



**EGE UNIVERSITY**

**MASTER'S THESIS**

**ELUCIDATION AND CHARACTERIZATION  
OF GLYCOSIDIC COMPOUNDS FROM  
*CEPHALARIA TUTELIANA* (DIPSACACEAE)**

**Merve DAĞLI**

**Supervisor : Assoc. Prof. Nazlı SARIKAHYA**

**Chemistry Department**

**Presentation Date: 16.01.2018**

**Bornova-İZMİR**

**2018**



**EGE UNIVERSITY GRADUATE SCHOOL of  
NATURAL and APPLIED SCIENCES**

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Sayın Merve Dağılı tarafından Yüksek Lisans tezi olarak sunulan “Elucidation and characterization of glycosidic compounds from *Cephalaria tuteliana* (Dipsacaceae)” başlıklı bu çalışma EÜ Lisansüstü Eğitim ve Öğretim Yönetmeliği ile EÜ Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi’nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve **16.01.2018** tarihinde yapılan tez savunma sınavında aday oybirliği/~~oyçokluğu~~ ile başarılı bulunmuştur.

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.....  
.....



**EGE ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ****ETİK KURALLARA UYGUNLUK BEYANI**

EÜ Lisansüstü Eğitim ve Öğretim Yönetmeliğinin ilgili hükümleri uyarınca Yüksek Lisans Tezi olarak sunduğum “Elucidation and characterization of glycosidic compounds from *Cephalaria tuteliana* (Dipsacaceae)” başlıklı bu tezin kendi çalışmam olduğunu, sunduğum tüm sonuç, doküman, bilgi ve belgeleri bizzat ve bu tez çalışması kapsamında elde ettiğimi, bu tez çalışmasıyla elde edilmeyen bütün bilgi ve yorumlara atıf yaptığımı ve bunları kaynaklar listesinde usulüne uygun olarak verdiğimi, tez çalışması ve yazımı sırasında patent ve telif haklarını ihlal edici bir davranışımın olmadığını, bu tezin herhangi bir bölümünü bu üniversite veya diğer bir üniversitede başka bir tez çalışması içinde sunmadığımı, bu tezin planlanmasından yazımına kadar bütün safhalarda bilimsel etik kurallarına uygun olarak davrandığımı ve aksinin ortaya çıkması durumunda her türlü yasal sonucu kabul edeceğimi beyan ederim.

16 / 01/ 2018

Merve DAGLI



## ÖZET

**CEPHALARIA TUTELIANA (DIPSACACEAE) TÜRÜ  
ÜZERİNDEKİ GLİKOZİDİK BİLEŞİKLERİN  
KARAKTERİZASYONU VE YAPILARININ  
AYDINLATILMASI**

**DAĞLI, Merve**

Yüksek Lisans Tezi, Kimya Anabilim Dalı

Tez Danışmanı: **Doç. Dr. Nazlı SARIKAHYA**

Ocak 2018, 80 sayfa

Bu çalışmada endemik *Cephalaria tuteliana* (Dipsaceae) bitkisinden biyolojik aktif bileşenlerin izolasyonu, saflandırılması, yapı tayinleri ve biyolojik aktivitelerinin araştırılması amaçlanmıştır.

Araştırmaya konu olan bitki materyali çiçeklenme döneminde toplanıp uygun şartlar altında kurutularak, çalışmaya hazır hale getirilmiştir. Kuru bitki materyali metanol ile ekstrakte edildikten sonra *n*-butanol:su ekstraksiyonu yapılmıştır. Saponin içeriğinin zengin olduğu bilinen *n*-butanol fazı yağı ve apolar kısımları uzaklaştırmak için *n*-hegzan ile ekstraksiyon yapılmıştır. *n*-butanol fazına kromatografi yöntemlerinden vakum likit kromatografisi, orta basınçlı sıvı kromatografisi, açık kolon kromatografisi ve ince tabaka kromatografisi uygulanarak 2 sapogenin, 1 iridoit glikozit ve 10 triterpen saponin olmak üzere toplam 13 adet saf bileşik elde edilmiştir. İzole edilen bileşiklerin yapıları NMR (1 ve 2 boyutlu) kullanılarak belirlenmiştir.

Bileşiklerden iki sapogenin (**1-2**) ve bir saponin (**12**), *Cephalaria* cinsinin dahil olduğu Caprifoliaceae familyasında ilk kez bulunmuştur. Elde edilen iki aglikonun sitotoksik aktiviteleri yapı aktivite ilişkisini belirlemek için farklı hücre panellerinde test edilmiştir.

**Anahtar Kelimeler:** Caprifoliaceae, *C. tuteliana*, fitokimya, izolasyon, kromatografi, saponin, sitotoksik aktivite



**ABSTRACT****ELUCIDATION AND CHARACTERIZATION OF  
GLYCOSIDIC COMPOUNDS FROM  
*CEPHALARIA TUTELIANA* (DIPSACACEAE)****DAGLI, Merve**

MSc in Department of Chemistry

Supervisor: **Assoc. Prof. Dr. Nazli SARIKAHYA**

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In this study, it is aimed isolation, purification, structural determination studies and to investigate biological activities of endemic *Cephalaria tuteliana* (Dipsacaceae).

The plant material was collected during flowering period and dried under suitable conditions to be ready for research. The dried plant material was extracted with methanol and then separated into fractions by *n*-butanol:water extraction. The *n*-butanol phase, known to be rich in saponins, was extracted with *n*-hexane to remove apolar and oily parts. The chromatographic techniques such as vacuum liquid chromatography, medium pressure liquid chromatography, open column chromatography and thin layer chromatography were applied to butanol phase and 2 triterpenoid saponins, 1 iridoid glycoside and 10 saponin glycosides, totally 13 compounds were obtained. The exact structures of the isolated compounds were determined using (1D- and 2D-) NMR.

Among isolated compounds, two sapogenins, (**1-2**) and a saponin glycoside (**12**) were obtained from Caprifoliaceae family which include *Cephalaria* species, for the first time. The cytotoxic activities of two obtained saponins were examined against different cell lines for discussing structure activity relationship.

**Key words:** Caprifoliaceae, *C. tuteliana*, phytochemistry, isolation, chromatography, saponin, cytotoxic activity



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## CONTENTS

	<u>Page</u>
ÖZET.....	vii
ABSTRACT.....	ix
ACKNOWLEDGEMENT.....	xi
LIST OF FIGURES.....	xv
LIST OF TABLES.....	xvii
LIST OF SCHEMES.....	xviii
ABBREVIATIONS.....	xix
<b>1. INTRODUCTION .....</b>	<b>1</b>
1.1. Importance of Natural Products.....	1
1.1.1. Natural Products as Cosmetics .....	1
1.1.2. Natural Products as Textiles and Dyes .....	2
1.1.3. Natural Products in Agriculture.....	3
1.1.4. Natural Products as Folk Medicine.....	4
1.2. Primary and Secondary Metabolites .....	6
1.2.1. Types of secondary metabolites.....	6
1.2.1. Phenolics.....	6
1.2.2. Nitrogen containing compounds ‘Alkaloids’ .....	8
1.2.3. Terpenes.....	9
1.3. Saponins.....	12

xiv  
**CONTENTS (continue)**

1.4. The <i>Cephalaria</i> Species.....	14
<b>2.MATERIAL &amp; METHOD.....</b>	<b>16</b>
2.1.General.....	16
2.2.Plant Material .....	17
2.3.Extraction, Isolation and Purification.....	17
2.4.Cytotoxic activity assays .....	23
<b>3.RESULTS and DISCUSSION .....</b>	<b>25</b>
3.1.Compound 1 .....	27
3.2.Compound 2 .....	33
3.3.Compound 3 .....	39
3.4.Compound 4 .....	42
3.5.Compound 5 .....	45
3.6.Compound 6 .....	48
3.7.Compound 7 .....	49
3.8.Compound 8 .....	52
3. 9.Compound 9 .....	55
3.10. Compound 10.....	59
3.11. Compound 11 .....	61
3.12. Compound 12 .....	64
3.13. Compound 13 .....	67
<b>4. CONCLUSION.....</b>	<b>70</b>

xv  
**LIST OF FIGURES**

<b><u>Figure</u></b>	<b><u>Page</u></b>
1.1. Cosmetic applicatons of natural product...	1
1. 2. Structure of Kinetin .....	2
1.3. Idigofera tinctoria .....	3
1.4. Indigo chemical structure .....	3
1.5. Pyrethrin .....	4
1.6. Willow tree .....	4
1.7.Acetylsalicylic acid... ..	4
1.8. <i>Thymus vulgaris</i> and Thymol molecule .....	8
1.9. <i>Papaver somniferum</i> .....	9
1.10.Structure of Morphine .....	9
1.11. Pine tree.....	10
1.12. Turpentine .....	10
1.13. Pacific Yew Tree .....	12
1.14. Structure of Paclitaxel... ..	12
1.15. Parts of saponins.....	13
1.16. Glycone and Aglycone Parts of Saponins .....	14
2.1. <i>Cephalaria tuteliana</i> .....	18
3.1. Pomolic acid .....	27
3.2. <sup>13</sup> C spectrum of Compound 1 .....	28
3.3. <sup>1</sup> H spectrum of Compound 1 .....	29
3.4.HMBC spectrum of Compound 1 .....	30
3.5.COSY spectrum of Compound 1 .....	31
3.6.HMQC spectrum of Compound 1 .....	32
3.7. Tormentic Acid .....	33
3.8. <sup>1</sup> H spectrum of Compound 2.....	34
3.9. <sup>13</sup> C spectrum of Compound 2.....	35
3.10.HMQC spectrum of Compound 2 .....	36
3.11.HMBC spectrum of Compound 2 .....	37
3.12.COSY spectrum of Compound 2 .....	38
3.13.Elmalienoside A .....	39

xvi  
LIST OF FIGURES(Continue)

<b><u>Figure</u></b>	<b><u>Page</u></b>
3.14. $^{13}\text{C}$ spectrum of Compound 3.....	40
3.15. $^1\text{H}$ spectrum of Compound 3.....	41
3.16.Davisianoside A .....	42
3.17. $^{13}\text{C}$ spectrum of Compound 4.....	43
3.18. $^1\text{H}$ spectrum of Compound 4.....	44
3.19.Alpha Hederin .....	45
3.19. $^{13}\text{C}$ spectrum of Compound 5.....	46
3.20. $^1\text{H}$ spectrum of Compound 5.....	47
3.21.Elmalienoside B .....	48
3.22.3-O- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabynopyranosyl-hederagenin 28-O- $\beta$ -D-glycopyranosyl ester .....	49
3.23. $^{13}\text{C}$ spectrum of Compound 7.....	50
3.24. $^1\text{H}$ spectrum of Compound 7.....	51
3.25.Davisianoside B .....	52
3.26. $^{13}\text{C}$ spectrum of Compound 8.....	53
3.27. $^1\text{H}$ spectrum of Compound 8.....	54
3.28.3-O- $\beta$ -D- glycopyranosyl-(1 $\rightarrow$ 3)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2) $\alpha$ -L- rhamnopyranosyl hederagenin 28-O- $\beta$ -D-glycopyranosyl ester .....	55
3.29. $^{13}\text{C}$ spectrum of Compound 9.....	56
3.30. $^1\text{H}$ spectrum of Compound 9.....	57
3.31.Dipsacoside B .....	58
3.32. $^{13}\text{C}$ spectrum of Compound 10.....	59
3.33. $^1\text{H}$ spectrum of Compound 10.....	60
3.34.Macranthoidin I.....	61
3.35. $^{13}\text{C}$ spectrum of Compound 11.....	62
3.36. $^1\text{H}$ spectrum of Compound 11.....	63
3.37. 3-O- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)]- $\alpha$ -L-arabinopyranosyl hederagenin .....	64
3.38. $^{13}\text{C}$ spectrum of Compound 12.....	65
3.39. $^1\text{H}$ spectrum of Compound 12.....	66
3.40. Laciniatoside I... ..	67
3.41. $^{13}\text{C}$ spectrum of Compound 13.....	68
3.42. $^1\text{H}$ spectrum of Compound 13.....	69
3.43. Oleanoic acid, Hederagenin, Pomolic acid and Tormentic acid .....	73

## LIST OF TABLES

<b><u>Table</u></b>	<b><u>Page</u></b>
<b>1.1.</b> Some biological active compounds and their source .....	5
<b>1.2.</b> Classification of Phenolic Compounds .....	7
<b>1.3.</b> Classification of Terpenoids .....	11
<b>3.1.</b> The cytotoxic activities of <i>n</i> -butanol extract, compounds <b>1-2</b> and aglycones hederagenin, oleanoic acid .....	27
<b>4.1.</b> Biological Activities of Saponins .....	72

**LIST OF SCHEME**

<b><u>Scheme</u></b>	<b><u>Page</u></b>
<b>2.1.</b> Application steps for extraction.....	20
<b>2.2.</b> VLC application steps.....	21
<b>2.3.</b> Application steps for Open CC.....	22
<b>2.4.</b> Application steps for MPLC.....	23



**ABBREVIATIONS**

UV	Ultraviolet
NMR	Nuclear Magnetic Resonance
COSY	Correlation ( $^1\text{H}$ - $^1\text{H}$ ) Spectroscopy
HMQC	Heteronuclear Multiple Quantum Coherence
HMBC	Heteronuclear Multiple Bond Correlation
DMSO	Dimethylsulfoxide
TLC	Thin Layer Chromatography
CC	Column Chromatography
VLC	Vacuum Liquid Chromatography
MPLC	Medium Pressure Liquid Chromatography
TMS	Tetramethylsilan
HMDS-TMCS	Hexamethyldisilazane–Trimethylchlorosilane
HEK-293	Human Embryonic Kidney Cell 293
A-549	Adenocarcinomic Human Alveolar Basal Epithelial Cells
HeLa	Human Uterine Cervical Carcinoma
PANC1	Pancreas Ductal Adeno Carcinoma
SHSY5Y	Human Neuroblastoma Cells



## 1. INTRODUCTION

### 1.1. Importance of Natural Products

Natural compounds are important compounds that form themselves in living metabolism. In ancient times, people benefited from natural sources to make their lives easier. Old people have endorsed from them by applying them on their skin, adding them to their foods, giving them to their harvest and their animals. People used plants, animals, microorganisms and marine animals for cosmetics, agriculture, foods, textile, dyes and especially folk medicine since ancient times to today. The usage areas and sources of natural products were given in detail under sub-headings.

#### 1.1.1. Natural Products as Cosmetics

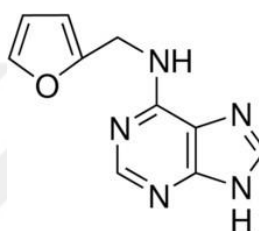
Since ancient times, people have been searching for ways to stay young. They have tried beauty rituals by using natural resources to prevent aging (see Figure 1.1). For example, Old Greek goddess Aphrodite, has been greased with olive oil to protect her skin. Honey was also mixed with olive oil to help lighten the appearance of skin. Almond oils, apple cores, sea salt honey and milk were indispensable for the beauty rituals of Cleopatra, the last active pharaoh of Egypt (Stoecker, 2017).



**Figure 1.1.** Cosmetic applications of natural products

Nowadays, many cosmetic products have kinetin component due to aging retarding effect. Kinetin, (Figure1.2) a plant growth hormone of the cytokinin type, is a molecule that supports cell division. (Levy, 2017) It is a compound first isolated from herring sperm DNA in 1955 (Miller et.al., 1955).

There are still many patented literatures about the cosmetic activity of kinetin and its derivatives (Kumari et. al., 2015;Xie et.al., 2008).



**Figure 1. 2.** Structure of Kinetin

### 1.1.2. Natural Products as Textiles and Dyes

Plants and animals have been used as dyes since centuries. Some examples for these, indigo is a fabric that has been used since ancient times.

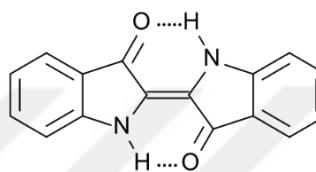
The medicinal *Rocella tinctoria* in the Mediterranean sea seaweed also contains stain (Robiquet, 1829). *Dactylopius coccus* is an insect species that survives as parasitic in different cacti from the Dactylopiidae family. It is a bug that is known and used in ancient times, giving a red color (Greenfield, 2005).

In ancient Egyptian mummies, indigo blue stained wraps were found. The source of this dye is the tropical *Indigofera tinctoria* (see Figure 1.3.) plant found in Eastern India (Kamal and Mangla, 1993).

Today, Indigo (see Figure 1.4.) blue is the color of jeans that find a great place among our clothes and in recent years, the annual production of synthetic indigo has reached thousands of tons in the world.



**Figure 1.3.** *Indigofera tinctoria*

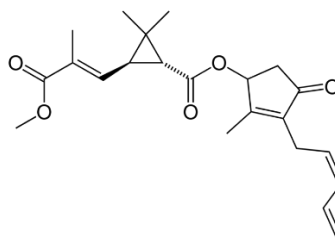


**Figure 1.4.** Indigo chemical structure

### 1.1.3. Natural Products in Agriculture

Since ancient times, people have benefited from natural sources to preserve and protect their crops and fields. It is known that plant powders and extracts were used as insecticides during the Ancient Roman period. In the epoch of the Persian king Xerxes, the powder of the *Pyrethrum* plant was used to control the head lice in children (Addor, 1995).

If we look again today, plant-derived pyrethrin is still used as an insecticide in agriculture (Aydin and Mammadov, 2017) (Figure 1.5).



**Figure 1.5.** Pyrethrin

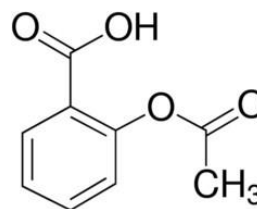
Nowadays, one of these protection methods is to used against diseases and harmful plants by using plants and plant extracts (Erler, 2000). For example, every part of the *Nimba* plant is active on humans and microorganisms which is used as a household pesticide (Prajapatil , 2016).

#### 1.1.4. Natural Products as Folk Medicine;

One of the earliest examples of the use of natural compounds as a medicine is the willow tree (see Figure 1.6.). In ancient Egypt, Assyrian and Greek manuscripts, former doctors Galen, Hippocrat and Dioscorides have described pain reliever and fever reducing properties of this tree. Nation Americans used for headache, fever, rheumatism, tremor and muscle pain, as well. In 1700s, it was also used in the treatment of malaria disease. Another example is aspirin which is known that the source of it is still the willow tree today (Stone, 1763).



**Figure.1.6.** Willow tree



**Figure.1.7.**Acetylsalicylic acid

Many other studies on the use of natural compounds as medicine have been reported (Cragg and Newman, 2014). Humans have benefited not only plants but also many different natural sources, to get rid of their diseases. Some examples of different sources, isolated active compounds and their using areas are listed in Table 1.1.

**Table 1.1.** Some biological active compounds and their source

Source	Source's Name	Compound	Using area
<b>Plant</b>	Pacific yew tree	Paclitaxel	Breast and ovarian cancer
	<i>Camptotheca acuminata</i>	Camptothecin	Cancer treatment
	<i>Papaver somniferum</i>	Morphine	Pain Reliever
	<i>Parthenium integrifolium</i>	Quinnine	Malaria treatment
<b>Microorganisms</b>	<i>Penicillium notatum</i>	Penicillin	Infactions
	<i>Cephalosporium acremonium</i>	Cephalosporins	Infactions
<b>Animals</b>	<i>Epipedobates anthony</i>	Epibatidine	Pain Reliever
	<i>Bothrops jararaca</i>	Captopril	Hypertension, congestive heart failure
<b>Marines</b>	<i>Tethya crypta</i>	Spongouridine	Cytostatic activity on cancer treatment
	<i>Discodermia species</i>	Discodermolide	Cancer treatment

## 1.2. Primary and Secondary Metabolites

Metabolites are molecules that are spontaneously formed in living metabolism, usually in a small structure. The compounds required for the formation and maintenance of viability are called **primary metabolites**. These compounds are responsible for the basic needs of the organism such as growth, development and reproduction. Unlike primary metabolites, **secondary metabolites** do not directly affect life but in long-term decline or absence, affect adapt to life, fertility and aesthetics (Cooper, 2015).

The secondary metabolites play an important role in the vital defense and they have a limited distribution in the plant kingdom. This property separates these compounds from the primary metabolites (amino acids, sugars, nucleotides and acyl lipids) (Seigler, 1998). Because primary metabolites are found in all individuals of the plant kingdom, while secondary metabolites are often found only in a certain species or close species.

### 1.2.1. Types of secondary metabolites

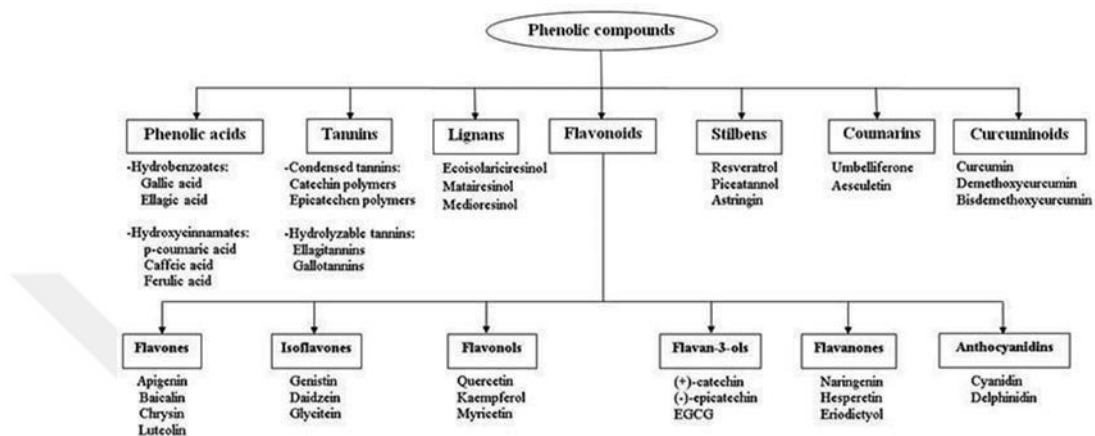
There are three main classes of secondary metabolites as given under sub-headings in detail.

#### 1.2.1. Phenolics

Phenolics are compounds having one or more hydroxyl groups and aromatic rings. Generally, they can be classified (Table 1.2.) as phenolic acids and analogues, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones (Fresco et.al, 2006).

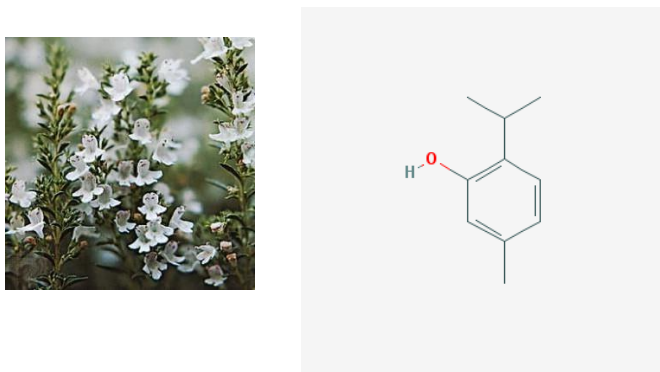
Many vegetables, fruits and phenolic compounds found in medicinal plants have been the subject of many studies on the prevention of diseases due to their antioxidant properties (Cai et. al., 2004; Basli et. al., 2017).

**Table.1.2.** Classification of Phenolic Compounds



Phenolic compounds generally have antimicrobial and antibacterial activities due to the phenol in their structures. An example of this is the thymol molecule. Thymol has antimicrobial and antibacterial activities because of its phenolic structure. In the markets, this molecule which is a phenolic compound isolated from *Thymus vulgaris* plant species used in the treatment of diseases such as cough, asthma, bronchitis (Figure 1.8).

As well as the usage of it as a pesticide (European Commission Health & Consumers Directorate, 2013) and food additive (Commission Implementing Regulation, 2012).



**Figure 1.8.** *Thymus vulgaris* and Thymol molecule

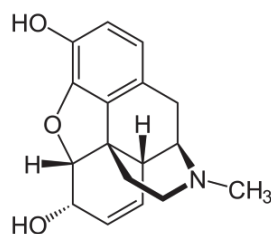
### 1.2.2. Nitrogen containing compounds ‘Alkaloids’

In 1805 A German pharmacist Friederich VWilhelm named Serturmer obtained a crystallized substance during the studies on *Papaver somniferum* (Figure 1.9.) (Busse et.al., 2006). This substance, called "morphinum" (Figure 1.10), showed a basic reaction, unlike what was known until then. The findings of Serturmer have opened a new era in chemical research in plants. The Other, other basic substances were also obtained as well. All these substances are called alkaloids in a similar meaning alkaline. Subsequently, it was understood that this alkaline reaction was leading to molecular azotites and thus alkaloids were described as organic compounds containing nitrogen and alkaline reaction. A simple recipe was not enough to describe alkaloids. Because, this recognition, it would be necessary to count physiological amines (histamine, serotonin, etc.) from alkaloids obtained from plants or animals. The following studies showed that the alkaloids were nitrogen

containing heterocyclic those compounds. It was then necessary to make an addition to recognition (Tanker, 1990 ; Aniszewski, 2007).



**Figure 1. 9.** *Papaver somniferum*



**Figure 1.10.** Structure of morphine

Alkaloids are substances from plants that are more or less basic reactive substances carrying strong physiological and pharmacodynamic activity, carrying one or more nitrogens in the ring (Mueller-Harvey, 1992). They are compounds that are commonly used as a drug starting ingredient, usually acting on the central nervous system. So that, some countries in which Turkey is also included have legalized alkaloid fields in particular as a starting material (Toprak Mahsulleri Ofisi Genel Müdürlüğü, 2001).

### 1.2.3. Terpenes

Turpentine is (Figure 1.12) a balsam obtained by cutting and carving the shells of various pine trees, is the basis of the word 'terpen'. Turpentine is a volatile liquid isolated from pine trees (Figure1.11).

Simple mono and sesquiterpenes are essential oils obtained from various parts of plants. But di and triterpenes are not volatile, so they are derived from the resins of plants. Unlike these, tetraterpenes also form a separate group as carotenoids ( Bano, 2007).



**Figure 1.11.** Pine tree



**Figure 1.12.** Turpentine

Terpenes are the largest class of secondary metabolites. They can be called natural compounds, which contain isoprene units on their surface. The number of isoprene units in the structure determines the type of terpenes can be seen in Table 1.3.

Terpenoids are used in many areas because of their biological activities. Many commercial uses are also the consequences of these activities such as terpenes have been found in many cosmetic products due to their pleasant smell, medicines which are important for human health and pest control agents (Zwenger, 2008).

