UNIVERSITY OF KWAZULU-NATAL

FACULTY OF SCIENCE AND AGRICULTURE

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THE ISOLATION, STRUCTURE ELUCIDATION AND BIOLOGICAL TESTING OF COMPOUNDS FROM *PLECTRANTHUS HADIENSIS*

BY

SHIKSHA DUKHEA

2010

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CO-SUPERVISOR: DR. B. MOODLEY

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SHIKSHA DUKHEA

Submitted in partial fulfilment of the requirements for the degree of Master of Science in the School of Chemistry, Faculty of Science and Agriculture, University of KwaZulu-Natal.

Supervisor: Dr N. A. Koorbanally

Co-Supervisor: Dr. B. Moodley

Preface

The experimental work described in this dissertation was carried out from March 2007 to January 2009 in the School of Chemistry, Howard College and Westville campuses, Durban, under the supervision of Dr. N.A. Koorbanally and Dr. B. Moodley.

This study represents original work by the author and has not been submitted in any other form to another university. Where use was made of work of others it has been duly acknowledged in the text.

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Declaration

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List of Abbreviations

NMR	-	nuclear magnetic resonance
^{1}H NMR	-	proton nuclear magnetic resonance
^{13}C NMR	-	carbon nuclear magnetic resonance
DEPT	-	distortionless enhancement by polarization transfer
HSQC	-	heteronuclear single quantum coherence
HMBC	-	heteronuclear multiple bond coherence
COSY	-	correlation nuclear magnetic spectroscopy
NOESY	-	nuclear overhauser effect spectroscopy
J	-	coupling constant
s	-	singlet
d	-	doublet
dd	-	doublet of doublets
t	-	triplet
brs	-	broad singlet
m	-	multiplet
td	-	triplet of doublets
TLC	-	thin layer chromatography
IR	-	infrared spectroscopy
UV	-	ultra-violet
$CDCl_3$	-	deuterated chloroform
CD_3OD	-	deuterated methanol
DMSO	-	dimethylsulphoxide
m/z	-	mass-to-charge ratio
$[M]^+$	-	molecular ion peak
IPP	-	isopentenyl diphosphate
GPP	-	geranyl pyrophosphate
FPP	-	farnesyl pyrophosphate
DMAPP	-	dimethylallyl diphosphate

GC-MS	-	gas chromatography-mass spectrometry
LC-MS	-	liquid chromatography-mass spectrometry
Hz	-	Hertz
$W_{½}$	-	half-width
GI_{50}	-	growth inhibition - the concentration at which the growth of the cell is inhibited by 50%
TGI	-	total growth inhibition
LC_{50}	-	lethal dose concentration - concentration at which 50% of the cells are killed
LC_{100}	-	lethal dose concentration - concentration at which 100% of the cells are killed
RPMI	-	Roswell Park Memorial Institute
ATCC	-	American Type Culture Collection
SRB	-	sulforhodamine B
MIC	-	minimum inhibitory activity
TCA	-	trichloroacetic acid
MRSA	-	methicillin resistant *Staphylococcus aureus*
VRE	-	vancomycin-resistant *Enterococcus faecalis*
I.U.P.A.C	-	international union of pure and applied chemistry
Δ	-	delta (to indicate the position of the double bond)

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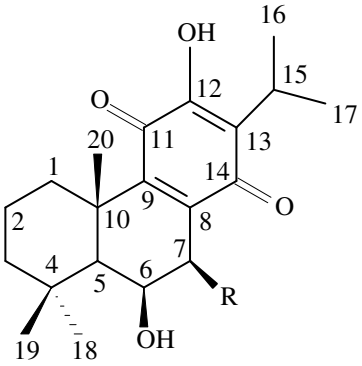
Abstract

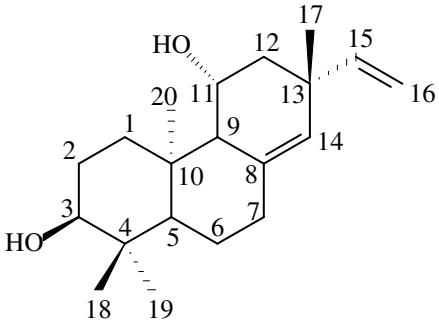
Three diterpenes of the abietane class, 7β-acetoxy-6β-hydroxyroyleanone (**I),** 6β,7β-dihydroxyroyleanone (**II**) and *ent*-pimara-8(14),15-diene-3β,11α-diol (**III**) and three triterpenes, 2α,3α,19α-trihydroxyurs-12-en-28-oic acid (**IV**), stigmasterol (**V**) and lupeol (**VI**) were isolated from the stem and leaf material of *Plectranthus hadiensis*. The structures of the compounds were elucidated using 2D NMR spectroscopy and Mass spectrometry. All six compounds have been isolated previously, but this is the first occurrence of compounds **III-VI** in *Plectranthus hadiensis*. This is also the first report of the isolation of a pimarene from *Plectranthus*, which provides a biochemical link to other genera in the family Lamiaceae where this class of compounds exist.

Compounds **I** to **IV** were tested for their antibacterial activity against *Enterococcus faecalis* and *Pseudomonas aeruginosa* as well as their anticancer activity against breast (MCF-7), renal (TK-10) and melanoma (UACC-62) cell lines. Compounds **I** and **II** exhibited good antibacterial activity against *Enterococcus faecalis* and *Pseudomonas aeruginosa* and although the *ent*-pimara-8(14),15-diene-3β,11α-diol (**III**), was inactive against *E. faecalis,* it was very active against *P. aeruginosa.* Compound **IV**, the triterpenoid, was structurally different to **I-III** and did not show any anti-bacterial activity. Compounds **I-III** were weakly active toward the cancerous renal (TK-10), melanoma (UACC-62) and breast (MCF-7) cell lines, while **IV** was inactive in all of the cell lines.

Structures of compounds isolated from *Plectranthus hadiensis*



	R
(I)	$OC(O)CH_3$
(II)	OH



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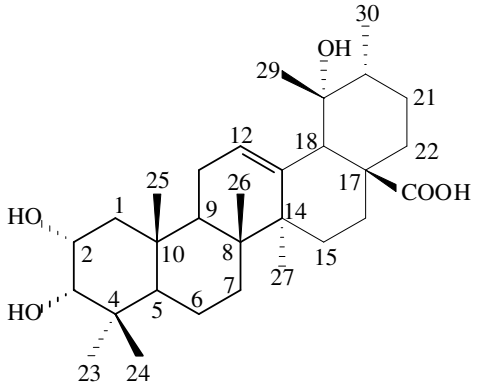

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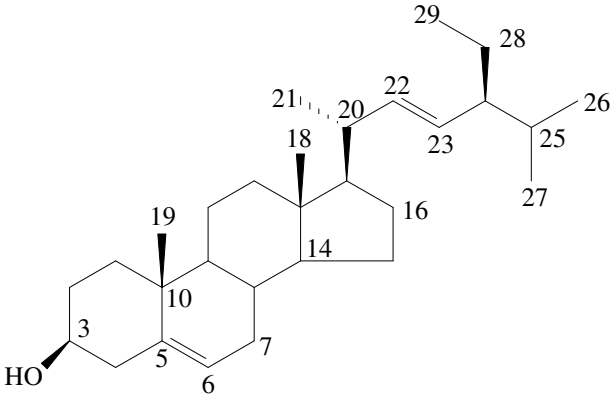

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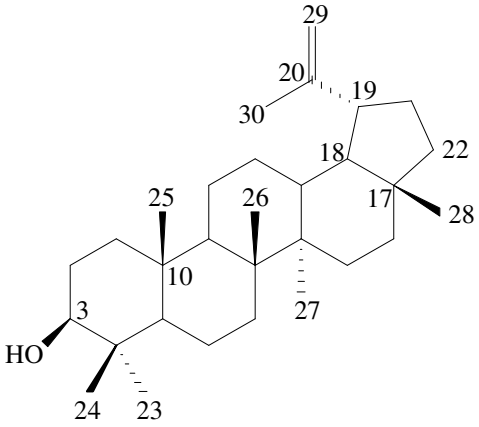

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Chapter 1 Introduction to diterpenoids

1.1 Classification of diterpenoids

Diterpenes consist of four isoprene units (C_{20}) and are by far the most diverse group of compounds. They are placed into different classes (termed acyclic, monocyclic, bicyclic, tricyclic, tetracyclic and macrocyclic) based on the number of carbocyclic rings in the structure (figures 1a-d).

The acyclic and monocyclic compounds (figure 1a) do not have much variation in that they are either straight chain structures or contain one ring and a side chain respectively, such as phytane (**1**) and vitamin A (**2**).

Bicyclic compounds belonging to Class 3 (figure 1a), contain two six-membered carbocyclic rings with alkyl groups attached at either C-8 or C-9 or both. Labdanes and clerodanes are differentiated because of the different positions of the methyl groups. In clerodanes these methyl groups occur at C-4, C-5, C-8, C-9 and C-13 while in labdanes there are two methyl groups at C-4 and one each at C-8, C-10 and C-13.

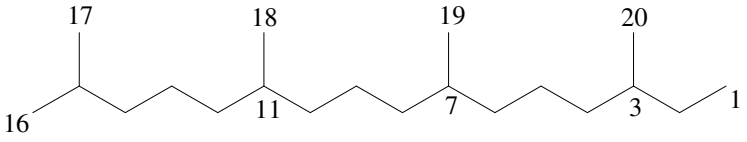
Class 4 diterpenes (figure 1b) are the tricyclic diterpenoids and have three six-membered rings but differ in the substitution pattern on these rings. Abietane and totarane diterpenoids are fairly similar in structure, the only difference being the position of the isopropyl group in ring C, which is situated at C-13 in abietanes and C-14 for totaranes due to different alkyl shifts, abietane being the precursor to totarane (Dewick, 2002; Nakanishi, 1974).

The structural difference between pimarane and cassane type diterpenes is the position of the methyl group (CH_3-17) on ring C, which occurs at C-13 in pimaranes and C-14 in cassanes, pimarane being the precursor to the cassanes (Nakanishi, 1974) and occurs by a methyl shift from C-13 in the pimaranes to C-14 in the cassanes when a carbocation at C-14 is created by a hydride shift in the sandaracopimarenyl cation (Figure 2). Taxane type

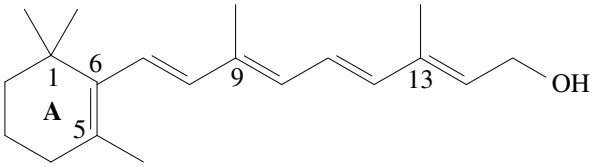
compounds are also classified as tricyclic, but have a different biosynthetic pathway (Dewick, 2002).

Within the class 5 diterpenoids (figure 1c), the kauranes, beyeranes and atisanes are all derived from the sandaracopimarenyl cation. This is shown later in figures 10 and 11. They are therefore similar in structure, the kauranes having a methylene group at C-16, the beyeranes having a methyl group at C-17 and the methylene bridge occurring between C-12 and C-8 in the atisanes. The gibberellins are similar to the kauranes, the difference being the five-membered ring in gibberellins arising from the biosynthesis (Figure 10). Phorbols and ginkolides follow different biosynthetic pathways (Dewick, 2002).

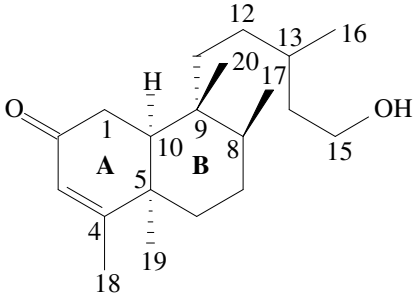
The macrocyclic compounds (figure 1d), the trachylobanes and the aconanes, are similar to the atisanes from which they are derived. This is shown later in figure 11.



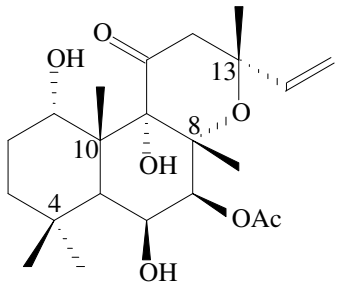
(**1**) phytane



(**2**) Vitamin A



(**3**) 15-hydroxy-3-cleroden-2-one (clerodane)



(**4**) Forskolin (labdane)

Figure 1a: Acyclic (class 1), monocyclic (class 2) and bicyclic (class 3) diterpenes

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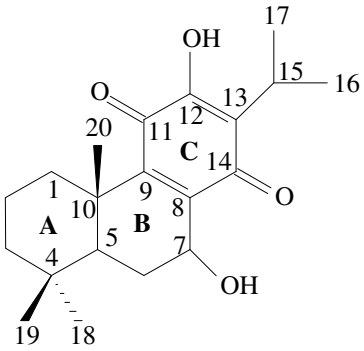
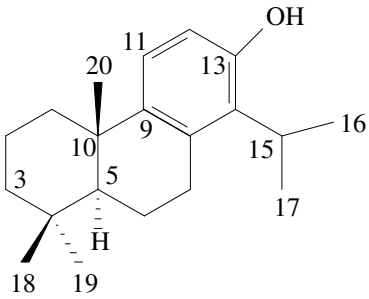
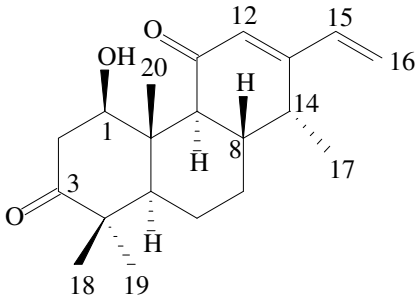


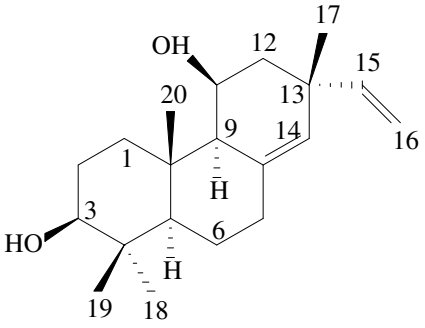
(**5**) Horminone (abietane)



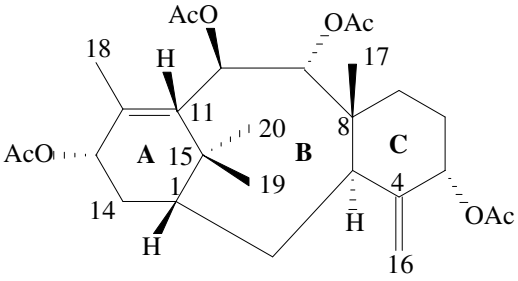
(**6**) Totarol (totarane)



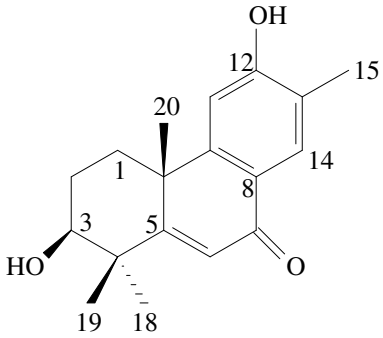
(**7**) Phytocassane E (cassane)



(**8**) 3β-dihydroxy-8(14),15-pimaradiene (pimarane)

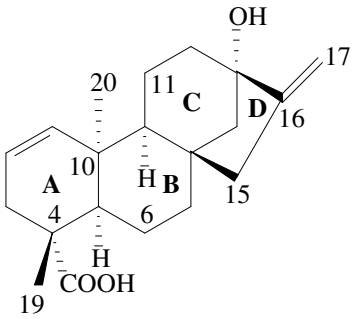


(**9**) Taxusin (taxane)

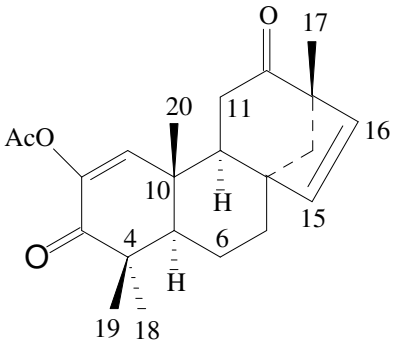


(**10**) 3β,12-dihydroxy-13-methyl-5,8,11,13-podocarpatetraen-7-one (podocarpane)

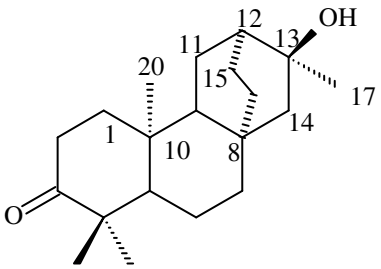
Figure 1b: Tricyclic (Class 4) diterpenes



(**11**) Steviol (kaurane)



(**12**) 2-acetoxy-1,15-beyeradiene-3,12-dione (beyerane)



(**13**) *Ent*-16α-hydroxyatisane-3-one (atisane)

Figure 1c: Tetracyclic (Class 5) diterpenes

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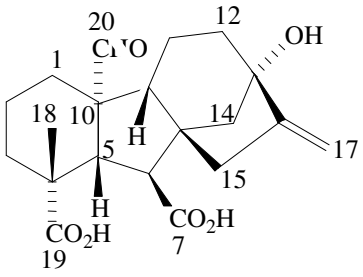
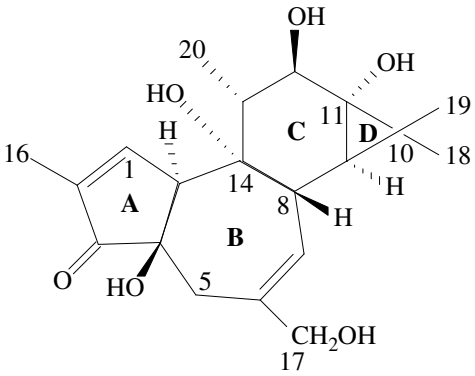
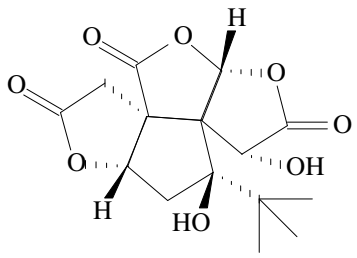


(**14**) GA_{19} (gibberellin)

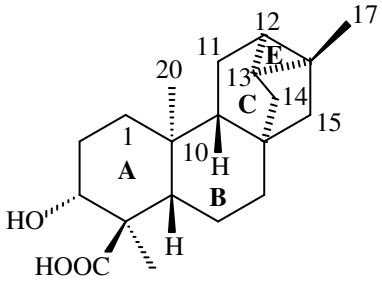


(**15**) phorbol

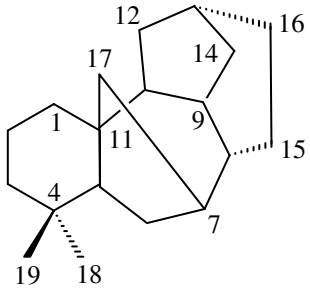


(**16**) Bilobalide (ginkgolide)

Figure 1c: Tetracyclic (Class 5) diterpenes

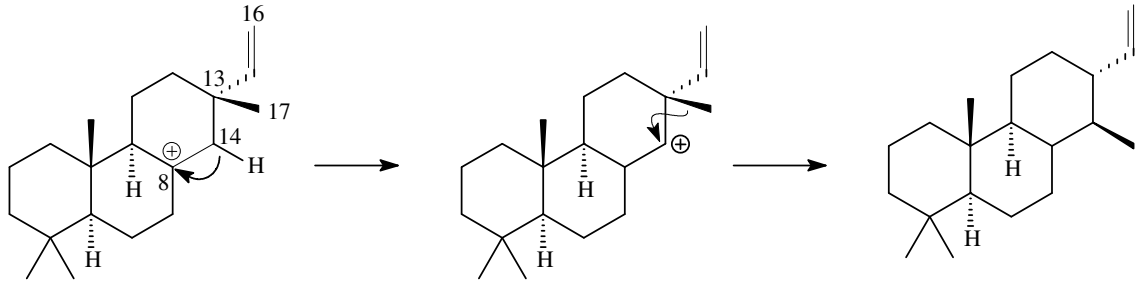


(**17**) hydroxytrachylobanic acid (trachylobane)



(**18**) aconane

Figure 1d: Macrocyclic (Class 6) diterepenes



sandaracopimarenyl cation

cassane type diterpenoid

Figure 2: Formation of cassane type diterpenoids

1.2 Classification of the abietane diterpenoids

Abietane diterpenoids consist of approximately twenty carbon atoms and commonly have three tertiary methyl groups (two at C-4 and one at C-10), and one isopropyl group at C-13. Their numbering system is based on the nomenclature of natural product hydrides as recommended by the I.U.P.A.C. in 1993 (Figure 3). These abietanes can be further classified into eight types based on structural differences through reductions (addition of hydrogen across a double bond) or oxidations such as hydroxylations and dehydrations as well as cyclisations between different oxygenated groups.

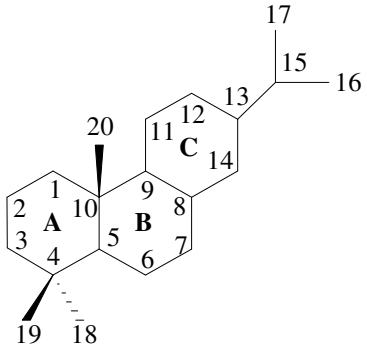
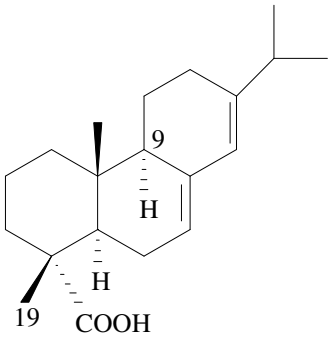


Figure 3: Numbering system for abietanoids

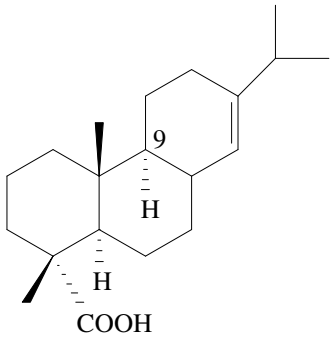
Acidic abietanes (e.g. abietic acid **(19),** figure 4) are characterised by the presence of a carboxylic acid in the compound. In levopimaric acid **(20)** there is a double bond in ring C (figure 4). In ferruginol **(21)** and carnosol **(22)** type abietanes, ring C is phenolic in character. Due to the presence of a lactone ring which extends across ring B from C-7 to C-10, carnosol type abietanes are differentiated from ferruginol type abietanes.

Callicarpone **(23)** abietanes are based on the epoxide ring at Δ^{12} and the presence of a hydroxy isopropyl group attached to C-15 as well as α,β-unsaturated ketone groups in rings B and C. The royleanones **(24)** have a benzoquinone ring C. Tanshinone **(25)** abietanes consist of highly oxidised rings where two of the rings, A and B are aromatic, ring C contains two adjacent α,β-unsaturated systems and a fourth ring, a furan ring is present as ring D. Fichtelite **(26)** closely resembles the abietane skeletal structure and

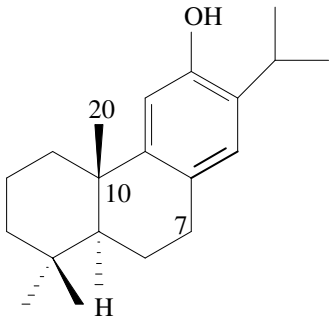
may be referred to as a norabietane due to the absence of the methyl group at C-4. Also present in the structure is a double bond at Δ^4.



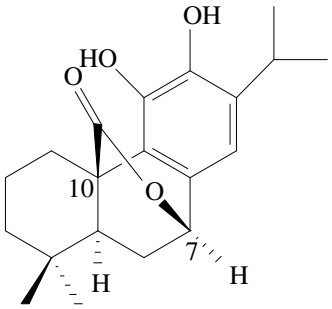
(**19**) abietic acid



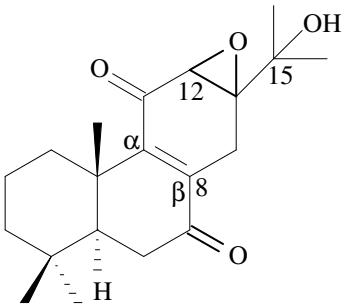
(**20**) levopimaric acid



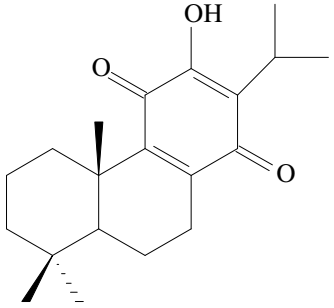
(**21**) ferruginol



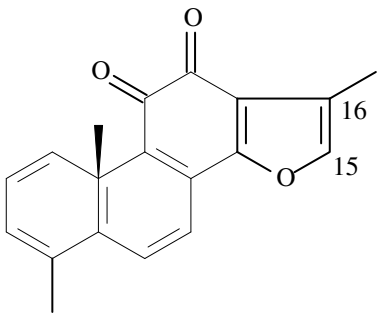
(**22**) carnosol



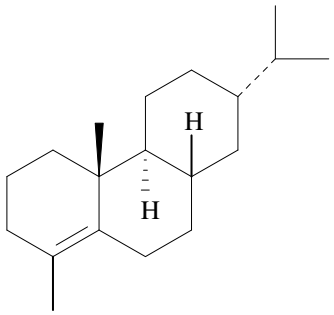
(**23**) callicarpone



(**24**) royleanone



(**25**) tanshinone

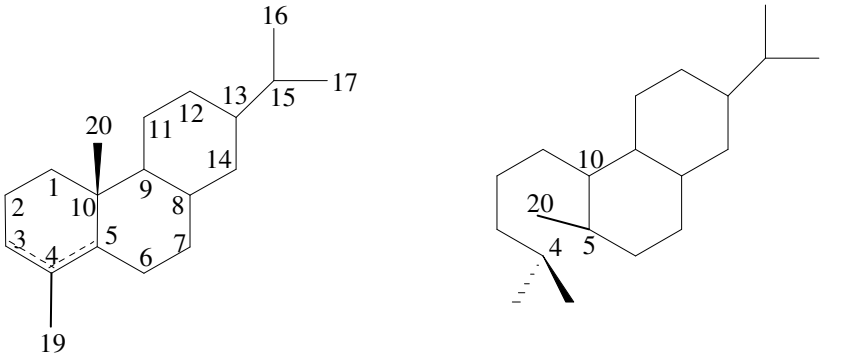


(**26**) *dl*-fichtelite

Figure 4: Representative structures of the different types of abietane diterpenoids (Nakanishi *et al*., 1974)

The terms “nor”, “abeo” and “seco” are used widely in the abietanes. The use of “nor” occurs when a methyl group is eliminated from the structure. The number preceding “nor” refers to the carbon atom which has been eliminated. The prefix “abeo” is used

when a methyl group migrates to an adjacent carbon atom and "seco" refers to a bond that has been broken on the skeletal structure. The numbers preceding "abeo" refers to the carbon atoms involved in the migration e.g. (10→5) abeo indicates that a methyl has migrated from C-10 to C-5 and the numbers preceding "seco" refers to the carbon atoms in which the bond has been broken e.g. in 4,5-seco the bond has been broken between C-4 and C-5. Figure 5 includes examples of these.



(a) 18-nor (b) 4,5-seco-20(10→5)*abeo*abietane

Figure 5: Examples of the use of the prefix "nor", "*abeo*" and "seco"

1.3 Biosynthesis

The acyclic diterpenes (Class 1), for example phytane **(1)** (Figure 1a) is formed by the addition of four molecules of IPP (isopentenyl diphosphate). The formation of IPP can follow either one of two biosynthetic pathways, the methylerythritol phosphate pathway (MEP), also known as the 1-deoxy-D-xylulose (DOX) pathway and the mevalonic acid (MVA) pathway. The MVA pathway is the only pathway used by animals, while both pathways are present in plants. The enzymes involved in the MVA pathway are found in the cytosol while those in the MEP or DOX pathway are found in the chloroplast of the plant.

In the mevalonic acid pathway, the biosynthesis starts with the Claisen condensation reaction where two molecules of acetyl-CoA react to produce acetoacetyl-CoA which reacts further with an additional molecule of acetyl-CoA in a stereospecific aldol reaction producing HMG-CoA (β-hydroxy-β-methylglutaryl-CoA) (figure 6). The carbonyl group in HMG-CoA is then reduced with NADPH to the primary alcohol producing mevaldic acid hemithioacetal, then mevaldic acid and further reduction to mevalonic acid (MVA). The addition of two ATP molecules leads to the sequential phosphorylation of MVA to mevalonic acid diphosphate and a further molecule of ATP results in the release of CO_2 producing isopentenyl disphosphate (IPP) which is subsequently isomerised to DMAPP in a reversible reaction. The forward reaction is favoured due to the electrophilic nature of DMAPP.

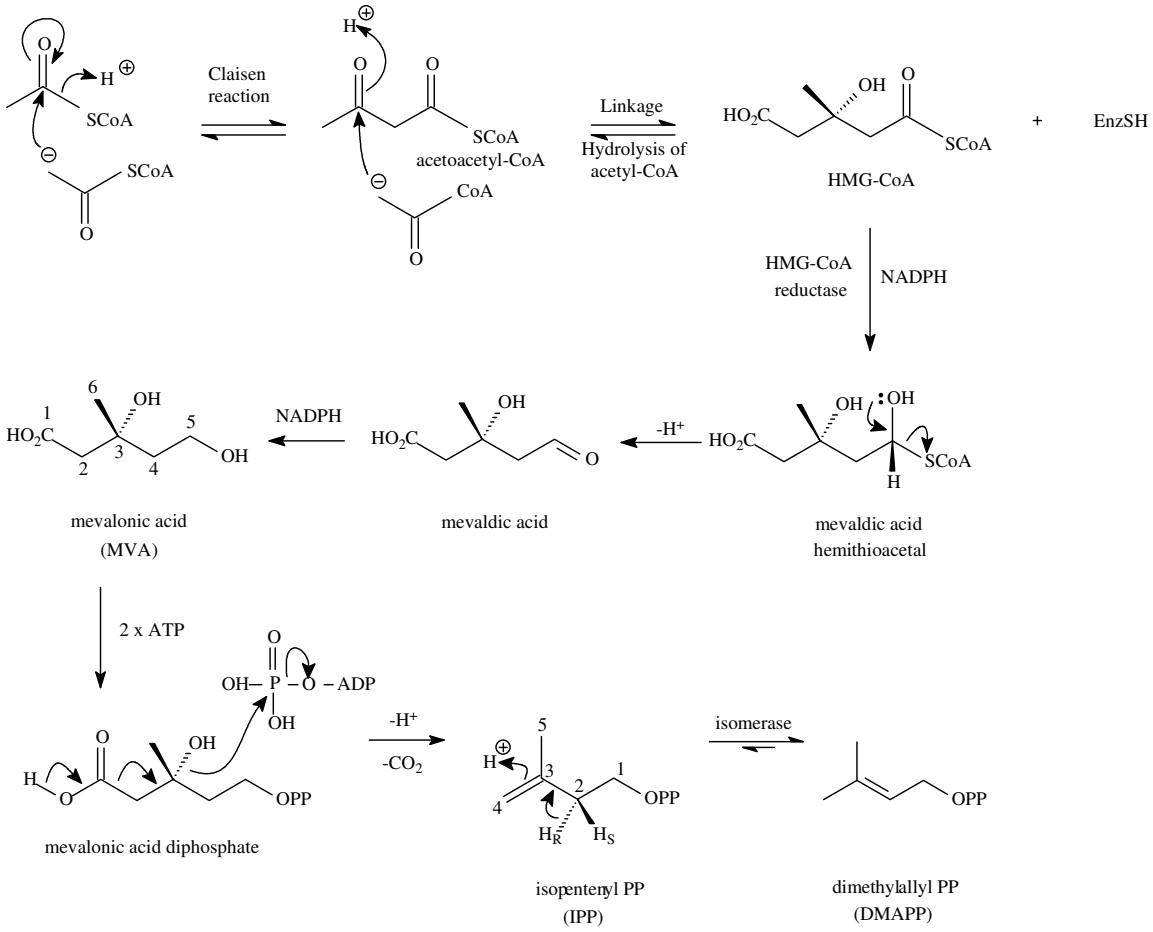


Figure 6: IPP derived from mevalonic acid (MVA), (reproduced from Dewick, 2002)

In the MEP pathway, 1-deoxy-D-xylulose-5-phosphate is derived from pyruvic acid. Thiamine diphosphate bonds to the pyruvic acid, which leads to decarboxylation producing a TPP/pyruvate enamine which then reacts with D-glyceraldehyde, followed by the loss of TPP to yield 1-deoxy-D-xylulose 5-P (DXP). DXP undergoes a pinnacol like rearrangement followed by a reduction to produce 2-methyl-D-erythritol 4-P. This is then transformed into 4-(CDP)-2-methyl-D-erythritol due to its reaction with cytidine triphosphate (CTP) and then phosphorylated with ATP. Cyclisation involving the oxygen of the terminal phosphate group with the phosphorus of the cytidine bound phosphate results in a phosphoanhydride. Opening of the ring and subsequent reduction and dehydration may lead to IPP, however these latter steps still need to be determined.

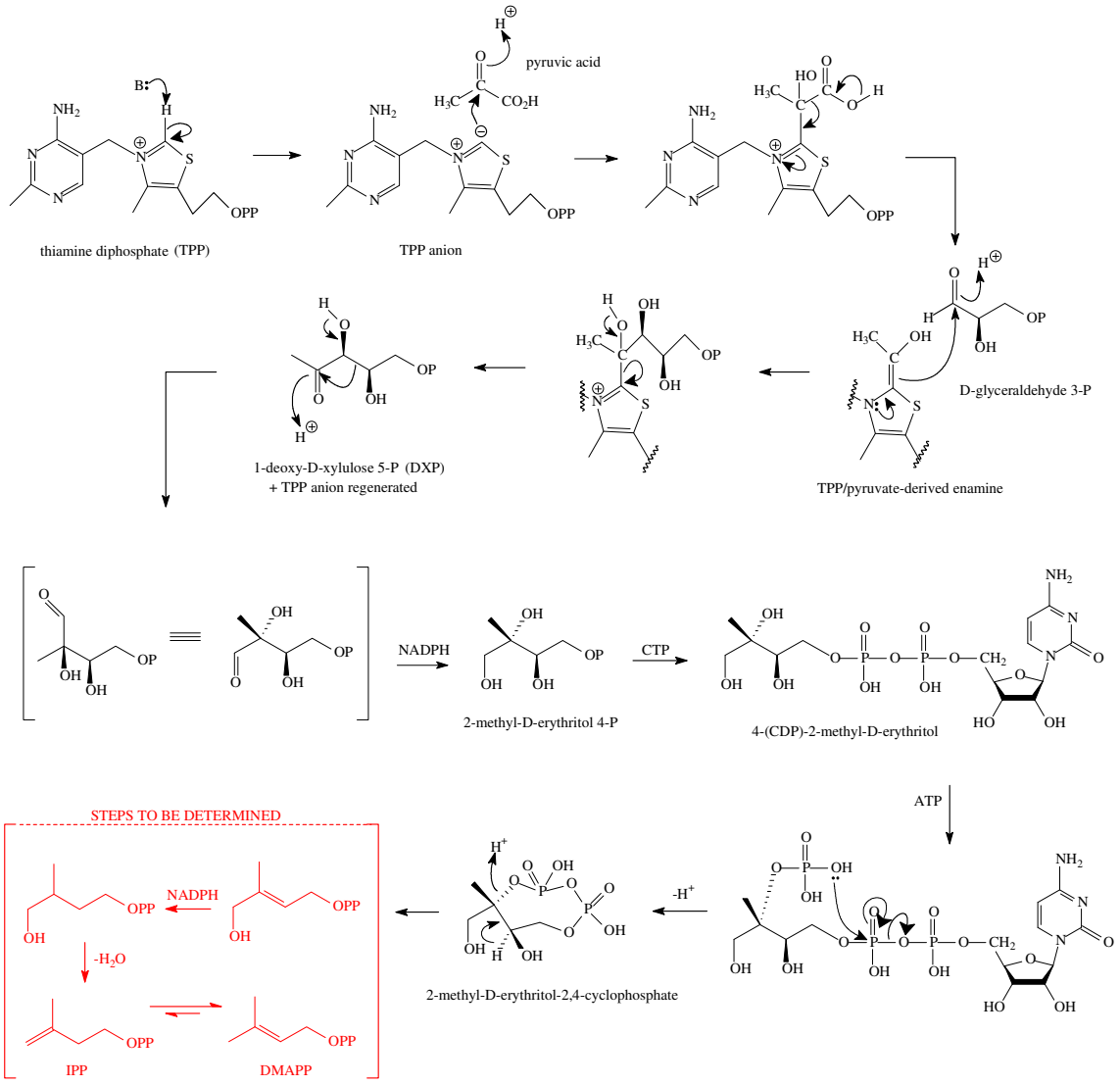


Figure 7: IPP derived from deoxyxylulose phosphate (DXP), (reproduced from Dewick, 2002)

DMAPP contains a diphosphate anion which acts as a good leaving group resulting in the isoprene unit being electrophilic. DMAPP yields an allylic cation by means of a S_N1 reaction. IPP then adds to this allylic cation and with a stereospecific loss of a proton (H_R) which then forms geranyl pyrophosphate (GPP). Addition of two further IPP molecules result in the formation of first farnesyl diphosphate (FPP) and then geranylgeranyl diphosphate (GGPP), the precursor to diterpenoid biosynthesis (figure 8).

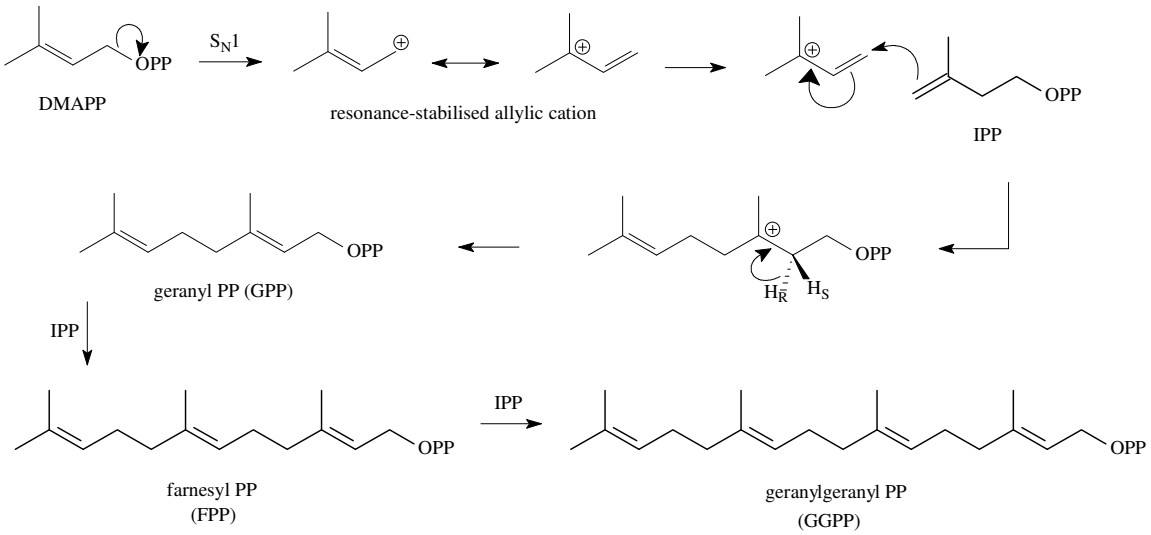


Figure 8: Derivation of GGPP from DMAPP (reproduced from Dewick, 2002)

Monocyclic diterpenes e.g. vitamin A **(2)** are formed via the first cyclisation step after the formation of GGPP, which is initiated by the protonation of the isopropylidene unit in GGPP.

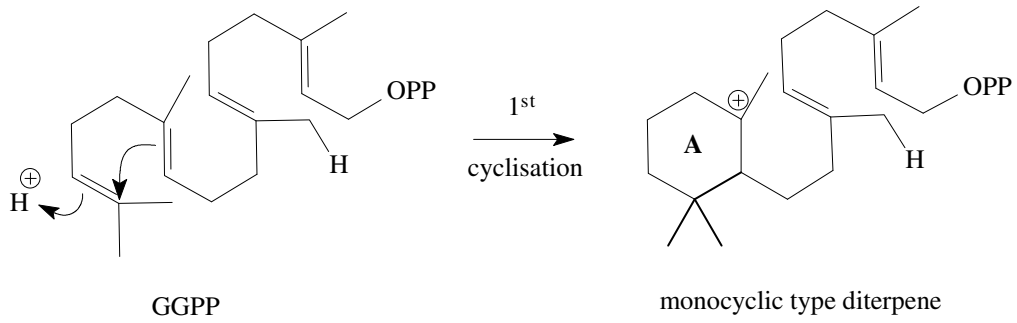


Figure 9: Formation of ring A from GGPP

A series of cyclisations as in figure 10 yields the bicyclic intermediate, copalyl diphosphate. The subsequent loss of pyrophosphate and cyclisation of copalyl PP results in the formation of the tricyclic diterpenes as in the sandaracopimarenyl cation (intermediate I). Attack by the double bond on the carbocation in the sandaracopimarenyl cation results in a fourth ring (intermediate II). Stabilisation of the cation in intermediate II, by forming a tertiary cation followed by loss of a proton result

in the kauranes. From the kauranes, loss of a hydride ion in ring B followed by ring contraction and further oxidations result in the gibberellins (figure 10) (Dewick, 2002).

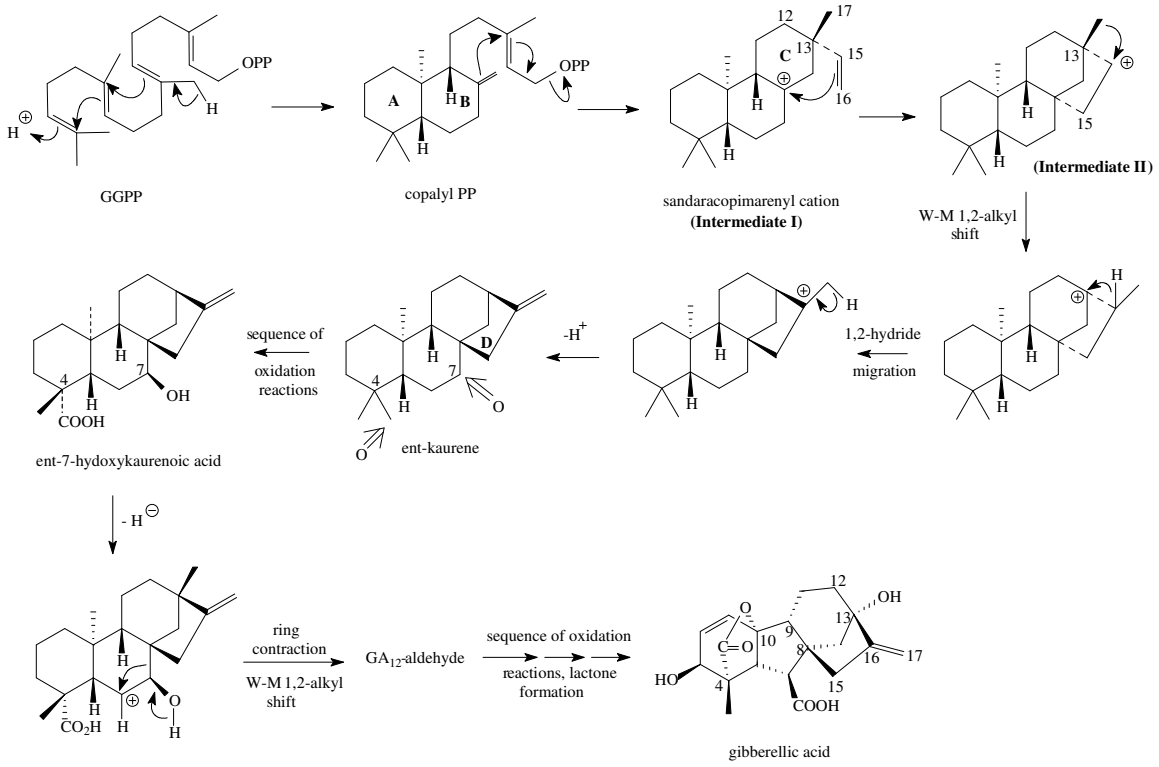


Figure 10: Biosynthetic pathway for bi-, tri- and tetracyclic diterpenes (reproduced partially from Dewick (2002), Nakanishi (1974) and Hanson (1968))

Loss of a proton at C-16 (in intermediate I) with retention of the methyl at C-13 would lead to the beyeranes (figure 11). Formation of a carbocation at C-12 from intermediate II followed by an alkyl migration of the methylene bridge gives rise to the atisane type compounds (Figure 11). The macrocyclic type compounds, the trachylobanes and aconanes form pentacyclic and tetracyclic diterpenes respectively. The bond between C-12 and C-15 is formed with loss of a proton producing the trachylobanes and rearrangement of the 8,9 bond to the 9,16 position with loss of the C-17 methylene group (Hanson, 1968) result in the aconanes (Figure 11).

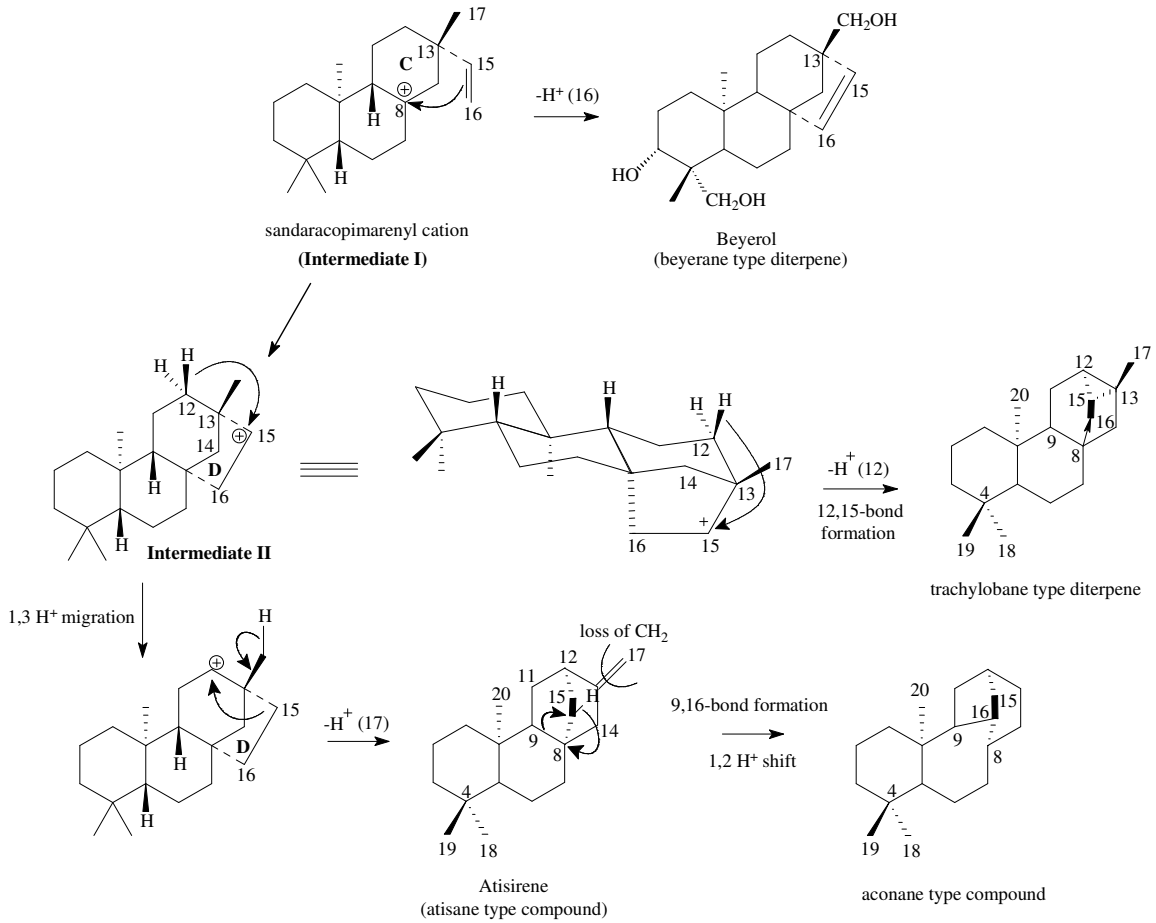


Figure 11: Biosynthesis of beyerane, trachylobane, atisane and aconane diterpenes (Manitto, 1981)

The loss of a proton at C-14 in the sandaracopimarenyl cation leads to the formation of a double bond between C-8 and C-14 after which a methyl migration from C-17 to C-15 yields an abietenyl cation, which loses a proton at C-7 to produce abietadiene (figure 12).

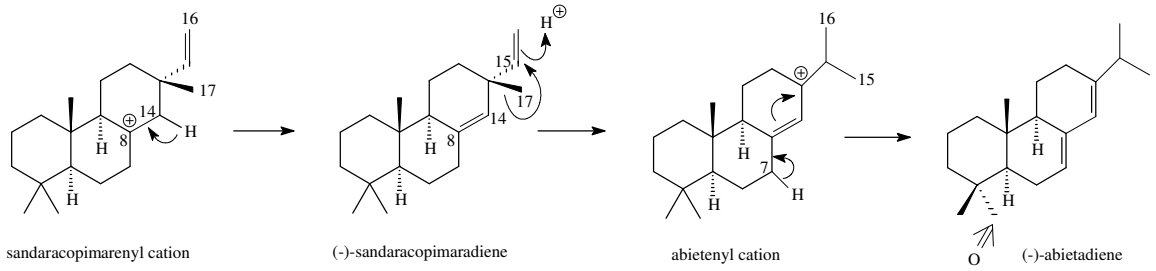


Figure 12: Synthesis of abietadiene (reproduced from Dewick, 2002)

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Chapter 2 An introduction to the *Plectranthus* in South Africa

2.1 Phylogeny, occurrence and description

The genus *Plectranthus* is one of twenty-five genera in the subfamily Nepetoideae of the family, Lamiaceae. *Plectranthus* is an "Old World" genus belonging to the Mint/Sage family. It contains about three-hundred species found in tropical Africa, Asia, India, Madagascar, Australia and a few Pacific islands (Rijo *et al*., 2007; Lukhoba *et al*., 2006; van Jaarsveld, 2006). The name *Plectranthus* literally means 'spur flower' due to the characteristic spurred corolla tube present in the first *Plectranthus* species that was discovered. This physical attribute however, is not consistent throughout the genus and may easily be confused for belonging to the *Solenostemon* or *Thorncroftia* species.

Within Africa, *Plectranthus* can be found in the southern part for example, Namibia, Swaziland, Lesotho and South Africa. It grows abundantly in eight out of the nine provinces within South Africa, with the largest concentration being found in the north-eastern part of the Eastern Cape and southern KwaZulu-Natal which has as much as thirty-six species. *Plectranthus* is desirable as a garden plant because it suppresses weed growth and prevents erosion (van Jaarsveld, 1988) and is simple and inexpensive to maintain. It is also of ornamental interest and is cultivated for their attractive foliage. They are however susceptible to attack by the eel-worm which, if not treated appropriately can lead to complete deterioration of the plant (van Jaarsveld, 1988).

They are usually found growing in shade under trees where the soil is rich in humus and well drained. However, not all *Plectranthus* species thrive under these conditions. Species with succulent leaves prefer growth in drier regions such as the dry bushveld or in rockeries (Figure 13). Even though *Plectranthus* species perish or wilt under extreme weather conditions such as frost and heat, they recover relatively quickly after a shower of rain or sprout again in spring (van Jaarsveld, 1988).

The flower colour varies among the species in *Plectranthus*, either being white, blue or mauve to pink. At the end of February, the plants begin to develop flowerbuds and reach full bloom between March and April. While some species of *Plectranthus* grow as upright shrubs to a height of approximately 1.5 meters, for example, *ecklonii, fruticosus* and *hadiensis*, others occur as groundcover plants, varying between 10 to 30 centimetres in height, for example *madagascariensis* and *saccatus*. *Plectranthus hadiensis* can be easily identified because of its large, hairy leaves.

Figure 13: *Plectranthus hadiensis* (picture courtesy of Prof. N. Crouch)

Species of *Plectranthus* are used as ornamental, economic and medicinal plants.

The phylogeny of *Plectranthus* was well documented by Paton *et al*. (2004) and Lukhoba *et al.* (2006) based on its DNA sequence and augmented morphological data of the genus. This information was presented in the form of a cladogram (figure 14) which divided the *Plectranthus* genus into two clades or groups. Clade 1 contains *Plectranthus* species formally known as *Coleus* and are grouped together based on their ethnobotanical/medicinal uses with Clade 1b containing a greater number of medicinally active species than Clade 1a. The groups in bold in figure 14 all have reported medicinal uses. Of the three subclades, subclade 1b seems to be the most widely used medicinally,

followed by subclade 2 and lastly subclade 1a where only groups 2 and 8 are used medicinally. Species within Clade 1 are also sources of food and are used as food flavourants, fodder for domestic animals, ornamental displays in homes and gardens as well as other uses such as building material (Lukhoba *et al*., 2006). For example, the wood from *Plectranthus insignis* can be used to build huts or temporary houses (Cheek *et al.*, 2000; Lukhoba *et al*., 2006).

There are further species that fit into clades 1 and 2 but could not be placed into any of the subclades as they were morphologically different species within these subclades. These are listed as unplaced groups A-E in each clade.

Plectranthus is a synonym for *Coleus* (Lukhoba *et al*., 2006) and therefore in searching the literature for phytochemical reviews of *Plectranthus*, one has to also consider the *Coleus* species. *Plectranthus amboinicus* itself has synonymns of *Plectranthus aromaticus* Roxb*., Coleus aromaticus* Benth. and *Coleus amboinicus* Lour. (Lukhoba *et al*., 2006).

Beside morphological characteristics and DNA sequencing, it would also be useful to have chemotaxonomic data linking the different species together and hence, phytochemical studies on the different species of *Plectranthus* is important to build up a database of the secondary metabolites linking these species together.

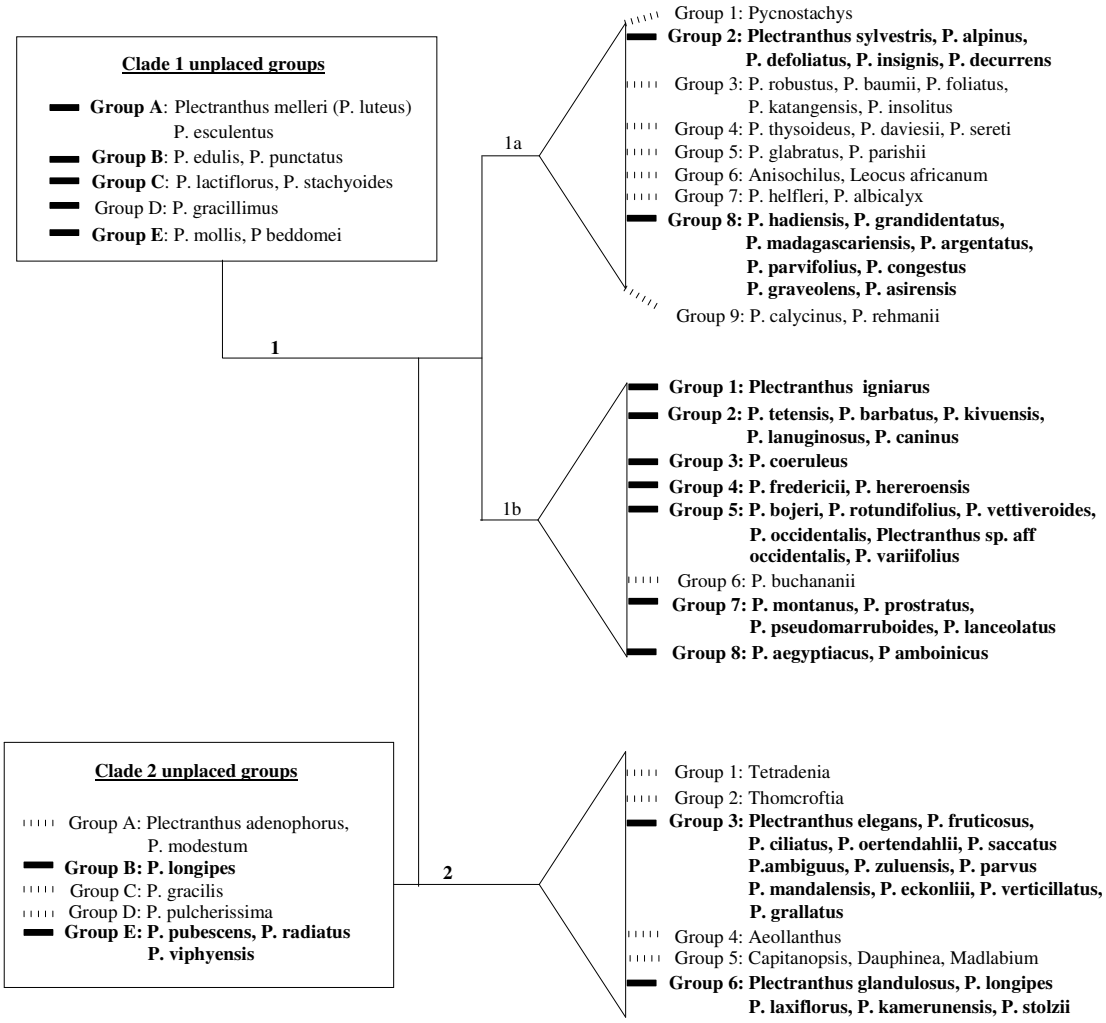

Figure 14: Illustration of the 2 clades within the *Plectranthus* species, categorized according to their phylogeny (reproduced from Lukhoba *et al*., 2006)

2.2 Ethnobotanical uses

A comprehensive review was done by Lukhoba *et al*. (2006), which contains ethnomedicinal information of a variety of *Plectranthus* species between 1934 and 2005. This is summarised in tables 1 and 2 with additional information added for published work between 2006 and 2010.

Table 1 is an inventory of *Plectranthus* species that are used by traditional healers to help alleviate and/or heal skin, digestive, respiratory, muscular-skeletal and genito-urinary conditions as well as infections, fever and pain, while table 2 contains an inventory of species used to treat heart, circulatory and blood disorders, ailments affecting the sensory and nervous system, treatment of poisonous substances in the body, inflammation and medical conditions which could not be assigned to any of the other categories, labelled as 'unspecific'.

A total of twenty-one *Plectranthus* species are used for digestive conditions, nineteen used for skin conditions and sixteen and fifteen species respectively used for respiratory conditions and infections and fever (Table 1). This in itself is evidence of the genus' widespread use ethnomedicinally.

The conditions listed in table 2 are not treated by as many species as are those listed in table 1 with the highest being eight species used for nervous conditions, followed by six species to treat sensory conditions, five species for heart, circulatory and blood disorders and four species each for treatment against poisons and inflammation.

Plectranthus barbatus and *Plectranthus amboinicus* have proven to be the most widely used species, being used in all of the medical conditions listed in Tables 1 and 2 with *Plectranthus laxiflorus* being used in all conditions in table 1 and three of the categories as reflected in Table 2.

It must be noted that digestive conditions as categorised by Lukhoba *et al*. (2006) contain nausea, vomiting and diarrhoea and that "pain" can be associated with a number of

different medical conditions and in many cases, the treatment of inflammation will also result in the relief of pain. Respiratory and genito-urinary conditions could also lead to infection and fever. It is thus unclear from the information presented in Lukhoba *et al*. (2006) or the papers cited therein whether or not the plant extracts are treating a certain condition or the symptoms arising from these conditions as pain and fever are the symptoms of a wide variety of diseases. The same can be said about nausea, vomiting and diarrhoea. They are the symptoms of a wide variety of viral and bacterial infections.

Examples of heart, circulatory and blood disorders are congestive heart failure, hypertension and angina. Epilepsy, convulsions and depression are related to the nervous system while ear and eye problems are grouped as 'sensory' conditions. Poisons treatment refers to the treatment of a person who has been bitten or stung by an insect or animal which posesses venom.

Even though in many instances, these ethnomedicinal uses are unsubstantiated and unclear, they can be useful leads in the testing of plant extracts or compounds isolated from them.

Table 1: Summary of the medicinal uses of various *Plectranthus* species (Lukhoba *et al*., 2006*)

***Plectranthus* species**	**Medical conditions**						
	Skin conditions	**Digestive conditions**	**Respiratory conditions**	**Infections and fever**	**Genito-urinary conditions**	**Pain**	**Muscular –skeletal conditions**
P. aegypticus (Forssk.) C. Chr		✓	✓	✓	✓		
P. alpinus (Vatke) O. Ryding	✓					✓	
P. ambiguus (Bolus) Codd			✓				
P. amboinicus (Lour.) Spreng	✓	✓	✓	✓	✓	✓	✓
P. asirensis J.R.I Wood	✓						
P. barbatus Andr.	✓	✓	✓	✓	✓	✓	✓
P. beddomei Raiz.	✓						
P. bojeri (Benth.) Hedge	✓		✓				
P. caninus Roth		✓	✓				
P. coeruleus (Gurke) Agnew				✓			
P. coleoides						✓[2]	
P. congestus R.Br.	✓			✓			
P. decurrens (Gurke) J.K. Morton		✓					
P. defoliatus Hochst. Ex Benth		✓					
P. eckonlii Benth	✓						
P. edulis (Vatke) Agnew		✓	✓				
P. elegans Britten		✓	✓		✓		
P. esculentus N.E.Br.		✓				✓	
P. fruticosus L'Her.	✓						
P. glandulosus Hook.f.			✓				
P. hadienis (Forssk.) Schweinf. Ex Spreng.	✓+	✓+	✓+				
P. hereroensis Engl.		✓					
P. ignarius (Schweinf.) Agnew	✓					✓	
P. insignis Hook.f.		✓					
P. kamerunensis (Gurke)	✓	✓					
P. lactiflorus (Vatke) Agnew		✓					
P. lanceolatus Bojer ex Benth			✓	✓			

Continued on next page.....

Plectranthus species	Medical conditions						
	Skin conditions	**Digestive conditions**	**Respiratory conditions**	**Infections and fever**	**Genito-urinary conditions**	**Pain**	**Muscular –skeletal conditions**
P. lanuginosus (Benth.) Agnew		✓					
P. laxiflorus Benth.	✓	✓	✓	✓	✓	✓	✓
P. longipes Baker		✓					
P. madagascariensis Benth.	✓		✓				
P. mandalensis Baker							✓
P. melleri Baker				✓			
P. mollis (Aiton) Spreng.	✓	✓[1]	✓	✓			✓
P. montanus Benth.		✓	✓	✓		✓	
P. parviflorus (Poir.) Henckel				✓			
P. prostratus Gurke					✓		
P. pseudomarrubiodes Willemse	✓						
P. pubescens Baker							✓
P. punctatus L'Her				✓			
P. rogosus						✓[3]	
P. stachyoides Oliv.	✓						
P. stolzii Gilli			✓				
P. sylvestris Gurke	✓	✓		✓		✓	
P. tetensis (Bak.) Agnew				✓			
P. vettiveroides (K.C. Jacob) H.I Maass	✓	✓		✓	✓		
Total	**19**	**21**	**16**	**15**	**7**	**10**	**6**

* Where data was not taken from Lukhoba *et al*. (2006) the references are denoted by superscripts in the table and the references are given below

[+] No mention in the references cited within Lukhoba *et al*. (2006) to these conditions, however Lukhoba *et al*. (2006) lists these as being prevalent

[1] Ayyanar and Ignacimuthu, 2005

[2] Ignacimuthu *et al*., 2006

[3] Khan *et al*., 2007

Table 2: Summary of uses of various *Plectranthus* species (Lukhoba *et al*., 2006)

***Plectranthus* species**	**Medical conditions**					
	Heart, circulatory and blood	**Nervous**	**Sensory**	**Poisons Treatment**	**Inflammation**	**Unspecific**
P. aegypticus (Forssk.) C. Chr.			✓			✓
P. alpinus (Vatke) O. Ryding		✓				
P. amboinicus (Lour.) Spreng	✓	✓	✓	✓	✓	✓
P. barbatus Andr.	✓	✓	✓	✓	✓	✓
P. bojeri (Benth.) Hedge			✓	✓		
P. congestus R.Br.						✓
P. edulis (Vatke) Agnew						✓
P. fruticosus L'Her.						✓
P. glandulosus Hook.f.						✓
P. grallatus Briq.						✓
P. grandidentatus Gurke	✓					
P. hadienis (Forssk.) Schweinf. Ex Spreng.					✓+	
P. ignarius (Schweinf.) Agnew			✓			
P. kivuensis (Lebrun & Touss.) R.H. Willemse						✓
P. laxiflorus Benth.		✓	✓		✓	
P. longipes Baker						✓
P. madagascariensis Benth.						✓
P. mandalensis Baker		✓				
P. mollis (Aiton) Spreng	✓	✓		✓		✓
P. montanus Benth.						✓
P. occidentalis B.J. Pollard						✓
P. pubescens Baker	✓					
P. punctatus L'Her		✓				

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Plectranthus species	Medical conditions					
	Heart, circulatory and blood	**Nervous**	**Sensory**	**Poisons Treatment**	**Inflammation**	**Unspecific**
P. sp. *aff. occidentalis*						✓
P. stolzii Gilli						✓
P. vettiveroides (K.C. Jacob) H.I Maass		✓				✓
P. viphyensis Brummit & J.H. Seyani						✓
Total	**5**	**8**	**6**	**4**	**4**	**18**

[+] No mention in the references cited within Lukhoba *et al*. (2006) to these conditions, however Lukhoba *et al*. (2006) lists these as being prevalent

The modes of administration of the plant extracts differ according to the ailment being treated. The treatment of skin conditions such as burns, wounds, allergies and insect bites are treated by applying ground plant material on the affected area whilst bathing in herbal extracts may help alleviate the irritation associated with measles and chicken pox. Digestive and respiratory conditions are treated by drinking teas, infusions or decoctions to alleviate constipation, indigestion and dyspepsia as well as asthma, bronchitis and other respiratory conditions and the inhalation of steam or smoke is used for the treatment of colds and influenza. Enemas and injections are also used as a means of administering plant extracts to the patient (Gurib-Fakim, 2006). For instance, the leaf infusion of *Plectranthus defoliatus* Hochst. ex Benth. is either drunk or admininstered as an enema (Neuwinger, 2000). With regard to injections being administered, no information is given as to how the extracts are injected and whether they use conventional needles and syringes or home-made equivalents as there is doubt as to where and how traditional healers would get access to needles and syringes. Furthermore, there is doubt as to whether traditional healers are trained to administer injections, and the improper use of needles and syringes, for example the repeated use of these could do the patient more harm than the illness itself.

2.3 Pharmacological/biological uses of the plant extract

Extracts of *Plectranthus* species have shown to be active in a wide range of bioassays. These include antibacterial, antifungal, antiparastitic, anti-inflammatory, antioxidant, antitumour and insect antifeedant activity. Of these, they find most widespread activity in antibacterial and antifungal assays. References are given in the subchapters that follow.

Methods used for the determination of antibacterial activity

The two most common qualitative methods for testing antimicrobial activity of extracts are performed by either using the agar well assay or the disc diffusion assay. In the agar well method, wells or holes are made into the solidified, sterile agar plate once the test inoculum has been evenly distributed on the agar surface. These wells are then filled with solutions of the plant extracts and test controls. After incubation, the antimicrobial activity is determined by measuring the diameters of zones of inhibition in milimeters.

In the disc diffusion assay, filter paper discs are dissolved in solutions of plant extracts and placed onto the inoculated plate. Once incubation of the plate is complete the antimicrobial activity is determined by measuring the diameters of zones of inhibition in milimeters. Wells or holes are not required for this method of testing. Since the unit of measurement is the same for both test methods, results can be compared.

The bioautography agar overlay method is derived from the disc diffusion assay/method and uses thin layer chromatography (TLC) plates instead of filter paper. In this method the plant extract or compound of interest is adsorbed onto the TLC plate followed by the introduction of a thin layer of inoculum onto the very same plate. The plant extract or compound being evaluated then diffuses from the TLC/adsorbent into the inoculum. After incubation of the TLC plate, the R*f* values of the observed spots are recorded and zones of inhibition are measured in mm.

All three methods described above, are performed in triplicate to ensure reproducible results and are measured against control standards.

Use of the minimum inhibitory concentration (MIC) assay allows one to determine the lowest concentration at which an extract will be active. This is normally represented in µg/ml and is carried out by serial dilutions of the plant extracts or compounds under study.

Antibacterial activity of *Plectranthus* and *Coleus* plant extracts

It is observed from Table 3 that the *Plectranthus* species are active against a variety of bacterial strains but being more active toward *Staphylococcus* and *Bacillus* species with sixteen of the twenty-one *Plectranthus* species being active against *Staphylococcus aureus* and *Bacillus subtilis* listed in table 3, below. Although the *Pseudomonas* bacterial strain was also a popular choice for antibacterial testing, only eight *Plectranthus* species was active against this pathogen.

