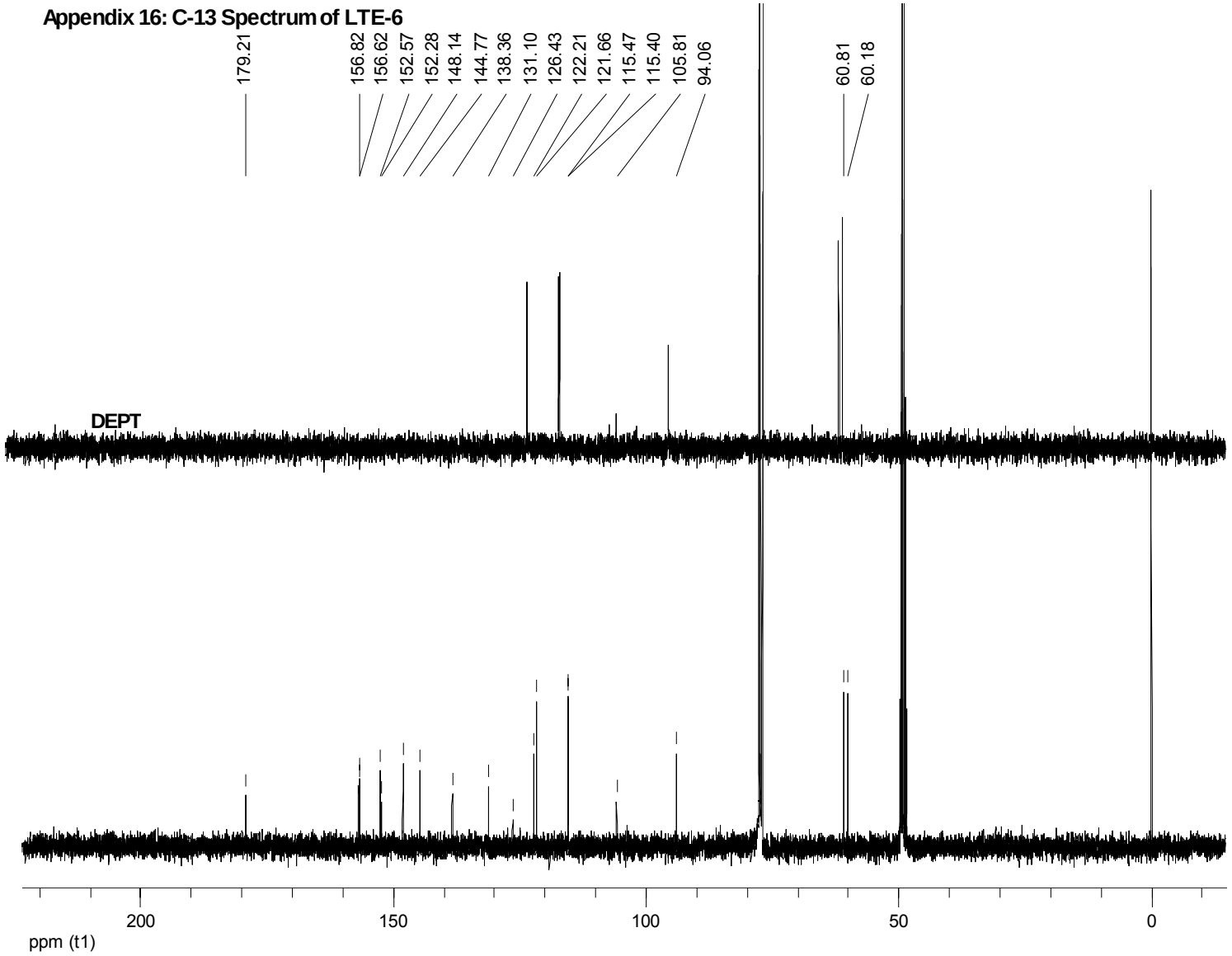
Appendix 14: UV spectrum of LTE- 5

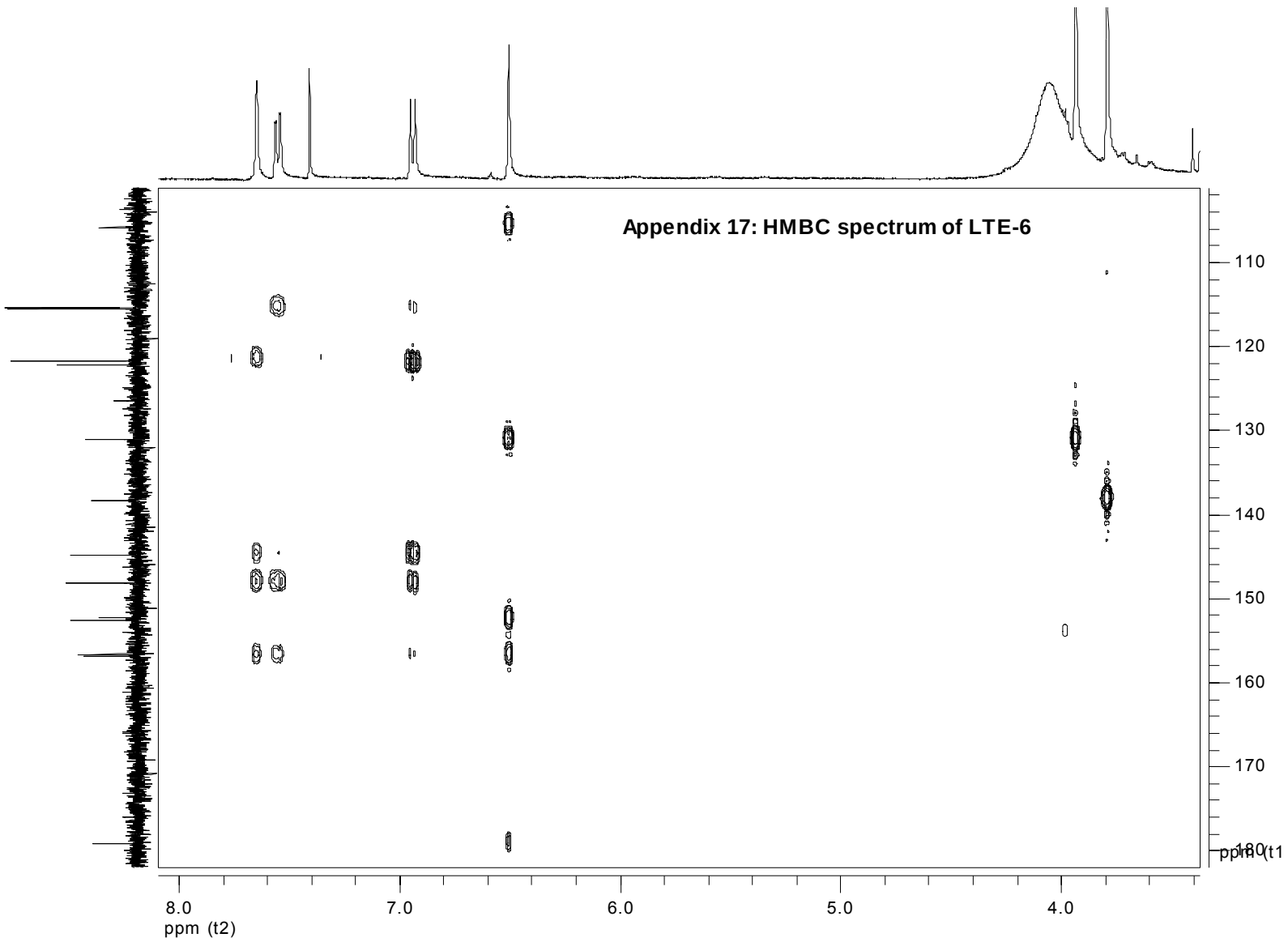


Appendix 15: Proton NMR of LTE-6