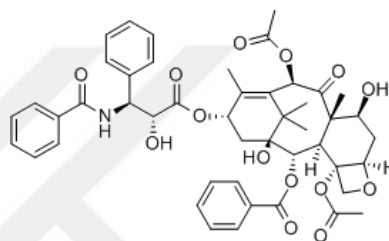
Table 1.3. Classification of Terpenoids

<i>Isoprene units</i>	<i>Type of Terpenoids</i>	<i>Number of Carbons</i>
1	<i>Hemiterpene</i>	5
2	<i>Monoterpene</i>	10
3	<i>Sesquiterpene</i>	15
4	<i>Diterpene</i>	20
5	<i>Sesterterpene</i>	25
6	<i>Triterpene</i>	30
8	<i>Tetraterpene (Carotenoids)</i>	40
<i>N</i>	<i>Polyterpene</i>	5( <i>n</i> )

Paclitaxel (Figure 1.14) (trade name Taxol®) used in the treatment of breast, ovarian and lung cancer is a diterpenoid isolated from Pacific yew tree (Figure 1.13). However, the level of paclitaxel in the bark of a tree will not be needed for treatment because the total amount of paclitaxel needed for treatment is 3 trees. This has also caused total or partial synthesis of the structure. So we can say that the isolation method provides a good reference for drug synthesis. (Sell, 2003)



**Figure 1.13.** Pacific Yew Tree



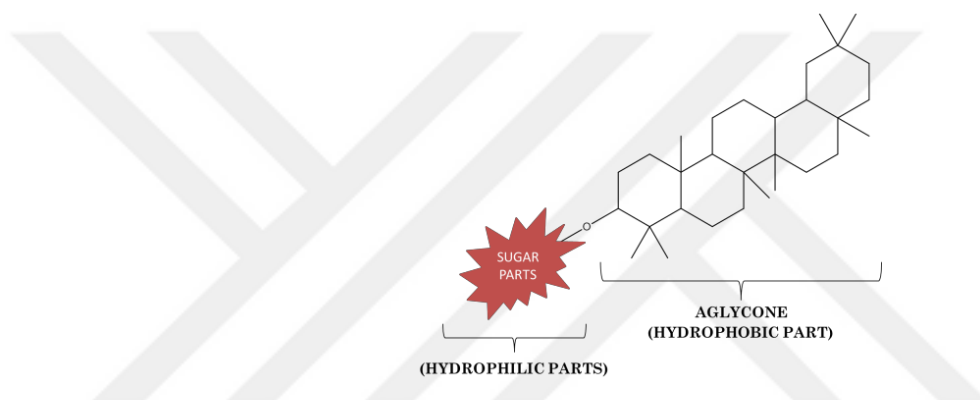
**Figure 1.14.** Structure of Paclitaxel

Besides all these, there are many benefits for vital functions within the plant. Studies have shown that pollinating beetles are attracted to plants by terpenoid release (Maimone et.al., 2007). There are many different modification products and derivatives generally characterized as terpenoids. These are mainly sterols, saponins and mero-terpenes (Kiyama, 2017).

In this thesis we mainly focused on the saponins which have become a source of hope for many issues, which are subject to much work.

### 1.3. Saponins

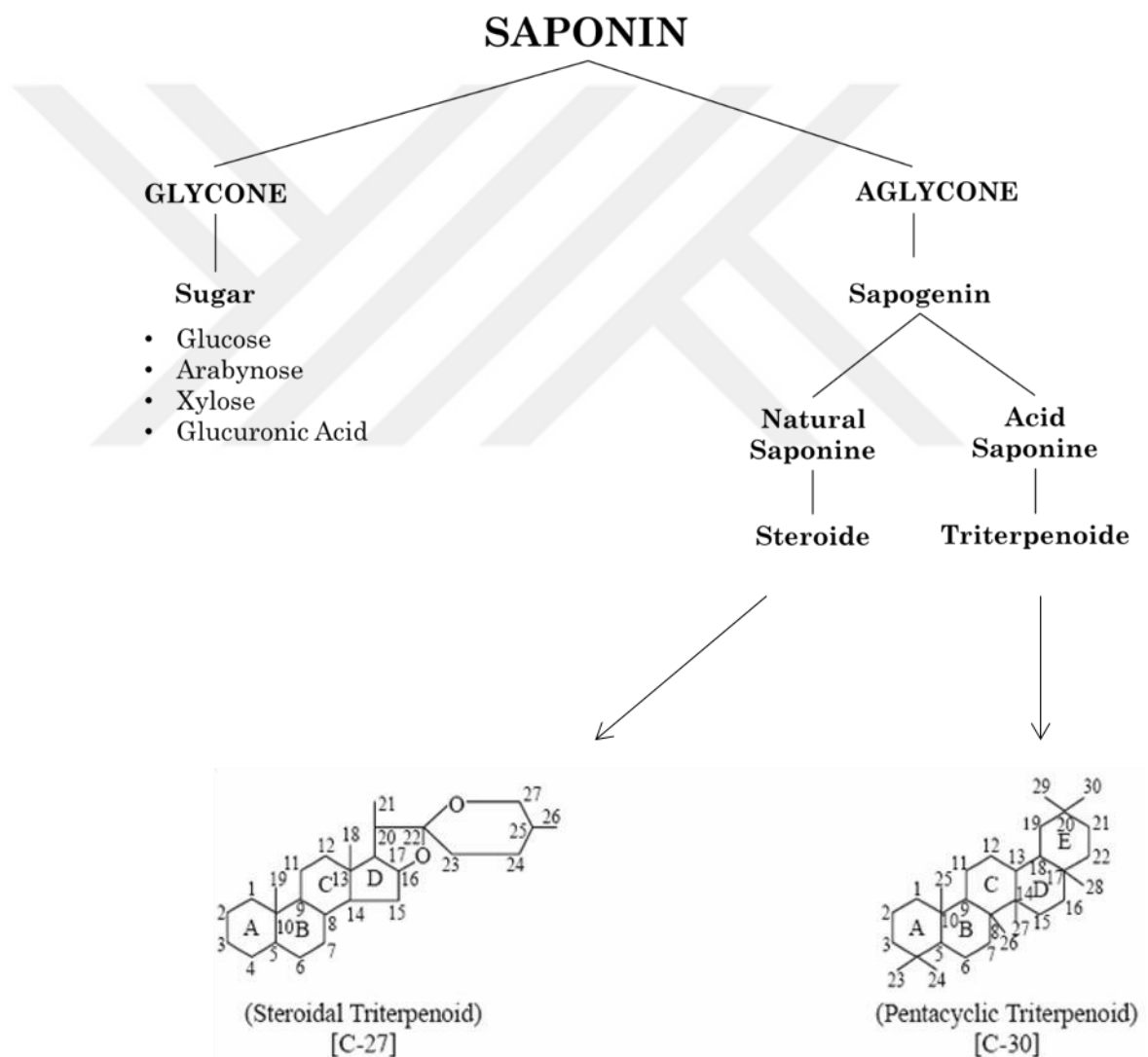
The word *saponin* is derived from *sapo*, Latin for “soap.” Because saponins form foams in aqueous solutions similar to soap. The cause of this foaming is the molecular structure of saponins. Because they contain both a hydrophobic (water-hating) sapogenin (non-sugar part) and a hydrophilic (water-loving) glycoside (sugar part) (see Figure 1.15) (Tamura et.al., 2012).



**Figure 1.15.** Parts of saponins

Saponins are phytochemicals found in many vegetables, beans and herbs. They play an important role in living metabolism. It can be said that the saponins in the 'triterpen' class of terpenes are triterpenoids which are generally have steroid or triterpenoid aglycone (see Figure 1.16) (Desai, 2009). In the past many plants have provided alternative methods due to the saponin content. Still, saponins continue to be used in different areas. Some of those are soaps, cosmetics, detergents, insecticides and pesticides. Even some plant extracts which are rich in saponins have been using in different industrial areas. Besides, saponins have become the targets of many researchers in medicine (Tamura, 2012; Moghimipour and Handali, 2015).

It was mentioned in the previous section that the saponins have a structure similar to soap. This also means that saponins can hold onto oils. This property allows saponins to emulsify oil soluble molecules in the digestive tract. It binds to bile acids and helps to throw them away. So it helps balance your cholesterol level in blood. A study found that giving a certain saponin extract to rats with high cholesterol reduced ‘bad’ (LDL) cholesterol without affecting ‘good’ (HDL) cholesterol (Malinow, 1977).



**Figure 1.16.** Glycone and Aglycone Parts of Saponins Structure of Steroidal (a) and Triterpenoidal (b) Aglycones

Cancer cell membranes are cholesterol type compounds. Saponins bind to cancer cell membranes as it binds cholesterol cells and prevent the reproduction of cancer cells (Rao, 1995; Yan et.al, 2009). In addition, many studies have supported the antimicrobial effect of saponins. Plants and other living organisms use saponins for defense against harm. Likewise, it is possible to play an important role for defense in human and animal metabolism, and a lot of research has been done for it.

As it interacts with cholesterol, it also causes hemolysis by forming complexes in the membranes of red blood cells. This property can also be used to enhance penetration of macromolecules (such as proteins) into the cell membrane. Saponins are also used as adjuvants in vaccines and serums (Simone, 2017; Sun, 2011).

#### **1.4. The *Cephalaria* Species**

Totally, there are 94 species of *Cephalaria* in the worldwide, located in Europe, the Eastern Mediterranean, East Asia, Central and North Africa. A total of 40 species found in Turkey and 24 of them are endemic (Davis, 1972).

As a result of many studies, some of these species has been reported to be used as dye (Szabó, 1940), food additive (Baytop, 1994), and folk medicine (Gunes and Ozhatay, 2011) in old times. Based on all these studies, it has been determined that this species are rich in secondary metabolites such as flavonoids (Godjevac et al., 2004; Movsumov et al., 2009), iridoids (Gođevac et al., 2000; Mustafaeva et al., 2008), alkaloids (Aliev et al., 1975) and especially saponins (Kayce et. al., 2014; Braca et.al., 2004).

Secondary metabolites abundance in *Cephalaria* species requires studies on the biological activities of this plant. As a result of these studies, it has been reported that essential oils of this plant species and compounds obtained as a result of isolation have cytotoxic, antimicrobial, antifungal, antioxidant, hemolytic and immunomodulatory properties (Sarıkahya et.al.,2018; Kayce and Kirmizigul, 2017; Sarıkahya et.al., 2015; Mutlu et.al., 2017).



## 2.MATERIAL & METHOD

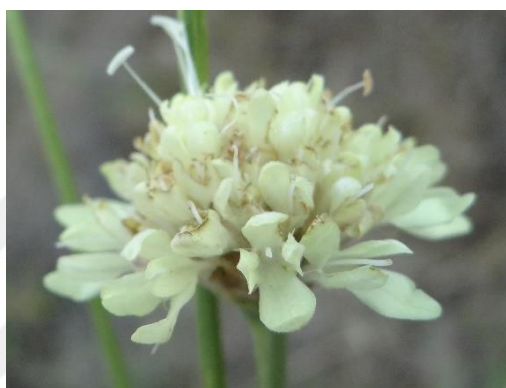
### 2.1.General

Chromatographic methods have been used extensively for isolation and purification studies that make up a large part of the experimental part of our work. These methods are VLC (Vacuum Liquid Chromatography), MPLC (Medium Pressure Liquid Chromatography), TLC (Thin Layer Chromatography) and open column chromatography. In the first step, the VLC method was applied with a Lichroprep RP18 (Merck 9303-25- 40 $\mu$ m) silica filler to roughly fractionate. In the second step, Buchi brand pumps (C-605) and glass columns (15 / 460 and 49 / 230) were used for the MPLC method. Silica gel 60 (0.063-0.200, Merck 7734) was used as the fill material for MPLC and open column chromatography. Thin Layer Chromatography (TLC) was used to monitor the components, to determine if they were pure or not, and to identify them. TLC plates (F<sub>254</sub> Merck 5554 silica gel) were run with different polarities (CHCl<sub>3</sub> / MeOH / H<sub>2</sub>O). In the dried layers, the UV active components were observed under the UV lamp at wavelengths of 254 nm and 366 nm. Components were visualized with 20% H<sub>2</sub>SO<sub>4</sub> solution and heated (120 °C).

The structures of the purified compounds were determined using spectroscopic methods. In the structure determination, values of *j* were recorded as Hz DMSO-*d*<sub>6</sub> as a solvent , and TMS as internal standard using Varian AS 400 MHz spectroscopy for 1D-(<sup>13</sup>C and <sup>1</sup>H) and 2D-NMR (HMBC, HMQC,COSY,TOCSY)analysis.

## 2.2. Plant Material

*Cephalaria tuteliana* (Caprifoliaceae) (Figure 2.1) (Kus and Gokturk, 2005) is an endemic species collected from Istanbul, Kirac, Bahcesehir in July 2012 at about 90 m by H. Sumbul and R.S. Gokturk (Akdeniz University Herbarium Research and Application Centre No:7526). Every part of the plant was studied above ground.



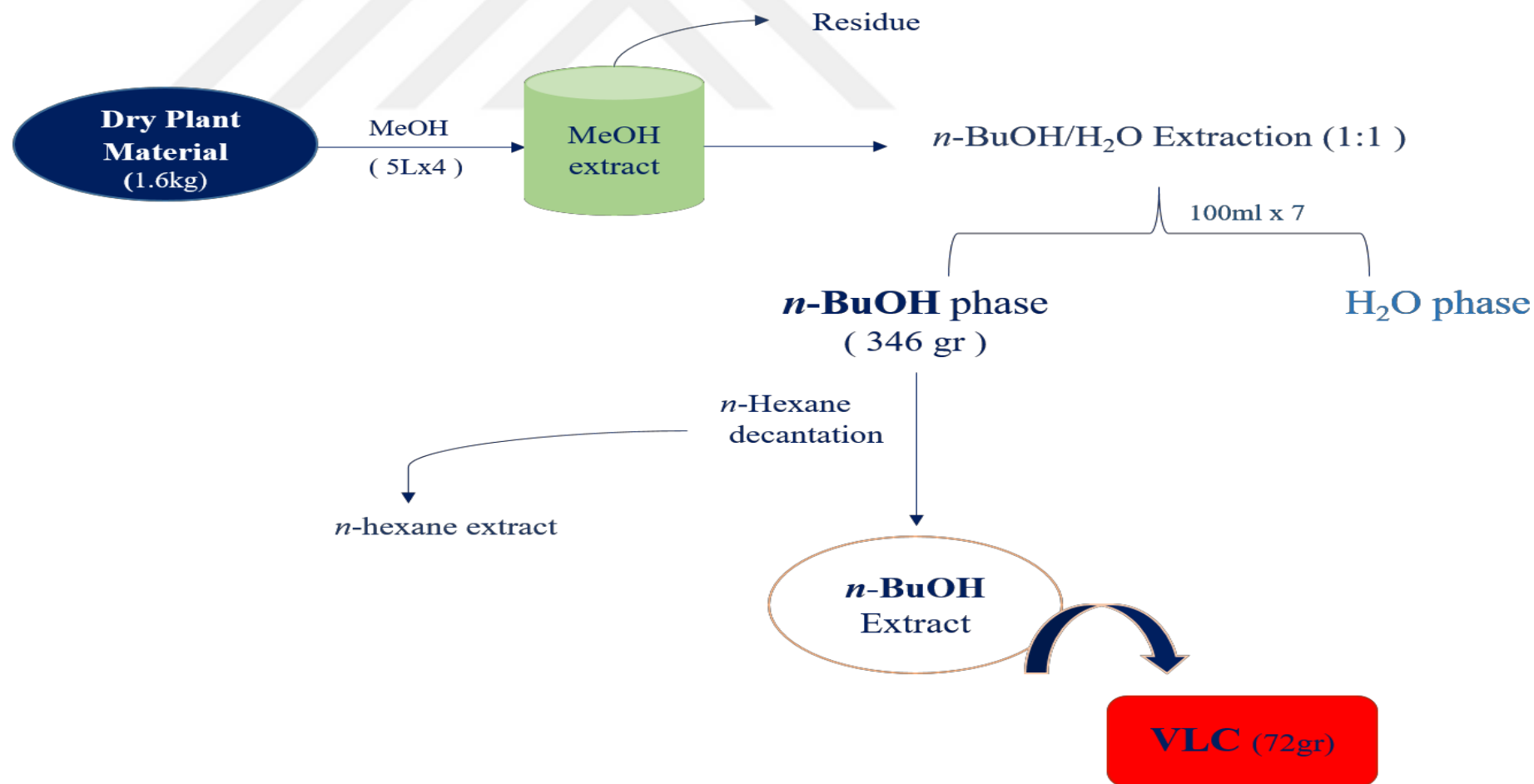
**Figure 2.1.** *Cephalaria tuteliana*

## 2.3. Extraction, Isolation and Purification

The *C. tuteliana* plant material was dried in dark and dry conditions and grinded. The plant material (1.6 kg) was mixed with MeOH at room temperature to prepare MeOH extract (5L x 4). MeOH was evaporated and then the concentrated material (388 g) was kept for the next step. In the second step, *n*-BuOH / H<sub>2</sub>O(1:1) extraction was performed to separate the biologically active components. With a few repetitions, the two phases were completely separated from each others (100 mL x 7 ). The BuOH phase riched in secondary materials, was evaporated and an intense consistency was achieved (346 g). The *n*-BuOH fraction was decanted with *n*-hexane (100mL x 20) to separate oily substances and then

the dried BuOH phase (72 g) was applied to VLC (Scheme 2.1). RP silicagel and MeOH / H<sub>2</sub>O (gradient from 0% to 100% MeOH) solvent system were used for the VLC method (Scheme 2.2). After that, fractions 20:80 and 10:90 (H<sub>2</sub>O:MeOH) were combined (25 g) and applied to open column chromatography with suitable solvent system with CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O (from 90:10:0.5 to 61:32:7). During this column 30 subfractions were obtained. Compound **1** (150 mg), compound **2** (58 mg), compound **5** (38 mg) and compound **6** (175mg) were obtained purely directly from this column (Scheme 2.3). The fractions 22<sup>nd</sup> (0.80 g), 23<sup>rd</sup> (0.41 g) and 25<sup>th</sup> (0.9 g) were separately subjected to another open column chromatography. Compounds **12** (40 mg), **7** (106 mg), **8** (63 mg) and **9** (108 mg) were obtained from these fractions by CC, respectively.

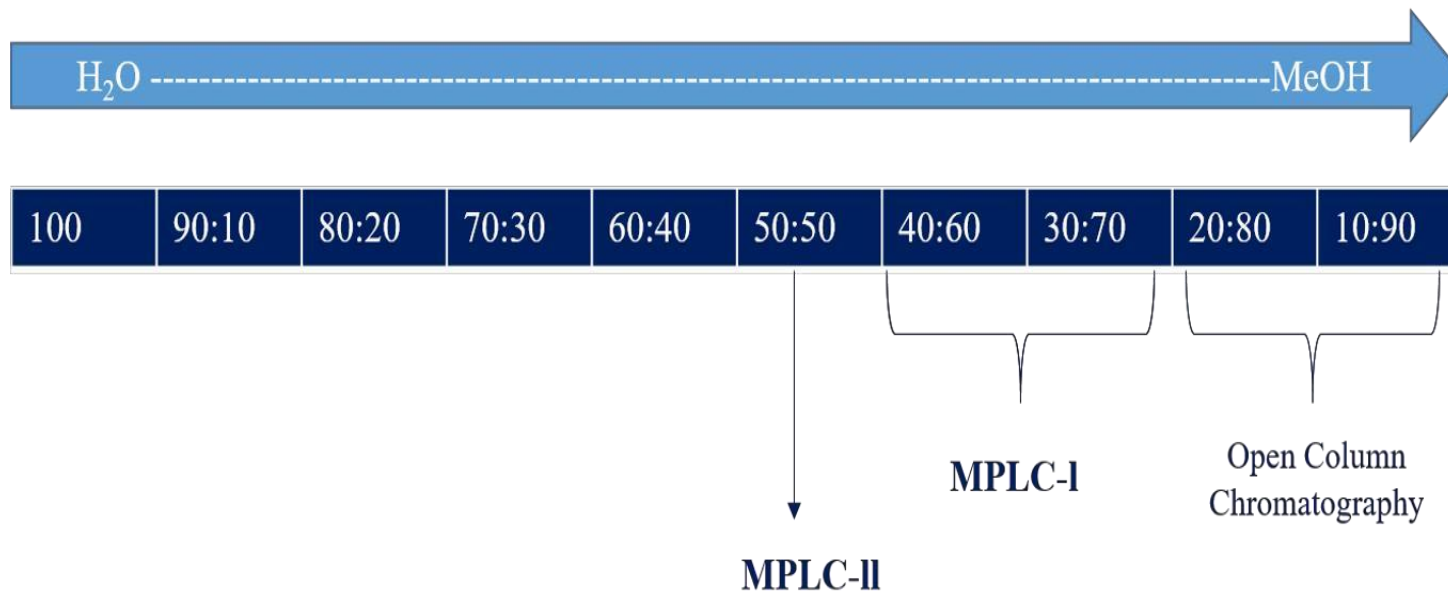
VLC fractions 40:60 and 30:70 were combined (9g) and MPLC was applied to this fraction. Fractionation was performed using the appropriate adsorbent (silicagel), buchi column (26 x 920 mm) and solvent system ( 90:10:0.5-61:32:7 / CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O) flow rate 25 mL/min and max. pressure:40barr (Scheme 2.4). 13 subfractions were obtained and fractions 8-10 were combined (2.8 g) and subjected to open column chromatography. Compound **10** (110 mg) was obtained form directly from this column. After this column, the fractions 8-10 were subjected to a series of more open column chromatography. Compounds **3** (60 mg), **4** (47 mg) and **11** (26 mg) were obtained from these columns. Compound **13** (120 mg) was obtained by MPLC application of a 50:50 VLC fraction (12 g) (adsorbent:silicagel, column:26 x 920 mm, flow rate 25 ml/min and solvent system 90:10:1-61:32:7 / CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O, max. pressure:40 bar).



**Scheme 2.1.** Application steps for extraction

**VLC (72gr)**

Adsorbent: RPSilica  
Solvent system: H<sub>2</sub>O:MeOH



**Scheme 2.2.** VLC application steps



### Open Column Chromatography

← VLC (10:90+20:80)

Silica gel  
CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O (from 90:10:0.5 to 61:32:7)

Compound 1  
(120 mg)

Compound 2  
(58 mg)

Compound 5  
(38 mg)

Compound 6  
(175 mg)

Fr22

Fr23

Fr25

Open Column Chromatography  
Silica gel  
CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O (from 90:10:0.5 to 61:32:7)

Open Column Chromatography  
Silica gel  
CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O (from 90:10:0.5 to 61:32:7)

Open Column Chromatography  
Silica gel  
CHCl<sub>3</sub>:MeOH:H<sub>2</sub>O (from 90:10:0.5 to 61:32:7)

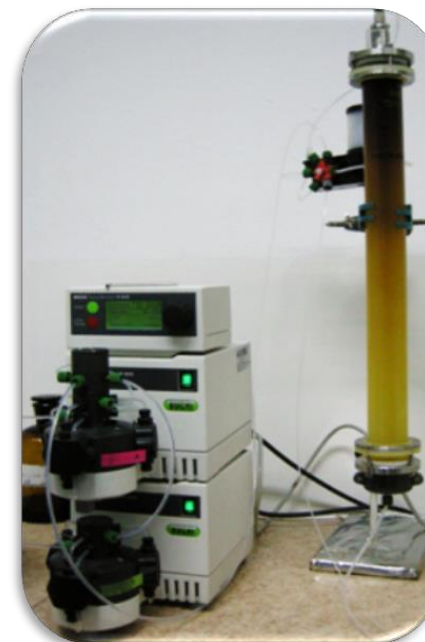
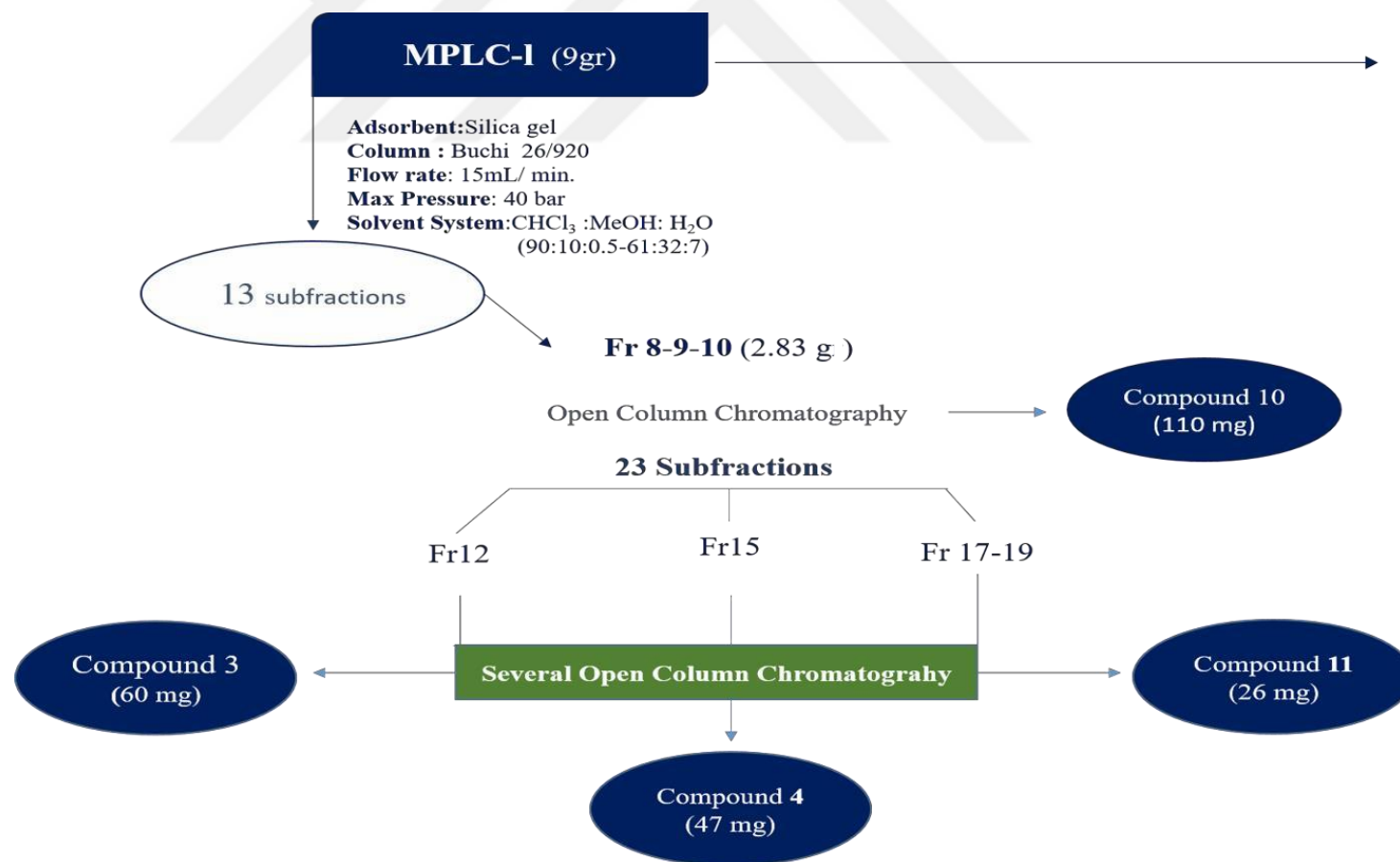
Compound 12  
(40 mg)

Compound 7  
(106 mg)

Compound 8  
(63 mg)

Compound 9  
(108 mg)

**Scheme 2.3.** Application steps for Open CC



**Scheme 2.4.** Application steps for MPLC

## 2.4. Cytotoxic activity assays

A549, HeLa, PANC1, SHSY5Y and a normal cell line HEK293 were used for testing cytotoxicity. All cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, U.S.A.). The cell lines were maintained in Dulbecco's modified Eagle's medium F12 (DMEM/F12), supplemented with 10 % fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL of penicillin, and 100 µg/mL of streptomycin (Gibco, NY, U.S.A.). The cells were incubated at 37 °C in a humidified atmosphere of 5 % CO<sub>2</sub>. The cells were sub-cultured twice a week, and cells in the exponential growth phase were used in the experiments.