The leaves, roots and aerial parts were the most common parts of *Plectranthus* which have been tested for antibacterial activity with there being one report on the activity of the stems and the flowers against bacteria (Rabe and van Staden, 1998; Matu and van Staden, 2003). In one study, the root and flower extracts displayed higher antibacterial activity than the leaf extracts (Rabe and van Staden, 1998; Matu and van Staden, 2003; Mothana *et al*., 2008) suggesting that further antimicrobial studies of the root and flower extracts in *Plectranthus* should also be carried out.

The solvents used to prepare the extracts for bioassays, were predominantly polar with there being just two instances where hexane was used as the solvent medium. Ideally, a high activity of the water extracts is desired as water is readily available, inexpensive and safe for human consumption. An alternative to using solvents to prepare plant extracts for bioassays, is the hydrodistillation of the plant or certain parts of the plant producing an essential oil (Ascensao *et al*., 1998; Alsufyani *et al*., (see footnote X under table 3); Marwah *et al*., 2007; Oliveira *et al*., 2007; Vagionas *et al*., 2007). This method however, excludes the secondary metabolites which are not volatile at the temperatures being used. The polar extracts are more active than the non-polar extracts at inhibiting the growth of bacteria (Matu and van Staden, 2003).

P. barbatus and *P. madagascariensis* were the two most commonly tested plant species, with extracts from both plants being more susceptible to gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis)* than gram-negative bacteria (Ascensao *et al*., 1998; Rabe and van Staden, 1998; Matu and van Staden, 2003; Wellsow *et al*., 2006; Kisangau *et al*., 2007; Figueiredo *et al*., 2010). *Coleus kilimandschari* was found to be active against a wide variety of gram-negative and -positive bacteria (Vagionas *et al*., 2007).

Table 3: Antibacterial activity of *Plectranthus* and *Coleus* extracts

***Plectranthus* species**	**Extraction medium and plant part/s used**	**Antibacterial activity**	**Reference**
Coleus kilimandschari	80% methanol[lv]	*Bacillus cereus, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoneae, Mycobacterium fortuitum, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Streptococcus pyogenes*	Cos *et al*., 2002
P. aff. puberulentus	acetone[lv]	*Bacillus subtilis, Pseudomonas syringae*	Wellsow *et al*., 2006
P. argentatus		*B. subtilis*	
P. barbatus	petroleum ether[lv], dichloromethane[lv] and water[lv]	*S. aureus, B. subtilis, E. coli, P. aeruginosa*	Kisangau *et al*., 2007
	water[w]	Cariogenic bacteria *(Streptococcus sobrinus* and *Streptococcus mutans)*	Figueiredo *et al*., 2010
	hexane[rt] and methanol[rt]	*B. subtilis, S. aureus, Micrococcus luteus*	Matu and van Staden, 2003
	hexane[s], methanol[s] and water[s]		
	methanol[lv]		
P. ciliatus (3 different collections)	methanol[lv]	*S. aureus, B. subtilis*	Rabe and van Staden, 1998
P. ciliatus (1 collection)		*S. aureus, B. subtilis, M. luteus*	
P. cylindraceus	oil[a]	*E. coli, P. aeruginosa, K. pneumoniae, S. aureus, B. subtilis, Salmonella choleraesuis*	Marwah *et al*., 2007
P. eckonlii	water[w]	Cariogenic bacteria *(S. sobrinus* and *S. mutans)*	Figueiredo *et al*., 2010
	methanol[lv]	*S. aureus, B. subtilis*	Rabe and van Staden, 1998
	methanol[f]	*S. aureus, B. subtilis, M. luteus, Staphylococcus epidermis*	

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***Plectranthus* species**	**Extraction medium and plant part/s used**	**Antibacterial activity**	**Reference**
P. forsteri 'marginatus'	acetone[lv]	*B. subtilis, P. syringae*	Wellsow *et al*., 2006
P. fruticosus (2 different collections)	methanol[lv]	*S. aureus, B. subtilis*	Rabe and van Staden, 1998
P. fruticosus	methanol[f]	*S. aureus, B. subtilis, M. luteus*	
P. hadiensis	water[rt]	multi-resistant strains of *S. epidermidis, Staphylococcus haemolyticus* and *S. aureus,* the North German epidemic strain	Mothana *et al*., 2008
	methanol[rt+lv] and water[lv]	*S. aureus, B. subtilis, Micrococcus flavus* and multi-resistant strains of *S. epidermidis, S. haemolyticus* and *S. aureus,* the North German epidemic strain	
	hexane[a]	8 *bacillus* formulations	Laing *et al*., 2006
	dichloromethane[a]	*B. subtilis, Xanthomonas campestris*	
P. hereroensis	acetone[rt]	*S. aureus, Vibrio cholera, Streptococcus faecalis*	Batista *et al*., 1994
P. laxiflorus	essential oil[a]	*S. aureus, S. epidermis, E. coli, E. cloacae, K. pneumoniae, P. aeruginosa*	Vagionas *et al*., 2007
P. madagascariensis	acetone[lv]	*B. subtilis, P. syringae*	Wellsow *et al*., 2006
	essential oil[a]	*B. subtilis, *Micrococcus sp., S. aureus, Yersinia enterocolitica*	Ascensao *et al*., 1998
P. madagascariensis (2 different collections)	methanol[lv]	*S. aureus, B. subtilis, M. luteus, S. epidermis*	Rabe and van Staden, 1998
P. oribiensis		*S. aureus, B. subtilis, M. luteus, S. epidermis*	
P. oribiensis		*S. aureus, B. subtilis, M. luteus, S. epidermis*	

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***Plectranthus* species**	**Extraction medium and plant part/s used**	**Antibacterial activity**	**Reference**
P. ornatus	essential oil[lv]	*S. aureus, S. pyogenes, E. coli, S. typhimurium*	Oliveira *et al*., 2007
P. petiolaris	methanol[lv]	*S. epidermis, B. subtilis*	Rabe and van Staden, 1998
P. puberulentus	acetone[lv]	*P. syringae*	Wellsow *et al*., 2006
P. rubropunctatus	methanol[lv]	*S. aureus, B. subtilis, S. epidermis*	Rabe and van Staden, 1998
**Plectranthus* sp. (3 hybrids)		*S. aureus, B. subtilis*	
**Plectranthus* sp. (1 hybrid)		*S. aureus, B. subtilis, M. luteus*	
**Plectranthus* sp. (1 hybrid)		*S. aureus, B. subtilis, S. epidermis*	
P. strigosus		*S. aureus, B. subtilis, M. luteus, S. epidermis*	
P. tenuiflorus	essential oil[lv]	*S. aureus, S. pyogenes, E. coli, K. pneumoniae, P. aeruginosa*	[x]Alsufyani *et al*.,
P. verticillatus	methanol[lv]	*S. aureus, B. subtilis, S. epidermis*	Rabe and van Staden, 1998

NB. A species of *Coleus* is also included in this table because of the uncertain relationship to the *Plectranthus* species

* Unknown species

[x] available electronically on http://www.kau.edu.sa, date accessed 27/09/2010; the date of the publication is not apparent.

Key: lv: leaves, rt: roots, s: stems, a: aerial parts, w: whole plant, f: flowers

Table 4 contains antifungal activity of eight *Plectranthus* species and one *Coleus* species. All of the species in table 4 were active against at least one *Candida* species with six *Plectranthus* species being active against *Candida albicans*, the most widely tested fungal pathogen. *P. amboinicus* and *P. barbatus* were the two most tested species for antifungal activity from all the *Plectranthus* species.

From the studies reviewed, hydrodistillation seemed to be the most popular method of extraction, followed by solvent extraction with water, methanol and more uncommonly solvents such as ether, hexane and dichloromethane.

There were two comparative studies on the solvent extracts with regard to antifungal activity (Laing *et al*., 2006; Kisangau *et al*., 2007). In one study, the ether extract showed higher activity than the dichloromethane and aqueous extracts while in the other study, the hexane extract was more effective than the dichloromethane extract. This indicates that future studies on ether and hexane extracts need to be conducted since these extracts were shown to be active.

The leaves are the most popular part of the plant used to make extracts for antifungal activity. Other studies mention aerial parts being used for extraction but these parts are not specified and could be the leaves, flowers, seeds or fruit. There is also one report on the roots being used. There is thus a need for more studies on the roots and stem material to be carried out for antifungal activity as not many studies have been done on these plant parts.

There is also a need for comparative studies to be done with the different plant parts as well as with the solvent used for extraction. This would then provide a much clearer indication of which plant part as well as which solvent is best suited for antifungal activity and would be a good guide for phytochemists to use when choosing a suitable plant part to study.

Table 4: Antifungal activity of *Plectranthus* and *Coleus* extracts

***Plectranthus* species**	**Extraction medium and plant part/s used**	**Antifungal activity**	**Reference**
Coleus kilimandschari	80% methanol[lv]	*Candida albicans, Microsporum canis, Trichophyton rubrum, Epidermophyton floccosum*	Cos *et al*., 2002
P. amboinicus	essential oil[lv]	*Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Aspergillus oryzae, Candida versatilis, Fusarium moniliforme, *Penicillium* sp., *Saccharomyces cerevisiae,* in stored food commodities	Murthy *et al*., 2009
	methanol[lv]	*Candida krusei*	Tempone *et al*., 2008
	essential oil[lv]	*C. albicans, Candida tropicalis, Candida guilliermondii, Candida krusei*	de Oliveira *et al*., 2007
P. barbatus	methanol[lv]	*Candida krusei*	Tempone *et al*., 2008
	80% methanol[rt+lv]	*C. albicans*	Runyoro *et al*., 2006
	petroleum ether[lv], dichloromethane[lv] and water[lv]	*C. albicans*	Kisangau *et al*., 2007
P. cylindraceus	oil[a]	*C. albicans, M. canis, Microsporum gypseum, T. rubrum, Trichophyton mentagrophytes*	Marwah *et al*., 2007
		Inhibits fungal spore germination and growth of **Bipolaris* sp., *Alternaria alternate, Fusarium oxysporum, Curvularia lunata, Stemphyllum solani*	
P. grandis	methanol[lv]	*Candida krusei*	Tempone *et al*., 2008

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***Plectranthus* species**	**Extraction medium and plant part/s used**	**Antifungal activity**	**Reference**
P. hadiensis	hexane[a]	*Sclerotinia sclerotiorum, Rhizoctonia solani, *Candida* species	Laing *et al*., 2006
	dichloromethane[a]	*S. sclerotiorum, *Candida* species	
P. laxiflorus	essential oil[a]	*C. albicans, C. tropicalis, C. glabrata*	Vagionas *et al*., 2007
P. neochilus	methanol[lv]	*Candida krusei*	Tempone *et al*., 2008
P. tenuiflorus	essential oil[lv]	*C. albicans*	[x]Alsufyani *et al*.,

NB. A species of *Coleus* is also included in this table because of the uncertain relationship to the *Plectranthus* species
* Unknown species
[x] available electronically on http://www.kau.edu.sa, date accessed 27/09/2010; the date of the publication is not apparent.
Key: lv: leaves, rt: roots, s: stems, a: aerial parts, w: whole plant, f: flowers

Besides posessing antifungal and antibacterial activity, *Plectranthus* and *Coleus* species also have anti-tumor, antiparasitic, antioxidant, antifeedant and anti-inflammatory properties, as shown in Table 5a.

P. amboinicus appears to be the most commonly used *Plectranthus* species, being active against a wide variety of pharmacological activities. *P. barbatus* was also a popular plant species used, being active in three of the five pharmacological activities contained in table 5a. Other *Plectranthus* species are used less frequently.

The leaves seem to be the most popular plant part used to inhibit the growth of parasites, to act as an antioxidant and antifeedant as well as for the treatment of inflammation and tumors. There has been only two reports on root extracts being used and just one report on the stem being used. More research on the root, stem and aerial parts is required as it is still unclear as to which plant part is the most active.

Methanol, ethanol and water seem to be used more frequently than hexane and acetone for making extracts to be tested in these bioassays. There has been one report where hexane and acetone extracts were used. This may limit the studies to polar extracts and more studies using hexane, dichloromethane and acetone is needed to ascertain whether the non-polar fractions are active in these same bioassays.

Table 5a: Antiparasitic, anti-inflammatory, antioxidant, antitumour and insect antifeedant activity of polar extracts from seven *Plectranthus* species

***Plectranthus* species**	**Solvent of Extraction**	**Pharmacological details**	**Reference**
Antiparastic activity			
P. amboinicus	aqueous[lv]	inhibits growth of *Plasmodium berghei yoelii*	Periyanayagam *et al*., 2008
	methanol[lv]	antileishmanial activity against *Leishmania chagasi* and *Leishmania amazonensis*	Tempone *et al*., 2008
P. barbatus	methanol[lv]	antileishmanial activity against *Leishmania chagasi* and *Leishmania amazonensis*	Tempone *et al*., 2008
P. cylindraceus	essential oil[a]	nematicidal activity against *Meloidogyne javanica*	Onifade *et al*., 2008
P. grandis	methanol[lv]	antileishmanial activity against *Leishmania chagasi* and *Leishmania amazonensis*	Tempone *et al*., 2008
P. marruboides	essential oil[a, lv]	toxic fumigant against *Anopheles gambiae*	Omolo *et al*., 2005
P. neochilus	methanol[lv]	antileishmanial activity against *Leishmania chagasi* and *Leishmania amazonensis*	Tempone *et al*., 2008
P. punctatus	aqueous[lv] and 80% methanol[lv]	ovicidal and larvicidal activity against *Haemonchus contortus*	Tadesse *et al*., 2009

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Plectranthus species	Solvent of Extraction	Pharmacological details	Reference
Antiviral activity			
Coleus kilimandschari	80% methanol[lv]	Antiviral activity against DNA-virus Herpes simplex virus type 1	Cos *et al*., 2002
Antioxidant Activity			
P. amboinicus	essential oil[lv]	antiradical activity	Murthy *et al*., 2009
P. barbatus	aqueous[lv]	antioxidant activity confirmed by DPPH and β-carotene/linoleic acid test assays	Fale *et al*., 2009
P. eckonlii			
P. fruticosus			
P. hadiensis	methanol[rt+lv] and water[rt+lv]	antiradical activity	Mothana *et al*., 2008
P. lanuginosus	aqueous[lv]	antioxidant activity confirmed by DPPH and β-carotene/linoleic acid test assays	Fale *et al*., 2009
P. laxiflorus	essential oil[a]	antiradical activity	Vagionas *et al*., 2007
P. verticillatus	aqueous[lv]	antioxidant activity confirmed by DPPH and β-carotene/linoleic acid test assays	Fale *et al*., 2009
Anti-inflammatory activity			
P. amboinicus	70% ethanol[lv]	anti-inflammatory	Gurgel *et al*., 2009
	composition		Wong *et al*., 2009
P. barbatus	hexane[rt, s+lv], water[rt, s+lv] and methanol[rt, s+lv]		Matu and van Staden, 2003

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Plectranthus species	Solvent of Extraction	Pharmacological details	Reference
Anti-tumor activity			
P. amboinicus	70% ethanol[lv]	anti-tumor (Sarcoma-180 ascite and Ehrlich ascite carcinoma) activity in mice	Gurgel *et al*., 2009
	leaf juice composition (orally, topically or injections)	Inhibits growth of carcinoma (HepG2, Huh7) and melanoma (Bowes) cells	Cheng-Yu *et al*., 2006
Insect-antifeedant activity			
P. aff. puberulentus	acetone[lv]	antifeedant activity against *S. littoralis*	Wellsow *et al*., 2006
P. forsteri 'marginatus'			
P. puberulentus			
P. saccatus			
P. zuluensis			

NB. The term 'mixture' refers to the testing of a composition comprising of a *Plectranthus* plant extract with either another plant extract/essential oil (not necessarily belonging to the *Plectranthus* species) or an appropriate pharmaceutical drug.
Key: lv: leaves, rt: roots, s: stems, a: aerial parts, w: whole plant, f: flowers

Plectranthus and *Coleus* species act as abortificients (Almeida and Lemonica, 2000), prevent the formation of plaque build-up on teeth (Figueiredo *et al*., 2010), are used topically as a barrier against harmful UV rays (Chen *et al*., 2009), act as a hair growth stimulant (Kiyoshi *et al*., 2005) and inhibitor (Kazuo *et al*., 2006), are used in the treatment of rheumatoid arthritis (Rey-Yuh *et al*., 2008; Jui-Yu *et al*., 2009), act as a scorpion venom antidote (Uawonggul *et al*., 2006) and are used to treat Alzheimers disease (Fale *et al*., 2009) as well as many other ailments, disorders and diseases (table 5b).

P. amboinicus and *P. barbatus* seem to be the most commonly tested species each having widespread application. Once again the leaves appear to be the most popular plant part used. There has been just one study on the root extract. The stems and roots need to be studied further.

There are plant extracts in table 5b which have been tested as mixtures either in combination with other plant extracts or pharmaceutical agents, which highlights the synergistic effect of various *Plectranthus* and *Coleus* species.

Table 5b: Other important inhibitory activity of polar extracts from *Plectranthus* and *Coleus* species

***Plectranthus* species**	**Solvent of Extraction**	**Other Activities**	**Reference**
Other inhibitory activity			
C. barbatus	70% ethanol[lv]	anti-implantation effects in rats	Almeida and Lemonica, 2000
	mixture	prevents foul breath	Sonoko *et al*., 2005
C. foskohlii	mixture	prevention of progression of diabetic complications and skin aging	Masayuki *et al.*, 2007
		antispasmodic effect on respiratory tract smooth muscle	Rongqiu *et al*., 2005
		treat all forms of obesity and associated metabolic syndromes	Marcin *et al*., 2005; Han *et al*., 2005
		hair growth stimulant	Kiyoshi *et al*., 2005
		body hair growth inhibitor	Kazuo *et al*., 2006
	food mixture	improves bowel movement	Motoyuki *et al*., 2007
	composition	antiasthmatic, cough-relieving, phlegm-expelling, treats acute and chronic bronchitis	Jian *et al*., 2006
	food/cosmetic compostions, powder	promotes longevity and increases bone mass and decreases body weight	Sayuri *et al*. 2008
**Coleus* species	mixture	remedy for menopause disorder	Chiyoko *et al*., 2005
P. amboinicus	aqueous[lv]	acts as scorpion venom antidote against *Heterometrus laoticus*	Uawonggul *et al*., 2006
	mixture	treat rheumatoid arthritis	Jui-Yu *et al*., 2009
	essential oil mixture	relaxing uterus and alleviating dysmenorrhea	Jiali *et al*., 2007

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***Plectranthus* species**	**Solvent of Extraction**	**Other Activities**	**Reference**
P. amboinicus	composition	treatment of skin disorders and healing of wounds especially in diabetic patients	Rey-Yuh *et al*., 2007
	essential oil mixture (applied on belly)	Relieves menstrual pain by relaxing the uterus, tested in mice	Chia-Li *et al*., 2007
	composition (taken orally or applied topically)	Treat rheumatoid arthritis (tested in animals)	Rey-Yuh *et al*., 2008
P. barbatus	aqueous[lv]	helps in treatment of Alzheimers disease	Fale *et al*., 2009
	water[lv]	prevents dental caries	Figueiredo *et al*., 2010
	70:30 ethanol:propylene glycol[rt]	used topically as a mixture for UV protection. Tested (in vitro) on human and guinea pig skin	Chen *et al*., 2009
P. eckonlii	aqueous[lv]	helps in treatment of Alzheimers disease	Fale *et al*., 2009
	waterlv	prevents dental caries	Figueiredo *et al*., 2010
P. fruticosus	aqueous[lv]	helps in treatment of Alzheimers disease	Fale *et al*., 2009
	essential oil (diethyl ether extract)	antifertility activity by inhibiting implantations, in rats	Chamorro *et al*., 1991
	essential oil[lv]	embryotoxic properties, in rats	Pages *et al*., 1988
P. grandis	leaves	possesses gastroprotective properties	Rodrigeus *et al*., 2010
P. lanuginosus	aqueous[lv]	helps in treatment of Alzheimers disease	Fale *et al*., 2009
**Plectranthus species*	hot water	increases blood vessel elasticity in stroke prone rats	Tetsuji *et al*., 2007
P. striatus	mixture	promotes function of gallbladder	Yaoliang *et al*., 2006

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Plectranthus species	Solvent of Extraction	Other Activities	Reference
P. ternifolius	mixture	treat liver function impairment	Guo et al., 2006
P. verticillatus	aqueouslv	helps in treatment of Alzheimers disease	Fale et al., 2009

NB. *Coleus* species are also included in this table because of the uncertain relationship to the *Plectranthus* species
* Unknown species
Key: lv: leaves, rt: roots, s: stems, a: aerial parts, w: whole plant, f: flowers

2.4 The phytochemistry of *Plectranthus*

The main phytochemical constituents of *Plectranthus* are diterpenoids, with about one-hundred and forty-seven of these compounds being isolated from the coloured leaf-glands of *Plectranthus* species, the majority of which were highly modified abietanoids. Essential oils, monoterpenoids, sesquitepenoids and phenolics have also been isolated from *Plectranthus* species (Abdel-Mogib *et al*., 2002; Lukhoba *et al.*, 2006).

Compounds **27** to **174** are grouped into five categories, the royleoanone-type abietanes (compounds **27** to **69**), the spirocoleons (compounds **70** to **108**), vinylogous quinones (compounds **109** to **130**), coleon-type abietanes (compounds **131** to **167**) and the miscellaneous abietanes (compounds **168** to **174**) which do not resemble any of the abietanes in the previous four categories.

Twenty royleanone type abietanes with the presence of a hydroxybenzoquinone or *p*-quinoid ring C system and an isopropyl group at C-13 have been isolated from eleven known *Plectranthus* species and three *Coleus* species (Table 6a). These compounds all have methyl groups at C-4 and C-10 with the exception of compound **42** where the methyl group at C-10 is oxidized to an alcohol. Compound **45** is notably different from compounds **27** to **46**, as the substituent at C-12 is an acetyl group. At C-6 and C-7 of compounds **27** to **42**, there are either hydroxy, aldehyde or acetyl groups attached to these positions, with the substituent at C-6 being in the beta position. In compounds **43** to **46**,

variation is observed within ring B with a double bond being present either at the Δ^5 or Δ^6 positions. Compound **46** has an epoxide ring in place of the olefinic bond at Δ^8.

Table 6a: Royleanone-type abietanes with an isopropyl side chain at C-13, isolated from *Plectranthus* and *Coleus* species

Compound	**Name**	**Synonym**	**Isolated from *Plectranthus (P)* or *Coleus (C)* species**	**Reference**
27	12-hydroxy-8,12-abietadiene-11,14-dione	royleanone	*P. grandidentatus; C. carnosus; *Plectranthus* species	Gaspar-Marques *et al*., 2006; Cerqueira *et al*., 2004; Yoshizaki *et al*., 1979; Hensch *et al*., 1975
28	7α,12-dihydroxy-8,12-abietadiene-11,14-dione	horminone	*P. hereroensis; P. grandidentatus; C. carnosus; *Plectranthus* species; *P. sanguineus*	Gaspar-Marques *et al*., 2006; Batista *et al*., 1994; Yoshizaki *et al*., 1979; Hensch *et al*., 1975; Matloubi-Moghadam *et al*., 1987
29	7β,12-dihydroxy-8,12-abietadiene-11,14-dione	taxoquinone	*Plectranthus* species	Hensch *et al*., 1975
30	7-formyl-12-hydroxy-8,12-abietadiene-11,14-dione	7-*O*-formylhorminone	*P. sanguineus*	Matloubi-Moghadam *et al*., 1987
31	7α-acetoxy-12-hydroxy-8,12-abietadiene-11,14-dione	7α-acetoxyroyleanone	*C. carnosus*	Yoshizaki *et al*., 1979
32	6β,12-dihydroxy-8,12-abietadiene-11,14-dione	6β-hydroxyroyleanone	*P. grandidentatus; C. carnosus; P. sanguineus*	Gaspar-Marques *et al*., 2006; Yoshizaki *et al*., 1979; Matloubi-Moghadam *et al*., 1987

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Compound	Name	Synonym	Isolated from *Plectranthus (P)* or *Coleus (C)* species	Reference
33	6β,7α,12-trihydroxy-8,12-abietadiene-11,14-dione	6β,7α-dihydroxyroyleanone	*P. grandidentatus; C. carnosus; P. argentatus; *Plectranthus* species; P. sanguineus; P. edulis; P. hadiensis; P. fasciculatus*	Gaspar-Marques *et al*., 2006; Gaspar-Marques *et al*., 2002; Cerqueira *et al*., 2004; Yoshizaki *et al*., 1979; Alder *et al*., 1984a; Hensch *et al*., 1975; Matloubi-Moghadam *et al*., 1987; Kunzle *et al*., 1987; Laing *et al*., 2006; Rasikari, 2007
34	6β,7β,12-trihydroxy-8,12-abietadiene-11,14-dione	6β,7β-dihydroxyroyleanone	*C. zeylanicus*	Mehrota *et al*., 1989
35	7α-acyloxy-6β,12-dihydroxy-8,12-abietadiene-11,14-dione	7α-acyloxy-6β-hydroxyroyleanone	*P. grandidentatus*	Cerqueira *et al*., 2004; Gaspar-Marques *et al*., 2002
36	7α-acetoxy-6β,12-dihydroxy-8,12-abietadiene-11,14-dione	7α-acetoxy-6β-hydroxyroyleanone	*P. grandidentatus; C. carnosus; P. argentatus; *Plectranthus species; C. zeylanicus; P. sanguineus; P. hadiensis; P. actites*	Teixera *et al*., 1997; Gaspar-Marques *et al*., 2006; Gaspar-Marques *et al*., 2002; Cerqueira *et al*., 2004; Yoshizaki *et al*., 1979; Alder *et al*., 1984a; Hensch *et al*., 1975; Mehrotra *et al*., 1989; Matloubi-Moghadam *et al*., 1987; van Zyl *et al*., 2008; Rasikari, 2007
37	7β-acetoxy-6β,12-dihydroxy-8,12-abietadiene-11,14-dione	7β-acetoxy-6β-hydroxyroyleanone	*C. zeylanicus*	Mehrotra *et al*., 1989
38	7α-formyloxy-6β,12-dihydroxy-8,12-abietadiene-11,14-dione	7α-formyloxy-6β-hydroxyroyleanone	*P. myrianthus; P. argentatus; P. hadiensis; P. sanguineus*	Miyase *et al*., 1977a; Alder *et al*., 1984a; van Zyl *et al*., 2008; Matloubi-Moghadam *et al*., 1987

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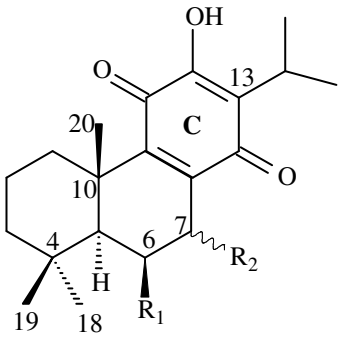
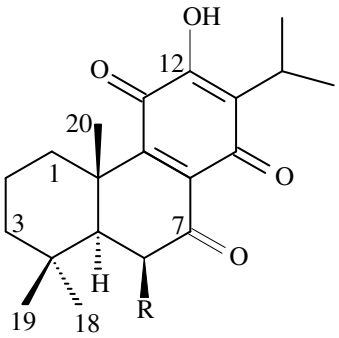
Compound	Name	Synonym	Isolated from *Plectranthus (P)* or *Coleus (C)* species	Reference
39	6β-formyloxy-7α,12-dihydroxy-8,12-abietadiene-11,14-dione	6β-formyloxy-7α-hydroxyroyleanone	*P. argentatus*	Alder *et al*., 1984a
40	12-hydroxy-8,12-abietadiene-7,11,14-trione	7-oxoroyleanone	**Plectranthus species*	Hensch *et al*., 1975
41	6β,12-dihydroxy-8,12-abietadiene-7,11,14-trione	5,6-dihydrocoleon U	*P. sanguineus*	Matloubi-Moghadam *et al*., 1987
42	7α-acetoxy-6β,12,20-trihydroxy-8,12-abietadiene-11,14-dione	7α-acetoxy-6β,20-dihydroxyroyleanone	*C. carnosus*	Yoshizaki *et al*., 1979
43	12-hydroxy-6,8,12-abietatriene-11,14-dione	6,7-dehydroroyleanone	*P. grandidentatus;* *C. carnosus;* **Plectranthus species;* *P. graveolens*	Gaspar-Marques *et al*., 2006; Yoshizaki *et al*., 1979; Hensch *et al*., 1975; Rasikari, 2007
44	6,12-dihydroxy-5,8,12-abietatriene-7,11,14-trione	Coleon U quinone	*P. forsteri 'marginatus';* *C. xanthanthus;* *P. argentatus;* *P. sanguineus*	Wellsow *et al*., 2006; Mei *et al*., 2002; Alder *et al*., 1984a; Matloubi-Moghadam *et al*., 1987
45	12,16-diacetoxy-6-hydroxy-5,8,12-abietatriene-7,11,14-trione	Xanthanthusin H	*C. xanthanthus*	Mei *et al*. 2002
46	8α,9α-epoxy-6,12-dihydroxy-5,12-abietadiene-7,11,14-trione	8α,9α-epoxycoleon U quinone	*C. xanthanthus;* *P. argentatus;* *P. sanguineus*	Mei *et al*. 2002; Alder *et al*., 1984a; Matloubi-Moghadam *et al*., 1987

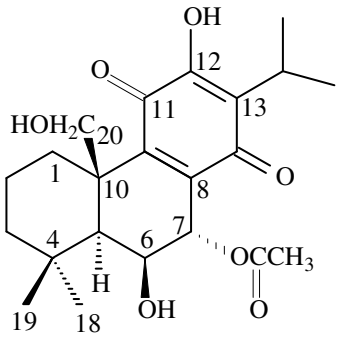
* unknown species



	R_1	R_2
(27)	H	H
(28)	H	α-OH
(29)	H	β-OH
(30)	H	α–OCH(O)
(31)	H	α-$OC(O)CH_3$
(32)	OH	H
(33)	OH	α-OH
(34)	OH	β-OH
(35)	OH	Fatty acid ester
(36)	OH	α-$OC(O)CH_3$
(37)	OH	β-$OC(O)CH_3$
(38)	OH	α-OCH(O)
(39)	OCH(O)	α-OH



	R
(40)	H
(41)	OH



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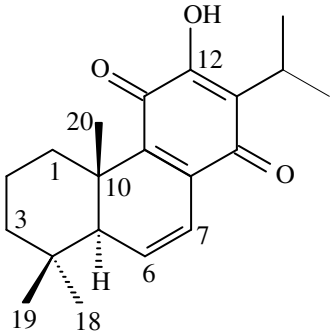

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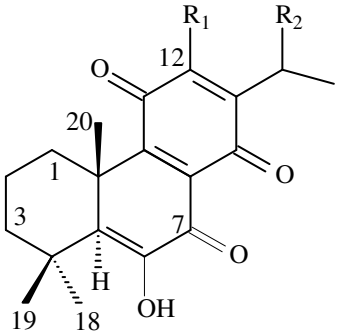
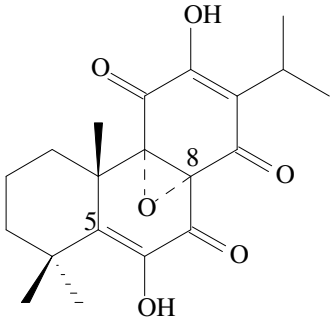


	R_1	R_2
(44)	OH	CH_3
(45)	$OC(O)CH_3$	$CH_2OC(O)CH_3$



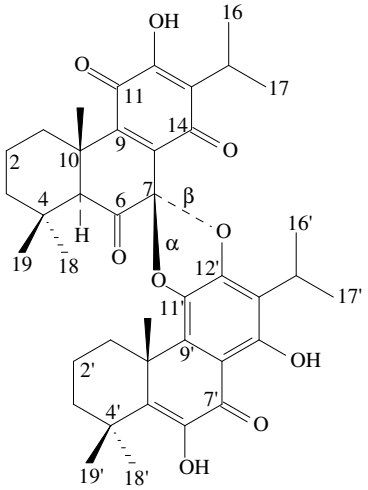
(46)

Seven compounds **47** to **53** consisting of two abietane compounds joined either by oxygen or directly by carbon atoms from C-7 in one abietane to C-11' and C-12' (as in compound **47**) or to C-7' and C-14' (as in compound **51**) in the other have also been isolated from *Plectranthus* species. These have been isolated from *P. grandidentatus*, *P. sanguineus*, *P. myrinathus* and *C. carnosus* with *P. grandidentatus* yielding all seven

compounds (Table 6b). Depending on the orientation at C-7 and how this carbon atom joins to the next abietane, isomers result as in compounds **47** and **48**; **49** and **50;** and **51** and **52**. Compound **53** is a dimer joined at C-7 of both compounds. These compounds are difficult to name systematically and therefore only the common names are given in Table 6b.

Table 6b: Royleanone-type abietane dimers isolated from *Plectranthus* and *Coleus* species

Compound	**Common name**	**Isolated from *Plectranthus (P)* or *Coleus (C)* species**	**Reference**
47	Grandidone A	*P. grandidentatus; P. sanguineus*	Cerqueira *et al*., 2004; Gaspar-Marques *et al*., 2002
48	7-epigrandidone A	*P. grandidentatus; P. myrianthus; C. carnosus; P. sanguineus*	Uchida *et al*., 1981; Matloubi-Moghadam *et al*., 1987
49	Grandidone B		
50	7-epigrandidone B		
51	Grandidone D	*P. grandidentatus; P. myrianthus; C. carnosus*	Uchida *et al*., 1981
52	7-epigrandidone D		
53	Grandidone C		



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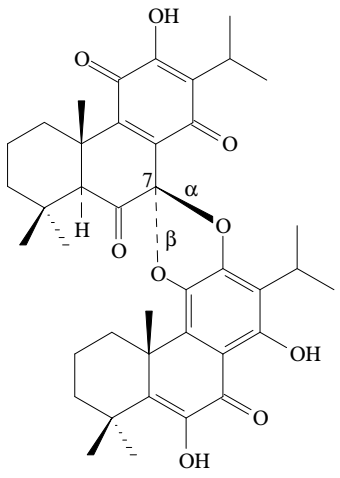

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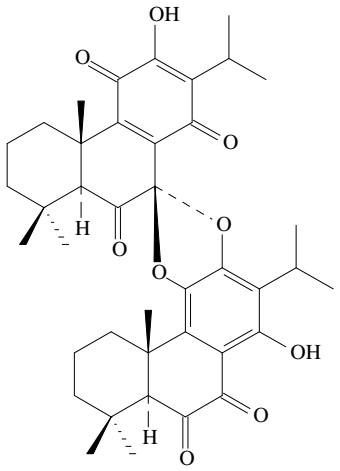

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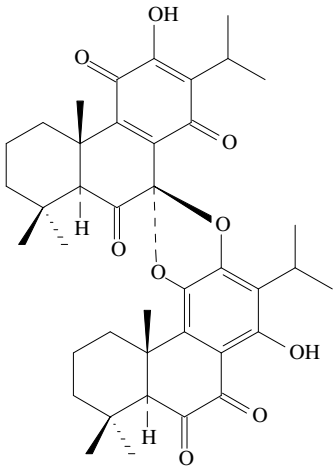

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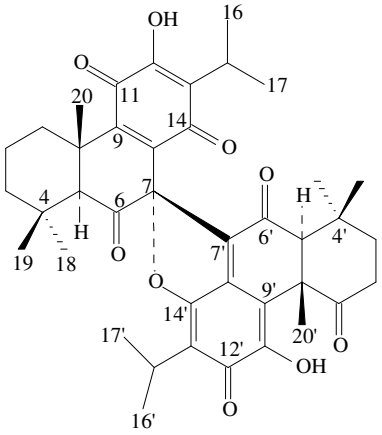

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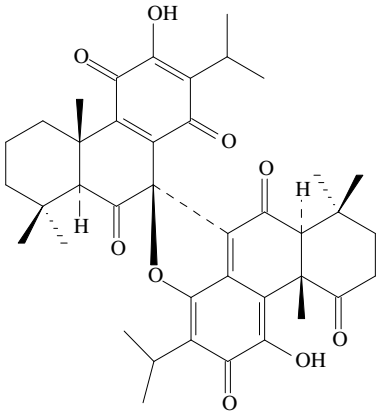

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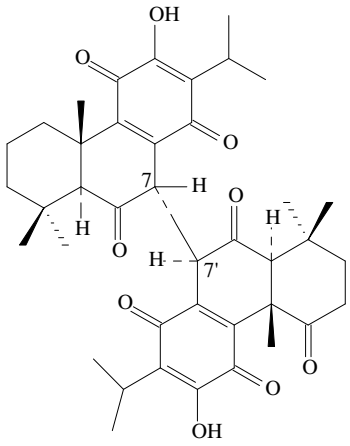

(53)

Seventeen compounds with linear side-chains at C-13 instead of the isopropyl group, compounds **54** to **69** were isolated from *P. hereroensis*, *P. edulis*, *P. lanuginosus*, *P. sanguineus* and *C. coerulescens* (Table 6c). *P. lanuginosus* yielded the highest number of compounds compared to the other species which yielded less than five compounds. The side chains are either saturated as in **54**, contain a double bond as in compounds **55**-**63**, an acetyl group as in compound **64** or an alcohol as in compounds **65**, **68** and **69**. Compounds **57** and **61** and **65**-**69** have functional groups at C-3 with **66**-**69** being *abeo*-abietanes where a methyl from C-4 has migrated to C-3.

Table 6c: Royleanone-type abietanes with a linear side chain at C-13 isolated from *Plectranthus* and *Coleus* species

Compound	Name	Synonym	Isolated from *Plectranthus (P)* or *Coleus (C)* species	Reference
54	16-acetoxy-7α,12-dihydroxy-8,12-abietadiene-11,14-dione		*P. hereroensis*	Gaspar-Marques *et al*., 2006; Batista *et al*., 1995
55	7α,12-dihydroxy-17(15→16)-*abeo*abieta-8,12,16-triene-11,14-dione		*P. hereroensis*	Gaspar-Marques *et al*., 2006; Batista *et al*., 1994
56	6β,7α,12-trihydroxy-17(15→16)-*abeo*abieta-8,12,16-triene-11,14-dione	6β,7α-dihydroxy(allyl) royleanone	*P. sanguineus*	Matloubi-Moghadam *et al*., 1987
57	3α-formyloxy-6β,7α,12-trihydroxy-17(15→16)-*abeo*abieta-8,12,16-triene-11,14-dione		*P. edulis*	Kunzle *et al*., 1987
58	19-formyloxy-6β,7α,12-trihydroxy-17(15→16)-*abeo*abieta-8,12,16-triene-11,14-dione	Lanugone D	*P. lanuginosus*	Schmid *et al*., 1982
59	7α-ethoxy-6β,12,19-trihydroxy-17(15→16)-*abeo*abieta-8,12,16-triene-11,14-dione	Lanugone E		
60	12-hydroxy-17(15→16)-*abeo*abieta-6,8,12,16-tetraene-11,14-dione	Lanugone A	*P. edulis; P. lanuginosus*	Kunzle *et al*., 1987; Schmid *et al*., 1982
61	3α-formyloxy-12-hydroxy-17(15→16)-*abeo*abieta-6,8,12,16-tetraene-11,14-dione		*P. edulis*	Kunzle *et al*., 1987
62	12,19-dihydroxy-17(15→16)-*abeo*abieta-6,8,12,16-tetraene-11,14-dione	Lanugone B	P. lanuginosus	Schmid *et al*., 1982
63	12-hydroxy-19-formyloxy-17(15→16)-*abeo*abieta-6,8,12,16-tetraene-11,14-dione	Lanugone C		
64	16-acetoxy-7α,12-dihydroxy-8,12-abietadiene-11,14-dione		*P. hereroensis*	Batista *et al*., 1995

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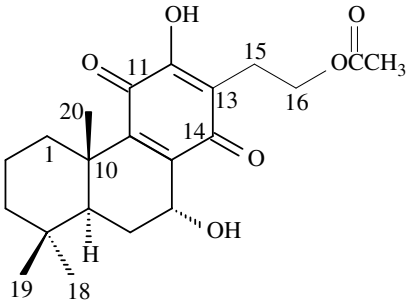
Compound	Name	Synonym	Isolated from *Plectranthus (P)* or *Coleus (C)* species	Reference
65	17(15→16)*abeo*-3α,18-diacetoxy-6β,7α,12,16-tetrahydroxy-8,12-abietadiene-11,14-dione		*C. coerulescens*	Grob *et al*., 1978
66	3β-acetoxy-6β,7α,12-trihydroxy-17(15→16),18(4→3)-*bisabeo*-abieta-4(18),8,12,16-tetraene-11,14-dione		*P. grandidentatus*	Gaspar-Marques *et al*., 2006
67	17(15→16),19α(4→3)-*bisabeo*-6β,7α,12-trihydroxy-4(18),8,12,16-abietatetraene-11,14-dione		*P. edulis*	Kunzle *et al*., 1987
68	17(15→16),19β(4→3)-*bisabeo*-6β,7α,12,16-tetrahydroxy-4(18),8,12-abietatriene-11,14-dione	17(15→16),19(4→3)-*bisabeo*-6β,7α,16-trihydroxyroyleanone	*C. coerulescens*	Grob *et al*., 1978
69	17(15→16),19β(4→3)-*bisabeo*-6β,12,16-trihydroxy-7α-methoxy-4(18),8,12-abietatriene-11,14-dione	17(15→16),19(4→3)-*bisabeo*-6β,16-dihydroxy-7α-methoxyroyleanone		



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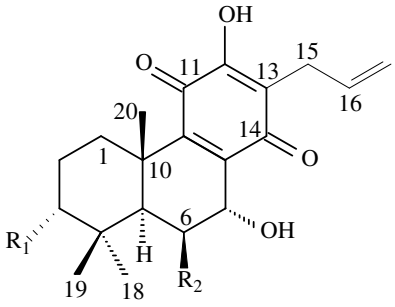
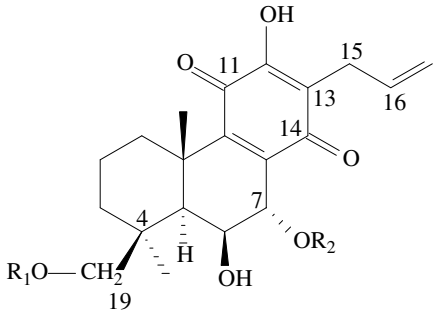
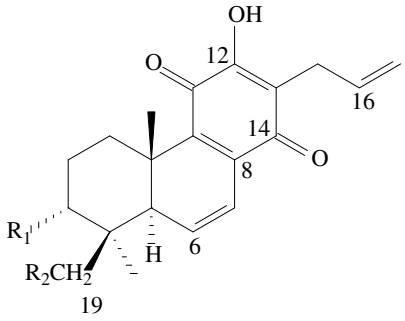


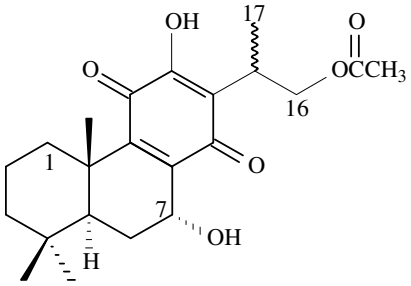
	R_1	R_2
(55)	H	H
(56)	H	OH
(57)	OCH(O)	OH



	R_1	R_2
(58)	CH(O)	H
(59)	H	C_2H_5



	R_1	R_2
(60)	H	H
(61)	OCH(O)	H
(62)	H	OH
(63)	H	OCH(O)



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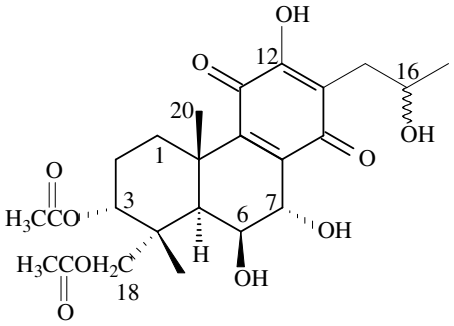

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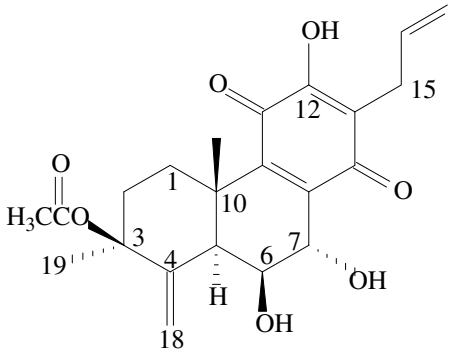

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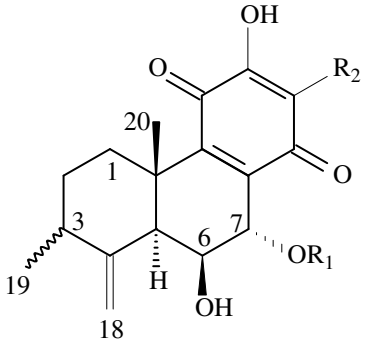

	R_1	R_2
(67)	H	$CH_2CH{=}CH_2$
(68)	H	$CH_2CH(OH)CH_3$
(69)	CH_3	$CH_2CH(OH)CH_3$

Thirty-eight compounds **70**-**108**, commonly referred to as spirocoleons were isolated from *P. edulis* (eleven compounds), *C. garckeanus* (nine compounds), *P. lanuginosus* and *C. coerulescens* (seven compounds), *C. barbatus* (three compounds), *P. grandis* and *C. somaliensis* (two compounds) and *P. barbatus* (one compound) (Table 7). These compounds are refered to as spirocoleons as C-13 is the common carbon atom which links the cyclopropyl ring to the coleon-type abietane. The cyclopropyl bridge is represented as being either in the α or β position with the methyl group at C-15 being oriented such that the molecule is either *R* or *S* with respect to C-15. In compounds **71**-**82**, R_1 to R_4 have either hydrogen, acetyl or formyloxy groups. The difference between **72** and **83** is the orientation/stereochemistry of the methyl group at C-15. Oxidised

functional groups occur at C-6 and C-7, with C-6 being predominantly hydroxyl. Compound **100** is the only compound which has an acetyl group present at C-17.

Table 7: Spirocoleons isolated from *Plectranthus* and *Coleus* species

Compound	**Name**	**Synonym**	**Isolated from *Plectranthus (P)* or *Coleus (C)* species**	**Reference**
70	*cannot be conventionally named due to the linkage between C-1 and C-11	Barbatusin	*C. barbatus; P. grandis*	Zelnik *et al*., 1977; Rodrigues *et al*., 2010
71	(15*S*)-6β,7α,12α-trihydroxy-13β,16-cycloabieta-8-ene-11,14-dione	Lanugone F	*P. lanuginosus*	Schmid *et al*., 1982; Kunzle *et al*., 1987
72	(15*S*)-7α-formyloxy-6β,12α-dihydroxy-13β,16-cycloabieta-8-ene-11,14-dione	Lanugone G		
73	(15*S*)-7α-formyloxy-6β,12α,19-trihydroxy-13β,16-cycloabieta-8-ene-11,14-dione	Lanugone H	*P. lanuginosus*	Schmid *et al*., 1982
74	(15*S*)-19-formyloxy-6β,7α,12α-trihydroxy-13β,16-cycloabieta-8-ene-11,14-dione	Lanugone I		
75	(15*S*)-7α,19-bis(formyloxy)-6β,12α-dihydroxy-13β,16-cycloabieta-8-ene-11,14-dione	Lanugone J		
76	(15*S*)-3α-formyloxy-6β,7α,12α-trihydroxy-13β,16-cycloabieta-8-ene-11,14-dione		*P. edulis*	Kunzle *et al*., 1987
77	(15*S*)-3α,7α-bis(formyloxy)-6β,12α-dihydroxy-13β,16-cycloabieta-8-ene-11,14-dione			
78	(15*S*)-3α-formyloxy-19-acetoxy-6β,7α,12α-trihydroxy-13β,16-cycloabieta-8-ene-11,14-dione	3-*O*-desacetyl-3-*O*-formyl coleon Y	*C. coerulescens*	Grob *et al*., 1978
79	(15*S*)-3α-formyloxy-6β-acetoxy-7α,12α-dihydroxy-13β,16-cycloabieta-8-ene-11,14-dione		*P. edulis*	Kunzle *et al*., 1987
80	(15*S*)-3α-acetoxy-6β,7α,12α-trihydroxy-13β,16-cycloabieta-8-ene-11,14-dione	6,12-bis(*O*-desacetyl) coleon R	*C. coerulescens*	Grob *et al*., 1978

Continued on next page….

Compound	Name	Synonym	Isolated from *Plectranthus (P)* or *Coleus (C)* species	Reference
81	(15*S*)-3α,6β-diacetoxy-7α,12α-dihydroxy-13β,16-cycloabieta-8-ene-11,14-dione	12-*O*-desacetyl coleon R	*C. coerulescens*	Grob *et al*., 1978
82	(15*S*)-3α,19-diacetoxy-6β,7α,12α-trihydroxy-13β,16-cycloabieta-8-ene-11,14-dione	Coleon Y		
83	(15*R*)-7α-formyloxy-6β,12α-dihydroxy-13β,16-cycloabieta-8-ene-11,14-dione	Lanugone K'	*P. lanuginosus*	Schmid *et al*., 1982
84	(15*R*)-7α-acetoxy-6β,12α-dihydroxy-13β,16-cycloabieta-8-ene-11,14-dione	Lanugone K		
85	(15*R*)-3α,7α-bis(formyloxy)-6β,12α-dihydroxy-13β,16-cycloabieta-8-ene-11,14-dione		*P. edulis*	Kunzle *et al*., 1987
86	(15*R*)-3α-formyloxy-6β,12α-diacetoxy-7α-hydroxy-13β,16-cycloabieta-8-ene-11,14-dione			
87	(15*S*)-6β,12α-acetoxy-7α-hydroxy-13β,16-cycloabieta-8-ene-3,11,14-trione	Barbatusin	*C. barbatus; P. grandis*	Zelnik *et al*., 1977; Rodrigues *et al*., 2010
88	(15*S*)-6β,12α-acetoxy-3,7α-dihydroxy-13β,16-cycloabieta-8-ene-11,14-dione	3β-hydroxy-3-deoxybarbatusin	*P. grandis*	Rodrigues *et al*., 2010
89	(15*S*)-19(4→3)-*abeo*-7α-acetoxy-6β,12α-dihydroxy-13β,16-cycloabieta-3,8-diene-11,14-dione	Coleon O	*C. coerulescens*	Grob *et al*., 1978
90	(15*R*)-19(4→3)-*abeo*-6β-acetoxy-7α,12α-dihydroxy-13β,16-cycloabieta-3,8-diene-2,11,14-trione	Plectrin	*P. barbatus*	Kubo *et al*., 1984
91	(15*S*)-19α(4→3)-*abeo*-6β,7α,12α-trihydroxy-13β,16-cycloabieta-4(18),8-diene-11,14-dione	Coleon J	*C. somaliensis; P. edulis*	Moir *et al*., 1973b; Kunzle *et al*., 1987
92	(15*S*)-19β(4→3)-*abeo*-6β,7α,12α-trihydroxy-13β,16-cycloabieta-4(18),8-diene-11,14-dione	7,12-bis(*O*-desacetyl) coleon N	*C. coerulescens*	Grob *et al*., 1978

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Compound	Name	Synonym	Isolated from *Plectranthus (P)* or *Coleus (C)* species	Reference
93	(15*S*)-19α(4→3)-*abeo*-7α-acetoxy-6β,12α-dihydroxy-13β,16-cycloabieta-4(18),8-diene-11,14-dione	Coleon G	*C. somaliensis; P. edulis*	Moir *et al*., 1973b; Kunzle *et al*., 1987
94	(15*S*)-19β(4→3)-*abeo*-7α-acetoxy-6β,12α-dihydroxy-13β,16-cycloabieta-4(18),8-diene-11,14-dione	12-*O*-desacetylcoleon N	*C. coerulescens*	Grob *et al*., 1978
95	(15*S*)-19α(4→3)-*abeo*-12α-acetoxy-6β,7α-dihydroxy-13β,16-cycloabieta-4(18),8-diene-11,14-dione		*P. edulis*	Kunzle *et al*., 1987
96	(15*S*)-19α(4→3)-*abeo*-7α-formyloxy-6β,12α-dihydroxy-13β,16-cycloabieta-4(18),8-diene-11,14-dione			
97	(15*S*)-19α(4→3)-*abeo*-7α-formyloxy-12α-acetoxy-6β-hydroxy-13β,16-cycloabieta-4(18),8-diene-11,14-dione		*P. edulis*	Kunzle *et al*., 1987
98	(15*S*)-19α(4→3)-*abeo*-7α,12α-diacetoxy-6β-hydroxy-13β,16-cycloabieta-4(18),8-diene-11,14-dione			
99	(15*S*)-3α,7α,19-triacetoxy-6β,12β-dihydroxy-13α,16-cycloabieta-8-ene-11,14-dione	12-O-desacetyl-7-O-acetyl-3β,19-diacetyloxy-coleon Q	*C. garckeanus*	Miyase *et al*., 1979
100	(15*S*)-6β,17-diacetoxy-7α,12β-dihydroxy-13α,16-cycloabieta-8-ene-11,14-dione	12-O-desacetyl-6-O-acetyl-17-acetyloxy coleon P		
101	(15*S*)-6β,12α,19-triacetoxy-7α-hydroxy-13α,16-cycloabieta-8-ene-11,14-dione	6-O-acetyl-19-acetyloxy-coleon Q		
102	(15*S*)-7α,19-diacetoxy-6β,12α-dihydroxy-13α,16-cycloabieta-8-ene-11,14-dione	12-O-desacetyl-7-O-acetyl-19-acetyloxy-coleon Q		
103	(15*S*)-7α,19-diacetoxy-6β,12β-dihydroxy-13α,16-cycloabieta-8-ene-11,14-dione	12-O-desacety-7-O-acetyl-19-acetyloxy-coleon P		

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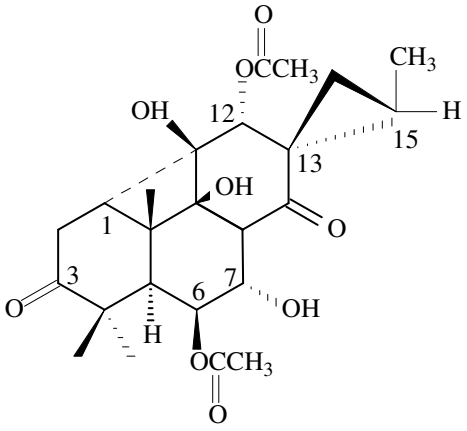
Compound	Name	Synonym	Isolated from *Plectranthus (P)* or *Coleus (C)* species	Reference
104	(15*S*)-19(4→3)-*abeo*-7α-acetoxy-6β,12α-dihydroxy-13α,16-cycloabieta-3,8-diene-11,14-dione	Coleon O	*C. garckeanus*	Miyase *et al*., 1979
105	(15*S*)-19(4→3)-*abeo*-7α,19-diacetoxy-6β,12α-dihydroxy-13α,16-cycloabieta-3,8-diene-11,14-dione	19-acetyloxy coleon O	*C. garckeanus*	Miyase *et al*., 1979
106	(15*S*)-19β(4→3)-*abeo*-3α-acetoxy-6β,7α,12α-trihydroxy-13α,16-cycloabieta-4(18),8-diene-11,14-dione	7-desoxy-12-O-desacetyl-3-acetyloxy coleon N		
107	(15*S*)-19β(4→3)-*abeo*-7α,3α-diacetoxy-6β,12α-dihydroxy-13α,16-cycloabieta-4(18),8-diene-11,14-dione		*P. edulis*	Kunzle *et al*., 1987
108	(15*S*)-19(4→3)-*abeo*-7α-acetoxy-6β,12α-dihydroxy-13α,16-cycloabieta-3(19),4(18),8-triene-11,14-dione	Coleon Z	*C. garckeanus; P. edulis*	Miyase *et al*., 1979; Kunzle *et al*., 1987



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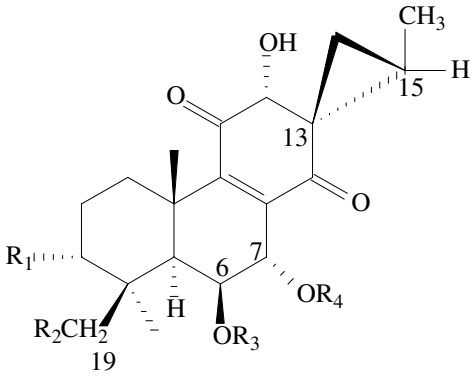
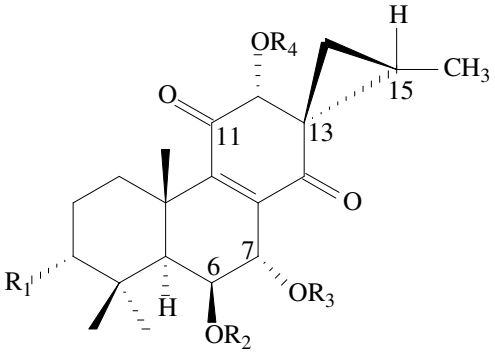
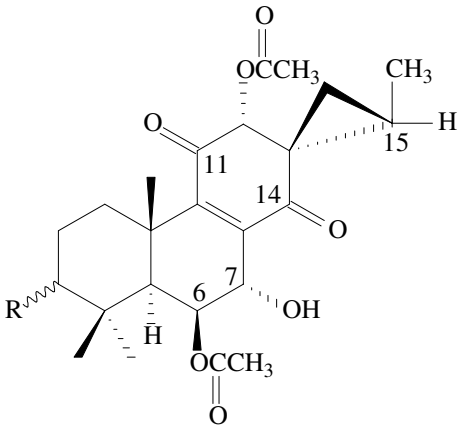


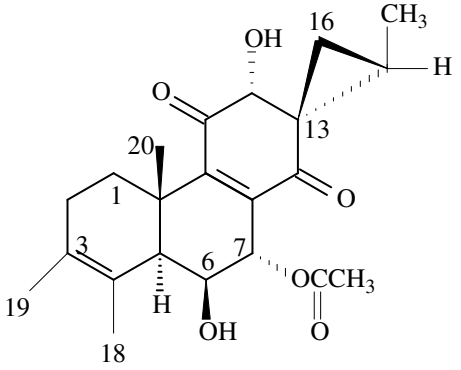
	R_1	R_2	R_3	R_4
(71)	H	H	H	H
(72)	H	H	H	CH(O)
(73)	H	OH	H	CH(O)
(74)	H	OCH(O)	H	H
(75)	H	OCH(O)	H	CH(O)
(76)	OCH(O)	H	H	H
(77)	OCH(O)	H	H	CH(O)
(78)	OCH(O)	$OC(O)CH_3$	H	H
(79)	OCH(O)	H	$C(O)CH_3$	H
(80)	$OC(O)CH_3$	H	H	H
(81)	$OC(O)CH_3$	H	$C(O)CH_3$	H
(82)	$OC(O)CH_3$	$OC(O)CH_3$	H	H



	R_1	R_2	R_3	R_4
(83)	H	H	CH(O)	H
(84)	H	H	$C(O)CH_3$	H
(85)	OCH(O)	H	CH(O)	H
(86)	OCH(O)	$C(O)CH_3$	H	$C(O)CH_3$



	R
(87)	=O
(88)	β-OH



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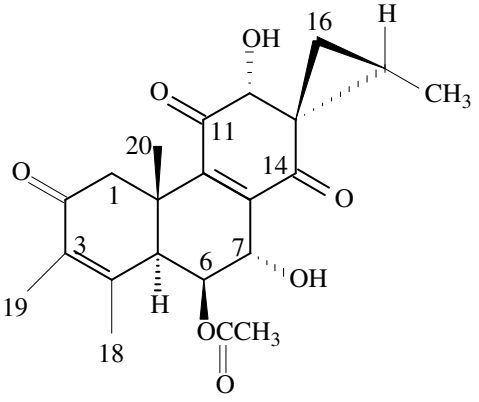

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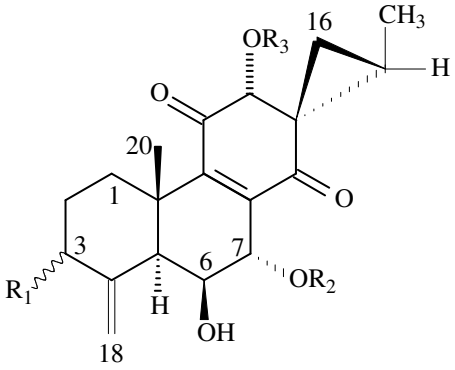
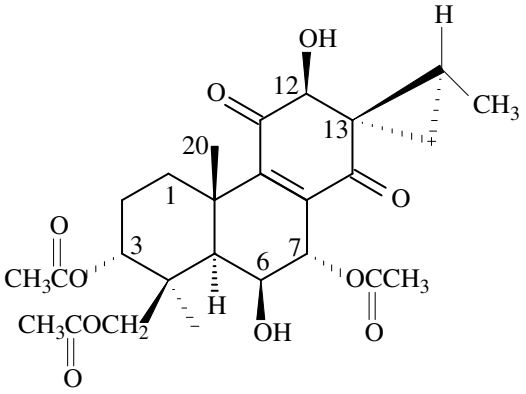


	R_1	R_2	R_3
(91)	α-CH_3	H	H
(92)	β-CH_3	H	H
(93)	α-CH_3	C(O)CH_3	H
(94)	β-CH_3	C(O)CH_3	H
(95)	α–CH_3	H	C(O)CH_3
(96)	α–CH_3	CH(O)	H
(97)	α–CH_3	CH(O)	C(O)CH_3
(98)	α–CH_3	C(O)CH_3	C(O)CH_3



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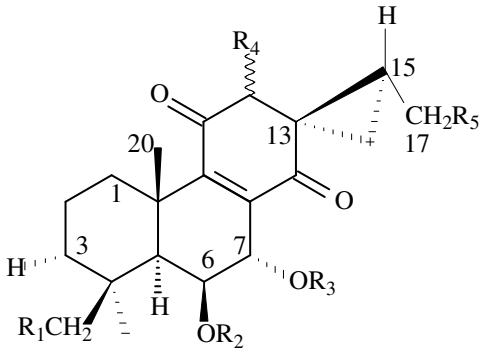
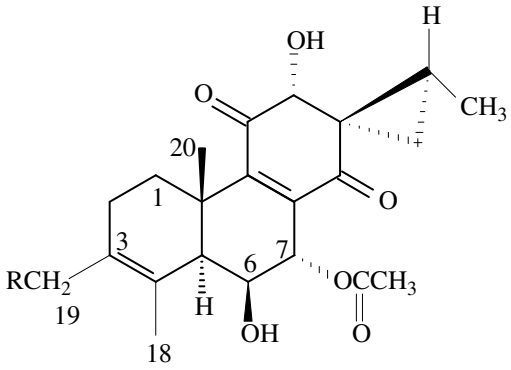
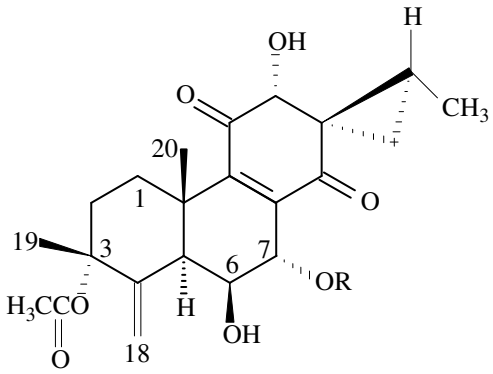


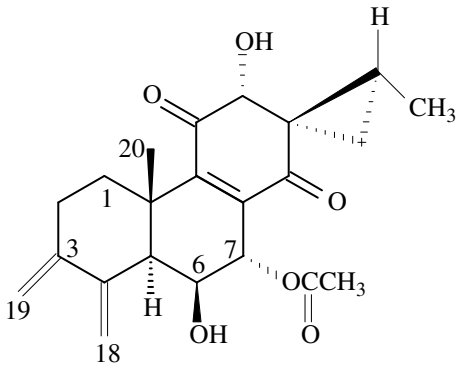
	R_1	R_2	R_3	R_4	R_5
(100)	H	$C(O)CH_3$	H	β-OH	$OC(O)CH_3$
(101)	$OC(O)CH_3$	$C(O)CH_3$	H	α-$OC(O)CH_3$	H
(102)	$OC(O)CH_3$	H	$C(O)CH_3$	α-OH	H
(103)	$OC(O)CH_3$	H	$C(O)CH_3$	β-OH	H



	R
(104)	H
(105)	$OC(O)CH_3$



	R
(106)	H
(107)	$C(O)CH_3$



(108)

Twenty-two quinone methides, **109-130** were isolated from ten *Plectranthus* species (Table 8). Eight compounds were isolated from *P. strigosus* and six from *P. lanuginosus* and *P. parviflorus* while the other species yielded less than four compounds each. Compounds **109-120** closely resemble benzoquinones, but there is only one carbonyl present in ring C, located at C-12 instead of C-11 and C-14 and has double bonds at Δ^7, C-9(11) and Δ^{13}. Compounds **121-130** have an additional double bond in ring B at Δ^5.

Yet again variation of the isopropyl group at C-13 is observed in compounds **109-117**. In compound **118** the isopropyl group has cyclised onto ring C, resulting in a partially

reduced furan ring. The linear side chains which have replaced the isopropyl group are either saturated as in **112,** oxidized to a double bond as in compound **116** or contain methoxy groups (**114)** and hydroxy groups (**111**, **112**, **115**, **117**, **119-122)**. The tertiary methyl group usually present at C-19, has been oxidized to a formyloxy (-OCHO) group in compounds **114** and **117**. In abietanes **126-130** the methyl group at position 19 has been modified to include either an aromatic group as in **126-129** or a prenylated group as in **130**. Compound **122** has an acetyl group present at C-3 as well as a hydroxy group at C-15 while compounds **123-125** have aromatic substituents at C-2.

