EGE UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

(MASTER OF SCIENCE THESIS)

SEMI SYNTHESIS OF GYPSOGENIN-CHALCONE HYBRID COMPOUNDS AND THEIR ANTIMICROBIAL ACTIVITY

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Presantation Date:13.07.2018

Bornova-İZMİR

2018

Nafia Gökçe ULUSOY tarafından Yüksek Lisans tezi olarak sunulan "*Semi Synthesis of Gypsogenin-Chalcone Hybrid Compounds and Investigation of Their Biological Activity*" başlıklı bu çalışma E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E.Ü. Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi'nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve 13.07.2018 tarihinde yapılan tez savunma sınavında aday oybirliği/oyçokluğu ile başarılı bulunmuştur.

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EGE ÜNİVERSİTESİ FEN BİLİMLERİ ENSTİTÜSÜ

ETİK KURALLARA UYGUNLUK BEYANI

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13/07/2018

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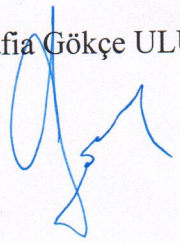

ÖZET

GYPSOGENİN-KALKON HİBRİT BİLEŞİKLERİNİN YARI SENTEZİ VE ANTİMİKROBİYAL AKTİVİTELERİNİN İNCELENMESİ

Nafia Gökçe ULUSOY

Yüksek Lisans Tezi, Kimya Anabilim Dalı

Danışman: Prof. Dr. Özgen ALANKUŞ

Temmuz 2018, 68 sayfa

Caryophyllaceae ailesinde bulunan ve halk arasında çöven olarak bilinen *Gypsophila arrostii* bitkisinin kökü başlıca saponin, şeker ve reçine içermektedir. *Gypsophila arrostii* bitkisinin köklerinden izole edilen aglikon olan Gypsogenin pentasiklik yapıda triterpenik doğal bir bileşiktir. Gypsogenin yapısı içeren doğal bileşiklerin antiviral, antitümör, antikarkinojenik, antioksidan ve antikanser gibi biyolojik aktiviteleri bilinmektedir. Literatürde pek çok bitkiden gypsogenin aglikon eldesi bulunmakla birlikte, en fazla elde etme yöntemi çöven suyundan yapılmaktadır. Bu tez çalışmasında, Gypsogenin aglikonunun 3 nolu karbonuna bağlı hidroksil grubunun asetik anhidrit ve tetrahidrofuran ile etkileşmesi sonucu asetilleme ürünü elde edilmiştir. Ayrıca birbirinin yapı izomeri olan dokuz farklı kalkon türevi de (**C1-9**) sentezlenmiş ve gypsogenin 28 nolu karbonuna esterleşme tepkimeleri ile bağlanarak Asetil-Gypso ve kalkon hibrit bileşikleri sentezlenmiştir. Saf olarak elde edilen (**1-9**) yeni 9 adet gypsogenin-COO-kalkon hibridi bileşiklerin yapıları çeşitli spektroskopik analiz (FT-IR, UV, MR, LC-MS) yöntemleriyle belirlenip antimikrobiyal aktivite çalışmaları yapılmıştır.

Anahtar Kelimeler: Gypsogenin, kalkon, semi-sentez, antimikrobiyal aktivite.

ABSTRACT

SEMI SYNTHESIS OF GYPSOGENIN-CHALCONE HYBRID COMPOUNDS AND THEIR ANTIMICROBIAL ACTIVITY

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M. Sc. Thesis, Chemistry Department

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July 2018, 68 pages

Gypsophila arrostii which is colloquially referred to as "çöven", is a member of Caryophyllaceae family and its roots contain mainly saponin, sugar and resin. Gypsogenin is a triterpenic natural compound in the pentacyclic structure and isolated from *Gypsophila arrostii* roots. Biological activities of natural compounds containing gypsogenin structure are known such as antiviral, antitumor, anticarkinogenic, and antioxidant and anticancer. In this thesis, acetylated derivative of gypsogenin was synthesized by the reaction of acetic anhydride and tetrahydrofuran with hydroxyl group on the C-3 position of gypsogenin. Besides, nine isomeric chalcone derivatives (**C1-9**) were synthesized and esterification reaction took place between chalcone derivatives and hydroxyl group on the C-28 position of Acetyl-Gypso in order to obtained nine new gypsogenin-COO-chalcone hybride compounds (**1-9**). All synthesized compounds (**1-9**) were in pure form and identified by various spectroscopic methods (FT-IR, UV, NMR, LC-MS) and their antimicrobial activity were studied.

Keywords: *Gypsogenin, chalcone, semi-synthesis, antimicrobial activity.*

ACKNOWLEDGEMENT

This study was carried out at the Department of Chemistry, Ege University, Science Faculty, İzmir from 2015 to 2018.

I am deeply grateful to Assoc. Prof. Dr. Safiye ÖZTÜRK for their precious suggestion, support, and patience, and for enlightening me via their deep knowledge and experience.

I am also very grateful to Prof. Dr. Özgen ALANKUŞ ÇALIŞKAN and Assoc. Prof. Dr. Tamer KARAYILDIRIM for his valuable advice and support. Our discussions on the completion of the thesis have been of great value.

I am grateful to Prof. Dr. Nurettin YAYLI, Assoc. Prof. Dr. Nuran KAHRİMAN and my workfellow Vildan SERDAROĞLU for their support and help during my study.

I want to thank all the staff and my lovely friends of the Department of Chemistry, Science Faculty, Ege University, for their helps.

This project was granted by TUBITAK (114R077). I would like to thank them for their financial support. I would also like to thank TUBITAK for MSc. fellowship.

I am deeply thankful to my parents for their love, patience and supports in my life.

Nafia Gökçe ULUSOY

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ABBREVIATIONS

UV	Ultraviolet
IR	Infra-red
MS	Mass Spectroscopy
NMR	Nuclear Magnetic Resonance
HMQC	^{1}H-detected Heteronuclear One-bond Spectroscopy
COSY	Correlation (^{1}H-^{1}H) spectroscopy
TLC	Thin Layer Chromatography
HPLC	High Performance Liquid Chromatography
ATCC	American Type Culture Collection
RSKK	Refik Saydam Culture Collection
DMSO	Dimethylsulfoxide
TMS	Tetramethylsilan
DCC	N,N′-Dicyclohexylcarbodiimide
DMAP	4-(Dimethylamino)pyridine

1. Introduction

1.1. Natural Products (Seconder Metabolites)

It has been understood that most chemicals, especially by the development of the industry, pose risks to the ecological balance, human and animal health and plants, and the traditional medical trend that has been going on for centuries has increased. Many plants are accumulated important organic chemicals in their various forms of scientific, technological and commercial application. Natural plant products are used directly or indirectly in a large number of industries, especially oils, saponins, natural plastics, dyes, and pharmaceuticals.

Plant chemicals are divided into primary and secondary metabolites. Primer metabolites (carbohydrates, lipids, proteins, etc.) are prevalent in nature and are quite abundant in the tissues of plants and are essential for the physiological development of the plant due to its essential functions in cell metabolism. It has been observed that plants synthesize some chemicals called secondary metabolites (Francis et al., 2002), which protect themselves against the attacks of insects and harmful organisms underground and survive, except for the plants, and there is increased interest in the high amount of saponins that are active.

1.1.1. Saponins

When the chemical structure of saponins is examined, it is composed of two structures called glycone and aglycone, and it is a glycosides with high molecular weight which is usually triterpenic or steroidal aglycone (Hosstettman and Marston, 1995).