Cytotoxicity of *n*-butanol extract, compounds **1-2**, hederagenin and oleanoic acid was determined using a modified MTT assay, which detects the activity of mitochondrial reductase of viable cells. The assay principle is based on the cleavage of MTT that forms formazan crystals by cellular succinate-dehydrogenases in viable cells. DMSO is added to the wells to dissolve the formazan crystals. Briefly, all cell lines were cultivated for 24 h in 96-well microplates with an initial cell numbers of  $1 \times 10^5$  cells/mL in a humidified atmosphere with 5 % CO<sub>2</sub>, at 37 °C. Then, the cultured cells were treated with different concentration of compounds (0.5, 5, 50 µg/mL) followed by incubation for 48 h at 37 °C. Doxorubicin (Sigma, St. Lois, MO, U.S.A.) was used as a positive control. The optical density of the dissolved material was measured at 570 nm with UV-vis spectrophotometer (Thermo Multiskan Spectrum). The viability (%) was determined by the following formula:

$$\% \text{ viable cells} = \frac{[(\text{absorbance of treated cells with compound}) - (\text{absorbance of blank})]}{[(\text{absorbance of control}) - (\text{absorbance of blank})]} \times 100.$$

The mean  $IC_{50}$  is the concentration of agent that reduces cell growth by 50 % under the experimental conditions and it is the average from at least three independent measurements that will be reproducible and statistically significant. The  $IC_{50}$  values were reported at  $\pm 95$  % confidence intervals ( $\pm 95$  % CI). This analysis was performed with Graph Pad Prism 5 (San Diego, CA, U.S.A.).



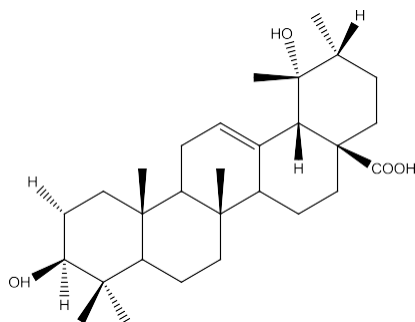
### 3. RESULTS and DISCUSSION

The secondary metabolite content of *Cephalaria tuteliana* was examined, for the first time. 2 sapogenins, 1 iridoid glycoside and 10 saponins, totally 13 known compounds were isolated, purified and structurally determined. Two sapogenins named pomolic acid (**1**) (Numata et.al., 1989), tormentic acid (**2**) (Villar et.al., 1986), ten known triterpene saponins which was named elmalienoside A (**3**) (Sarikahya and Kirmizigul, 2012), davisianoside A (**4**) (Kayce et.al., 2014),  $\alpha$ -hederin (**5**) (Aliev and Movsumov, 1976), elmalienoside B (**6**) (Sarikahya and Kirmizigul, 2012), 3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl hederagenin 28- *O*- $\beta$ -D-glucopyranosyl ester (**7**) (Kawai et. al., 1988), davisianoside B (**8**) (Kayce et.al., 2014), 3-*O*- $\beta$ -D-glucopyranosyl- (1 $\rightarrow$ 3)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl hederagenin 28-*O*- $\beta$ - D-glucopyranosyl ester (**9**) (Braca et.al., 2004), dipsacoside B (**10**) (Mukhamedzиеv et.al., 1971), macranthoidin A (**11**) (Mao et.al., 1993), 3-*O*- $\beta$ -[ $\alpha$ - L-rhamnopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-glucopyranosyl] hederagenin (**12**) ( Lemos et.al., 1992) and one iridoid glycoside namely laciniatoside I (**13**) (Kocsis et.al. 1993) were obtained. The structures of all compounds were elucidated by spectral methods including IR, 1D and 2D NMR methods Among these compounds pomolic acid (**1**), tormentic acid (**2**) and 3-*O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)]- $\alpha$ -L-arabinopyranosyl hederagenin (**12**) were detected in *Cephalaria* species and Caprifoliceaea family as well, for the first time. The cytotoxic activities of *n*-butanol extract, compounds **1-2** were examined against cancerous cells A549, Hela, PANC1, SHSY5Ycells and noncancerous cell HEK293 by MTT method. We compared the cytotoxicity results with common aglycones hederagenin and oleanoic acid to discuss structure activity relationship Table 3.1 .

**Table 3.1.** The cytotoxic activities of *n*-butanol extract, compounds **1-2** and aglycones hederagenin, oleanoic acid

Sample	Cell Lines ( $\mu\text{M}$ )				
	A549	Hela	PANC1	SHSY5Y	HEK293
pomolic acid ( <b>1</b> )	-	-	>50	79.35 $\pm$ 4.037	-
tormentic acid ( <b>2</b> )	-	-	-	-	-
hederagenin	44.48 $\pm$ 0.912	30.78 $\pm$ 1.555	31.40 $\pm$ 1.499	23.05 $\pm$ 0.509	37.05 $\pm$ 0.898
oleanoic acid	31.61 $\pm$ 0.954	68.88 $\pm$ 1.117	44.54 $\pm$ 2.503	18.94 $\pm$ 1.156	>50
<i>n</i> -butanol extract	-	-	-	-	-
Doxorubicin	13.97 $\pm$ 0.639	10.45 $\pm$ 0.308	15.89 $\pm$ 1.988	4.69 $\pm$ 0.036	25.85 $\pm$ 3.973

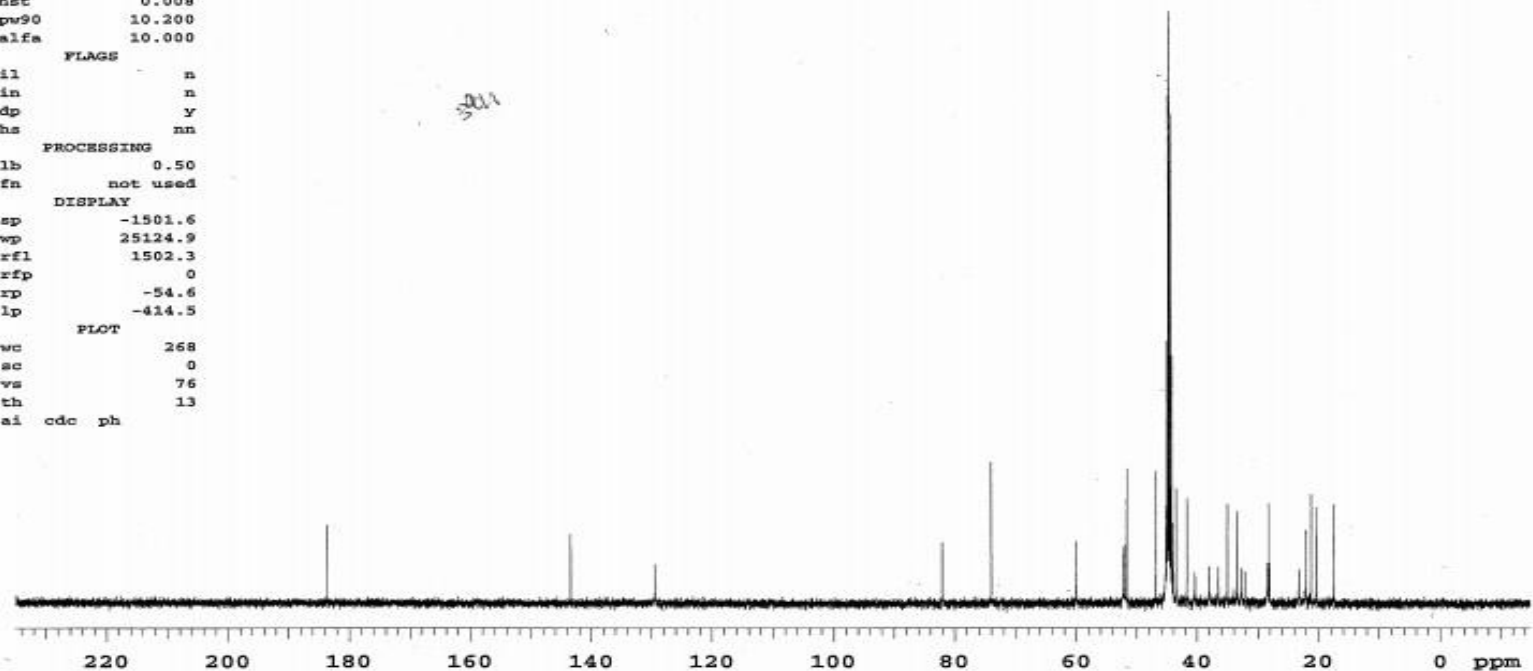
- *not active*

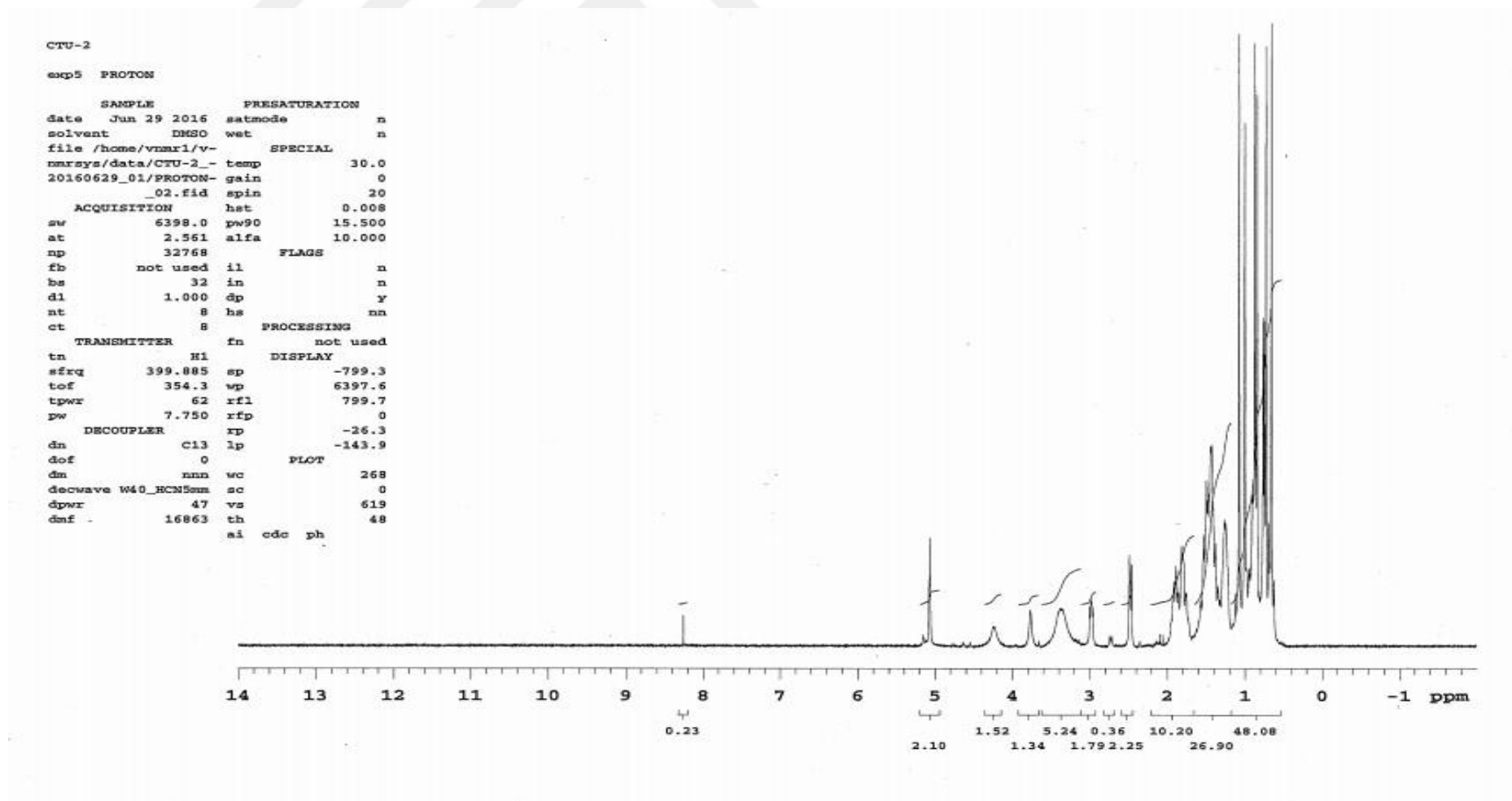
**3.1.Compound 1 : Pomolic Acid** (Numata et.al., 1989)**Figure 3.1.** Pomolic acid

CTU-2-C13

exp6 CARBON

SAMPLE		PRESATURATION	
date	Jun 29 2016	satmode	n
solvent	CDC13	wet	n
file	/home/vnmr1/v-	SPECIAL	
nmrays/Automation/-		temp	30.0
auto_20160629_03/e-		gain	30
nterQ.macdir/loc0_-		spin	20
004/current		hst	0.008
ACQUISITION		pw90	10.200
sw	25125.6	alfa	10.000
at	1.304	FLAG	
np	65536	il	n
fb	13800	in	n
bs	8	dp	y
dl	2.000	hs	nn
nt	2000	PROCESSING	
ct	280	lb	0.50
TRANSMITTER		fn	not used
tn	C13	DISPLAY	
sfrq	100.561	sp	-1501.6
tof	1519.6	wp	25124.9
tpwr	61	rfl	1502.3
pw	5.100	rfp	0
DECOUPLER		rp	-54.6
dn	H1	lp	-414.5
dof	0	PLOT	
dm	YYY	wc	268
decwave	w	sc	0
dpwr	44	vs	76
dmf	8117	th	13
	ai	cdc	ph

Figure 3.2.  $^{13}\text{C}$  spectrum of Compound 1

Figure 3.3.  $^1\text{H}$  spectrum of Compound 1

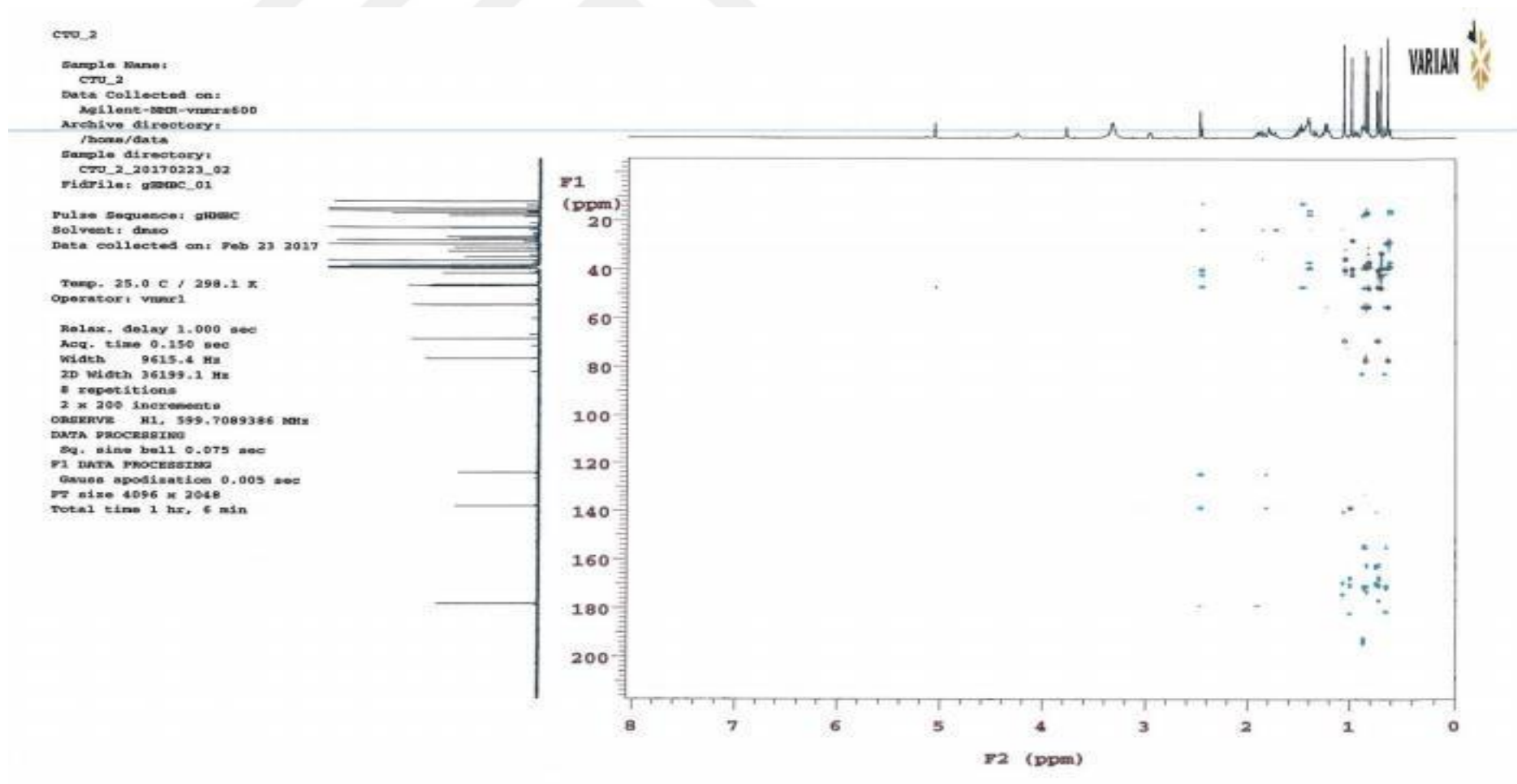


Figure3.4.HMBC spectrum of Compound 1

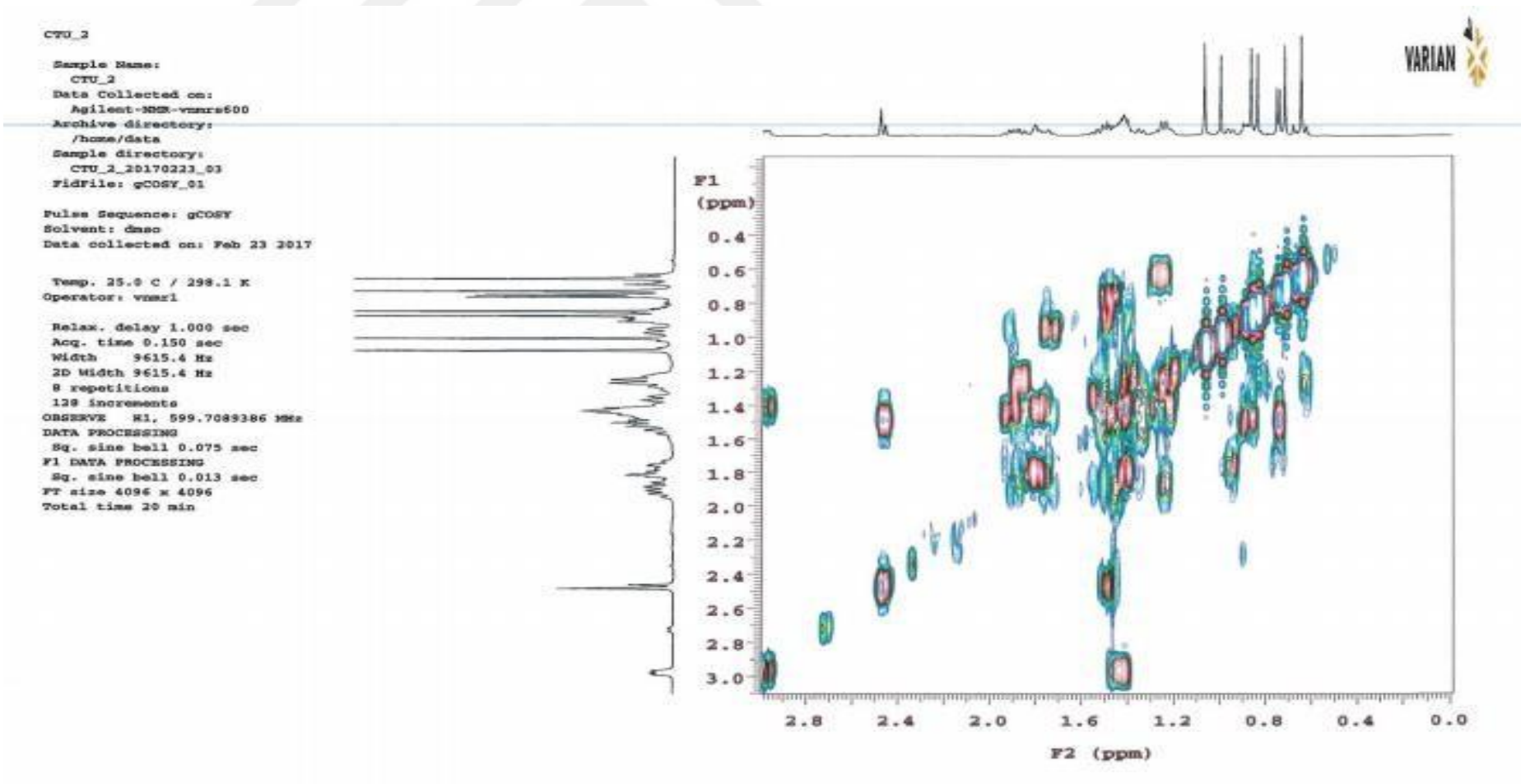


Figure 3.5. COSY spectrum of Compound 1

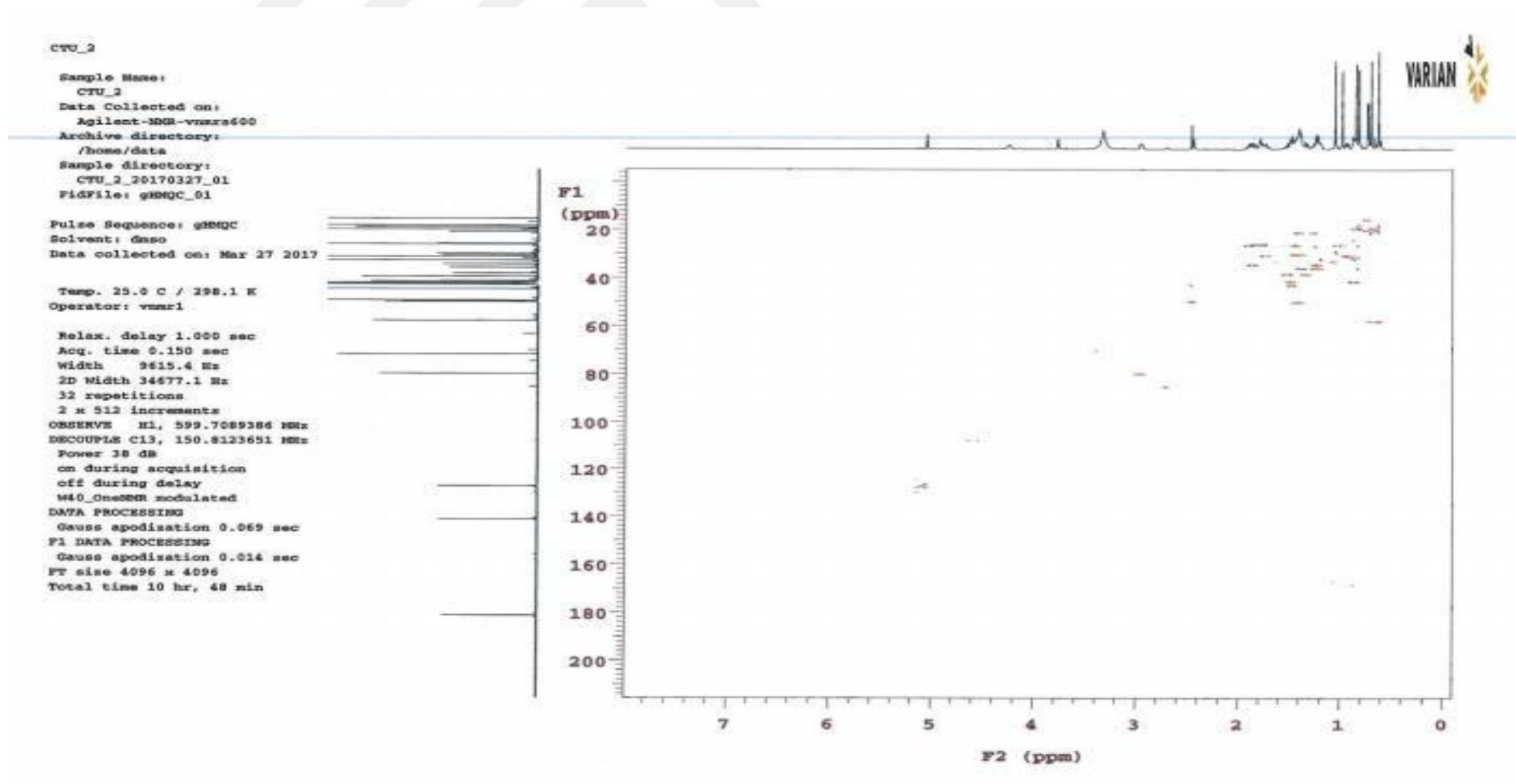
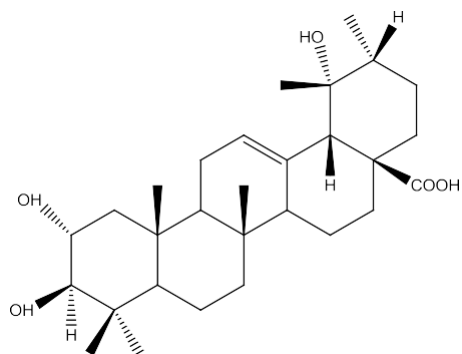