Table 8: Vinylogous quinones isolated from *Plectranthus* species

Compound	**Name**	**Synonym**	**Isolated from *Plectranthus (P)* species**	**Reference**
109	11-hydroxy-7,9(11),13-abietatriene-12-one		*P. elegans*	Dellar *et al*., 1996
110	11,14-dihydroxy-7,9(11),13-abietatriene-6,12-dione	14-hydroxytaxodione	*P. grandidentatus*	Uchida *et al*., 1981
111	11,14,16-trihydroxy-7,9(11),13-abietatriene-6,12-dione	Lanugone O	*P. lanuginosus; P. edulis*	Schmid *et al*., 1982; Kunzle *et al*., 1987
112	11,14,16-trihydroxy-17(15→16)-*abeo*abieta-7,9(11),13-triene-6,12-dione		*P. edulis*	Kunzle *et al*., 1987
113	16-acetoxy-11,14-dihydroxy-17(15→16)-*abeo*abieta-7,9(11),13-triene-6,12-dione			
114	19-formyloxy-6β,11,14-trihydroxy-16-methoxy-17(15→16)-*abeo*abieta-7,9(11),13-triene-12-one	Lanugone N		
115	6β,19-epoxy-11,14-dihydroxy-13-(2-hydroxypropyl)-7,9(11),13-abietatriene-12-one	Lanugone L		
116	13-allyl-6β,19-epoxy-11,14-dihydroxy- 7,9(11),13-abietatriene-12-one	Lanugone M		

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Compound	Name	Synonym	Isolated from *Plectranthus (P)* species	Reference
117	19-formyloxy-11,14,16-trihydroxy-17(15→16)-*abeo*abieta-7,9(11),13-triene-6,12-dione	Lanugone P	*P. edulis*	Kunzle *et al*., 1987
118	(15S)-14,16-epoxy-11-hydroxy-7,9(11),13-abietatriene-6,12-dione	Lanugone Q		
119	*Too complex to name systematically	Nilgherron A	*P. nilgherricus*	Miyase *et al*., 1977b
120	*Too complex to name systematically	Nilgherron B		
121	11,15-dihydroxy-5,7,9(11),13-abietatetraene-12-one	Fuerstione		
122	3β-acetoxy-11,15-dihydroxy-5,7,9(11),13-abietatetraene-12-one	3β-Acetoxyfuerstione		
123	2-(3-hydroxybenzoyl)-11-hydroxy-5,7,9(11),13-abietatetraene-12-one	Parvifloron D	*P. parviflorus; P. strigosus; P. eckonlii; P. lucidus*	Ruedi *et al*., 1978; Alder *et al*., 1984b; van Zyl *et al*., 2008; Nyila *et al*., 2009
124	2-(3,4-dihydroxybenzoyl)-11-hydroxy-5,7,9(11),13-abietatetraene-12-one;	Parviflorone F	*P. nummularius; P. parviflorus; P. strigosus; P. eckonlii*	Narukawa *et al*. 2001; Ruedi *et al*., 1978; Alder *et al*., 1984b; van Zyl *et al*., 2008; Nyila *et al*., 2009
125	2-(4-hydroxy-3-methoxybenzoyl)-11-hydroxy-5,7,9(11),13-abietatetraene-12-one	Parviflorone G	*P. strigosus*	Alder *et al*., 1984b
126	19-(3-methyl-2-butenoyl)-11-hydroxy-5,7,9(11),13-abietatetraene-12-one	Parviflorone A	*P. parviflorus; P. strigosus; P. purpuratus subsp. purpuratus; P. lucidus*	Ruedi *et al*., 1978; Alder *et al*., 1984b; van Zyl *et al*., 2008
127	19-(4-hydroxybenzoyl)-11-hydroxy-5,7,9(11),13-abietatetraene-12-one	Parviflorone C	*P. parviflorus; P. strigosus; P. purpuratus subsp. tongaensis*	Ruedi *et al*., 1978

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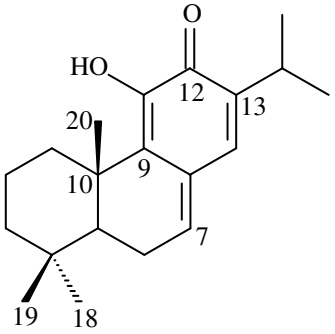
Compound	Name	Synonym	Isolated from *Plectranthus (P)* species	Reference
128	19-(3,4-dihydroxybenzoyl)-11-hydroxy-5,7,9(11),13-abietatetraene-12-one	Parviflorone E	*P. nummularius; P. parviflorus; P. strigosus; P. purpuratus subsp. tongaensis*	Narukawa *et al*. 2001; Ruedi *et al*., 1978; Alder *et al*., 1984b; van Zyl *et al*., 2008
129	19-(4-hydroxy-3-methoxybenzoyl)-11-hydroxy-5,7,9(11),13-abietatetraene-12-one	Parviflorone B	*P. parviflorus; P. strigosus*	Ruedi *et al*., 1978; Alder *et al*., 1984b;
130	19-(3-methyl-2-butenoyl)-2,11-dihydroxy-5,7,9(11),13-abietatetraene-12-one	Parviflorone H	*P. strigosus*	Alder *et al*., 1984b



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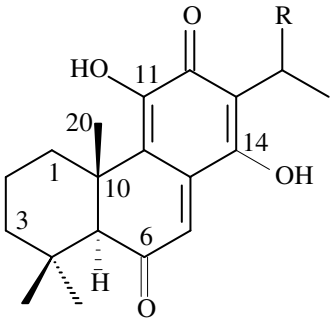
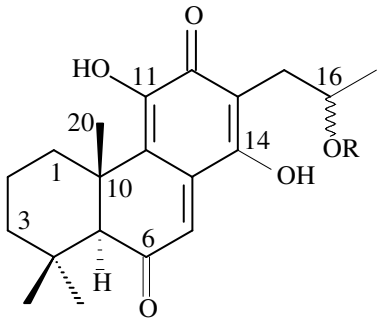
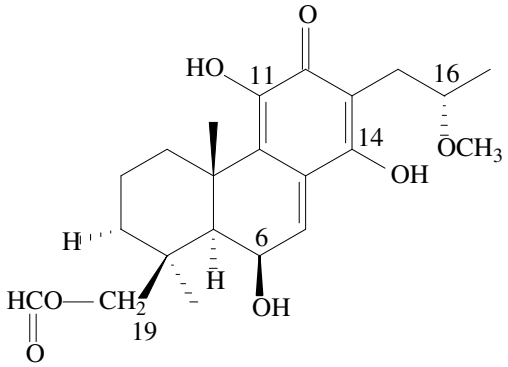


	R
(110)	CH_3
(111)	$CH_2(OH)$



	R
(112)	H
(113)	$C(O)CH_3$



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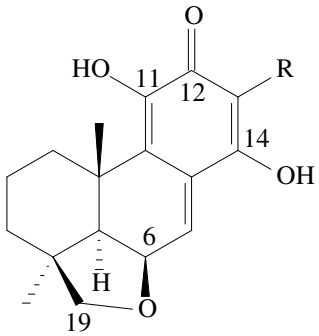
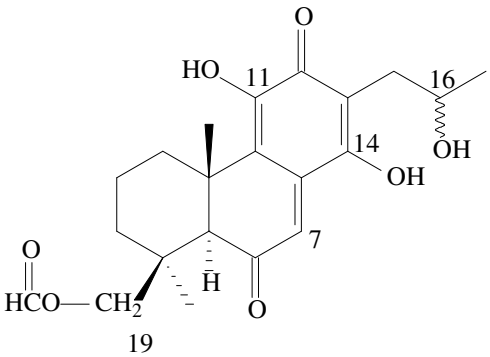


	R
(115)	$CH_2CH(OH)CH_3$
(116)	CH_2-CH=CH_2



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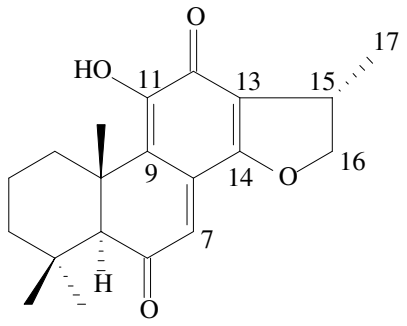

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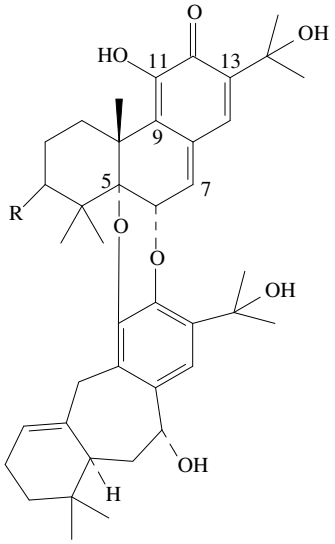
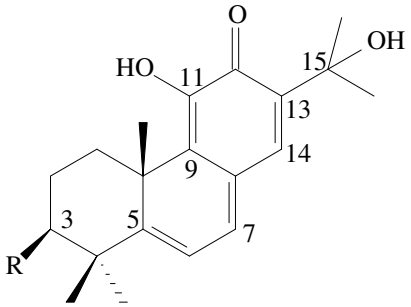
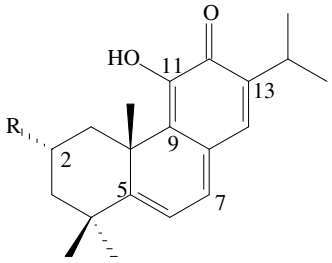


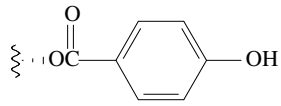
	R
(119)	H
(120)	$OC(O)CH_3$



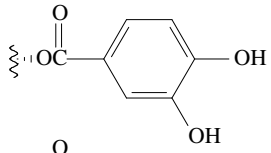
	R
(121)	H
(122)	$OC(O)CH_3$



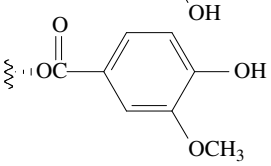
(123) R =

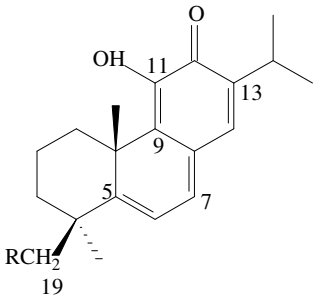


(124) R =



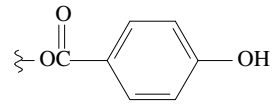
(125) R =



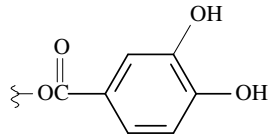


(126) R =

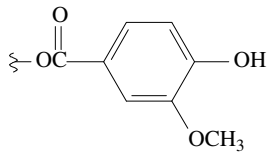
(127) R =

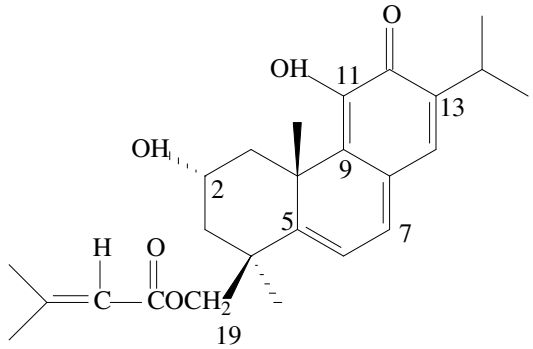


(128) R =



(129) R =





(130)

Thirty-six compounds (**131-167)** having a coleon-type structure were isolated from eleven *Plectranthus* and ten *Coleus* species, where most of the compounds have been isolated from *P. edulis* and *C. coerulescens* (Table 9a-c). The coleon-type compounds differ from the royleanones in that ring C is aromatic in coleon-type compounds as apposed to having a hydroxybenzoquinone or *p*-quinoid ring system.

Compounds **131-148** (Table 9a) differ in ring B with regard to positions 6 and 7 as they both could be saturated as in **131,** or oxidized, with either a hydroxy or carbonyl group (**132-135**) or two carbonyl groups (**136-145).** Alternatively either C-6 or C-7 could be oxidized as in **132-134**. Compounds **138** to **141** are the only compounds where the methyl group/s at C-15 have been oxidized to an alcohol or acetyl group, while **134** is the only compound where the methyl group at C-20 has been oxidized to an alcohol. Plectranthol B (**131**), has an aromatic substituent at C-19 with a prenyl group occurring at C-12. In compounds **146** to **148**, an oxygen bridge is formed between C-20 and either C-6 or C-7. Compound **147** has a methoxy group present at C-20 while **148** has a carbonyl function at C-7.

Table 9a: Coleon-type abietanes isolated from *Plectranthus* and *Coleus* species

Compound	**Name**	**Synonym**	**Isolated from *Plectranthus (P)* or *Coleus (C)* species**	**Reference**
131	12-*O*-(3-methyl-2-butenoyl)-19-*O*-(3,4-dihydroxybenzoyl)-11-hydroxy-8,11,13-abietatriene	Plectranthol B	*P. nummularius*	Narukawa *et al*. 2001
132	7α,11-dihydroxy-12-methoxy-8,11,13-abietatriene		*P. elegans*	Dellar *et al*., 1996
133	11,12-dihydroxy-8,11,13-abietatriene-7-one	11,20-dihydroxysugiol	*P. cyaneus*	Horvath *et al*., 2004
134	11,12,20-trihydroxy-8,11,13-abietatriene-7-one	11-hydroxysugiol	*P. cyaneus; C. forskohlii*	Horvath *et al*., 2004; Lingling *et al*., 2005
135	6β,11,12,14-tetrahydroxy-8,11,13-abietatriene-7-one	5,6-dihydrocoleon U	*P. argentatus*	Alder *et al*., 1984a

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Compound	Name	Synonym	Isolated from *Plectranthus (P)* or *Coleus (C)* species	Reference
136	11,12,14-trihydroxy-8,11,13-abietatriene-6,7-dione	Coleon V	*P. myrianthus; C. carnosus; C. coerulescens; P. sanguineus*	Miyase *et al*., 1977a; Yoshizaki *et al*., 1979; Grob *et al*., 1978; Matloubi-Moghadam *et al*., 1987
137	14-formyloxy-11,12-dihydroxy-8,11,13-abietatriene-6,7-dione	14-*O*-formyl-coleon -V	*P. myrianthus*	Miyase *et al*., 1977a
138	11,12,14,16-tetrahydroxy-8,11,13-abietatriene-6,7-dione	Coleon D	*C. aquaticus; C. coerulescens*	Ruedi *et al*., 1972; Grob *et al*., 1978; Schmid *et al*., 1982; Kunzle *et al*., 1987
139	16-acetoxy-11,12,14-trihydroxy-8,11,13-abietatriene-6,7-dione	16-*O*-acetylcoleon D	*C. coerulescens; P. edulis*	Grob *et al*., 1978; Kunzle *et al*., 1987
140	3α-acetoxy-11,12,14,16-tetrahydroxy-8,11,13-abietatriene-6,7-dione	Coleon I	*C. somaliensis*	Moir *et al*., 1973a
141	16,17-diacetoxy-3α,11,12,14-tetrahydroxy-8,11,13-abietatriene-6,7-dione	Coleon K		
142	16-acetoxy-11,12,14,17-tetrahydroxy-8,11,13-abietatriene-6,7-dione	Coleon X	*C. garckeanus*	Miyase *et al*., 1979
143	16,17-diacetoxy-11,12,14-trihydroxy-8,11,13-abietatriene-6,7-dione	16-*O*-acetyl coleon X		
144	11,12,16-trihydroxy-8,11,13-abietatriene-6,7-dione		*P. edulis*	Kunzle *et al*., 1987
145	3β,11,12,14-tetrahydroxy-8,11,13-abietatriene-6,7-dione	Coleon T	*P. caninus*	Arihara *et al*., 1977
146	7β,20β-epoxy-11,12-dihydroxy-8,11,13-abietatriene	20-deoxocarnosol	*C. barbatus*	Kelecom *et al*., 1984

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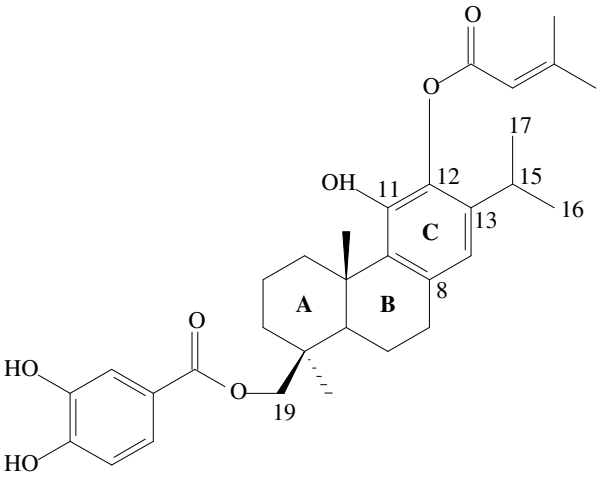
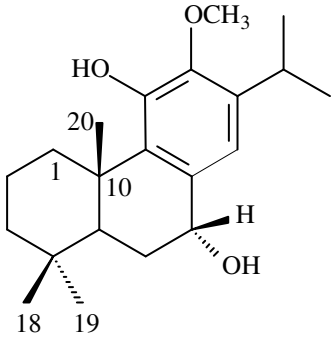
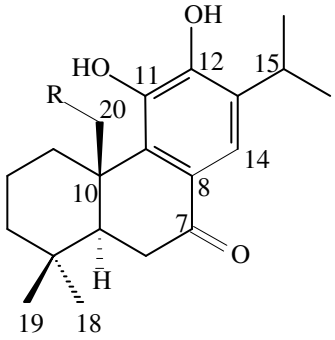
Compound	Name	Synonym	Isolated from *Plectranthus (P)* or *Coleus (C)* species	Reference
147	7β,20β-epoxy-20*S*-methoxy-11,12-dihydroxy-8,11,13-abietatriene	Esquirolin D	*C. esquirolii*	Li *et al*., 1991
148	6β,20β-epoxy-6α,11,12-trihydroxy-8,11,13-abietatriene-7-one	Carnosolone	*P. cyaneus; C. carnosus*	Horvath *et al*., 2004; Yoshizaki *et al*., 1979



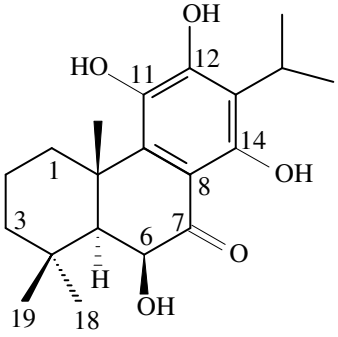
(131)



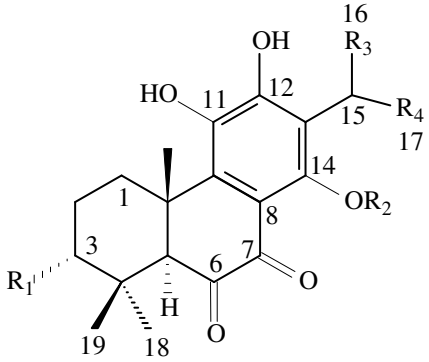
(132)



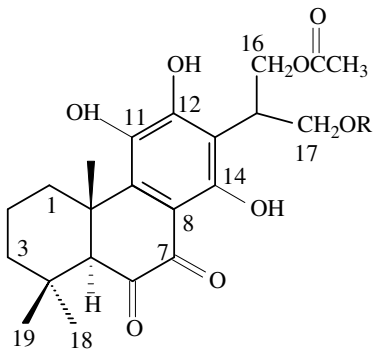
	R
(133)	H
(134)	OH



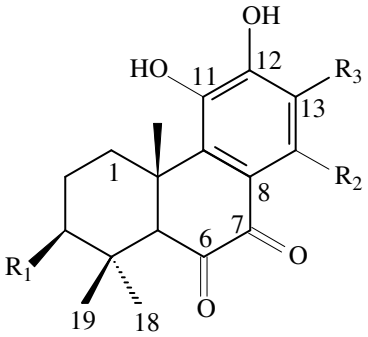
(135)



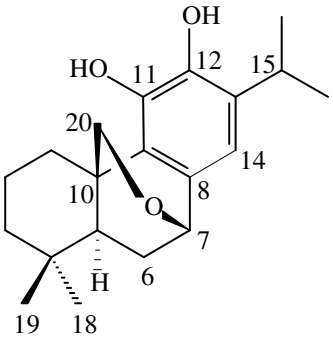
	R_1	R_2	R_3	R_4
(136)	H	H	CH_3	CH_3
(137)	H	CHO	CH_3	CH_3
(138)	H	H	$CH_2(OH)$	CH_3
(139)	H	H	$CH_2OC(O)CH_3$	CH_3
(140)	$OC(O)CH_3$	H	$CH_2(OH)$	CH_3
(141)	OH	H	$CH_2OC(O)CH_3$	$CH_2OC(O)CH_3$



	R
(142)	H
(143)	$C(O)CH_3$



	R_1	R_2	R_3
(144)	H	H	$CH_2CH(OH)CH_3$
(145)	OH	OH	$CH(CH_3)_2$



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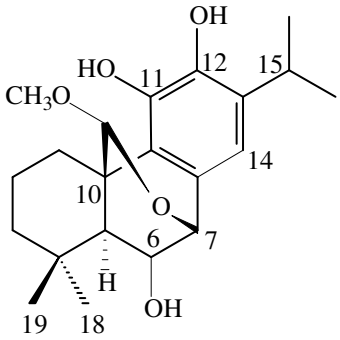
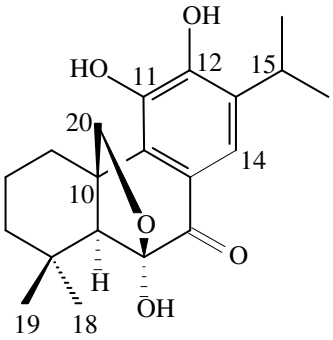


(147)



(148)

In compounds **149-165** (Table 9b), an olefinic bond is present at C-5 while oxygenated substituents occur at C-11, C-12 and C-14. With the exception of compound **157**, compounds **153**-**165** have either one or both of the isopropyl methyl groups at C-15, being oxidized to an acetyl and/or alcohol function. Compounds **152** and **159** have a hydroxy and acetoxy group present at C-3 respectively while compounds **160** and **161** have an acetyl group at C-2. Abietanes **163** and **164** have a formyloxy (-OCHO) group at C-19 while the isopropyl group at C-13 has been replaced by a linear, 3-carbon side-chain containing either a double bond or alcohol in compounds **162**-**164**.

Table 9b: Coleon-type abietanes with an olefinic bond at Δ^5 isolated from *Plectranthus* and *Coleus* species

Compound	**Name**	**Synonym**	**Isolated from *Plectranthus (P)* or *Coleus (C)* species**	**Reference**
149	6,11,12,14-tetrahydroxy-5,8,11,13-abietatetraene-7-one	Coleon U	*P. myrianthus; P. grandidentatus; P. forsteri 'marginatus'; C. carnosus; P. sanguineus; P. edulis; P. fasciculatus*	Wellsow *et al*., 2006; Gaspar-Marques *et al*., 2006; Gaspar-Marques *et al*., 2002; Cerqueira *et al*., 2004; Miyase *et al*., 1977a; Yoshizaki *et al*., 1979; Matloubi-Moghadam *et al*., 1987; Kunzle *et al*., 1987; Coutinho *et al*., 2009; Rasikari, 2007
150	14-acetoxy-6,11,12-trihydroxy-5,8,11,13-abietatetraene-7-one	14-*O*-acetylcoleon U	*P. grandidentatus*	Rijo *et al*., 2007
151	11-acetoxy-6,12,14-trihydroxy-5,8,11,13-abietatetraene-7-one	Coleon U 11 acetate	*C. xanthanthus*	Mei *et al*. 2002
152	3β,6,11,12,14-pentahydroxy-5,8,11,13-abietatetraene-7-one	Coleon S	*P. caninus*	Arihara *et al*., 1977:

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Compound	Name	Synonym	Isolated from *Plectranthus (P)* or *Coleus (C)* species	Reference
153	6,11,12,14,16-pentahydroxy-5,8,11,13-abietatetraene-7-one	Coleon C	*C. aquaticus, C. coerulescens; P. edulis; C. forskohlii*	Ruedi *et al*.,1971; Grob *et al*., 1978; Schmid *et al*., 1982; Kunzle *et al*., 1987; Yanwen *et al*., 2007; Xiu *et al*., 2008
154	16-acetoxy-6,11,12,14-tetrahydroxy-5,8,11,13-abietatetraene-7-one	16-*O*-acetyl coleon C	*C. coerulescens; P. edulis*	Grob *et al*., 1978; Kunzle *et al*., 1987
155	16-acetoxy-6,11,12,14,17-pentahydroxy-5,8,11,13-abietatetraene-7-one	Coleon W	*C. coerulescens; P. myrianthus; C. garckeanus*	Grob *et al*., 1978; Miyase *et al*., 1977a, Miyase *et al*., 1979
156	16,17-diacetoxy-6,11,12,14-tetrahydroxy-5,8,11,13-abietatetraene-7-one	16-*O*-acetyl coleon W	*C. garckeanus*	Miyase *et al*., 1979
157	11-acetoxy-6,12,14-trihydroxy-5,8,11,13-abietatetraene-7-one	11-acetoxy-coleon U	*C. xanthanthus*	Mei *et al*., 2000
158	11,16-diacetoxy-6,12,14-trihydroxy-5,8,11,13-abietatetraene-7-one	11,16-diacetoxy-coleon U		
159	3α-acetoxy-6,11,12,14,16-pentahydroxy-5,8,11,13-abietatetraene-7-one	Coleon H	*C. somaliensis*	Moir *et al*., 1973a
160	2α,17-diacetoxy-6,11,12,14,16-pentahydroxy-5,8,11,13-abietatetraene-7-one		*C. blumei*	Ragasa *et al*., 2001
161	2α,6,11,12,16,17-hexacetoxy-14-hydroxy-5,8,11,13-abietatetraene-7-one			
162	6,11,12,14,16-pentahydroxy-17(15→16)-*abeo*abieta-5,8,11,13-tetraene-7-one		*P. edulis*	Kunzle *et al*., 1987

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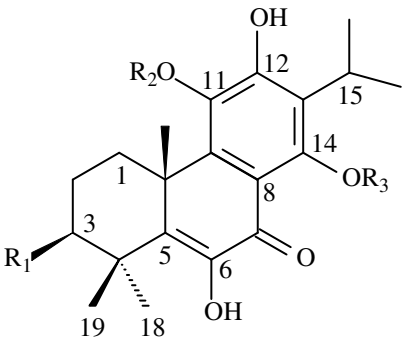
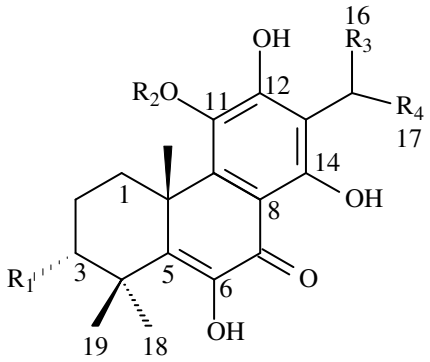
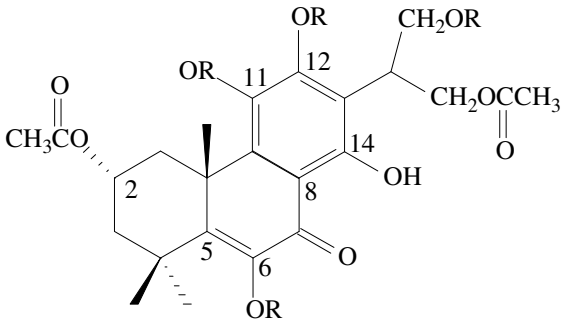
Compound	Name	Synonym	Isolated from *Plectranthus (P)* or *Coleus (C)* species	Reference
163	19-formyloxy-6,11,12,14-tetrahydroxy-17(15→16)-*abeo*abieta-5,8,11,13,16-pentaene-7-one	Lanugone R	*P. lanuginosus*	Schmid *et al*., 1982
164	19-formyloxy-6,11,12,14,16-pentahydroxy-17(15→16)-*abeo*abieta-5,8,11,13-tetraene-7-one	Lanugone S		
165	16-acetyl-11,12,14-trihydroxy-5,8,11,13-abietatetraene-7-one(6,18-lactone)		*P. edulis*	Kunzle *et al*., 1987



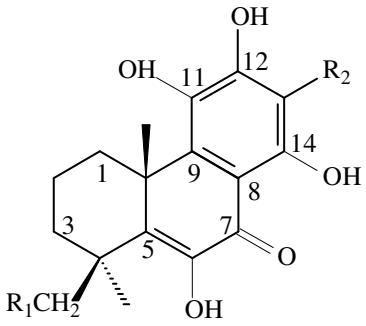
	R_1	R_2	R_3
(149)	H	H	H
(150)	H	H	$C(O)CH_3$
(151)	H	$C(O)CH_3$	H
(152)	OH	H	H



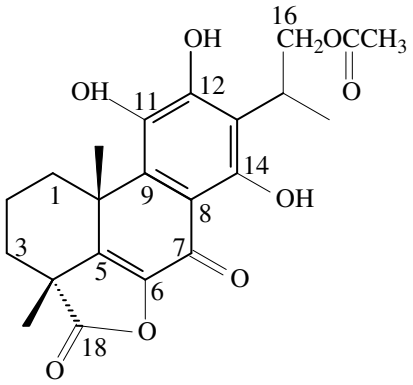
	R_1	R_2	R_3	R_4
(153)	H	H	CH_2OH	CH_3
(154)	H	H	$CH_2OC(O)CH_3$	CH_3
(155)	H	H	$CH_2OC(O)CH_3$	CH_2OH
(156)	H	H	$CH_2OC(O)CH_3$	$CH_2OC(O)CH_3$
(157)	H	$C(O)CH_3$	CH_3	CH_3
(158)	H	$C(O)CH_3$	$CH_2OC(O)CH_3$	CH_3
(159)	$OC(O)CH_3$	H	$CH_2(OH)$	CH_3



	R
(160)	H
(161)	$C(O)CH_3$



	R_1	R_2
(162)	H	$CH_2CH(OH)CH_3$
(163)	OCH(O)	$CH_2CH{=}CH_2$
(164)	OCH(O)	$CH_2CH(OH)CH_3$

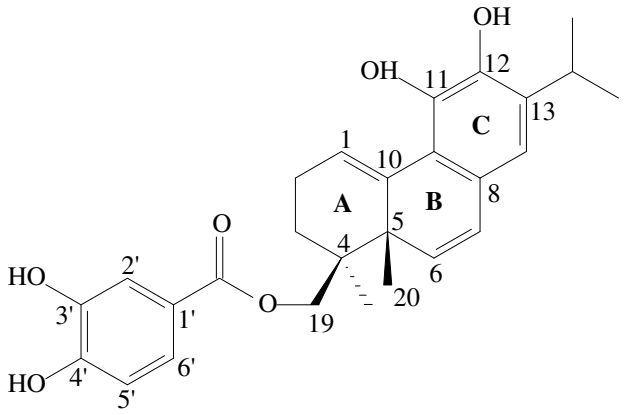


(165)

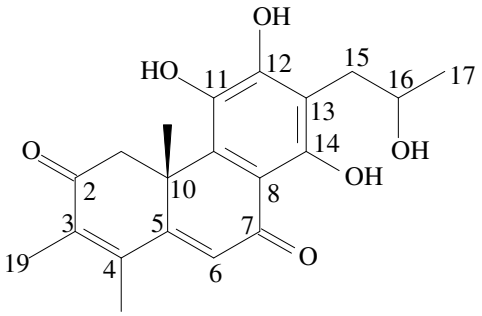
Compounds **166** and **167** (Table 9c) have double bonds within rings A and B. In compound **166**, the double bonds are located at C-1(10) and Δ^6 respectively while the methyl group is no longer at C-10 but at C-5. In **167** the double bonds occur at Δ^3 and Δ^5 and the methyl group at C-19 has migrated from C-4 to C-3. The linear, 3-carbon side-chain in this compound has been oxidized to an alcohol. In compound **166**, the substituent at C-19 is aromatic.

Table 9c: Coleon-type abietanes with double bonds within ring A and B isolated from *Plectranthus* species

Compound	Name	Synonym	Isolated from *Plectranthus (P)* species	Reference
166	19-*O*-(3',4'-dihydroxybenzoyl)-11,12-dihydroxy-20(10→5)-*abeo*abieta-1(10),6,8,11,13-pentene	Plectranthol A	*P. nummularius*	Narukawa *et al*., 2001
167	19(4→3),17(15→16)-*bisabeo*abieta-11,12,14,16-tetrahydroxy-3,5,8,11,13-pentene-2,7-dione	Plectrinone A	*P. barbatus*	Schultz *et al*., 2007



(166)



(167)

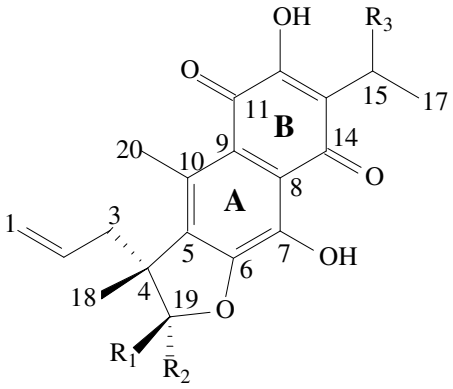
Miscellaneous constituents

Eight compounds **168-174** (table 10) do not resemble the abietanes **27-167** as they contain carbocyclic and heterocyclic rings which are fused differently from the other abietanes. Compounds **168** to **171** contain a highly conjugated system with ring A being aromatic and ring B resembling the hydroxybenzoquinone system. An oxidised furan ring also forms a part of structures **168** to **171**. In compound **172** rings A and B are linked by a carbon-to-carbon bond. Ring A resembles that of the normal ring A in abietanes, with methyl groups at C-4 and C-6 and a ketone at C-5, and ring B has the hydroxybenzoquinone structure. Compound **173** is similar to the royleanones but B ring

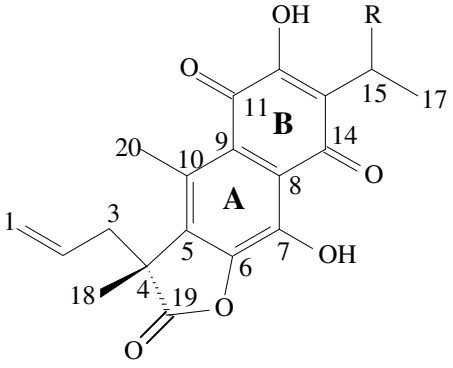
has been expanded by an extra oxygen atom forming a seven membered ring with carbonyl groups flanking the oxygen at either side. Compound **174** has four rings fused together including an aromatic and lactone ring. Both compounds **173** and **174** have highly conjugated systems.

Table 10: Miscellaneous abietanes isolated from *Plectranthus* and *Coleus* species

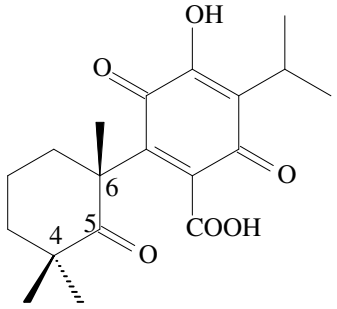
Compound	**Name**	**Common name**	**Isolated from *Plectranthus (P)* species**	**Reference**
168	(4*R*,19*S*)-7,12,19α-trihydroxy-1,5(10),6,8,12-abietapentene-11,14-dione	Coleon A (19*S*)	*P. aff puberulentus*	Wellsow *et al*., 2006
169	(4*R*,19*R*)-7,12,19β-trihydroxy-1,5(10),6,8,12-abietapentene-11,14-dione	Coleon A (19*R*)		
170	7,12-dihydroxy-1,5(10),6,8,12-abietapentene-11,14,19-trione	Coleon A lactone	*P. puberulentus; P. edulis*	Wellsow *et al*., 2006; Kunzle *et al*., 1987
171	16-acetoxy-7,12-dihydroxy-1,5(10),6,8,12-abietapentene-11,14,19-trione		*P. edulis*	Kunzle *et al*., 1987
172	*Too complex to name systematically	Xanthanthusin E	*C. xanthanthus*	Mei *et al*., 2002
173	*Too complex to name systematically	Sanguinon A	*P. sanguineus*	Matloubi-Moghadam *et al*., 1987
174	*Too complex to name systematically	Edulon A	*P. edulis*	Buchbauer *et al*., 1978; Kunzle *et al*., 1987



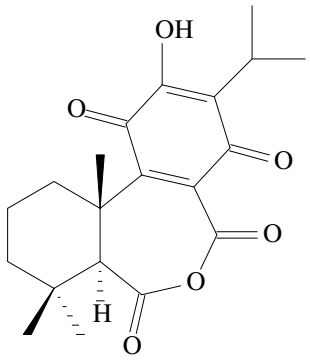
	R_1	R_2	R_3
(168)	H	OH	CH_3
(169)	OH	H	CH_3



	R
(170)	CH_3
(171)	$CH_2OC(O)CH_3$



(172)



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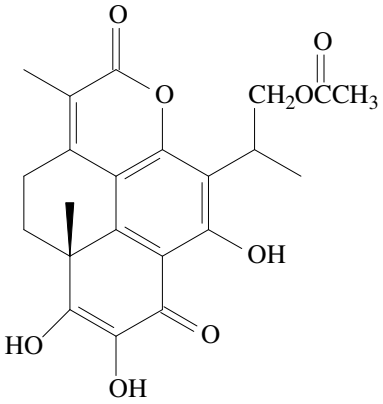

(174)

2.5 Biological activity of the phytochemical constituents of *Plectranthus*

The antibacterial and antifungal activities of abietanes isolated from *Plectranthus* species are listed in table 11. Of the one-hundred and fifty abietanes isolated from *Plectranthus* and *Coleus* species, only twenty are reported as having showed antimicrobial activity. These twenty abietanes were isolated from eight *Plectranthus* species with horminone (**28**), 7α-acetoxy-6β-hydroxyroyleanone (**36**), 7α,12-dihydroxy-17(15→16)-*abeo*abieta-8,12,16-triene-11,14-dione (**55**), 16-acetoxy-7α,12-dihydroxy-8,12-abietadiene-11,14-dione (**54**) and Coleon U (**149**) being isolated from more than one species.

Of all the abietanes isolated from *Plectranthus* and *Coleus* species the highest antibacterial and antifungal activities were shown by the royleanone and coleon type compounds. These were compounds **28**, **36**, **38** (7α-formyloxy-6β-hydroxyroyleanone), **44** (coleon U quinone) and **149**. All these compounds are structurally related and have the same basic skeleton with different functional groups located in the molecules. However, coleon U has an aromatic ring and the others have a hydroxyquinoid ring C.

Bacillus subtilis and *Staphylococcus aureus* were the most commonly tested bacterial strains while the anti-fungal pathogens tested were *Cladosporium cucumerinum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Pythium ultimum* and *Candida albicans*.

Acetone was the most frequently used solvent for extraction, while dichloromethane, chloroform and ethyl acetate were reportedly used only once for the extraction of abietanes from the plant material.

Of the eight antimicrobial studies carried out on abietanes, two were done on the roots only and two on the entire plant. The rest of the studies were carried out on the aerial parts of the plant and the leaves (aerial parts in many instances mean leaves and flowers). Comparative studies need to be done on the different plant parts, to ascertain where the largest concentration of abietane diterpenoids exist.

The majority of the abietanes isolated remain untested against microbes (bacteria and fungi) and there is thus a need to carry out antimicrobial assays on the remainder of the abietanes.

It was found that the coleon-type compound (where ring C is aromatic) are more active than royleanone-type compounds (where ring C has a 12-hydroxy-*p*-benzoquinone moeity) (Gaspar-Marques *et al*., 2006; Wellsow *et al*., 2006).

It has been observed that the oxidation pattern at the C-6 and C-7 positions in compounds having a royleanone-type structure plays a role in the antibacterial activity of the compound with compounds where C-7 is oxidised being more active than those oxidised at C-6 or not oxidised at all (Teixeira *et al*., 1997; Gaspar-Marques *et al*., 2006). Another important observation is that the presence of an isopropyl group at C-13 in royleanone-type compounds results in better activity than those compounds having an oxidised isopropyl group or allylic group at the same position (Gaspar-Marques *et al*., 2006; Batista *et al*., 1995; Batista *et al*., 1994).

Table 11: Antibacterial and antifungal activity of compounds extracted from *Plectranthus* species

***Plectranthus* species**	**Plant part**	**Compound**	**Antimicrobial Activity**	**Reference**
P. aff. puberulentus	acetone[lv]	**(168), (169)**	*B. subtilis*	Wellsow *et al*., 2006
P. ecklonii	ethyl acetate[w]	**(123), (124)**	Drug resistant *Mycobacterium tuberculosis* *Listeria monocytogenes*	Nyila *et al.*, 2009
P. elegans	chloroform[a]	**(109), (132)**	*B. subtilis, S. aureus, Streptomyces scabies, P. aeruginosa, Pseudomonas syringae, Erwinia carotovora, *Cladosporium cucumerinum*	Dellar *et al*., 1996

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***Plectranthus* species**	**Plant part**	**Compound**	**Antimicrobial Activity**	**Reference**
P. forsteri 'marginatus'	acetone[lv]	**(44)**, **(149)**	*Bacillus subtilis, Pseudomonas syringae*	Wellsow *et al*., 2006
P. grandidentatus	acetone[w]	**(36)**	*Staphylococcus aureus, Vibrio cholera*	Teixera *et al*., 1997
	acetone[a+rt]	**(27)**, **(28)**, **(32)**, **(33)**, **(36)**, **(43)**, **(149)**	Six strains of methicillin resistant *S. aureus,* two strains of vancomycin-resistant *E. faecalis*	Gaspar-Marques *et al*., 2006
P. hadiensis	dichloromethane[a]	[x]**(36)**, **(38)**	*B. subtilis, Xanthomonas campestris, *Rhizoctonia solani, *Sclerotinia sclerotiorum, *Pythium ultimum,* *unknown *Candida* species	Laing *et al*., 2006
		[x]**(33)**	**S. sclerotiorum,* *unknown *Candida* species	
P. hereroensis	acetone[a+rt]	**(28)**, **(54)**, **(55)**, **(64)**	Six strains of methicillin resistant *S. aureus*, two strains of vancomycin-resistant *E. faecalis*	Gaspar-Marques *et al*., 2006
	acetone[rt]	**(55)**	*S. aureus*, *V. cholerae*	Batista *et al*., 1995
		(28), **(54)**	*S. aureus*, *V. cholera*, **Candida albicans*, *P. aeruginosa*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhimurium*, *Steptococcus faecalis*	Batista *et al*., 1994

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Plectranthus species	Plant part	Compound	Antimicrobial Activity	Reference
P. puberulentus	acetone[lv]	**(170)**	*Bacillus subtilis*, *Pseudomonas syringae*	Wellsow *et al*., 2006

* denotes anti-fungal pathogens
[x] Laing *et al*. (2006) reported the isolation of 7α-acetoxy-6-hydroxyroyleanone and 7α,6-dihydroxyroyleanone without including the stereochemistry in the name. It is thus assumed that these compounds are 7α-acetoxy-6β-hydroxyroyleanone (**36**) and 7α,6β-dihydroxyroyleanone (**33**), respectively
Key: lv: leaves, rt: roots, s: stems, a: aerial parts, w: whole plant, f: flowers

Three abietanes have also been found in *Salvia* species where antimicrobial studies were carried out (Table 12). All three of these abietanes have a royleanone-type structure.

Compounds **28** (horminone) and **31** (7α-acetoxyroyleanone) exhibited similar and in some cases stronger antibacterial activity to commonly used antibiotics such as cefoperazone, amicain, kanamycin and vancomycin (Ulubelen *et al*., 2001; 2003).

Table 12: Antibacterial and antifungal activity of abietanes isolated from *Salvia* species

Plant species	Compound	Activity	Reference
Salvia sclarea	**(40)**	*S. aureus, *C. albicans*	Ulubelen *et al*., 1994
Salvia blepharochlaena; Salvia amplexicaulis; *Salvia eriophora*	**(28)**	*S. aureus, S. epidermis, B. subtilis, E. faecalis*	Kolak *et al*., 2001; Ulubelen *et al*., 2001; 2003
	(31)	*S. aureus, S. epidermis, B. subtilis*	

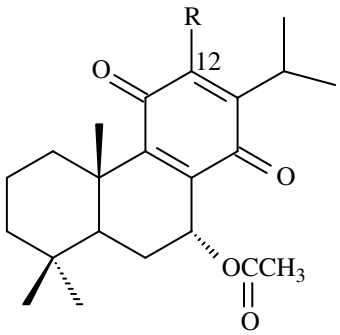
* denotes anti-fungal pathogens

Table 13 lists twenty-seven abietanes with antioxidant, anticancer, antifeedant and other activity which have been isolated from thirteen *Plectranthus* species and two *Coleus* species.

Compounds **36** (7α-acetoxy-6β-hydroxyroyleanone), **123** and **124** commonly known as Parvifloron D and F respectively, have been isolated the most and have exhibited both

anticancer (Cerqueira *et al*., 2004; Nyila *et al*., 2009; Gaspar-Marques *et al*., 2002) and antimalarial activity (van Zyl *et al*., 2008). Compound **124** also has antioxidant activity (Narukawa *et al*., 2001). Compound **36** has a royleanone-type structure while the other two compounds (**123-124**) have a vinylogous quinone-type structure. In the three studies that were done on coleon-type and royleanone-type compounds (Mei *et al*., 2002; Cerqueira *et al*., 2004; Gaspar-Marques *et al*., 2002), it was found that the coleon-type compounds were more effective as anticancer agents than the royleanone-type compounds.

When Kabouche *et al*. (2007) tested the antioxidant activity of royleanone and coleon-type compounds, they found that the presence of a carbonyl group at C-7 led to higher activity as apposed to compounds having no substituent at this position or having a substituent other than a carbonyl at C-7. When the hydroxyl group at C-12 is replaced by a methoxy group as in **176**, the activity is increased as well (Kabouche *et al*., 2007). In studies where coleon (compounds where ring C is aromatic) and royleanone-type (where ring C has a 12-hydroxy-*p*-benzoquinone moeity) compounds were isolated, the coleon-type compounds proved to be more active than the royleanone-type compounds (Kabouche *et al*., 2007; Cerqueira *et al*., 2004; Mei *et al*., 2002; Gaspar-Marques *et al*., 2002; Narukawa *et al*., 2001). However there has been one instance were a royleanone-type diterpene (Coleon U quinone (**44**)) showed potent antifeedant activity whereas the coleon-type diterpene (coleon U (**149**)) displayed no activity as an antifeedant (Wellsow *et al*., 2006).



	R
(175)	OH
(176)	OCH_3

The leaves seem to be the most frequently used plant part. There has been three instances where compounds from the entire plant has been tested but only one report on abietanes being isolated from the roots of *Plectranthus* species.

In ten of the eleven studies on *Plectranthus* species, polar solvents (ethyl acetate, dichloromethane, acetone, ether and water) were used for extraction with there being just one report of hexane and dichloromethane being used as a solvent for extraction. This is probably because abietanes are best isolated with a polar solvent medium as many of the abietanes are highly functionalised with carbonyl and hydroxyl groups.

Table 13: Inhibitory activity of abietanes isolated from *Plectranthus* and *Coleus* extracts

***Plectranthus* species**	**Plant part**	**Compound**	**Other Activity**	**Reference**
Anticancer activity				
C. forskohlii	NS	**(153)**	anti-tumor activity (*in vivo* and *in vitro*)	Xiu *et al*., 2008
	taken orally or applied topically		inhibits growth and proliferation of tumors	Yanwen *et al*., 2007
C. xanthanthus	70% acetone[a]	**(44)**, **(45)**,**(46)**, **(151)**, **(172)**	cytotoxic against K562 human leukemia cells	Mei *et al*., 2002
P. ecklonii	ethyl acetate[w]	**(123)**, **(124)**	active against vero cell line	Nyila *et al*., 2009
P. grandidentatus	acetone[w]	**(149)**	promising anti-cancer drug	Coutinho *et al*., 2009
	acetone extract[w]	**(35)**, **(36)**, **(47)**, **(149)**	antiproliferative activity against human lymphocytes	Cerqueira *et al*., 2004
		(36), **(149)**	antiproliferative activity against the expression of CD69 by T- and B-mouse lymphocytes and induces lymphocyte apoptosis	
P. grandidentatus	acetone[w]	**(33)**, **(35)**, **(36)**, **(47)**, **(149)**	anti-tumor activity (breast, lung, renal, melanoma and CNS)	Gaspar-Marques *et al*., 2002

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Plectranthus species	Plant part	Compound	Other Activity	Reference
Antioxidant activity				
P. nummularis	acetone[lv]	**(124)**, **(128)**, **(131)**, **(168)**	antioxidant activity	Narukawa *et al*., 2001
Antifeedant activity				
P. aff. puberulentus	acetone[lv]	**(168)**, **(169)**	antifeedent activity against *S. littoralis*	Wellsow *et al*., 2006
P. barbatus	ether[lv]	**(90)**	insect antifeedent activity against *S. gramium* and *P. gossypiella*	Kubo *et al*., 1984
P. forsteri 'marginatus'	acetone[lv]	**(44)**	antifeedent activity against *S. littoralis*	Wellsow *et al*., 2006
P. puberulentus		**(170)**		
Antimalarial activity				
P. ecklonii	dichloromethane[lv]	**(123)**, **(124)**	antimalarial activity; inhibits β-haematin formation	van Zyl *et al*., 2008
P. hadiensis	dichloromethane[lv]	**(38)**, **(36)**	antimalarial activity; inhibits β-haematin formation	van Zyl *et al*., 2008
P. lucidus	dichloromethane[lv]	**(123)**, **(126)**	antimalarial activity; inhibits β-haematin formation	van Zyl *et al*., 2008
P. purpuratus subsp. purpuratus		**(126)**		
P. purpuratus subsp. tongaensis		**(127)**, **(128)**		
Other activity				
P. barbatus	water[lv]	**(169)**	antisecretory and antiulcer activities	Schultz *et al*., 2007
P. ecklonii	ethyl acetate[w]	**(123)**, **(124)**	inhibits tyrosinase activity	Nyila *et al*., 2009
P. grandis	hexane[lv]	**(70)**, **(87)**	gastroprotective properties, in mice	Rodrigues *et al*., 2010
P. hereroensis	acetone[rt]	**(64)**	antiviral activity against Herpes virus ie. Herpes simplex type II	Batista *et al*., 1995

NB. *Coleus* species are also included in this table because of the uncertain relationship to the *Plectranthus* species
Key: lv: leaves, rt: roots, s: stems, a: aerial parts, w: whole plant, f: flowers, NS: not specified

Table 14 contains six abietanes isolated from five known *Salvia* species, one unknown *Salvia* species and one *Lepechinia* species. These compounds have been shown to possess anticancer, antioxidant or cardiovascular activity with compounds **27** (royleanone) having antitumor and antioxidant activity and **28** (horminone) having antitumor, anticancer and antioxidant activity.

The royleanone-type abietanes (**27**, **28**, **32** and **44**) have shown to exhibit anticancer, antioxidant and cardiovascular activity while the coleon-type compounds (**134** and **146**) have proved to be active only against cancer cell lines.

Table 14: Pharmacological activity of abietanes isolated from other plant species

Plant species	Compound	Activity	Reference
Salvia pachyphylla	**(146)**	Active against human cancer cell lines	Guerrero *et al*., 2006
Salvia hypargeia	**(134)**	Active against ovarian cancer cell line (A2780)	Topcu *et al*., 2008
**Salvia* species	**(27)**, **(28)**, **(32)**	Antitumor	Topcu and Goren, 2007
Lepechinia bullata	**(28)**, **(43)**	Anticancer	Jonathan *et al*., 1989
Salvia amplexicaulis	**(44)**	Cardiovascular activity	Kolak *et al*., 2001; Ulubelen *et al*., 2003
Salvia amplexicaulis; Salvia eriophora		Antihypertensive	Kolak *et al*., 2001; Ulubelen *et al*., 2002
Salvia barrelieri	**(27)**, **(28)**, **(31)**	Antioxidant activity	Kabouche *et al*., 2007

* unknown species

The distinguishing feature of an abietanoid structure which determines the activity of the compound, is the oxidation pattern within ring B and ring C. It has been observed that the oxidation patterns at C-6, C-7 and C-12 influence the activity of the abietanoid. Compounds having a coleon-type structure (where ring C is aromatic) have proven to be more active than royleanone-type compounds (where ring C has a 12-hydroxy-*p*-benzoquinone moeity) (Gaspar-Marques *et al*., 2006; Wellsow *et al*., 2006).

Two compounds, 7α-acetoxy-6β-hydroxyroyleanone (**36**) and coleon U (**149**) have shown to have the widest range of biological activity with compound **149** always being more active than compound **36** and occasionally having even stronger activity than the well known drugs used as controls.

Although much has been done on testing the abietanes for biological activity, there are still many abietanes which have not been tested for any biological or pharmacological activity. These assays will prove useful in determining the structure-activity relationships of the abietanes.

References

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Chapter 3 Extractives from *Plectranthus hadiensis*

3.1 Introduction

Plectranthus hadiensis is commonly known as the 'hairy spurflower' or 'imbozisa' by the Zulus. This plant is a shrubby perennial which grows to a height of 1.5 metres and flowers between March and June every year, peaking in May (van Jaarsveld, 2006). The flower blossoms are purple in colour (figure 15) and are pleasantly aromatic.

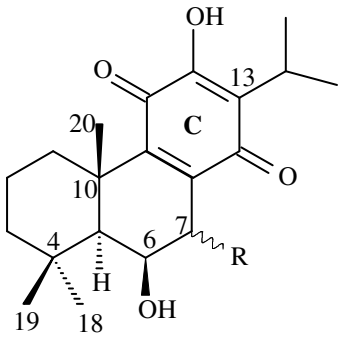
Figure 15: *Plectranthus hadiensis* in bloom (picture courtesy of Prof. N. Crouch)

P. hadiensis favours dry conditions for growth and can be found growing between the Kwa-Zulu Natal coastline and Gauteng, South Africa. The cuttings of this plant are used for propogation purposes (van Jaarsveld, 2006).

Ethnobotanically, *P. hadiensis* was reported by Lukhoba *et al*. (2006) as having been known to treat certain skin conditions, diarrhoea in South India and Sri Lanka (Mehrotra *et al*., 1989) respiratory conditions and inflammation (*loc cit* Sivarajan and Balachandran, 1986; Neuwinger, 2000; Hutchings *et al*, 1996). However the literature cited in Lukhoba *et al*. (2006) does not make mention of these reports.

There have been only four biological studies on *Plectranthus hadiensis* where the antiplasmodial, insect-antifeedant, antifungal, antiradical and antibacterial properties of this plant extract and the isolated compounds were evaluated for activity (van Zyl *et al*., 2008; Laing *et al*., 2006; Mothana *et al*., 2008; Wellsow *et al*., 2006). The acetone extract of this plant was found to be inactive as an insect-antifeedant (Wellsow *et al*., 2006). The hexane extract showed good activity against *Sclerotinia sclerotiorum*, *Rhizoctonia solani* and an unknown *Candida* species while the dichloromethane extract showed moderate activity against bacterial strains, *B. subtilis* and *X. campestris* and the fungal microorganism, *Sclerotinia sclerotiorum* as well as an unknown *Candida* species (Laing *et al*., 2006).

Three royleanones **33**, **36** and **38** were isolated previously from the dichloromethane extract of the aerial parts of *P. hadienis* (van Zyl *et al*., 2008) and the isomers of **33** and **36**, **34** and **37** respectively, were isolated from the ethanol extract of *Coleus zeylanicus* (a synonym for *P. hadiensis*) (Mehrotra *et al*., 1989). Compounds **33** and **36** exhibited anticancer activity against breast, lung, renal, melanoma and central nervous system cell lines (MCF-7, NCI-H460, TK-10, UACC-62 and SF-268, respectively) with both compounds proving to be less potent than the positive control, Cyclosporin A (Cerqueira *et al*., 2004; Gaspar-Marques *et al*., 2002). These three compounds (**33**, **36** and **38)** have also proven to be active against six strains of methicillin resistant *Staphylococcus aureus,* two strains of vancomycin-resistant *Enterococcus faecalis, Bacillus subtilis, Vibrio cholera* and *Xanthomonas campestris* (Laing *et al*., 2006; Teixera *et al*., 1997; Gaspar-Marques *et al*., 2006). Compounds **36** and **38** (van Zyl *et al*., 2008) exhibited antimalarial activity with **38** displaying synergistic properties when combined with quinine (Tables 11 and 13 on pages 72 and 76, respectively). There were no reports of biological activity in compounds **34** and **37**.



	R
(33)	α-OH
(34)	β-OH
(36)	α-$OC(O)CH_3$
(37)	β-$OC(O)CH_3$
(38)	α-OCH(O)

The purpose of this study was to perform phytochemical analyses on the stem and leaf material of *Plectranthus hadiensis* in the hope of isolating abietane diterpenes to test against *Enterococcus faecalis* and *Pseudomonas aeruginosa* bacterial microorganisms and breast (MCF-7), renal (TK-10) and melanoma (UACC-62) cancer cell lines. Since there has been no reports of compounds **34** and **37** possessing any biological activity, it was decided to evaluate these compounds for antibacterial and anticancer activity. We hope to compare our activity against *E. faecalis* with that reported in the literature and to report for the first time the antibacterial activity. We also hope to compare the anticancer activity of known compounds from *P. hadiensis* with that in the literature and report on the anticancer activity of any new compounds isolated from *P. hadiensis*.

Research has been conducted previously on the aerial parts, roots and leaves (Mothana *et al*., 2008; Laing *et al*., 2006), but there has been no phytochemical reports on the stem material. Since the aerial parts, roots and leaves of *P. hadiensis* were found to possess antiradical, antibacterial and antifungal activity (Mothana *et al*., 2008; Laing *et al*., 2006), this plant made an interesting phytochemical and pharmacological subject.

3.2 Foreword to Experimental

Nuclear Magnetic Resonance (NMR) Spectroscopy

The NMR spectra (^{1}H, ^{13}C, DEPT, HETCOR, COSY, NOESY, HSQC and HMBC) were recorded on either the Bruker 400MHz or 600MHz NMR spectrometer at the University of Kwa-Zulu Natal, Westville campus. TMS was used as an internal standard and chemical shifts were given as δ (ppm), and the coupling constants (*J*) were reported in Hz. In the case of the 400 MHz NMR instrument, the operating frequency for the ^{1}H NMR spectra was 400 MHz and for the ^{13}C NMR spectra was 100 MHz and in the 600 MHz NMR instrument, the operating frequencies were 600 and 150 MHz respectively for the ^{1}H and ^{13}C NMR spectra.

General chromatography

Column chromatography was carried out with silica gel (Merck 60, 0.040-0.063 mm) as the stationary phase. All crude extracts were separated on 4.5 cm diameter columns with step gradients of binary solvent systems and 100 ml fractions were collected with ten fractions for each step of the gradient. Elution of the columns was initiated with the least polar organic solvent, hexane, followed by dichloromethane, ethyl acetate and methanol in a stepwise gradient until the column had been eluted with 100% methanol. Thin layer chromatography (TLC) was done concurrently with column chromatography in order to monitor the elution of compounds. Merck 20 x 20 cm silica gel 60 F_{254} aluminium plates were used and visualized with anisaldehyde spray reagent [anisaldehyde: concentrated hydrochloric acid: aqueous methanol (1:2:97)] by spraying the plates and then heating. UV light (254 and 366 nm) was also used to detect compounds with conjugated double bonds.

Similar fractions were combined and purified on columns of varying diameter (1.5-2.5 cm) depending on the amount of the material to be purified and are referred to as 2cm columns (meaning 2cm diameter columns) in the text that follows. With the exception of

a few cases (specifically stated in the experimental) all purifications were carried out on 2cm diameter columns collecting 50 ml fractions. Where 3cm and 1cm columns were used, 100 ml fractions and 10 ml fractions were collected respectively. Any deviations to these conditions are mentioned specifically in the experimental.

Infrared Spectroscopy (IR Spectroscopy)

Infrared spectra were recorded on a PerkinElmer™ Spectrum 100. The sample was dissolved in either dichloromethane or methanol before being loaded onto the surface of potassium bromide discs.

Ultraviolet Absorption Spectroscopy (UV)

Ultraviolet absorption spectra were recorded with a PerkinElmer™ Lambda 35 UV/VIS spectrophotometer. Samples were dissolved in either dichloromethane or methanol and made to concentrations between 0.0052g/100ml and 1.2g/100ml.

Optical Rotation

Optical rotations were performed with a PerkinElmer™, Model 341 Polarimeter. The samples were dissolved in dichloromethane or methanol and were measured at room temperature. Concentrations were calculated in g/100mL.

Gas Chromatography-Mass Spectrometry (GC-MS)

The mass spectra were registered on an Agilent MS 5973 instrument which was coupled to an Agilent GC 6890 instrument equipped with a DB-5MS (30m x 0.25 id, 0.25 µm stationary phase) column. Helium (at 0.7ml/min) was used as the carrier gas. Optimal results required that the oven temperature be maintained at 200^{o}C for one minute followed by 5^{o}C increments in temperature every minute until an oven temperature of

280^{o}C was attained. This temperature was then held constant for a further eight minutes. The MS was operated in the EI mode at 70eV, in *m/z* range 100-500.

Liquid Chromatography-Mass Spectrometry (LC-MS)

The mass spectra were recorded on an Agilent 1100 Series LC/MSD Trap instrument operating in the negative ion mode and equipped with an electrospray interface. Helium (at 9ml/min) was used as the carrier gas. Separation was achieved using a mobile phase consisting of 95:5 acetonitrile:water containing 0.1% TFA at a flow rate of 0.3ml/min. The mass spectrometer was run in a partial scan mode range *m/z* 100-500.

Melting Point Determination

Melting points were determined on a Kofler micro hotstage melting point apparatus and are uncorrected. Compounds for melting point determination were recrystallised from dichloromethane or methanol before determining the melting point.

General extraction procedure

Plants were air dried, the stems milled and the leaves ground. Using a soxhlet, the plant material was extracted successively using hexane, dichloromethane, ethyl acetate and methanol. The plant material was extracted for forty-eight hours each with each of the four solvents and the extracts concentrated using a Buchi rotary evaporator. Crude extracts were stored in the fridge to prevent degradation of compounds and fungal contamination until they were ready to be purified further.

3.3 Experimental

Leaf and stem materials of *Plectranthus hadiensis* were collected in May 2007 in Kloof, Kwa-Zulu Natal by Professor Neil Crouch. A voucher specimen (N. Crouch 1126, NH) was retained at the National Botanic Institute, Berea Road, Durban, South Africa.

The leaves (184.63g) were air-dried, shredded in a domestic blender and extracted using the general extraction procedure above. Concentration of the extracts resulted in masses of 9.42 g, 1.72 g, 5.67 g and 17.86 g respectively for hexane, dichloromethane, ethyl acetate and methanol. The stems (511.29g) were milled and extracted as above, yielding extracts of 3.96 g, 1.39 g, 0.98 g and 9.32 g from hexane through to methanol respectively.

Separation of the leaf extract

The hexane extract (9.42g) was eluted with a step gradient of hexane:dichloromethane (3:2; 1:1; 2:3; 1:4; 0:1), dichloromethane:ethyl acetate (4:1; 3:2; 1:1; 2:3; 1:4; 0:1) and ethyl acetate:methanol (4:1; 1:1; 0:1). TLC and NMR analysis of the crude fractions indicated that fractions 20-22, 23-40, 61-66 and 67-71, each contained compounds that showed characteristic ^{1}H NMR resonances for either abietane diterpenes or sterols and were chosen to be purified further.

Fractions 20-22 was purified with 80% dichloromethane in hexane on a 2cm diameter column collecting 20 ml fractions, where fractions 11-12 contained stigmasterol (**V**). This compound was identified by its ^{1}H NMR spectrum and by comparison with an authentic sample.

Fractions 23-40, eluted with 60% dichloromethane in hexane and needed no further purification (compound **I**).

Fractions 61-66 was first purified with ethyl acetate:dichloromethane (20:80) on a 3cm column collecting 50 ml fractions. Fractions 8-15 of this column was further purified with ethyl acetate:hexane (60:40) on a 2cm column collecting 20 ml fractions where the pure compound **III** eluted in fractions 6-8.

Fraction 67-71 was purified with 20% ethyl acetate in dichloromethane on a 2 cm diameter column collecting 20 ml fractions where fraction 25-42 contained the pure compound **III**.

The dichloromethane, ethyl acetate and methanol extracts were chromatographed with step gradients from hexane through to methanol but did not result in the isolation of any compounds that could be identified.

Separation of the stem extract

The hexane extract (3.96g) was eluted with dichloromethane:hexane (1:4; 2:3; 3:2; 4:1; 1:0), ethyl acetate:dichloromethane (1:4; 2:3; 3:2; 4:1; 1:0) and ethyl acetate:methanol (4:1; 1:1; 0:1). Two fractions of interest were obtained; fractions 13-16 and 17-23.

Fraction 13-16 was purified further on a 2 cm diameter column with 20% dichloromethane in hexane collecting 20 ml fractions. Fraction 18-20 of this column was further purified with 20% ethyl acetate in hexane as above where fraction 6 contained more stigmasterol (**V**).

Fractions 17-23 of the crude column was purified on a 3cm column with dichloromethane:hexane (3:7 for fractions 1-20 and 7:3 for fractions 21-40), where fractions 27-30 was purified with 30% ethyl acetate in hexane and then fraction 6 of this column was purified with 20% ethyl acetate in hexane where fraction 10-11 resulted in the pure compound **II** and fraction 12 contained lupeol (**VI**). The purifications were done in 2cm columns, collecting 20 ml fractions. Lupeol was identified from its ^{1}H NMR spectrum and in comparison with an authentic sample contained in the laboratory.

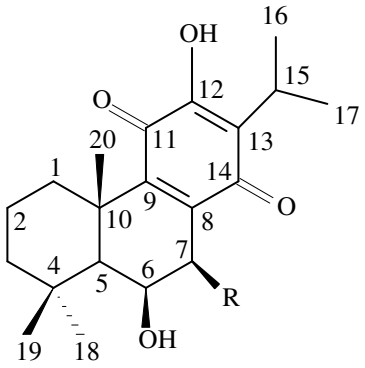
The dichloromethane extract (1.39g) was eluted with dichloromethane:hexane (2:3; 3:2; 4:1; 1:0), ethyl acetate:dichloromethane (1:4; 2:3; 3:2; 4:1; 1:0) and ethyl acetate:methanol (4:1; 1:1; 0:1) on a 3 cm column collecting 100 ml fractions at each stage. Fraction 53-60 was purified with ethyl acetate:dichloromethane (1:1) on a 3cm column collecting 50 ml fractions, where fractions 4-6 yielded compound **IV.**

The ethyl acetate extract (0.98g) was eluted with dichloromethane:hexane (1:4; 2:3; 3:2; 4:1; 1:0), ethyl acetate:dichloromethane (1:4; 2:3; 3:2; 4:1; 1:0) and ethyl acetate: methanol (4:1; 1:1; 0:1). The ^{1}H NMR spectrum of the combined fractions 27-31 indicated the presence of stigmasterol (**V**).

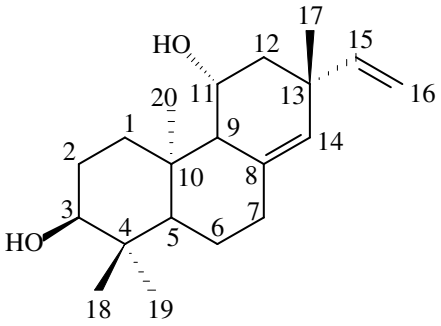
The methanol extract was chromatographed with a step gradient of 10%-40% methanol in dichloromethane but no compounds were isolated for which structural elucidation could be carried out. NMR spectroscopy of the crude extracts showed mainly the presence of sugars.

3.4 Results and Discussion of the compounds isolated

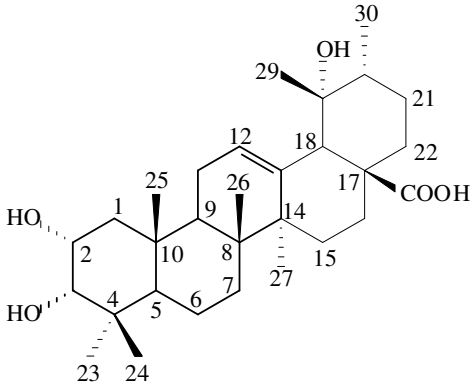
We have isolated the two previously found royleanone compounds (7β-acetoxy-6β-hydroxyroyleoanone (**I** or compound **37** as listed in table 6a) and 6β,7β-dihydroxyroyleaonone (**II** or compound **34** as listed in table 6a) from the hexane extract of the leaves and the hexane extract of the stem of *P. hadiensis*, respectively. This was the first occurrence of compound **III** (*ent*-pimara-8(14),15-diene-3β,11α-diol) in *Plectranthus*, which was isolated from the hexane extract of the leaves. Compound **III** was found previously in *Erythroxylum cuneatum* which belongs to the Erythroxylaceae family (Ansell, 1989). Further to this, three triterpenoids (**IV**-**VI**) were isolated from the dichloromethane extract of the stem. All six compounds isolated from *P. hadiensis* are known compounds.



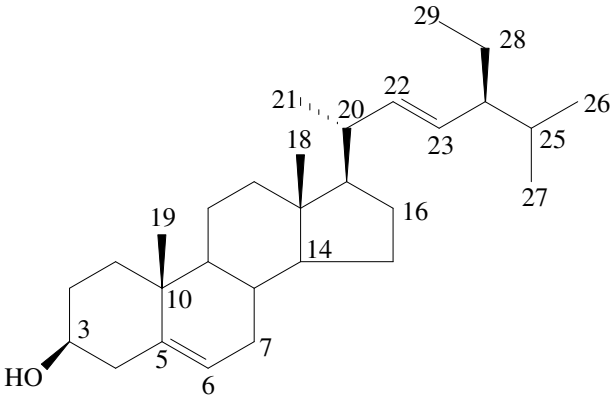
	R
(I)	$OC(O)CH_3$
(II)	OH



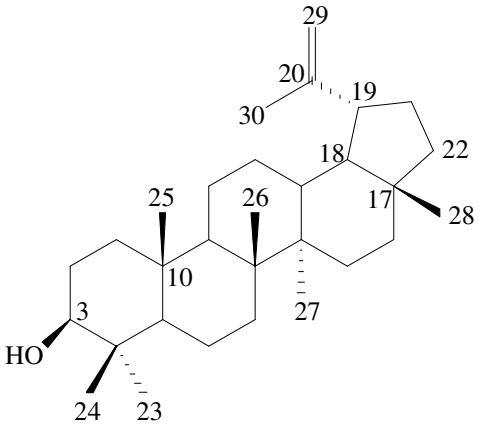
(III)



(IV)

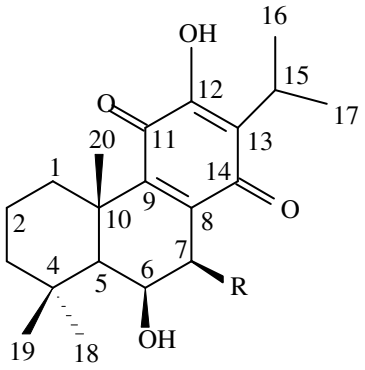


(V)



(VI)

3.4.1 Structural elucidation of Compounds I and II



	R
I	$OC(O)CH_3$
II	OH

Compound **I** is a yellow crystalline compound soluble in dichloromethane and methanol with a melting point of 197 °C and an optical rotation of $[\alpha]^{20}_{D}$ +29° (*c* 0.0052g/100ml, $CHCl_3$) [Mehrotra *et al*., 1989, optical rotation = +23°]. The mass spectrum showed a molecular ion peak at *m/z* 390 corresponding to a molecular formula of $C_{22}H_{30}O_6$ and fragment ion peaks at *m/z* 372 (M^+ - H_2O) and 357 (M^+ - H_2O - CH_3). The IR spectrum showed carbonyl stretching bands at 1734 cm^{-1}, O-H stretching bands at 3377 cm^{-1}, alkene stretching bands at 1641 cm^{-1} and C-H bending vibrations of the isopropyl group at 1375 cm^{-1}. The UV spectrum showed a maximum absorbance at a wavelength of 275nm (log ε = 1.61).