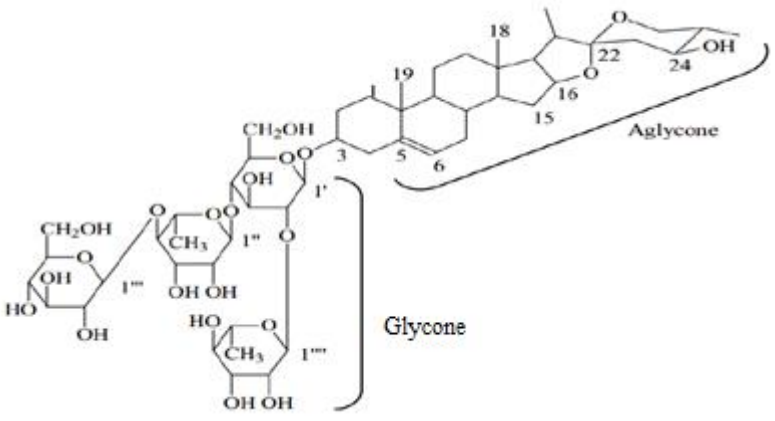

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Figure 1.1. General structure of saponins*

*:

Saponins are spread out widely in nature (Yücekutlu and Bildaci, 2008) and are generally known as surface-active substances. The classical definitions of saponins are derived from the soap which means "sapo" in Latin, depending on the foaming nature when it is agitated in aqueous solution. It has been used as soap for years (Vincken, 2007) such as soapwort (*Saponaria officinalis*) and soapbark tree (*Quillaia saponaria*) were used as detergents. (Hostettmann and Marston, 1995).

Saponins are found in nature and culturally grown plants (Yücekutlu and Bildaci, 2008). They are found in a wide variety of plant species and are distributed throughout the crust, leaves, stalks, roots and even flowers. Saponin containing plants have been suggested to have many biological effects, mainly hypocholesterolemic, anticarcinogenic, antioxidant, anti-inflammatory, antimicrobial, antiprotozoal, antiviral, antioxidant, antitumor and antihypertensive effects. Furthermore, saponins against cancer cells have been evaluated for anticancer activity. Thus, plants are a tremendous resource for the discovery of new medicinal products for drug development.

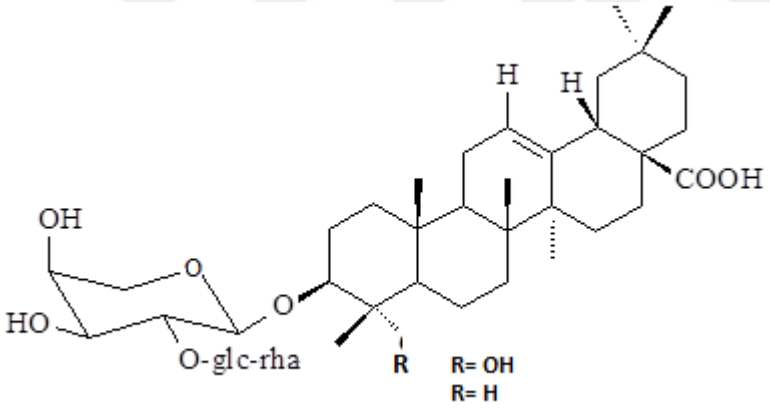
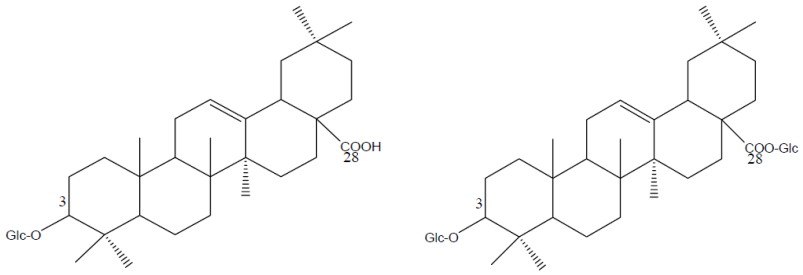


Figure 1.2. Sample of saponins compounds

For example, the saponin compound (**Figure 1.2)** is isolated from *Pulsatilla Koreana* plant roots, which is present in the literature as having antitumor activity (Bang, 2005).

In addition to all these, extracts of saponin-containing plants or isolated saponins are used as cosmetics, detergents and foaming agents in soft drinks (Güçlü-Üstündağ and Mazza, 2007).

Saponins generally have several glycoside groups covalently attached to the C3 position, but some saponins may contain two sugar chains attached to position C3 and C17 (via C28). Sugars are called monodesmosidic and bidesmosidic saponins, respectively, when they are added to agar, also called sapogenin in one or two different positions.



Monodesmosidic saponin Bidesmosidic saponin

Figure 1.3. Monodesmosidic and Bidesmosidic saponins

Depending on the modification of the ring structure of the aglycone and the number of sugars added, it may be possible to obtain a number of different saponin variants by producing different biological properties. Saponin compounds are shown in three main groups.

1) Triterpene saponins

2) Steroid saponins

3) Steroid alkaloid saponins

1.1.1.1 Triterpene Saponins

Triterpenes are terpenes of 30 carbon atoms. From an isoprene unit with five carbons, the cytosolic mevalonate pathway is combined to form a thirty-carbon compound. Cholesterol, phytosterols, and phytoecdysteroids are triterpenes. The triterpenes are divided into 20 groups depending on their specific structure. Some triterpenoid compounds are found as glycosides of saponin which express the binding of various sugar molecules to the triterpene unit. These sugars can be broken down by bacteria in the intestine, sometimes allowing the aglycone (triterpenes) to be absorbed into the bloodstream or attached to cell membranes.

Several examples of triterpenes saponins in mono-, bi- or even tridermicidic structures are given in **Figure 1.4**.

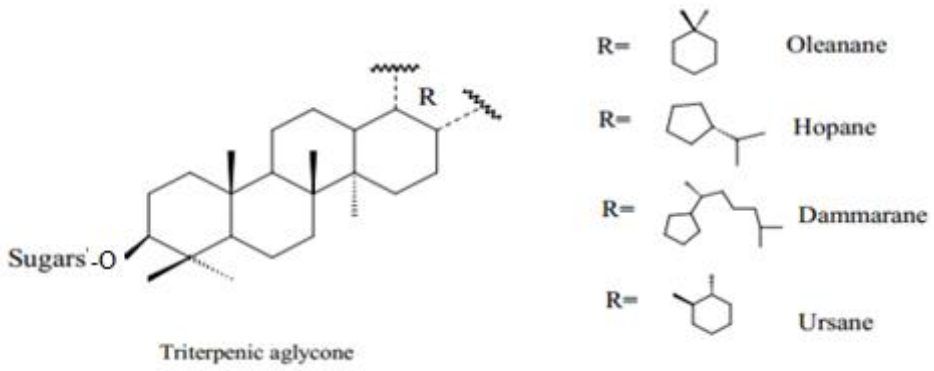


Figure 1.4. Triterpenic aglycone and its derivatives

1.1.1.2 Steroid Saponins

Steroidal saponins are found less in nature than saponins in the triterpenic structure. They are divided into four groups in terms of structure and properties.

The most common spirostanol type saponins in the soil, followed by furostanols, nuatigenins and polypodosaponins, respectively.

Steroidal saponins, sex hormones, cortisone, diuretic steroids, vitamin D and cardiac heritable are similar of the structures. Some are used as starting materials in the semi-synthesis of these compounds. The sarsapogenin obtained from the *Yucca schidigera* plant can be used in the synthesis of corticosteroids. Sarsapogenin and smilagenin form a large part of the *Y. schidigera* saponins. As a result of the screening, it is understood that Dioscorea, Agave and Yucca species are sources of the rich steroid saponin.

The steroidal core structure consists of seventeen carbon atoms attached in four "fused" rings. Steroids depend on the oxidation groups of the functional groups and rings associated with these quadrivalent nuclei. Sterols are steroid forms that contain a hydroxy group and cholestane-derived skeleton at three positions.

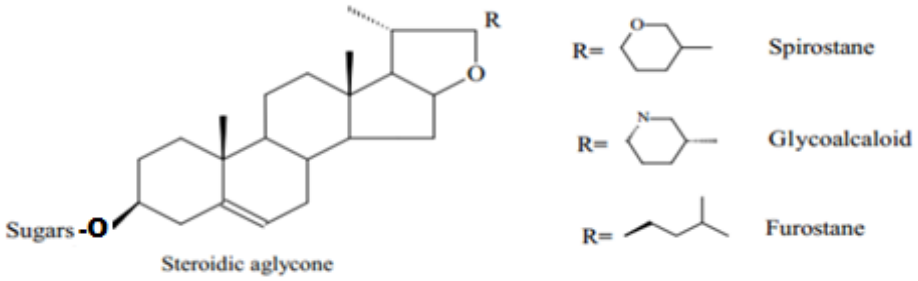


Figure 1.5. Stereoidic aglycone and its derivatives

1.1.1.3 Steroidal Alkaloid Glycosides

Steroidal alkaloids have organic ring backbones with nitrogen-based functional groups. More specifically, they are distinguished by the tetracyclic cyclopentanophenanthrene backbone, which is closely related to sterols.