Figure 3.6. HMQC spectrum of Compound 1

**3.2.Compound 2 : Tormentic Acid (Villar et.al., 1986)****Figure 3.7.** Tormentic Acid

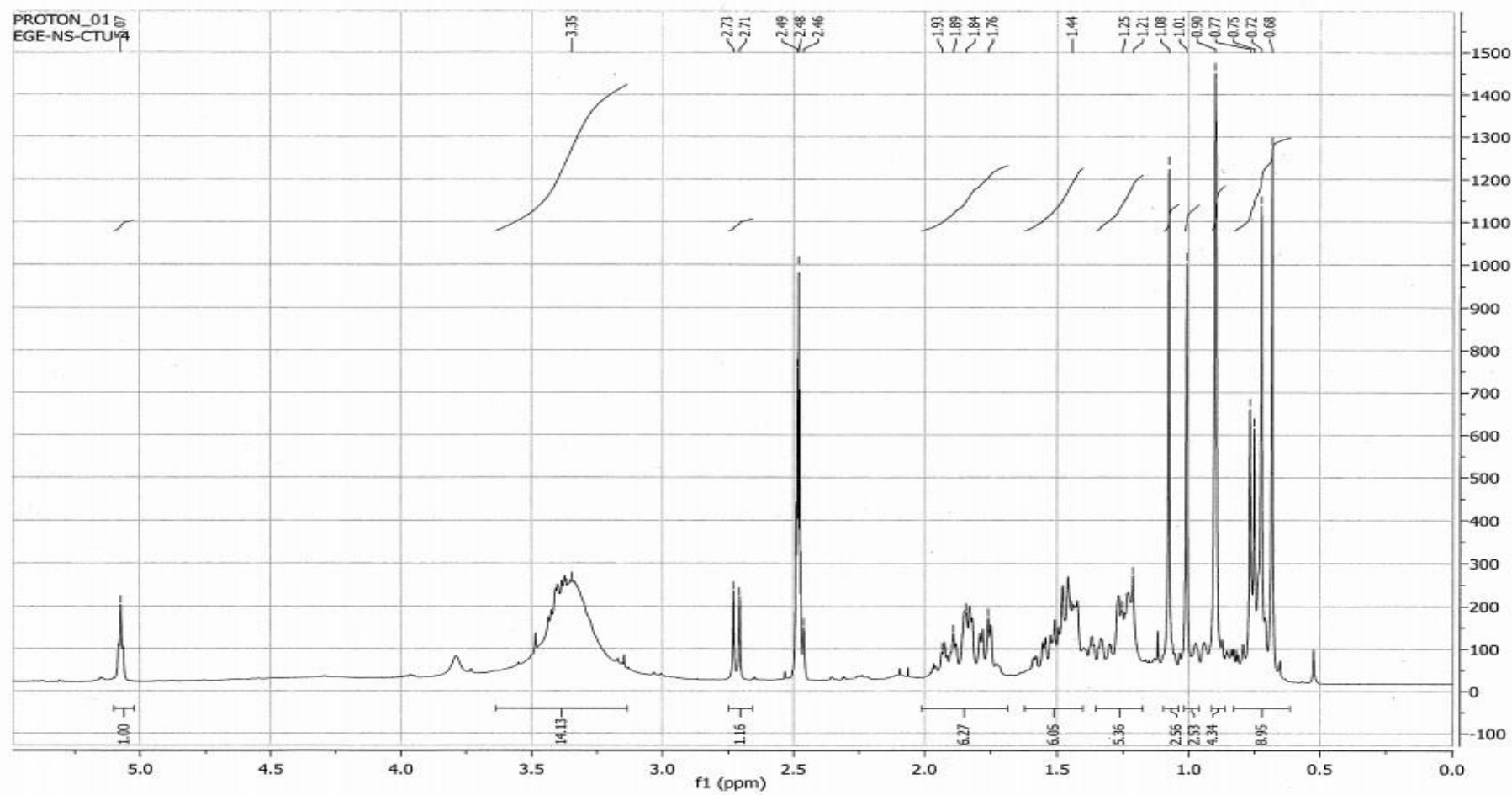


Figure 3.8.  $^1\text{H}$  spectrum of Compound 2

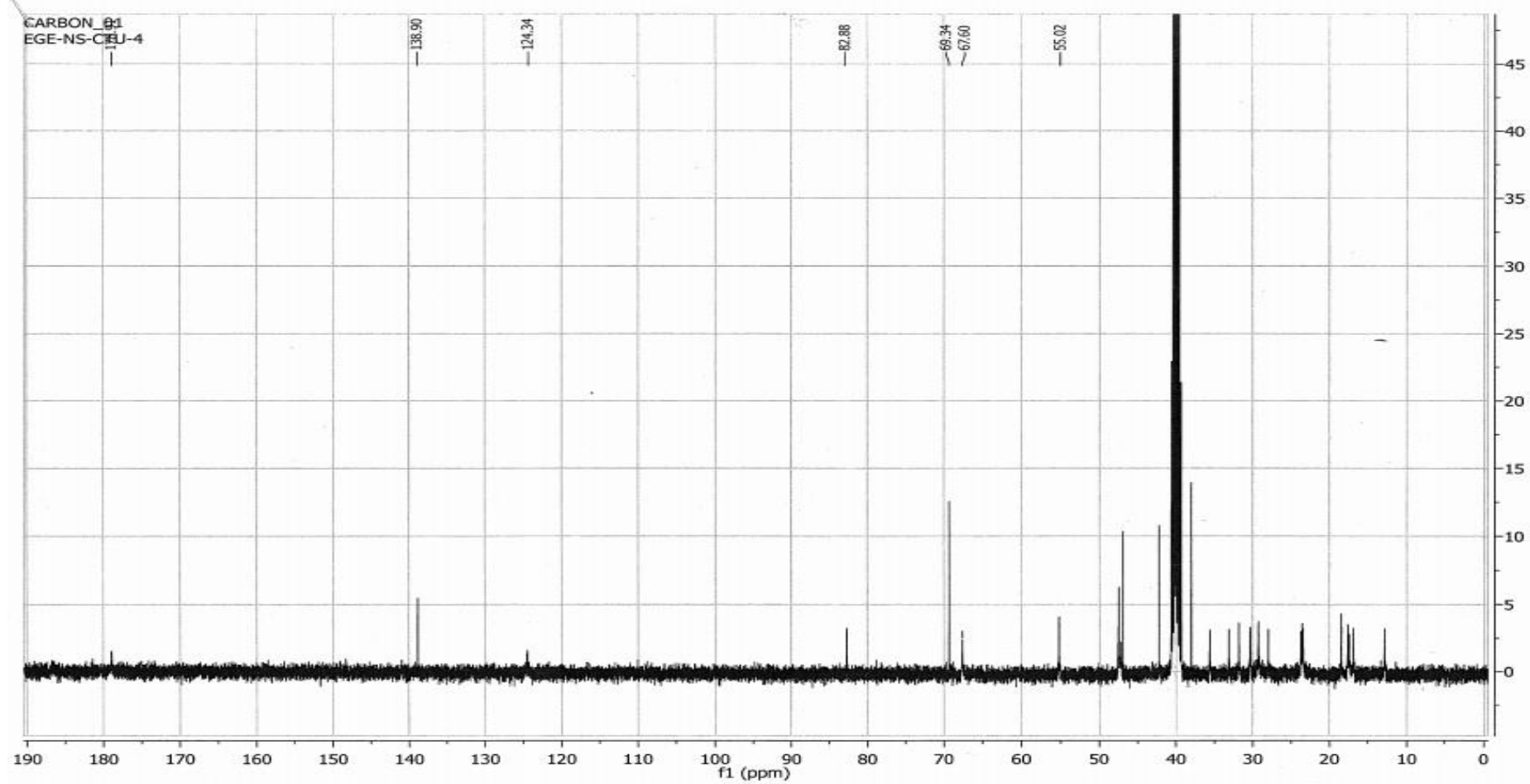


Figure 3. 9.  $^{13}\text{C}$  spectrum of Compound 2

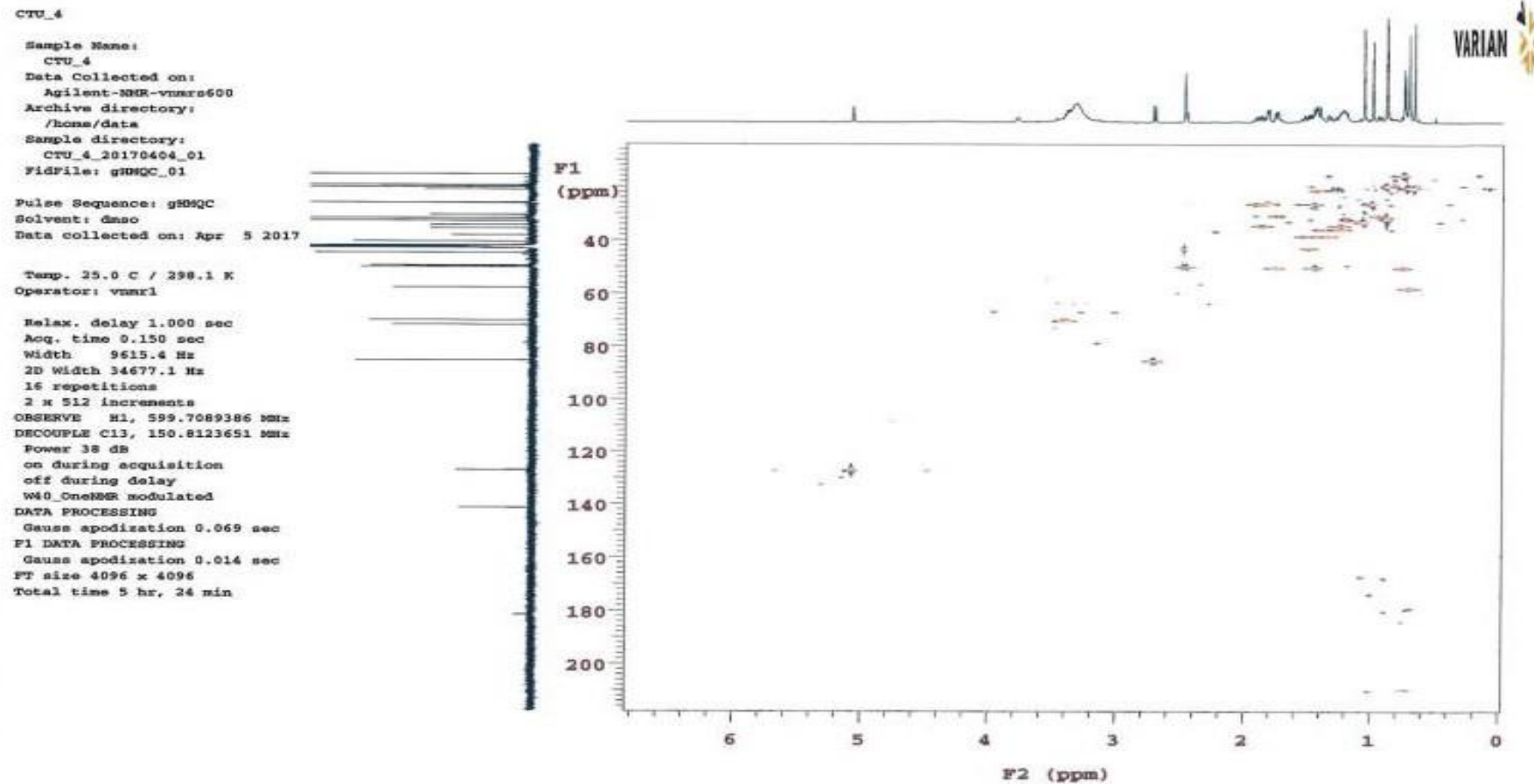


Figure 3.10.HMQC spectrum of Compound 2

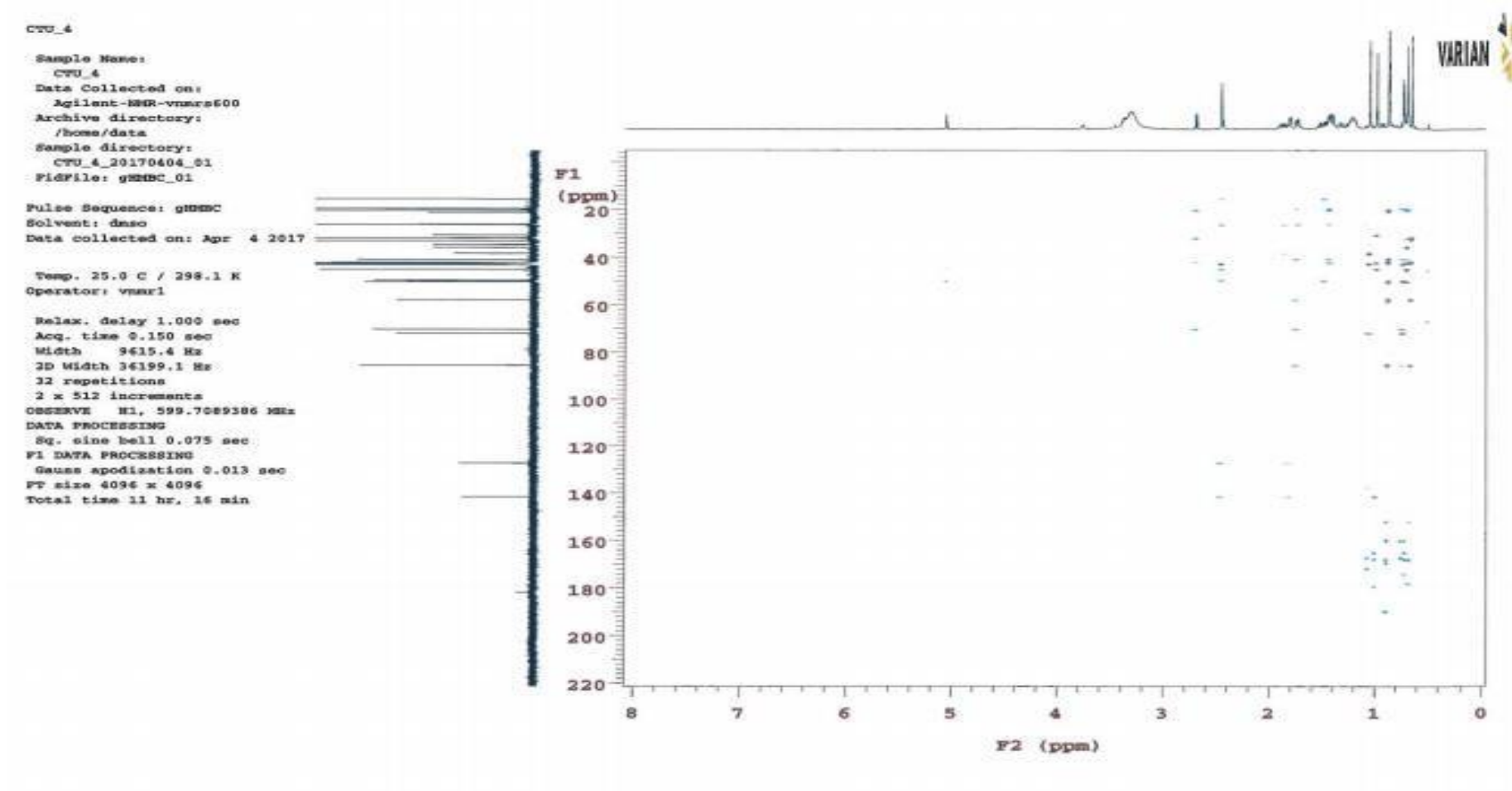


Figure 3.11. HMBC spectrum of Compound 2

CTU\_4

Sample Name:  
CTU\_4  
Data Collected on:  
Agilent-MMR-vnmr600  
Archive directory:  
/home/data  
Sample directory:  
CTU\_4\_20170404\_01  
FidFile: gCOSY\_01

Pulse Sequence: gCOSY  
Solvent: dmsc  
Data collected on: Apr 4 2017

Temp. 25.0 C / 298.1 K  
Operator: vnmr1

Relax. delay 1.000 sec  
Acq. time 0.150 sec  
Width 5481.8 Hz  
2D Width 5481.8 Hz  
32 repetitions  
312 increments  
OBSERVE H1, 599.7089386 MHz  
DATA PROCESSING  
Sq. sine bell 0.075 sec  
F1 DATA PROCESSING  
Sq. sine bell 0.053 sec  
FT size 2048 x 2048  
Total time 5 hr, 34 min

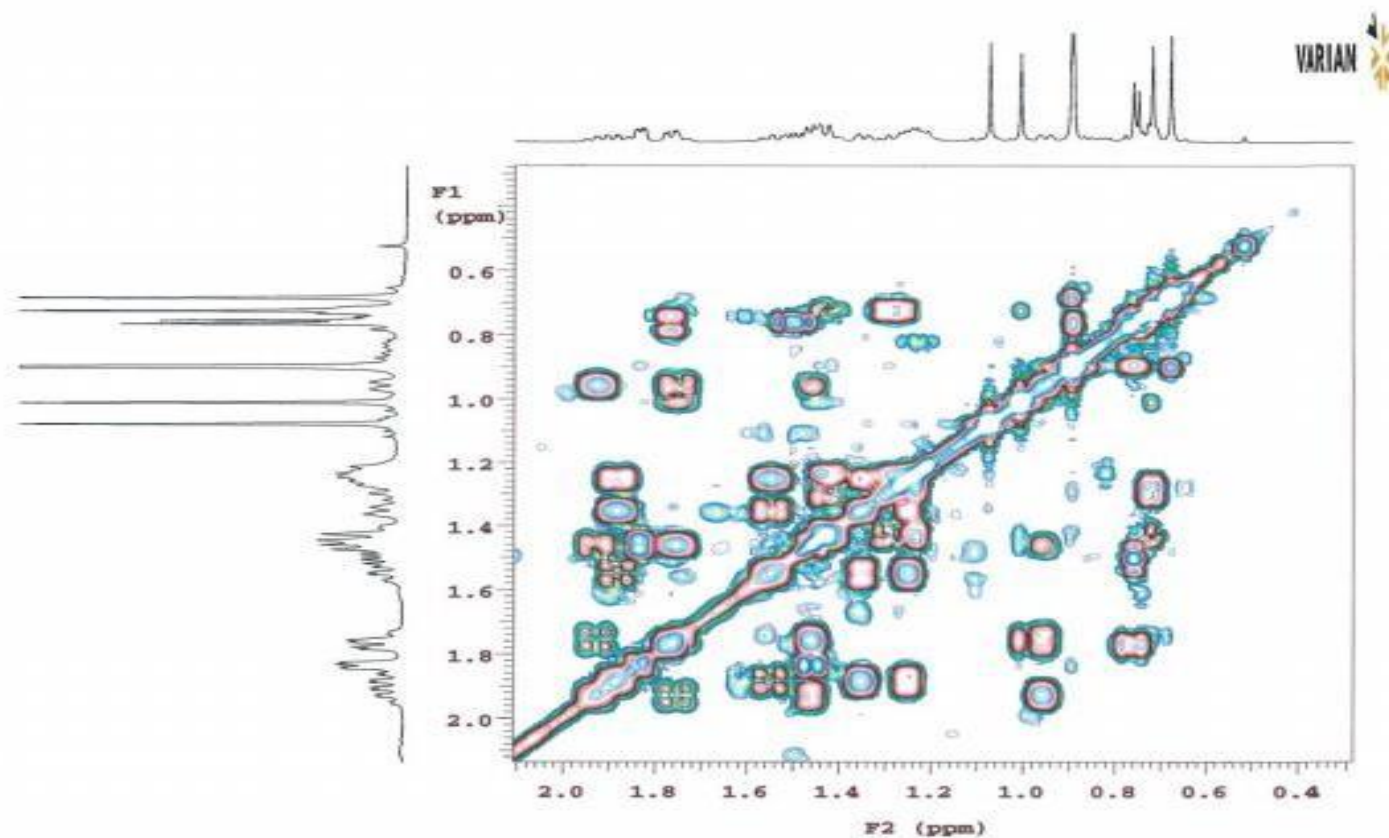
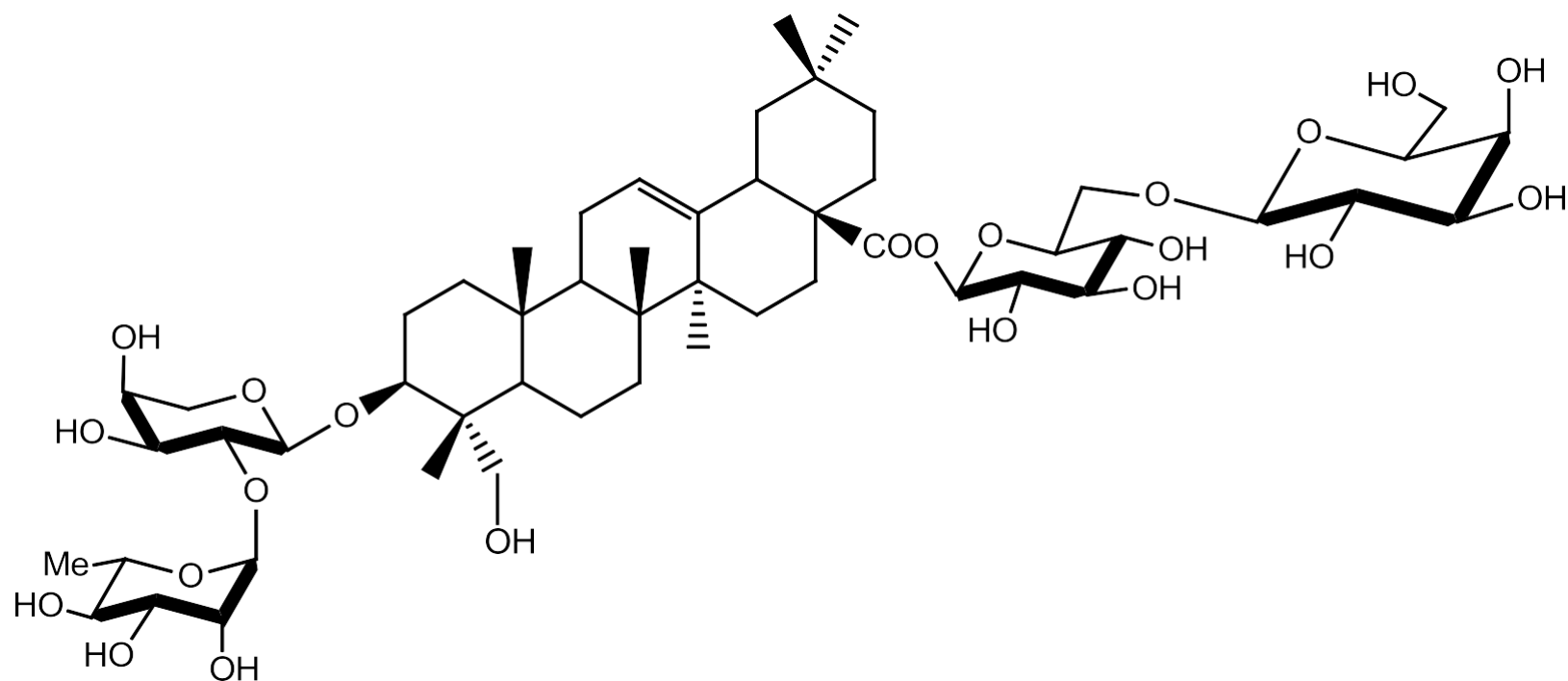


Figure 3.12. COSY spectrum of Compound 2

**3.3. Compound 3:** Elmalienoside A (Sarikahya and Kirmizigul, 2012)

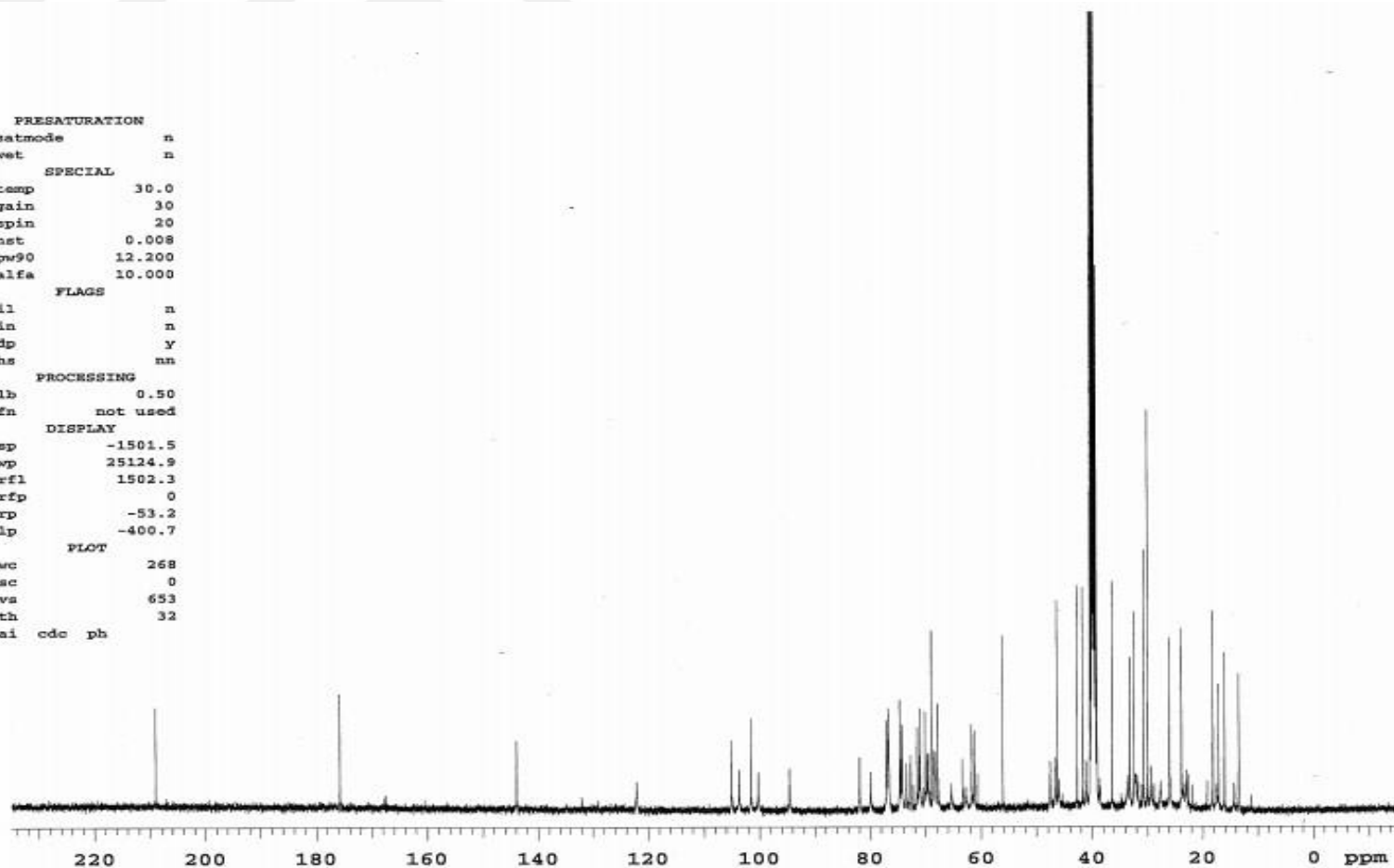


**Figure 3.13.**Elmalienoside A

CTU-1-C13

exp7 CARBON

SAMPLE		PRESATURATION	
date	Aug 3 2017	satmode	n
solvent	DMSO	wet	n
file	/home/vmr1/v-	SPECIAL	
nmr	Automation/-	temp	30.0
auto	20170803_02/e-	gain	30
nterQ	macdir/loc0_-	spin	20
003/current		hst	0.008
ACQUISITION		pw90	12.200
sw	25125.6	alfa	10.000
at	1.304	FLAGS	
np	65536	il	n
fb	13800	in	n
bs	8	dp	y
dl	2.000	hs	nn
nt	25600	PROCESSING	
ct	16984	lb	0.50
TRANSMITTER		fn	not used
tn	C13	DISPLAY	
sfrq	100.562	sp	-1501.5
tof	1519.6	wp	25124.9
tpwr	60	rfl	1502.3
pw	6.100	rfl	0
DECOUPLER		rp	-53.2
dn	H1	lp	-400.7
dof	0	PLOT	
dm	YYY	wc	268
decuave	w	sc	0
dpwr	44	va	653
daf	8100	th	32
		ai	cdc ph