The ^1H NMR spectrum of compound **I** showed deshielded methine resonances at δ_H 5.64 (H-7) and δ_H 4.30 (H-6) as well as a methine resonance of an isopropyl group (H-15) at δ_H 3.14. One of the protons of the methylene group at C-1 resonating at δ_H 2.62 could also be clearly seen as well as three methyl group resonances at δ_H 0.92 (3H-18_{ax}), 1.62 (3H-20_{eq}) and 2.02 ($OC(O)CH_3$). Three other methyl groups were also present in the ^1H NMR spectrum, but were not clearly distinguishingable as they overlapped with each other. These were the methyl resonances at positions 16, 17 and 19 which occurred at δ_H 1.18 and δ_H 1.23.

The ^{13}C NMR spectrum showed the presence of twenty-two carbon resonances of which three were attributed to carbonyl groups (C-11, 14 and the acetyl carbonyl), four to substituted olefinic carbon atoms (C-8, 9, 12 and 13) (indicating two double bonds in the structure), two to quaternary carbon resonances (C-4 and 10), four to methine (C-5,6,7 and 15), three to methylene (C-1, 2 and 3) and six to methyl (C-16, 17, 18, 19, 20 and the acetyl methyl carbon). The methine and methyl resonances were identified using both the DEPT and the HSQC spectra. It must be noted that the C-18 methyl carbon resonance is more deshielded than the methine carbon resonance of C-15. This can be be distinguished clearly in the HSQC spectrum with δ_C 24.17 (C-15) showing a correlation to the methine proton resonance at δ_H 3.14 (H-15) and δ_C 33.68 (C-18) showing a correlation to the methyl proton resonance at δ_H 0.92 (3H-18_{ax}).

The resonances at δ_H 4.30 and δ_H 5.64 were both coupled in the COSY spectrum. Since both these resonances were deshielded, both these groups had an oxygen substituent attached to them. The H-7 resonance showed HMBC correlations to six carbon resonances (δ_C 49.75, 67.05, 137.08, 149.90, 169.61 and 185.76), two of which are aliphatic, two are olefinic and two are carbonyl. The aliphatic carbon resonance at δ_C 49.75 also shows HMBC correlations to three methyl resonances and was therefore attributed to C-5, with the three methyl resonances at δ_H 0.92, δ_H 1.23 and δ_H 1.62 being 3H-18, 3H-19 and 3H-20. Both the olefinic carbon resonances which showed HMBC correlations to H-7 were fully substituted and were therefore attributed to C-8 (δ_C 137.08) and C-9 (δ_C 149.90), which could be distinguished because the resonance at δ_C 137.08 showed an additional HMBC correlation to H-6. Of the two carbonyl resonances which showed HMBC correlations to H-7, one was assigned to the acetyl carbonyl at δ_C 169.61 as this is coupled to the acetyl methyl group at δ_H 2.02 in the HMBC spectrum. The other carbonyl group at δ_C 185.76 was assigned to C-14.

The C-14 carbonyl group showed HMBC correlations to the methine proton resonance at δ_H 3.14 (H-15). This resonance was the methine resonance of the isopropyl group and showed COSY correlations to the equivalent methyl resonances at δ_H 1.18. The H-15

resonance showed HMBC correlations to the other two olefinic resonances at δ_C 150.90 and δ_C 124.67 as well as the carbonyl resonance at δ_C 185.76. These resonances were therefore assigned to C-12 (δ_C 150.9), C-13 (δ_C 124.67) and C-14 (δ_C 185.76). The C-12 and C-13 resonances were distinguished based on chemical shift as the more deshielded resonance of C-12 at δ_C 150.90 was assigned to the olefinic carbon where the hydroxyl group is situated. The hydroxyl group proton resonance at δ_H 7.21 showed HMBC correlations to both C-12 and C-13 and to the other carbonyl resonance at δ_C 183.28, which was then assigned to C-11.

One of the 2H-1 resonances was present at δ_H 2.62 and the other overlapped with the 3H-16 and 3H-17 methyl resonances at δ_H 1.18, determined by the HSQC correlation with C-1. The other two non equivalent methylene resonances at C-2 and C-3 were assigned due to the C-3 carbon resonance at δ_C 42.27 showing a HMBC correlation with the two methyl resonances at δ_H 1.23 (3H-19_{ax}) and δ_H 0.92 (3H-18_{eq}). The C-2 carbon resonance was assigned to the remaining methylene resonance. The resonance at δ_H 1.62 was assigned to 3H-20 since C-5 showed correlations to all three methyl resonances, 3H-18_{eq}, 3H-19_{ax} and 3H-20_{ax}.

Of the remaining two quaternary resonances at δ_C 33.54 and δ_C 38.63, the resonance at δ_C 38.63 was assigned to C-4 based on a HMBC correlation with H-6.

The stereochemistry of H-5, H-6 and H-7 were ascertained from coupling constants and using a molecular model to determine their dihedral angles and assuming the H-5 proton to be axial. Since the H-5, H-6 and H-7 proton resonances were all slightly broadened singlets, we were looking for a model where the dihedral angles between H-5 and H-6, and H-6 and H-7 were close to 90°. This was possible if the H-5 proton was axial (alpha), H-6 was equatorial (alpha) and H-7 was axial (alpha). This indicates that the hydroxyl group at C-6 is axial (beta) and that the acetoxy group at C-7 is equatorial (beta) (Figure 16). This also supports the downfield chemical shift of δ_H 1.62 of 3H-20 as the

1,3-diaxial interaction between 3H-20 and the 6β-hydroxy group results in a paramagnetic shift of the methyl resonance, supporting this assignment.

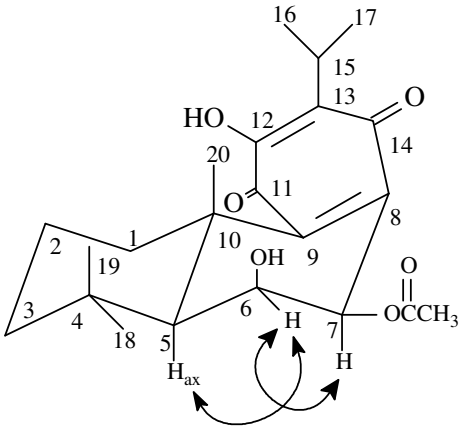


Figure 16: Chair conformation of compound I showing the relationship between H-5, H-6 and H-7

There have been two reports of compound **I** being isolated previously (Mehrotra *et al*., 1989; Rasikari, 2007). The NMR data compares well with literature (Rasikari, 2007), however the C-4 and C-10 resonances are switched around, but we have assigned C-4 based on an HMBC correlation with H-6 and are confident in our assignment. With regard to the stereochemistry of the molecule, in Rasikari (2007) it is important to point out that the stereochemistry in the name of the compound does not correspond with the structure. We think that the compound was incorrectly named as 7α-acetoxy instead of 7β-acetoxy as a coupling constant of 3.5 Hz is used as a basis for this assignment. Mehrotra *et al.* (1989) is also used as a comparison in the identification in Rasikari *et al*. (2007). However, Mehrotra *et al*. (1989) observed a $J_{\text{H-6, H-7}}$ of 3.5 Hz, close enough to that observed by Rasikari (2007) of $J_{\text{H-6, H-7}}$ of 2.0 Hz, and concluded a 6β-axial, 7β-equatorial configuration. The structure of the molecule in Rasikari (2007) is however correctly depicted as the 6β-ax, 7β-eq form.

While the ^{1}H NMR data also compares well to that in Mehrotra *et al*. (1989), the carbon resonances of C-6, 13, 15-17, 19 and 20 all differ by 1-3 ppm and the carbon resonance of C-18 is very different (33.68 as opposed to 21.00 in the literature). The carbonyl

resonance at δ_C 185.76 of C-14 is also different to that of 179.00 and that of the acetyl carbon at δ_C 169.61 is 3ppm higher than the acetyl carbon at 166.00. The C-2 carbon resonance in compound **I** is also 5ppm lower than that cited in Mehrotra *et al*. (1989).

Although Hensch *et al.* (1975) report the 7α-axial acetoxy isomer, the coupling constant $J_{H\text{-}6,\,H\text{-}7}$ is reported to be 2.0 Hz and we think that this is the 7β-equatorial acetoxy isomer. The NMR data is therefore used for comparison. The NMR data for compound **I** compares well with that in Hensch *et al.* (1975).

Compound **II** is a yellow compound soluble in dichloromethane and methanol with a melting point of 125 °C and an optical rotation of $[\alpha]^{20}_{D}$ -47° (*c* 0.0063g/100ml, $CHCl_3$). The GC-MS did not show a molecular ion peak at *m/z* 348 but the LC-MS showed a peak at *m/z* 347 in the negative ion mode which supports the molecular formula of $C_{20}H_{28}O_5$ with a molecular mass of 348 amu. The UV spectrum showed a maximum absorbance at a wavelength of 192nm (log ε = 2.73).

The ^{1}H and ^{13}C NMR data for compound **II** is similar to that of compound **I**, with a few notable differences. The H-7 resonance moved upfield from δ_H 5.64 to δ_H 4.54. The methyl acetyl resonance at δ_H 2.02 was also absent and a new one-proton resonance appeared as a broad singlet at δ_H 3.00. Another broad singlet could now be distinguished at δ_H 1.70. In the ^{13}C NMR spectrum of compound **II**, the acetyl carbonyl resonance was absent as well as the acetyl methyl resonance. Furthermore, the carbonyl stretching band in the IR spectrum was also absent. All these changes were consistent with the acetyl group at C-7 being replaced by a hydroxyl group.

The broad singlet resonance at δ_H 3.00 was attributed to the hydroxyl group proton at C-7 because of a COSY correlation to H-7. This proton resonance did not correlate to any of the carbon resonances in the HSQC spectrum. The resonance at δ_H 1.70 also did not correlate to any of the carbon resonances and was attributed to the hydroxyl group at C-6. This resonance could have also been present in compound **I**, but could not be

distinguished from the other resonances that occurred along with it. The C-9 carbon resonance showed a HMBC correlation with H-7 and C$\underline{H}_3$-20 and the C-8 carbon resonance showed a HMBC correlation to the H-7 resonance as well as the H-6 resonance. These correlations confirmed the assignments of H-6 and H-7 as well as C-8 and C-9.

The H-5, H-6 and H-7 resonances had the same slightly broadened singlet profile as that of compound **I** and therefore the same stereochemistry would be prevalent here as well with a 6β-ax, 7β-eq-dihydroxy form. The paramagnetic shift for the 3H-20 resonance is also observed in compound **II** as well as for 3H-19β-ax methyl group which is more deshielded at δ_H 1.62 than that of 3H-18 at δ_H 1.06.

Compound **II** was isolated previously from Mehrotra *et al.* (1989). Rasikari (2007) and Hensch *et al.* (1975) report the 7α-axial-hydroxy isomer. However, the coupling constants of $J_{H\text{-}6,\ H\text{-}7}$ in both Rasikari (2007) and Hensch *et al.* (1975) are 2.0 Hz and therefore we think that these compounds may also be the 7β-equatorial-hydroxy and not the 7α-axial-hydroxy isomer. The NMR data of all three references are thus used for comparison. The ^{1}H NMR data compare well, with a consistent 6-7 ppm difference for Hensch *et al.* (1975). The ^{13}C NMR data compared well with that of Rasikari (2007). No ^{13}C NMR data is given in both Mehrotra *et al.* (1989) and Hensch *et al.* (1975).

Table 15: ^{1}H NMR data for compound **I** compared with three reference compounds ($CDCl_3$, 400MHz)

Position	**Moiety**	**Compound I**	Rasikari, 2007 (500MHz)	(Mehrotra. *et al*., 1989) (80MHz)	(Hensch *et al*., 1975) (60MHz)
1	CH_2	2.62, *d* (*J* = 12.72 Hz)	2.65, *dt* (*J* = 12.8 Hz)		2.64, *m*
		1.18	1.22, *m*		
2	CH_2	1.57	1.59, *dt* (*J* = 13.4, 3.5 Hz)		
		1.83	1.85, *qt* (*J* = 13.4, 3.5 Hz)		

Continued on next page…..

Position	Moiety	Compound I	Rasikari, 2007 (500MHz)	(Mehrotra. *et al*., 1989) (80MHz)	(Hensch *et al*., 1975) (60MHz)
3	CH_2	1.49	1.49, *dt* (*J* = 12.8 Hz)		
		1.18	1.24, *m*		
5	CH	1.32, *s* ($w_{½}$ = 3.6 Hz)	1.35, *s*		
6	CH	4.30, *s*	4.33, *s*	4.25, *dd* (*J* = 3.5, 2.0 Hz)	4.34, *m*
	-OH				2.46, *s*
7	CH	5.64, *s*		5.62, *d* (*J* = 3.5 Hz)	5.70, *d* (*J* = 2 Hz)
12	-OH	7.21, *s*	7.20	7.20, *s*	7.25, *s*
15	CH	3.14, *sep*	3.18, *hept* (*J* = 7.1 Hz)	3.15, *sep* (*J* = 7 Hz)	3.16, *m* (*J* = 7 Hz)
16	CH_3	1.18, *d* (*J* = 7.08 Hz)	1.24, *d* (*J* = 7.1 Hz)	1.15, *d* (*J* = 7 Hz)	1.20, *d* (*J* = 7 Hz)
17	CH_3				
18	CH_3	0.92, *s*	0.96, *s*	0.90, *s*	0.88,*s*
19	CH_3	1.23, *s*	1.24, *s*	1.20, *s*	1.24, *s*
20	CH_3	1.62, *s*	1.63, *s*	1.60, *s*	1.62, *s*
7-$OCO\underline{C}H_3$		2.02, *s*	2.05, *s*	2.00, *s*	2.02, *s*

Table 16: ^{13}C NMR data for compound **I** compared with three reference compounds ($CDCl_3$, 400MHz)

Position	Moiety	Compound I	(Rasikari., 2007) (125MHz)	(Mehrotra. *et al*., 1989) (20MHz)	(Hensch *et al*., 1975) (60MHz)
1	CH_2	38.35	38.6	38.50	38.7
2	CH_2	18.97	19.2	24.00	24.2
3	CH_2	42.27	42.5	42.50	42.5
4	C	38.63	33.9	39.00	38.4
5	CH	49.75	50.0	49.50	49.9
6	CH	67.05	67.3	68.00	67.2
7	CH	68.74	69.0	70.00	68.9
8	C=C	137.08	137.3	137.50	137.3
9	C=C	149.90	150.1	150.50	150.1
10	C	33.54	38.9	34.50	33.6
11	C=O	183.28	183.5	183.50	185.9
12	C=C	150.90	151.1	151.00	151.1
13	C=C	124.67	124.9	126.00	124.8
14	C=O	185.76	186.0	179.00	183.6
15	CH	24.17	24.4	23.00	23.8

Continued on next page….

Position	Moiety	Compound I	(Rasikari., 2007) (125MHz)	(Mehrotra. *et al*., 1989) (20MHz)	(Hensch *et al*., 1975) (60MHz)
16	CH_3	19.71	19.9	22.00	*19.7
17	CH_3	19.85	20.1	22.50	*19.8
18	CH_3	33.68	33.7	21.00	#33.7
19	CH_3	23.82	24.1	20.00	*19.1
20	CH_3	21.51	21.8	23.50	*21.5
7-$OCO\underline{C}H_3$		20.95	21.1	20.50	*20.9
7-$O\underline{C}OCH_3$		169.61	169.8	166.00	169.9

* Resonance for each methyl group not specified

Resonance was assigned to C-19 by Hensch et al. (1975) but we suspect that it is the resonance for C-18

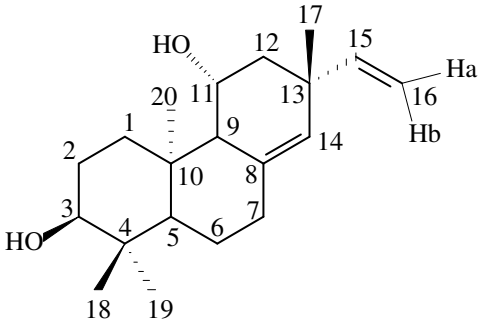
Table 17: ^{1}H NMR data for compound **II** compared with three reference compounds ($CDCl_3$, 400MHz)

Position	Moiety	Compound II	Rasikari, 2007 (500 MHz)	(Hensch *et al*., 1975) (60MHz)	(Mehrotra *et al*., 1989) (80MHz)
1	CH_2	2.61, *d* (*J* = 3.08 Hz)	2.59, *dt* (*J* = 10.2, 3.5 Hz)	2.53, *m*	
		1.17	1.19, *td* (*J* = 12.7, 3.6 Hz)		
2	CH_2	1.57	1.61, *m*		
		1.85	1.82, *qt* (*J* = 13.7, 3.6 Hz)		
3	CH_2	1.27	1.27, *td* (*J* = 13.3, 4.0 Hz)		
		1.48	1.48, *dt* (*J* = 13.1, 2.6 Hz)		
5	CH	1.46, *s* ($w_{½}$ = 4.4 Hz)	1.45, *s*		
6	CH	4.48, *s*	4.45, *s*	4.39, *m*	4.50, *m*
	-OH	1.70, *brs*			
7	CH	4.54, *s*	4.50, *d* (*J* = 1.8 Hz)	4.43, *d* (*J* = 2 Hz)	4.50, *m*
	-OH	3.00, *brs*			
12	-OH	7.34, *s*	7.25, *s*		
15	CH	3.18, *sep*	3.17, *hept* (*J* = 7.1 Hz)	3.10, *m* (*J* = 7 Hz)	3.15, *sep* (*J* = 7 Hz)
16	CH_3	1.24, *d* (*J* = 6.92 Hz)	1.21, *d* (*J* = 7.1 Hz)	1.14, *d* (*J* = 7 Hz)	1.30, *d* (*J* = 7 Hz)
17	CH_3				
18	CH_3	1.06, *s*	1.04, *s*	0.99, *s*	1.05, *s*
19	CH_3	1.27, *s*	1.26, *s*	1.23, *s*	1.20, *s*
20	CH_3	1.62, *s*	1.60, *s*	1.56, *s*	1.65, *s*

Table 18: ^{13}C NMR data for compound **II** compared with one reference compound ($CDCl_3$)

Position	Moiety	Compound II (400MHz)	Rasikari, 2007 (125 MHz)
1	CH_2	38.43	38.8
2	CH_2	19.03	19.3
3	CH_2	42.31	42.6
4	C	33.75	34.0
5	CH	49.50	49.8
6	CH	69.31	69.6
7	CH	69.13	69.4
8	C=C	140.94	141.2
9	C=C	147.54	147.7
10	C	38.56	38.8
11	C=O	183.44	183.7
12	C=C	151.20	151.4
13	C=C	124.26	124.5
14	C=O	189.14	189.4
15	CH	24.02	24.3
16	CH_3	19.86	20.0
17	CH_3	19.80	20.0
18	CH_3	33.52	33.7
19	CH_3	24.27	24.5
20	CH_3	21.62	21.9

3.4.2 Structure elucidation of Compound III



Compound **III** is a colourless compound soluble in both dichloromethane and methanol with a melting point of 89°C and an optical rotation of $[\alpha]^{20}_D$ -79° (*c* 0.0082g/100ml, $CHCl_3$). The mass spectrum showed a molecular ion peak at *m/z* 304 corresponding to a

molecular formula of $C_{20}H_{32}O_2$ and fragment ion peaks at *m/z* 286 and 268. These fragment ion peaks are brought about when compound **III** loses one and two water molecules consecutively. The IR spectrum showed O-H stretching bands at 3396 cm^{-1} and C-H stretching bands at 2930 cm^{-1}. The UV spectrum showed a maximum absorbance at a wavelength of 228 nm (log ε = 1.70).

The ^1H NMR spectrum indicated the presence of four tertiary methyl groups with resonances at δ_H 1.01, 0.99, 0.78 and 0.75, two double bonds with four olefinic proton resonances at δ_H 5.72, δ_H 5.20, δ_H 4.90 and δ_H 4.88 and two oxygenated methine groups with resonances at δ_H 3.26 and δ_H 3.85. The resonances at δ_H 5.72 (*dd*, *J* = 17.21, 10.57 Hz), δ_H 4.90 (dd, *J* = 10.57, 1.16 Hz) and δ_H 4.88 (*dd*, *J* = 17.21, 1.16 Hz) was typical of a vinyl group (H-15, H-16a and H-16b, respectively) (Lyder *et al*., 1998). A deshielded methine resonance at δ_H 3.25 is typical for compounds with a hydroxyl group at the 3-position (H-3, *dd*, *J* = 3.84, 10.84 Hz) (Kalauni *et al*., 2005).

The ^{13}C NMR spectrum showed the presence of twenty carbon resonances typical of a diterpenoid of which four were olefinic 146.18 (CH=), δ_C 137.46 (C), 127.64 (CH=) and 112.69 (CH_2) indicating two double bonds, three were quaternary (δ_C 39.11, 39.05 and 39.57), four were aliphatic methine carbon groups, five were methylene and four were methyl.

The interesting point about this molecule is that the methyl resonance at δ_H 1.01 showed HMBC correlations to the vinylic carbon resonance (C-15), the olefinic resonance at δ_C 127.64 (C-14) and a methylene resonance at δ_C 45.85 (C-12). Since this methyl group was aliphatic, indicated by its chemical shift, the two double bonds could not be conjugated and had to be separated by an aliphatic carbon. Ring C and its constituents could then be constructed as in figure 17 and the methyl proton resonance was assigned to 3H-17.

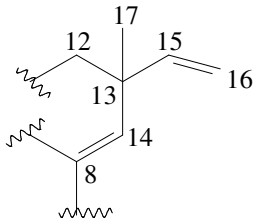


Figure 17: Fragment of Ring C

The equatorial methyl group at C-4, usually resonates at approximately δ_H 28 and the axial methyl group at this position resonates at approximately δ_H 15 (Ansell, 1989; Kang *et al*., 2005; Meragelman *et al*., 2003). This was used to assign the resonances at δ_H 28.43 and δ_H 15.59 to 3H-18β_{ax} and 3H-19α_{eq}, respectively. In the HMBC spectrum, 3H-18β_{ax} and 3H-19α_{eq} showed correlations to C-3 as well as a quartenary carbon at δ_C 39.11 (C-4) and an aliphatic methine at δ_C 53.99 (C-5). The C-5 resonance in turn showed a HMBC correlation to another methyl group at δ_H 0.75 which could only be assigned to 3H-20. This 3H-20 resonance was then used to assign C-10 and C-9 at δ_C 39.05 and δ_C 59.83 respectively because of HMBC correlations to them.

The tertiary carbon atom, C-13 could be assigned to δ_C 39.57 because of a HMBC correlation to H-16a and H-16b. The methine resonance at δ_H 3.85 could then be assigned to H-11 because of a HMBC to C-13. Since this proton resonance is situated downfield, it suggests the presence of a hydroxyl group at this position even though the corresponding carbon resonance at δ_C 66.19 is not as deshielded as other C-O carbon resonances (with chemical shifts between 72-80).

The methylene carbon resonances could not be assigned from the spectra available, therefore, literature with NMR data of similar compounds was used to assign them (Tables 19 and 20) (Ansell, 1989; Kang *et al*., 2005).

We have placed the proton at C-3 in the axial/alpha position and the hydroxyl group in the beta/equatorial position in accordance with the literature (Ansell, 1989). The stereochemistry in Ansell (1989) was determined using coupling constants of the ABX

system where coupling constants of 5.3 Hz and 9.7 Hz indicated an axial proton (α) and an equatorial hydroxyl group (β). The coupling constants of compound **III** compared well to this with values of 3.84 Hz and 10.84 Hz indicating that the hydroxyl group at position 3 in compound **III** was equatorial (β). This was supported by a NOESY correlation between H-3 and H-5 indicating a 1,3-diaxial interaction between the two protons.

The NOESY spectrum was then used to determine the configuration of the hydroxyl group at C-11 as well as the methyl group situated at C-13. The methyl group CH_3-20_{ax} showed a correlation to the proton at H-11 which puts this hydrogen in a beta/axial position and the hydroxyl group in the alpha/equatorial position (Figure 18). It is for this reason that the compound **III** has been named as *ent*-pimara-8(14),15-diene-3β,11α-diol.

The methyl group, CH_3-17 was assigned to the equatorial (alpha) position as it showed NOESY correlations with the H-12α proton at δ_H 1.82. The protons at H-12 was assigned using values from the literature of similar compound, **3.3** (Meragelman *et al*., 2003) (Table 20, page 117) as compound **III** in the literature does not distinguish between the H-12 protons.

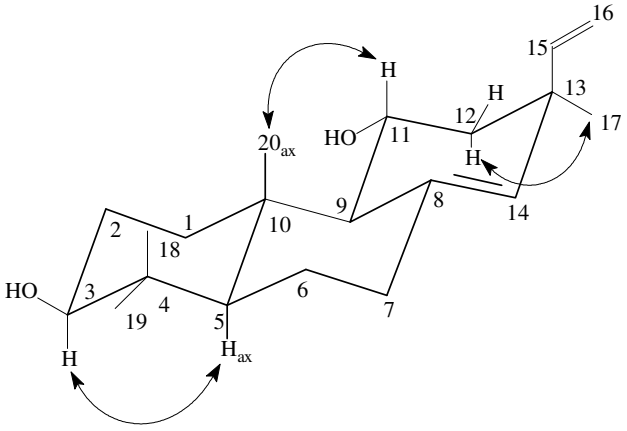


Figure 18: NOESY correlations for compound III

The proton NMR data for compound **III** was similar to that in the literature with the exception of the resonance for the methylene group at C-6 (Table 19). Ansell (1989) reported 2H-6 having a resonance of δ_H 2.37 (*ddd*, *J* = 2.3, 3.3, 13.2 Hz) while in compound **III**, the methylene group at C-6 resonated as singlets at δ_H 1.38 and δ_H 1.63 (Table 19). The ^{13}C NMR data was not reported in the literature and therefore the diacetylated compound was used for comparison (Table 19). Due to insufficient sample, compound **III** could not be acetylated for absolute comparison.

In comparing our compound to that of literature, we have had to make an assumption that the compound in the literature had the hydroxyl groups in the equatorial positions (3β and 11α) as concluded in the discussion and not by what was depicted in the structure. The discussion of the compound in Ansell (1989) did not match the structure (where the hydroxyl groups were axial, 3α and 11β). The ^{13}C NMR data also compared well to similar compounds **3.1** to **3.3** (Table 20, pages 118 and 119).

Table 19: NMR data of compound **III** ($CDCl_3$) and its acetylated equivalent

Position	**Moiety**	**^{1}H**		**^{13}C**	
		Compound III (400MHz)	Ansell, 1989 (80MHz)	**Compound III** (400MHz)	#Ansell, 1989 (20MHz)
1	CH_2	2.35		35.74	36.5
		2.02			
2	CH_2	1.69		27.59	24.0
		1.57			
3	CH-OH	3.26, *dd* (*J* = 3.84, 10.84 Hz)	3.26, *sx* (*J* = 5.3, 9.7, Hz)	78.86	80.6
*4	C			39.11	38.0
5	CH	1.08		53.99	54.0
6	CH_2	1.63	2.37, *ddd* (*J* = 2.3, 3.3, 13.2 Hz)	22.55	22.4
		1.38			
7	CH_2	1.48		38.38	35.6
		1.85			
8	C=C			137.46	136.4
9	CH	1.61		59.83	55.4
*10	C			39.05	38.7

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Position	Moiety	^{1}H		^{13}C	
		Compound III (400MHz)	Ansell, 1989 (80MHz)	**Compound III** (400MHz)	#Ansell, 1989 (20MHz)
11	CH-OH	3.85, *m*	3.86, *ddd* (*J* = 4.7, 7.1, 11.7 Hz)	66.19	69.3
12	CH_2	1.82 (α)		45.85	40.7
		1.35 (β)			
*13	C			39.57	38.7
14	CH=	5.20, *s*	5.21, *d* (*J* = 1.5 Hz)	127.64	128.4
15	CH=	5.72, *dd* (*J* = 10.57, 17.21 Hz)	5.74, *sx* (*J* = 9.5, 18.1 Hz)	146.18	145.4
16	CH_2	4.89, *dd* (*J* = 9.28, 17.4 Hz)	4.87, *dd* (*J* = 1.9, 18.1 Hz)	112.69	112.5
			4.91, *dd* (*J* = 1.9, 9.5 Hz)		
17	CH_3	1.01, *s*	1.02, *s*	29.51	29.5
18	CH_3	0.99, *s*	1.00, *s*	28.43	28.3
19	CH_3	0.78, *s*	0.79, *s*	15.59	15.7
20	CH_3	0.75, *s*	0.76, *s*	15.82	16.6
3	$O\underline{C}OCH_3$	---	---	---	170.2
3	$OCO\underline{C}H_3$	---	---	---	21.1
11	$O\underline{C}OCH_3$	---	---	---	170.6
11	$OCO\underline{C}H_3$	---	---	---	21.5

* these resonances can be used interchangeably
# The ^{13}C NMR data corresponds to the acetylated product of compound **III**

Table 20: ^{1}H NMR data for compound **III** ($CDCl_3$) compared with two similar compounds

Position	^{1}H			
	Compound III (400MHz)	**Compound 3.1** (80MHz)	**Compound 3.2** (300MHz)	**Compound 3.3** (600MHz)
		Ansell, 1989	Kang *et al*., 2005	Meragelman *et al*., 2003
1	2.35		2.68, *s*	1.71, *dt* (*J* = 13.1, 3.5 Hz)
	2.02			0.98
2	1.69			1.61, *m*
	1.57			1.61, *m*
3	3.26, *dd* (*J* = 3.84, 10.84 Hz)	3.31, *dd* (*J* = 4.5, 9.6 Hz)	4.0, *s*	3.21, *dd* (*J* = 11.1, 5,2 Hz)
5	1.08		1.68, *m*	0.82

Continued on next page….

Position	^{1}H			
	Compound III (400MHz)	**Compound 3.1** (80MHz)	**Compound 3.2** (300MHz)	**Compound 3.3** (600MHz)
		Ansell, 1989	Kang *et al*., 2005	Meragelman *et al*., 2003
6	1.63		1.73, *m*	1.63, *qd* (*J* = 13.4, 3.2 Hz)
	1.38			1.49
7	1.48		2.13, *m*	1.22
	1.85		2.35, *m*	1.78, *dt* (*J* = 13.4, 3.2 Hz)
9	1.61		2.02, *d* (*J* = 5.7 Hz)	0.85
11	3.85		4.0, *m*	1.47 (α), *qd* (*J* = 13.4, 3.1 Hz)
				1.47 (β), *m*
12	1.82		1.63, *m*	2.01, *dq* (*J* = 13.7, 3.1 Hz)
15	5.72, *dd* (*J* = 10.57, 17.21	5.74, *septet* (*J* = 18.4, 8.6 Hz)	5.83, *dd* (*J* = 10, 17.8 Hz)	5.98, *dd* (*J* = 17.9, 11.0 Hz)
16	4.89, *dd* (*J* = 9.28, 17.4 Hz)	4.90, *dd* (*J* = 2.1, 18.4 Hz)	4.94, *d* (*J* = 10 Hz)	5.09, *dd* (*J* = 11.0, 1.2 Hz)
		4.96, *dd* (*J* = 2.2, 8.5 Hz)	5.00, *d* (*J* = 17.8 Hz)	5.14, *dd* (*J* = 17.9, 1.2 Hz)
17	1.01, *s*	*1.09, *s*	1.05, *s*	0.91, *s*
18	0.99, *s*	*1.10, *s*	1.19, *s*	0.99, *s*
19	0.78, *s*	*0.84, *s*	0.69, *s*	0.81, *s*
20	0.75, *s*	*0.92, *s*	0.80, *s*	0.93, *s*

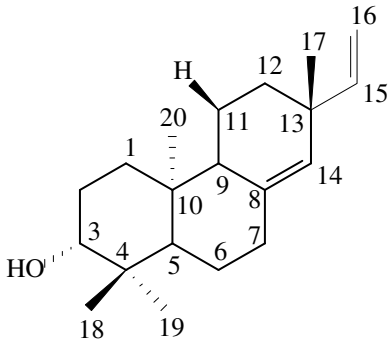
* Resonance for each methyl group not specified

Table 21: ^{13}C NMR data for compound **III** ($CDCl_3$) compared with two similar compounds

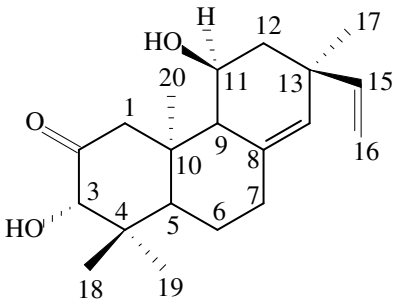
Position	Compound III	**Compound 3.1** (80MHz)	**Compound 3.2** (75MHz)	**Compound 3.3** (50MHz)
		Ansell, 1989	Kang *et al*., 2005	Meragelman *et al*., 2003
1	35.74	37.3	52.2	37.8
2	27.59	27.7	211.0	27.2
3	78.86	79.2	82.2	79.1
4	39.11	38.3	45.3	38.9
5	53.99	54.3	53.3	55.6
6	22.55	22.3	22.3	17.8
7	38.38	35.8	35.2	42.0
8	137.46	137.9	134.6	72.3
9	59.83	51.3	59.1	56.2

Continued on next page…

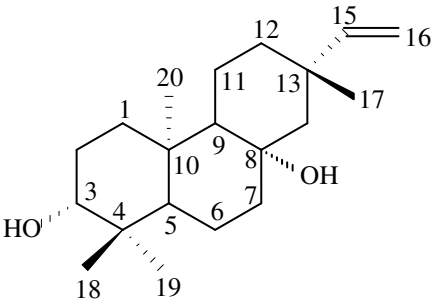
Position	Compound III	**Compound 3.1** (80MHz)	**Compound 3.2** (75MHz)	**Compound 3.3** (50MHz)
		Ansell, 1989	Kang *et al*., 2005	Meragelman *et al*., 2003
10	39.05	38.6	44.7	37.0
11	66.19	19.2	65.8	17.4
12	45.85	35.8	44.0	36.1
13	39.57	38.6	37.8	36.5
14	127.64	128.3	129.1	53.4
15	146.18	147.4	148.3	147.5
16	112.69	112.8	110.9	112.0
17	29.51	29.5	26.5	28.3
18	28.43	28.5	29.3	32.4
19	15.59	15.7	16.4	15.5
20	15.82	14.8	16.5	15.5



3.1

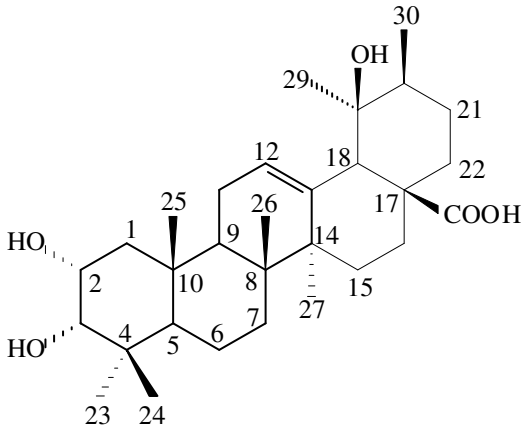


3.2



3.3

3.4.3 Structure elucidation of Compound IV



Compound **IV** is a white compound soluble in methanol with a melting point of 255°C and an optical rotation of $[\alpha]_D^{20}$ +32.26° (*c* 0.0186g/100ml, MeOH). A molecular ion peak of *m/z* 488 was anticipated as this molecular weight corresponded to the molecular formula of compound **IV**, $C_{30}H_{48}O_5$. This peak could not be detected on the mass spectrum but a fragment ion peak of *m/z* 426 and a base peak of *m/z* 218 were documented. The loss of one of the hydroxyl (-OH) group together with the carboxylic acid (-COOH) group, accounts for the fragment ion peak of *m/z* 426. Tertiary alcohols and carboxylic acids seldom show the molecular ion peaks in the mass spectrum. The IR spectrum showed O-H stretching bands at 3424 cm^{-1}, C-H stretching vibrations at 2927 cm^{-1} and a stretching band at 1687 cm^{-1} which is suggestive of a carboxylic acid group since this group has less double bond character than an aliphatic ketone. The UV spectrum showed a maximum absorbance at a wavelength of 211nm (log ε = 2.05). The UV spectra of pentacyclic triterpenoids with a single double bond, a carboxylic acid group and several hydroxyl groups normally show absorbance values at approximately 205-210 nm.

The ^1H NMR spectrum in conjuction with the HSQC and DEPT spectrum showed the presence of seven methyl resonances at δ_H 0.78 (s), δ_H 0.86 (s), δ_H 0.92 (d, *J* = 7.8 Hz), δ_H 0.98 (s, 2 x CH_3), δ_H 1.19 (s) and δ_H 1.35 (s), one broadened triplet olefinic proton

resonance at δ_H 5.29 and two oxygenated methine resonances at δ_H 3.33 (*d, J* = 2.28 Hz) which overlapped with the solvent peaks and δ_H 3.93, a broad doublet of triplets respectively.

The two characteristic olefinic resonances at δ_C 127.95 (CH=) and δ_C 138.66 (C=) suggested an urs-12-ene skeleton. However the tertiary methyl group (3H-28) which is usually present at C-17 in urs-12-ene triterpenes was replaced by a carboxylic acid group evidenced by the carbonyl resonance of the carboxyl group at δ_C 180.88. The C-17 carbon resonance overlapped with the resonances of the deuterated methanol solvent peak between δ 47 and δ 48 and showed HMBC correlations to a methylene and methine resonance at δ_H 2.57 (2H-16) and δ_H 2.49 (H-18), respectively. The proposed resonance for quartenary carbon C-17, falls within the range as documented for similar compounds 4.1, 4.4 and 4.5 (Table 23).

The two proton resonances at δ_H 3.93 and δ_H 3.33 were attributed to H-2_{ax} and H-3_{eq} respectively based on a comparison with the C-17 methylated compounds, where H-2 and H-3 are axial and equatorial respectively (Lontsi *et al*., 1992) and where H-2 and H-3 are both axially situated (Lontsi *et al*., 1998). Our compound compared favourably with the H-2_{ax} and H-3_{eq} configuration. The corresponding carbon resonances at δ_C 65.90 (C-2) and δ_C 78.70 (C-3) are deshielded owing to the oxygenation at these carbons.

In addition to C-2 and C-3, a further deshielded carbon resonance at δ_C 72.18 suggested the presence of another oxygenated group. This carbon resonance was however quaternary and was placed at C-19 with a hydroxyl and methyl attached to it. Literature supported this assignment as C-19 in the literature with the same substitution pattern resonates between δ 71 and δ 74, Table 22 (da Graca Rocha *et al*., 2007; Fang *et al*., 1991; Lontsi *et al*., 1992; 1998).

The C-3 carbon resonance showed HMBC correlations to two tertiary methyl resonances, 3H-23_{eq} (δ_H 0.98) and 3H-24_{ax} (δ_H 0.86) whose corresponding methyl proton resonances

showed HMBC correlations to another methine resonance and one quartenary carbon resonance at δ_C 47.90 and δ_C 38.07, which were then attributed to C-5 and C-4, respectively.

The quartenary carbon resonance at δ_C 39.86 (C-8) showed correlations to two tertiary methyl resonances at δ_H 1.35 and δ_H 0.78 which were assigned as 3H-27 and 3H-26, respectively. The 3H-27 methyl resonance was then used to assign the quaternary carbon resonance of C-14 and the methylene resonance of C-15 to δ 41.35 and δ 28.21 due to HMBC correlations.

The C-19 carbon resonance showed HMBC correlations to two methyl resonances at δ_H 1.19 and δ_H 0.92 (*d*, *J* = 7.8 Hz), which were then attributed to 3H-29_{eq} and 3H-30_{eq} respectively. The two methyl groups were distinguished as the resonance at δ_H 0.92 was a doublet and had to be situated at C-20, which was assigned to δ_C 41.73 because of a HMBC correlation to 3H-29 and 3H-30. The H-18 methine resonance could also be assigned because of a HMBC correlation to C-19.

In addition to the HMBC correlation to C-8, 3H-26 showed correlations to the carbon resonances at δ_C 32.68 and δ_C 46.79 which were then assigned to C-7 and C-9 respectively. The distinction between the two was made as δ_C 32.68 was a methylene carbon resonance and δ_C 46.79 was a methine carbon resonance. The C-9 carbon resonance in turn was used to assign 3H-25, the remaining methyl resonance as C-9 showed HMBC correlations to it. The 3H-25 methyl resonance then showed a HMBC correlation to δ_C 41.09 which was then attributed to C-1.

The carboxylic acid carbon resonance at δ_C 180.88 and the two olefinic carbon resonances at δ_C 127.95 and δ_C 138.66 showed correlations to H-18 and were assigned to C-28 of the carboxylic acid group and C-12 and C-13 of the double bond. The quaternary carbon atom, C-17 was assigned to a resonance underneath the solvent peak due to a

HMBC correlation with H-18. The H-18 resonance was also used to assign the methylene proton resonance of 2H-16 due to HMBC coupling with this resonance.

The remaining methylene carbon resonances of C-6, C-11, C-21 and C-22 were assigned using literature values (Table 22) (da Graca Rocha *et al*., 2007; Fang *et al*., 1991; Lontsi *et al*., 1992; 1998). We think that da Graca Rocha *et al*. (2007) has incorrectly assigned C-15 and C-21 and that these values are in fact switched around. We have based our assignment of C-15 on a HMBC correlation with 3H-27.

Compounds having the ursene skeletal structure posess seven methyl groups with the orientation of five methyl groups (3H-23 to 3H-27) being certain as a result of biosynthesis and two methyl groups (3H-29 and 3H-30) being either alpha or beta. To determine the stereochemistry of these two methyl groups as well as the two oxygenated methines (H-2 and H-3), correlations in the NOESY spectrum was used, in conjunction with a molecular model.

The H-2 proton resonance at δ_H 3.93 showed correlations to the H-3, 3H-24$_{ax}$ (δ_H 0.86) and the overlapping methyl group resonances of 3H-23$_{eq}$ and 3H-25$_{ax}$ at δ_H 0.98. We have assumed that H-2 correlates to the resonance of 3H-25$_{ax}$ since this 3H-25$_{ax}$ is closer in proximity to H-2. Thus, H-2, based on this correlation would be axial and beta and since there is a NOESY correlation between H-2 and H-3, H-3 must also be beta and equatorial. All these NOESY correlations were also observed as possible on a molecular model.

Assuming that all the rings in the molecule are in the more stable chair conformation, H-18 would be in the axial/alpha position. A NOESY correlation between H-18$_{ax}$ and 3H-27 confirms the methyl group (CH_3-27) to be axial/alpha and a further correlation between H-18$_{ax}$ and 3H-29 puts the methyl group (CH_3-29) in the alpha/equatorial position as well (Figure 19).

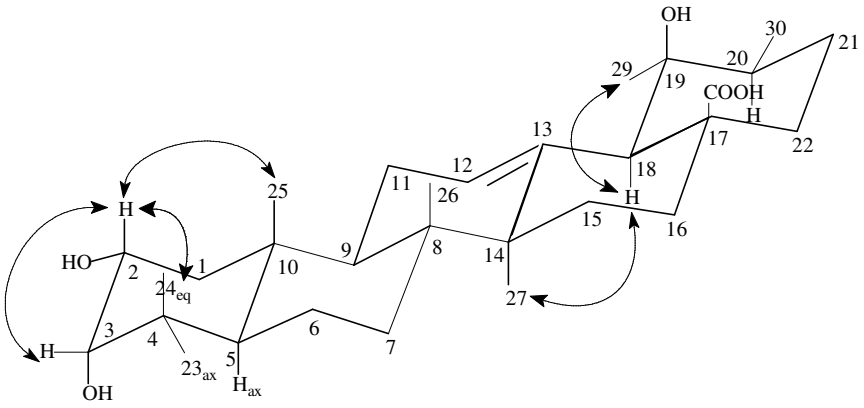


Figure 19: NOESY correlations for compound IV

Based on the analytical data reported for compound **IV** as well as the comparison of this data with literature, compound **IV** was identified as 2α,3α,19α-trihydroxyurs-12-en-28-oic acid (Table 22). A slight difference with regard to chemical shift has been obeserved between compound **IV** and the reference compound (Table 22).

Table 22: NMR data of compound **IV** (CD_3OD) compared with a reference compound

Position	**Moiety**	**^{1}H**		**^{13}C**	
		Compound IV (600MHz)	*da Graca Rocha *et al*., 2007[b] (200MHz)	**Compound IV** (600MHz)	da Graca Rocha *et al*., 2007[b] (200MHz)
1	CH_2	1.28		41.09	37.2
		1.56			
2	CH	3.93, *dt*	4.10, *m*	65.90	64.6
3	CH	3.33, *d* (*J* = 2.28 Hz)	3.90, *d*	78.70	77.9
4	C			38.07	41.6
5	CH	1.26		47.90	46.5
6	CH_2	1.39		17.93	17.7
		1.45			
7	CH_2	1.31		32.68	32.6
		1.58			
8	C			39.86	41.1
9	CH	1.85		46.79	47.6
10	C			37.99	38.1
11	CH_2	2.00		23.41	25.1
12	CH=	5.29, *t* (*J* = 3.72 Hz)	[a]5.20, *s*	127.95	126.9
13	C=C			138.66	138.6

Continued on next page…..

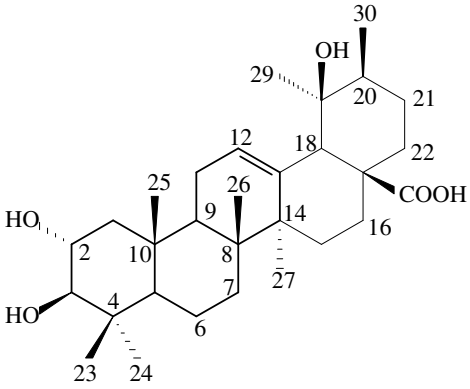
Position	Moiety	^{1}H		^{13}C	
		Compound IV (600MHz)	*da Graca Rocha *et al*., 2007[b] (200MHz)	**Compound IV** (600MHz)	da Graca Rocha *et al*., 2007[b] (200MHz)
14	C			41.35	40.7
15	CH_2	1.01		28.21	25.9
		1.79			
16	CH_2	1.51		25.25	23.1
		2.57			
17	C			47-48	46.8
18	CH	2.49		53.66	53.1
19	C			72.18	71.6
20	CH	1.35		41.73	41.4
21	CH_2	1.23		25.96	29.0
		1.73			
22	CH_2	1.61		37.65	38.0
		1.71			
23	CH_3	0.98	0.69, *s*	27.89	28.9
24	CH_3	0.86	1.30, *s*	21.09	21.8
25	CH_3	0.98	0.89, *s*	16.15	16.3
26	CH_3	0.78	0.95, *s*	15.51	16.1
27	CH_3	1.35	1.22, *s*	23.55	24.1
28	COOH			180.88	178.9
29	CH_3	1.19	1.06, *s*	25.68	26.4
30	CH_3	0.92, *d*	0.90, *d*	15.25	16.6

* coupling constants not documented

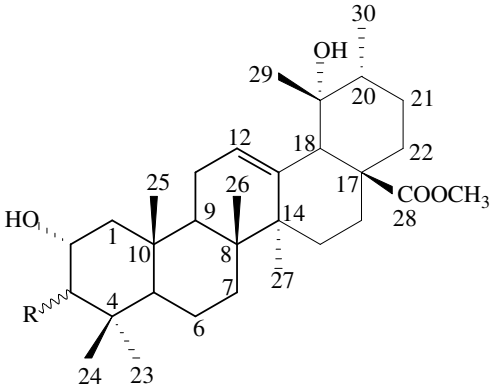
[a] Reported as resonance for H-5 by De Graca Rocha *et al*. (2007) but suspect that this resonance belongs to H-12

[b] DMSO

The ^{1}H and ^{13}C NMR data of three previously isolated compounds (da Graca Rocha *et al*., 2007; Lontsi *et al*., 1998; Fang *et al*., 1991) were compared with that of compound **IV** (Tables 23 and 24) as they closely resemble euscaphic acid, the differences being the orientation of the hydroxyl group at C-3 and C-11 as well as the substituent at the C-17 position. Once again it should be noted that reference compounds **4.1** to **4.3** were analysed in dimethylsulphoxide and chloroform and at different frequencies for ^{1}H and ^{13}C NMR analysis (Tables 23 and 22).



4.1



	R
4.2	β-OH
4.3	α-OH

Table 23: Comparison of ^{13}C NMR data of compound **IV** (CD_3OD) with two similar compounds

Position	**Compound IV** (600MHz)	**4.1**	**4.2**		**4.3**	
		[b]da Graca Rocha *et al*., 2007 (200MHz)	[a]Fang *et al*., 1991 (75MHz)	[a]Lontsi *et al*., 1992 (75MHz)	[a]Fang *et al*., 1991 (75MHz)	[a]Lontsi *et al*., 1998 (75MHz)
1	41.09	39.1	47.7	41.7	41.4	46.34
2	65.9	67.1	68.4	66.5	77.2	68.47
3	78.70	82.3	83.2	78.9	78.6	83.25
4	38.07	41.4	39.1	38.2	39.9	39.14
5	47.95	53.1	55.0	48.0	53.0	55.05
6	17.93	18.1	18.3	18.1	17.9	18.28
7	32.68	32.6	32.4	32.5	32.3	32.44
8	39.86	42.4	39.7	40.1	41.0	39.72
9	46.79	46.7	47.7	46.9	47.7	46.95
10	37.99	37.6	37.9	38.3	37.3	37.94
11	23.41	25.2	23.5	23.6	23.5	23.54
12	127.95	126.7	128.5	129.0	128.8	128.59
13	138.66	138.9	138.0	138.1	138.0	137.99
14	41.35	40.7	41.0	41.2	41.0	40.99
15	28.21	30.4	25.8	28.1	28.0	27.99
16	25.25	25.1	25.2	25.4	25.3	25.23
17	47-48	47.0	46.3	47.9	47.8	47.68
18	53.66	54.8	53.0	53.2	51.5	53.02
19	72.18	71.7	72.8	73.1	72.9	72.83
20	41.73	41.4	41.0	41.1	46.7	40.99
21	25.96	28.1	28.0	26.0	25.3	25.83
22	37.65	39.1	37.2	37.4	37.3	37.24

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Position	Compound IV (600MHz)	4.1	4.2		4.3	
		[b]da Graca Rocha *et al*., 2007 (200MHz)	[a]Fang *et al*., 1991 (75MHz)	[a]Lontsi *et al*., 1992 (75MHz)	[a]Fang *et al*., 1991 (75MHz)	[a]Lontsi *et al*., 1998 (75MHz)
23	27.89	28.8	28.5	28.5	28.5	28.55
24	21.09	17.1	16.7	21.8	21.8	16.72
25	15.51	16.6	16.4	16.2	16.1	16.36
26	16.15	23.3	16.7	16.6	17.9	16.42
27	23.55	24.0	224.3	24.7	24.6	24.37
28	180.88	179.0	178.2	178.2	178.4	178.26
29	25.68	26.4	28.0	27.4	27.2	27.18
30	15.25	16.5	15.9	16.1	16.5	15.97
$COOCH_3$	--	--	51.4	51.6	66.2	51.46

[a] $CDCl_3$
[b] DMSO

Table 24: Comparison of ^1H NMR data of compound **IV** (600MHz, CD_3OD) with three reference compounds

Position	Compound IV	4.1	4.2	4.3
		[b #]da Graca Rocha *et al*., 2007 (200MHz)	[a]Lontsi *et al*., 1992 (300MHz)	[a]Lontsi *et al*., 1998 (300MHz)
1	1.28		1.21, *m*	0.92, *m*
	1.56		1.62, *m*	1.96, *m*
2	3.93, *dt* (*J* = 3.72, 3.18 Hz)	4.10, *m*	3.97, *ddd* (*J* = 11.8, 4.4, 2.8 Hz)	3.36, *ddd* (*J* = 14.7, 9.5, 4.7 Hz)
3	3.33, *d* (*J* = 2.28 Hz)	3.91, *s*	3.40, *d* (*J* = 2.8 Hz)	2.98, *d* (*J* = 9.5 Hz)
5	1.26			0.81, *m*
6	1.39			1.32, *m*
	1.45			1.52, *m*
7	1.31			1.26, *m*
	1.58			1.50, *m*
9	1.85		1.70, *m*	1.65, *m*
11	2.00		1.96, *m*	1.98, *m*
12	5.29, *t* (*J* = 3.72 Hz)	*5.19, *s*	5.33, *dd* (*J* = 3.6, 3.5 Hz)	5.33, *dd* (*J* = 3.6, 3.6 Hz)
15	1.01			1.00, *m*
	1.79			1.60, *m*
16	1.51			1.56, *m*
	2.57		2.49, *ddd* (*J* = 13.7, 11.5, 4.7 Hz)	2.49, *ddd* (*J* = 14.5, 12.6, 4.8 Hz)
18	2.49		2.57, s	2.57, *s*

Continued on next page……

Position	Compound IV	4.1	4.2	4.3
		[b #]da Graca Rocha *et al*., 2007 (200MHz)	[a]Lontsi *et al*., 1992 (300MHz)	[a]Lontsi *et al*., 1998 (300MHz)
20	1.35			1.38, *m*
21	1.23			1.25, *m*
	1.73			1.66, *m*
22	1.61			1.52, *m*
	1.71			1.72, *m*
23	0.98		0.99, *s*	1.01, *s*
24	0.86		0.84, *s*	0.80, *s*
25	0.98		0.93, *s*	0.95, *s*
26	0.78		0.64, *s*	0.65, *s*
27	1.35		1.23, *s*	1.23, *s*
29	1.19		1.18, *s*	1.19, *s*
30	0.92, *d*		0.92, *d*	0.92, *d*
28-OCH_3			3.57, *s*	3.58, *s*

NB ^{1}H NMR data for compounds **4.2** and **4.3** were not documented by Fang *et al*. (1991)

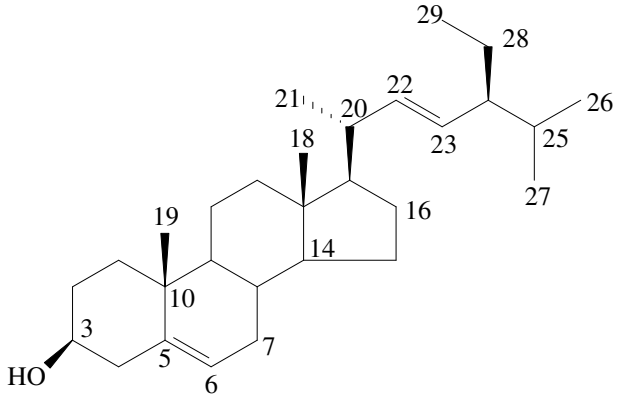
* Reported as resonance for H-5 by da Graca Rocha *et al*. (2007) but suspect that this resonance belongs to H-12

[#] coupling constants not documented

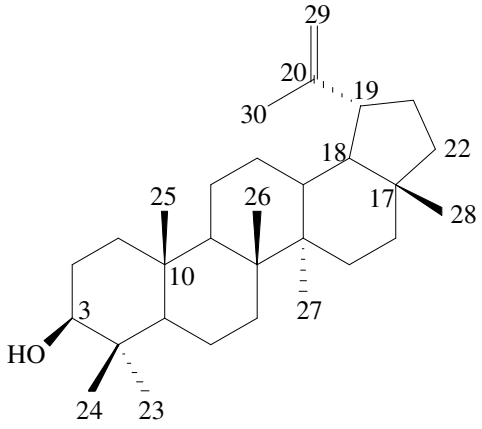
[a] $CDCl_3$

[b] DMSO

3.4.4 Structure elucidation of Compounds V and VI



(V)



(VI)

Compound **V** is a white compound soluble in both dichloromethane and methanol having a molecular formula of $C_{29}H_{42}O$. It has a melting point of 168°C and an optical rotation

of $[\alpha]^{20}_{D}$ -40.8° (*c* 0.12g/100ml, $CDCl_3$). The IR spectrum showed O-H stretching bands at 3305 cm^{-1}, C-H stretching vibrations at 2932 cm^{-1} and alkene stretching bands at 1641 cm^{-1}.

The ^1H NMR spectrum showed the characteristic pattern of the ubiquitous stigmasterol with the three olefinic proton resonances at δ_H 4.99 (*dd*, *J* = 8.64, 15.2 Hz, H-22), δ_H 5.13 (*dd*, *J* = 8.56, 15.2 Hz, H-23) and δ_H 5.32 (*bd*, *J* = 5.04 Hz, H-6) and the deshielded methine resonance at δ_H 3.49 (*m*, H-3), owing to the hydroxy group at this position. On closer examination of the methyl group region of the ^1H NMR spectrum and in comparison with the literature (Langlois, 2000), the methyl groups numbered as 18, 19, 21, 26, 27 and 29 were assigned to δ_H 0.65, 0.67, 0.99 (*d*, *J* = 8.12 Hz), 0.89 (*d*, *J* = 7.18 Hz), 0.80 (*d*, *J* = 6.24 Hz) and 0.82 (*t*, *J* = 6.28 Hz), respectively.

The ^{13}C NMR spectrum indicated the presence of characteristic olefinic resonances at δ_C 121.71 (C-6), δ_C 129.29 (C-23), δ_C 138.31 (C-22) and δ_C 140.76 (C-5). The deshielded oxygenated methine carbon resonance of C-3 occurred at δ_C 71.81. Compound **V** was positivley identified as stigmasterol by comparison with the ^1H and ^{13}C NMR spectra of an authentic sample in the laboratory. The NMR data also compared well with that in literature (Langlois, 2000; Kongduang *et al*., 2008)

Compound **VI** is a white compound soluble in both dichloromethane and methanol having a molecular formula of $C_{30}H_{50}O$. It has a melting point of 214°C and an optical rotation of $[\alpha]^{20}_{D}$ +25.3° (*c* 1.2g/100ml, $CDCl_3$). The IR spectrum showed O-H stretching bands at 3332 cm^{-1}, C-H stretching vibrations at 2941 cm^{-1} and alkene stretching bands at 1637 cm^{-1}.

The ^1H NMR spectrum showed characteristic resonances for the well known pentacyclic triterpenoid, lupeol with the characteristic olefinic proton resonances at δ_H 4.57 (*brs*, H-29_α) and δ_H 4.69 (*brs*, H-29_β), one deshielded methine group resonance at δ_H 3.19 (*dd*, *J* = 5.04, 11.17 Hz, H-3), the H-19 triplet of doublets at δ_H 2.38 (*J* = 5.88, 11.03 Hz), the

multiplet at δ_H 1.91 (H-21) and a very shielded doublet at δ_H 0.68 (J = 9.12 Hz, H-5) (Makatini, 2007). The methyl groups identified as 3H-23 (δ_H 0.97), 3H-24 (δ_H 0.76), 3H-25 (δ_H 0.83), 3H-26 (δ_H 1.03), 3H-27 (δ_H 0.94), 3H-28 (δ_H 0.79) and a methyl group bonded to an olefinic carbon at δ_H 1.68 (3H-30).

The ^{13}C NMR spectrum indicated the presence of thirty carbons with characteristic olefinic resonances at δ_C 150.98 (C-20) and δ_C 109.33 (C-29). The C-3 and C-5 carbon resonance could also be singled out at δ_C 79.01 and δ_C 55.30, respectively, Compound **VI** was identical to an authentic sample of lupeol with regard to physical appearance, melting point, optical rotation and IR spectroscopy. A comparison of the NMR data of compound **VI** with that documented by Burns *et al*. (2000) for lupeol, also proved that the two compounds were identical.

Table 25: ^{1}H and ^{13}C NMR data of stigmasterol (**V**) compared with reference data ($CDCl_3$, 400MHz)

Position	**^{1}H**		**^{13}C**	
	Compound V	Langlois, 2000	**Compound V**	Kongduang *et al*., 2008 (125MHz)
1			37.26	37.21
2			31.66	231.62
3	3.49, *m*	3.50, *m*	71.81	71.80
4			42.31	42.18
5			140.76	140.72
6	5.32, *bd*	5.35, *brs*	121.71	121.71
7			31.87	31.87
8			31.91	31.87
9			50.15	50.08
10			36.51	36.48
11			21.08	21.07
12			39.68	39.64
13			42.32	42.29
14			56.87	56.83
15			24.36	24.34
16			28.91	28.92
17			55.96	55.90
18	0.65, *s*	0.66, *s*	12.04	12.03
21	0.99, *d* (J = 8.12 Hz)	1.00, *d* (J = 6.65 Hz)	21.21	21.05

Continued on next page….

Position	^{1}H		^{13}C	
	Compound V	Langlois, 2000	**Compound V**	Kongduang *et al*., 2008
19	0.67, *s*	0.70, *s*	19.39	19.38
20			40.48	40.50
22	4.99, *dd* (*J* = 8.56, 15.12 Hz)	5.00, *dd* (*J* = 7.60, 15.38 Hz)	138.31	138.32
23	5.13, *dd* (*J* = 8.64, 15.16 Hz)	5.15, *dd* (*J* = 11.53, 15.38 Hz)	129.29	129.22
24	2.25, *m*	2.21, *m*	51.24	51.21
25			32.42	31.87
26	0.89, *d* (*J* = 7.18 Hz)	0.90, *d* (*J* = 6.53 Hz)	20.20	21.20
27	0.80, *d* (*J* = 6.24 Hz)	0.78, *d* (*J* = 6.35 Hz)	18.98	18.95
28			25.40	25.40
29	0.82, *t* (*J* = 6.28 Hz)	0.80, *t* (*J* = 6.5 Hz)	12.24	12.25

NB. Langlois (2000) did not report the ^{13}C NMR data for stigmasterol
Kongduang *et al*. (2008) did not report the ^{1}H NMR data for stigmasterol

Table 26: ^{1}H and ^{13}C NMR data of lupeol (**VI**) compared with reference data ($CDCl_3$, 400MHz)

Position	^{1}H		^{13}C	
	Compound VI	Burns *et al*., 2000 (500MHz)	**Compound VI**	Burns *et al*., 2000 (500MHz)
1		0.90	38.71	38.72
		1.67		
2		1.60	27.42	27.42
		1.56		
3	3.19, *dd* (*J* = 5.04, 11.17 Hz)	3.19	79.01	79.02
-OH		1.26		
4			38.86	38.87
5	0.68, *d* (*J* = 9.12 Hz)		55.30	55.31
6		1.51	18.32	18.33
		1.39		
7		1.39	34.29	34.29
8			40.84	40.84
9		1.27	50.44	50.35
10			37.17	37.18
11		1.41	20.93	20.94
		1.23		
12		1.07	25.15	25.16
		1.67		
13		1.66	38.06	38.07

Continued on next page….

Position	^{1}H		^{13}C	
	Compound VI	Burns *et al*., 2000 (500MHz)	**Compound VI**	Burns *et al*., 2000 (500MHz)
14			42.84	42.85
15		1.00	27.45	27.46
		1.68		
16		1.37	35.59	35.60
		1.47		
17			43.00	43.01
18		1.36	47.99	48.32
19	2.38, *dt* (J = 5.88, 11.03 Hz)	2.38	48.31	48.00
20			150.98	150.98
21		1.32	29.85	29.86
	1.91, *m*	1.92		
22		1.19	40.01	40.02
		1.38		
23	0.97, *s*	0.97	27.99	28.00
24	0.76, *s*	0.76	15.38	15.38
25	0.83, *s*	0.83	16.12	16.13
26	1.03, *s*	1.03	15.98	15.99
27	0.94, *s*	0.95	14.55	14.56
28	0.79, *s*	0.79	18.01	18.02
29	4.57, *brs*	4.56	109.33	109.32
	4.69, *brs*	4.69		
30	1.68, *s*	1.68	19.31	19.32

Physical data for compounds I-VI

Compound I

Chemical name: 7β-acetoxy-6β,12-dihydroxy-8,12-abietadiene-11,14-dione

Common name: 7β-acetoxy-6β-hydroxyroyleanone

Molecular formula: $C_{22}H_{30}O_6$

Physical description: yellow crystals

Melting point: 197°C (228°C, Mehrotra *et al*., 1989)

Optical rotation: $[\alpha]_D^{20}$ +29° (*c* 0.0052g/100ml, DCM) (lit. value = +23° ($CHCl_3$; Cl), Mehrotra *et al*., 1989)

Infrared spectrum ν_{max}^{KBr} cm^{-1}: 3377 (O-H stretching bands), 1734 (carbonyl stretching bands), 1375 (C-H stretching vibrations), 1641 (alkene stretching bands)

Ultraviolet spectrum (λ max): 275 nm (log ε = 1.61)

GC-MS (70eV, direct inlet), relative intensity %: *m/z* 390 (1.46) $[M]^+$, *m/z* 372 (4.85) $[M - H_2O]^+$, *m/z* 357 (0.97) $[M - H_2O - Me]^+$, *m/z* 71 (100)

^1H NMR and ^{13}C NMR data ($CDCl_3$, 400MHz): Refer to tables 15 and 16 on pages 109 and 110

Compound II:

Chemical name: 6β,7β,12-trihydroxy-8,12-abietadiene-11,14-dione

Common name: 6β,7β-dihydroxyroyleanone

Molecular formula: $C_{20}H_{28}O_5$

Physical description: yellow residue

Melting point: 125°C (203-205°C, Hensch *et al*., 1975)

Optical rotation: $[\alpha]_D^{20}$ -47° (*c* 0.0063g/100ml, DCM) (lit. value = -77°, Hensch *et al*., 1975)

Infrared spectrum ν_{max}^{KBr} cm^{-1}: 3369 (O-H stretching bands), 1376 (C-H bending vibrations), 1638 (alkene stretching bands)

Ultraviolet spectrum (λ max): 192nm (log ε = 2.73)

LC-MS (70eV, direct inlet): $[M]^+$ at 347 (negative ion mode)

^1H and ^{13}C NMR data ($CDCl_3$, 400MHz): Refer to tables 17 and 18 on pages 111 and 112

Compound III:

Chemical name: *ent*-pimara-8(14),15-dien-3α,11β-diol

Physical description: white residue

Molecular formula: $C_{20}H_{32}O_2$

Melting point: 89°C (125-130°C, Ansell, 1989)

Optical rotation: $[\alpha]_D^{20}$ -79° (*c* 0.0082g/100ml, DCM)

Infrared spectrum ν_{max}^{KBr} cm^{-1}: 3396 (O-H stretching bands), 2930 (C-H stretching vibrations)

Ultraviolet spectrum (λ max): 228nm (log ε = 1.70)

GC-MS (70eV, direct inlet), relative intensity %: *m/z* 304 (1.94) $[M]^+$, *m/z* 135 (100), *m/z* 286 (10.68) $[M - H_2O]^+$, *m/z* 268 (5.34) $[M - 2Me]^+$

^1H NMR and ^{13}C NMR data ($CDCl_3$, 400MHz): Refer to table 19 on page 116

Compound IV:

Chemical name: 2α,3α,19α-trihydroxyurs-12-en-28-oic acid

Common name: euscaphic acid, jacarandic acid

Molecular formula: $C_{30}H_{48}O_5$

Physical description: white residue

Melting point: 255°C

Optical rotation: $[\alpha]_D^{20}$ +32.26° (*c* 0.0186g/100ml, MeOH)

Infrared spectrum ν_{max}^{KBr} cm^{-1}: 3424 (O-H stretching bands), 2927 (C-H stretching bands), 1687 (C=O)

Ultraviolet spectrum (λ max): 211nm (log ε = 2.05)

GC-MS (70eV, direct inlet), relative intensity %: *m/z* 426 (8.56) $[M - COOH - OH]^+$, *m/z* 218 (100)

^1H NMR and ^{13}C NMR data (CD_3OD, 600MHz): Refer to table 22 on page 124

Compound V:

Chemical name: 5,22-Stigmastadien-3β-ol

Common name: Stigmasterol

Molecular formula: $C_{29}H_{42}O$

Physical description: white residue

Melting point: 168°C (165°C, Jamal *et al*., 2009)

Optical rotation: $[\alpha]_D^{20}$ -40.8° (*c* 0.12g/100ml, $CDCl_3$)

Infrared spectrum ν_{max}^{KBr} cm^{-1}: 3305 cm^{-1} (O-H stretching bands), 2932 cm^{-1} (C-H stretching vibrations), 1641 cm^{-1} (alkene stretching bands)

^1H NMR and ^{13}C NMR data: Refer to table 25 on page 130

Compound VI:

Chemical name: lup-20(29)-en-3β-ol

Common name: Lupeol

Molecular formula: $C_{30}H_{50}O$

Physical description: white residue/powder

Melting point: 214°C (213°C, Fotie *et al*., 2006; 214-215°C, Saeed *et al*., 2003)

Optical rotation: $[\alpha]_D^{20}$ +25.3° (*c* 1.2g/100ml, DCM) (lit. value = +25.7° (*c* 0.70, $CHCl_3$), Fotie *et al*., 2006)

Infrared spectrum ν_{max}^{KBr} cm^{-1}: 3332 cm^{-1} (O-H stretching bands), 2941 cm^{-1} (C-H stretching vibrations), 1637 cm^{-1} (alkene stretching bands)

^1H NMR and ^{13}C NMR data: Refer to table 26 on pages 131

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Chapter 4 Antibacterial and anticancer activity of the compounds isolated

The compounds isolated were tested for their antibacterial and anticancer activity in collaboration with the CSIR as these biological screens were available at the time. The bacterial strains used were the Gram-negative *Pseudomonas aeruginosa* American Type Culture Collection 25922 (ATCC25922) and the Gram-positive *Enterococcus faecalis* (ATCC29212) whilst breast (Michigan Cancer Foundation – Seventh sample (MCF-7)), renal (TK-10) and melanoma (UACC-62) cell lines were assayed for anticancer purposes.