Steroidal alkaloids can be broadly divided into two main groups;

(a) Solanum Alkaloids

(b) Veratrum Alkaloids

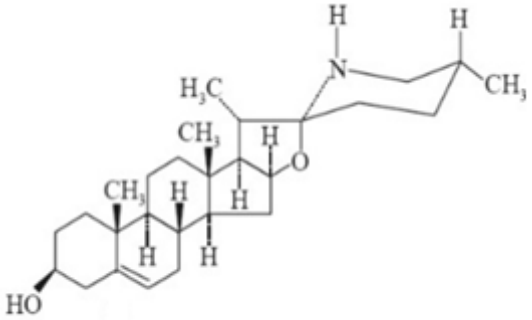
a) Solanum Alkaloids

Solanum alkaloids are essentially nitrogen analogs of steroidal saponins.

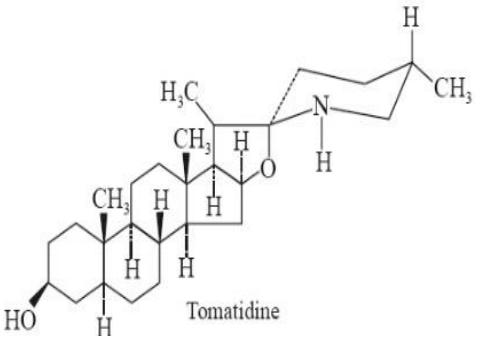
Unlike the oxygen counterparts, all of these N-containing alkaloids show the same stereochemistry at C-25 (always equivocal) but include C-22 isomers, such as solasodine and tomatidine.

These alkaloids generally occur in a wide variety of Solanum species, for example *Solanum laciniatum*; occur naturally in the plant.

However, two types of Solanum, *Solanum laciniatum* and *Solanum aviculare*, are a rich source of alkaloids (aglycone fragments) used only as starting materials for the synthesis of various hormones and adrenocortical steroids.



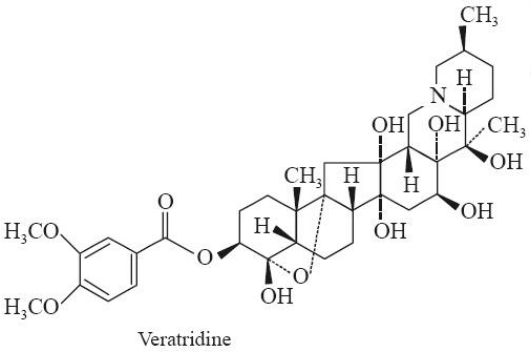
Solasodine



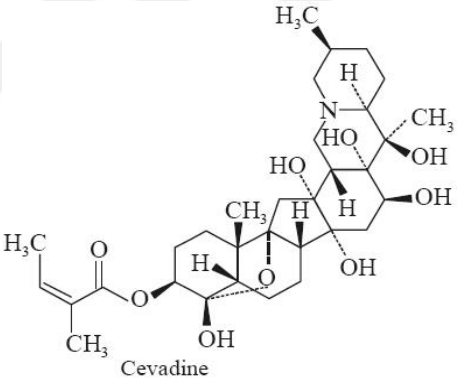
Tomatidine

b) Veratrum Alkaloids

Veratrum alkaloids are the most important class of steroidal alkaloids medically. However, it should be noted that the basic ring systems found in the veratrum alkaloids are not identical to those seen in the usual steroidal nucleus, as they are present in aglycone residues of cholesterol or cardiac glycosides.



Veratridine



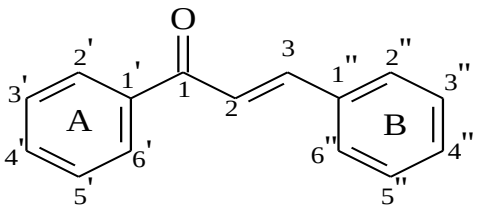
Cevadine

1.1.2. Chalcones

Most of the bioactive compounds found in nature and produced by plants form flavonoid type compounds. Flavonoids are responsible for the colors of the flowers, with phenolic compounds with a low molecular weight, and all the plants are in abundant quantities. This class of compounds is also an important influence on human health and is attributed to secondary metabolites of plants (Jaakola, 2003).

The broadest and the most basic members of the flavonoid-type compounds, which can be isolated from plants, have been shown to have a wide range of biological activity (Rajput, 2015; Anto, 1995).

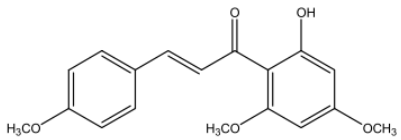
Chalcone derivative compounds are members of the flavonoids that do not have the heterocyclic C ring.



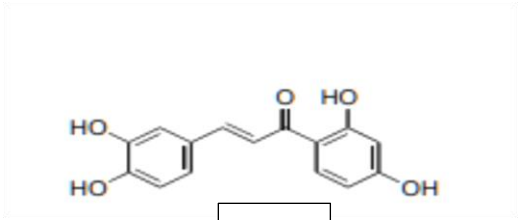
(2*E*)-1,3-difenilprop-2-en-1-on

Figure 1.6. General formula of chalcones

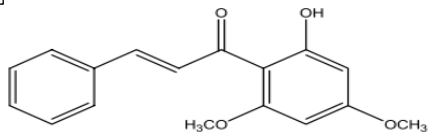
Some of the natural chalcone compounds with biological activities are shown below (**Figure 1.7.).** The methoxy and hydroxy substitute known as flavokavain has been reported in the literature in which flavokavain A has activity against bladder cancer and Flavokavain B has activity against prostate cancer (Kong, 2010; Zi, 2005; Tang, 2008). Bupatin, isolated from *Creopsis L*. plant, has been shown to be used as a therapeutic agent for breast cancer cells (Ying, 2012; Tang, 2010; Suo et al., 2014). *Caesalpinia sapan* ext. Sappankalkon isolated from the plant was found to have anti-inflammatory, antihyperlipidemic, sedative and anti-allergic effects (Yadav et al., 2011).



Flavokavain A



Butein



Flavokavain B

Figure 1.7. Natural chalcone compounds

One of the best known methods of chalcone synthesis is Claisen-Schmidt Condensation. (Rajput, 2015).

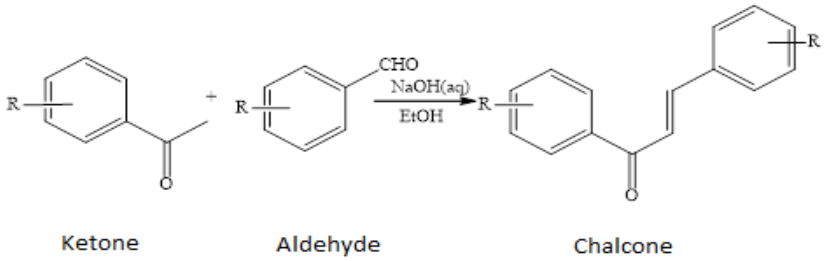


Figure 1.8. General synthesis scheme of chalcones

In addition, many studies in recent years have shown that these compounds show antiviral, antihepatotoxic, antioxidative, antisteoporotic, antiinflammatory, antimicrobial, antiulcerogenic, antiallergic, antispasmodic, hepatoprotective and hypolipemic properties (Gryglewski et al., 1987, Hertog et al., 1993, Di Carlo et al., 1999; Cuyckens and Claeys, 2004).