Figure 3.14.  $^{13}\text{C}$  spectrum of Compound 3

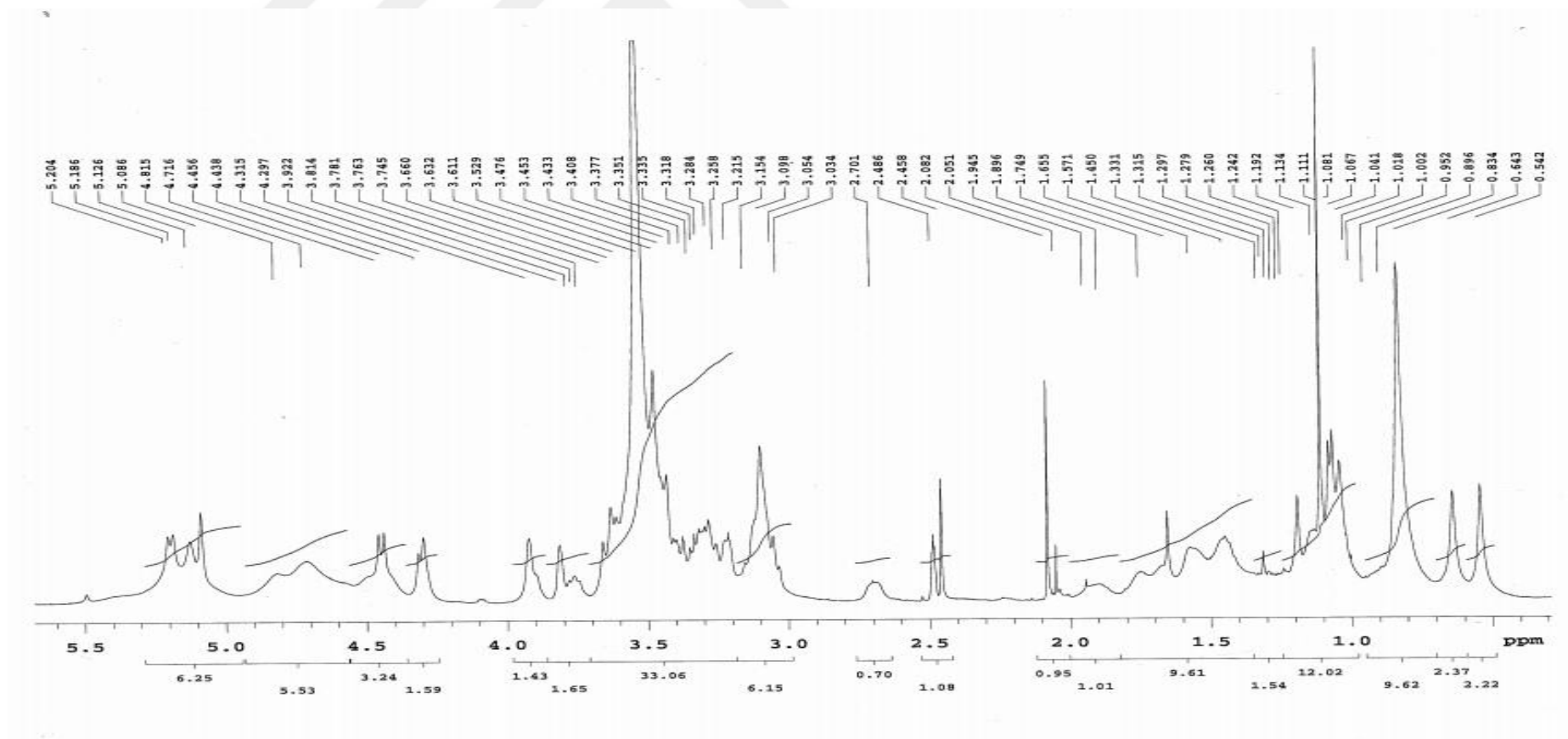
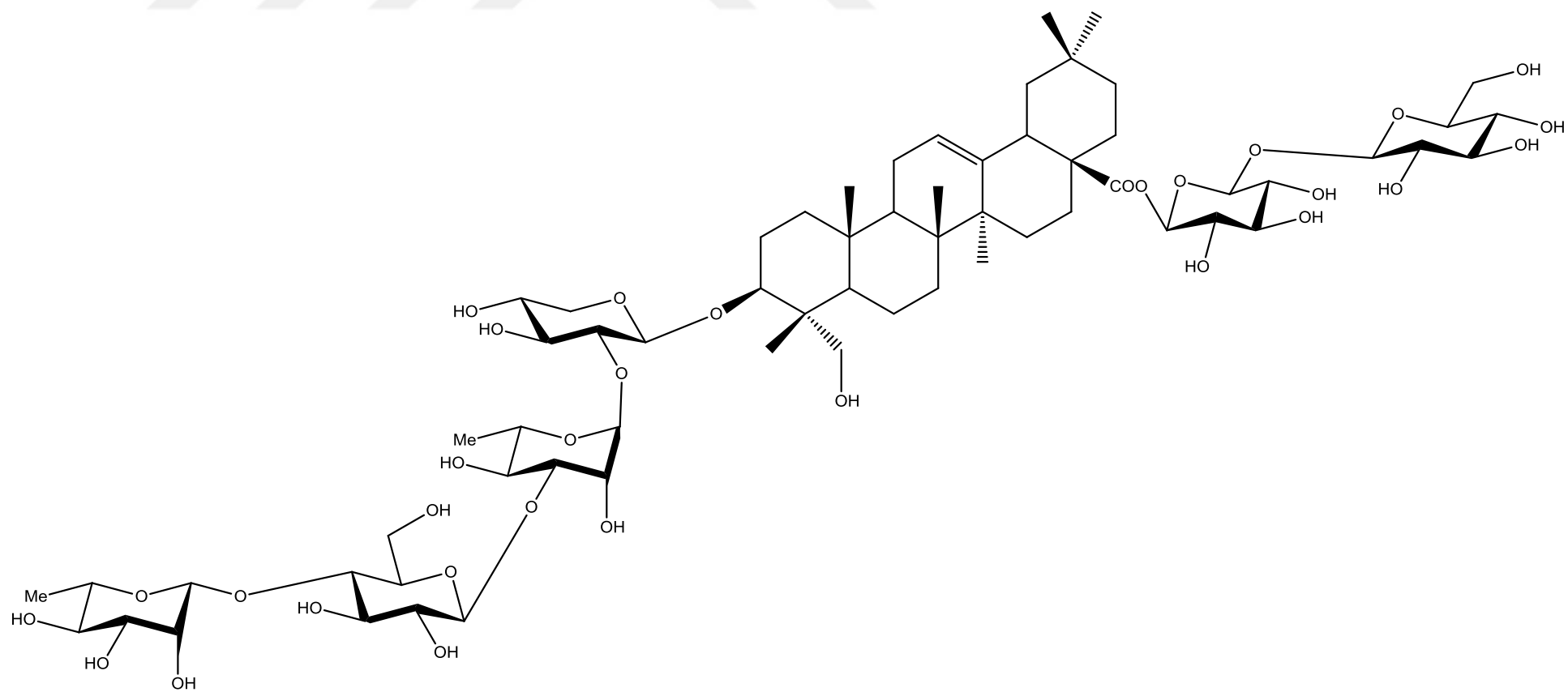
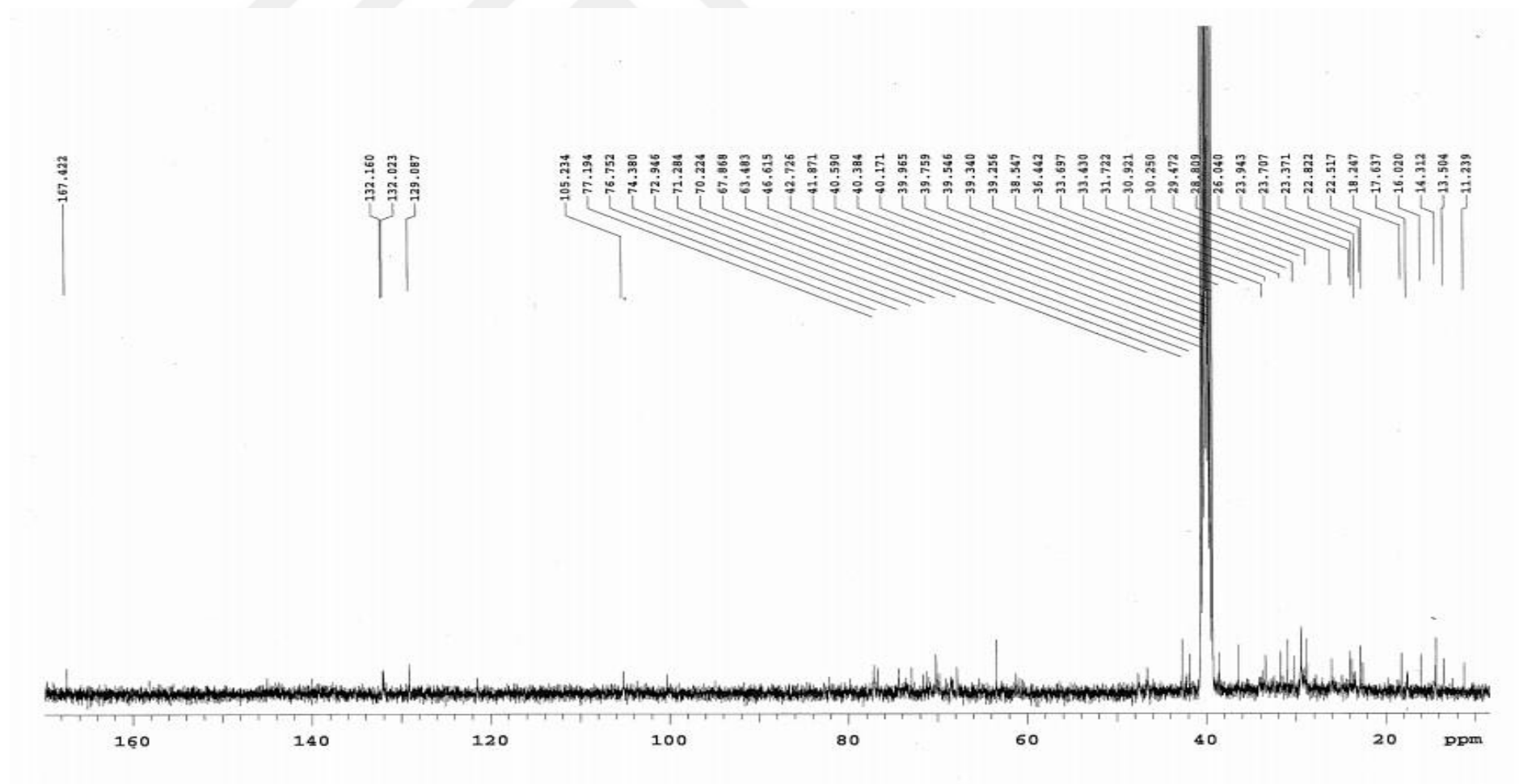


Figure 3.14.  $^1\text{H}$  spectrum of Compound 3

**3.4. Compound 4:** Davisianoside A (Kayce et.al., 2014)



**Figure 3.16.** Davisianoside A



Şekil 3.16. <sup>13</sup>C spectrum of Compound 4

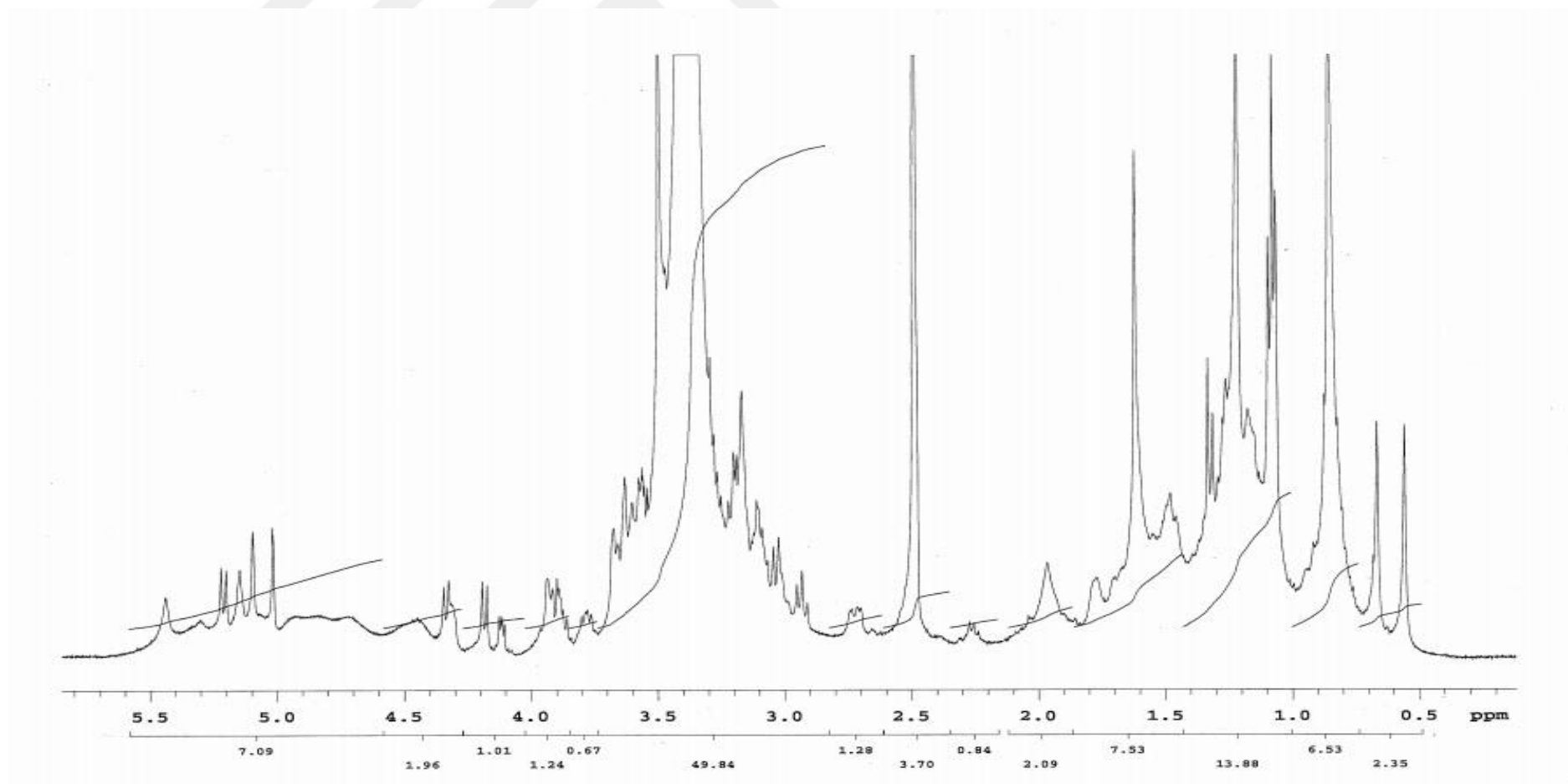
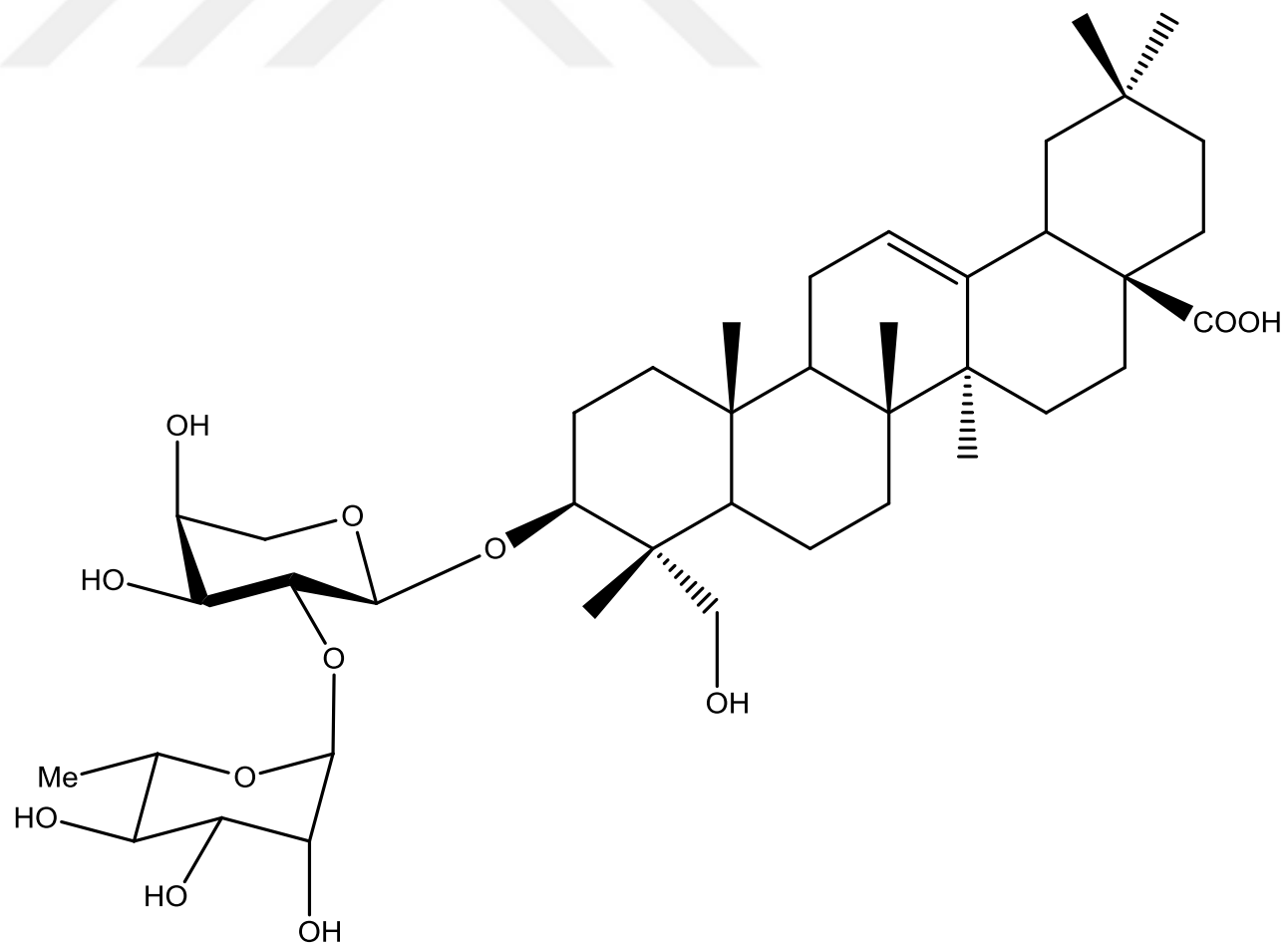


Figure 3.17.  $^1\text{H}$  spectrum of Compound 4

**3.5. Compound 5:** Alpha Hederin (Aliev and Movsumov, 1976 )



**Figure 3.18.**Alpha Hederin

CTU\_5

Sample Name:  
CTU\_5  
Data Collected on:  
Agilent-300-vmars600  
Archive directory:  
/home/data  
Sample directory:  
CTU\_5\_20170323\_01  
FidFile: CARBON\_01

Pulse Sequence: CARBON (s2pul)  
Solvent: dmsc  
Data collected on: Mar 23 2017

Temp. 25.0 C / 298.1 K  
Operator: vmars1

Relax. delay 1.000 sec  
Pulse 45.0 degrees  
Acq. time 0.865 sec  
Width 37878.8 Hz  
29500 repetitions  
OBSERVE C13, 150.7970006 MHz  
DECOUPLE H1, 599.7119372 MHz  
Power 43 dB  
continuously on  
WALTZ-16 modulated  
DATA PROCESSING  
Line broadening 0.5 Hz  
FT size 65536  
Total time 15 hr, 17 min

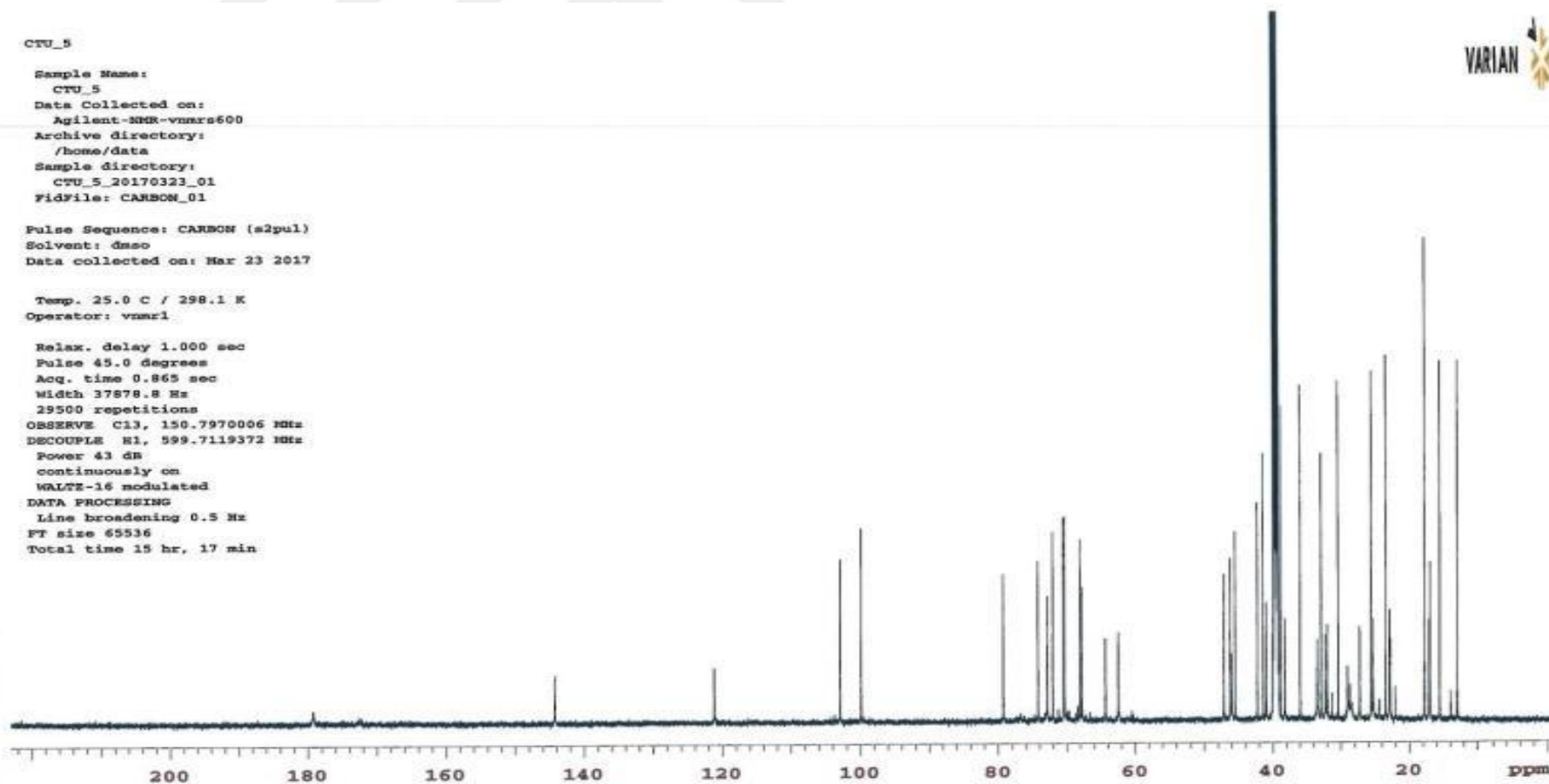


Figure 3.19. <sup>13</sup>C spectrum of Compound 5

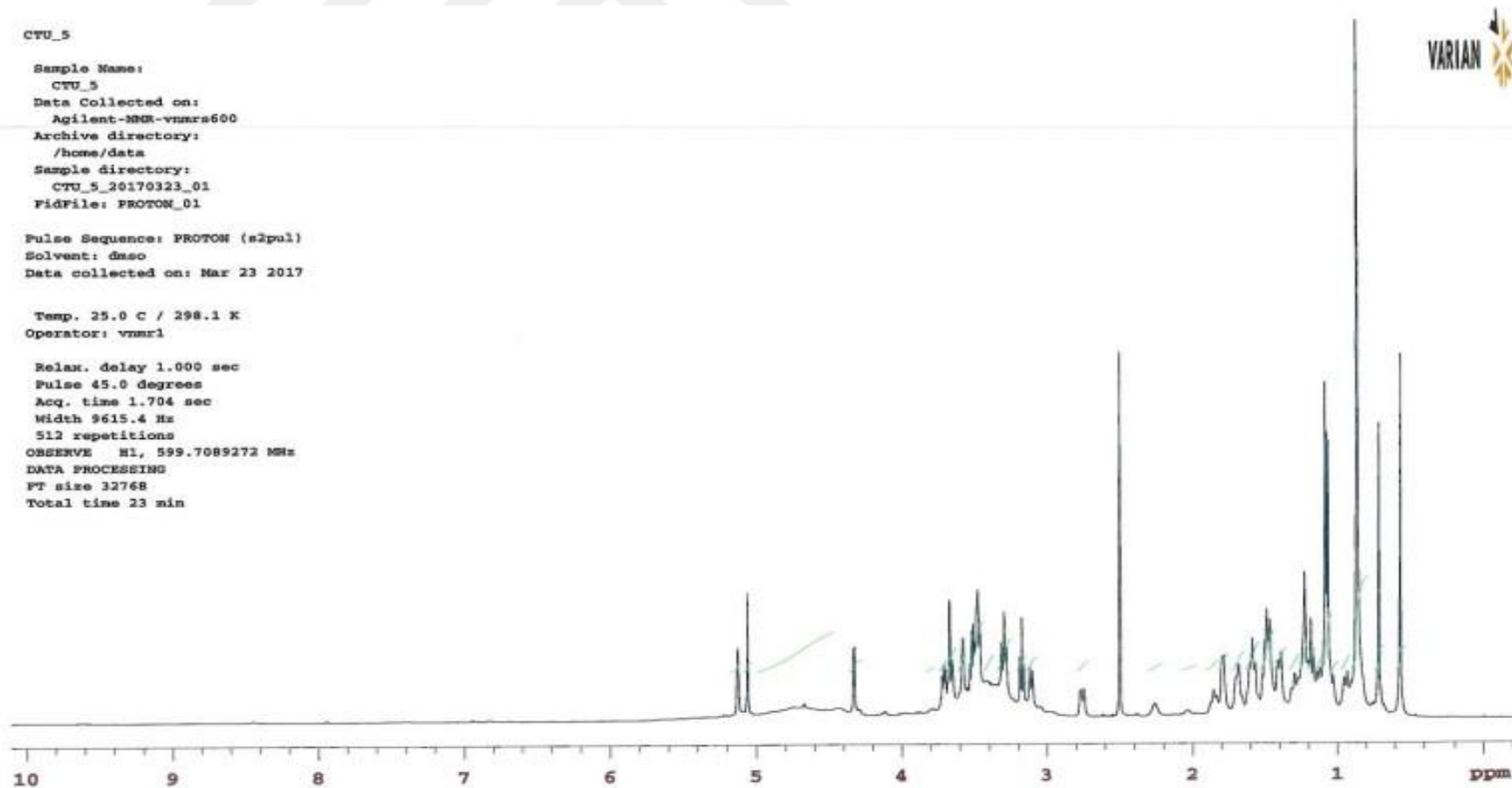
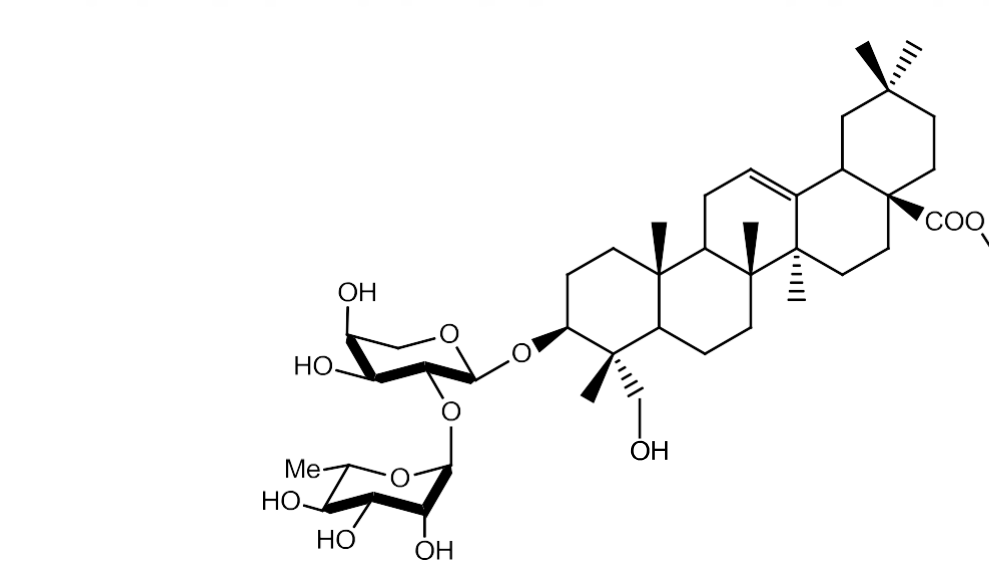
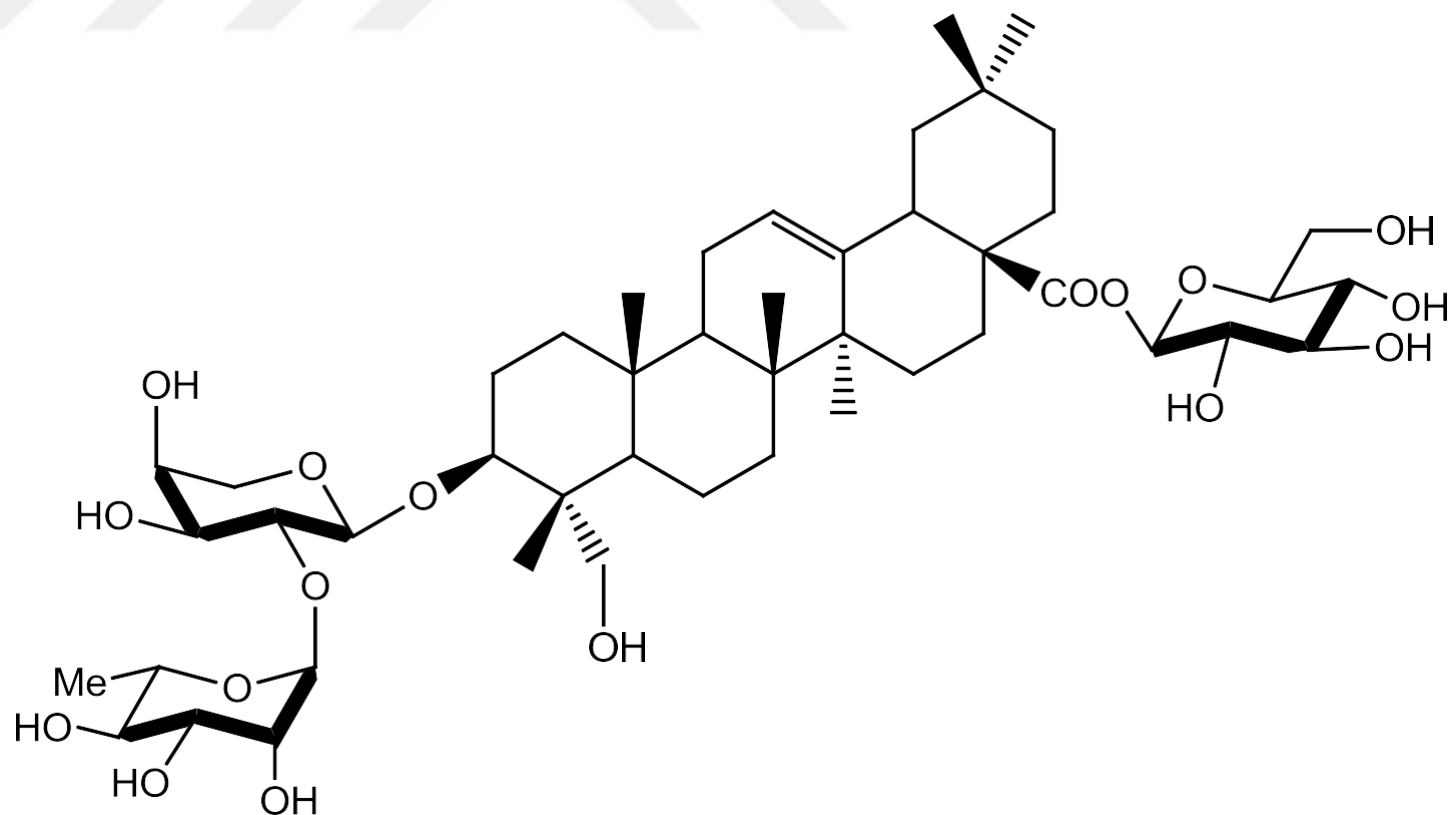