There is no record of antibacterial, antifungal or anticancer testing being done on compounds **I to IV**. However, compounds **33** (6β,7α-dihydroxyroyleanone) and **36** (7α-acetoxy-6β-hydroxyroyleanone) which are 7α-isomers of compounds **I** and **II** respectively, have proven to posess antibacterial activity against six strains of methicillin resistant *Staphylococcus aureus* (MRSA), two strains of vancomycin-resistant *Enterococcus faecalis* (VRE), *Bacillus subtilis, Staphylococcus aureus, Vibrio cholera* and *Xanthomonas campestris* (Laing *et al*., 2006; Teixera *et al*., 1997; Gaspar-Marques *et al*., 2006), antifungal activity against *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Pythium ultimum* and an unknown *Candida* species (Laing *et al*., 2006; Teixera *et al*., 1997; Gaspar-Marques *et al*., 2006), anticancer activity against human lymphocytes induced by phytohaemagglutinin (PHA) and expression of CD69 by T- and B- mouse lymphocytes, antitumor activity against human cancer cell ines MCF-7, NCI-H460, SF-268, TK-10 and UACC-62 (Cerqueira *et al*., 2004; Gaspar-Marques *et al*., 2002) and antimalarial activity against *Plasmodium falciparum* (van Zyl *et al*., 2008) (Tables 12 and 14).

Compound **V** has been isolated twice before from the *Plectranthus* species (Simoes, 2010; Yao, 2002) but this is the first time that it has been isolated from *P. hadiensis*. This is the first report of lupeol (**VI**) being isolated from the *Plectranthus* species.

Since the 7α-isomers of compounds **I** and **II** (**36** and **33**, respectively) exhibited the above mentioned activity, it was decided to determine the antibacterial activity and anticancer activity of these two compounds. The bacterial strains tested were that of *E. faecalis* and *P. aeruginosa* with the hope of these two compounds exhibiting greater activity than that reported in literature where **33** and **36** (having MIC values of 62.50µg/mL and 31.25µg/mL and 15.63µg/mL and 31.25µg/mL against two VRE strains, respectively) displayed stronger activity against VRE than vancomycin (having a minimum MIC value of 125µg/mL) and where **36** displayed more potent activity against MRSA (with MIC values averaging 7.8µg/mL) than oxacillin (having a MIC value of 15.63µg/mL) (Gaspar-Marques *et al*., 2006). The 7-acetoxy function also proved superior in activity to the 7-hydroxy function when tested for anticancer activity against MCF-7, NCI-H460, SF-268, TK-10 and UACC-62 cell lines (Gaspar-Marques *et al*., 2002). The purpose of the anticancer testing performed in this study was to see how the isomers of **36** and **33**, **I** and **II** respectively, faired in the anticancer assays.

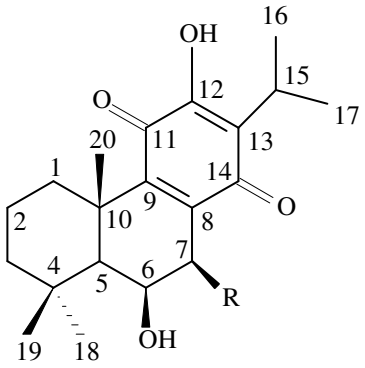
Even though, compound **III** has been isolated by Ansell (1989) there hasn't been any report of this compound showing biological activity. The only report of an *ent*-pimarene displaying antibacterial activity was by Porto *et al*. (2009) where *ent*-8(14),15-pimaradien-3β-ol was found to be active against *E. faecalis* having an an MIC value of 20µg/mL. It is still unknown as to whether *ent*-pimarene compounds possess anticancer activity as they have not been tested against any cell lines to date.

There were three reports of anticancer testing of compound **IV**, however none of these studies tested for activity against the TK-10 (renal) and UACC-62 (melanoma) cell lines. Cheng *et al*. (2010) tested compound **IV** for activity against four human cancer cell lines (MCF-7, tested in this work, NCI-H460, HT-29 and CEM) (Cheng *et al*., 2010). The results indicated that this compound was inactive as an anticancer agent based on cancer cell proliferation (Cheng *et al*., 2010). Studies by Ogura *et al*. (1977) and Mujovo (2010) proved that compound **IV** does possess anticancer activity by testing positive for activity against P-388 lymphocytic leukaemia cell lines (Ogura *et al*., 1977) and exhibiting strong

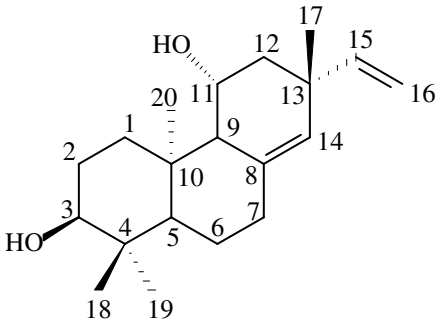
cytotoxic activity against monkey kidney Vero cell lines, having IC_{50} values ranging between 4.52µg/mL and 19.21µg/mL (Mujovo, 2010).

4.1 Antibacterial testing

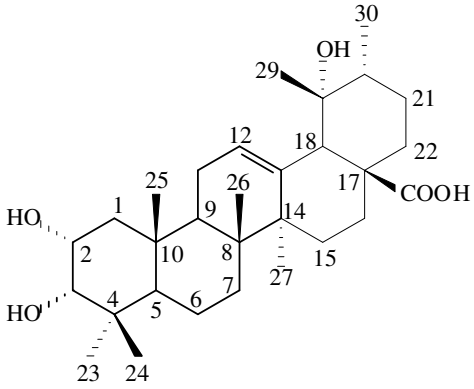
Compounds **I** to **IV** (7β-acetoxy-6β-hydroxyroyleanone (**I**), 7β,6β-dihydroxyroyleanone (**II**), *ent*-pimara-8(14),15-diene-3β,11α-diol (**III**) and 2α,3α,19α-trihydroxyurs-12-en-28-oic acid (**IV**)) were sent to the Council for Science and Industrial Research (CSIR) in Pretoria, South Africa to be assessed for their antibacterial activity. The bacterial strains used were the Gram-negative *Pseudomonas aeruginosa* (ATCC25922) and the Gram-positive *Enterococcus faecalis* (ATCC29212).



	R
(I)	$OC(O)CH_3$
(II)	OH



(III)



(IV)

4.1.1 Experimental

The samples were dissolved in acetone to a known concentration (1.0 mg/mL) prior to being tested, with the exception of compound **IV** which was prepared to a concentration of 0.8 mg/mL due to there being insufficient sample. The antibacterial assays followed the format of the serial microdilution assay (Eloff, 1998). In brief, two-fold serial dilutions of the samples were carried out and made up to 100µL in 96-well microtitre plates. Bacteria (100µL of an overnight culture) was then added to each well before

being incubated for 24 hours at 37°C. A volume of 40µL of 0.2mg/mL Iodonitrotetrazolium chloride (INT, Sigma) was added to each well as indicator of bacterial growth. INT, a colourless tetrazolium salt, is converted to a red-coloured formazan product by actively dividing cells. The minimum inhibitory concentration (MIC) was visually read as the lowest concentration of sample that inhibited microbial growth, as indicated by a visible reduction in the red colour of the INT formazan. In each assay, a negative solvent control and a positive control of the antibiotic was used. Gentamicin (Sigma) was used as the antibacterial control and the samples were tested in triplicate.

4.1.2 Results and discussion

The results of the Minimum Inhibitory Concentration (MIC) determinations of the four compounds against *Enterococcus faecalis* and *Pseudomonas aeruginosa* are given in Table 27. MIC values for the samples and the reference antibiotic, gentamicin are reported in µg/mL.

Table 27: Antibacterial activity of compounds **I** to **IV**

Compound	**Average MIC (µg/mL) 24 hours**	
	E. faecalis	***P. aeruginosa***
I	62.50	62.50
II	31.30	7.80
III	125.00	15.60
IV	200.00	200.00
Gentamicin	**0.39**	**0.78**

The antibacterial activity of compounds **I** to **IV** were evaluated relative to gentamicin. The lower the MIC value, the better the antibacterial activity of the compound. Since the MIC value for gentamicin is below 1µg/mL for both the bacterial strains tested, compounds with MIC values below 10µg/mL were noted to be potent antibacterial agents while compounds having MIC values between 10µg/mL and 100µg/mL were considered to be good antibacterial agents. Compounds exhibiting MIC values between 100µg/mL

and 125μg/mL were categorised as being moderately active. MIC values above 125μg/mL suggested poor activity.

Of the four compounds tested for activity against *E. faecalis* and *P. aeruginosa,* compound **IV** was the only compound which showed poor activity against both microorganisms, having MIC values of 200μg/mL. This compound however was structurally different to the other three, being a tetracyclic triterpenoid whilst the other three compounds were abietane diterpenoids. In the assay against *E. faecalis*, compound **III** exhibited moderate activity having a MIC value of 125μg/mL. Compounds **II** and **III** showed good antibacterial activity with royleanone **II,** having a hydroxy group at position 7, having the highest activity at 31.30μg/mL.

With regard to *P. aeruginosa* the highest activity (regarded as potent) was shown by compound **II**, the royleanone with the hydroxy group at position 7. In comparison to it's acetylated counterpart, compound **I**, it was eight times more active. The pimarane, *ent*-pimara-8(14),15-diene-3β,11α-diol (**III**), was also very active with a MIC value of 15.60μg/mL.

In general, the abietanes showed good to potent activity against both strains being more active against *P. aeruginosa* than *E. faecalis*, with the highest activity being shown by compound **II** (7β,6β-dihydroxyroyleanone). The acetyl group at position 7 of compound **I** seemed to lower the activity in both bacterial strains in relation to the unacetylated compound **II**.

In comparison to the antibiotic gentamicin, compounds **I**-**III** are less potent, however they may have less side effects and be less toxic than standard antimicrobials used in the drug industry and are worth being investigated for development into a new range of antibiotics.

Compounds **I** (7β-acetoxy-6β-hydroxyroyleanone) and **II** (7β,6β-dihydroxyroyleanone) exhibited MIC values of 62.50 μg/mL and 31.30 μg/mL against *E. faecalis*, respectively.

This indicated that the dihydroxy compound had better activity than its monoacetylated counterpart. This is different from that in the literature where the dihydroxy isomer (**33**) showed worse activity with MIC values of 62.50 and 31.25μg/mL than the isomer of the acetylated compound (**36**) with MIC values of 15.63 and 31.25μg/mL against two strains of vancomycin-resistant *E. faecalis*. Even though our compounds displayed weaker activity than the antibiotic, gentamicin, they do exhibit stronger activity than that reported for vancomycin in Gaspar-Marques *et al*. (2006).

4.2 Anti-cancer screening

An in-house test method which was developed by the CSIR was employed for the evaluation of the anticancer activity of compounds **I** to **IV**. This test method was implemented by the CSIR in 1999 and is known as the three cell prescreening method (Fouche *et al*., 2006; 2008). Breast (MCF-7), renal (TK-10) and melanoma (UACC-62) cell lines were three human cell lines chosen for anticancer testing due to their high sensitivity to detect anticancer activity (Fouche *et al*., 2008). Compounds need to demonstrate growth inhibitory activity in this pre-screen panel before proceeding to advanced testing in the full 60-cell-line screen.

Melanoma refers to cancer of the skin and occurs when a UV photon strikes a chromophore in a skin cell. A chromophore is the part of a molecule which gives it colour. When the chomophore is struck by the UV photon, a singlet oxygen ($^1O_2$) or hydroxyl (•OH) free radical is produced, which then travels about the body until it finds a home in a melanocyte, where DNA is mutated by means of oxidation. The damaged melanocyte then becomes a malignant tumour, which is apparent by a change in skin colour, usually to a darker shade. Genetic predisposition, excessive, unprotected exposure to the sun as well as the use of tanning or sun beds are some of the factors which lead to the formation of melanomas.

Breast cancer is a form of cancer which originates from breast tissue, commonly found in the inner lining of milk ducts or lobules, the ducts responsible for the supply of milk.

Breast cancer can develop in both males and females and is detectable by means of self-examination of the breast area. The presence of lumps or masses in this area is usually an indication of cancerous matter, however a mammogram which is performed at most medical centres, must be done to confirm the presence of such matter. Prognosis and survival rate varies greatly depending on cancer type and staging. Treatment includes surgery, drugs (hormonal therapy and chemotherapy) and radiation.

Renal cancer otherwise known as renal cell carcinoma refers to cancer of the kidney. Since the kidney is responsible for the filtration of blood and removal of waste products from the body, renal cell carcinoma affects the lining of the small tubes within the kidney which in turn affects the filtering system. Unfortunately renal cancer is not detected as easily as breast cancer is. Chronic fatigue, hypertension, fever, presence of blood in the urine, pain in the side or lower back as well as a mass or lump in the abdomen are a few of the signs and symptoms associated with this cancer. Chemotherapy, radiation therapy and surgery are some of the treatment options available to patients diagnosed with this particular cancer.

4.2.1 Experimental

In the three cell prescreening method, the three cell lines were grown in Roswell Park Memorial Institute 1640 (RPMI 1640) medium containing 5% fetal bovine serum and 2µM L-glutamine. The cells were then inoculated into 96-well microtiter plates with densities ranging between 5,000 and 40,000 cells per well. A volume of 100µL of the medium was introduced into the microtiter plates and subsequently incubated at 37^{o}C in a 5:95 (carbon dioxide:air) atmosphere with 100% relative humidity for 24 hours.

Compounds **I**-**IV** were dissolved in dimethyl suphoxide (DMSO) and then added to the cells at concentrations ranging between 0.001µg/ml and 100µg/ml. These cells were then incubated for a further 48 hours at 37^{o}C in a humidified atmosphere, followed by the fixing of the cells *in situ* with trichloroacetic acid (TCA) and staining with 100µL sulforhodamine B (SRB) solution. Unbound dye was removed by washing with 1%

acetic acid and air drying the plates. Bound stain was solubilized with 10µM trizma base and the optical density was read on an automated plate reader at a wavelength of 540nm.

The percentage growth of human tumor cells was determined spectrophotometrically by measuring the difference in optical density of the control (*C*) at the start (T_0) and end of drug exposure (*T*). If $T \geq T_0$ either no effect is experienced or inhibition occurs. Inhibition occurs if T<C and no effect is experienced if T=C (Monks *et al*., 1991; Guerrero *et al*., 2006). Dose-response parameters GI_{50} (the concentration at which the growth of the cell is inhibited by 50%) and LC_{50} (the concentration at which 50% of the cells are killed) are calculated using *T*, T_0 and *C* where GI_{50} is calculated as 100 x $[(T-T_0)/(C-T_0)] = 50$ and LC_{50} is calculated as 100 x $[(T-T_0)/(C-T_0)] = -50$ (Guerrero *et al*., 2006). The total growth inhibition (TGI) value symbolizes cytostatic activity and refers to the concentration at which total cell growth is inhibited. This value is calculated in much the same maner as the GI_{50} value: 100 x $[(T-T_0)/(C-T_0)] = 0$ (Fouche *et al*., 2008). The calculations of the dose-response parameters required for plotting the dose-response curves, were performed by the CSIR.

4.2.2 Results and discussion

The results are presented as dose-response curves where the net percentage growth (PG) is represented on the vertical (y) axis and the concentration for each cell line (expressed in µg/mL) is represented on the horizontal (x) axis. The response parameters GI_{50}, TGI and LC_{50} are interpolated values from these graphs, representing the concentrations at which net percentage growth is +50, 0 and -50, respectively (Moodley, 2004). The interpolated values have been determined by the CSIR and are listed in Table 28, page 153.

Compounds **I**-**III** exhibited non-dose-responsive inhibition up to 10µg/mL for all three cell lines with compound **IV** exhibiting non-dose-responsive inhibition up to 100µg/mL. The fact that compound **IV** behaved differently from the other three compounds is not

surprising since this compound, a pentacyclic triterepenoid, is structurally different to the other three abietane diterpenoids.

The criteria followed by the CSIR states that compounds with TGI > 50µg/mL are regarded as being inactive, TGI ranging between 15µg/mL and 50µg/mL are weakly active whilst TGI values between 6.25µg/mL and 15µg/mL are indicative of compounds which are moderately active. Any compound having a TGI value of less that 6.25µg/mL for at least two of the three cell lines, is considered to be potent (Fouche *et al*., 2008). According to these criteria, compounds **I** to **III** are weakly active towards the melanoma cell line (44.71 µg/mL (**I**), 42.18µg/mL (**II**) and 44.64µg/mL (**III**)) while the TGI values obtained for the breast (51.20µg/mL, 52.72µg/mL and 81.22µg/mL, respectively) and renal (49.10µg/mL, 55.84µg/mL and 53.99µg/mL, respectively) cell lines were only slightly above 50µg/mL suggesting that these compounds could also be regarded as weakly active anticancer agents against these cell lines (Table 28). Since **I** to **III** are regarded as weakly active, slight chemical modifications to the structure may enhance it's anticancer activity. The chemical modifications could not be carried out in this work since the samples isolated were insufficient for chemical derivatisation.

The standard used in the three cell pre-screening method was Etoposide having TGI values of >100µg/mL, 36.20µg/mL and 27.00µg/mL for breast (MCF-7), melanoma (UACC-62) and renal (TK-10) human cell lines, respectively. In comparison to the standard, compounds **I** to **III** were proven to be less active with regard to the renal and melanoma cell lines. Compounds **II** and **III** displayed TGI values of 55.84µg/mL and 53.99ug/mL respectively for the renal cell line. These values are twice that of etoposide having a TGI value of 27.00µg/mL against the same cell line. Compounds **I** to **III** were found to be more active against the breast cell line than the control with compounds **I** and **II** having TGI values measuring half at most of that of etoposide with values of 51.20µg/mL and 52.72µg/mL, respectively.

Compounds **I** to **III** inhibited the growth of the melanoma cell line (UACC-62) more than the other two cell lines having GI_{50} values ranging between 12.05µg/mL and 13.53µg/mL

whereas GI_{50} values for the renal and breast cell lines ranged between 21.02µg/mL and 26.50µg/mL, and 20.57µg/mL and 39.08µg/mL, repectively. These GI_{50} values indicate weak activity as considered by the NCI's criteria (Fouche *et al*., 2008).

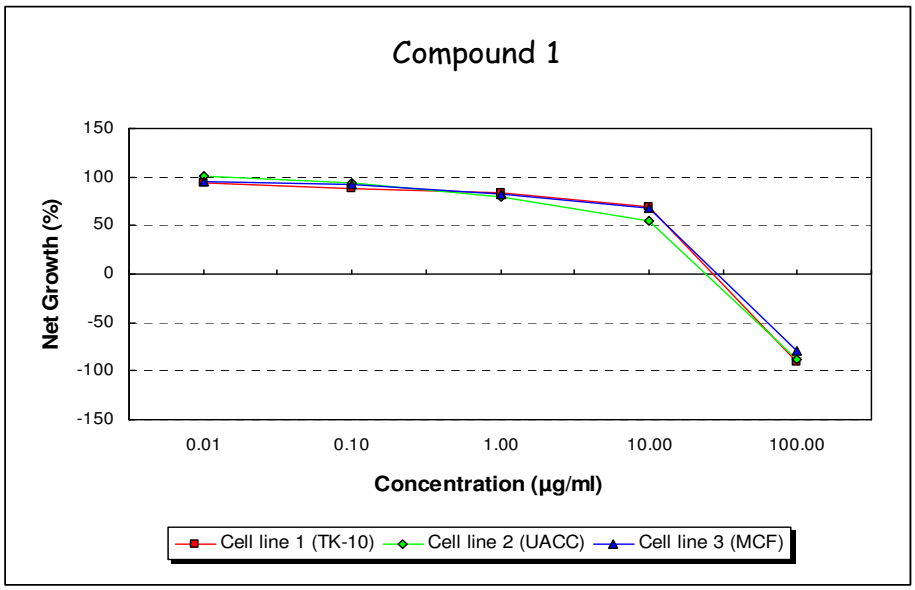


Figure 20: Dose response curve for 7β-acetoxy-6β-hydroxyroyleanone (I) against TK-10, UACC-62 and MCF-7 cell lines

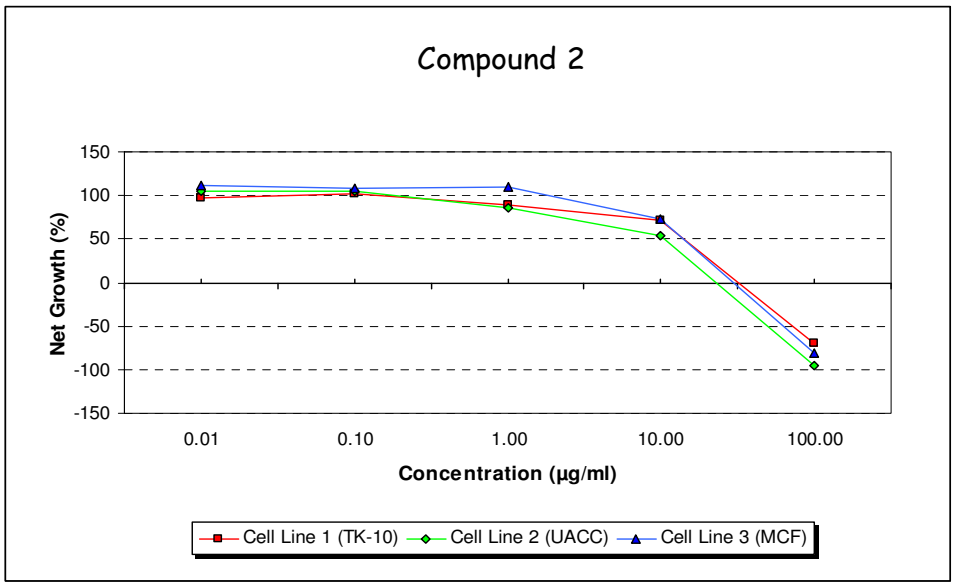


Figure 21: Dose response curve for 7β,6β-dihydroxyroyleanone (II) against TK-10, UACC-62 and MCF-7 cell lines

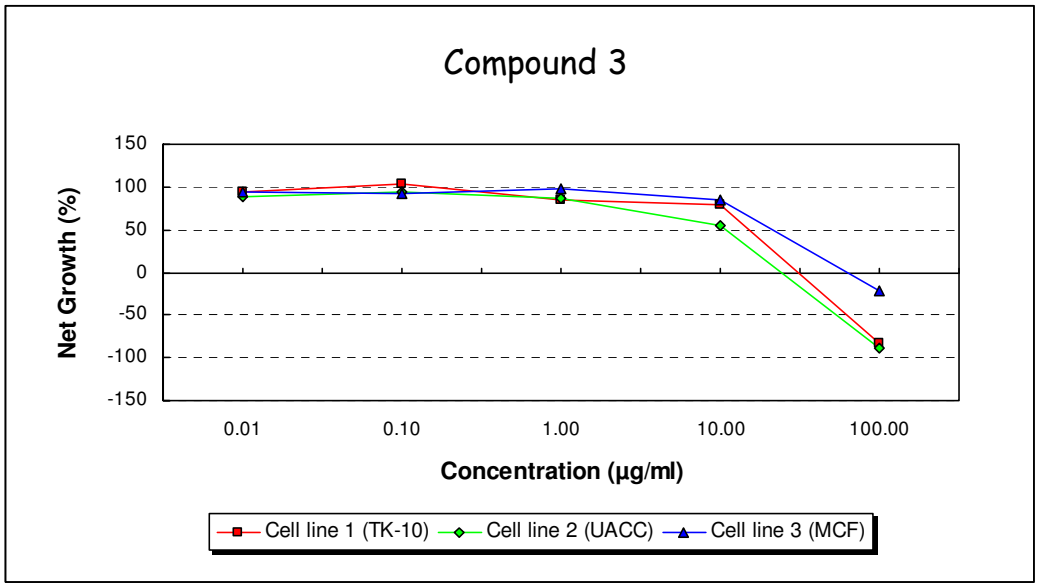


Figure 22: Dose response curve for *ent*-pimara-8(14),15-diene-3β,11α-diol (III) against TK-10, UACC-62 and MCF-7 cell lines

Compound **IV,** the only triterpenoid tested was only capable of inhibiting the growth (GI_{50}) of the melanoma and breast cancer cell lines at concentrations which classified this compound as inactive and showed no potential killing activity (LC_{50} or LC_{100}) even at 100µg/mL.

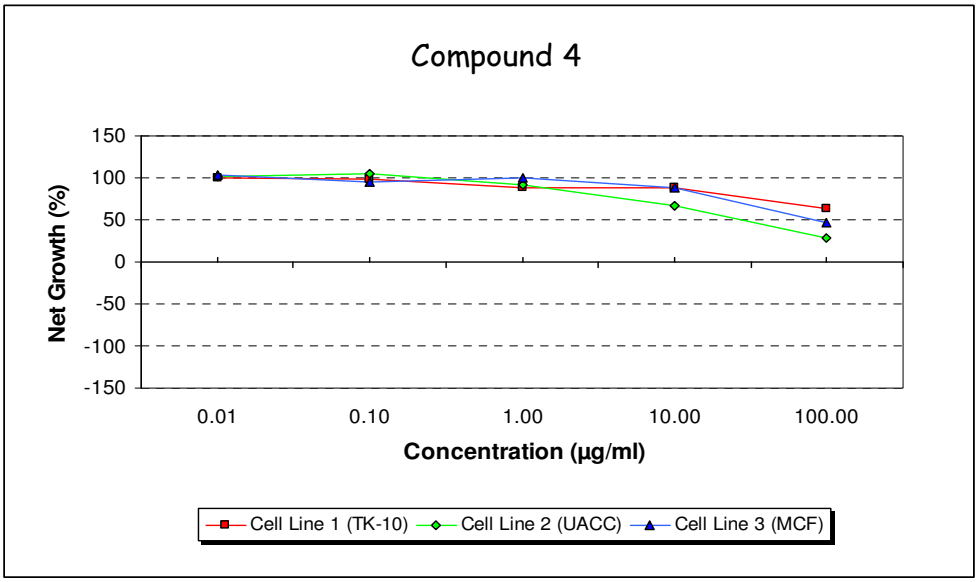


Figure 23: Dose response curve for 2α,3α,19α-trihydroxyurs-12-en-28-oic acid (IV) against TK-10, UACC-62 and MCF-7 cell lines

Table 28: Growth inhibition values for compounds **I**-**IV** against TK-10, UACC-62 and MCF-7 cell lines

Compound	Dose-response parameters	Line 1 (TK-10) renal	Line 2 (UACC-62) melanoma	Line 3 (MCF-7) breast
I	GI_{50}	21.02	13.42	20.57
	TGI	49.10	44.71	51.20
	LC_{50}	77.18	76.00	81.84
	LC_{100}	N/A	N/A	N/A
II	GI_{50}	24.06	12.05	23.63
	TGI	55.84	42.18	52.72
	LC_{50}	87.63	72.32	81.82
	LC_{100}	N/A	N/A	N/A
III	GI_{50}	26.50	13.53	39.08
	TGI	53.99	44.64	81.22
	LC_{50}	81.48	75.74	N/A
	LC_{100}	N/A	N/A	N/A
IV	GI_{50}	N/A	48.67	92.57
	TGI	N/A	N/A	N/A
	LC_{50}	N/A	N/A	N/A
	LC_{100}	N/A	N/A	N/A
Etoposide	TGI	27.00	36.20	>100

NB. N/A denotes inactivity

The GI_{50} values for compounds **I** and **II** are compared to that of their isomers (Gaspar-Marques *et al*., 2002) in Table 29. In our results, the GI_{50} values of both **I** and **II** are similar. However, this is not the case with the GI_{50} values for the isomers, compounds **33** and **36** reported in the literature. Compound **36** displayed significantly better activity than any of the other three compounds against the three cell lines used. Our two compounds **I** and **II** however showed better activity than the dihydroxy isomer (**33**).

Table 29: GI_{50} values (μM) of compounds **I** and **II** and their 7α-isomers

Compound	Cell line		
	MCF-7	TK-10	UACC-62
I	34.41	53.90	52.74
36	6.4	7.4	4.5
II	34.63	69.14	67.90
33	48.3	107.6	77.9
Doxorubicin	0.055	0.570	0.094

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Chapter 5 Conclusion

The aim of this research project was to isolate and identify the compounds extracted from *Plectranthus hadiensis* and to compare the phytochemistry with previous studies as well as to test the compounds isolated for antibacterial and anticancer proprties.

According to literature (Abdel-Mogib *et al*., 2002), abietane diterpenes are the most abundantly occurring compounds in the genus *Plectranthus* with there being two instances where ten triterpenes (oleanolic acid, ursolic acid, betulin, β-sitosterol, plectranthoic acid, plectranthoic acid A, plectranthoic acid B, acetylplectranthoic acid, plectranthadiol and hexacosanol) have been isolated (Misra *et al*., 1971; Razdan *et al*., 1982).

Compounds **I** and **II** have been isolated only once before (Mehrotra *et al*., 1989) from *Coleus zeylanicus* which is a synonym for *Plectranthus hadiensis*. This is the first report of compounds **III** to **VI** being isolated from *P. hadiensis*. Lupeol has never been isolated in the *Plectranthus* genus, however an isopimarane (7α,18-dihydroxy-isopimara-8(14),15-diene) was reported in *Plectranthus diversus* (Rasikari, 2007) and stigmasterol has been isolated twice before, once from *Coleus* and once from *Plectranthus*.

Three of the six compounds isolated from *P. hadiensis*, (7β-acetoxy-6β-hydroxyroyleanone (**I**), 7β,6β-dihydroxyroyleanone (**II**) and *ent*-pimara-8(14),15-diene-3β,11α-diol (**III**)) were abietanes while the other three were common pentacyclic triterpenes known as euscaphic acid (**IV**), stigmasterol (**V**) and lupeol (**VI**). These three triterpenoids add to the ten previously isolated from the *Plectranthus* species. Even though ethyl acetate and methanol were used as extraction solvents, nothing of interest was isolated from these solvents. The relatively non-polar hexane extract yielded five of the six compounds with euscaphic acid **IV** being isolated from the dichloromethane extract**.** The isolation of triterpenoids in *P. hadiensis* is not surprising as most plant species contain these common sterols.

The isolation of a pimarane in *Plectranthus* was an interesting find as pimarane-type diterpenes are abundant in the Lamiaceae family (to which *Plectranthus* belongs) being found predominantly in the *Salvia* and *Nepeta* genera and to a lesser extent in the *Rabdosia*, *Callicarpa*, *Premna*, *Amaracus*, *Orthosipho* and *Satureja* genera (Alvarenga *et al*., 2001). The finding of a pimarane from *Plectranthus* may now provide a biochemical link between *Plectranthus* and these genera. Pimarane-type diterpenes have also been isolated from the *Erythroxylum* genus (Ansell, 1989) which suggests a link between the Lamiaceae family to which *Plectranthus* is a member of and the Erythroxylaceae to which *Erythroxylum* belongs.

Compounds **I** and **II** showed good activity against *Enterococcus faecalis* and *Pseudomonas aeruginosa* with MICs for compound **I** being 62.5µg/mL against both bacterial strains and that of compound **II** being 31.30µg/mL and 7.8µg/mL for *E. faecalis* and *P. aeruginosa,* respectively. This result is unexpected as royleanones having a highly oxidised substituent at C-7 (such as the acetyl group in compound **I**) are usually more active than those bearing a hydroxyl group at positions C-6 and C-7 (Teixeira *et al*., 1997; Gaspar-Marques *et al*., 2006). The pimarane, *ent*-pimara-8(14),15-diene-3β,11α-diol (**III**), although inactive against *E. faecalis* was very active against *P. aeruginosa*.

Based on criteria set by the CSIR where the total growth inhibition (TGI) value is used to evaluate the anticancer activity of compounds, compounds **I** to **III** are considered to be weakly active against breast (MCF-7), renal (TK-10) and melanoma (UACC-62) cell lines. However when compared to the positive control, etoposide, compounds **I** to **III** exhibit better antitumor activity against the breast cell line than the control. Compound **IV** was inactive against all three cell lines.

Because compounds **I** to **IV** showed weak activity in the three cell prescreen against the breast, renal and melanoma cell lines, there was no need to to pursue further anticancer testing i.e. the 60-cell-line screen.

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Appendix

Spectra

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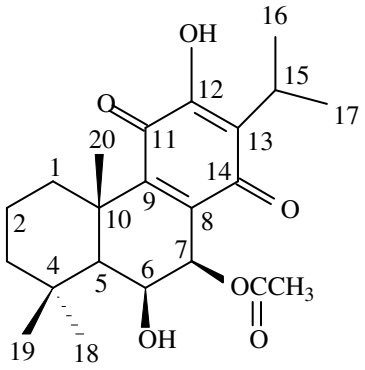
Compound I, 7β-acetoxy-6β-acetoxyroyleanone



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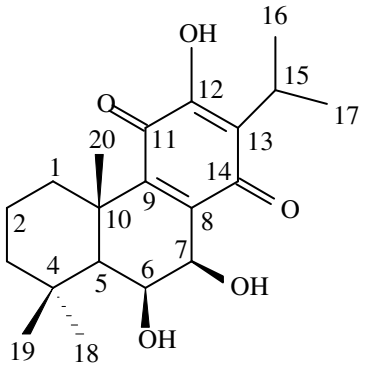

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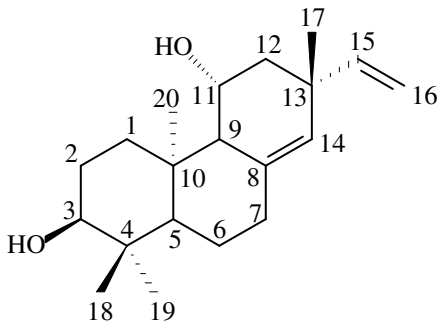

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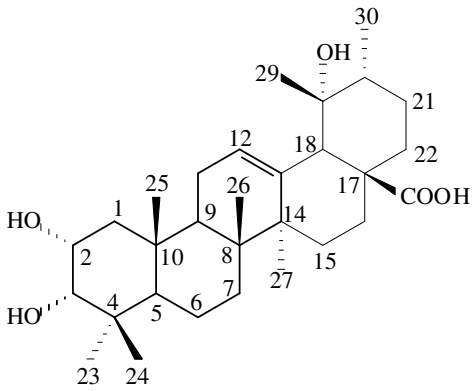
Compound IV, euscaphic acid



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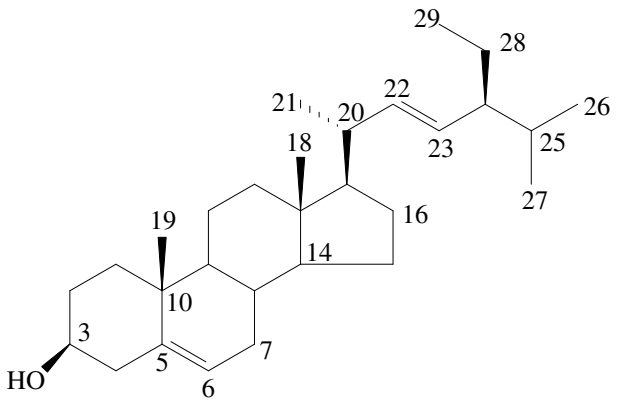
Compound V, stigmasterol



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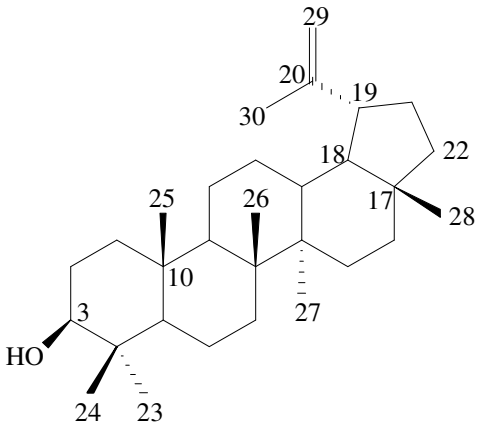
Compound VI, lupeol



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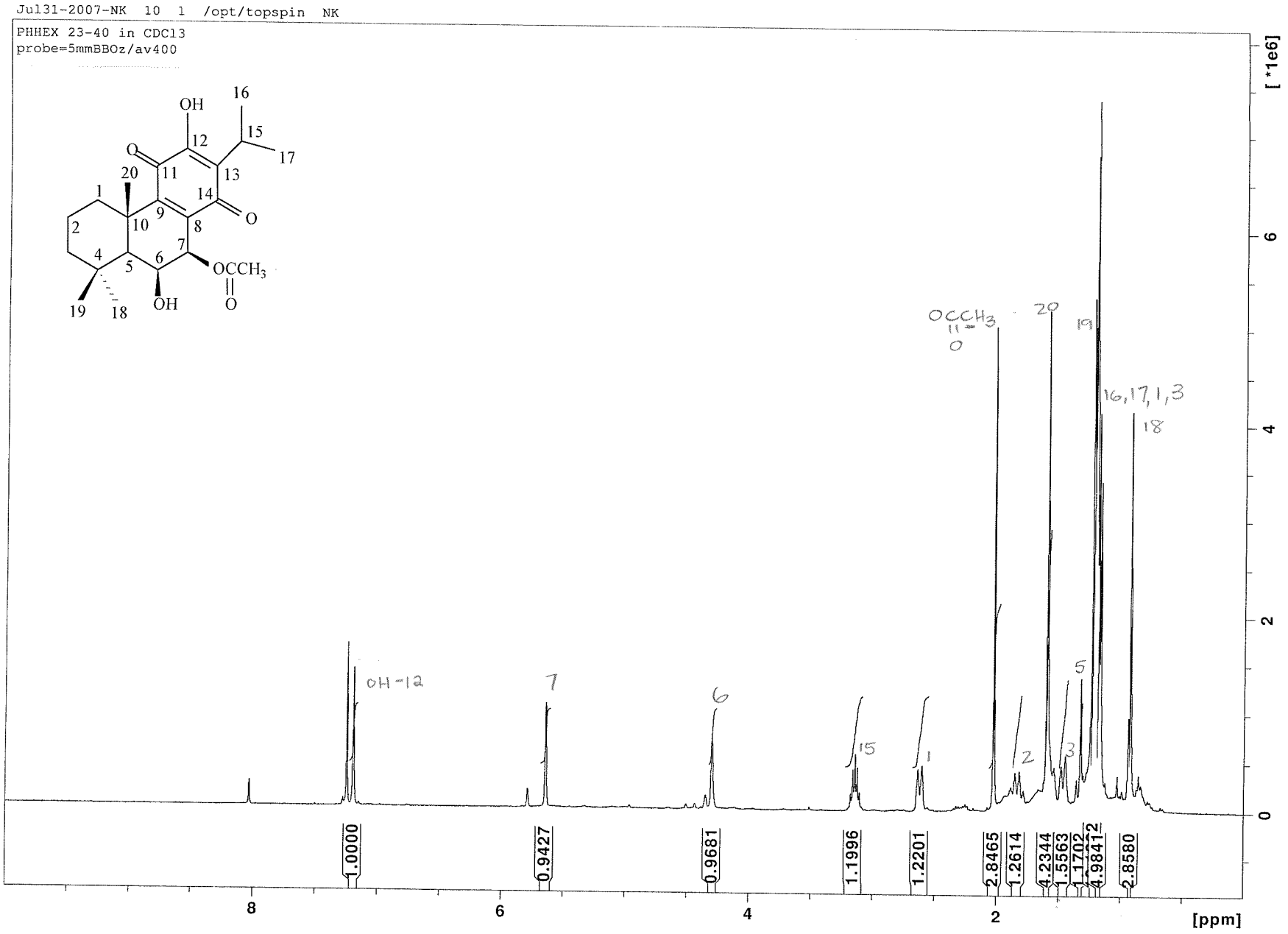
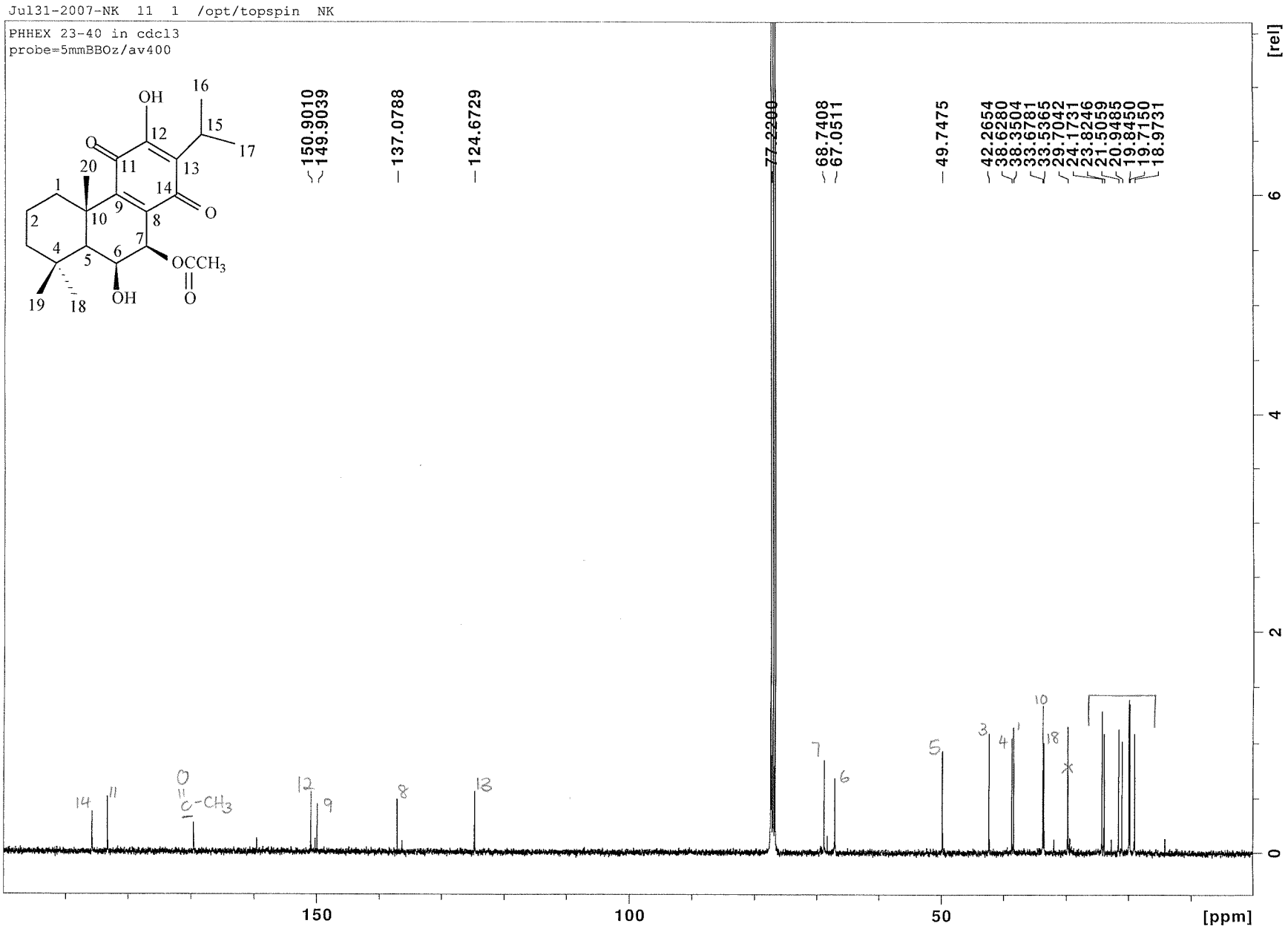


Spectrum 1a: ^{1}H NMR spectrum of compound **I**, 7β-acetoxy-6β-hydroxyroyleanone



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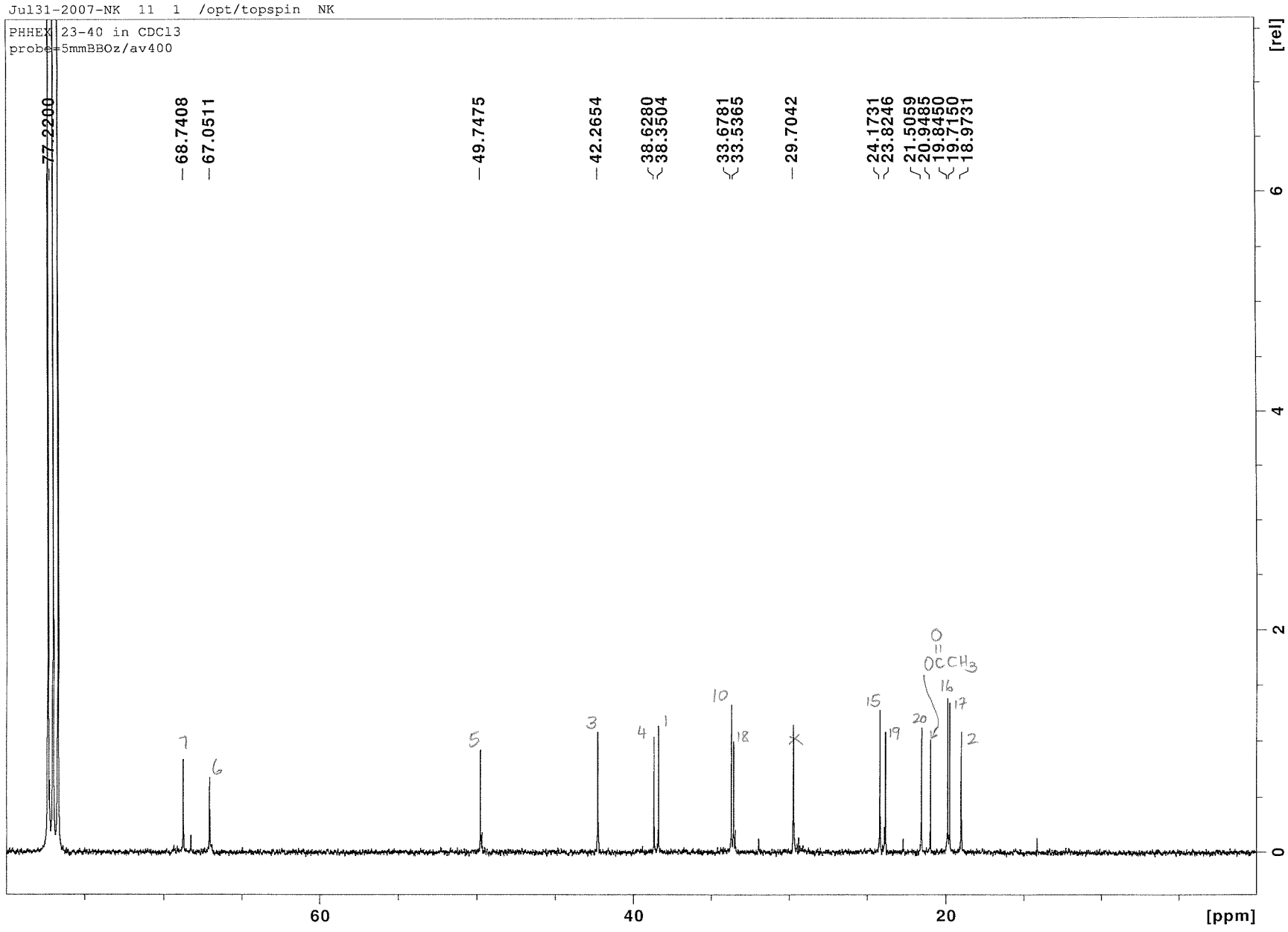
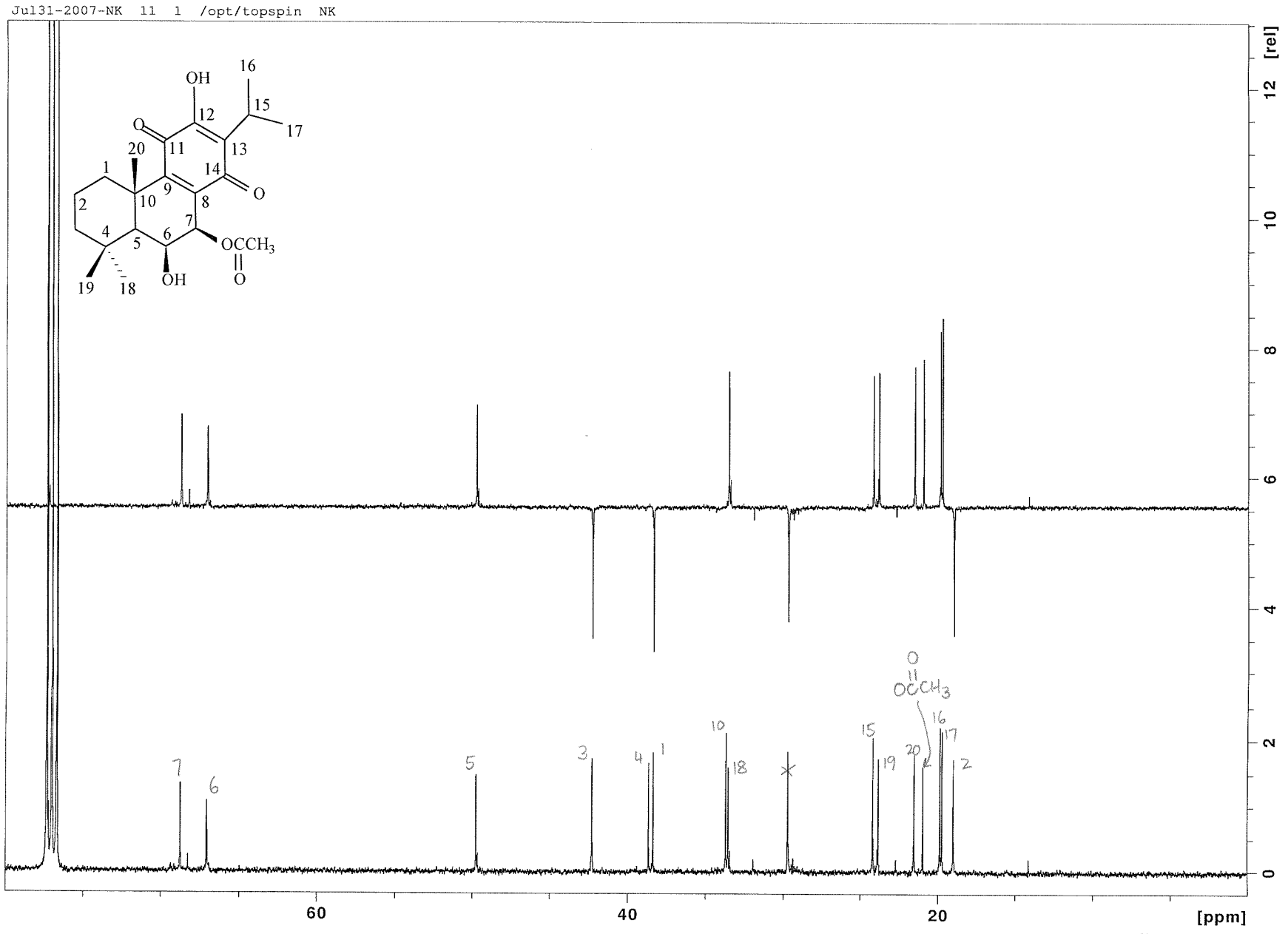
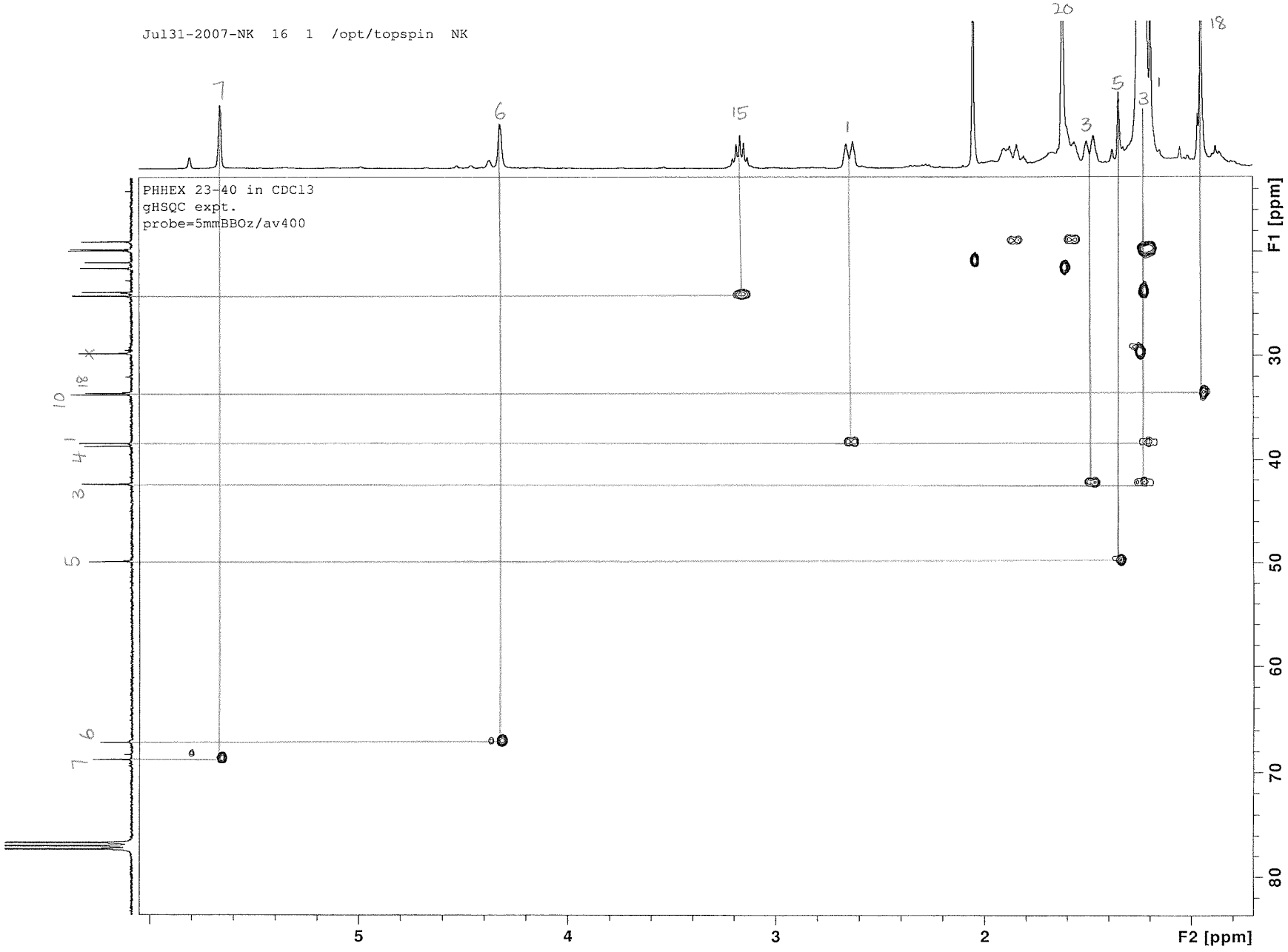


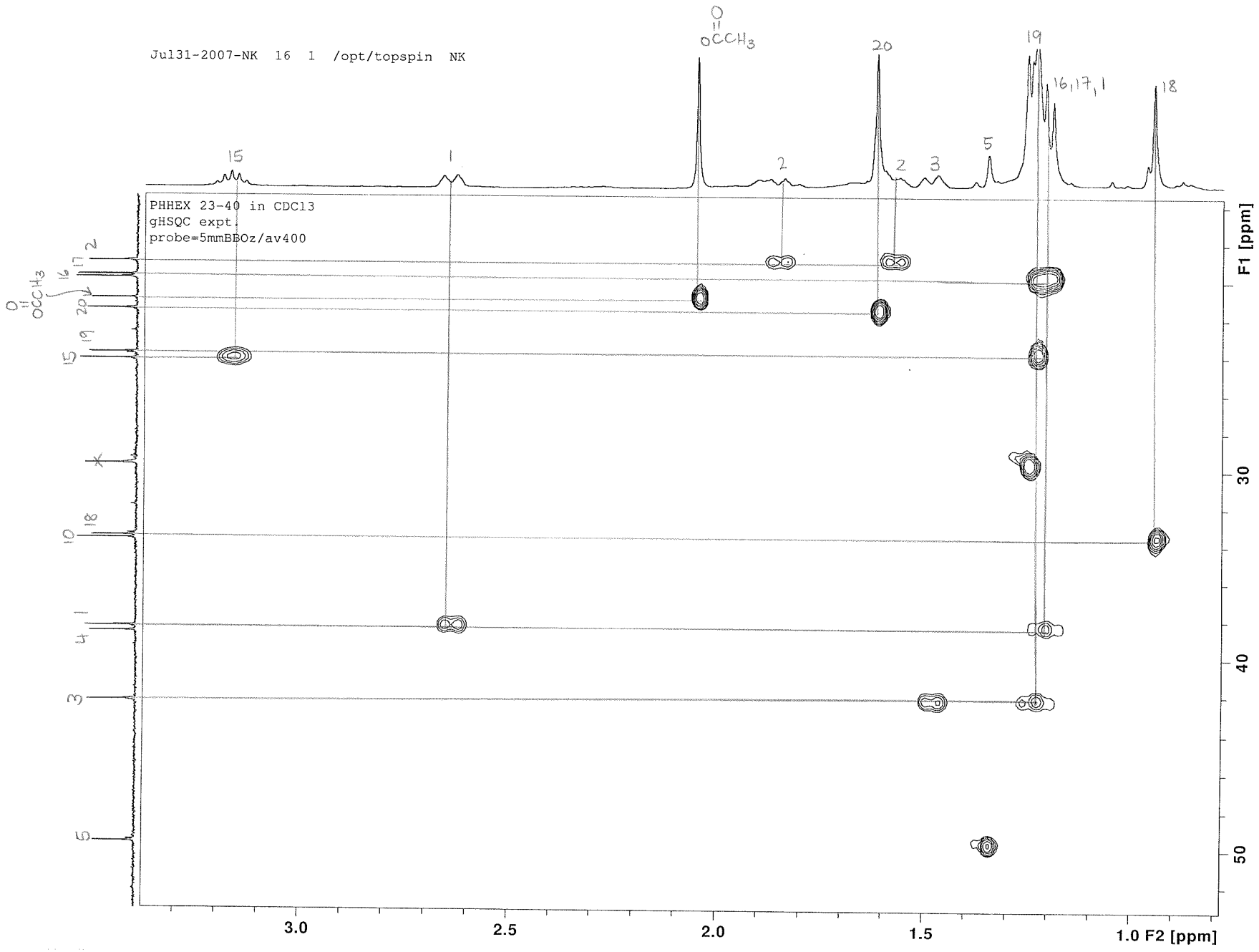
Spectrum 1c: Expanded ^{13}C NMR spectrum of compound **I**, 7β-acetoxy-6β-hydroxyroyleanone



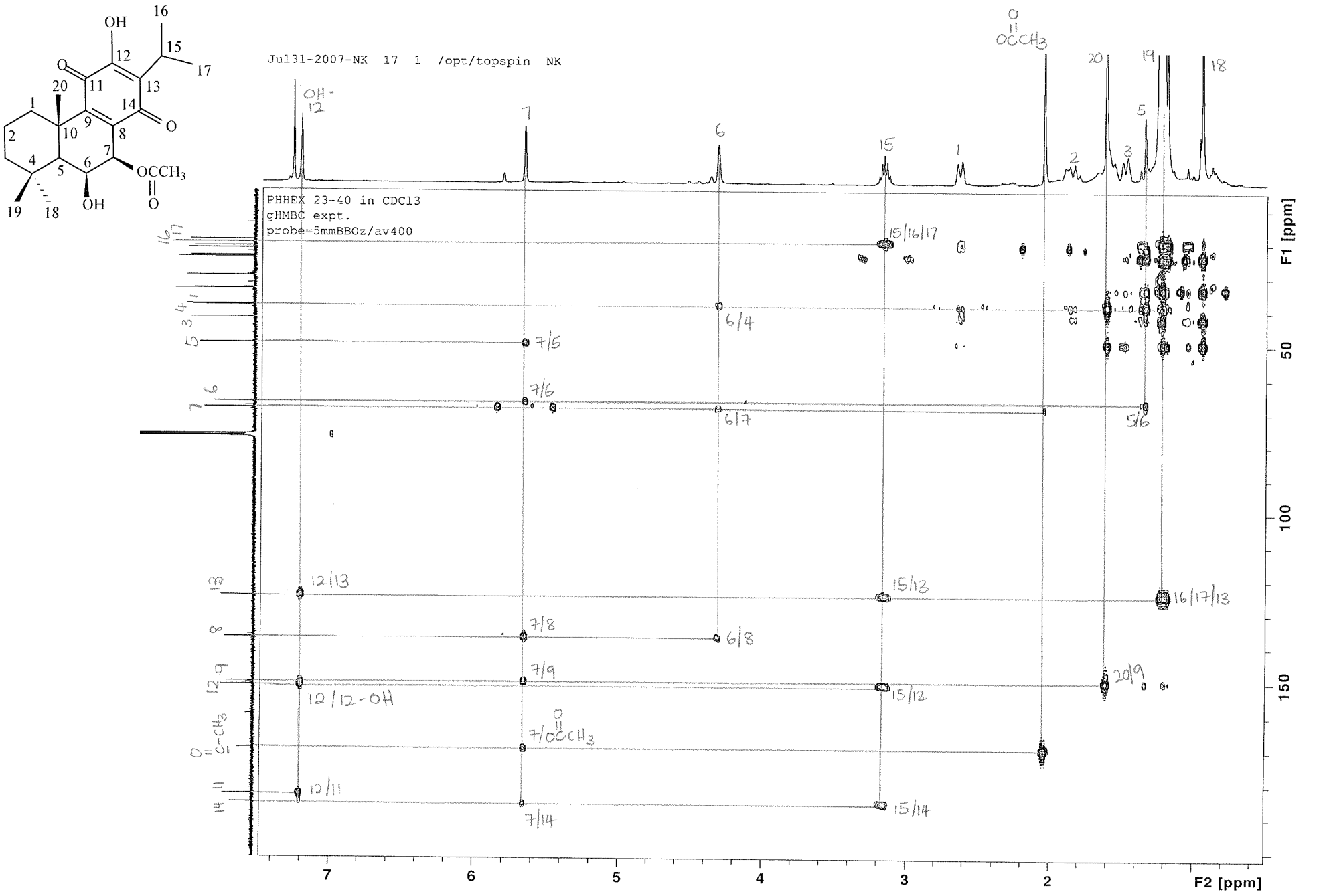
Spectrum 1d: Expanded DEPT spectrum of compound **I**, 7β-acetoxy-6β-hydroxyroyleanone



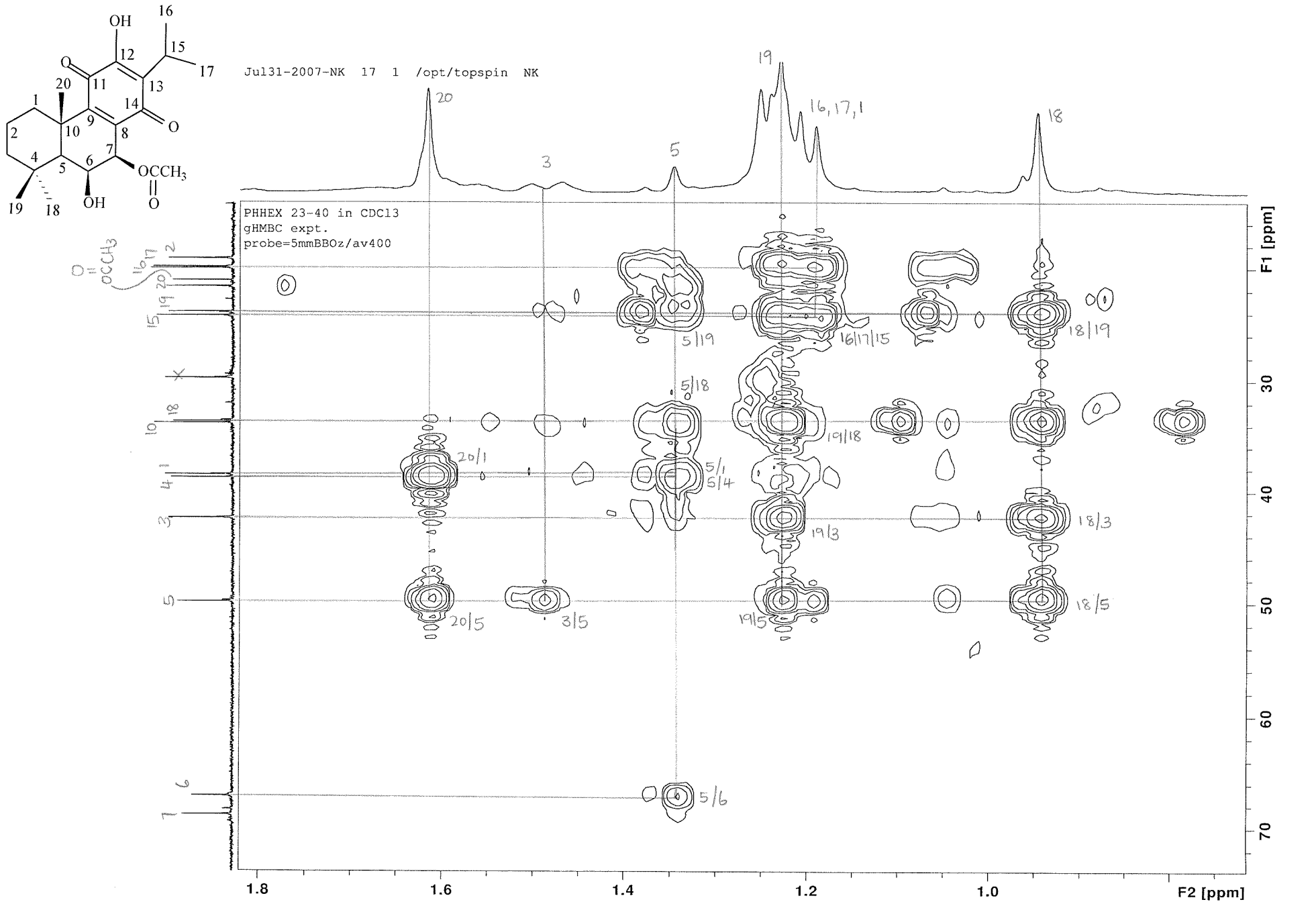
Spectrum 1e: HSQC spectrum of compound **I**, 7β-acetoxy-6β-hydroxyroyleanone