1.2. General Knowledge on Gypsogenin

Caryophyllaceae is a large flowering plant family that grows naturally in the Gypsophila, especially in Central Anatolia and Eastern Anatolia. (Davis, 1974, Baytop, 1997). The name Gypsophila is derived from the words "gypsus" meaning lime in Greek and "philos" meaning loving. Among the people is called "Çöven" these plant species were 55 reported are endemic which are located 33, in Turkey (Korkmaz and Ozcelik, 2011). They are annual, biennial or perennial plants.

It is well known that the genus Gypsophila contains saponins with industrial appeal due to its various applications. Today, some species of *Gypsophila* (*G. arrostii* Guss., *G. bicolor* Freyn & Sint.Grossh., *G. eriocalyx* Boiss.) are used in the food sector as additives in the production of halvah, liqueur and ice cream. The roots of some species (*G. arrostii* var. *nebulosa*) have been used as detergents due to the presence of saponin in its chemical containment and good foaming properties.

The isolation and characterization of saponins has led to many cancer-fighting effects on saponins from Gypsophila derivatives. In addition, Gypsophila

saponins have been found to possess a variety of biological activities such as antimicrobial and antioxidant activities.

Gypsogenin is the natural and bidesmosidic saponin, derived from the root of the *Gypsophila arrostii* plant. This saponin is used in the local name is "çöven suyu". Gypsogenin, obtained from this plant, may inhibit the growth and metastasis of lung cancer and induce apoptosis by increasing Bax levels. Momordica charantia, a famous medicinal plant used in various Asian traditional medicines, also contains gypsogenin and has anticancer activities so there is strong evidence that Gypsogenin is anticancer. Gypsogenin aglycons are found in high concentrations in Gypsophila.

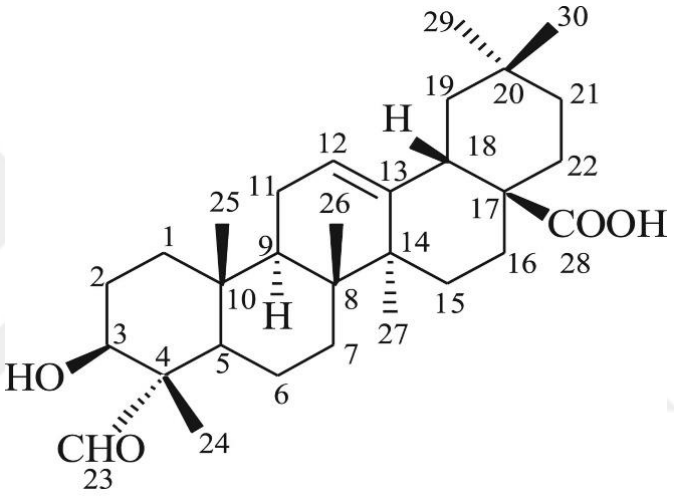


Figure 1.9. Gypsogenin aglycone

1.3. Analysis and Isolation of Gypsogenin

Isolation and characterization is important to determine the structure of a plants contents, to identify triterpenes and steroid glycosides, as well as to investigate their biological activity and potential toxic effects.

Different methods are used in qualitative and quantitative analysis of saponins; such as gravimetry, spectrophotometry, TLC, GC, HPLC, etc. (Price et al., 1987)

Saponins often have large molecular weights and high polarities, so isolation is quite challenging. The coloring of the stains on the TLC plates after extraction and chromatographic purification of a material with the appropriate reagents is an additional indication of identity.

2. MATERIAL AND METHODS

2.1. General

The melting points of all compounds were recorded on a Gallenkamp electrothermal melting point device. IR spectra were recorded on a Perkin-Elmer Frontier FT-IR spectrometer. NMR spectra were measured in $CDCl_3$ at 600 MHz for ^{1}HNMR, HMQC, ^{13}C NMR, APT and COSY experiments, using TMS as internal standard. LC-MS was recorded on a Thermo Scientific/ Surveyor MSQ spectrometer in the ESI mod. For column chromatography 60 Å silica gel (Merck 7734) was being used. TLC was carried out by using 60 Å silica gel on F_{254} aluminum plates (Merck 5554). Compounds were detected by UV and spraying with 20% H_2SO_4 solution followed by heating for 1-2 min.

During the isolation process and TLC controls the following solvent system were used:

8: 2 (hexane: etyl acetate)

7: 3 (hexane: etyl acetate)

6: 4 (hexane: etyl acetate)

1:1 (hexane: ethylether)

2:1 (hexane: ethylether)

2.2. Isolation, Purification and Reactions Steps

This study was occured by three main step.

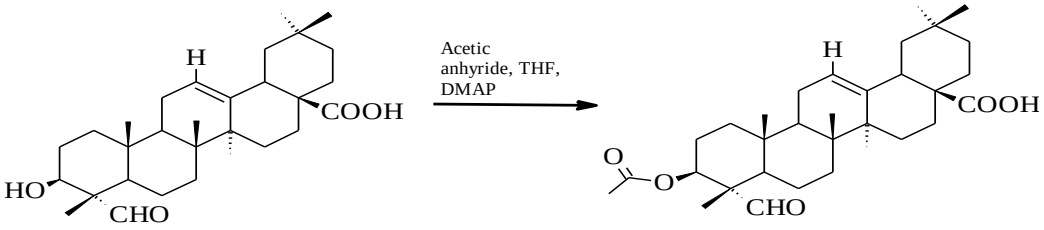
For the first step, the commercially available çöven water extract of *Gypsophila arrostii* roots (1000 mL) was mixed ethanol (250 mL) and hydrolyzed with 10% KOH base and two days later, this mixture was hydrolyzed with 10% HCl, respectively. After three days being neutralized with KOH and extracted with CH_2Cl_2**.** The CH_2Cl_2 phase was concentrated to give 3500g of crude gypsogenin. The crude product was purified by column chromatography with hexane:ethyl acetate (6:4) as the eluent to afford gypsogenin (1000 mg).

The acetylation reaction is preferred to protecting the -OH group at C-3 for the gypsogenin compound obtained. The route for the reaction is as follows: Gypsogenin (500 mg) was treated with acetic anhydride (2 mL) and DMAP in tetrahydrofuran (5 mL) at room temperature to provide Acetyl-Gypso compound. The mixture was allowed to stir for 48 h at room temperature. After stirring, the mixture was evaporated to dryness. The residue was purified by column chromatography with hexane:ethyl acetate (8:2) to give Acetyl-Gypso compound (300mg).

For second step, Chalcones were synthesized by base-catalyzed Claisen-Schmidt condensation using appropriate substituted acetophenone with commercially available. A mixture of methoxy acetophenone derivatives (10 mmol) and hydroxy benzaldehyde derivative (10 mmol) was dissolved in ethanol (30 mL), and added (40%, 15 mL) of alcoholic NaOH was stirred at 5–10 °C by using an ice bath. The progress of reaction was monitored by TLC. Thereafter the completion of reaction, the reaction mixture was poured into ice-water; acidified with dilute hydrochloric acid. The precipitated solid was filtered, washed thoroughly wih distilled water, and recrystallized from aqueous ethanol. Thus pure chalcone compounds were obtained (**C1-9**).

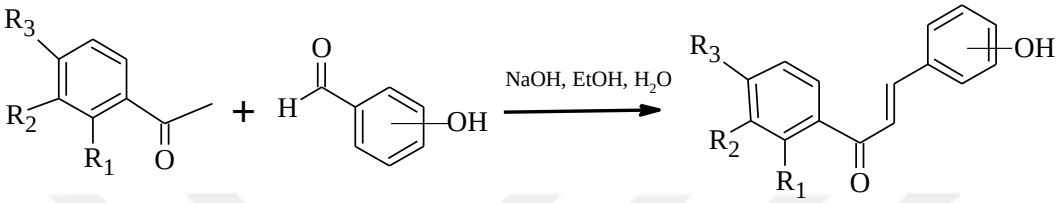
Final step was semi synthesis with Acetyl-Gypso compounds and chalcones. Acetyl-Gypso compound (150 mg, $2{,}9 \times 10^{-4}$ mol) in dry CH_2Cl_2 (10mL) was dissolved then added chalcones (37mg, 1.4×10^{-4}mol) and then added DMAP and DCC under N_2. The mixture was allowed to stir for 72 h at 0 ℃. After stirring, the mixture was evaporated to dryness. The residue was purified by silica gel chromatography using hexane:ethyl acetate (7:3) to give Gypsogenin-chalcone hybrid compounds respectively (**Compound 1-9**).