Figure 3.20.  $^1\text{H}$  spectrum of Compound 5



**Figure 3.21. Elmalienosid**

**3.7.Compound 7:** 3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl hederagenin 28-*O*- $\beta$ -D-glucopyranosyl ester (Kawai et. al., 1988)



**Figure 3.22.** 3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl hederagenin 28-*O*- $\beta$ -D-glucopyranosyl ester

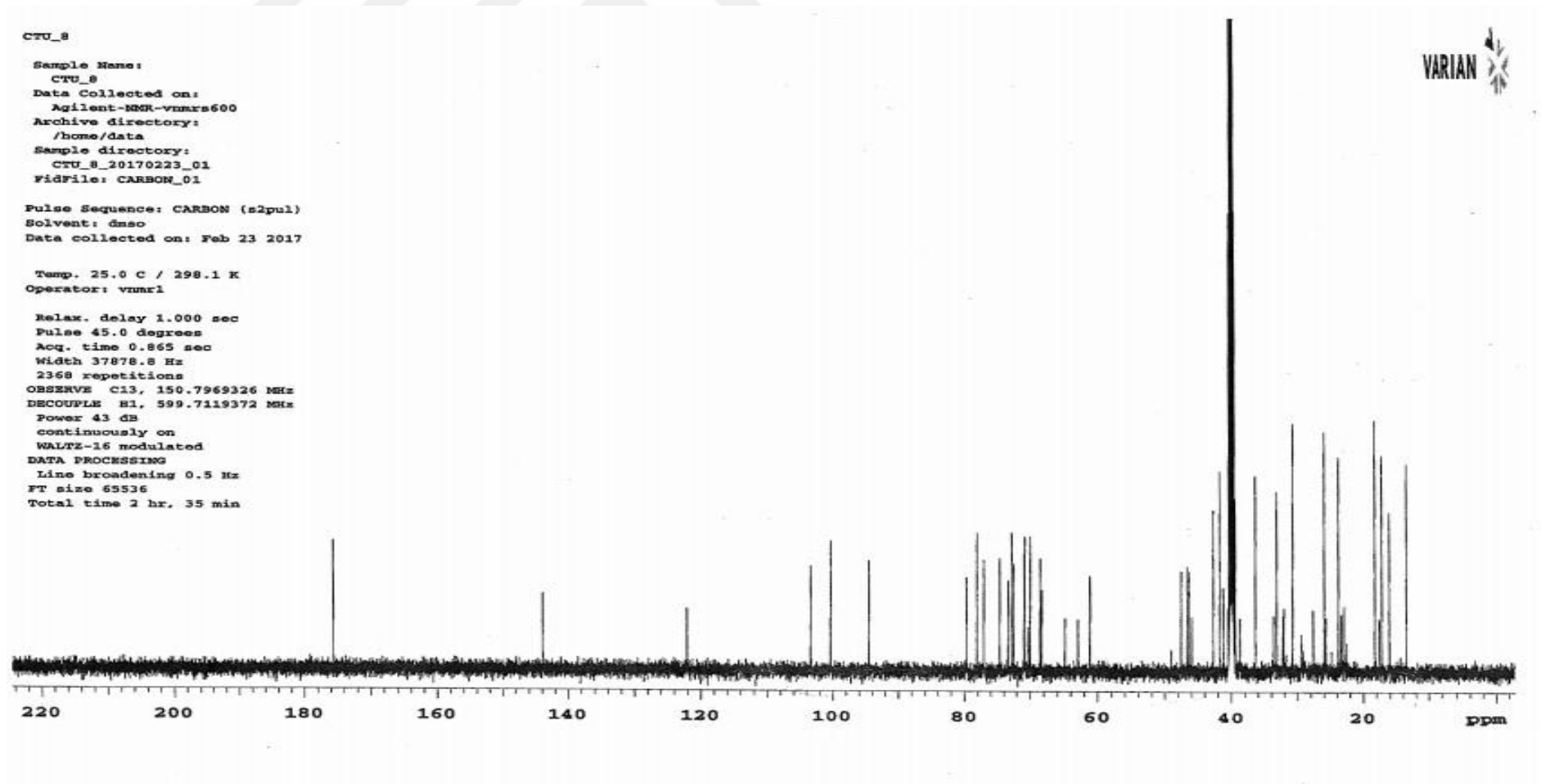


Figure 3.23.  $^{13}\text{C}$  spectrum of Compound 7

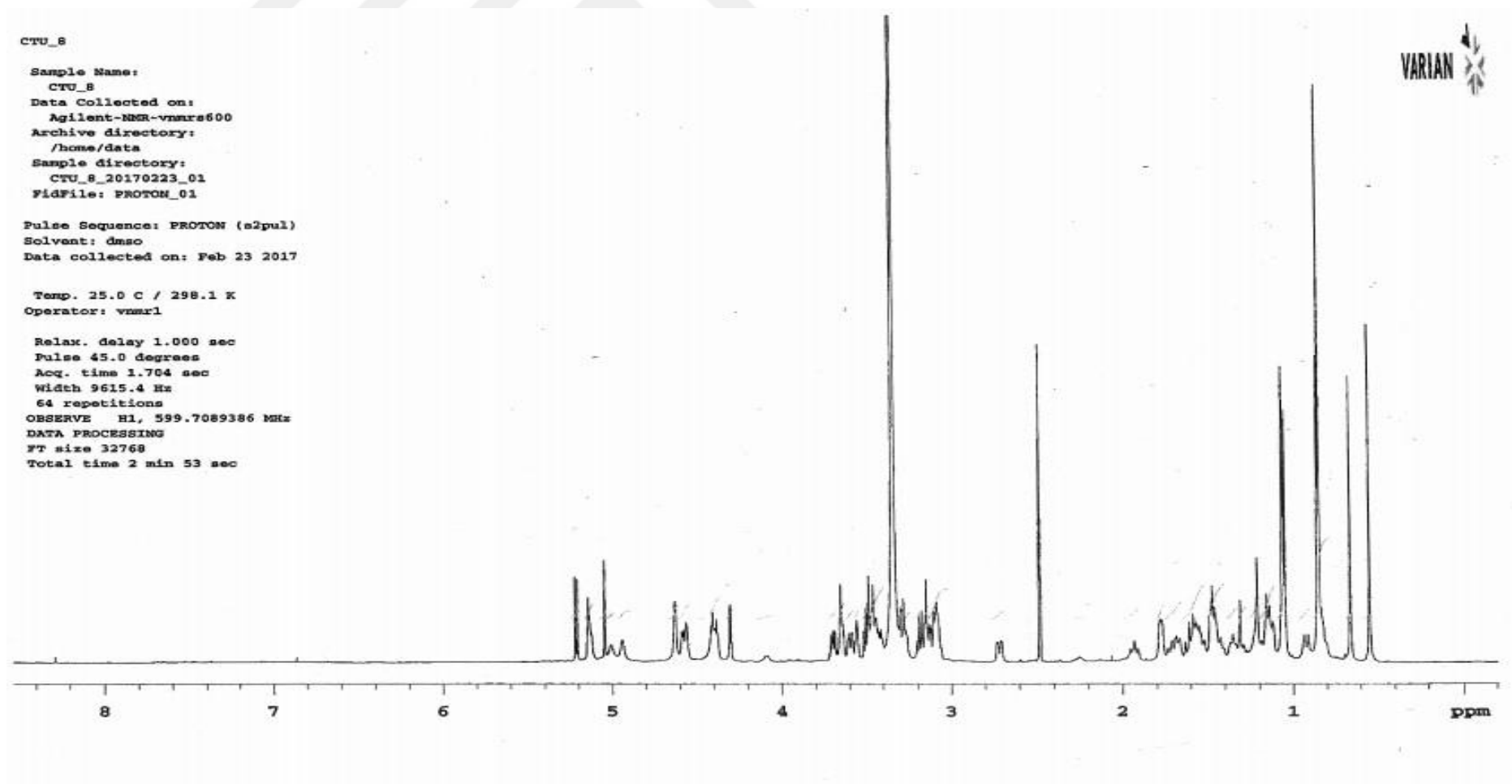
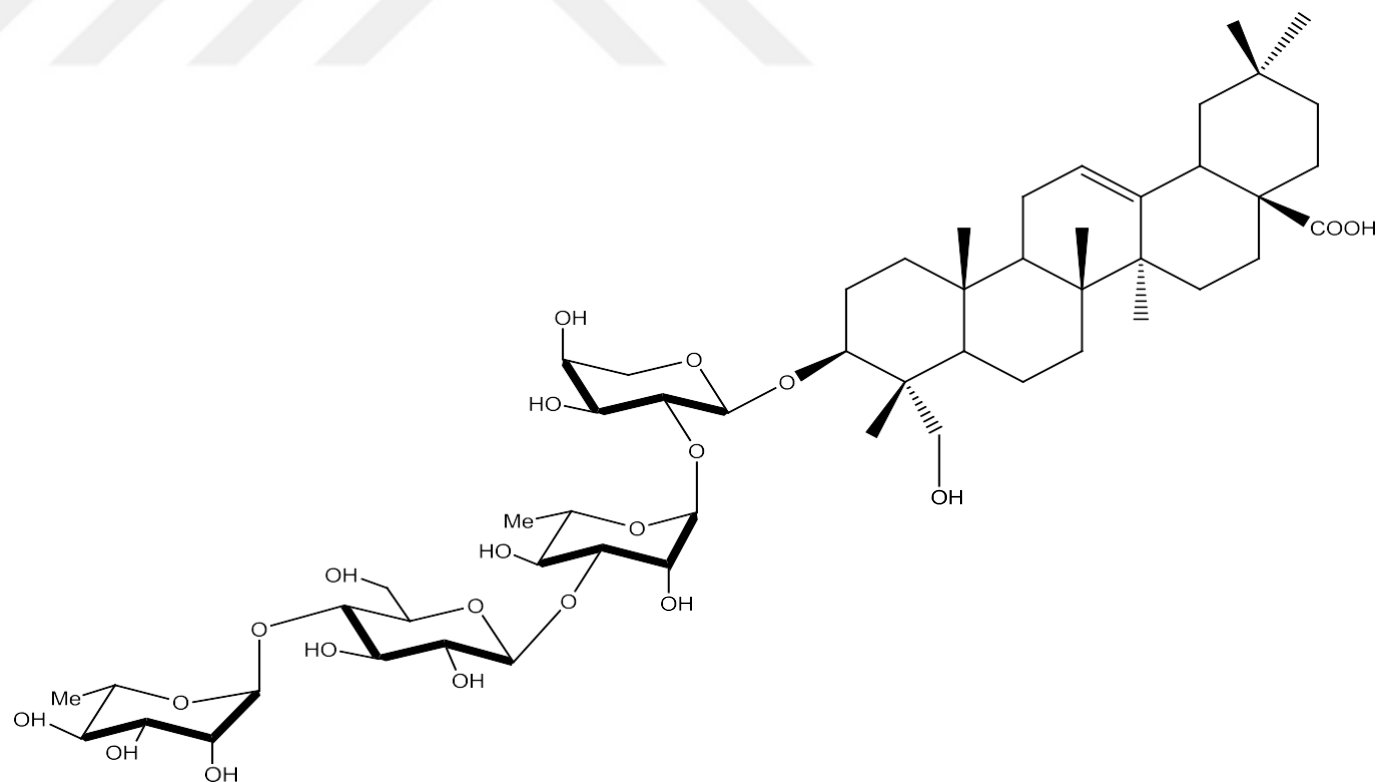


Figure 3.24.  $^1\text{H}$  spectrum of Compound 7

**3.8. Compound 8:** Davisianoside B (Kayce et.al., 2014)



**Figure 3.25.** Davisianoside B

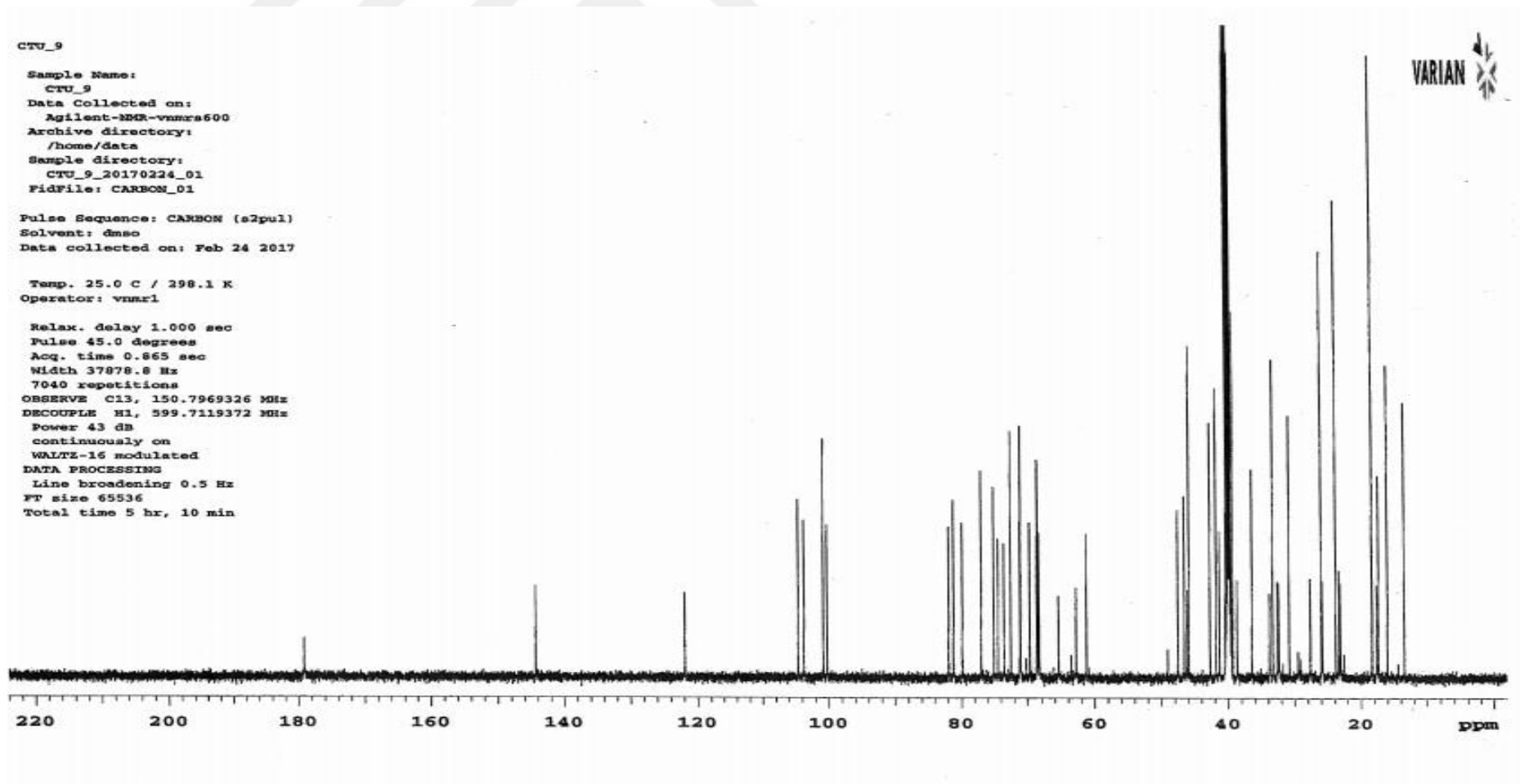


Figure 3.26.  $^{13}\text{C}$  spectrum of Compound 8

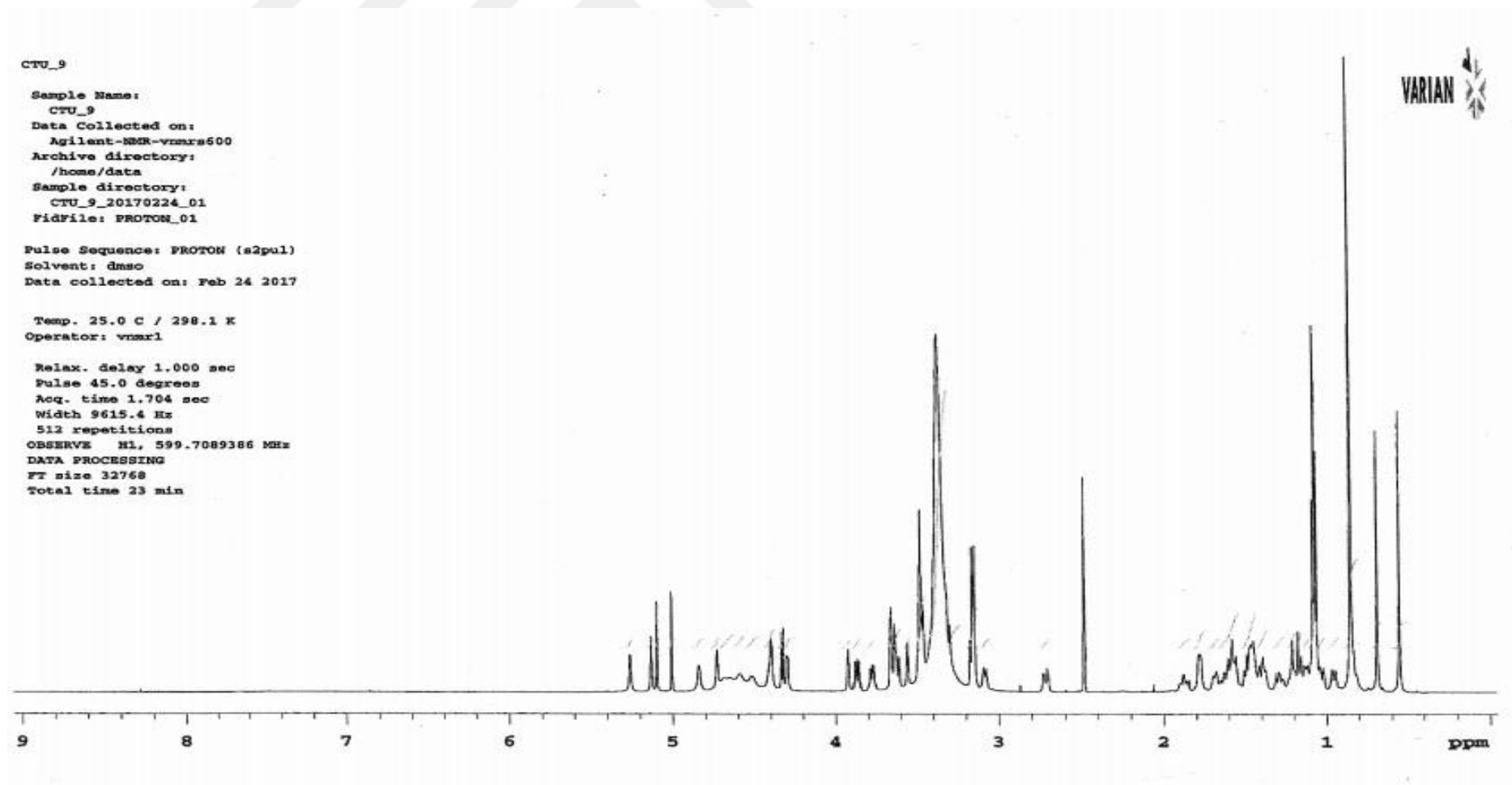
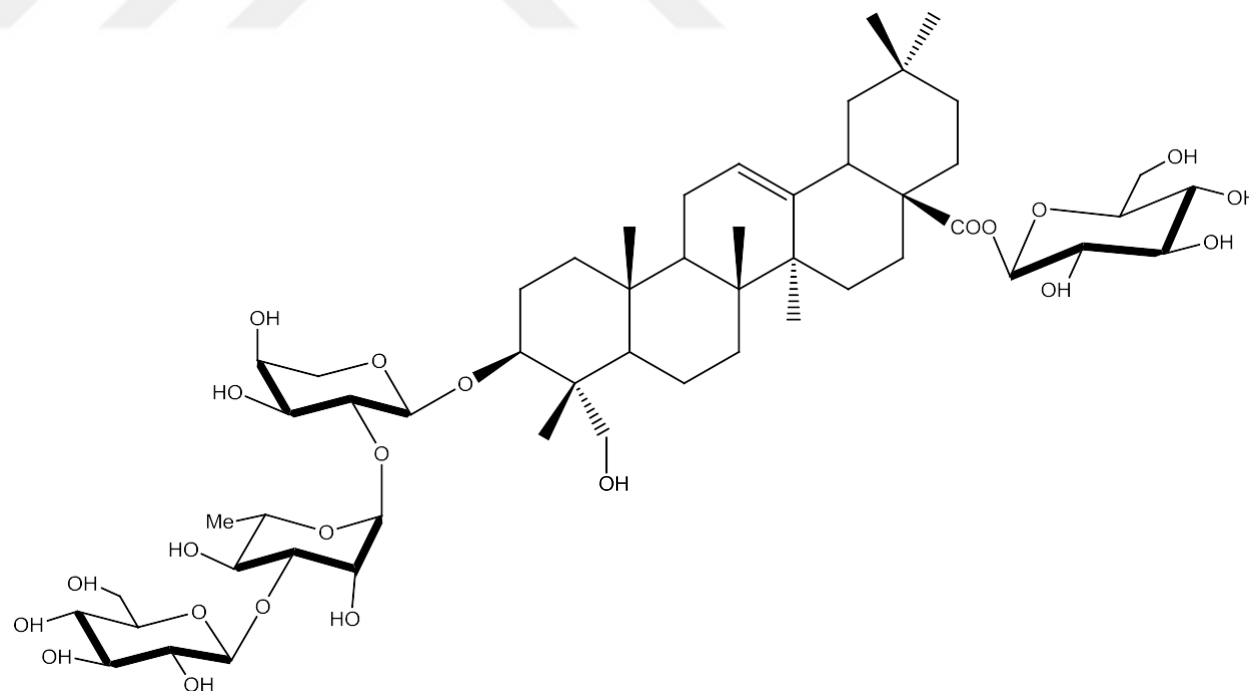


Figure 3.27.  $^1\text{H}$  spectrum of Compound 8

**3. 9. Compound 9:** 3-*O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl hederagenin 28-*O*- $\beta$ -D-glucopyranosyl ester. (Braca et.al., 2004)



**Figure 3.28.** 3-*O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl hederagenin 28-*O*- $\beta$ -D-glucopyranosyl ester

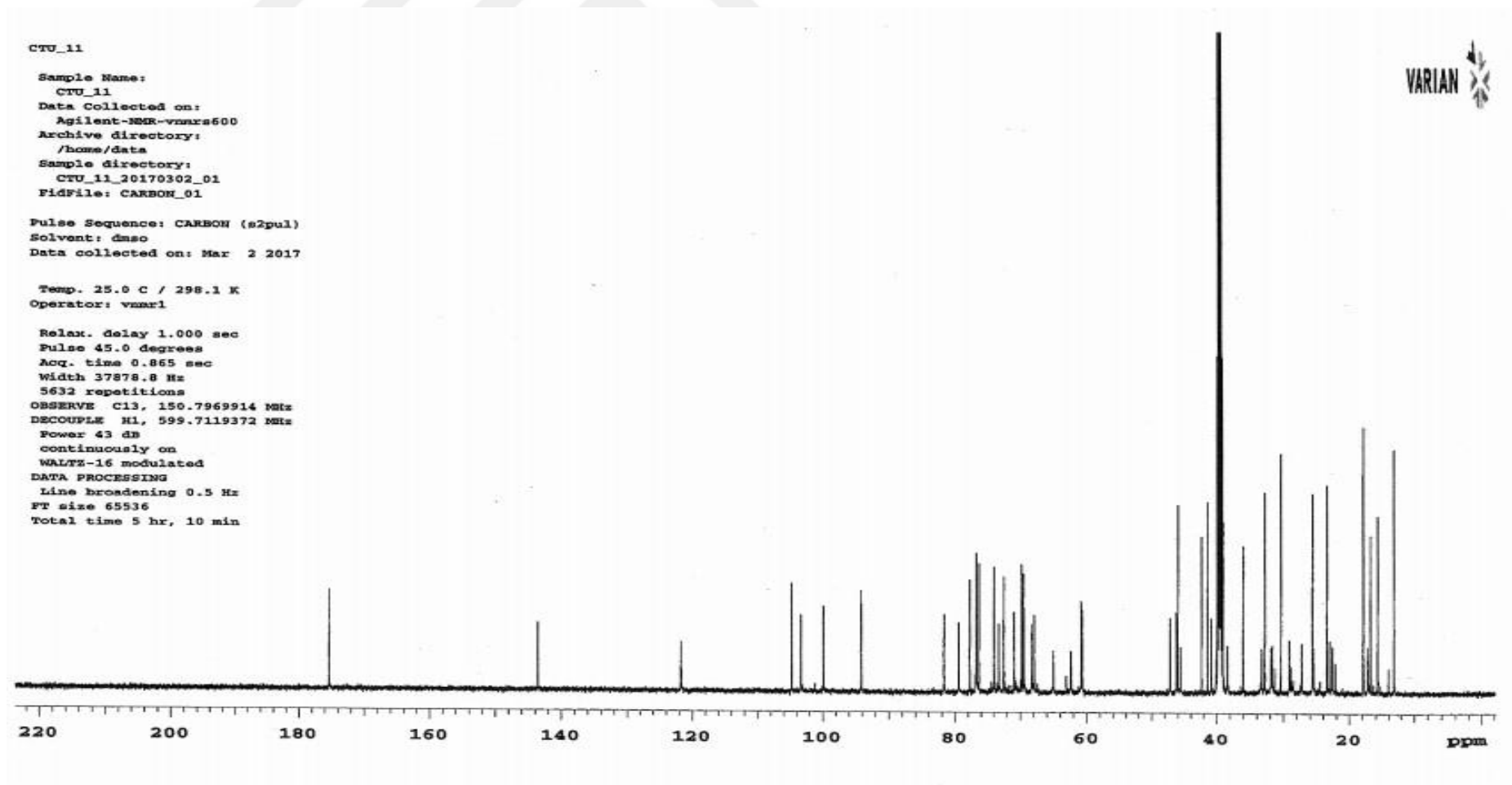


Figure 3.29.  $^{13}\text{C}$  spectrum of Compound 9

CTU\_11

Sample Name:  
CTU\_11

Data Collected on:  
Agilent-NMR-vnmr600

Archive directory:  
/home/data

Sample directory:  
CTU\_11\_20170302\_01

Fidfile: PROTON\_01

Pulse Sequence: PROTON (s2pul)  
Solvent: dmsc  
Data collected on: Mar 2 2017

Temp. 25.0 C / 298.1 K  
Operator: vnmr1

Relax. delay 1.000 sec  
Pulse 45.0 degrees  
Acq. time 1.704 sec  
Width 9615.4 Hz  
512 repetitions  
OBSERVE H1, 599.7089184 MHz  
DATA PROCESSING  
FT size 32768  
Total time 23 min