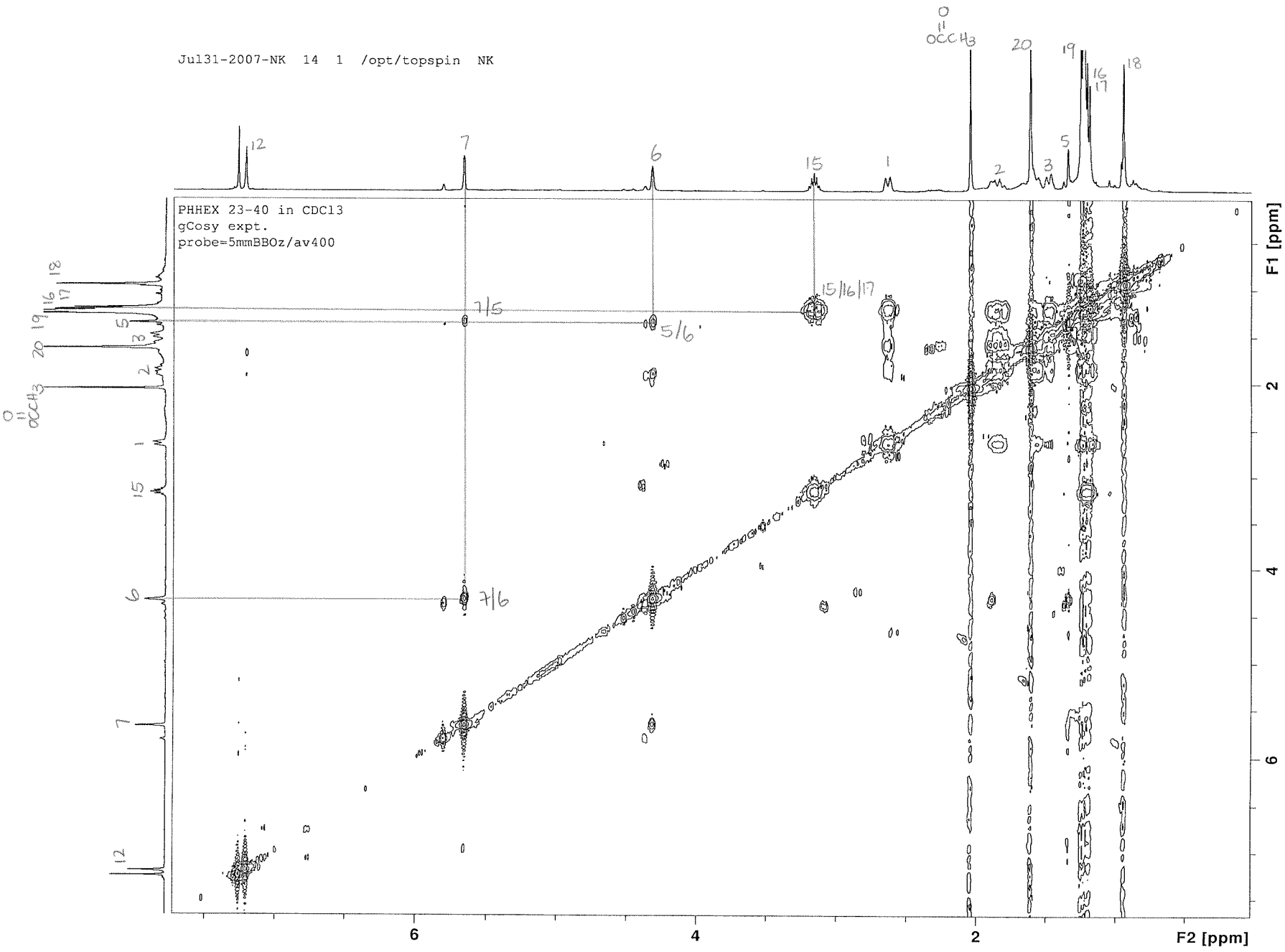
Spectrum 1f: Expanded HSQC spectrum of compound **I**, 7β-acetoxy-6β-hydroxyroyleanone



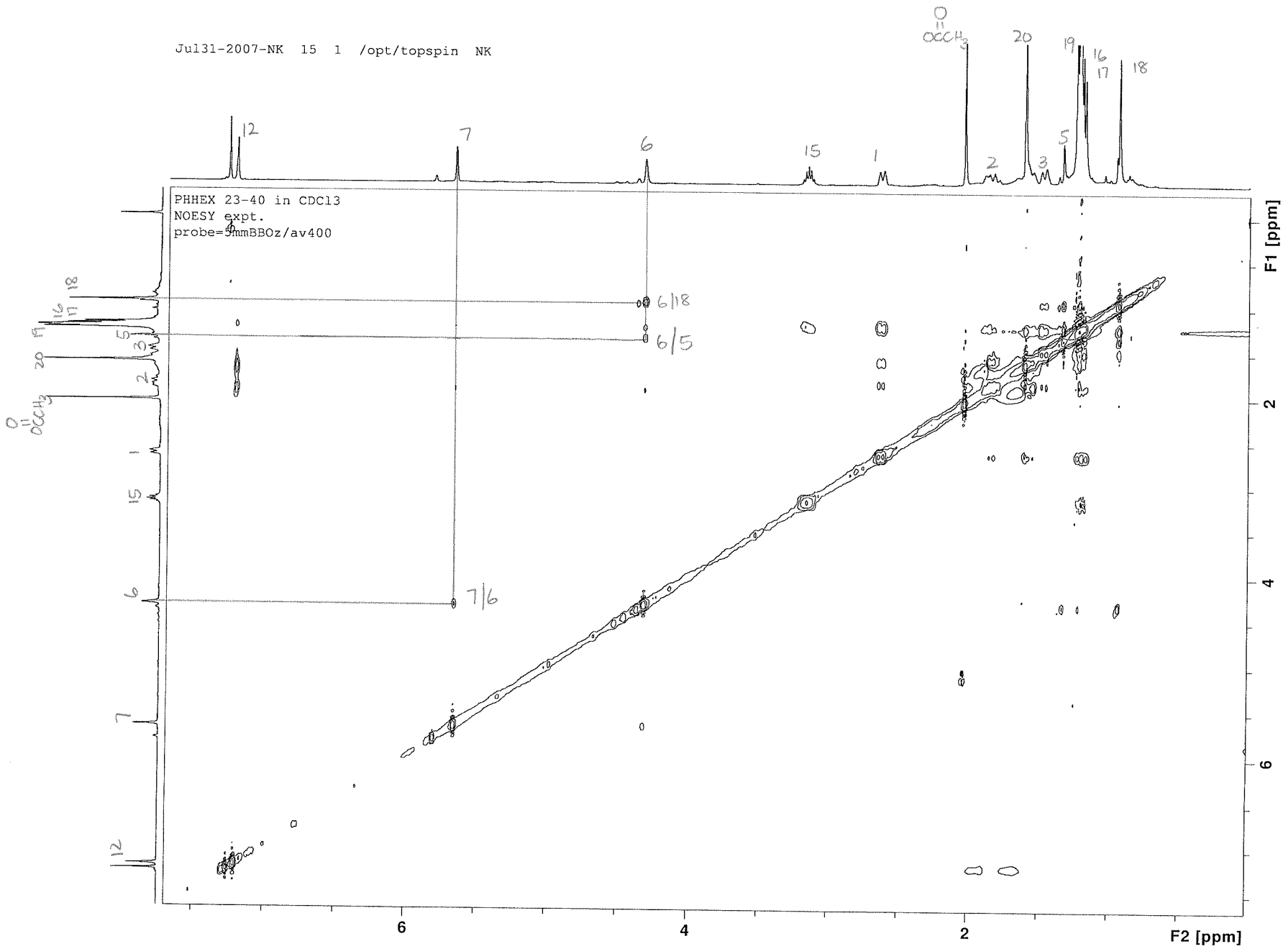
Spectrum 1g: HMBC spectrum of compound **I**, 7β-acetoxy-6β-hydroxyroyleanone



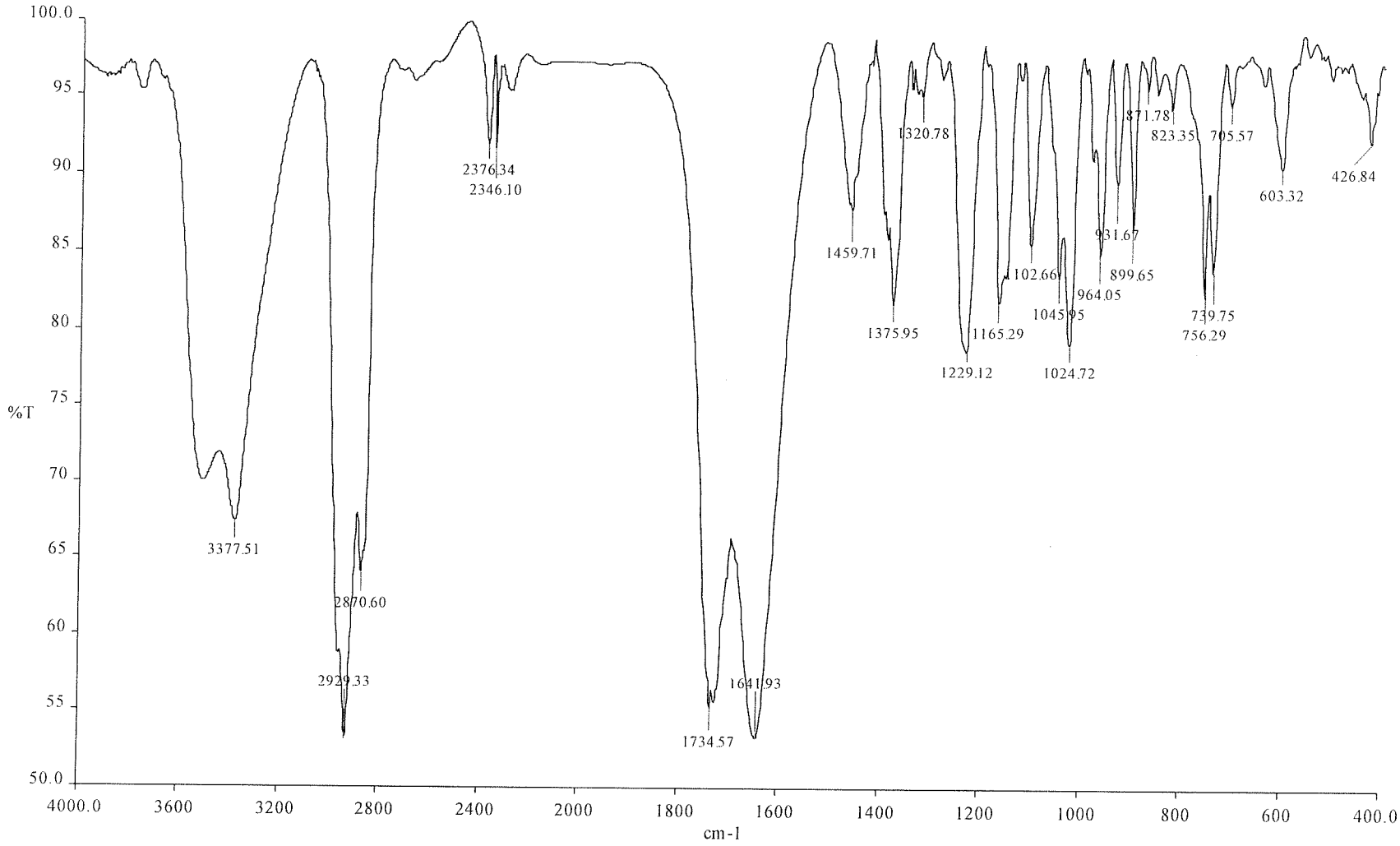
Spectrum 1h: Expanded HMBC spectrum of compound **I**, 7β-acetoxy-6β-hydroxyroyleanone



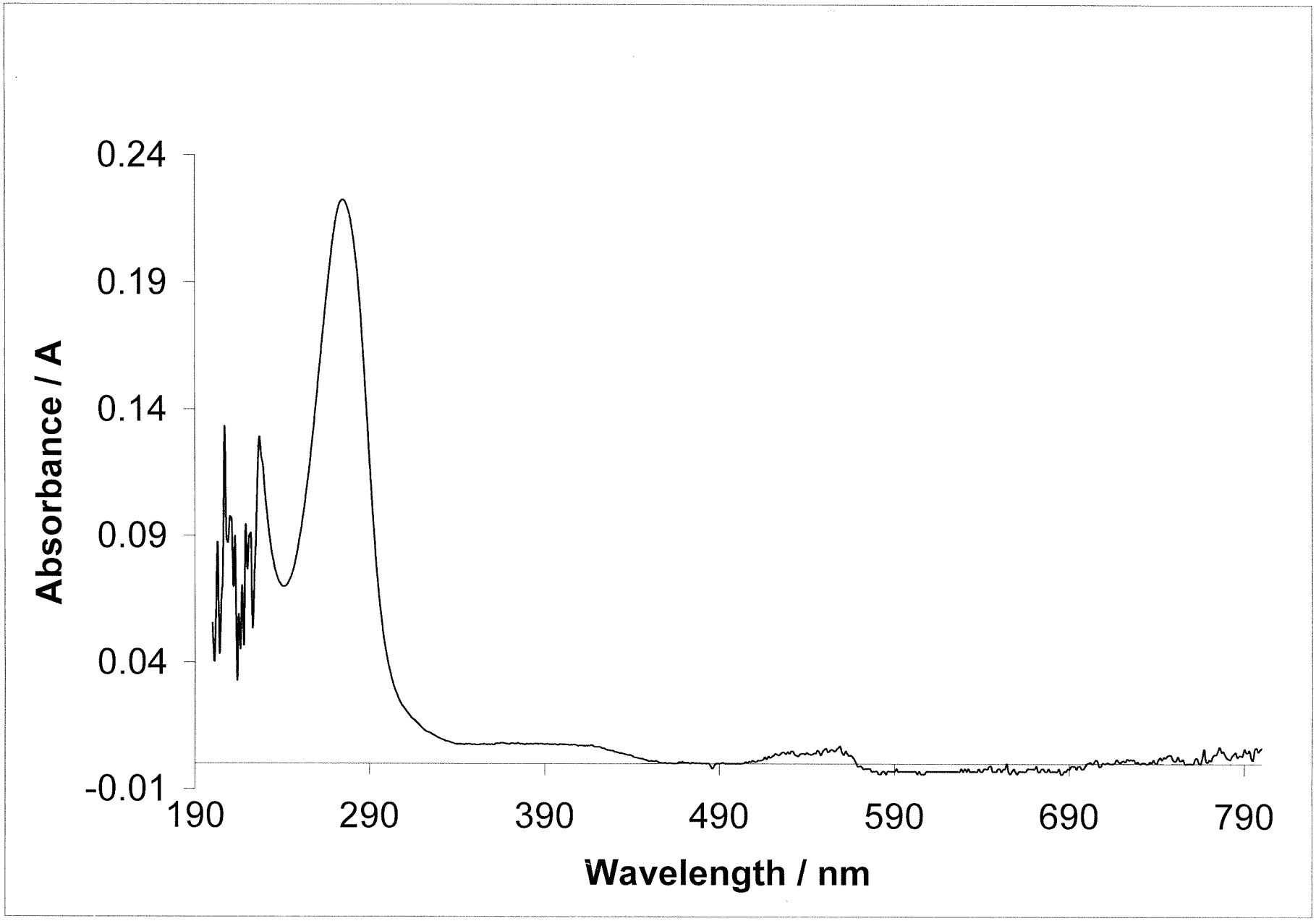
Spectrum 1i: COSY spectrum of compound **I**, 7β-acetoxy-6β-hydroxyroyleanone



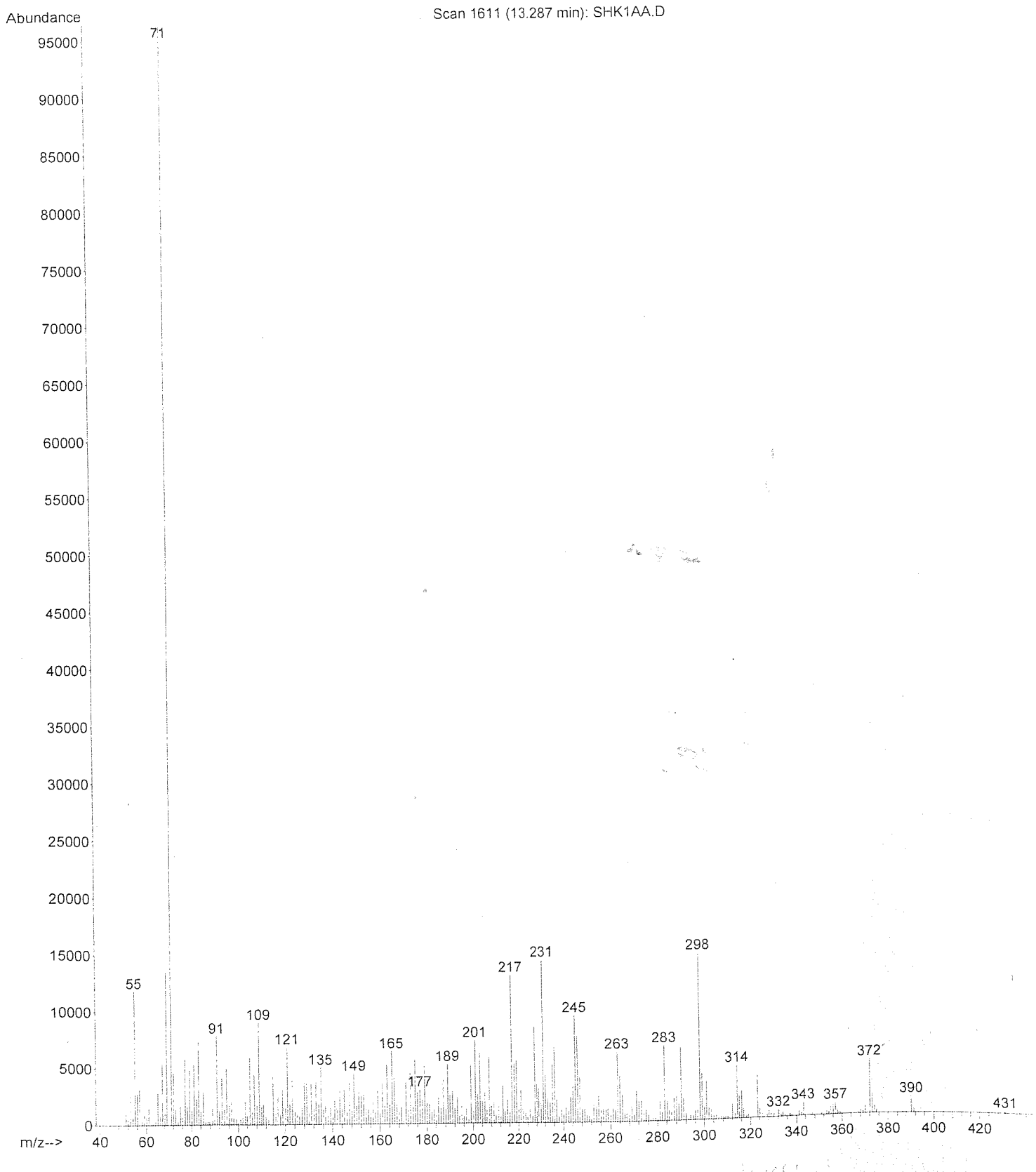
Spectrum 1j: NOESY spectrum of compound **I**, 7β-acetoxy-6β-hydroxyroyleanone



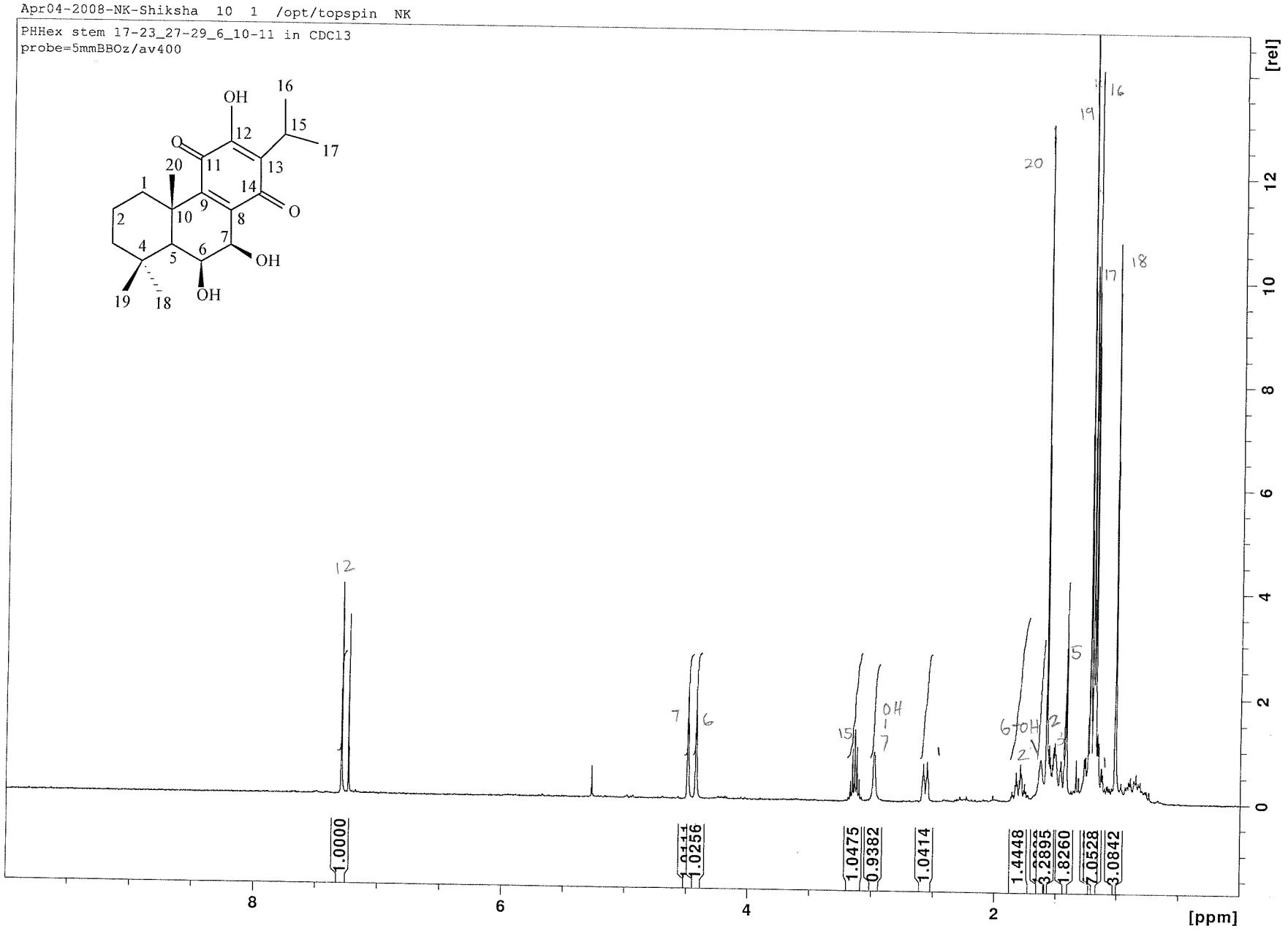
Spectrum 1k: IR spectrum of compound **I**, 7β-acetoxy-6β-hydroxyroyleanone



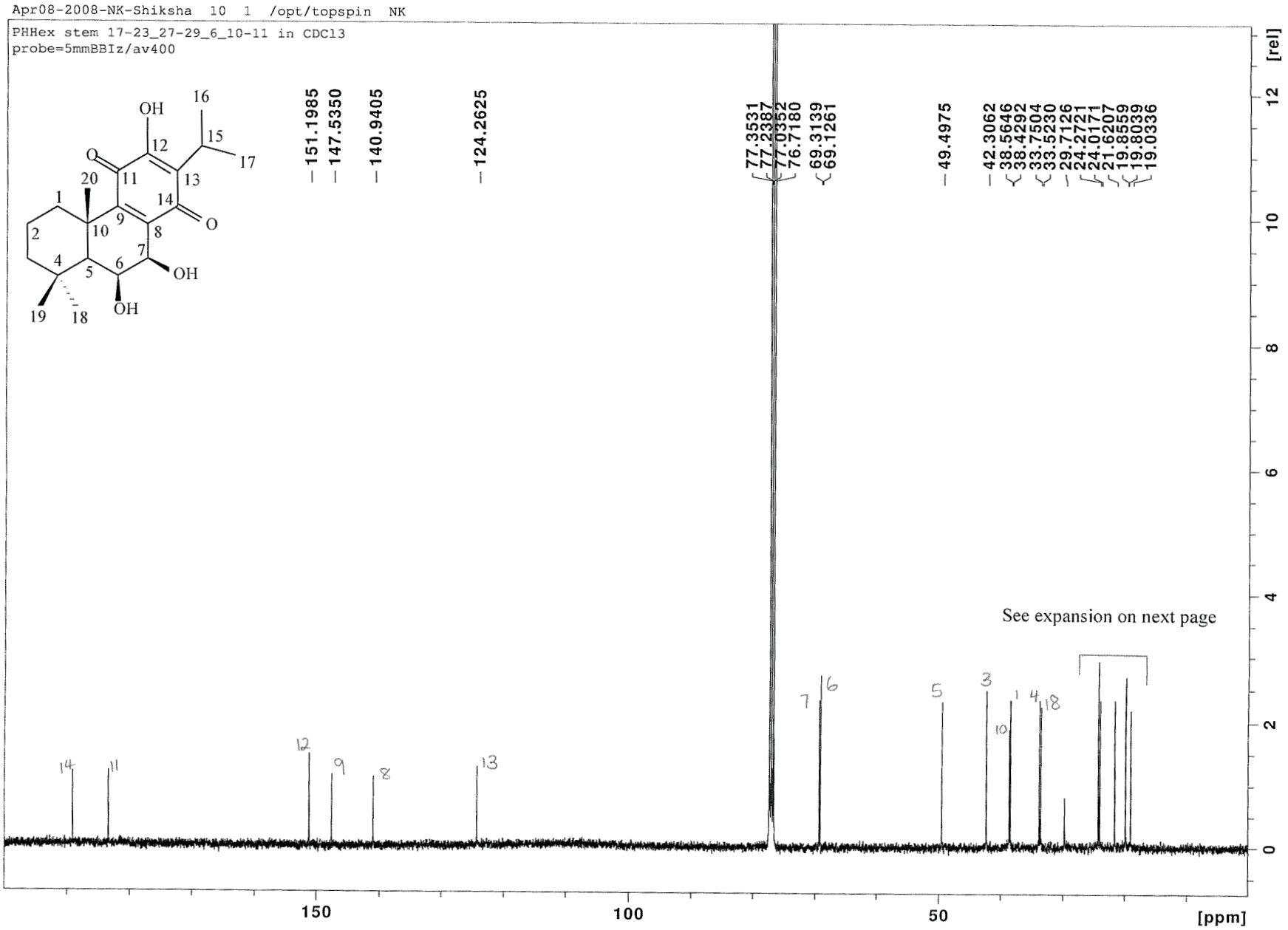
Spectrum 1l: UV spectrum of compound **I**, 7β-acetoxy-6β-hydroxyroyleanone



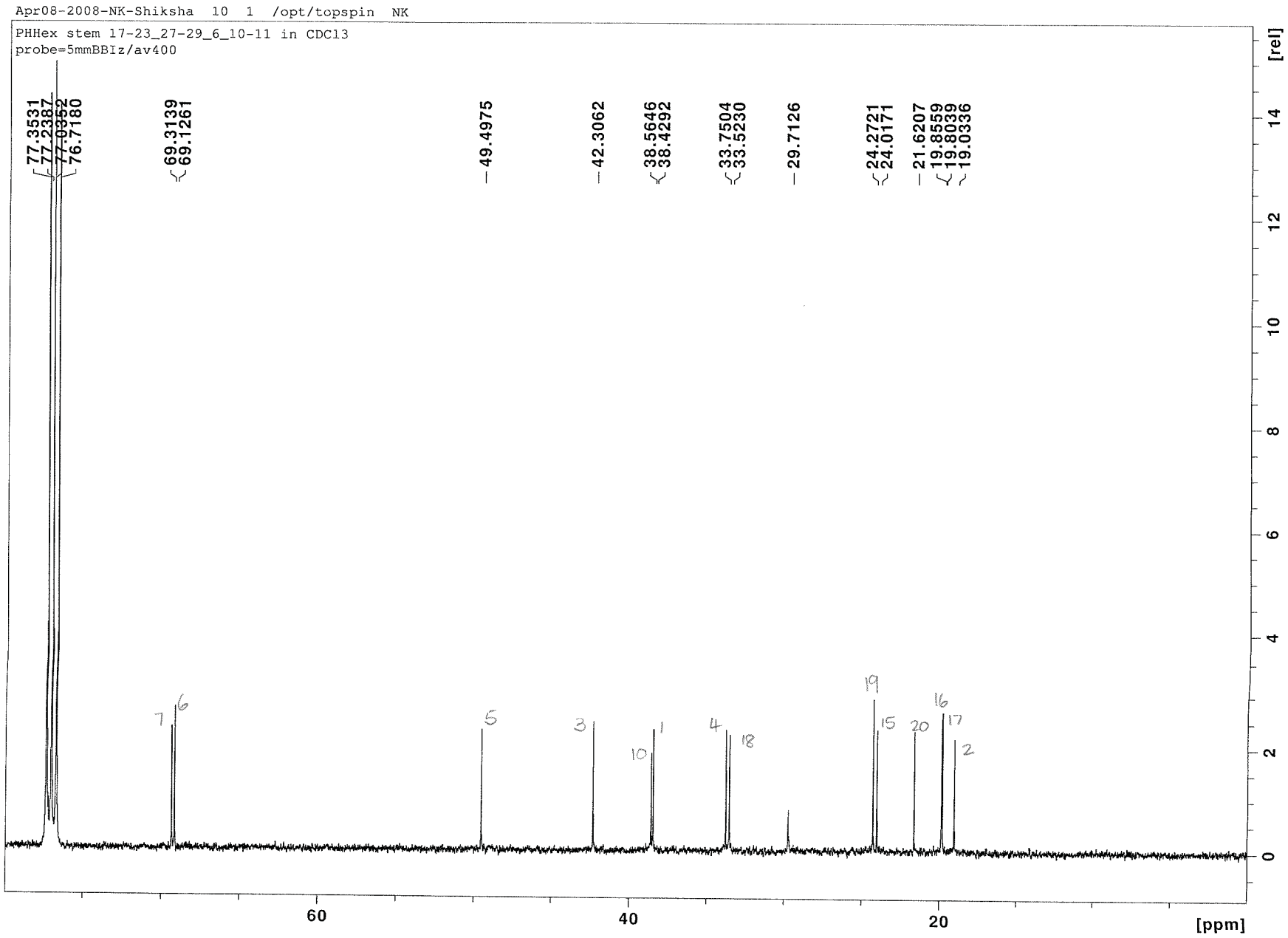
Spectrum 1m: GC-MS spectrum of compound **I**, 7β-acetoxy-6β-hydroxyroyleanone



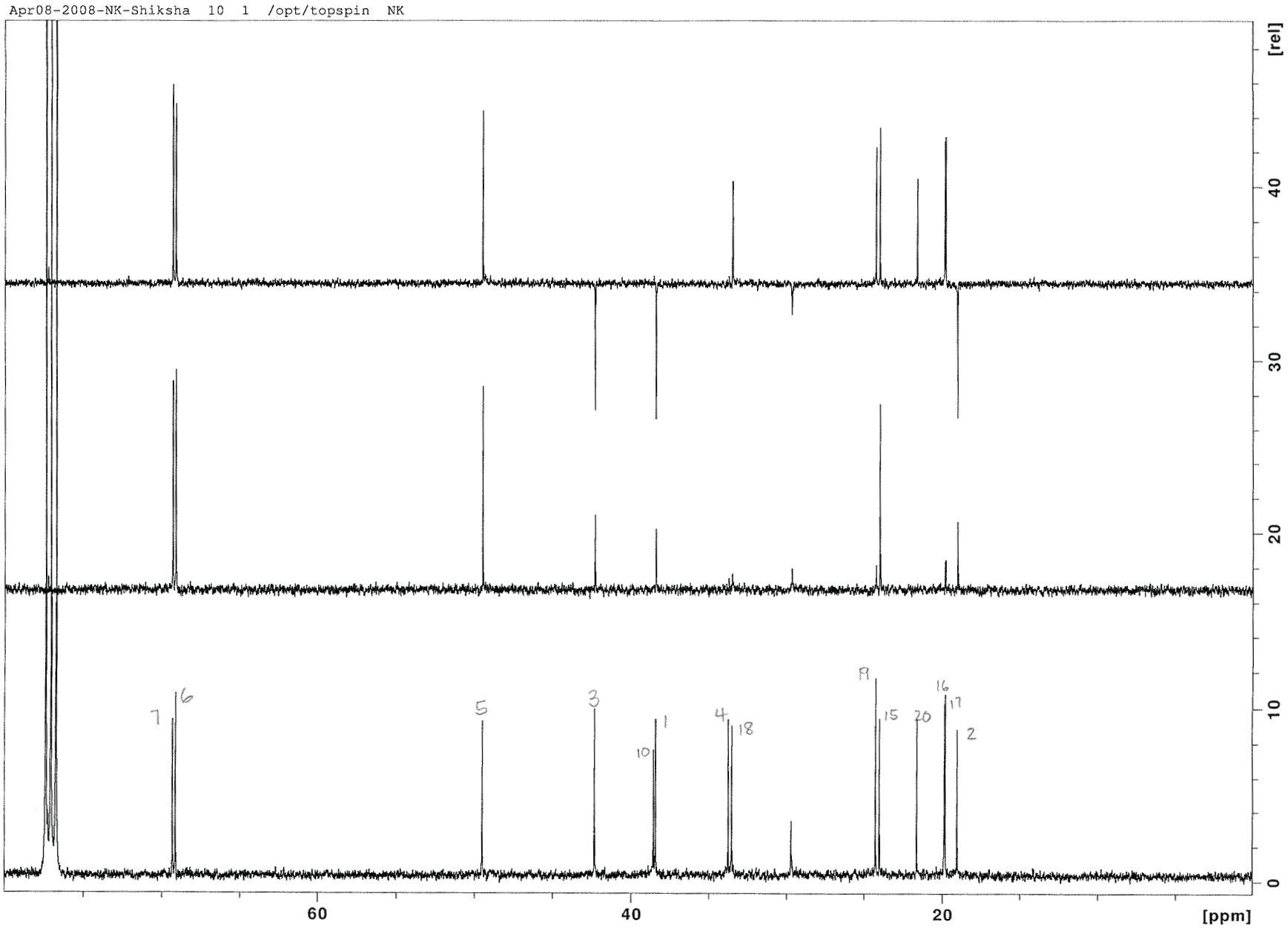
Spectrum 2a: ^{1}H NMR spectrum of compound **II**, 6β,7β-dihydroxyroyleanone



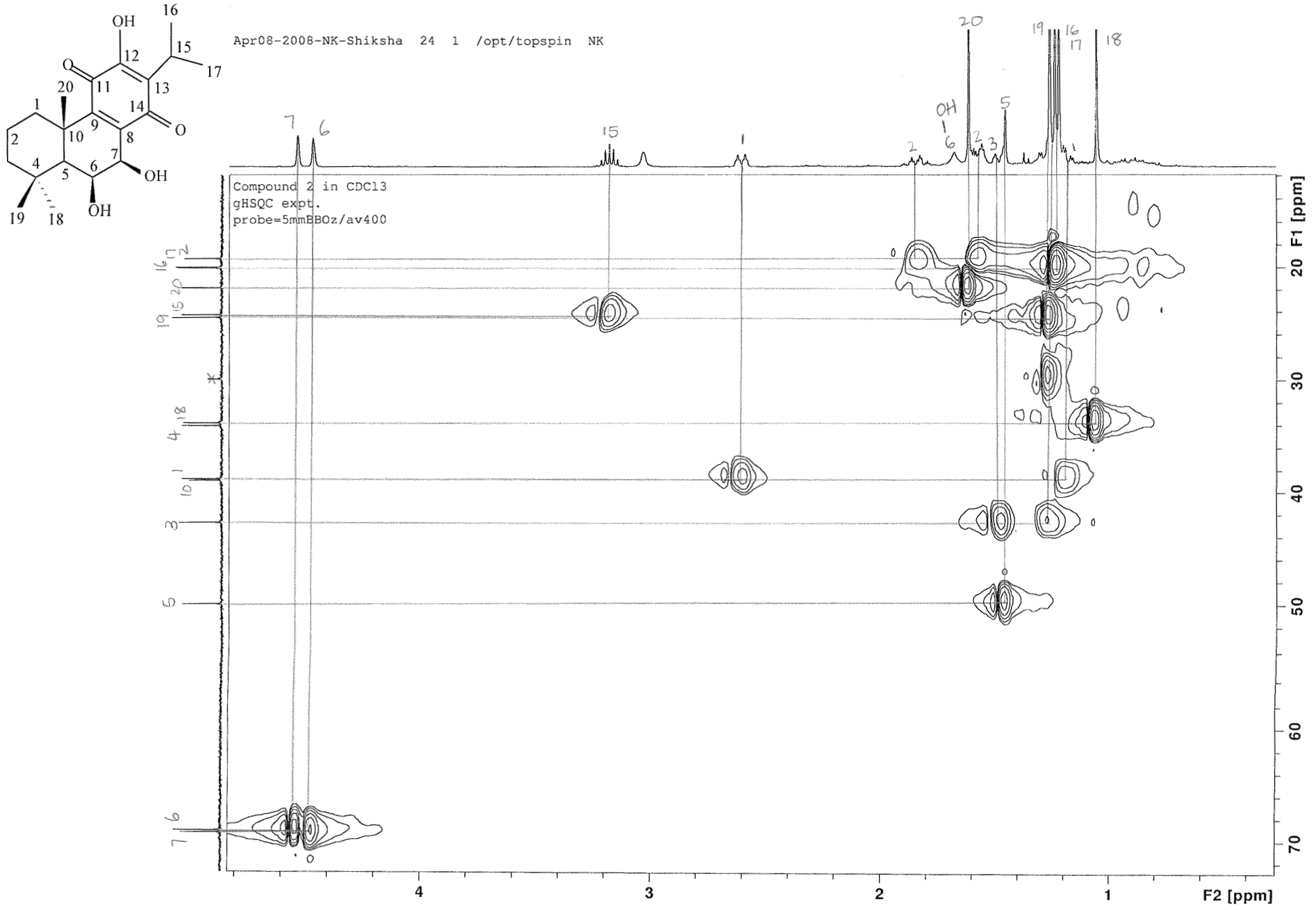
Spectrum 2b: ^{13}C NMR spectrum of compound **II**, 6β,7β-dihydroxyroyleanone



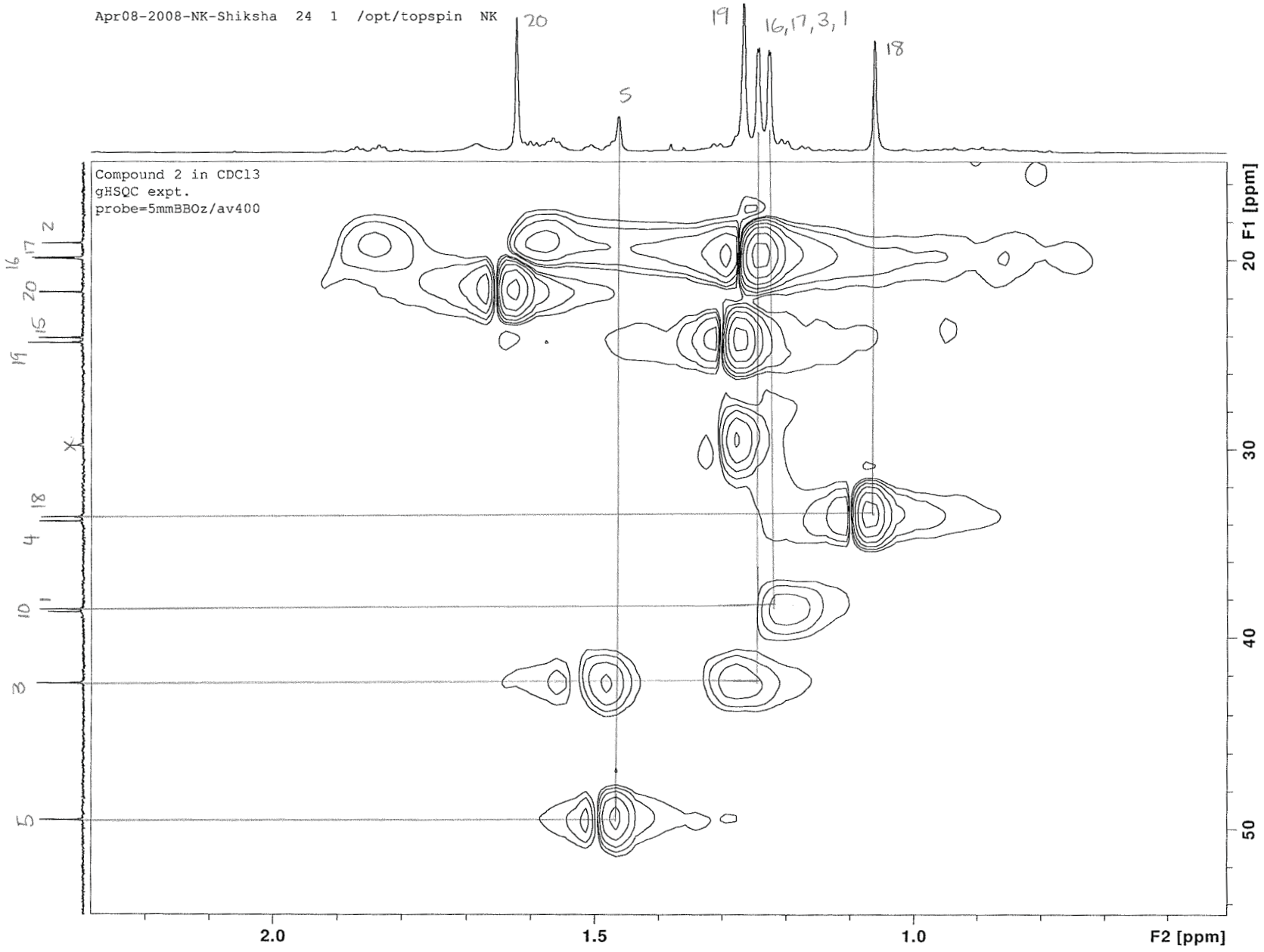
Spectrum 2c: Expanded ^{13}C NMR spectrum of compound **II**, 6β,7β-dihydroxyroyleanone



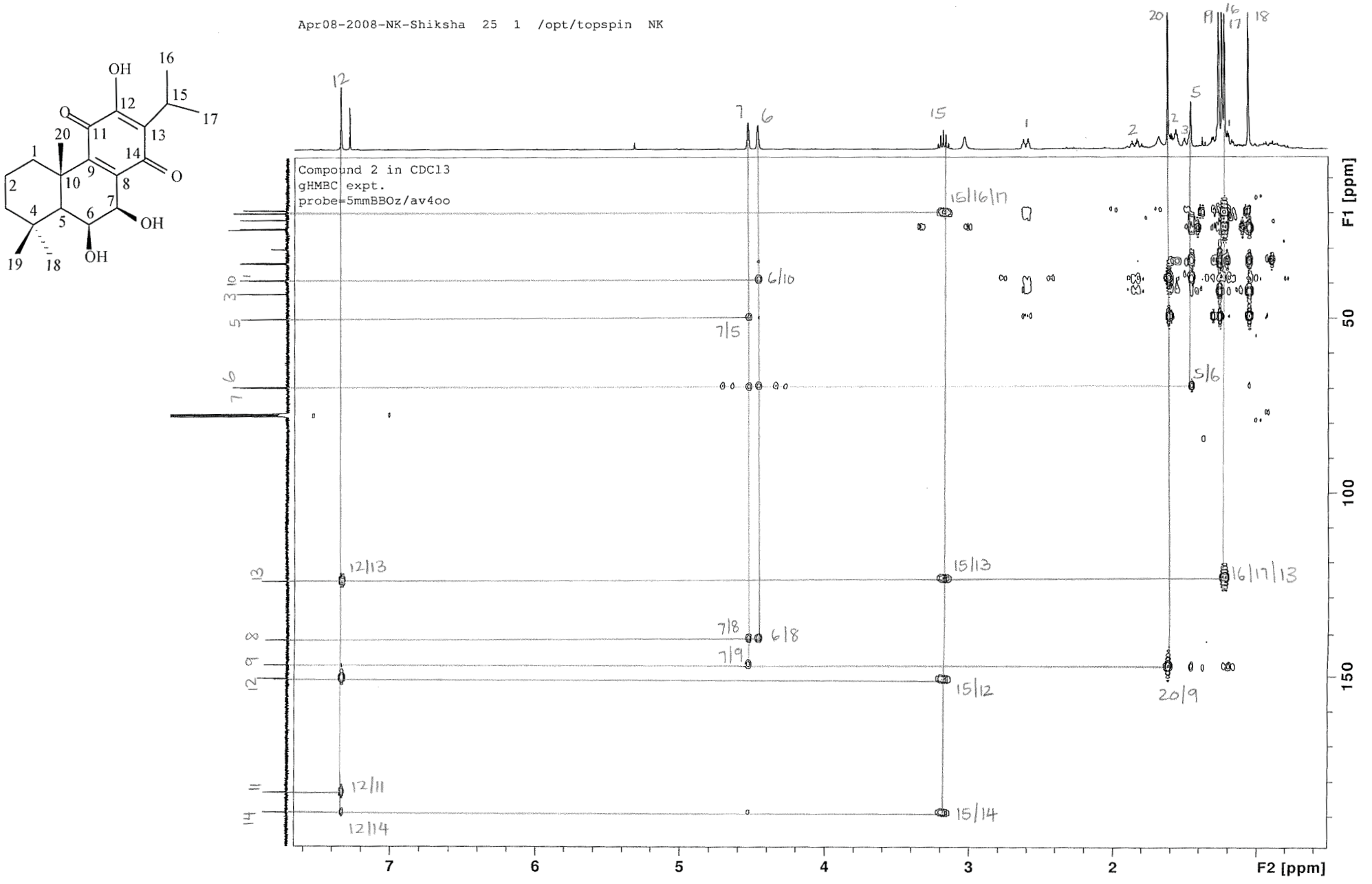
Spectrum 2d: Expanded DEPT spectrum of compound **II**, 6β,7β-dihydroxyroyleanone



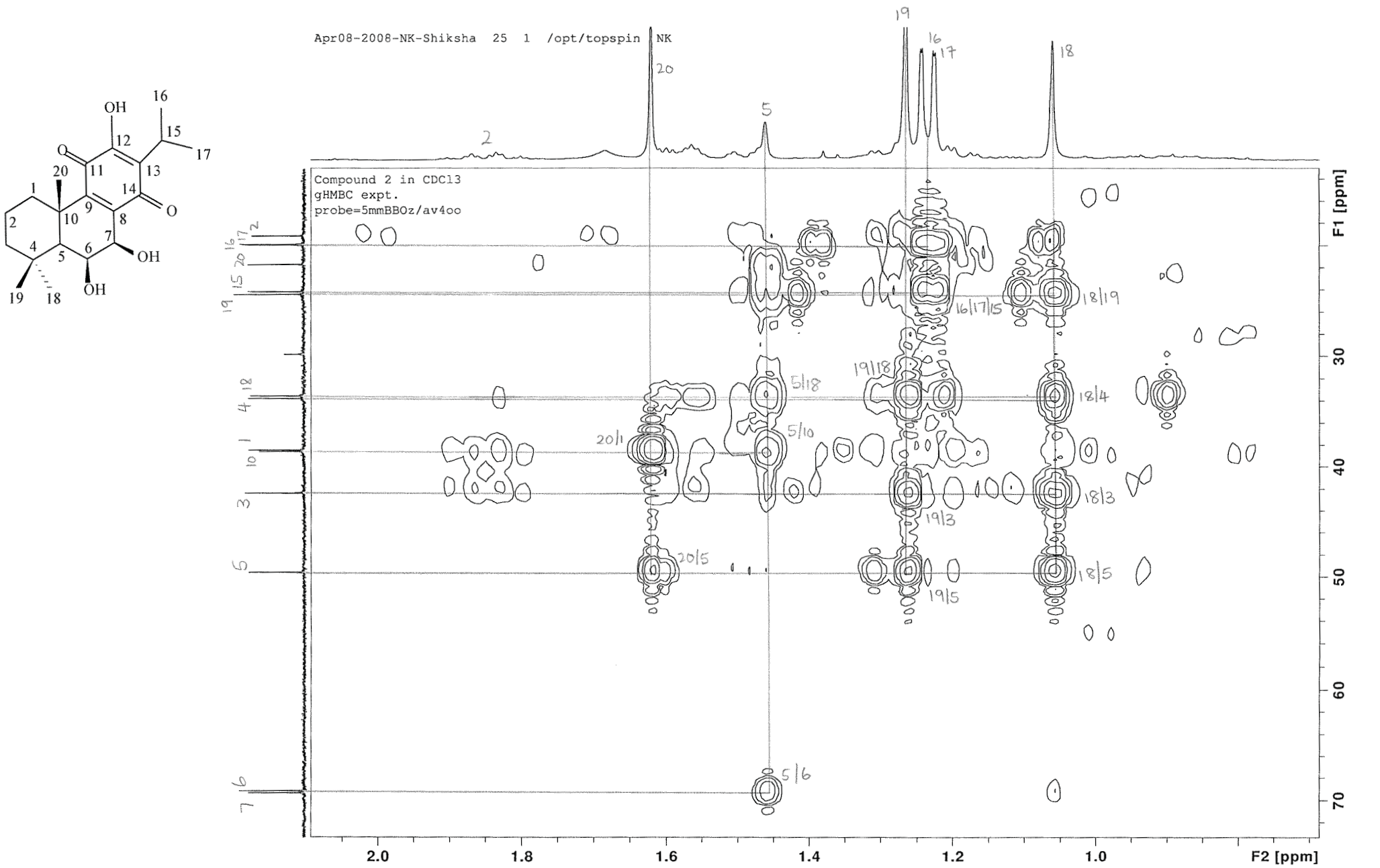
Spectrum 2e: HSQC spectrum of compound **II**, 6β,7β-dihydroxyroyleanone



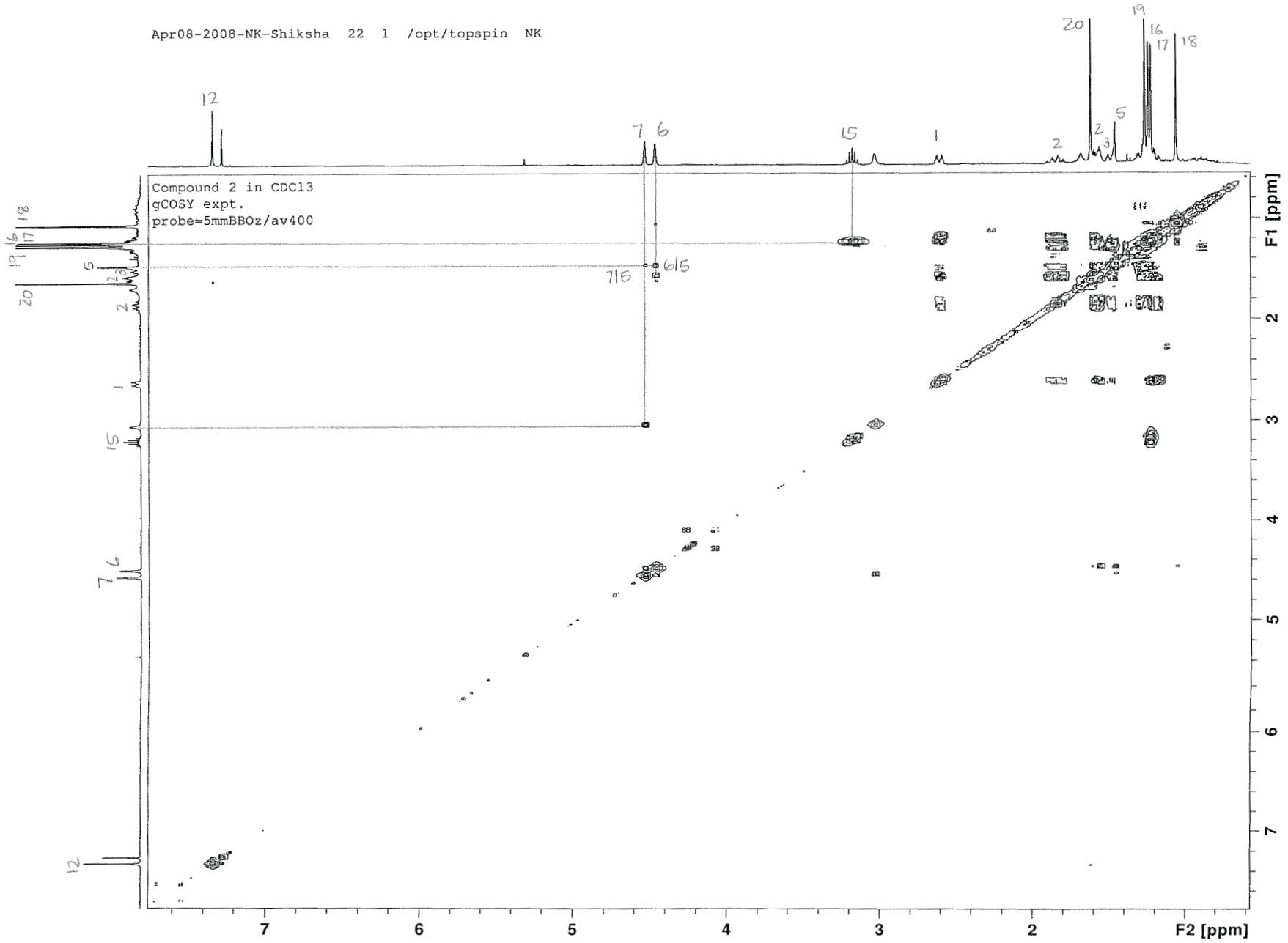
Spectrum 2f: Expanded HSQC spectrum of compound **II**, 6β,7β-dihydroxyroyleanone



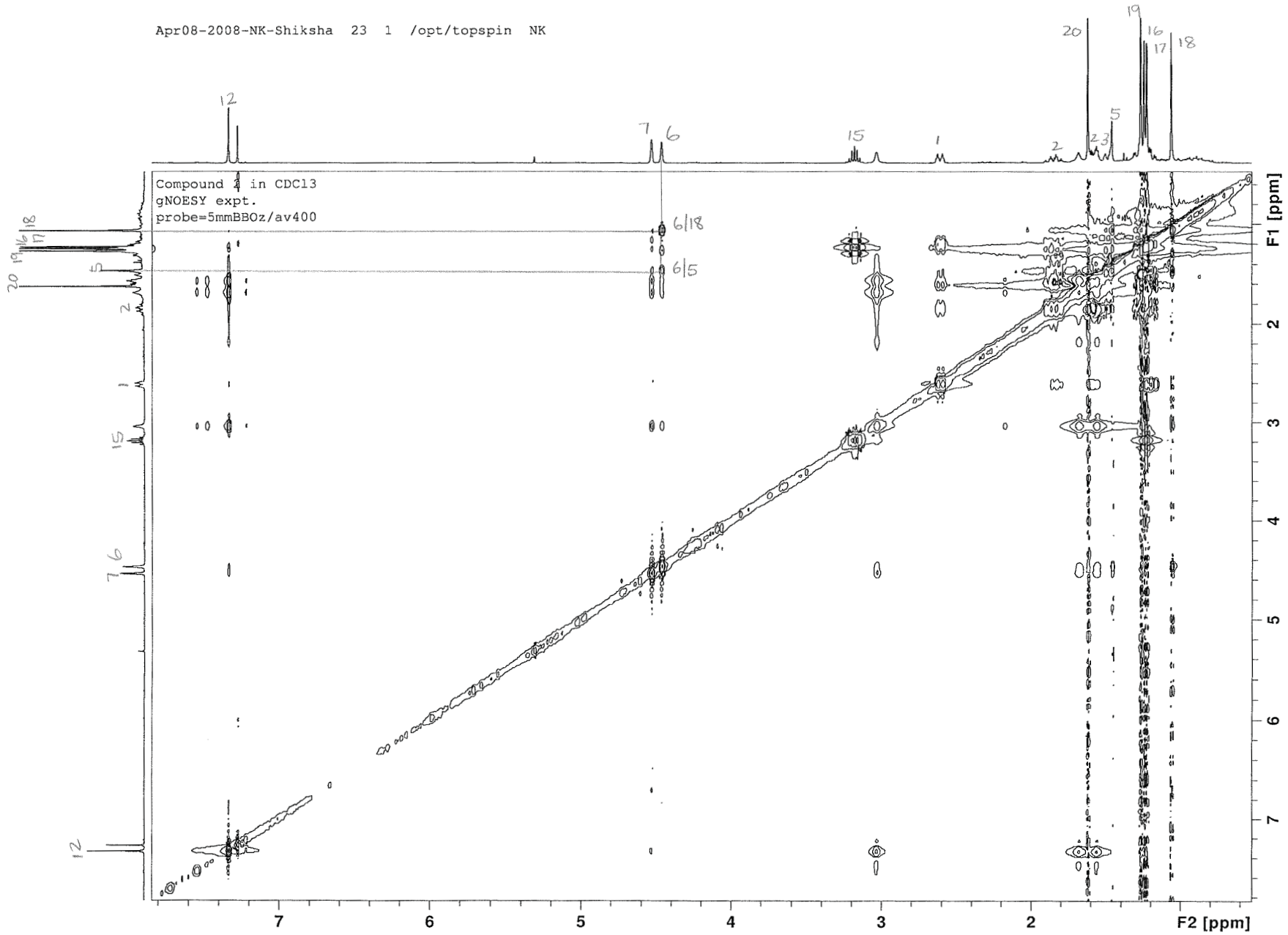
Spectrum 2g: HMBC spectrum of compound **II**, 6β,7β-dihydroxyroyleanone



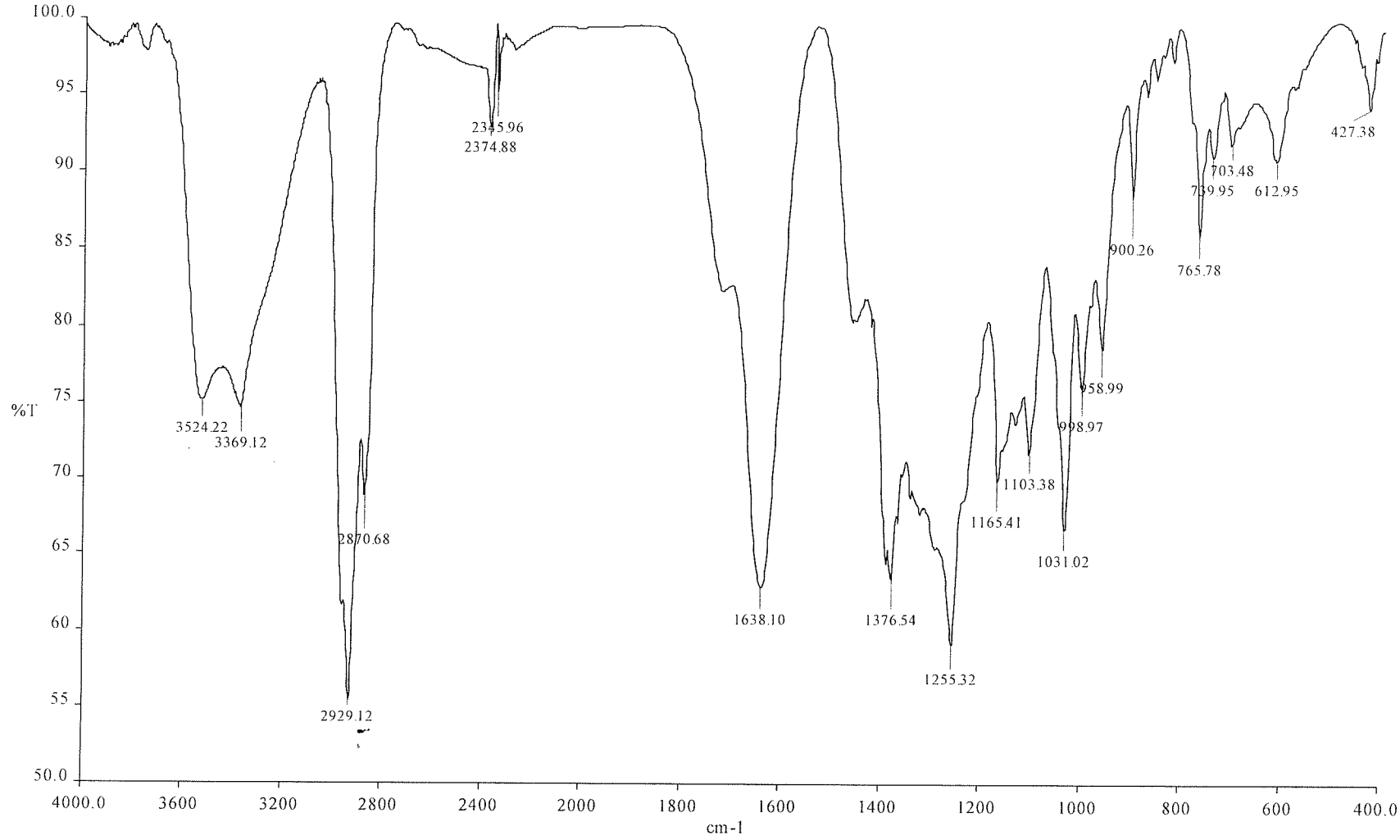
Spectrum 2h: Expanded HMBC spectrum of compound **II**, 6β,7β-dihydroxyroyleanone



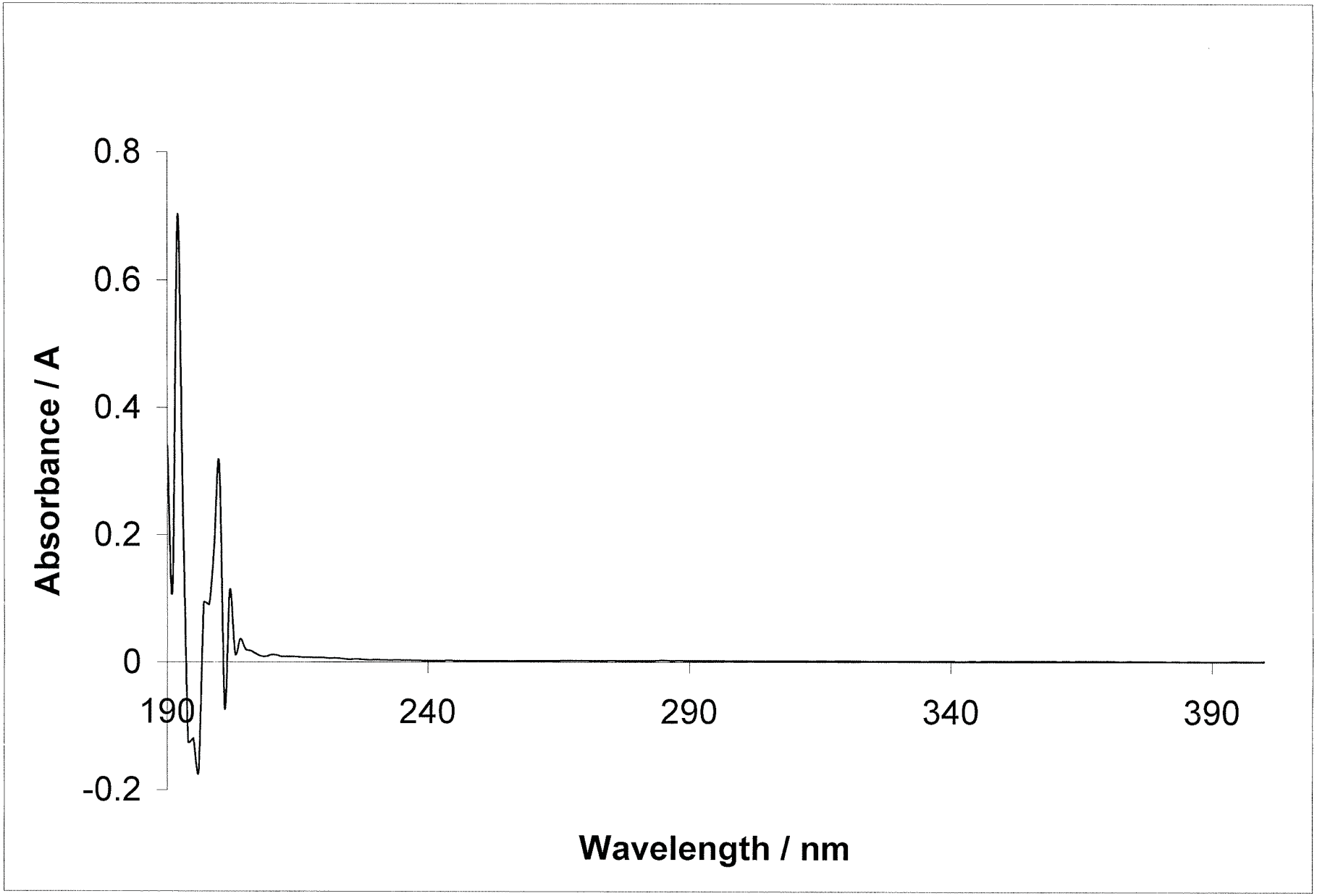
Spectrum 2i: COSY spectrum of compound **II**, 6β,7β-dihydroxyroyleanone



Spectrum 2j: NOESY spectrum of compound **II**, 6β,7β-dihydroxyroyleanone



Spectrum 2k: IR spectrum of compound **II**, 6β,7β-dihydroxyroyleanone



Spectrum 2l: UV spectrum of compound **II**, 6β,7β-dihydroxyroyleanone

Display Report - All Windows Selected Analysis

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Method: FIA.M
Sample Name: SHIKSHA
Analysis Info: 348

Instrument: LC-MSD-Trap-VL
Operator: Administrator

Print Date: 11/05/2009 02:19:56 PM
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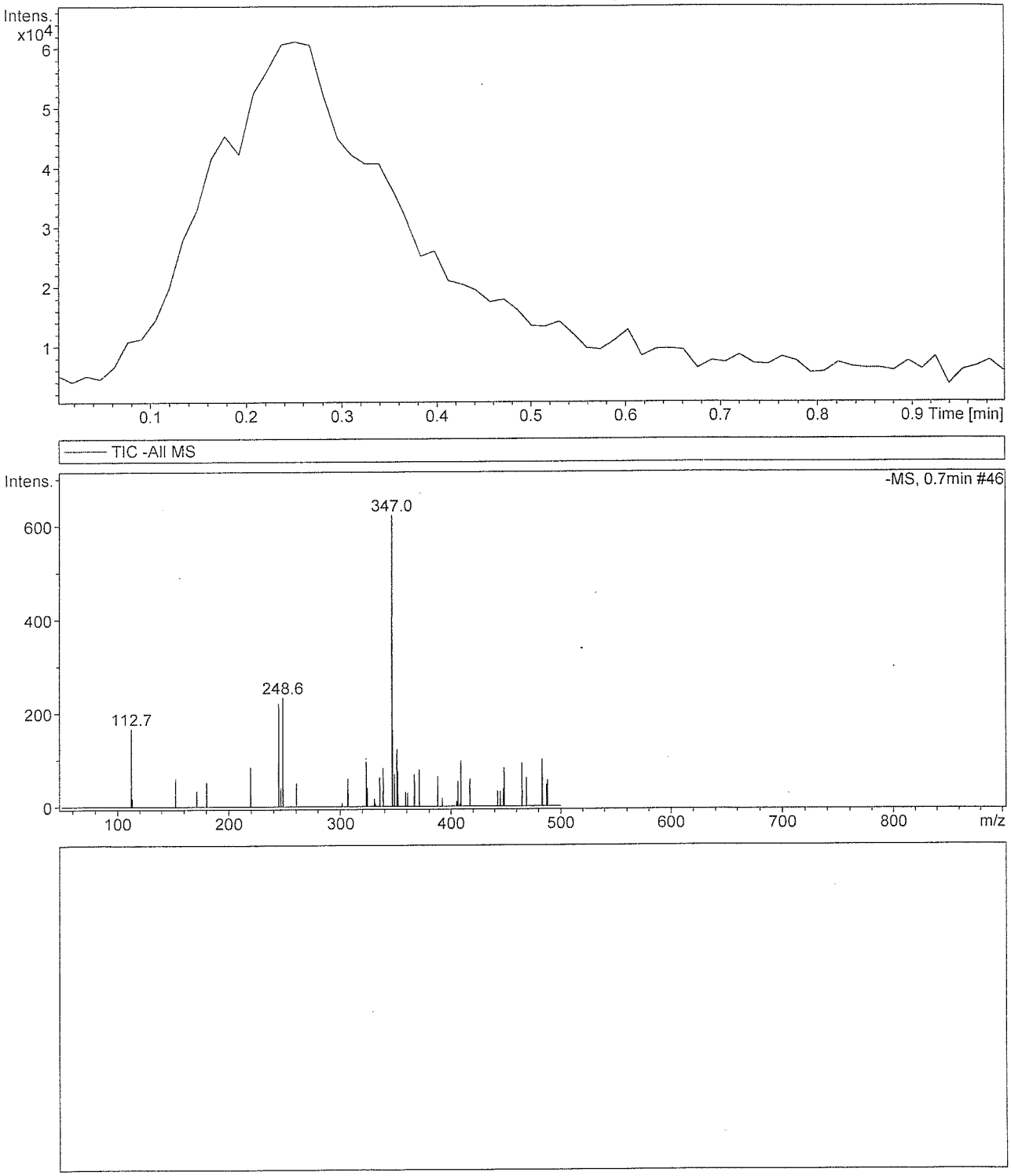