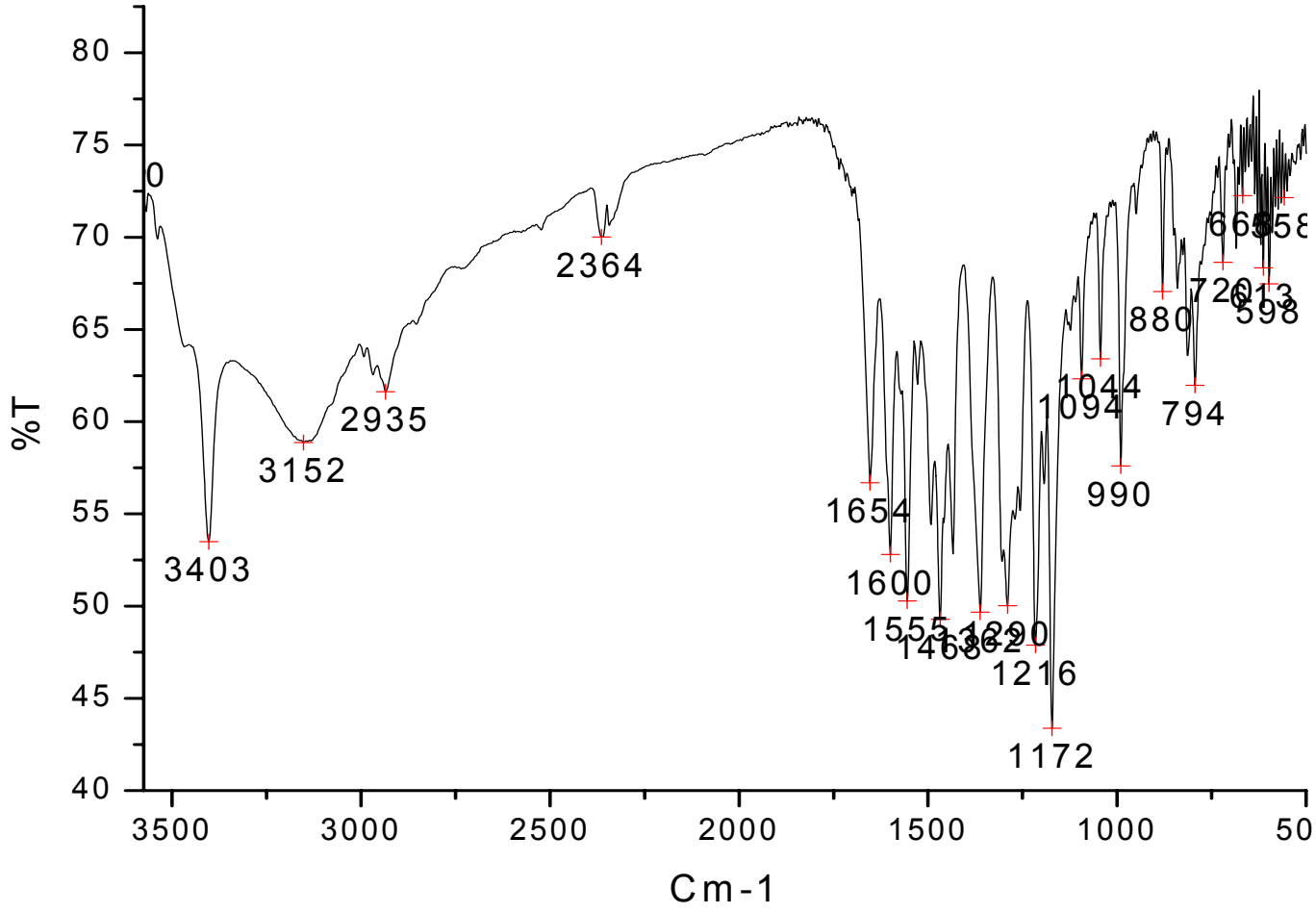


Appendix 16: C-13 Spectrum of LTE-6

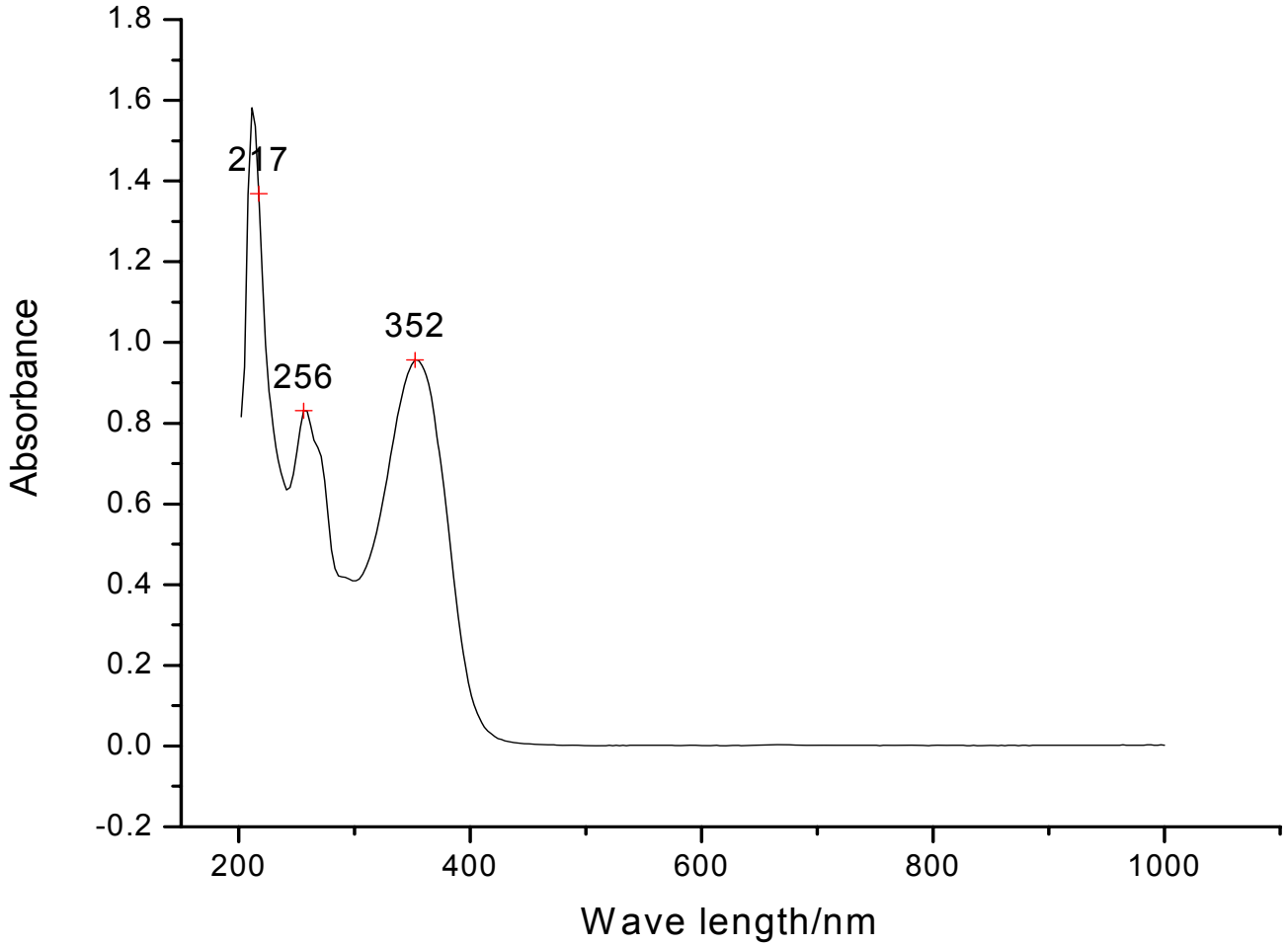



Appendix 17: HMBC spectrum of LTE-6
110
120
130
140
150
160
170
180
ppm (t1
8.0
7.0
6.0
5.0
4.0
ppm (t2)

Appendix 18: IR Spectrum of LTE-6



Appendix 19: UV Spectrum of LTE-6



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: Kibrom G/Heiwot

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