First Part of the Work:



Gypsogenin Acethyl-Gypso Compound

Second Part of the Work:

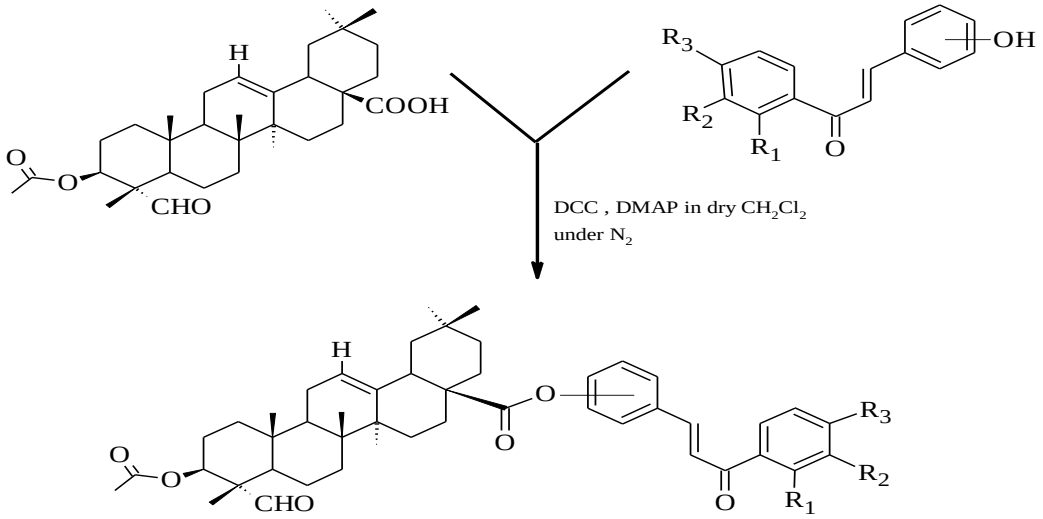


Chalcones (**C1-C9**)

-OH: *o-, m- ve p-*

Chalcones			
1-3	R^1=H	R^2=H	R^3=-OCH_3
4-6	R^1=H	R^2=-OCH_3	R^3=H
7-9	R^1=-OCH_3	R^2=H	R^3=H

Third Part of the Work:



Gypsogenin-COO-Chalcone Hybrid Compounds (**Compounds 1-9**)

Scheme 2.1. Synthetic pathway of Gypsogenin-COO-Chalcone hybrid compounds

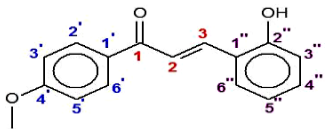
Eight new synthesized compounds 1-9 were established by IR, UV, ^{1}H NMR, ^{13}C NMR, APT and LC-MS analyses.

2.3. Activity Assay

In microdilution method applied for antimicrobial activity; bacterial strains were cultured on Mueller-Hinton agar (MHA) plaque media, yeast broth Sabouraud dekstroz agar (SDA) plaque media, and incubated at 37 ° C for 18-20 hours. Suspensions were prepared from fresh cultures with 0.9% NaCl solution (saline) and the suspensions were adjusted to 0.5 (1-2 × 108 CFU / ml) with the McFarland device (Den-1, Biosan). The suspensions were diluted 1/100. 50 µl of bacteria were distributed to the wells of sterile 96-well microplates, Mueller-Hinton II broth (cation-added) (MHIIB) for bacterial origins (*Staphylococcus aureus ATCC 29213,Enterococcus faecalis ATCC 29212,Escherichia coli ATCC 25922,Pseudomonas aeruginosa ATCC 27853,Bacillus subtilis RSKK 02021*) and RPMI-1640 (Buffered with MOPS) for yeast origins (*Candida albicans ATCC 90028*). First batches were added to 50 µl of active substance solutions (DMSO / distilled water, 1/1) and serial dilutions were prepared by transferring to the side wells. 50 µl of bacterial and fungal suspensions were added to the wells. The lowest concentrations without growth after 16-24 hours incubation at 37 ° C were accepted as MIC. Feeding and reproductive controls were made in every microplate. The control group used ciprofloxacin and fluconazole as antimicrobial agents. MIC interpretation limits and control limits were evaluated according to CLSI and EUCAST criteria.

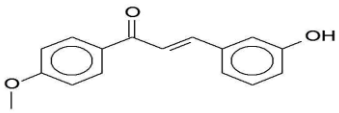
3. RESULT AND DISCUSSIONS

Chalcone 1



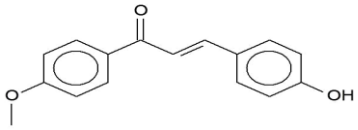
Matsushima, R., et al., 1988

Chalcone 2



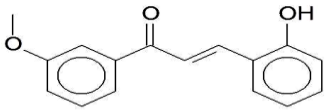
Moorthi, S., et al., 2005

Chalcone 3



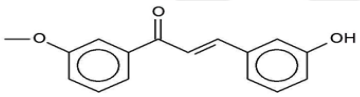
Susumu, I., et al., 2005

Chalcone 4



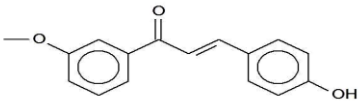
Masesane, I., et al., 2014

Chalcone 5



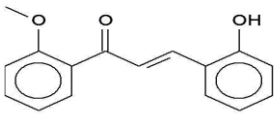
Ronald, R., et al, 1996

Chalcone 6



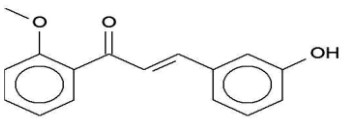
Gan, X., et al., 2017

Chalcone 7



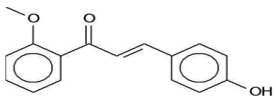
Matsushima, R., et al., 1988

Chalcone 8



Murphy, W.S., et al.,1980

Chalcone 9



Hu, D., et al, 2016

Figure 3.1. Chalcones 1-9 (C1-9)

3.1. Structural Identification of Compound 1

Compound **1** was synthesized from Acetyl-Gypso compound and chalcone (**C1**) according to procedure given in **2.2.**

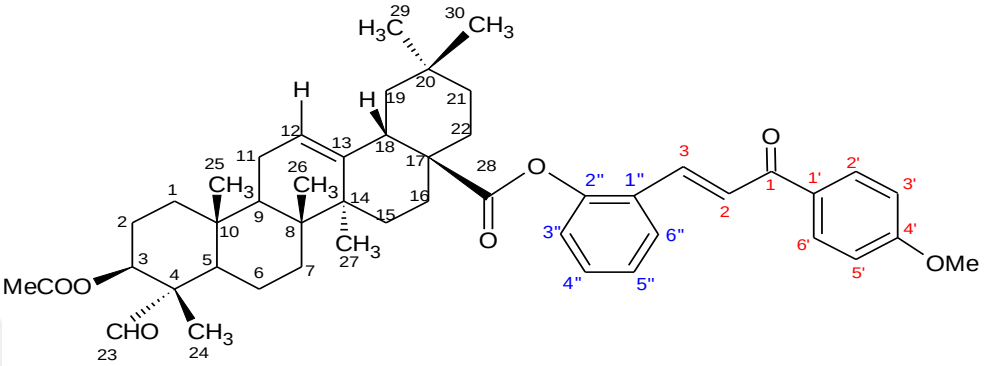
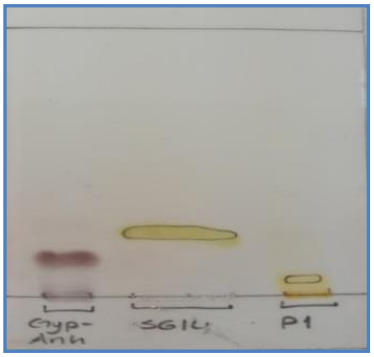


Figure 3.2. Structure of Compound 1

TLC (Thin Layer Chromatography)



R_f: 0.60 (Hexane:Ethyl acetate 7:3)

Yield (%): 51

Melting Point: (°C): 221-223.1

FT-IR (cm^{-1}): 3048, 2921, 2850, 1733, 1625, 1573, 1467, 1341, 1279, 1146, 1096, 1059, 960, 841.