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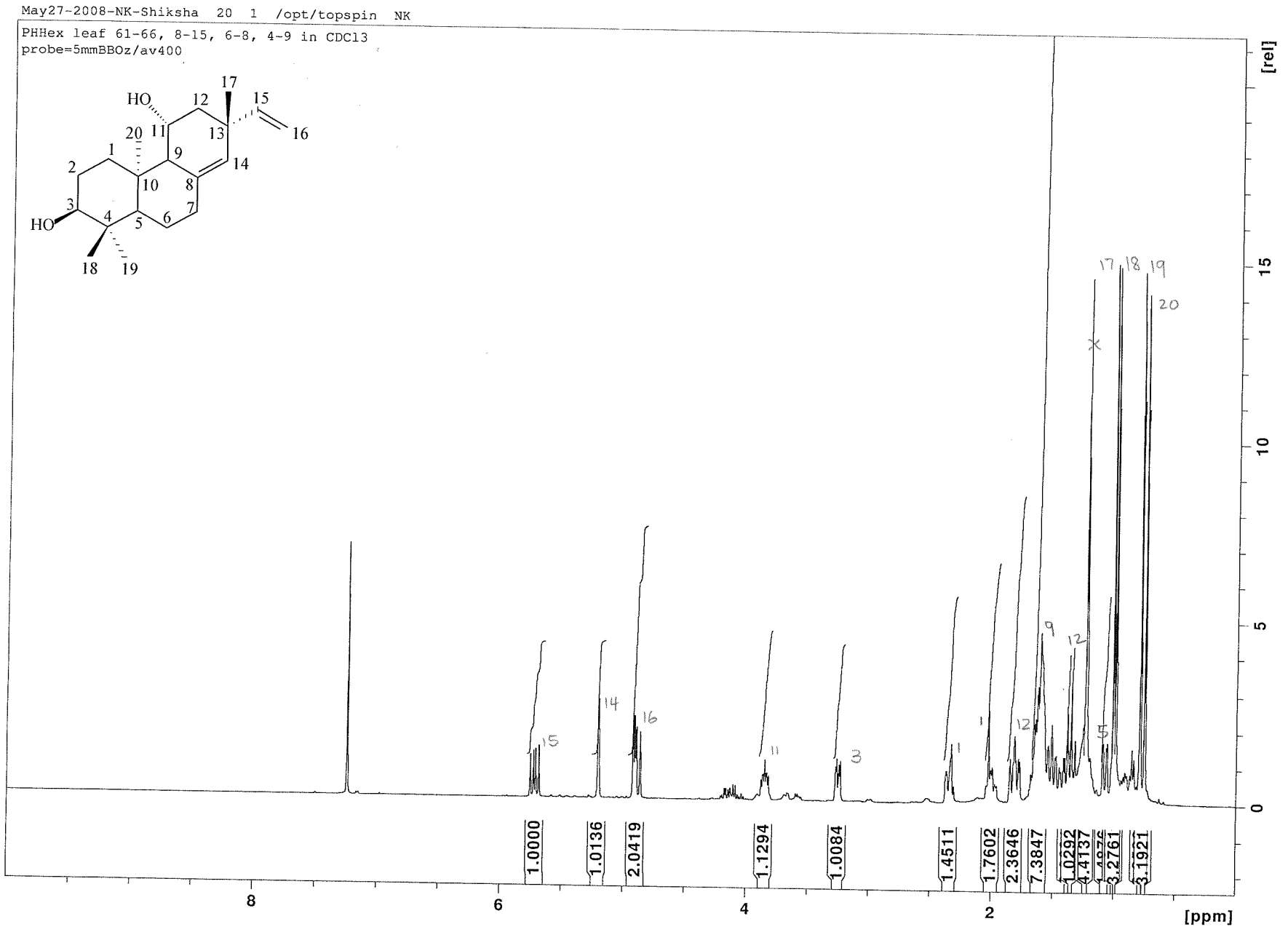
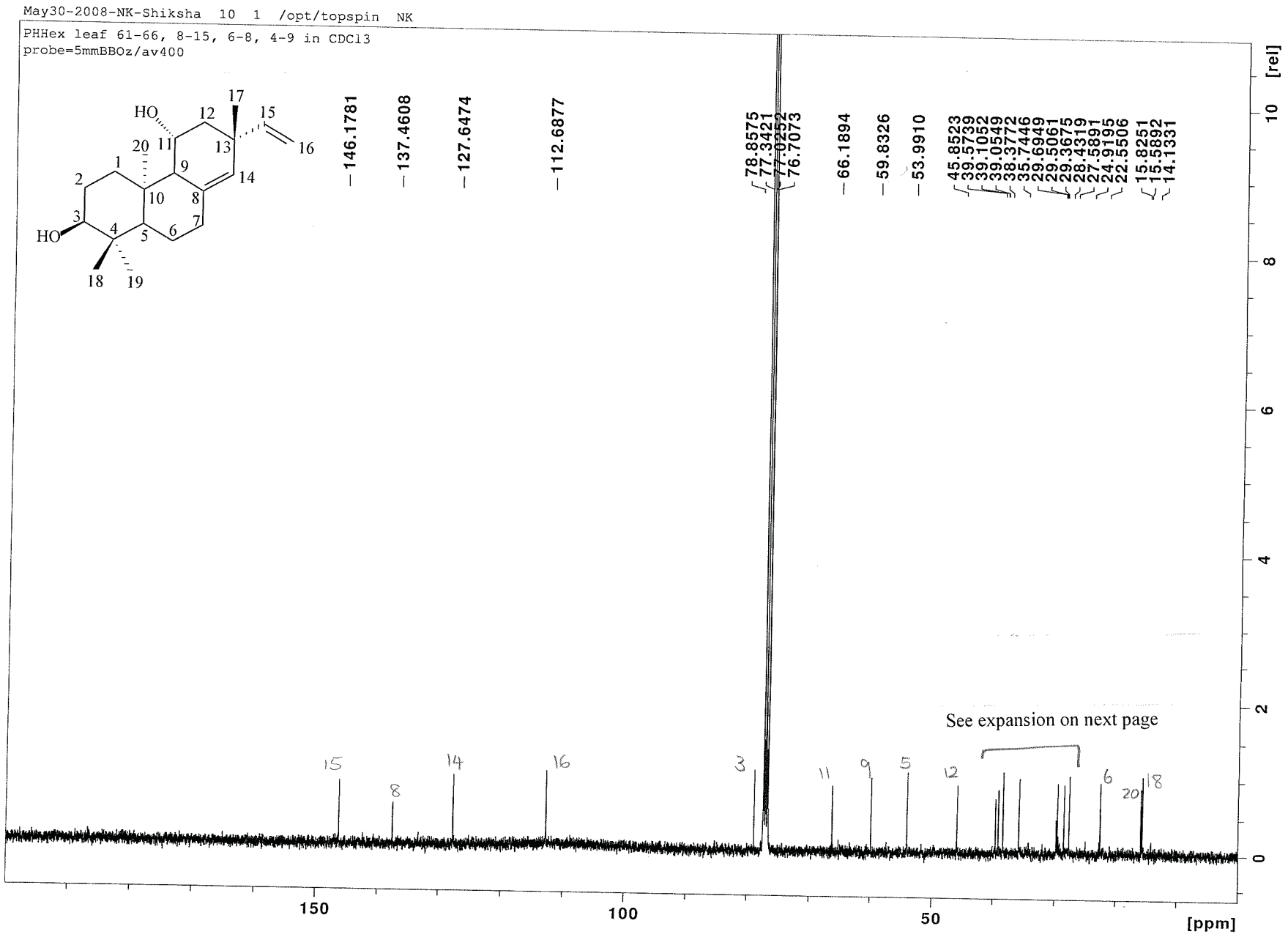
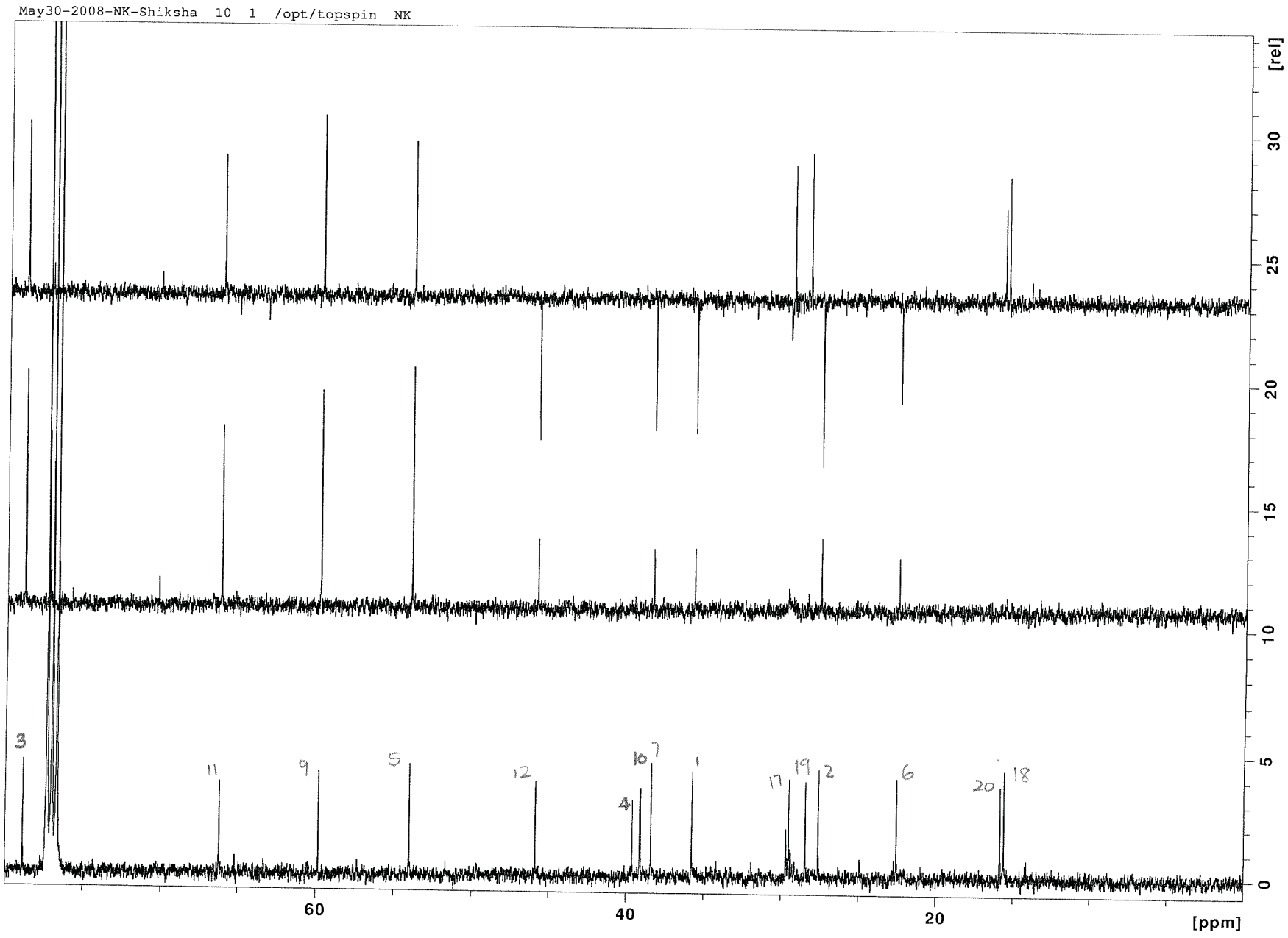
Spectrum 2m: LC-MS spectrum of compound **II**, 6β,7β-dihydroxyroyleanone



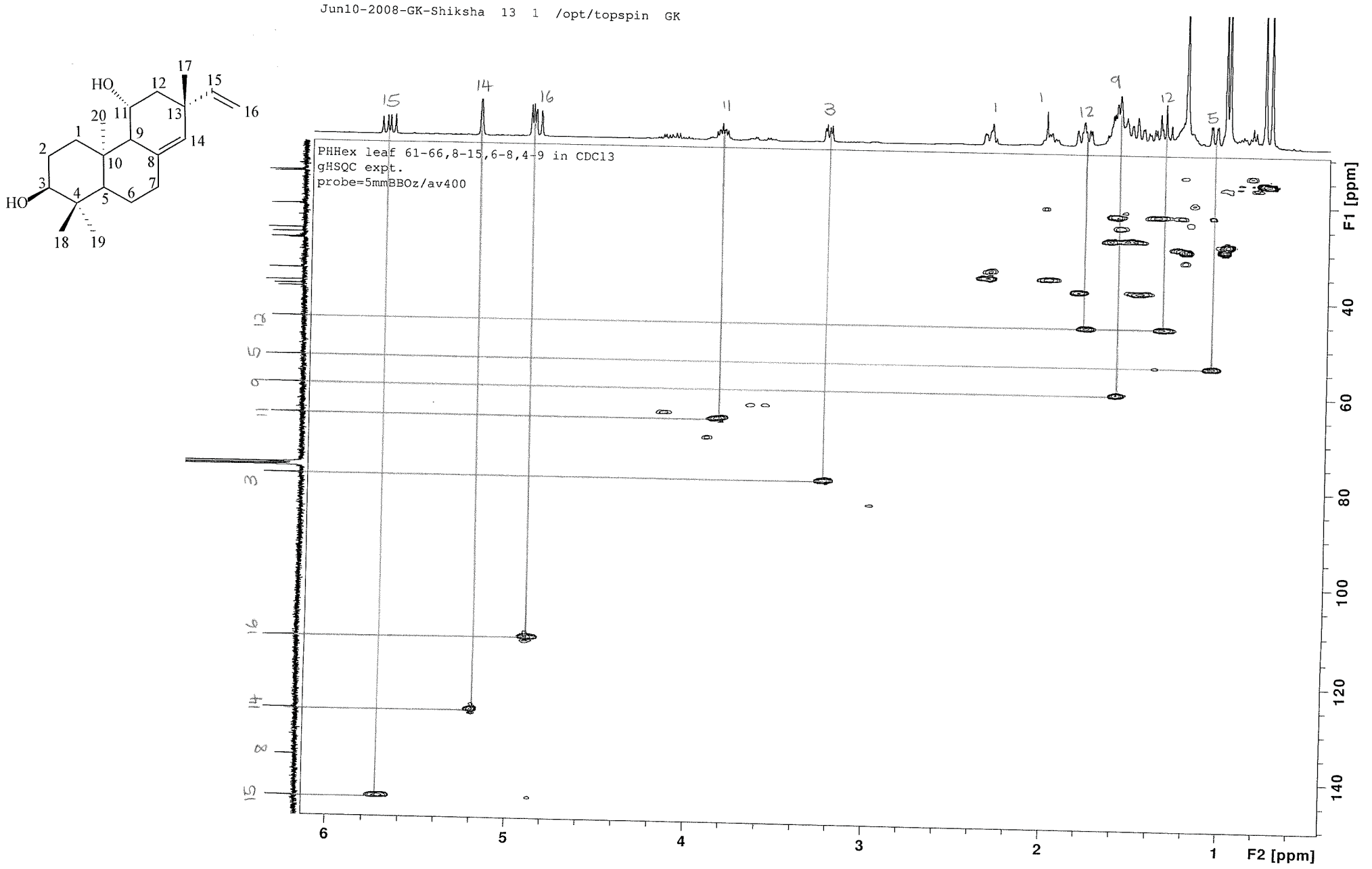
Spectrum 3a: ^{1}H NMR spectrum of compound **III**, *ent*-pimara-8(14),15-diene-3β,11α-diol



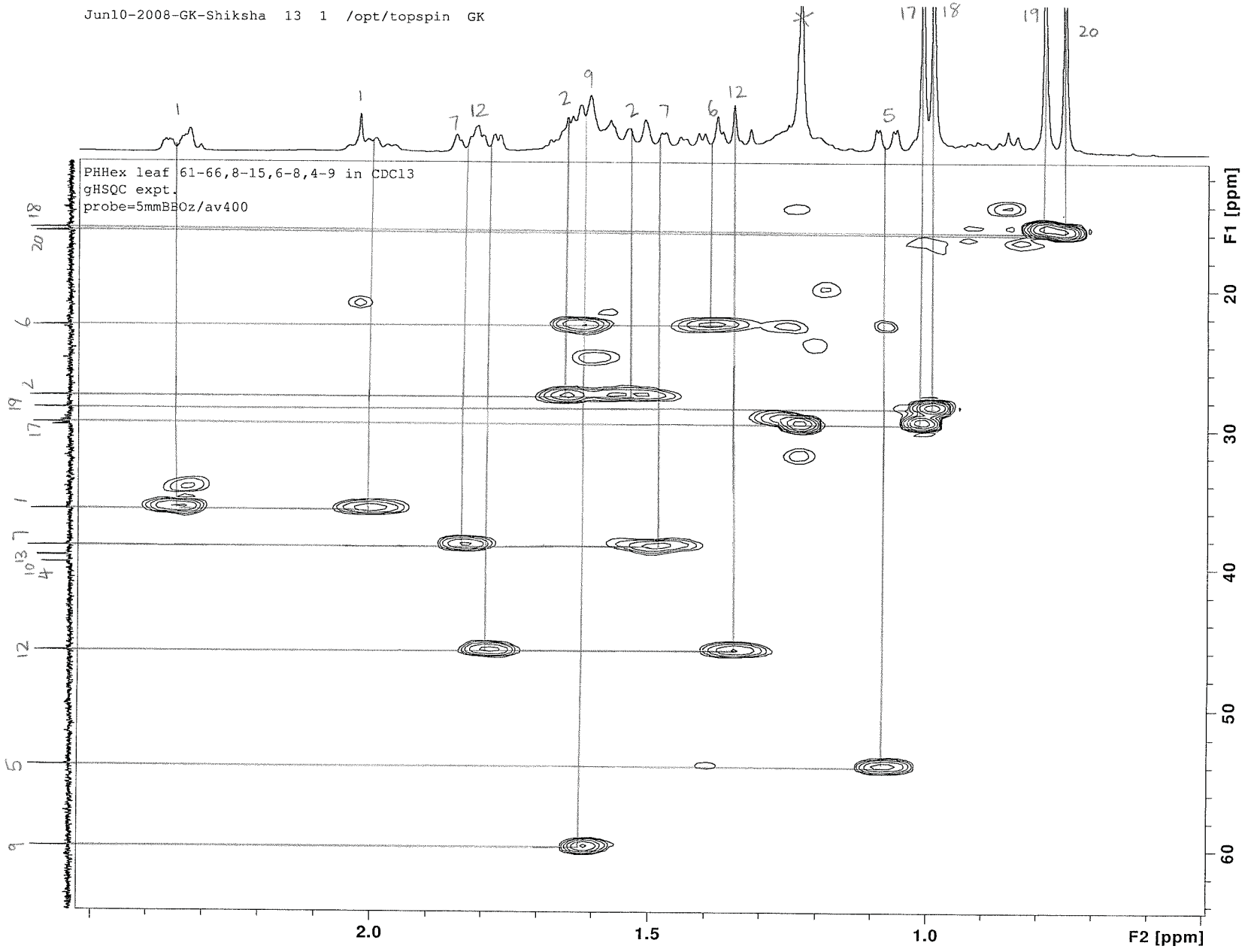
Spectrum 3b: ^{13}C NMR spectrum of compound **III**, *ent*-pimara-8(14),15-diene-3β,11α-diol



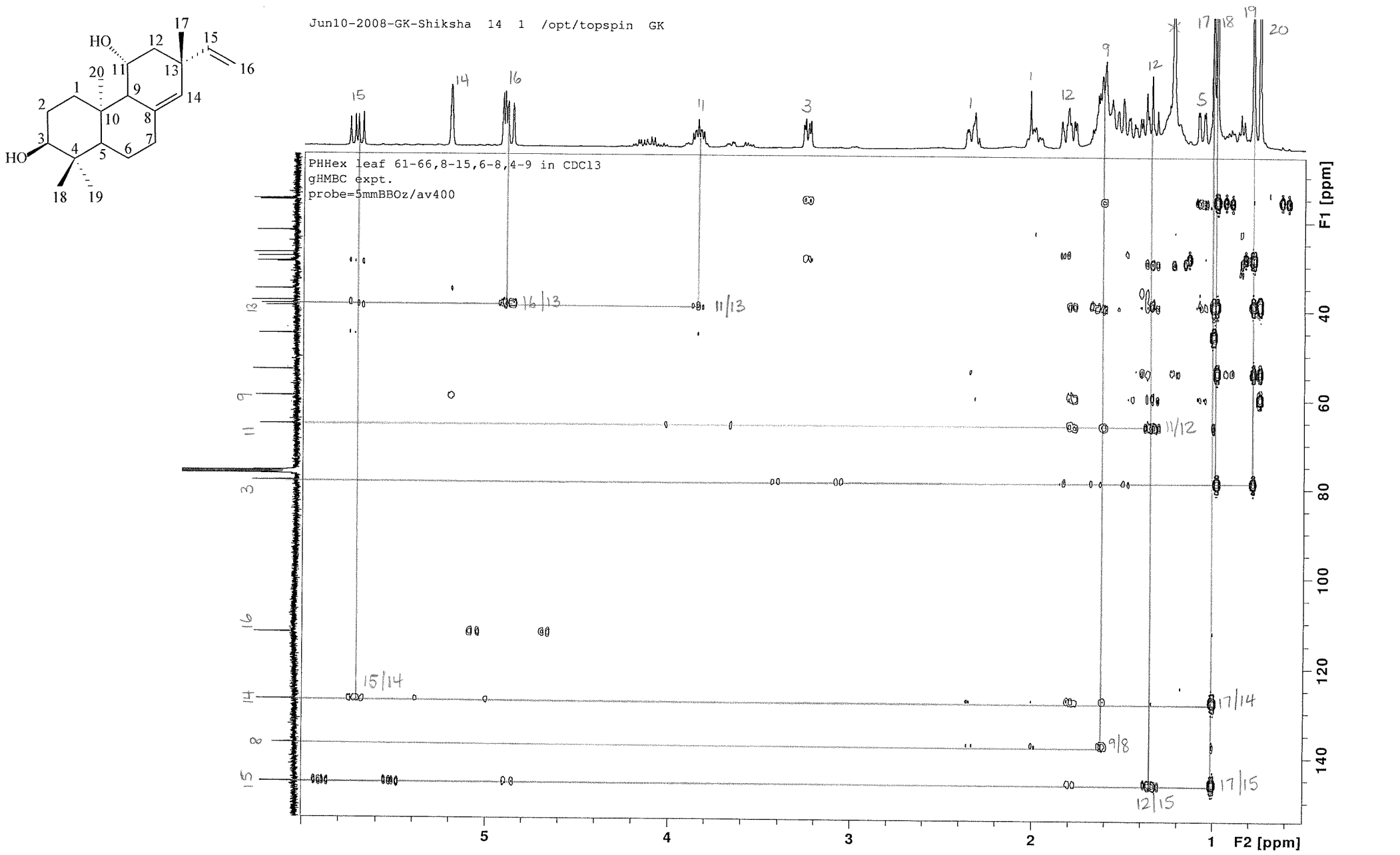
Spectrum 3c: Expanded DEPT spectrum of compound **III**, *ent*-pimara-8(14),15-diene-3β,11α-diol



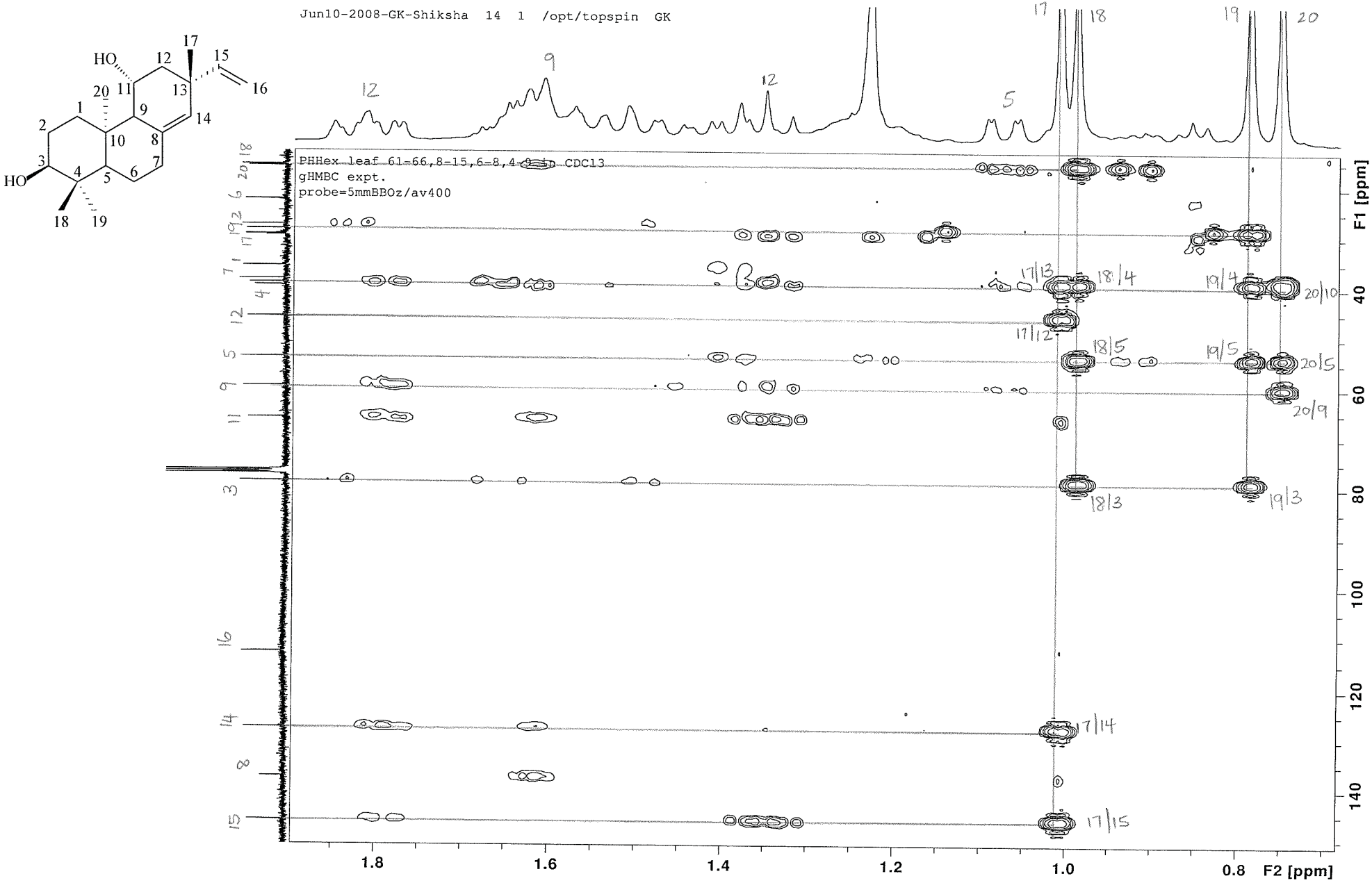
Spectrum 3d: HSQC spectrum of compound **III**, *ent*-pimara-8(14),15-diene-3β,11α-diol



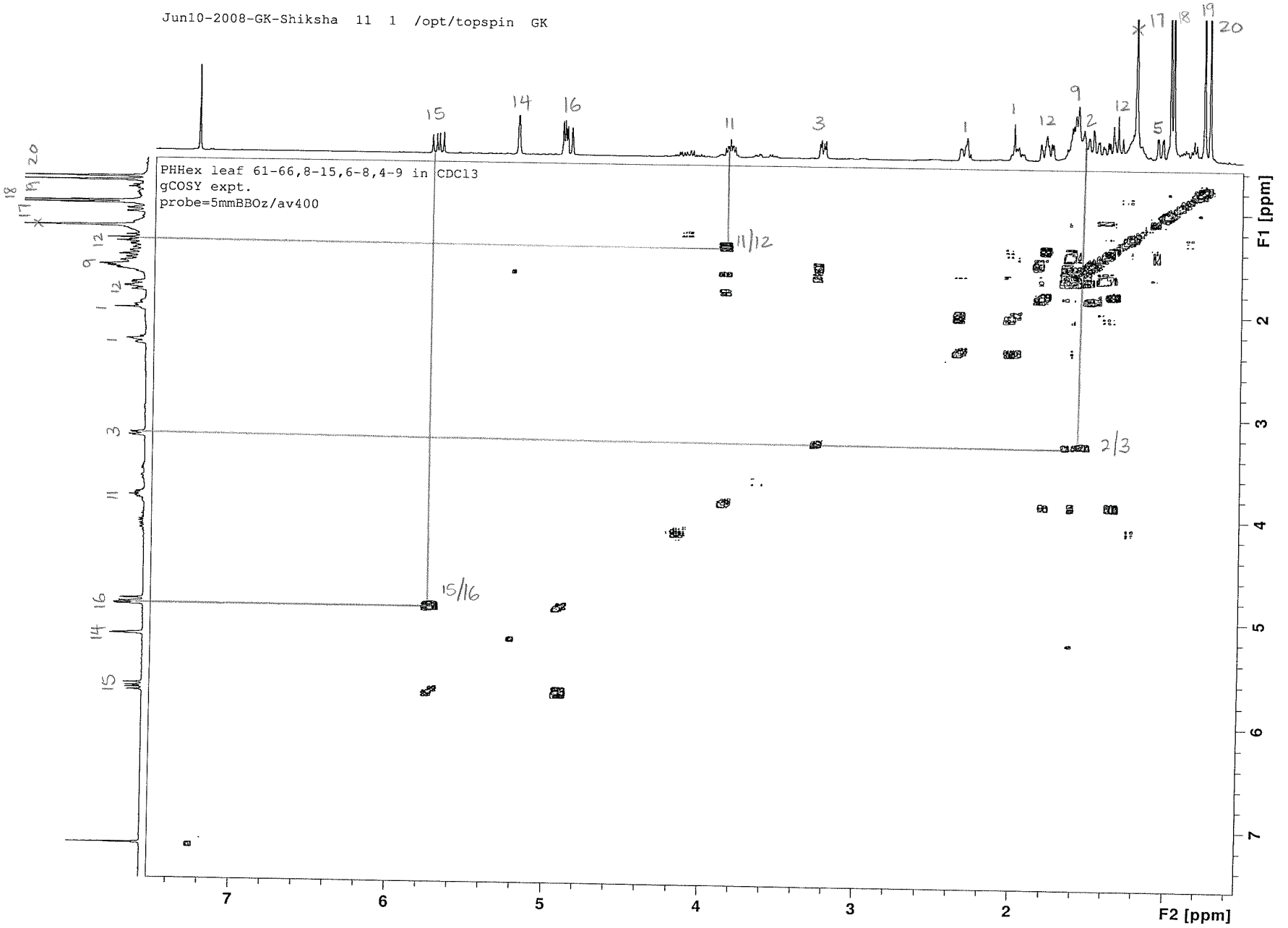
Spectrum 3e: Expanded HSQC spectrum of compound **III**, *ent*-pimara-8(14),15-diene-3β,11α-diol



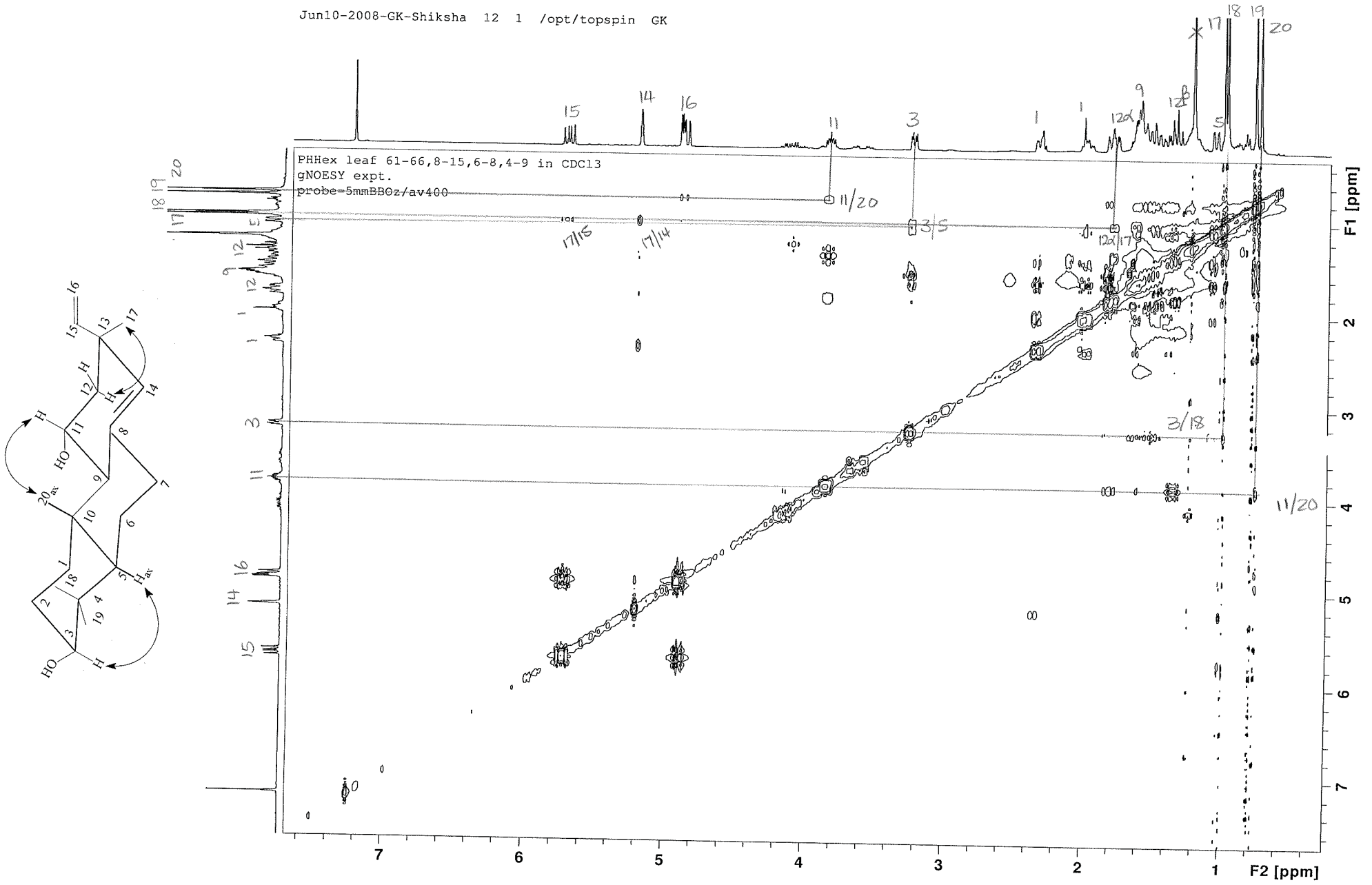
Spectrum 3f: HMBC spectrum of compound **III**, *ent*-pimara-8(14),15-diene-3β,11α-diol



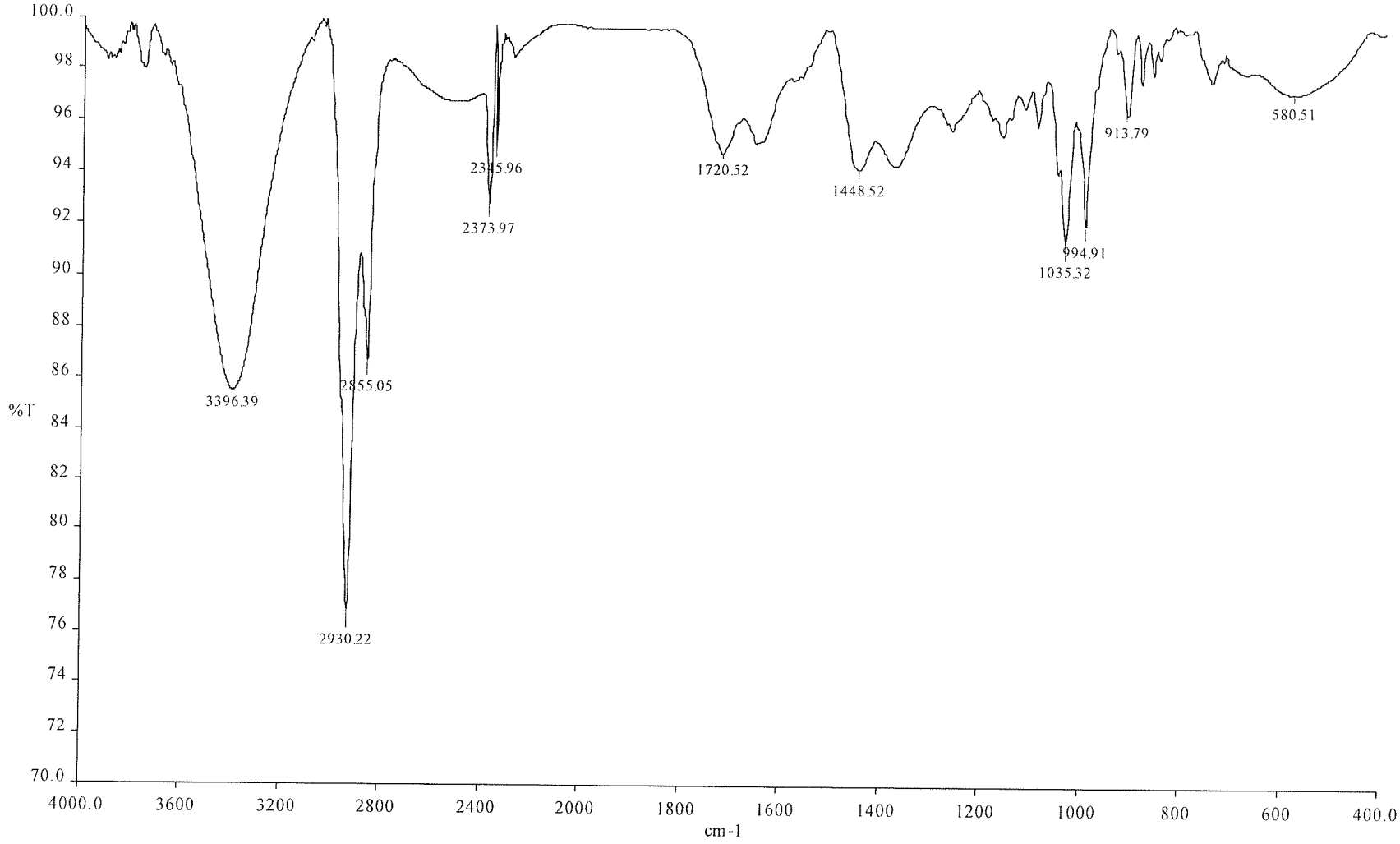
Spectrum 3g: Expanded HMBC spectrum of compound **III**, *ent*-pimara-8(14),15-diene-3β,11α-diol



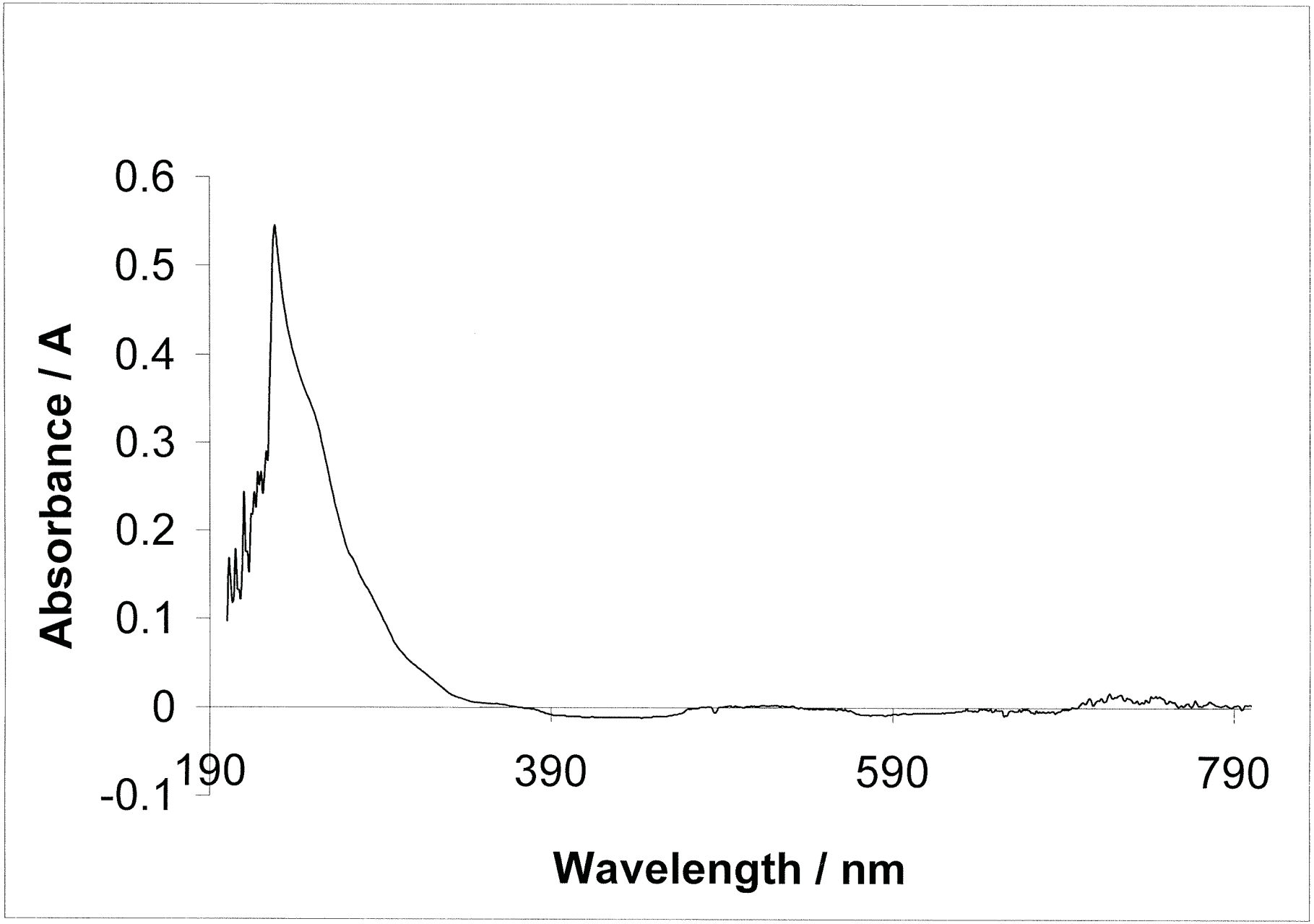
Spectrum 3h: COSY spectrum of compound **III**, *ent*-pimara-8(14),15-diene-3β,11α-diol



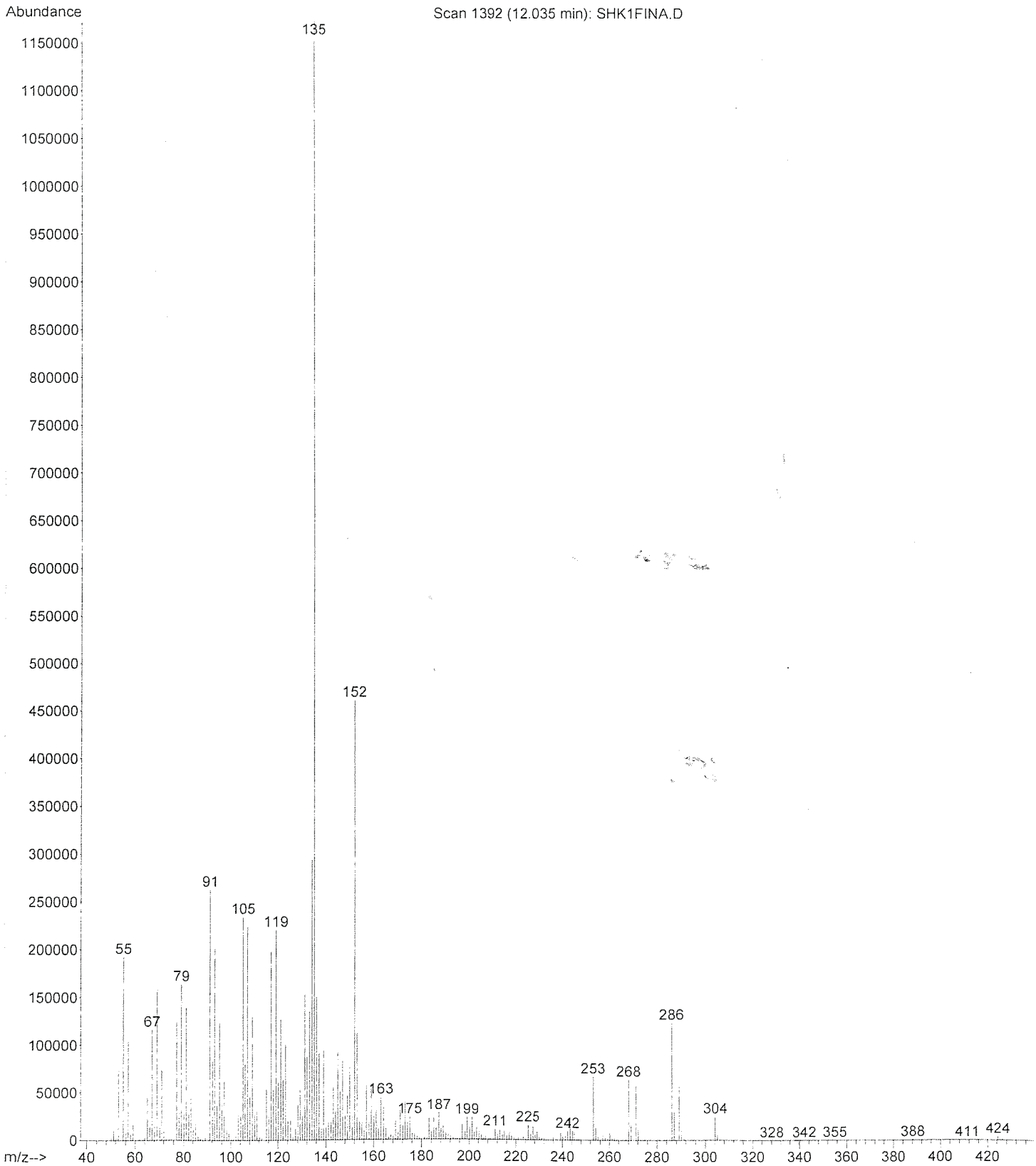
Spectrum 3i: NOESY spectrum of compound **III**, *ent*-pimara-8(14),15-diene-3β,11α-diol



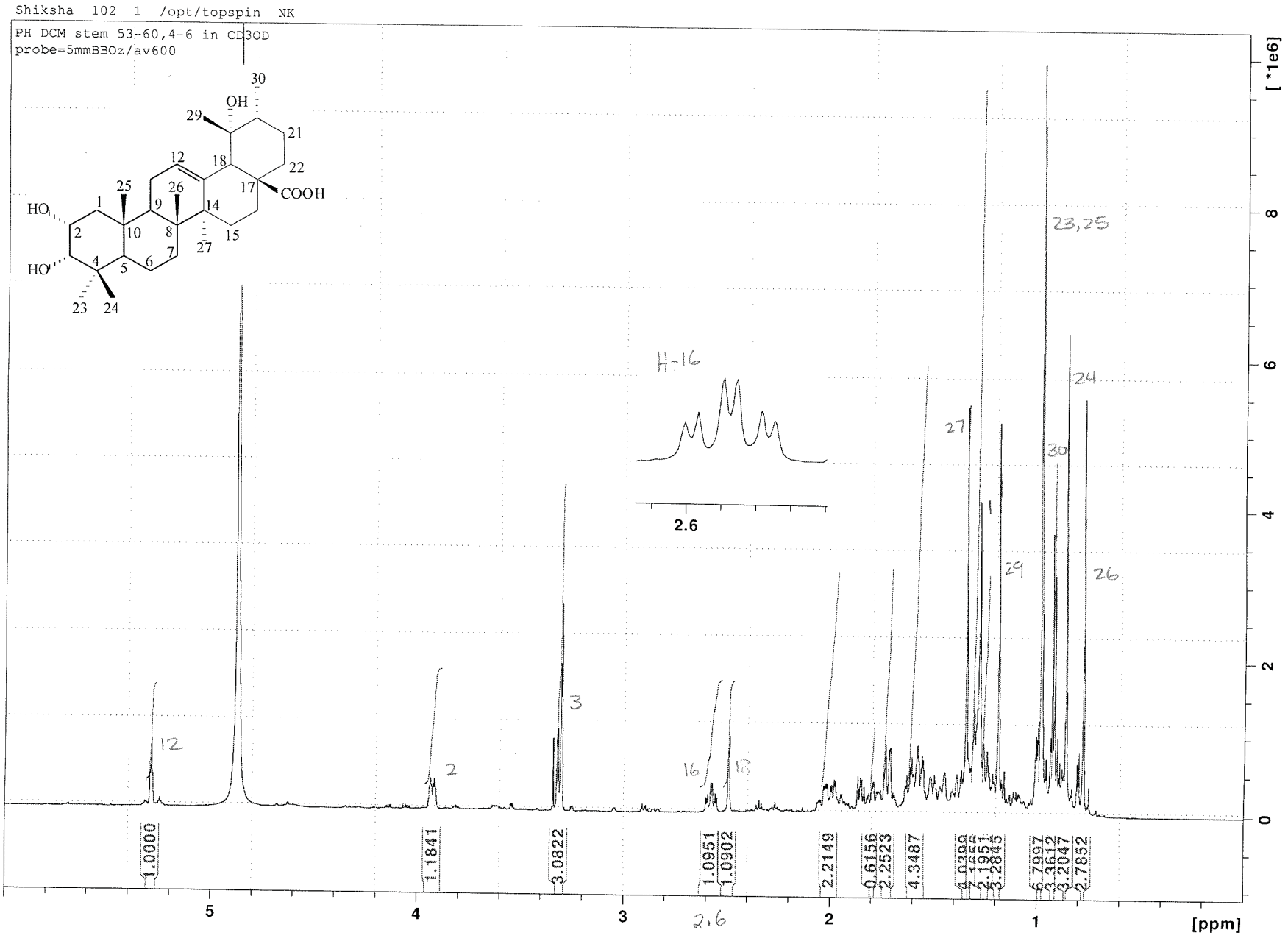
Spectrum 3j: IR spectrum of compound **III**, *ent*-pimara-8(14),15-diene-3β,11α-diol



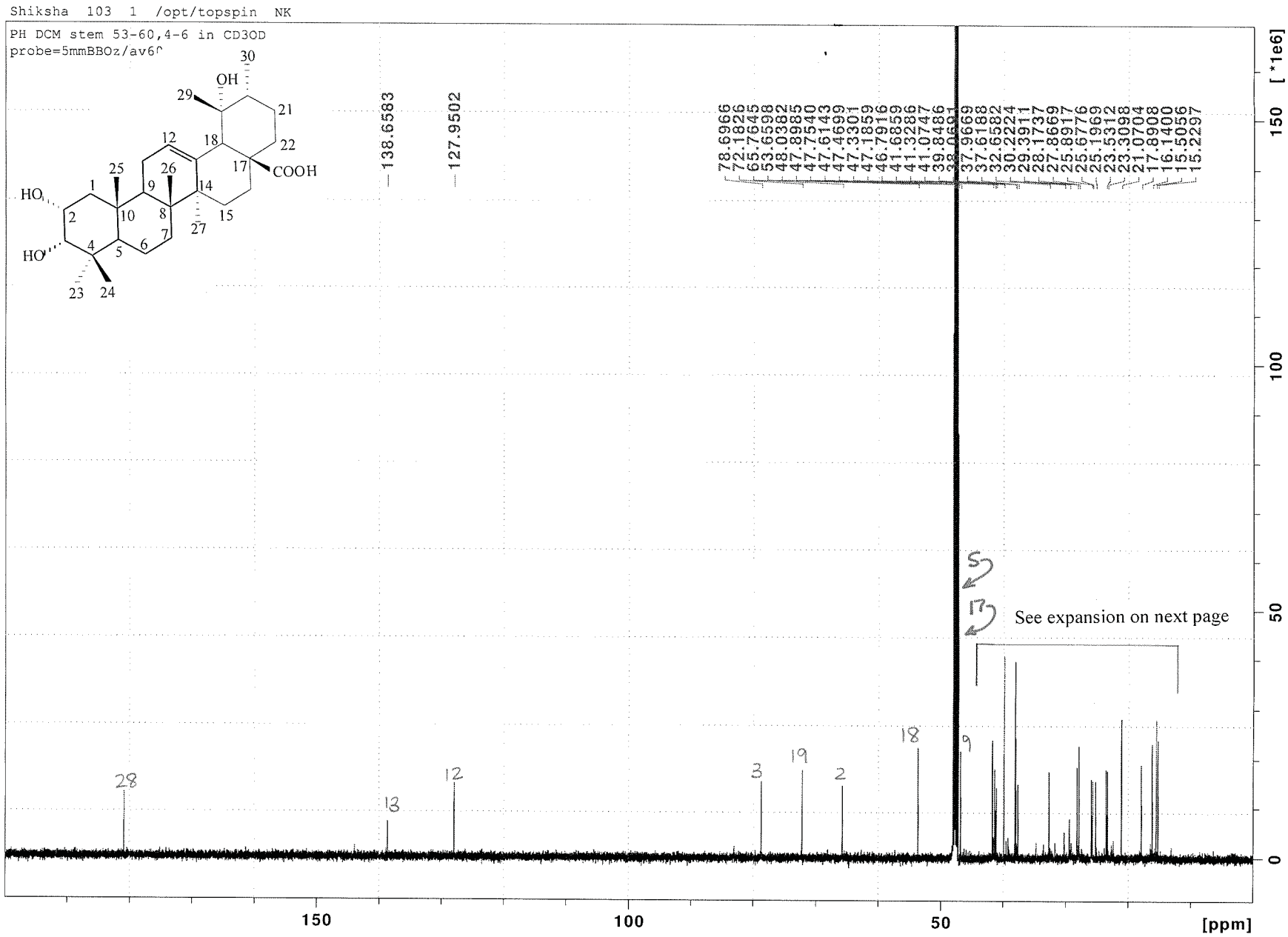
Spectrum 3k: UV spectrum of compound **III**, *ent*-pimara-8(14),15-diene-3β,11α-diol



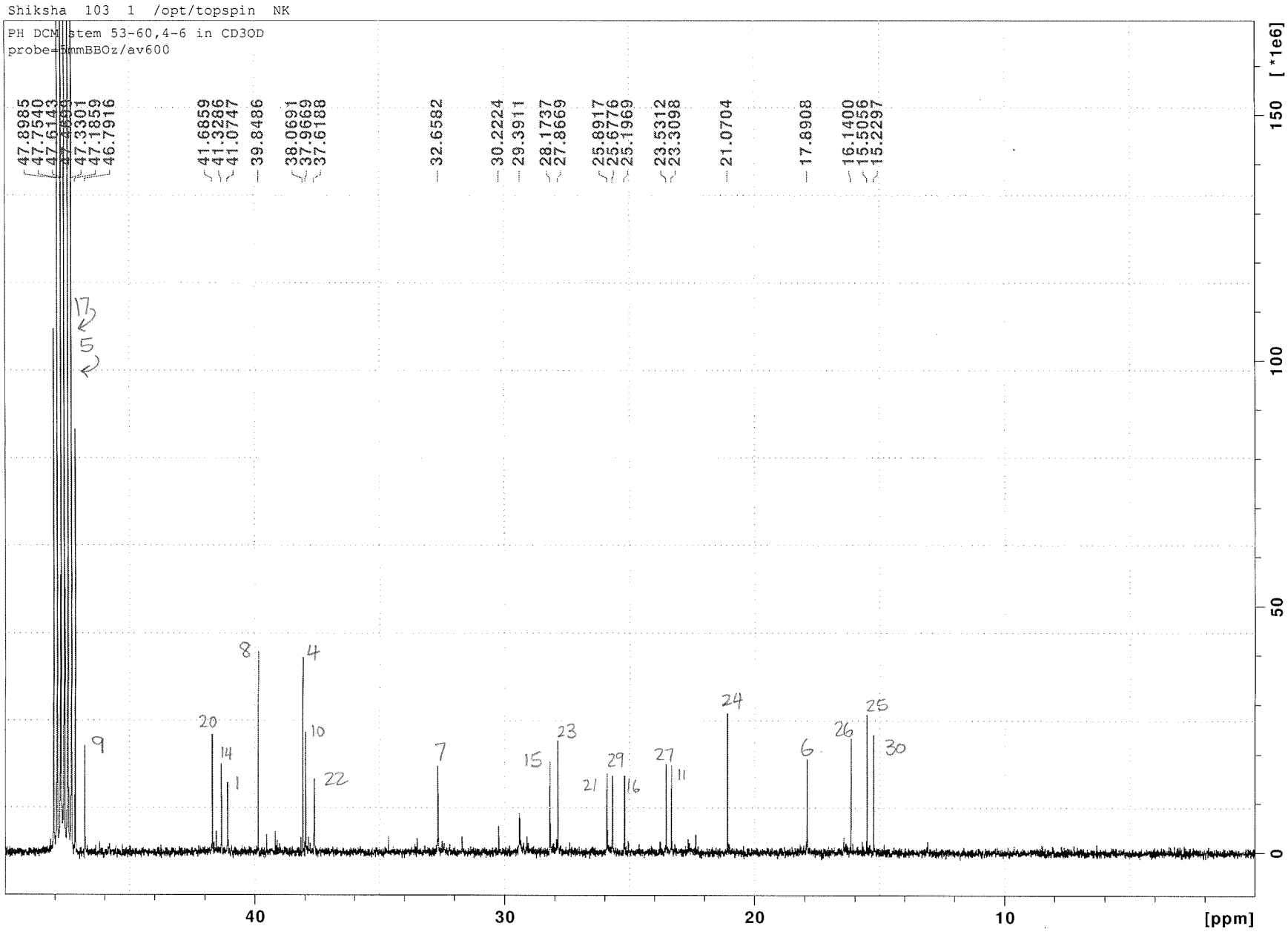
Spectrum 3l: GC-MS spectrum of compound **III**, *ent*-pimara-8(14),15-diene-3β,11α-diol



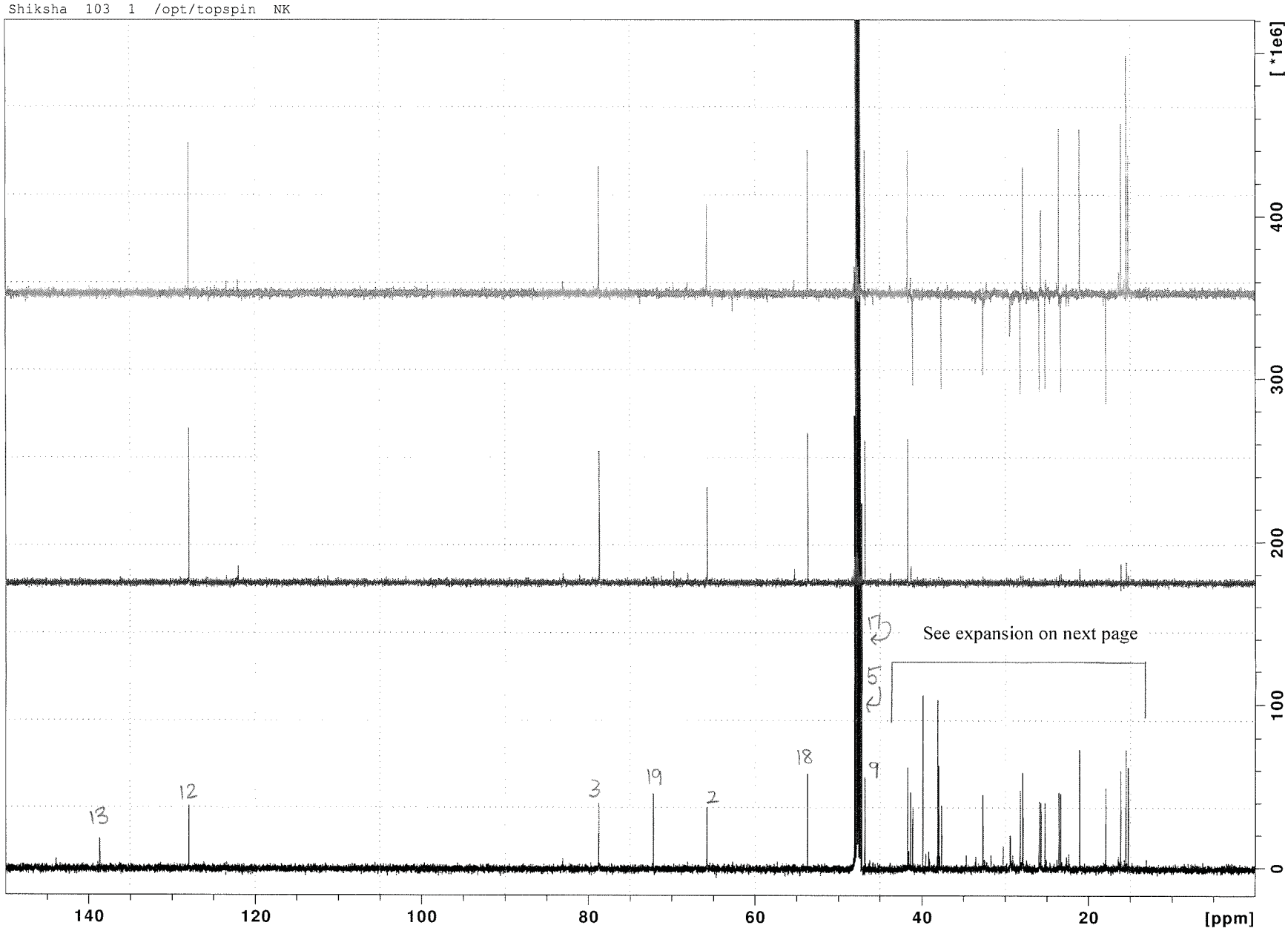
Spectrum 4a: ^{1}H NMR spectrum of compound **IV**, euscaphic acid



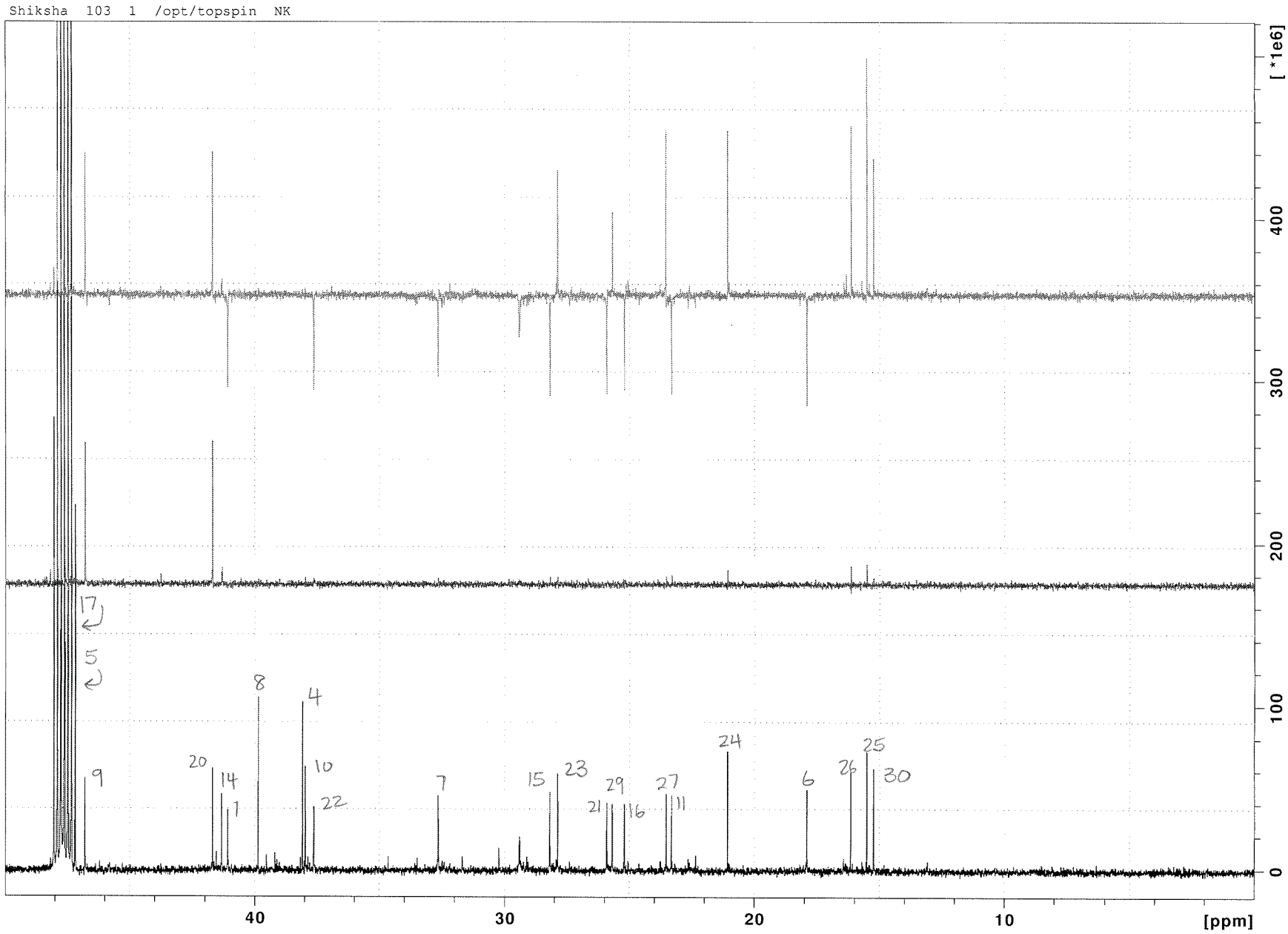
Spectrum 4b: ^{13}C NMR spectrum of compound **IV**, euscaphic acid



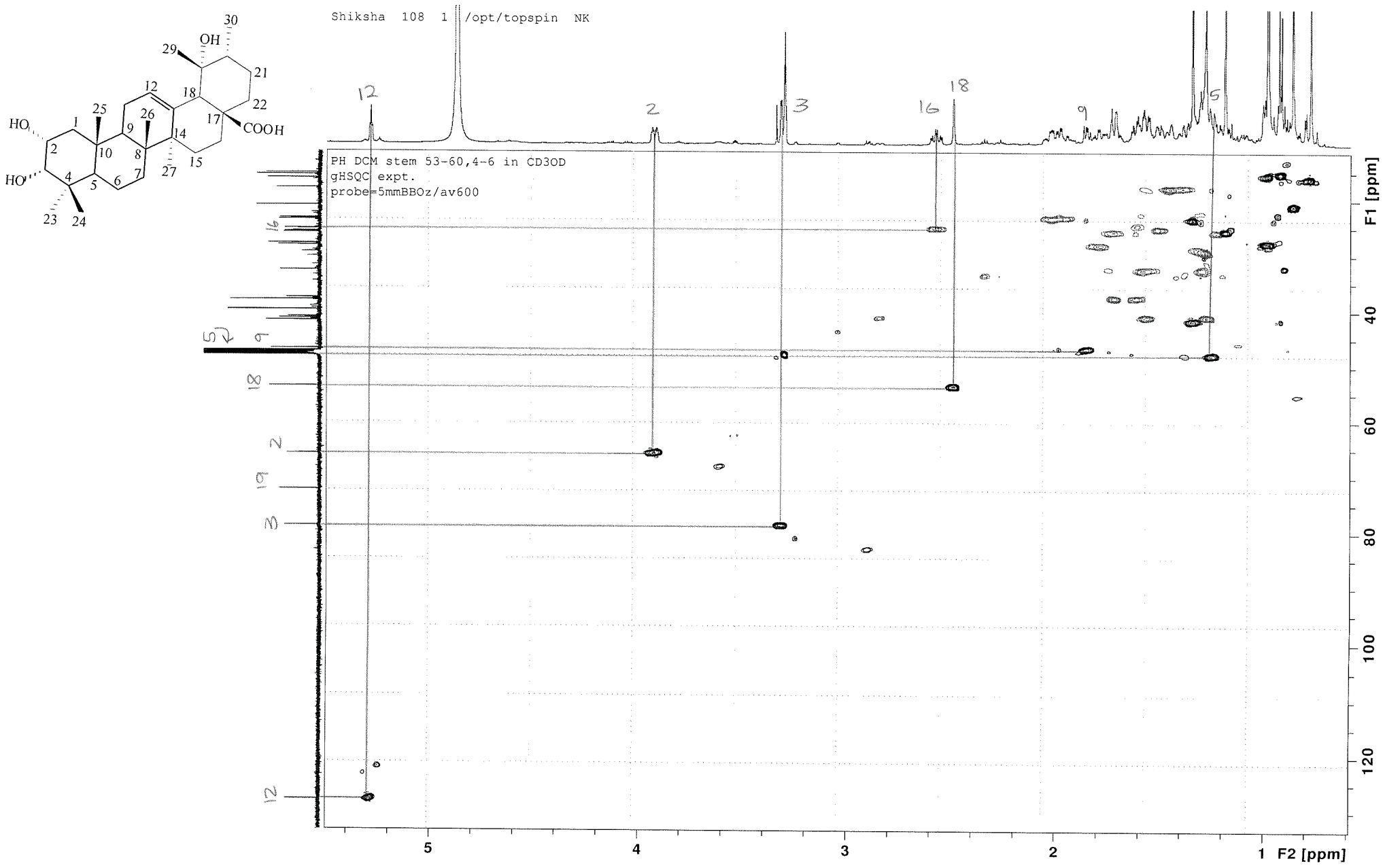
Spectrum 4c: Expanded ^{13}C NMR spectrum of compound **IV**, euscaphic acid