VARIAN

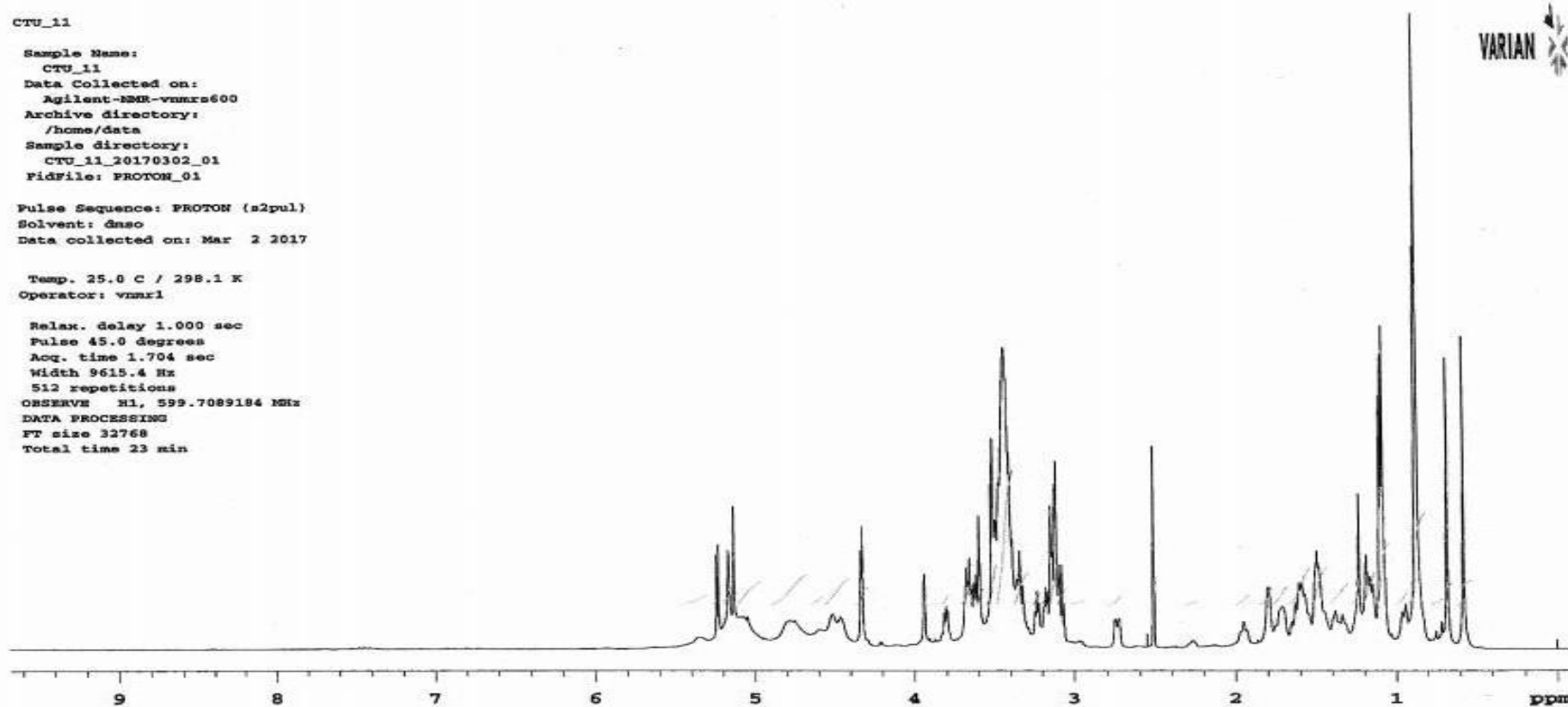
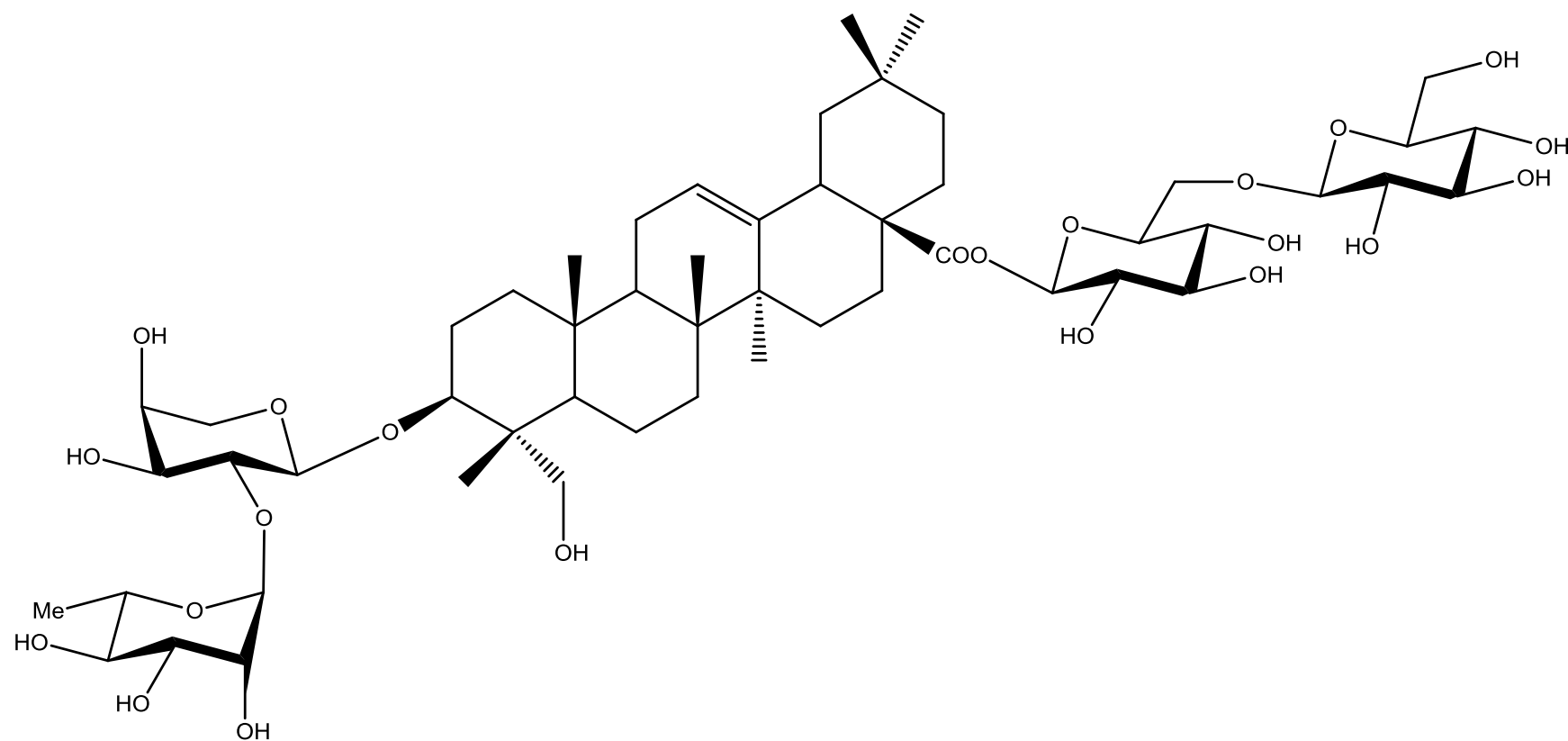


Figure 3.30.  $^1\text{H}$  spectrum of Compound 9

**3.10. Compound 10:** Dipsacoside B (Mukhamedziev et.al., 1971)



**Figure 3.31.**Dipsacoside B

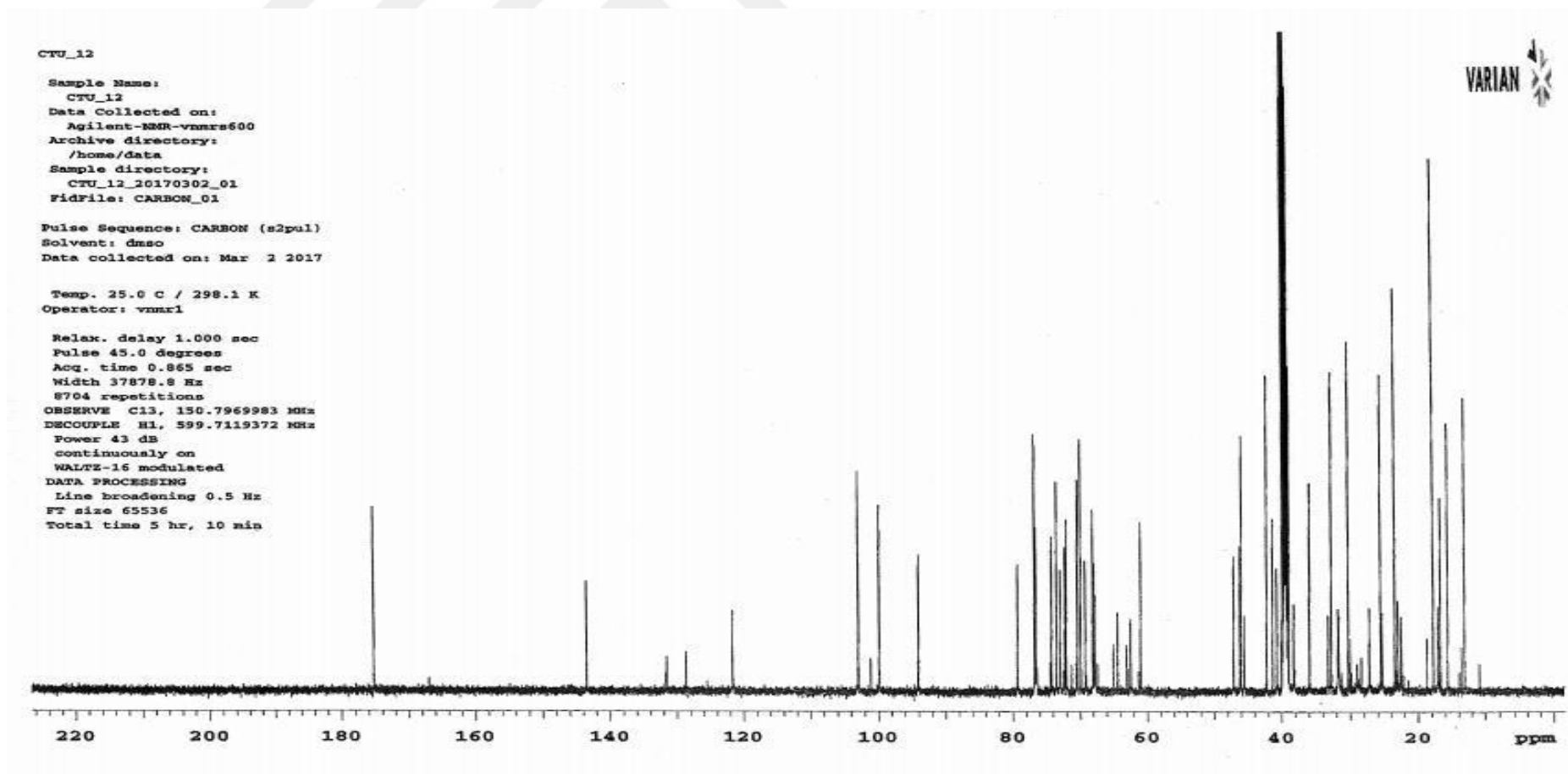


Figure 3.32.  $^{13}\text{C}$  spectrum of Compound 10

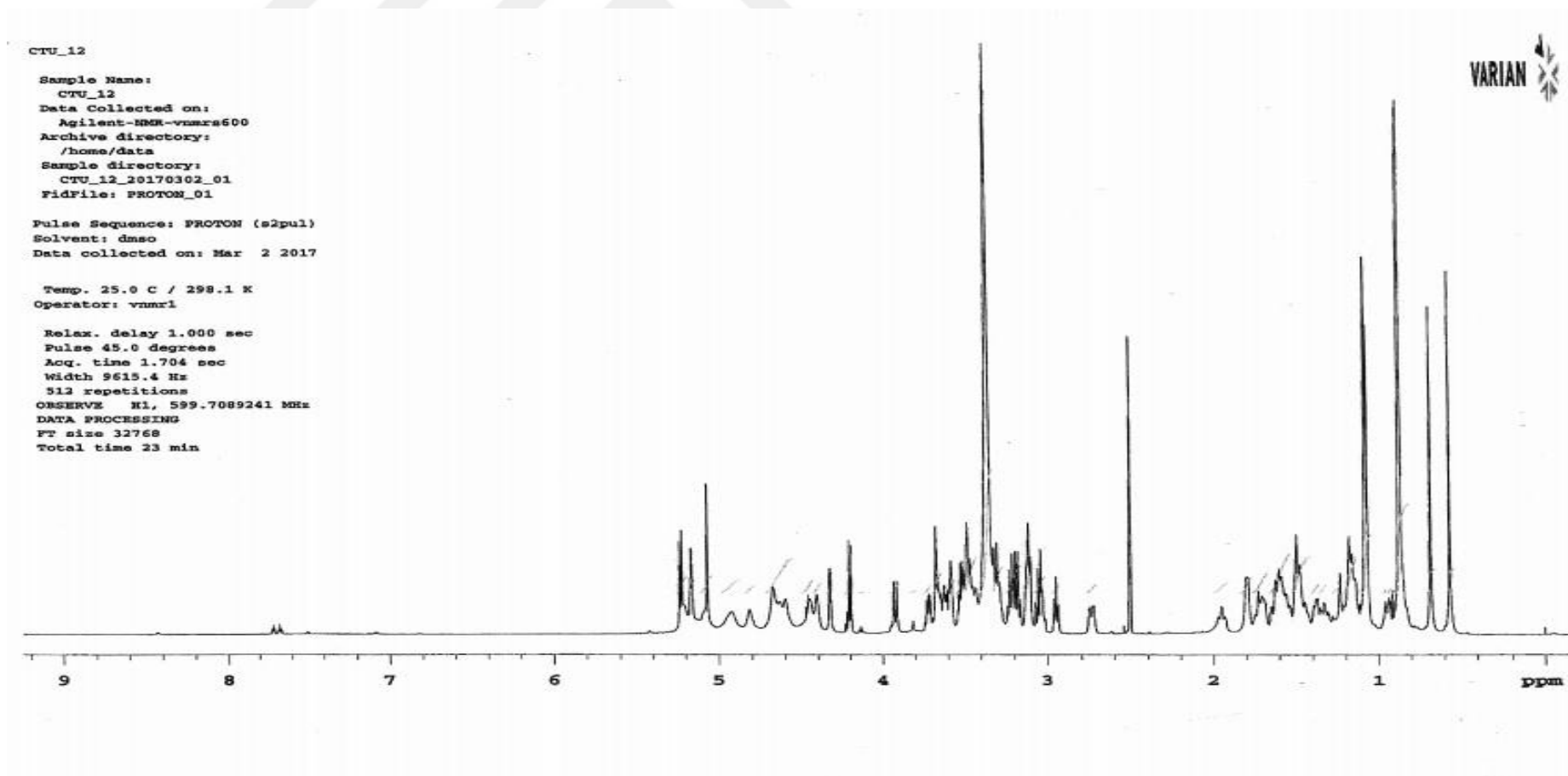
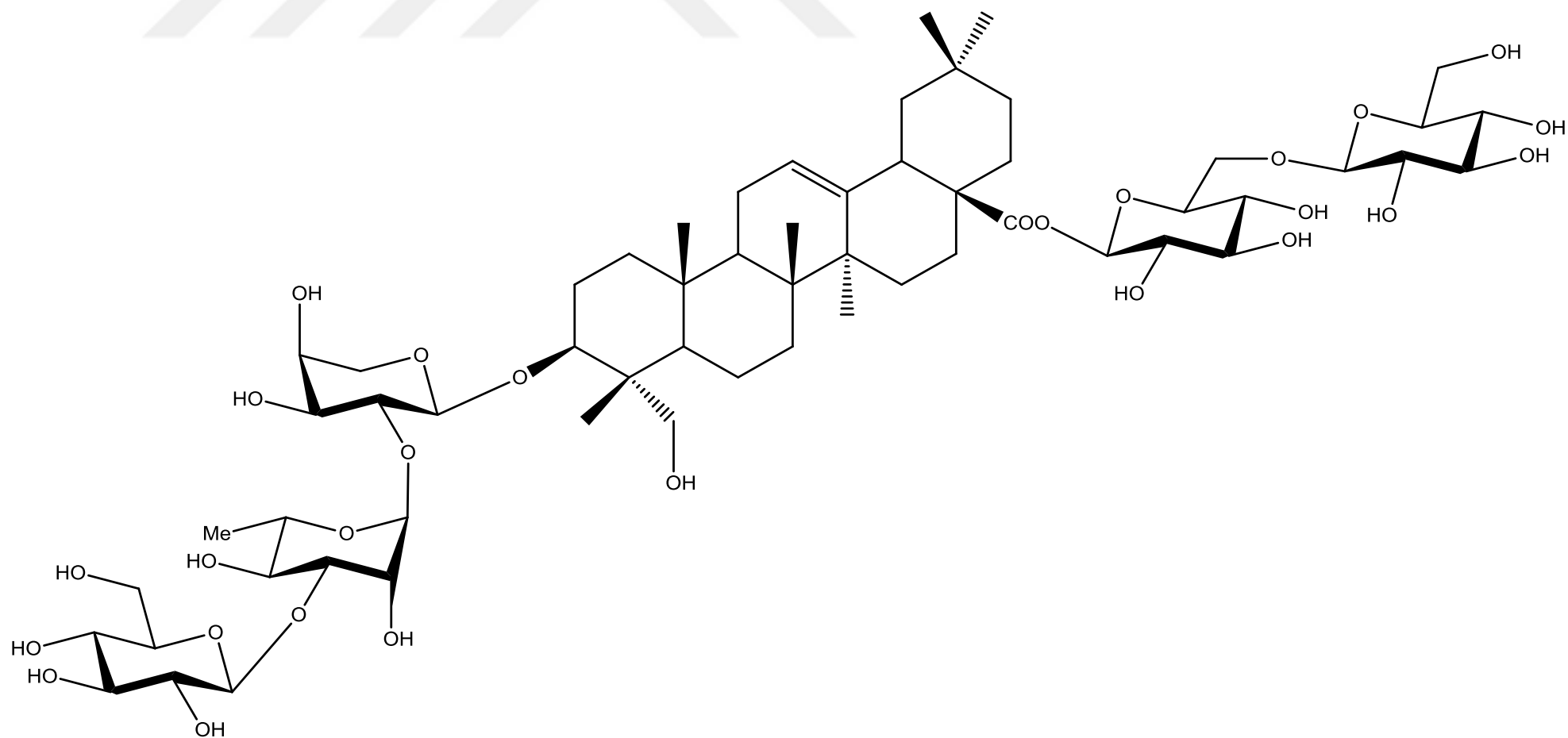


Figure 3.33.  $^1\text{H}$  spectrum of Compound 10

**3.11. Compound 11: Macranthoidin A (Mao et.al., 1993)**



**Figure 3.34.** Macranthoidin A

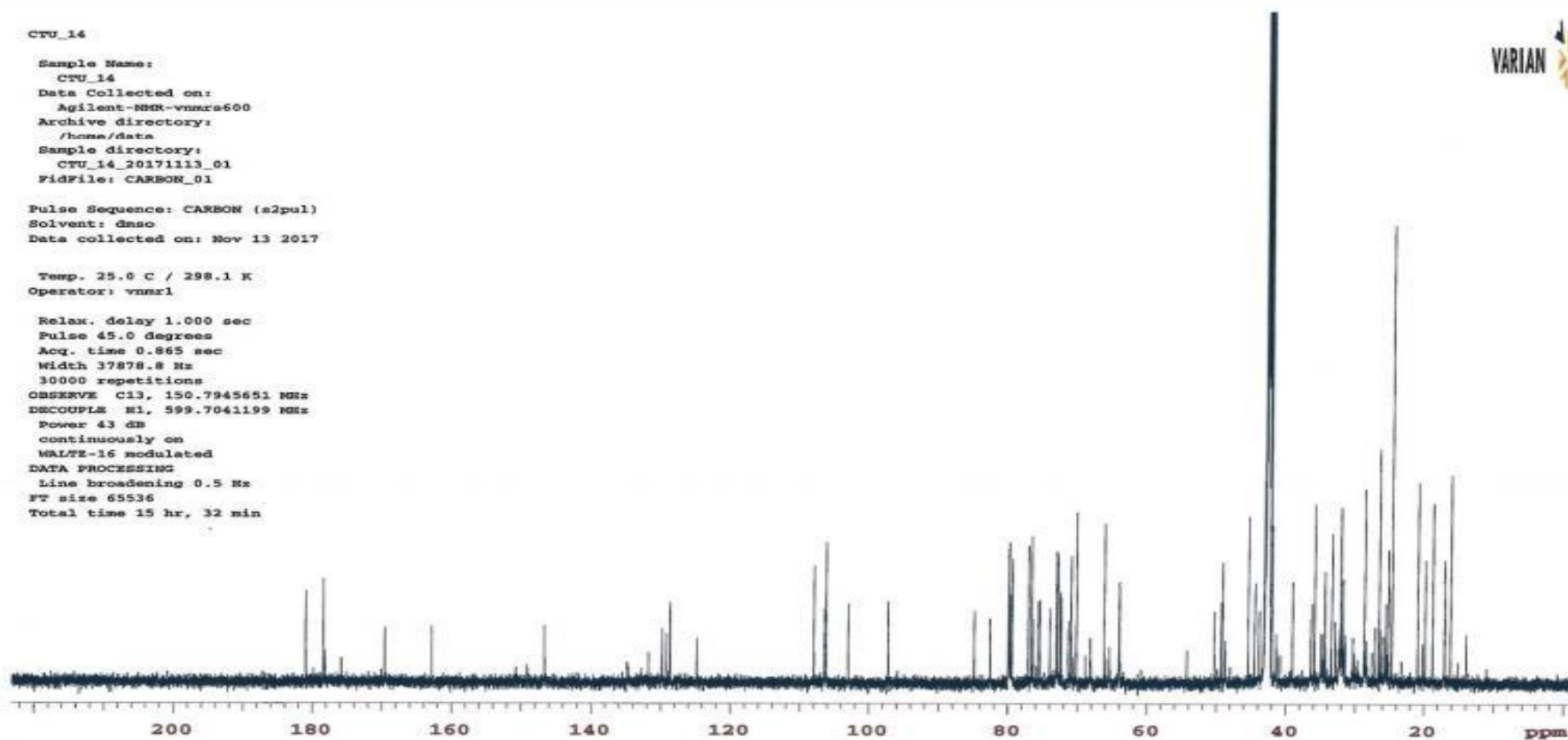


Figure 3.35.  $^{13}\text{C}$  spectrum of Compound 11

CTU\_14

Sample Name:  
CTU\_14  
Data Collected on:  
Agilent-300-vmr600  
Archive directory:  
/home/data  
Sample directory:  
CTU\_14\_20171113\_01  
FidFile: PROTON\_01

Pulse Sequence: PROTON (s2pul)  
Solvent: dmsc  
Data collected on: Nov 13 2017

Temp. 25.0 C / 298.1 K  
Operator: vmr1

Relax. delay 2.000 sec  
Pulse 45.0 degrees  
Acq. time 3.000 sec  
Width 9615.4 Hz  
512 repetitions  
OBSERVE H1, 599.7011214 MHz  
DATA PROCESSING  
Line broadening 0.5 Hz  
FT size 65536  
Total time 42 min