($C_{48}H_{60}O_7$) Calculated m/z (%): 748,98

LC-MS/MS m/z (%):749,00 (100) $[M]^+$

The H-4' is not observed for compound 1 at ^{1}H-NMR. It appears at 163.4 ppm in APT. There are no proton values for H-1" and H-2" for the chalcone. The peaks are (C-1″) 121.9 ppm and (C-2″) 148.5 ppm appeared in APT. Although at

the C-28, which is bound to the Gypsogenin compound, which normally appears in 180.4 ppm in APT, after the reaction it is observed in 175.9 ppm, we understand that the reaction has occurred with the ester linkage.

Table 3.1. ^{1}H- and APT Data of Compound 1 (600/150 MHz, $CDCl_3$)

Part A			**Part B**		
Position	^{1}HNMR δ (ppm)	APT (ppm)	**Position**	^{1}HNMR δ (ppm)	APT (ppm)
3	4.89	73.5	1	-	189.0
4	-	54.3	2	7.62	122.4
12	5.31	122.9	3	7.82	143.3
13	-	143.4	1′	-	130.1
18	2.95	41.2	2′/6′	7.92	130.3
23	9.20	204.9	3′/5′	6.77	113.7
24	1.00	9.3	4′	-	163.4
25	0.92	15.5	$-OCH_3$	3.83	55.4
26	0.80	17.5	1″	-	121.9
27	1.16	25.6	2″	-	148.5
28	-	175.9	3″	6.90	115.9
29	0.87	32.9	4″	7.13	132.9
30	0.93	23.3	5″	-	119.9
C**H**$_3$-COO-Anh	1.90	-	6"	7.64	-
CH$_3$-COO-Anh	-	20.8			
CH$_3$-**C**OO-Anh	-	170.9			

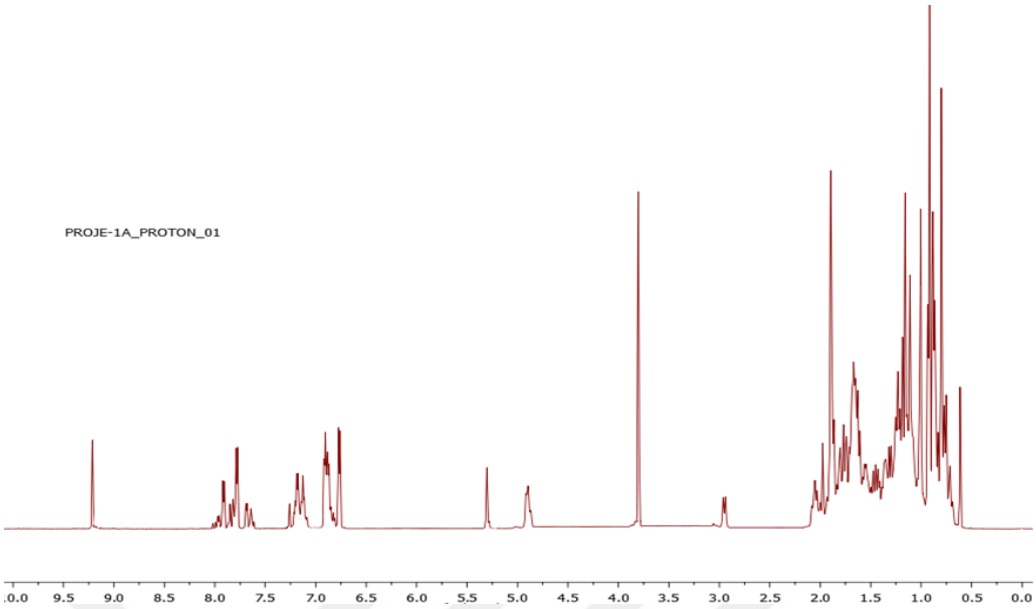


Figure 3.3. ^{1}H NMR Spectrum of Compound 1 (600 MHz,$CDCl_3$)

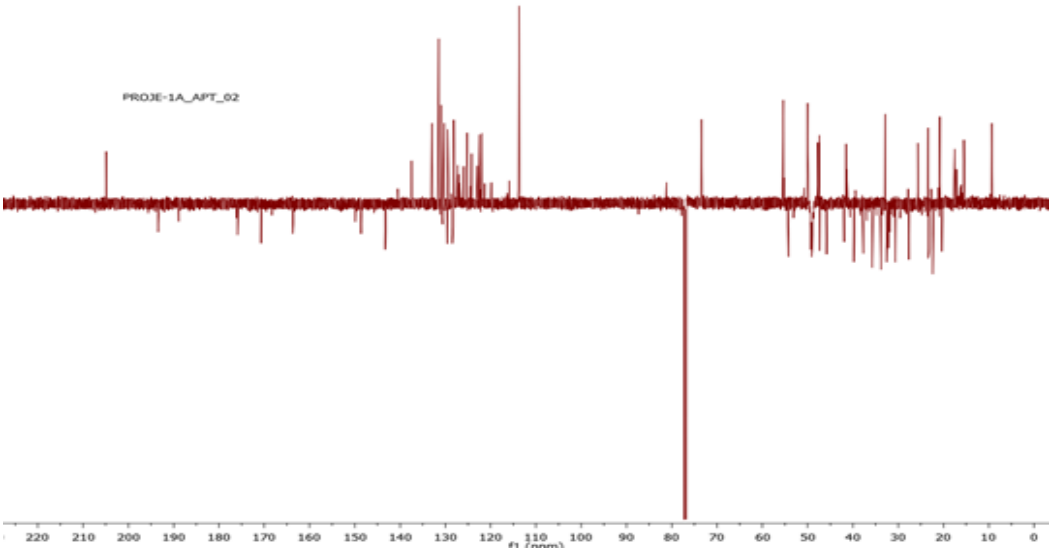


Figure 3.4. APT Spectrum of Compound 1 (150MHz, $CDCl_3$)

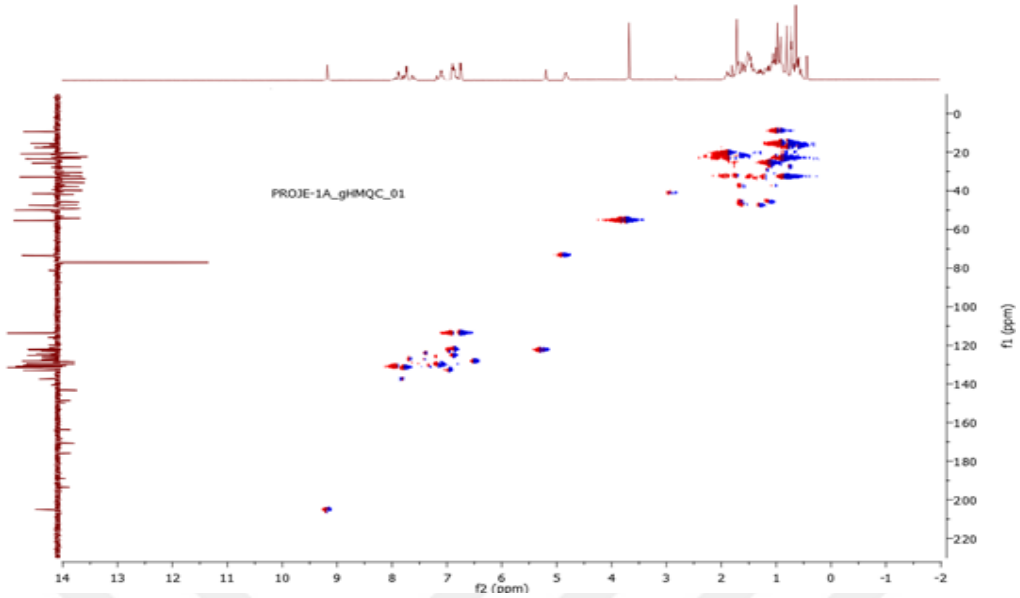


Figure 3.5. HMQC Spectrum of Compound 1 (600 MHz,$CDCl_3$)