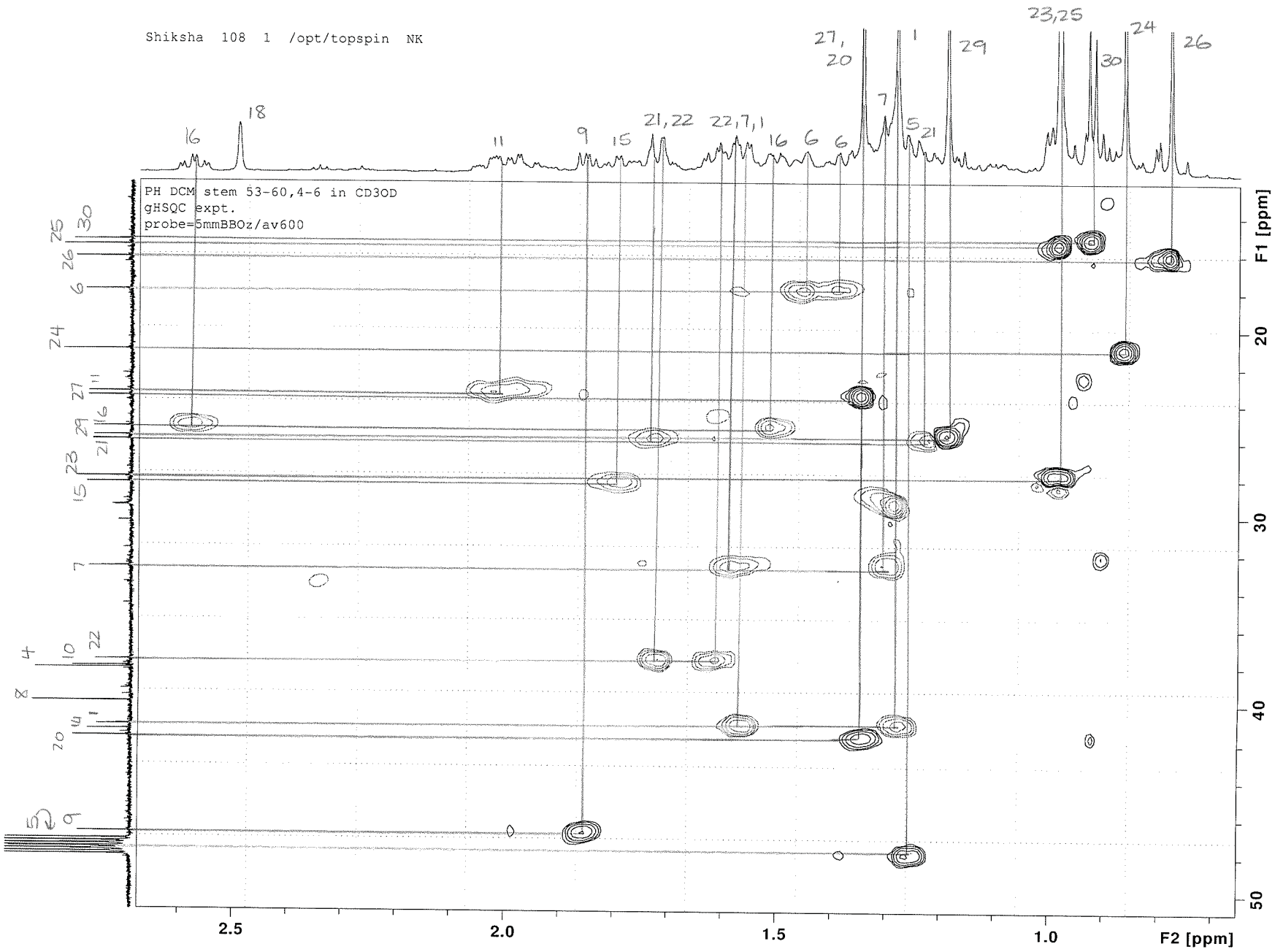
Spectrum 4d: DEPT spectrum of compound **IV**, euscaphic acid



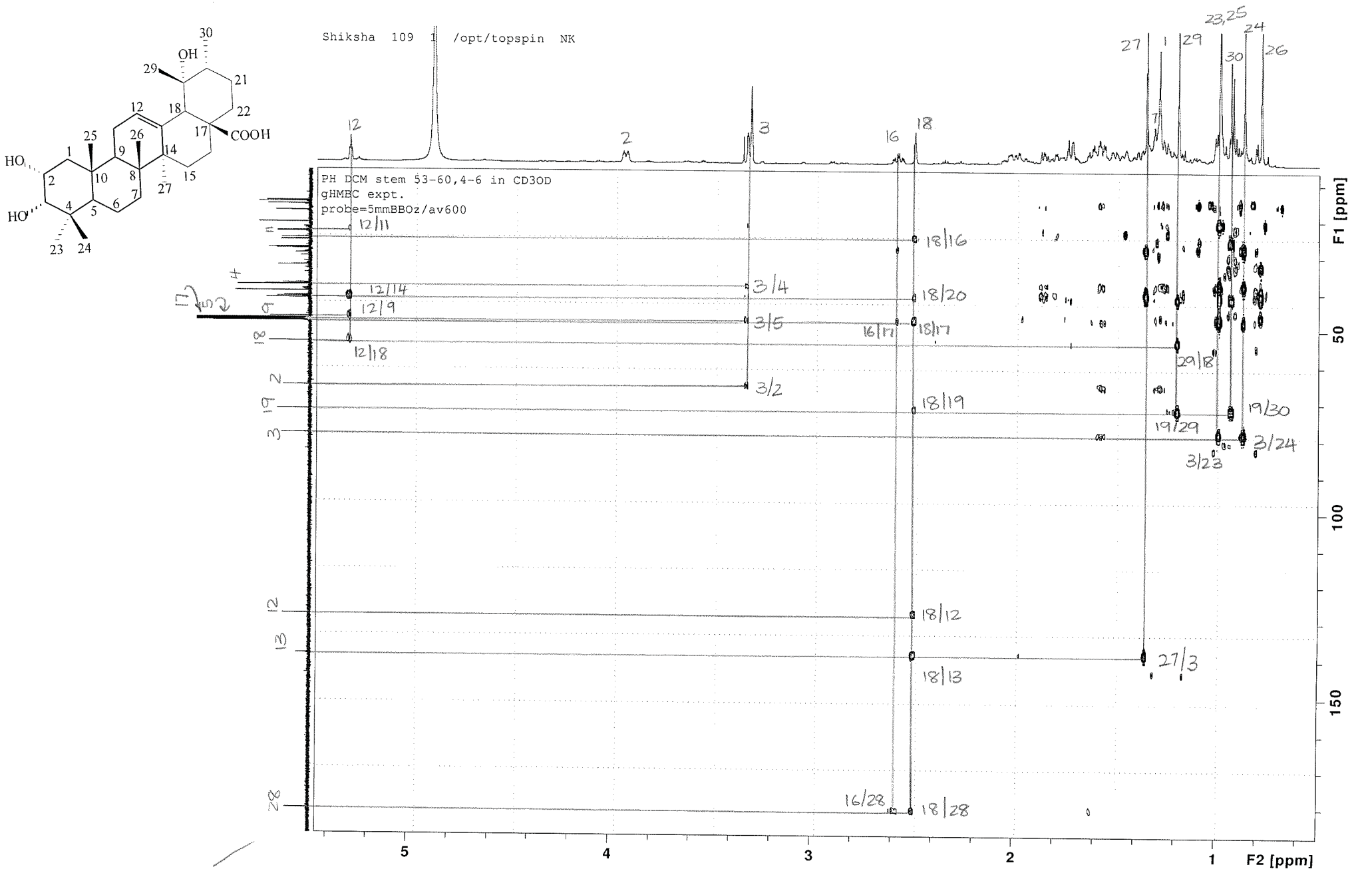
Spectrum 4e: Expanded DEPT spectrum of compound **IV**, euscaphic acid



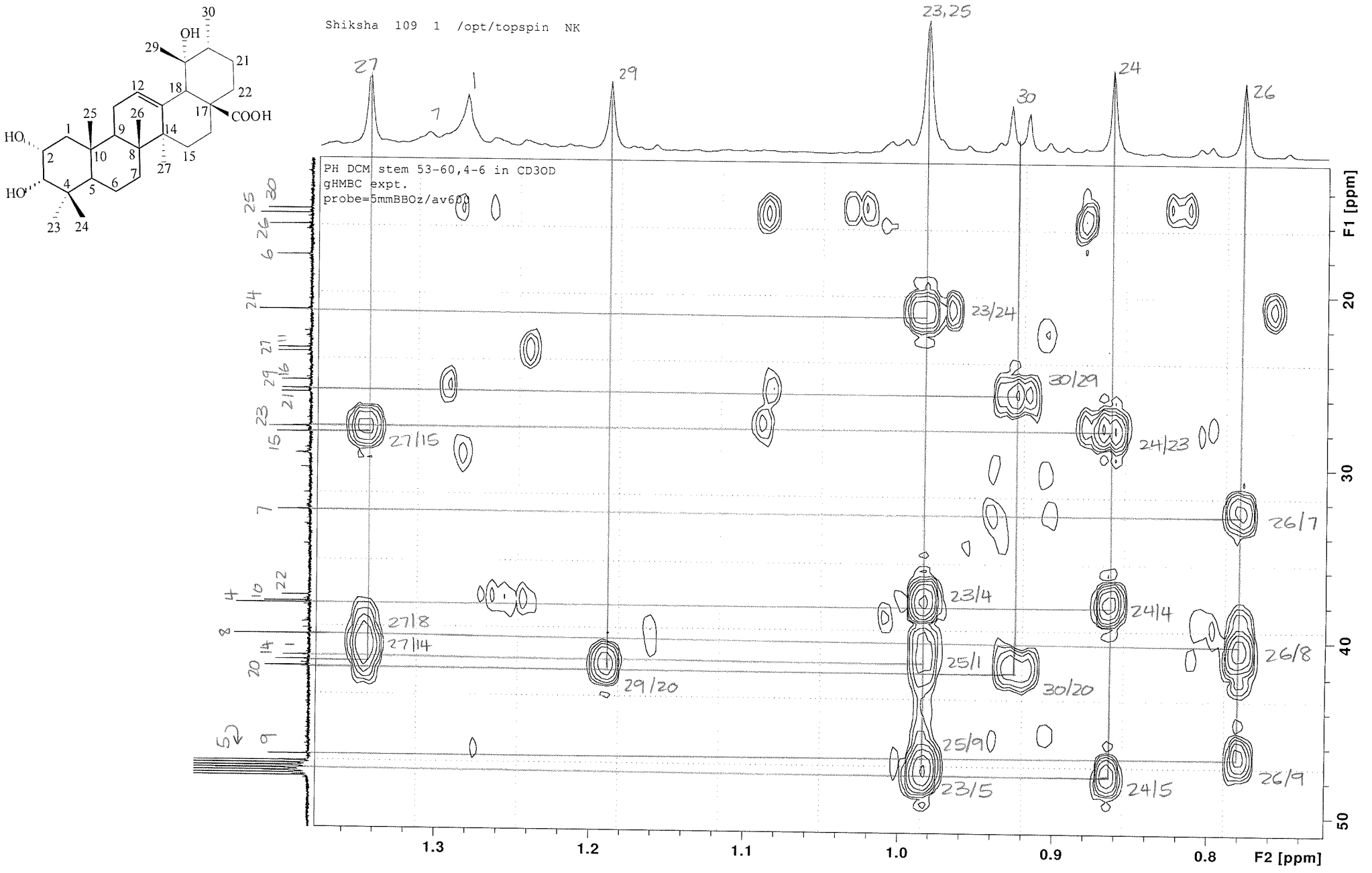
Spectrum 4f: HSQC spectrum of compound **IV**, euscaphic acid



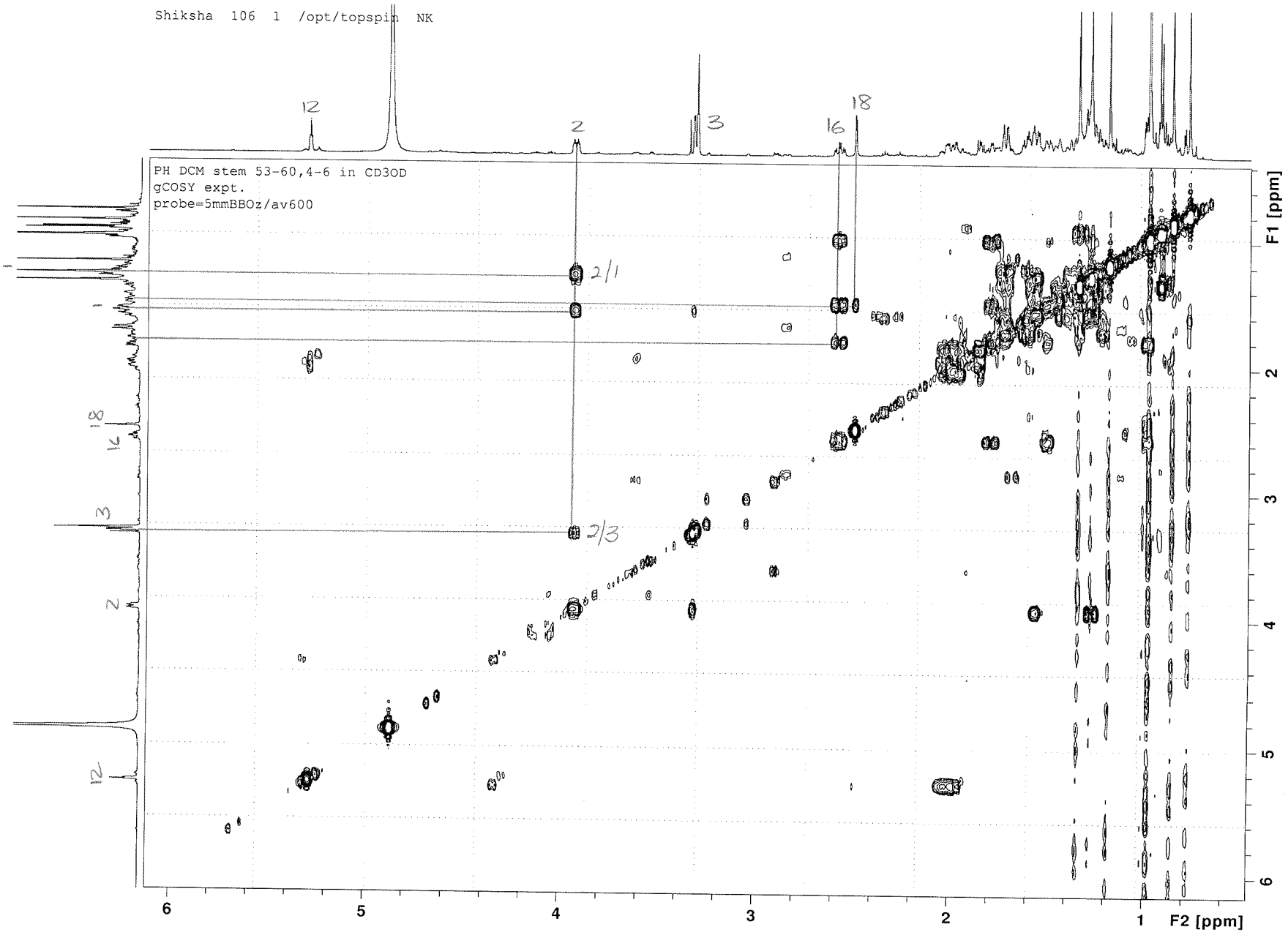
Spectrum 4g: Expanded HSQC spectrum of compound **IV**, euscaphic acid



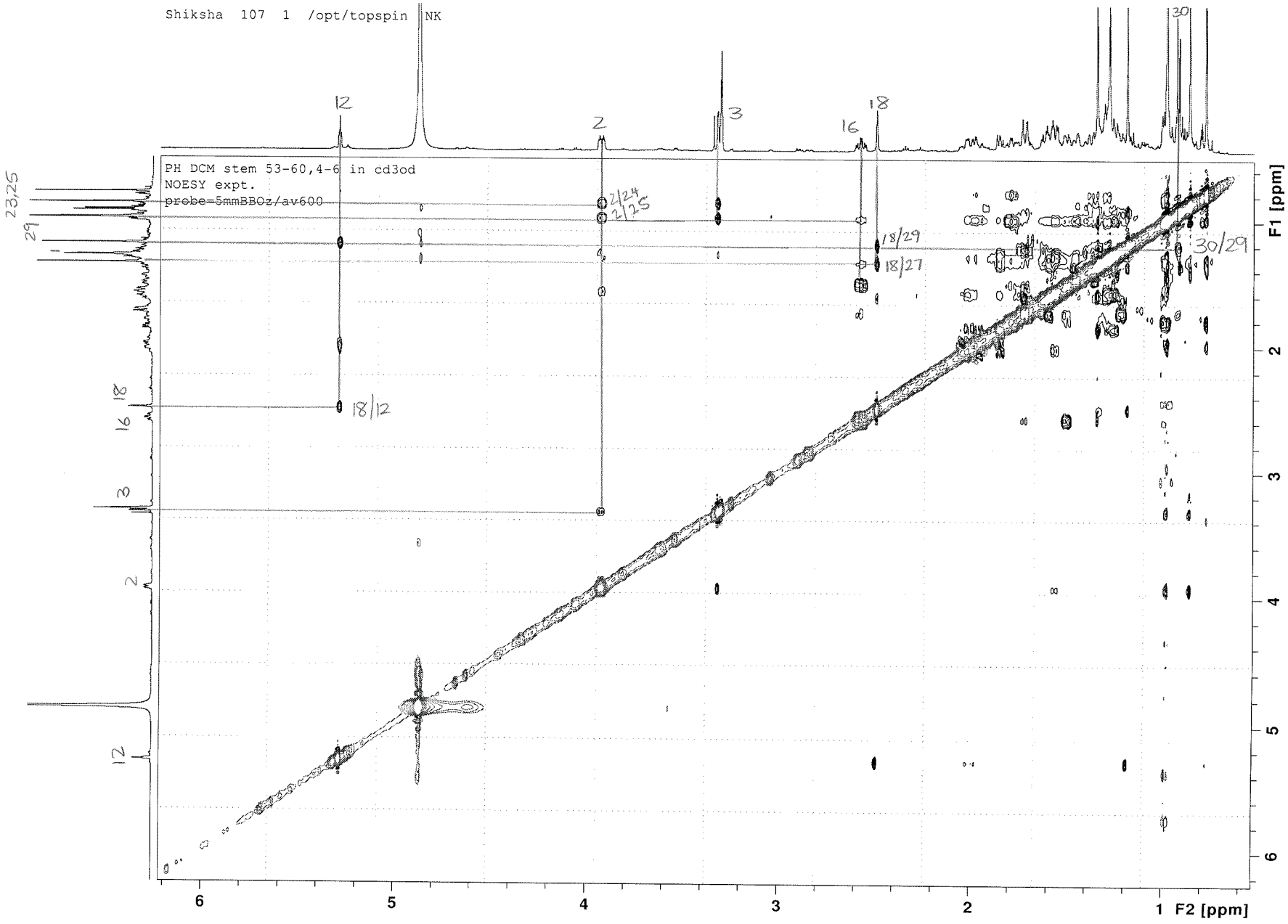
Spectrum 4h: HMBC spectrum of compound **IV**, euscaphic acid



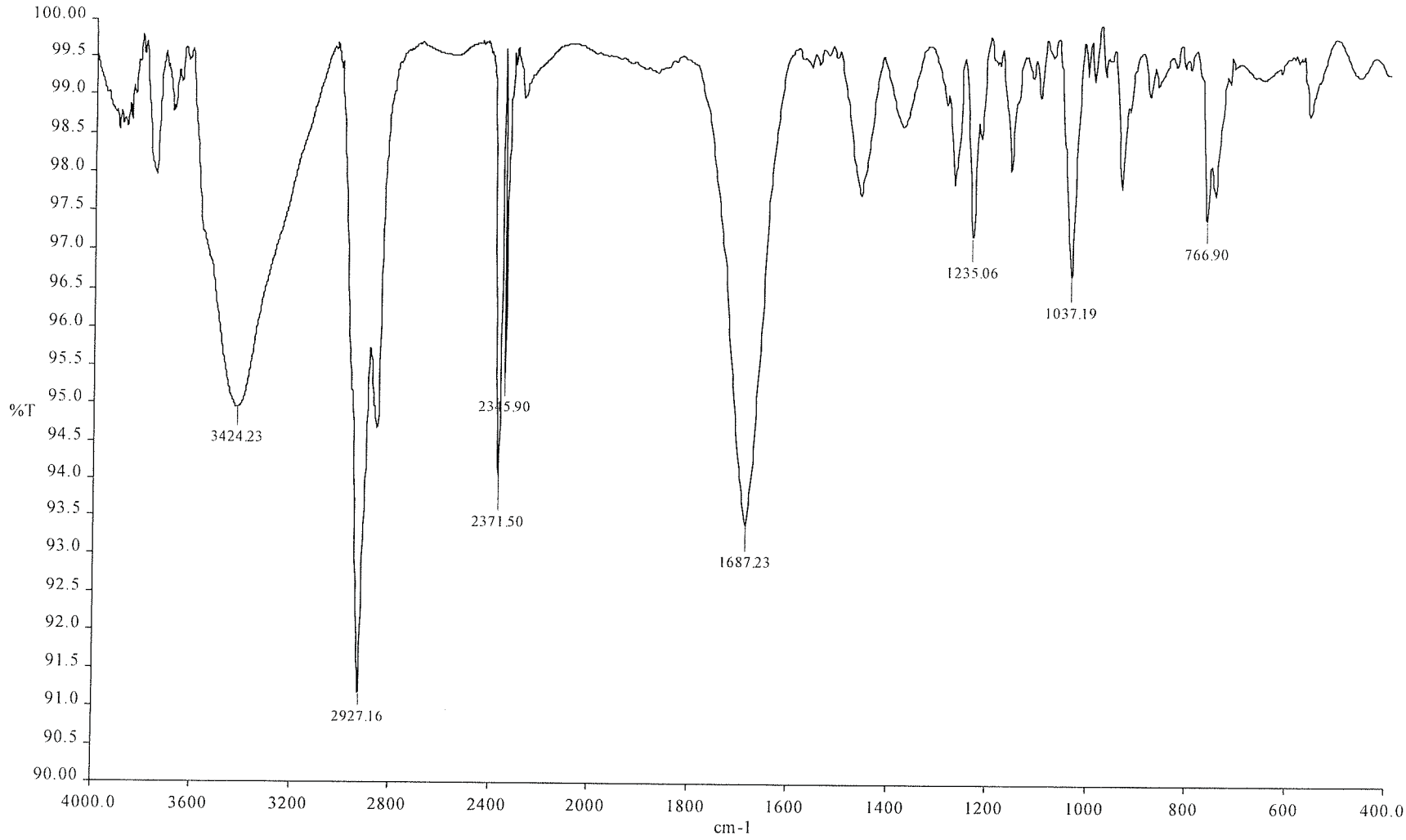
Spectrum 4i: Expanded HMBC spectrum of compound **IV**, euscaphic acid



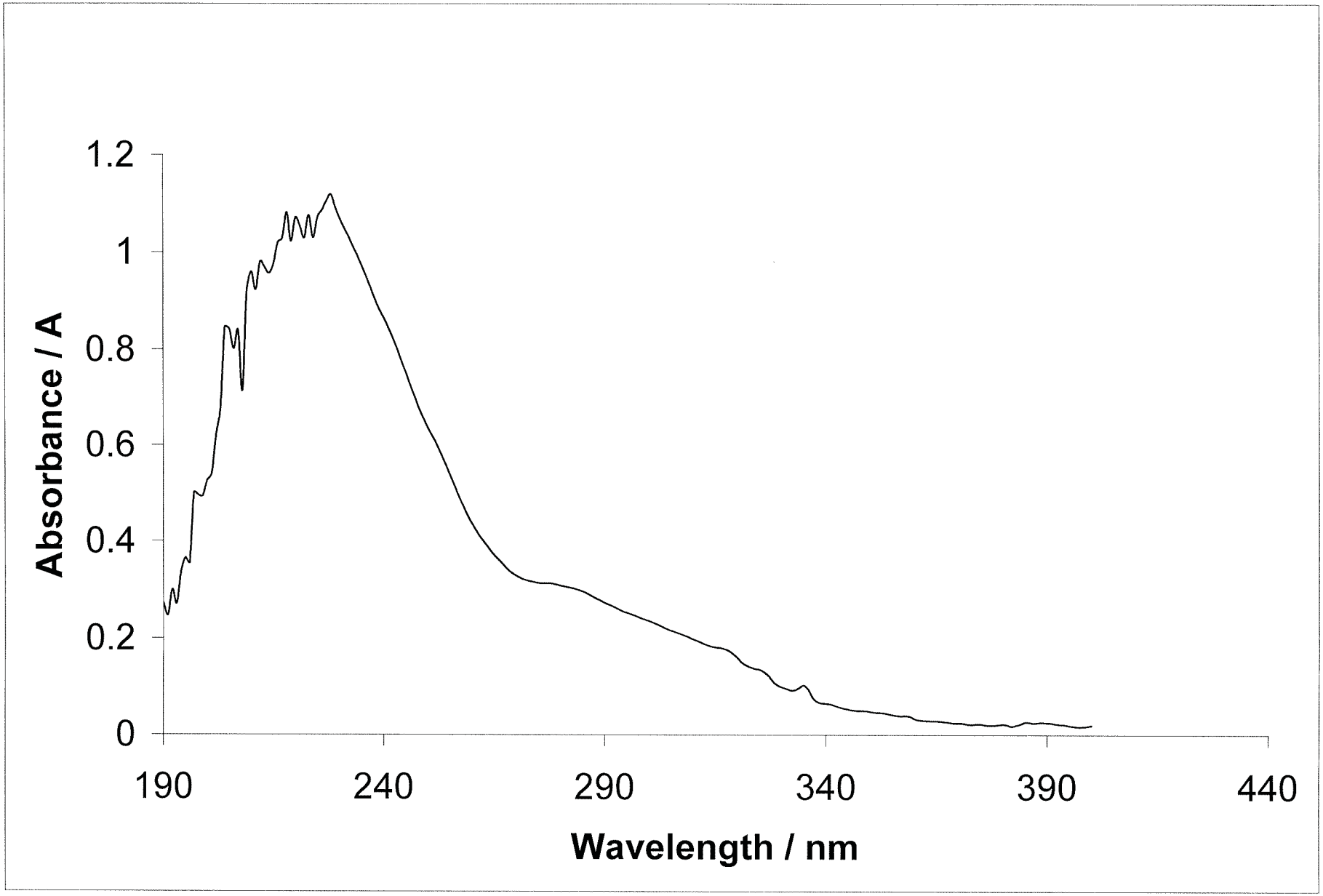
Spectrum 4j: COSY spectrum of compound **IV**, euscaphic acid



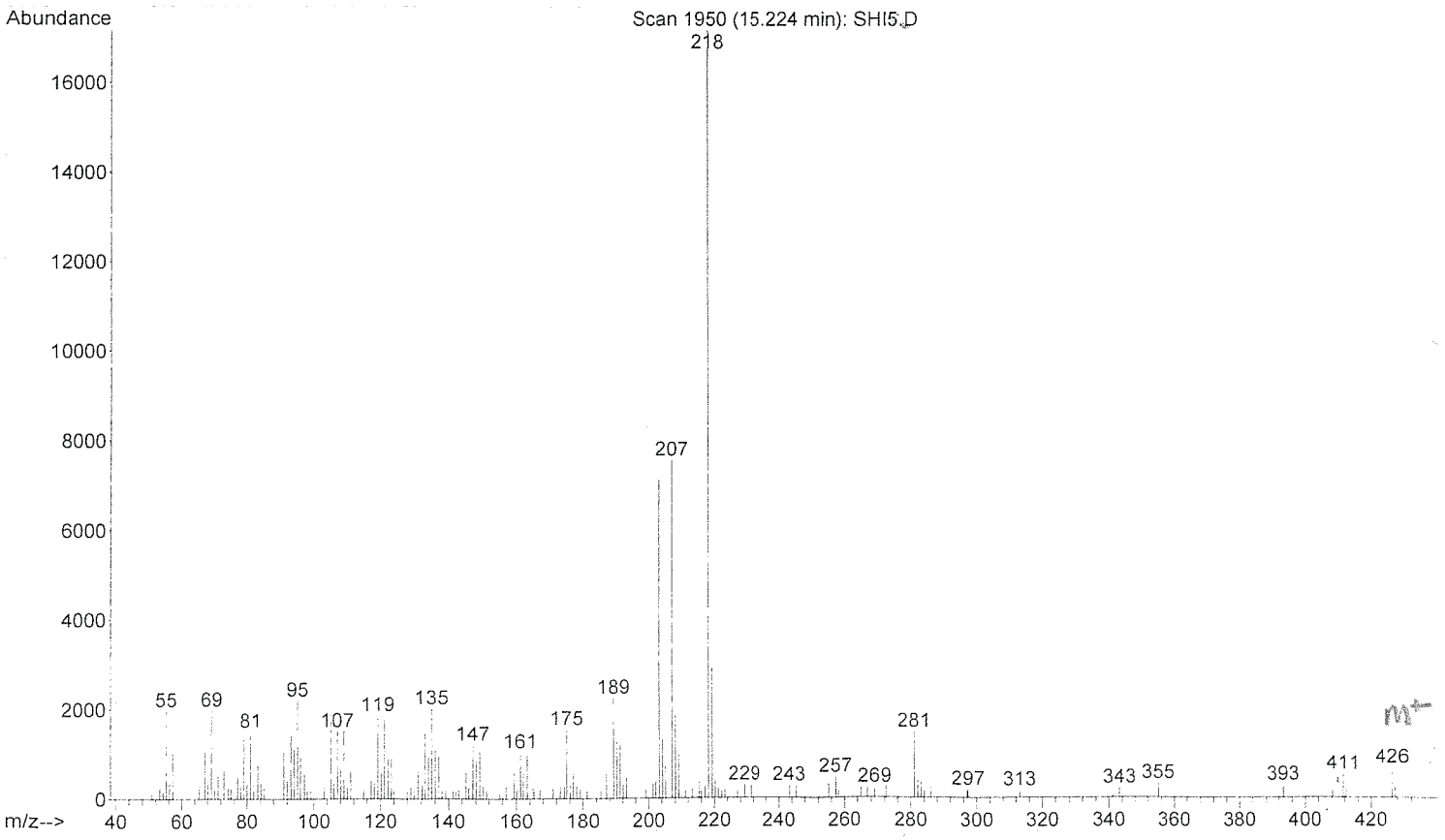
Spectrum 4k: NOESY spectrum of compound **IV**, euscaphic acid



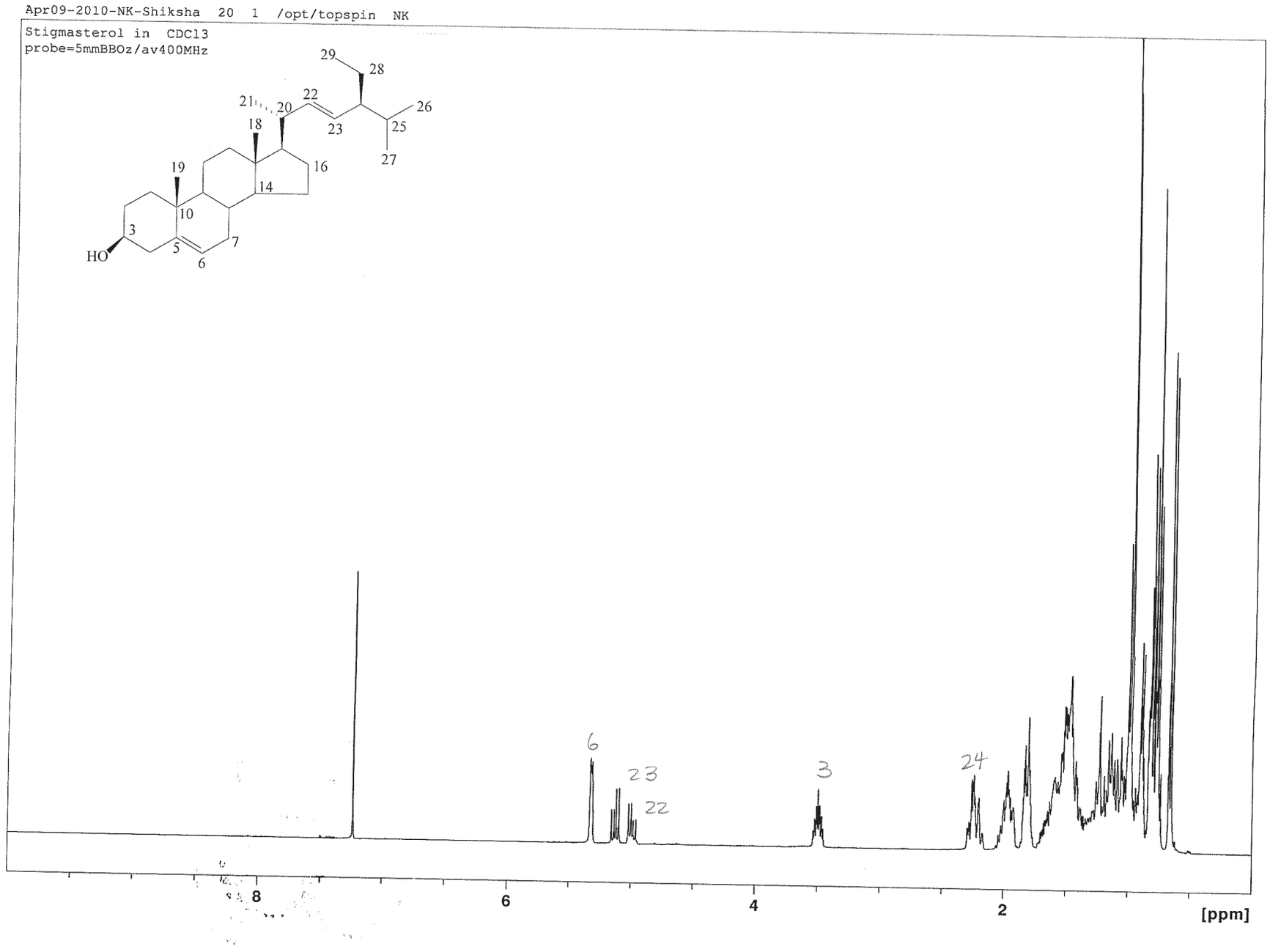
Spectrum 4l: IR spectrum of compound **IV**, euscaphic acid



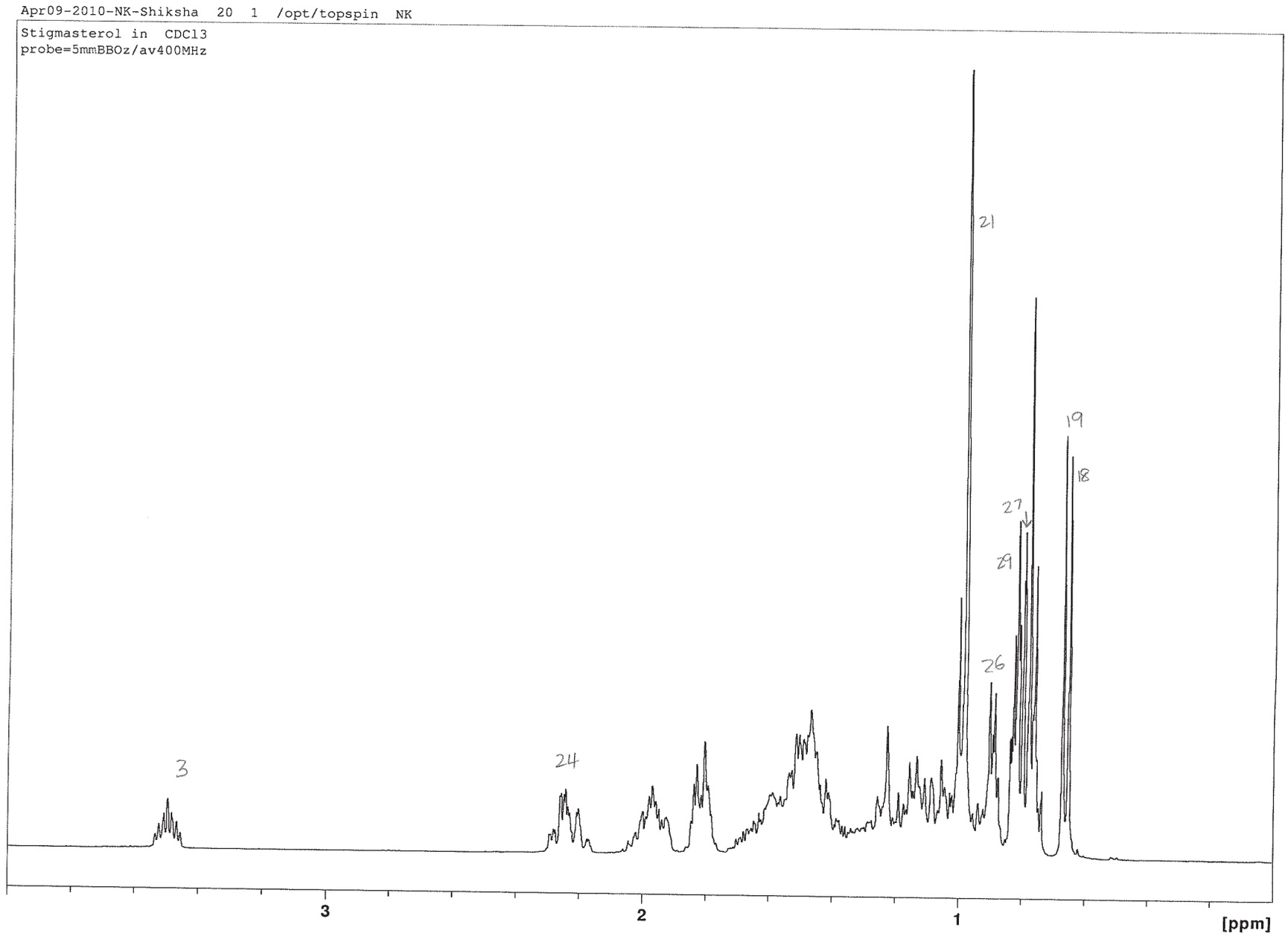
Spectrum 4m: UV spectrum of compound **IV**, euscaphic acid



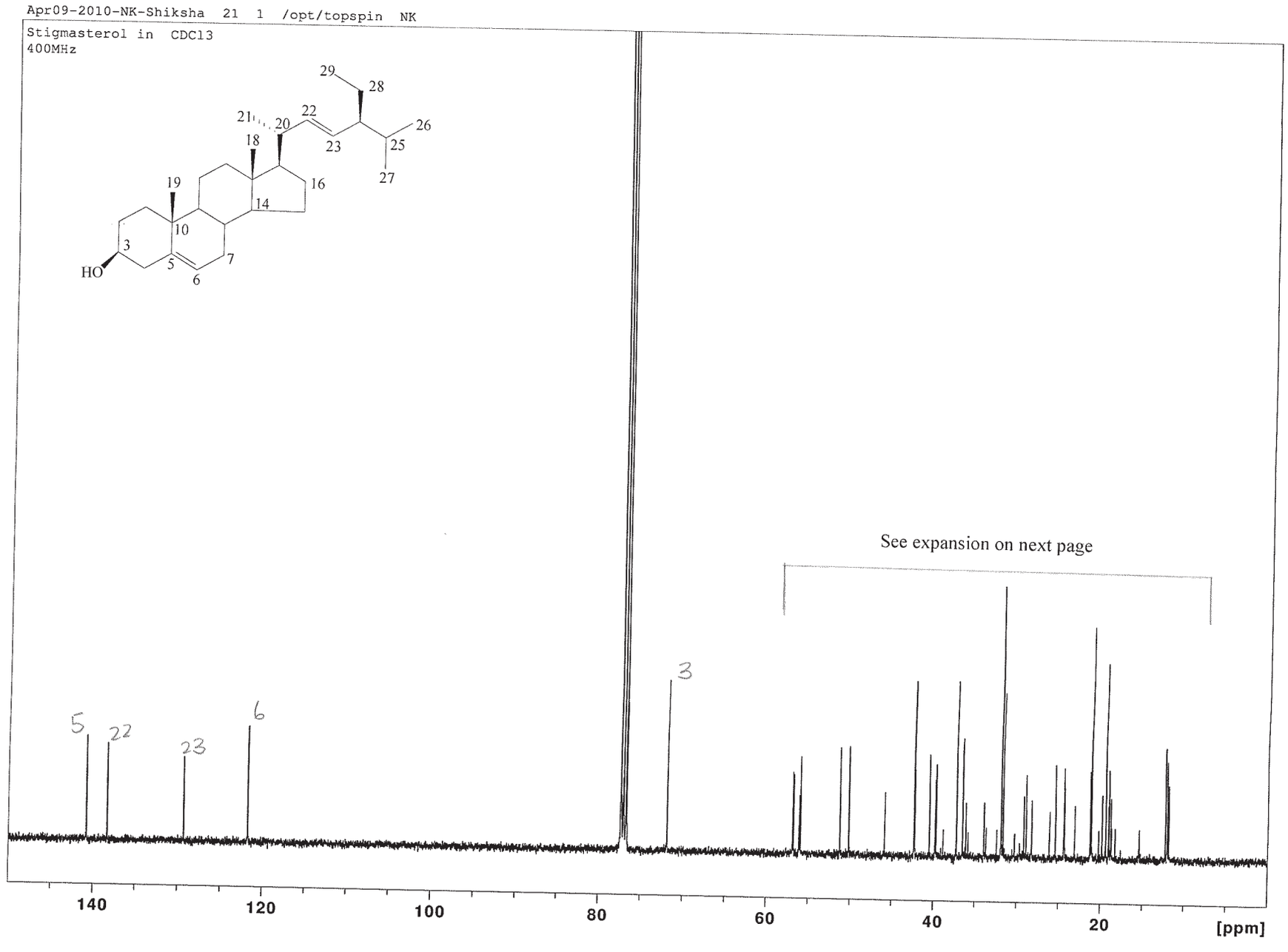
Spectrum 4n: GC-MS spectrum of compound **IV**, euscaphic acid



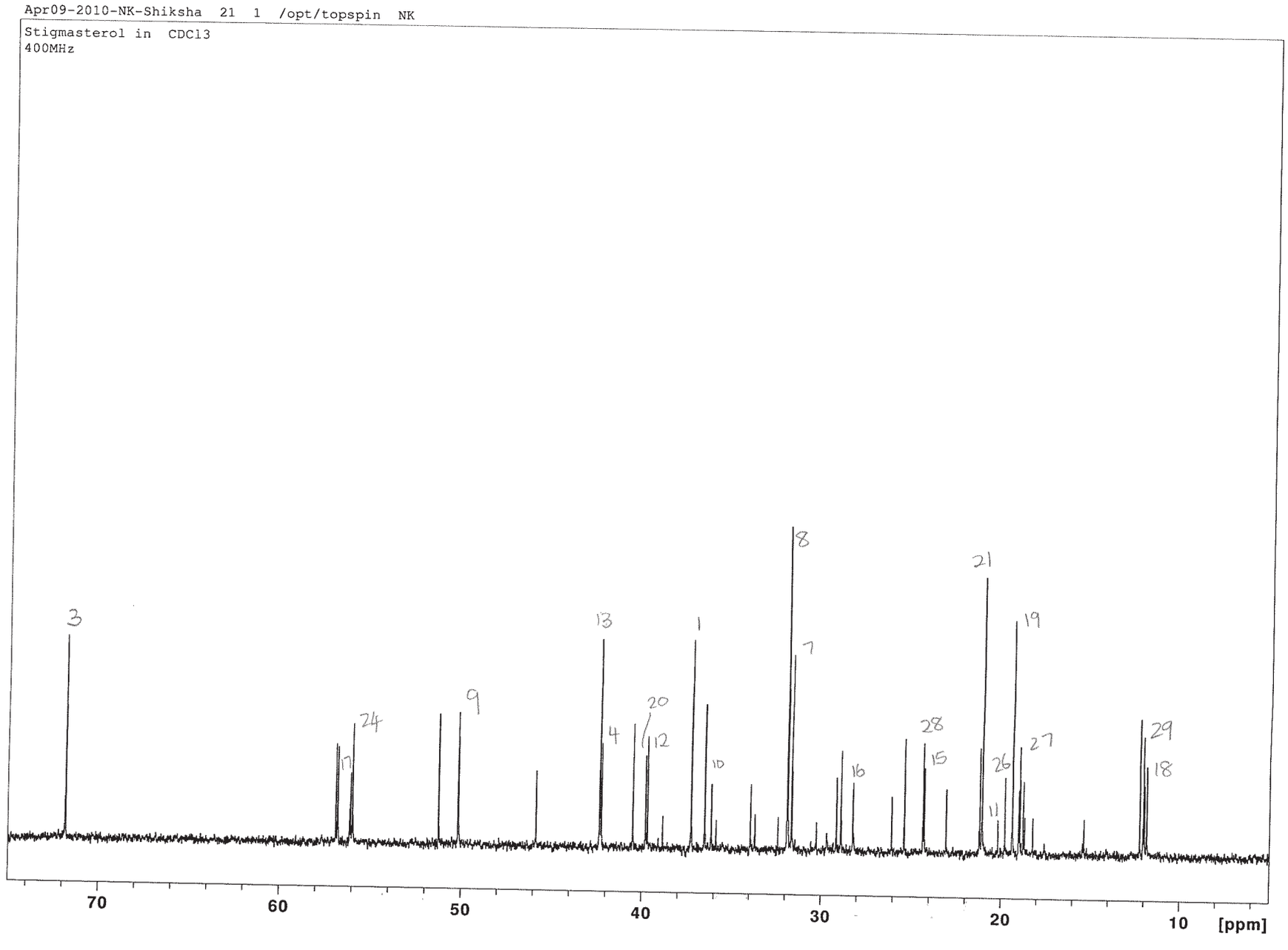
Spectrum 5a: ^{1}H NMR spectrum of compound **V**, stigmasterol



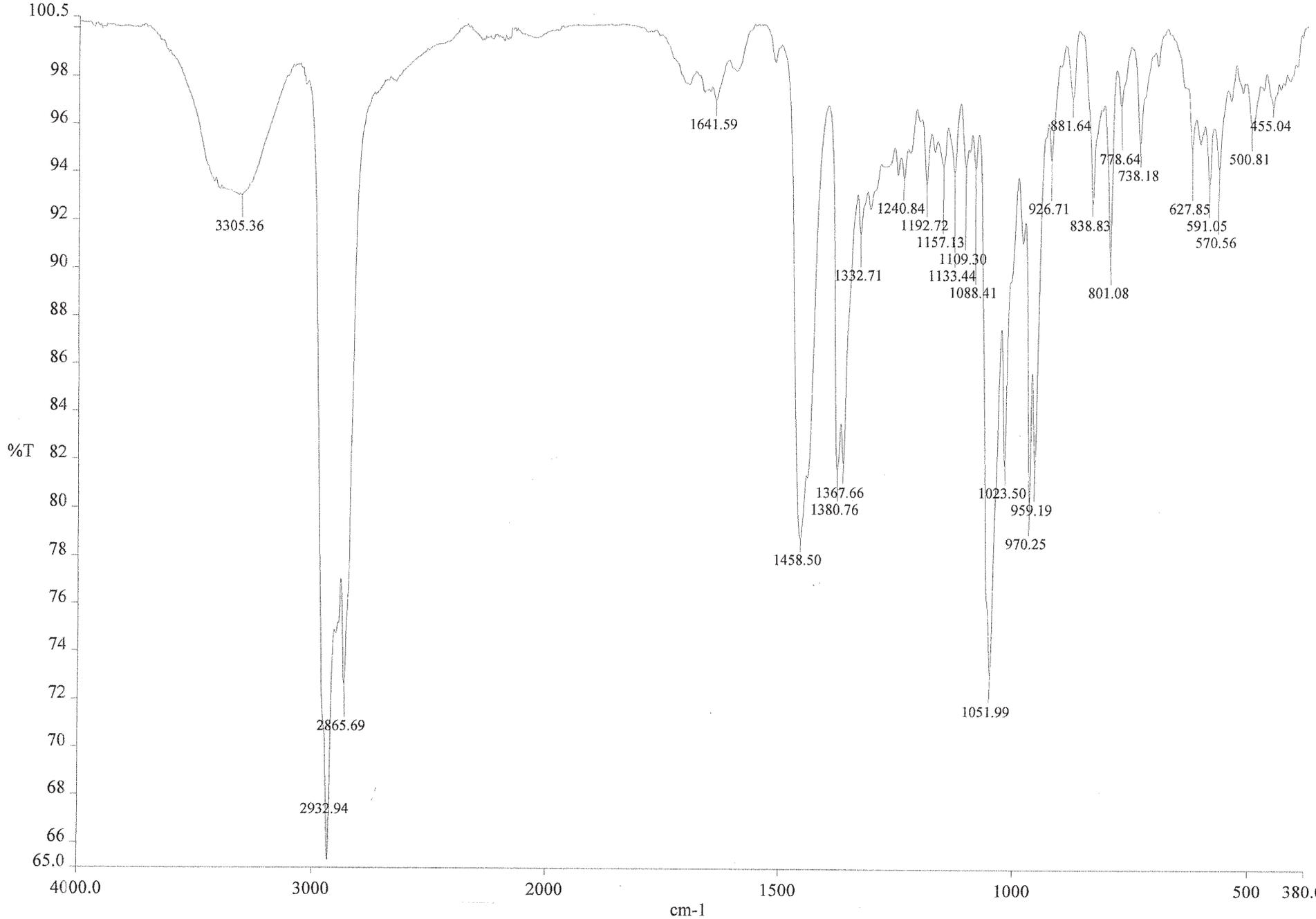
Spectrum 5b: Expanded ^{1}H NMR spectrum of compound **V**, stigmasterol



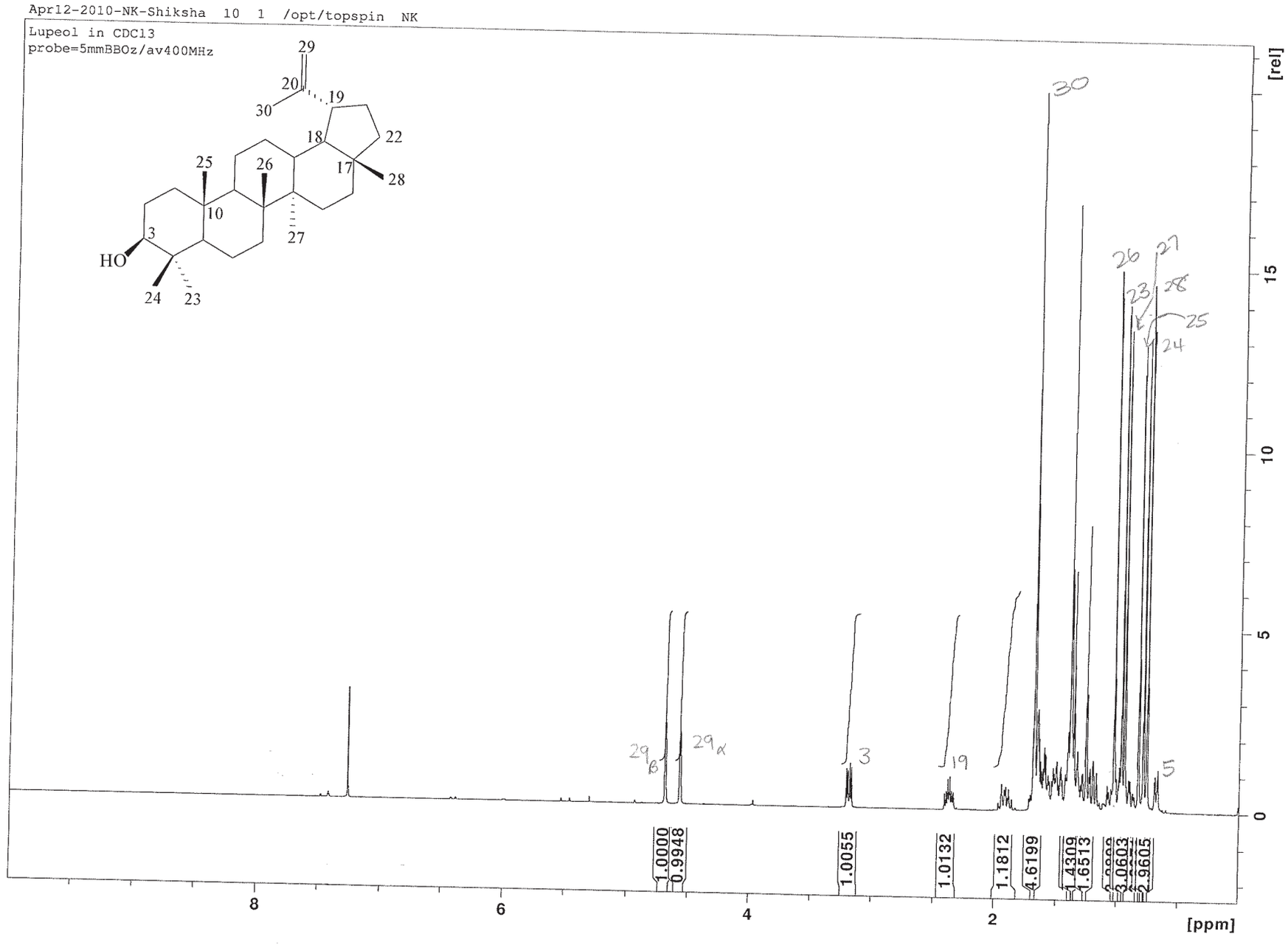
Spectrum 5c: ^{13}C NMR spectrum of compound **V**, stigmasterol



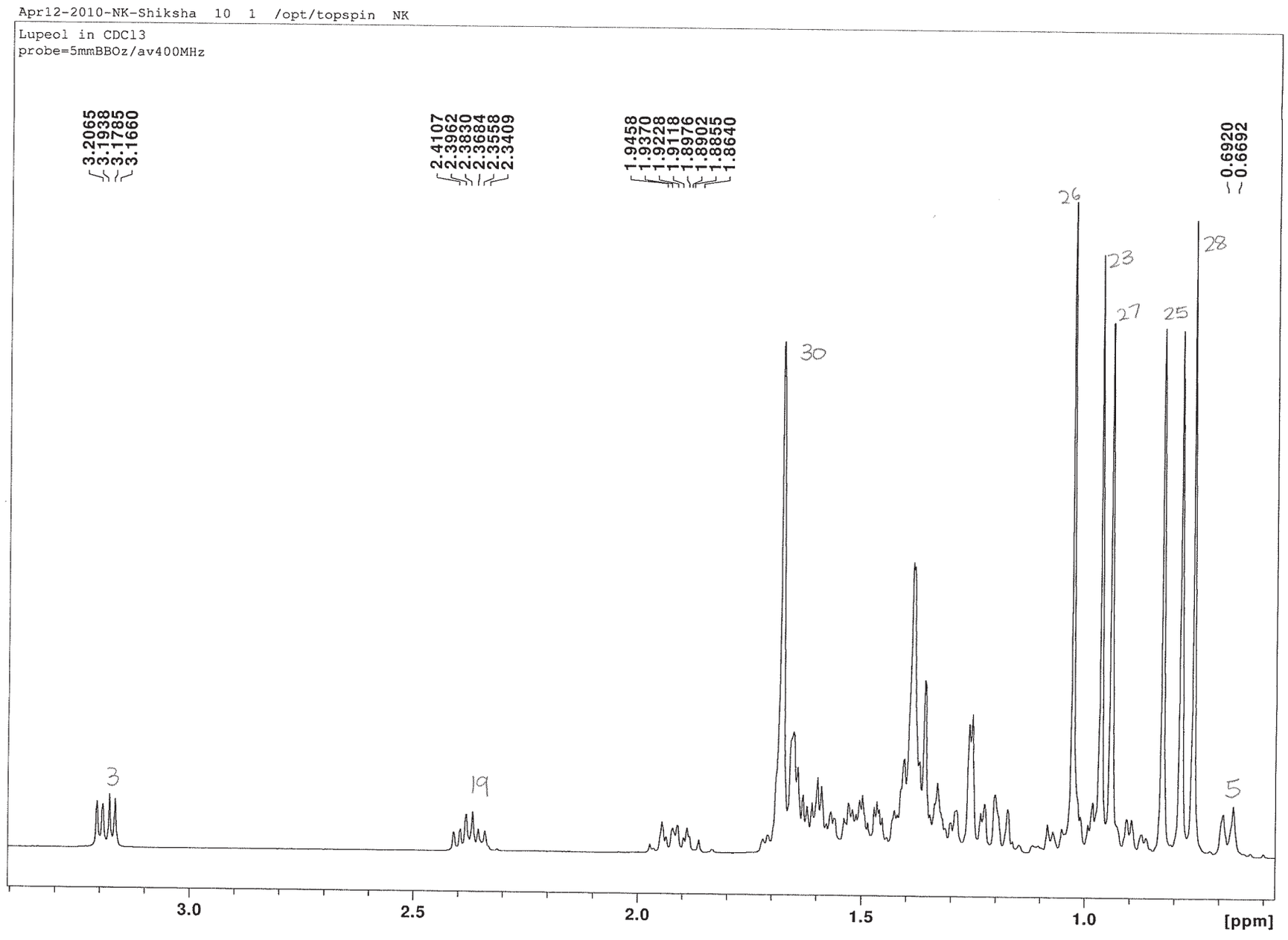
Spectrum 5d: Expanded ^{13}C NMR spectrum of compound **V**, stigmasterol



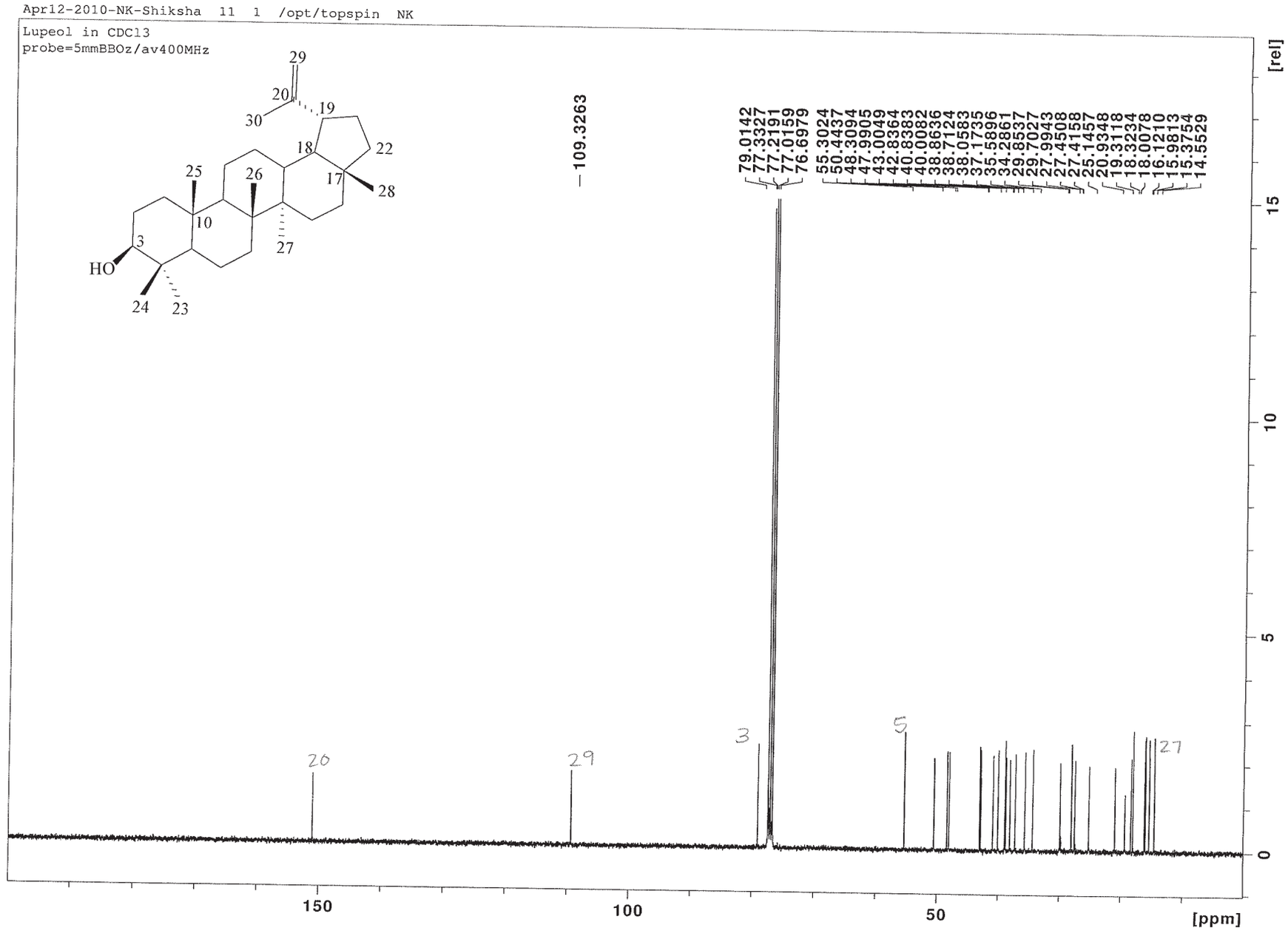
Spectrum 5e: IR spectrum of compound **V**, stigmasterol



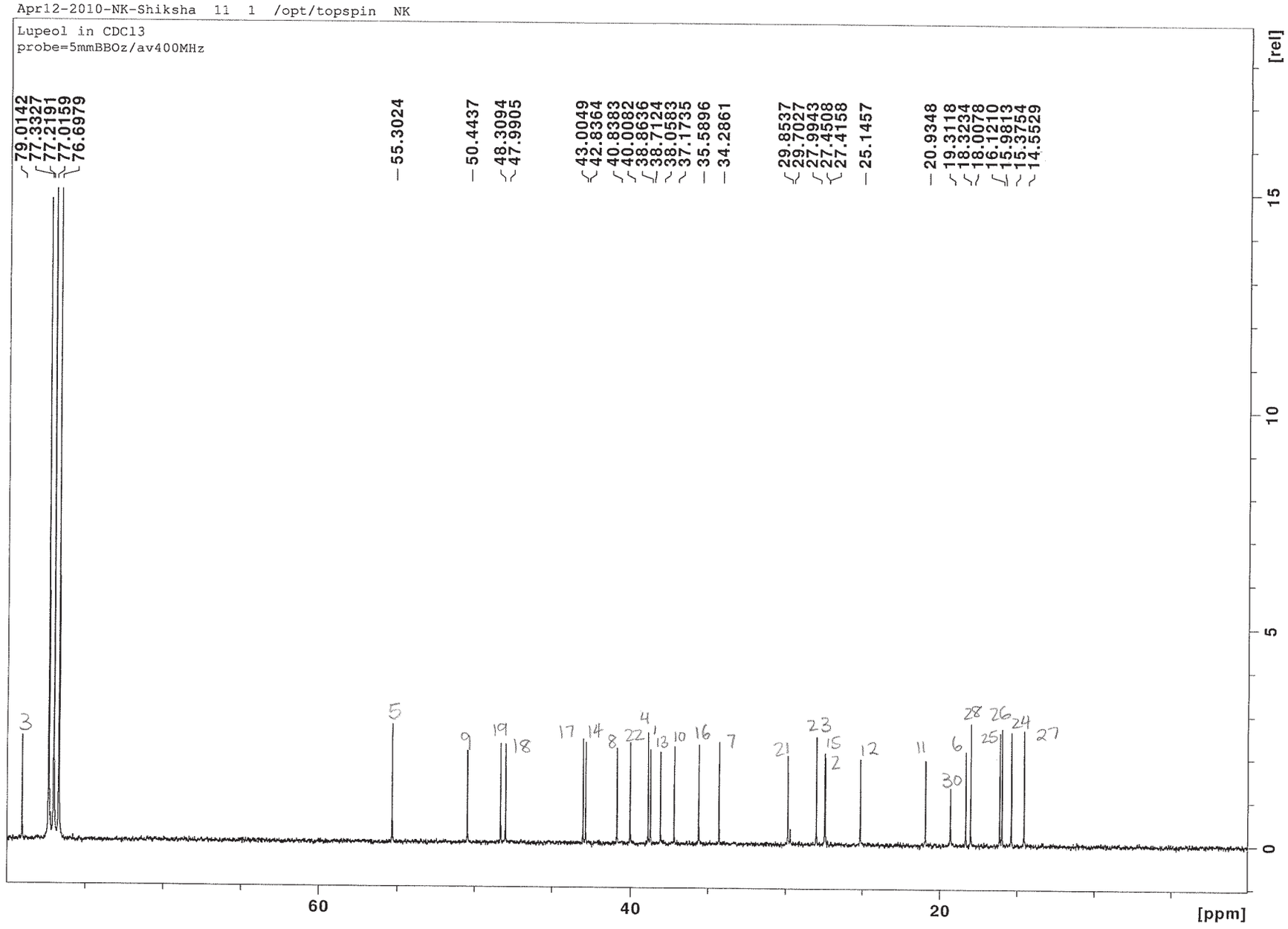
Spectrum 6a: ^{1}H NMR spectrum of compound **VI**, lupeol



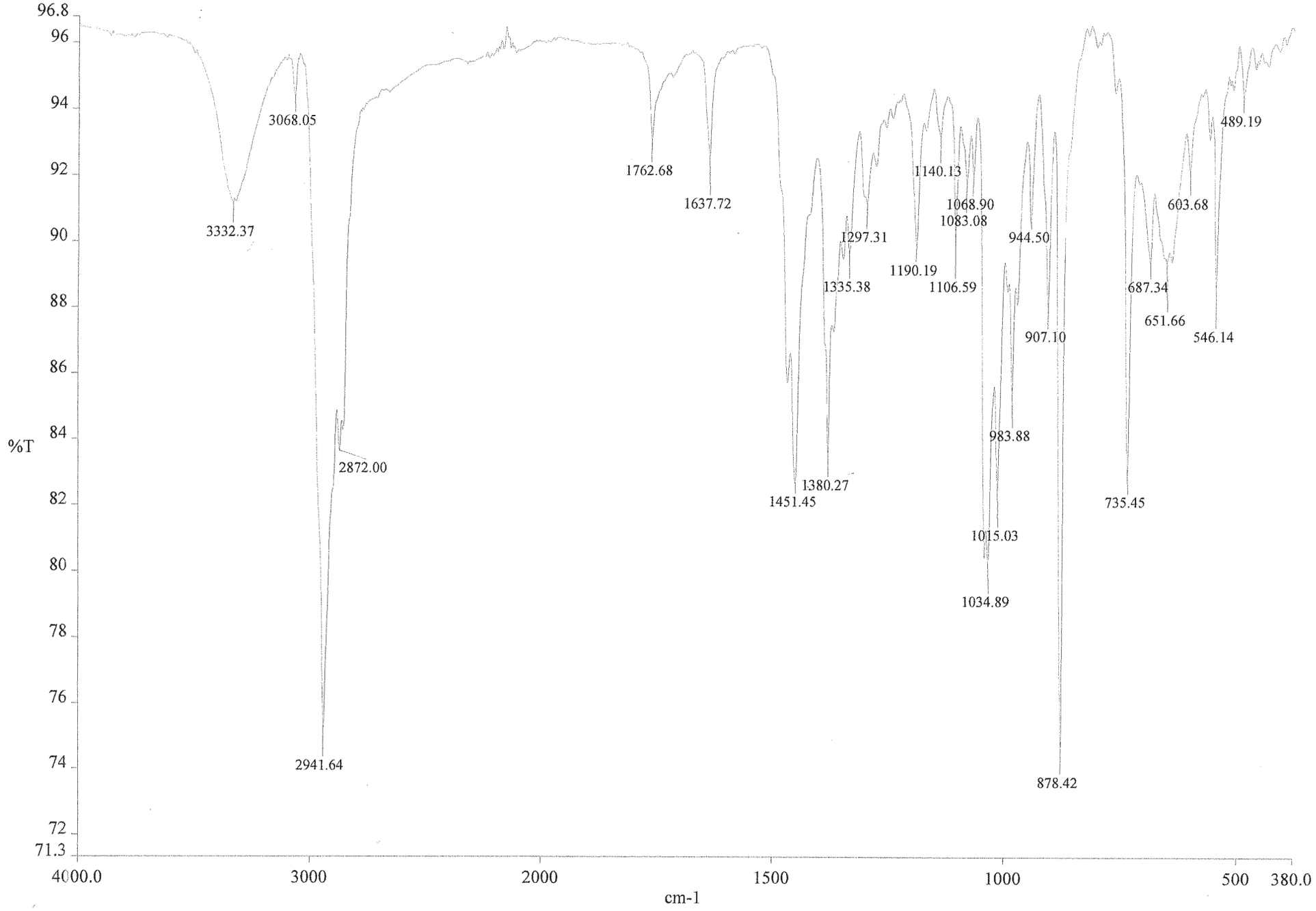
Spectrum 6b: Expanded ^{1}H NMR spectrum of compound **VI**, lupeol



Spectrum 6c: ^{13}C NMR spectrum of compound **VI**, lupeol



Spectrum 6d: Expanded ^{13}C NMR spectrum of compound **VI**, lupeol



Spectrum 6e: IR spectrum of compound **VI**, lupeol