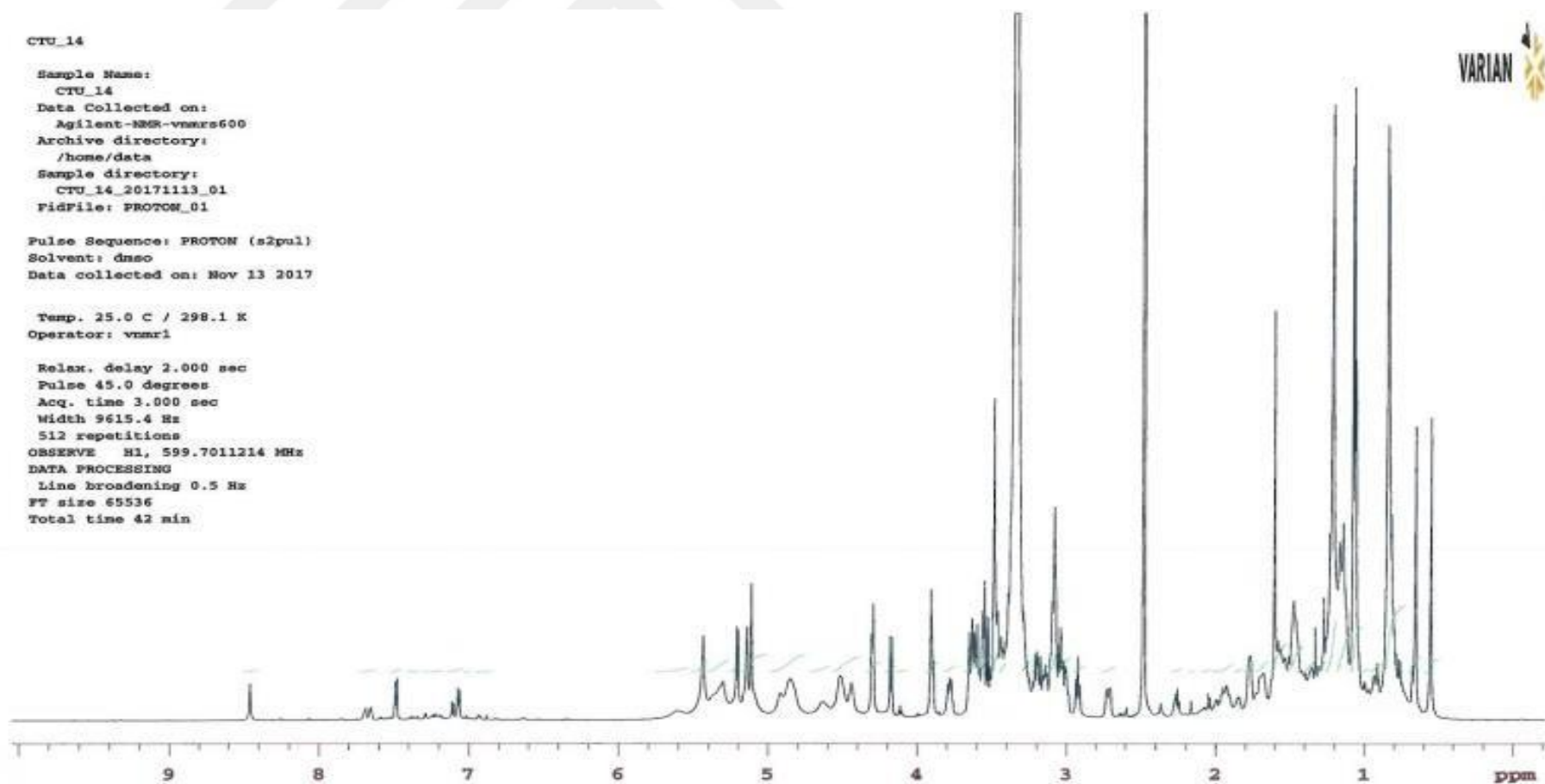
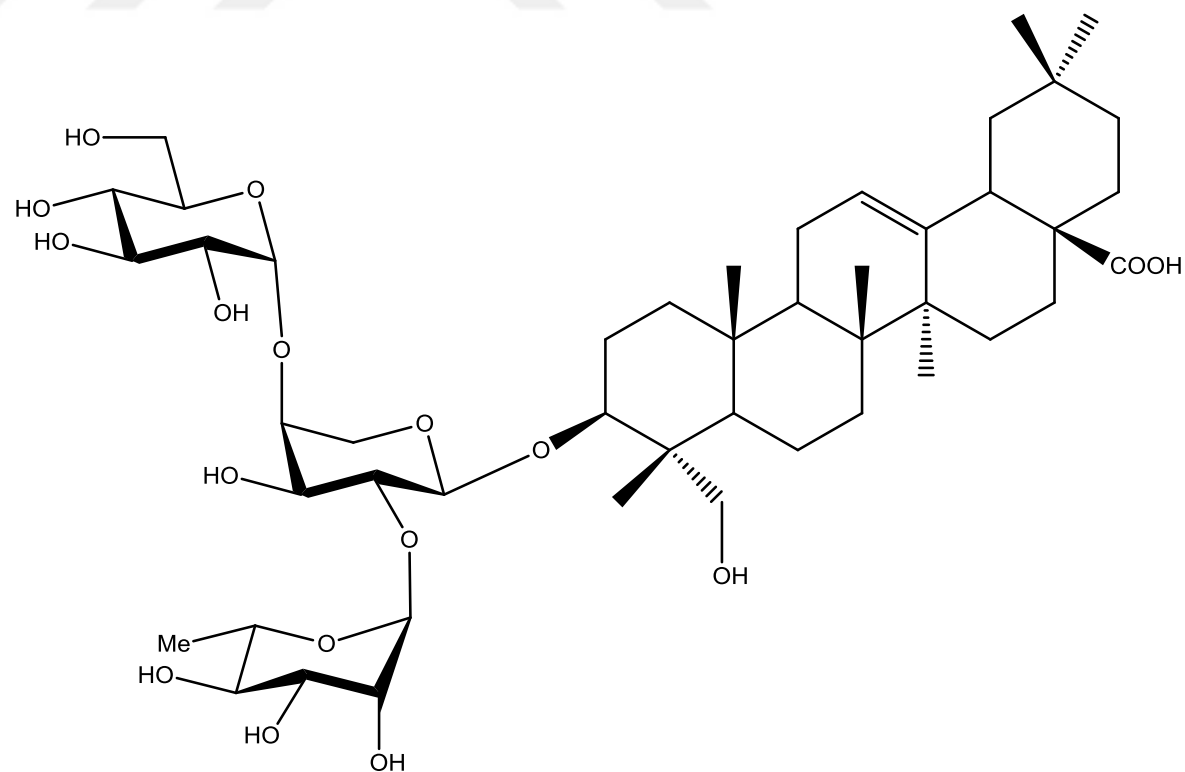


Figure 3.36.  $^1\text{H}$  spectrum of Compound 11

**3.12. Compound 12:** 3-*O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)]- $\alpha$ -L-arabinopyranosyl hederagenin ( Lemos et.al., 1992)



**Figure 3.37.** 3-*O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)]- $\alpha$ -L-arabinopyranosyl hederagenin

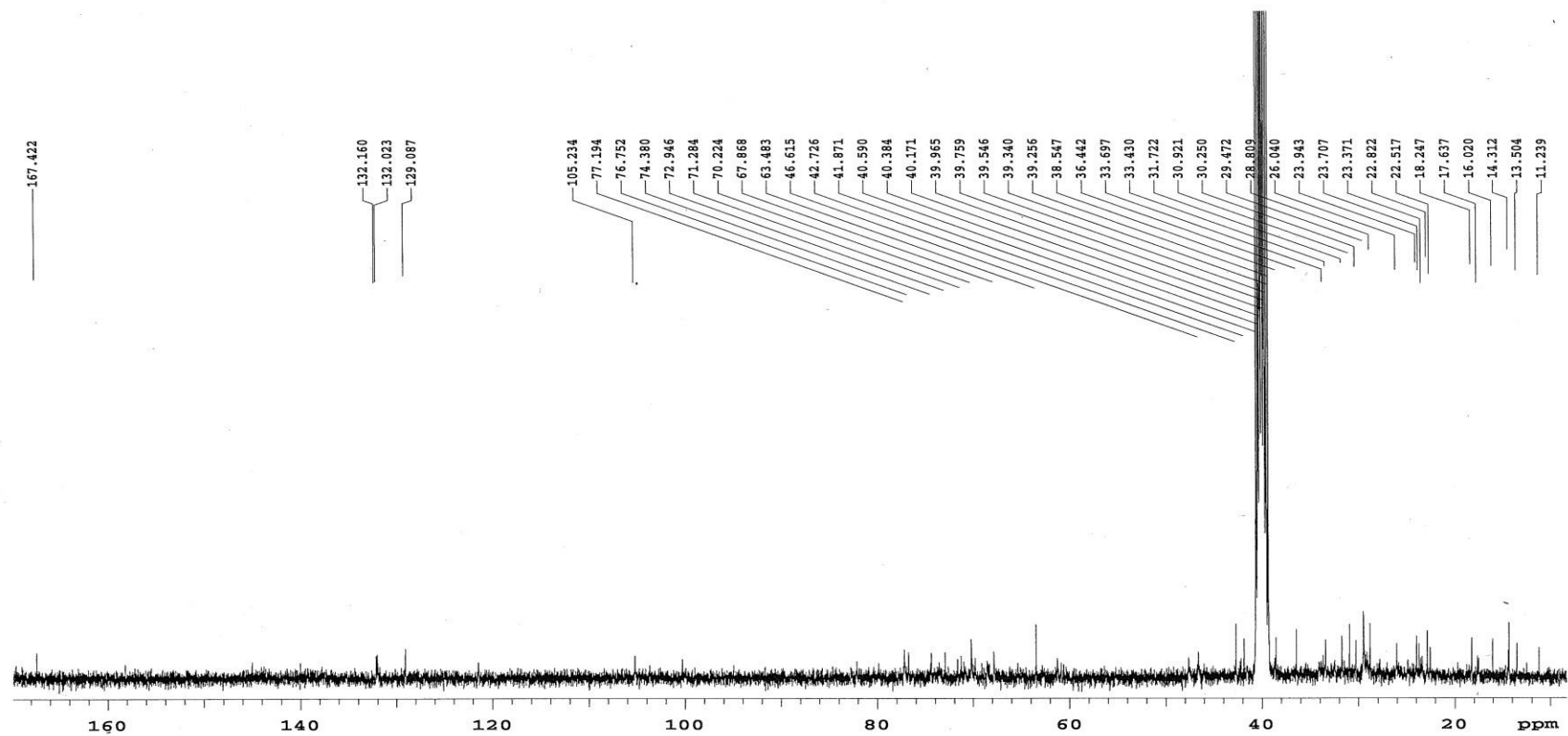
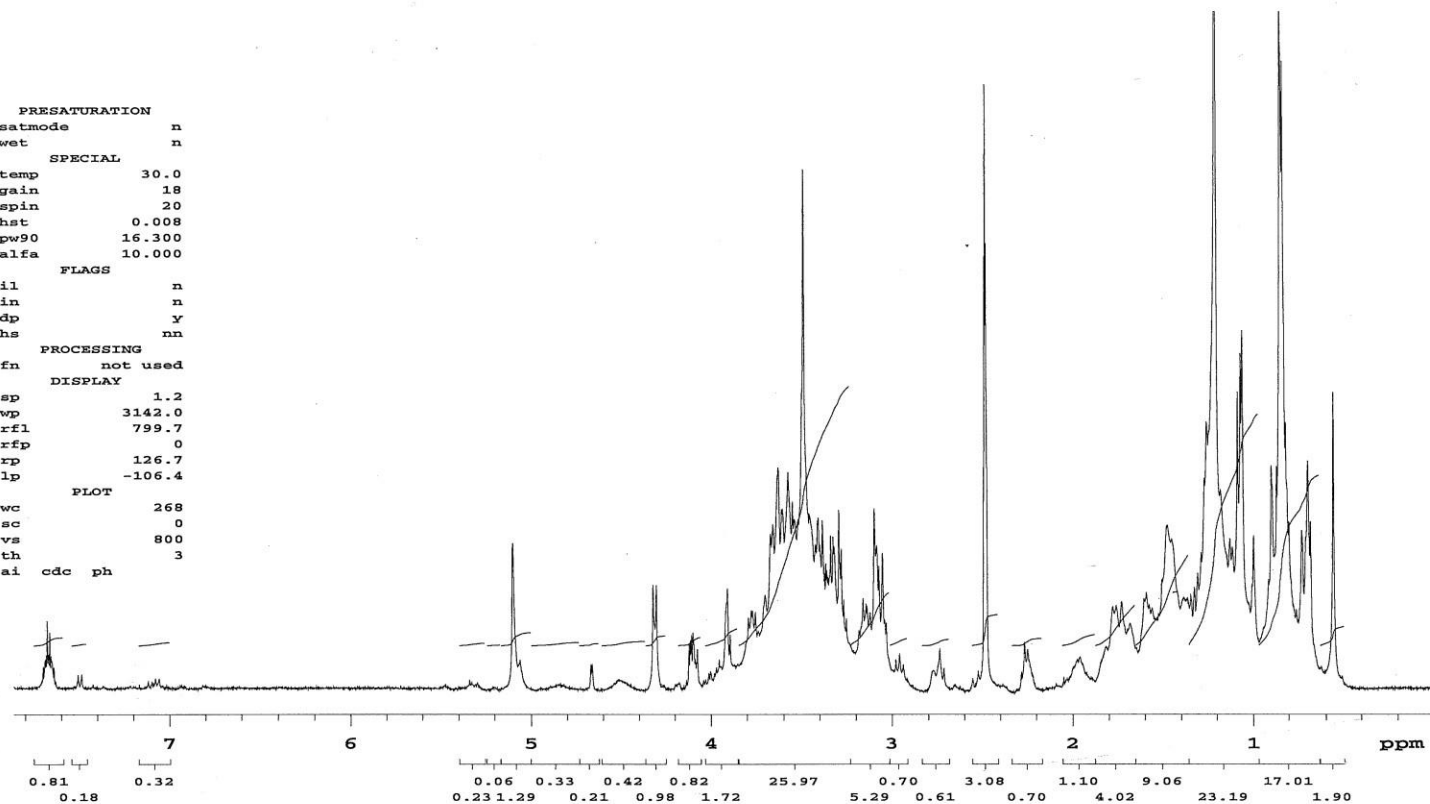


Figure.3.38. <sup>13</sup>C spectrum of Compound 12

CTU-7

exp7 PROTON

SAMPLE		PRESATURATION	
date	Aug 1 2017	satmode	n
solvent	DMSO	wet	n
file	/home/vnmr1/v-	SPECIAL	
nmr	sys/data/CTU-7_-	temp	30.0
20170801_02/CTU-7_-	gain	18	
PROTON_01	spin	20	
ACQUISITION		hst	0.008
sw	6398.0	pw90	16.300
at	2.561	alfa	10.000
np	32768	FLAGS	
fb	3600	il	n
bs	32	in	n
d1	1.000	dp	y
nt	32	hs	nn
ct	32	PROCESSING	
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	399.885	sp	1.2
tof	354.3	wp	3142.0
tpwr	61	rfl	799.7
pw	8.150	rfl	0
DECOUPLER		rp	126.7
dn	C13	lp	-106.4
dof	0	PLOT	
dm	nnn	wc	268
decwave	W40_HCN5mm	sc	0
dpwr	47	vs	800
dmf	16863	th	3
ai	cdc	ph	

Figure 3.39.  $^1\text{H}$  spectrum of Compound 12



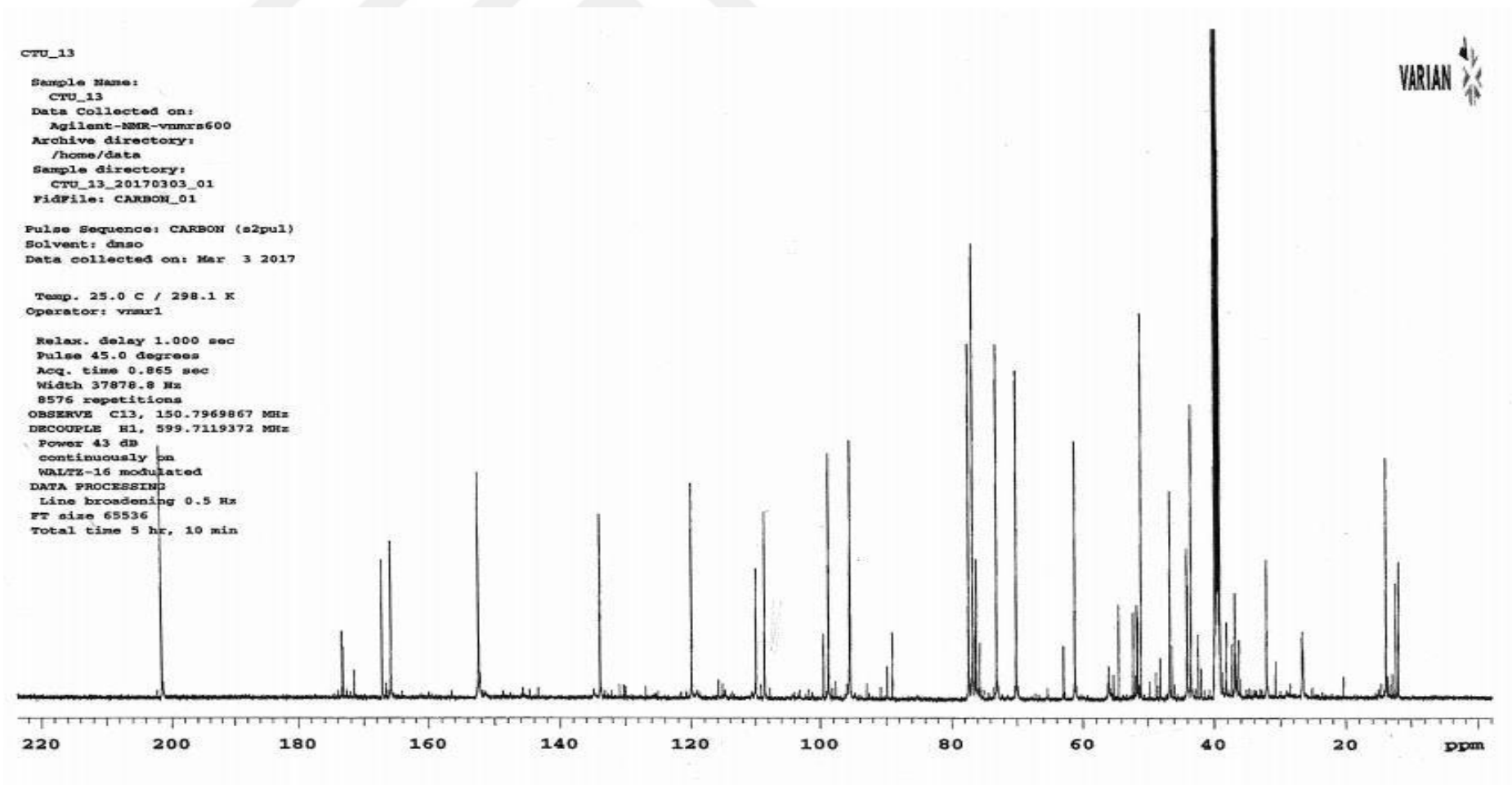


Figure 3.41.  $^{13}\text{C}$  Spectrum of Compound 13

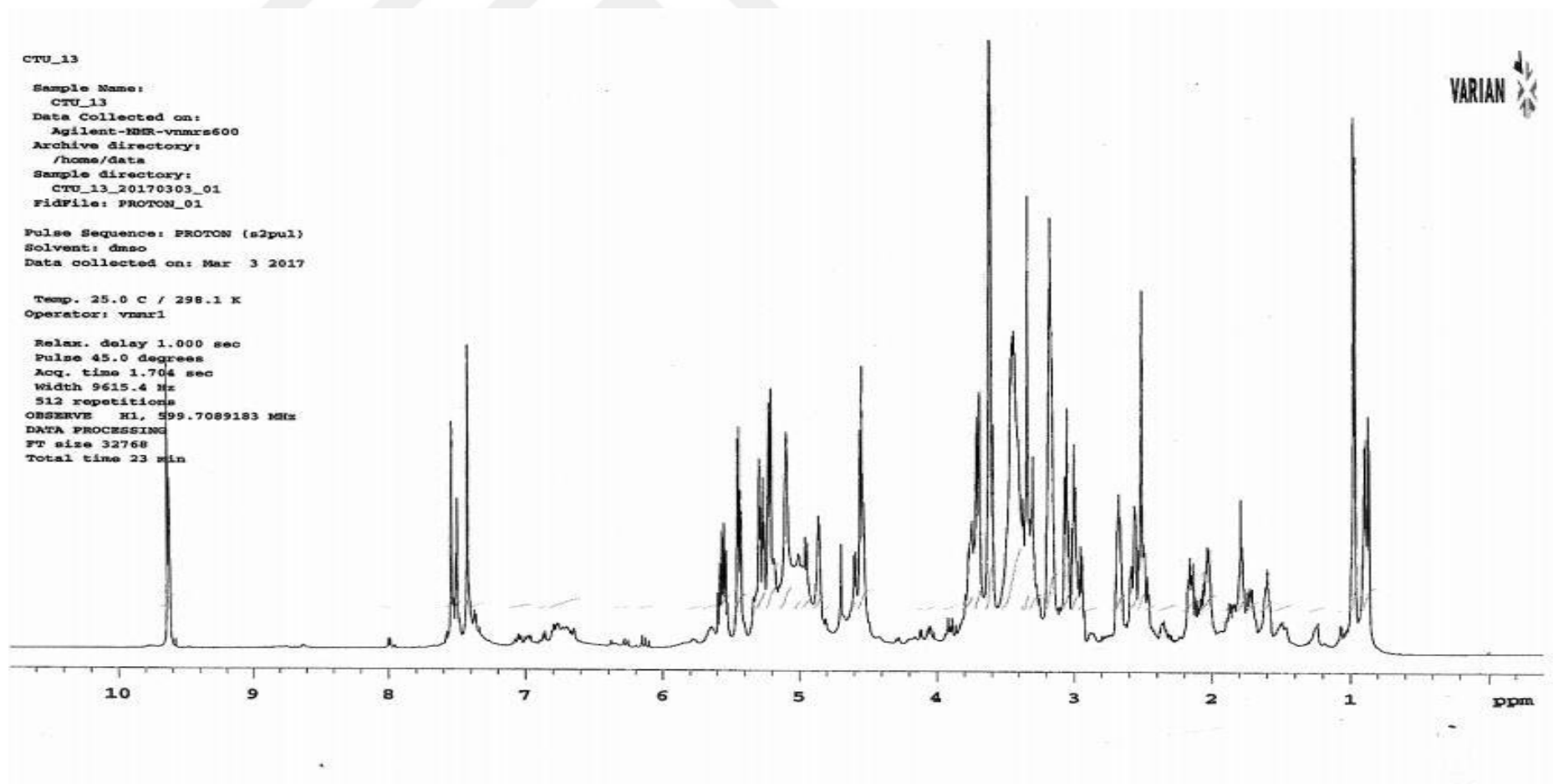


Figure 3.42.  $^1\text{H}$  spectrum of Compound 13

#### 4. CONCLUSION

The main purpose of our study is to examine the glycosidic compounds of *Cephalaria tuteliana* for the first time. According to bioassay-guided isolation and pre-chromatographic studies we focused on our phytochemical studies on *n*-butanol extract which were rich in triterpenoid glycosides.

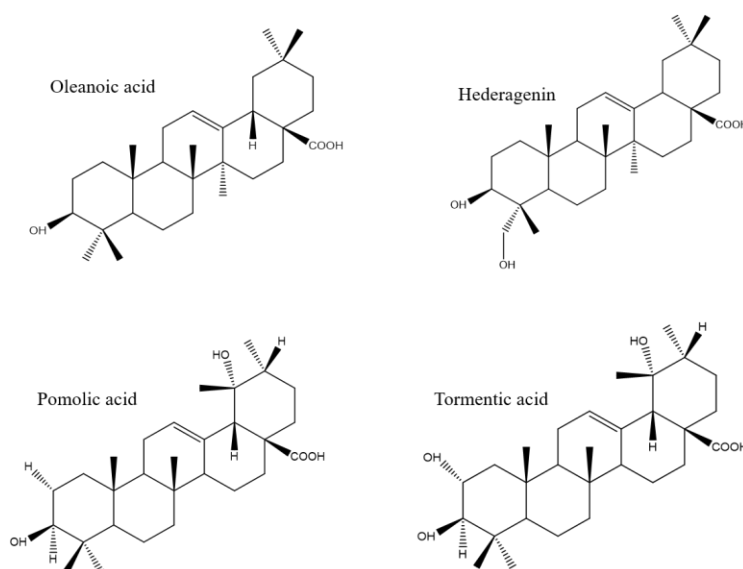
Isolation and purification studies on this extract of *Cephalaria tuteliana* yielded 13 substances 3 of which were new in Caprifoliceae family. Two sapogenins named pomolic acid (**1**) (Figure 3.1), tormentic acid (**2**) (Figure 3.7), ten known triterpene saponins which was named elmalienoside A (**3**) (Figure 3.11), davisianoside A (**4**) (Figure 3.13),  $\alpha$ -hederin (**5**) (Figure 3.18), elmalienoside B (**6**) (Figure 3.21), 3-*O*- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl hederagenin 28-*O*- $\beta$ -D-glucopyranosyl ester (**7**) (Figure 3.22), davisianoside B (**8**) (Figure 3.25), 3-*O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl hederagenin 28-*O*- $\beta$ -D-glucopyranosyl ester (**9**) (Figure 3.28), dipsacoside B (**10**) (Figure 3.31), macranthoidin A (**11**) (Figure 3.34), 3-*O*- $\beta$ -[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 3)- $\beta$ -D-glucopyranosyl] hederagenin (**12**) (Figure 3.37) and one iridoid glycoside namely laciniatoside I (**13**) (Figure 3.40) were obtained. The structures of all compounds were elucidated by a combination of spectral methods including IR, 1D and 2D NMR methods. Among these compounds pomolic acid (**1**), tormentic acid (**2**) and 3-*O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)]- $\alpha$ -L-arabinopyranosyl hederagenin (**12**) were detected in *Cephalaria* species and Caprifoliceaea family as well, for the first time.

Studies have demonstrated that saponins have many positive effects especially on cancer, stimulation of the immune system and blood cholesterol levels. According to these advantages, scientists have investigated many biological activities of saponins as seen in Table 4.1. (Hostettmann and Marston, 1995).

**Table 4.1.** Biological Activities of Saponins

<b>Biological activities of saponins</b>	
Antiulcer	Cytotoxic
Antioxidant	Diuretic
Adjuvant	Effects on ruminal fermentation
Analgesic activity	Effect on absorption of minerals and vitamins
Adaptogenic	Effect on animal growth (growth impairment), reproduction
Antiallergic	Effect on cognitive behavior
Antiedematous	Effect on ethanol induced amnesia
Antiexudative	Effect on morphine/nicotine induced hyperactivity
Antifeedant	Expectorant
Antifungal	Genotoxic
Anti-inflammatory	Haemolytic
Antimicrobial	Hepatoprotective
Antigenotoxic	Hepatocytotoxic
Antihepatotoxic inhibitory effect on ethanol absorption	Hypocholesterolemic
Antiprotozoal	Hypoglycemic
Antimutagenic	Increase permeability of intestinal mucosa cells
Antiparasitic	Immunostimulatory effects
Antiobesity	Inhibit active nutrient transport
Antiviral	Molluscicidal
Antipsoriatic	Nootropic
Antiphlogistic	Neuroprotective
Antipyretic	Reductions in stillbirths in swine
Antispasmodic	Reduction in fat absorption
Antithrombotic (effect on blood coagulability)	Reduction in ruminal ammonia concentrations
Antitussive (relieving or preventing cough)	Ruminant bloat
Chemopreventive	Sedative
Clastogenic	Inhibit active nutrient transport

In this study, in the light of our ongoing studies about cytotoxic and immunomodulatory activities of triterpenic compounds (Sarikahya et al., 2018), we investigated cytotoxic properties of two different aglycones which were isolated from *Cephalaria*, for the first time. For this reason, the cytotoxicity of compounds **1** and **2** and hederagenin, oleanoic acid which are two common aglycones in *Cephalaria* were compared against cancerous cells A549, Hela, PANC1, SHSY5Ycells and noncancerous cell HEK293 by MTT method. As it is seen in Table 3.1 while pomolic acid and tormentic acid did not show any cytotoxicity, hederagenin and oleanoic acid found as slightly active. Hederagenin showed cytotoxic activity on cancerous A-549, Hela, PANC1, SHSY5Ycells and non-cancerous HEK-293 cell with IC<sub>50</sub> values of 44.48, 30.78, 31.40, 23.05 and 37.05  $\mu$ M, respectively. Oleanoic acid displayed cytotoxicity on cancerous A-549, Hela, PANC1, and SHSY5Ycells with IC<sub>50</sub> values of 31.61, 68.88, 44.54 and 18.94, respectively. Beside that it did not any cytotoxicity against non-cancerous HEK-293 cell. When it comes to structure activity relationship, it can be concluded that extra hydroxyl group (-OH) and different locations of methyl (-CH<sub>3</sub>) groups on aglycone may not result more cytotoxicity. It can be seen clearly from the structures of four different aglycones in Figure 3.43.



**Figure 3.43.** Oleanoic acid, Hederagenin, Pomolic acid and Tormentic acid

The use of plants and their extracts as a folk medicine has a long history. The plant-derived compounds have also a long history of clinical usage. To date, 35.000-70.000 plant species have been investigated for their possible medicinal use. Current drug innovation from plants still depends on on bioactivity-guided fractionation and isolation of many important biologically active pure compounds. As it was stated before in Introduction section, one of these the pure compounds triterpene saponins play an important role in modern drug development in terms of biological activities. As a conclusion, the results of this investigation clearly show that *Cephalaria tuteliana* contain a large number of saponins. Thus, the extract of *C. tuteliana* which contains these saponins can be a folk medicine and additive materials for different industrial areas.

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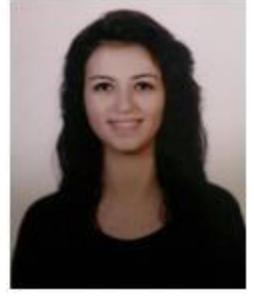
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