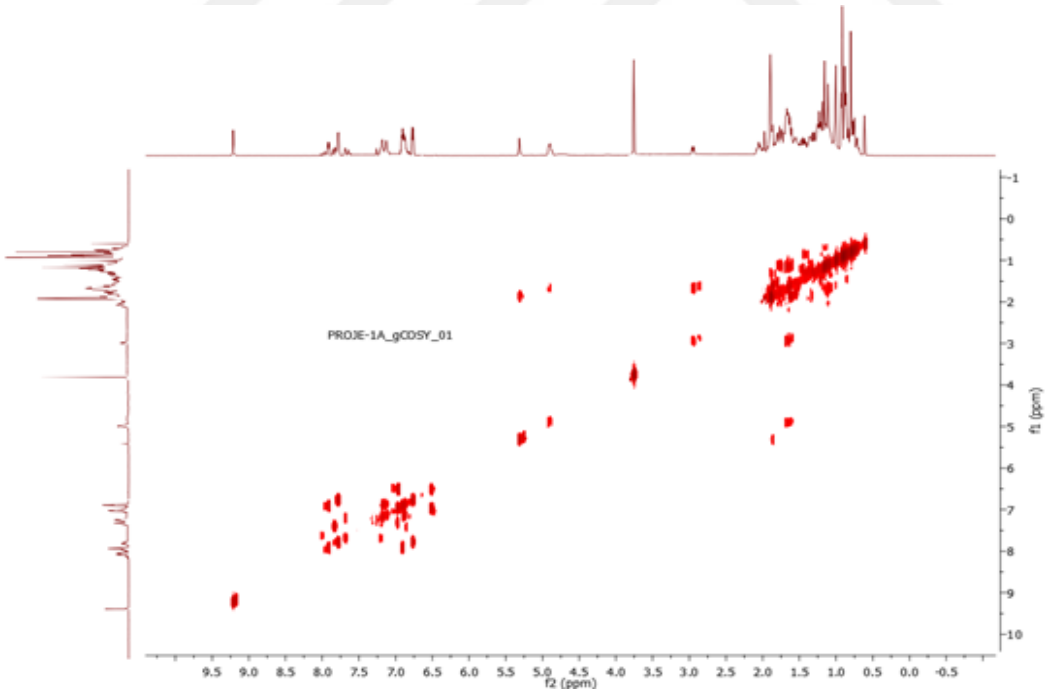


Figure 3.6. COSY Spectrum of Compound 1 (600 MHz,$CDCl_3$)

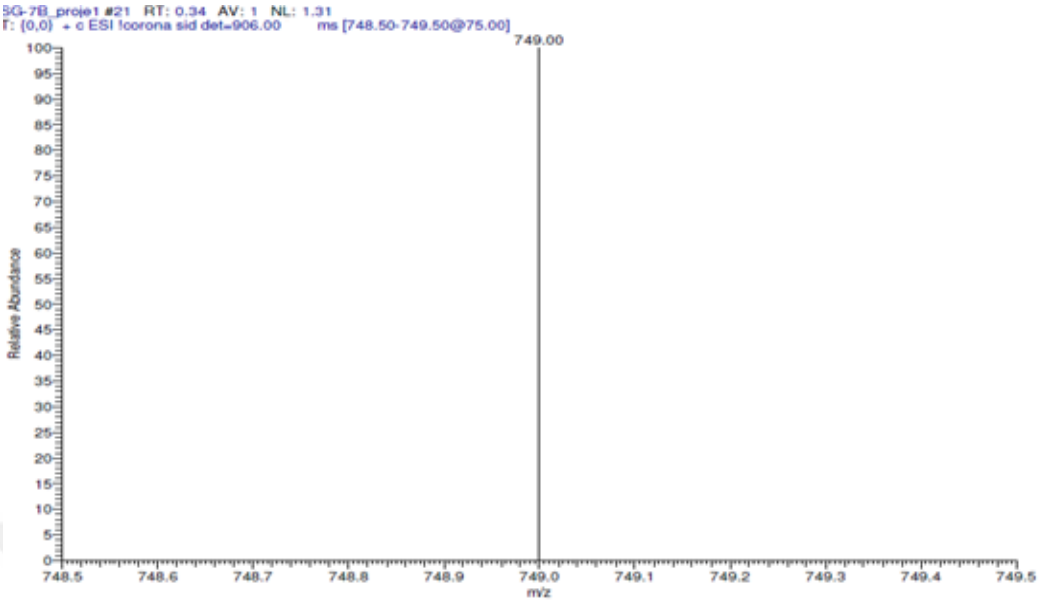


Figure 3.7. LC-Mass Spectrum of Compound 1

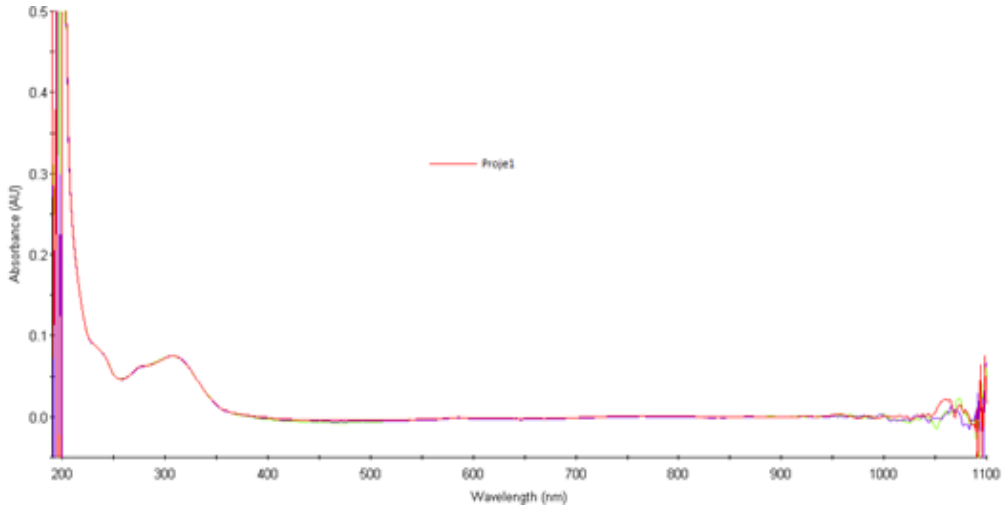


Figure 3.8. UV Spectrum of Compound 1

*:

3.2. Structural Identification of Compound 2

Compound **2** was synthesized from Acetyl-Gypso compound and chalcone (**C2**) according to procedure given in **2.2.**

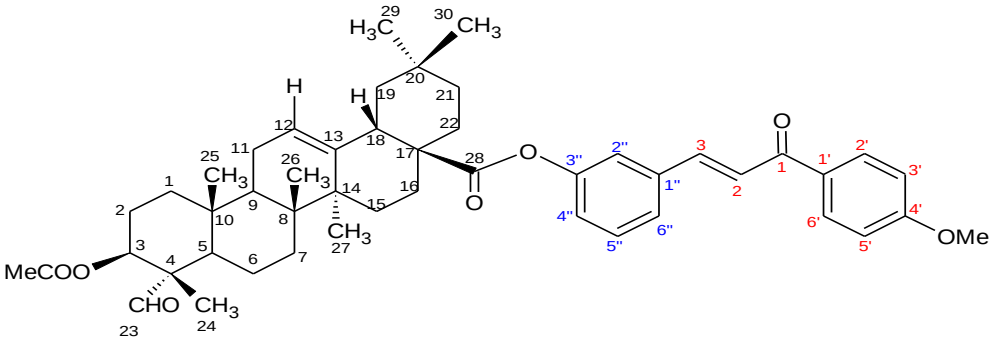
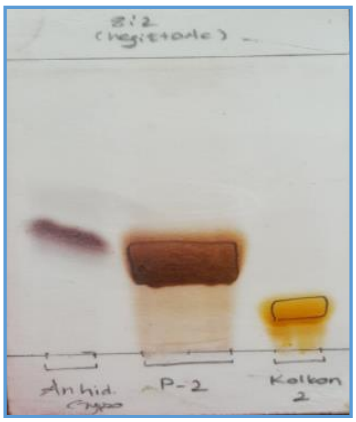


Figure 3.9. Structure of Compound 2

TLC (Thin Layer Chromatography)



R_f: 0.33 (Hexane-Ethyl acetate 8:2)

Yield(%): 30

Melting Point (ºC): 209-211

FT-IR (cm^{-1}): 3048, 2925, 2851, 1741, 1599, 1450, 1359, 1341, 1279, 1090, 1059, 960, 946, 841.

($C_{48}H_{60}O_7$) Calculated m/z (%): 748,98

LC-MS/MS m/z (%):749,00 (100) $[M]^+$

The H-4' is not observed for compound 2 at ^{1}H-NMR. It appears at 164.3 (C-4′) ppm in APT. There are no proton values for H-3" for the chalcone. The peak is 152.7 (C-3") ppm appeared in APT. Although at the C-28, which is bound

to the Gypsogenin compound, which normally appears in 180.4 ppm in APT, after the reaction it is observed in 176.6 ppm, we understand that the reaction has occurred with the ester linkage.

Table 3.2. ^{1}H- and APT Data of Compound 2(600/150 MHz, pyridine-d_5)

Part A			**Part B**		
Position	^{1}HNMR δ (ppm)	APT (ppm)	**Position**	^{1}HNMR δ (ppm)	APT (ppm)
3	5.31	73.8	1	-	188.3
4	-	55.0	2	7.92	122.3
12	5.53	123.3	3	8.23	143.1
13	-	144.1	1′	-	137.8
18	3.26	42.6	2′/6′	8.37	130.7
23	9.60	205.0	3′/5′	7.16	114.8
24	1.29	10.2	4′	-	164.3
25	0.92	15.9	-OCH_3	3.81	55.8
26	0.95	18.0	1″	-	144.1
27	1.35	28.5	2″	7.07	-
28	-	176.6	3″	-	152.7
29	1.02	34.3	4″	6.92	-
30	1.05	23.9	5″	7.57	129.9
C**H**$_3$-COO-Anh	2.01	-	6"	7.64	-
CH_3-COO-Anh	-	21.1			
CH_3-**C**OO-Anh	-	170.5			

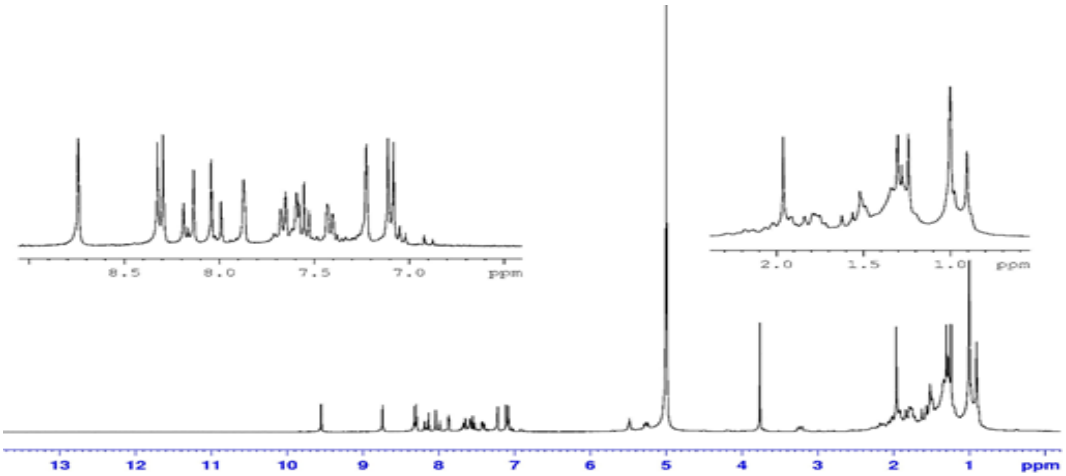


Figure 3.10. ^{1}H-NMR Spectrum of Compound **2 (600 MHz, C_5D_5N)**

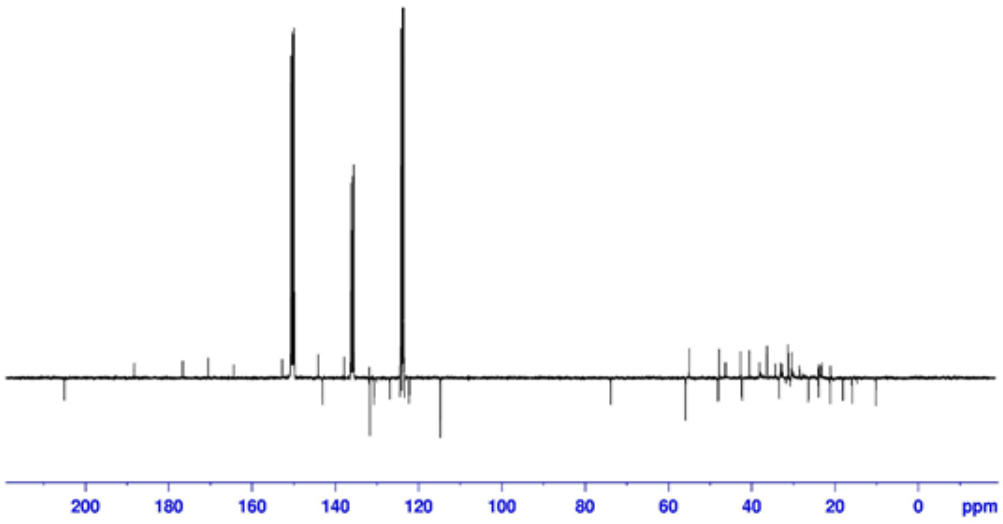


Figure 3.11. APT Spectrum of Compound 2 (150 MHz, C_5D_5N)

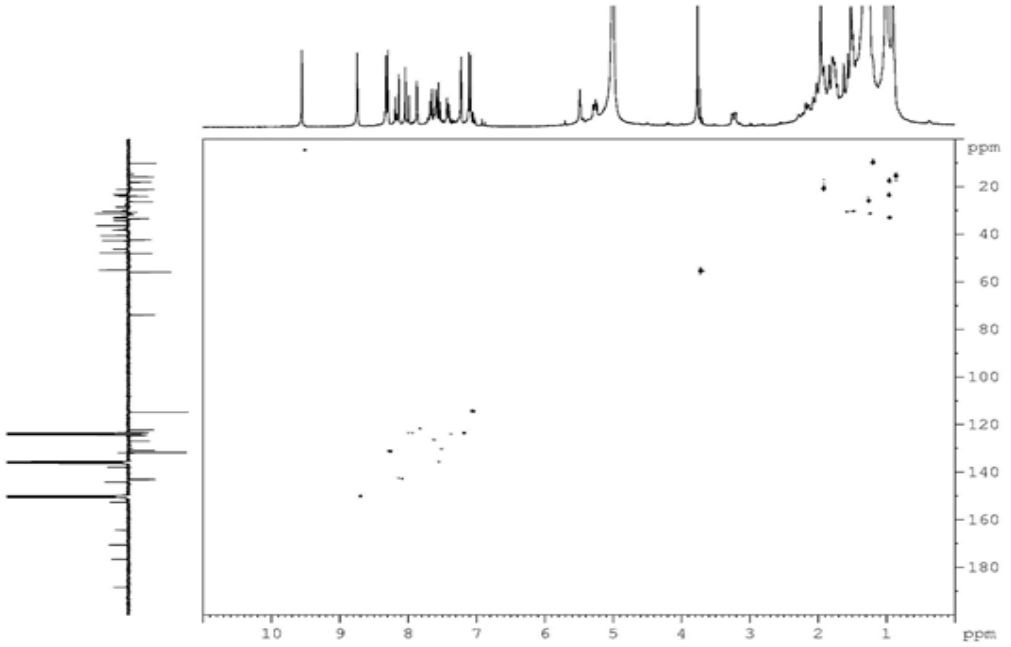


Figure 3.12. HMQC Spectrum of Compound 2 (600 MHz, C_5D_5N)

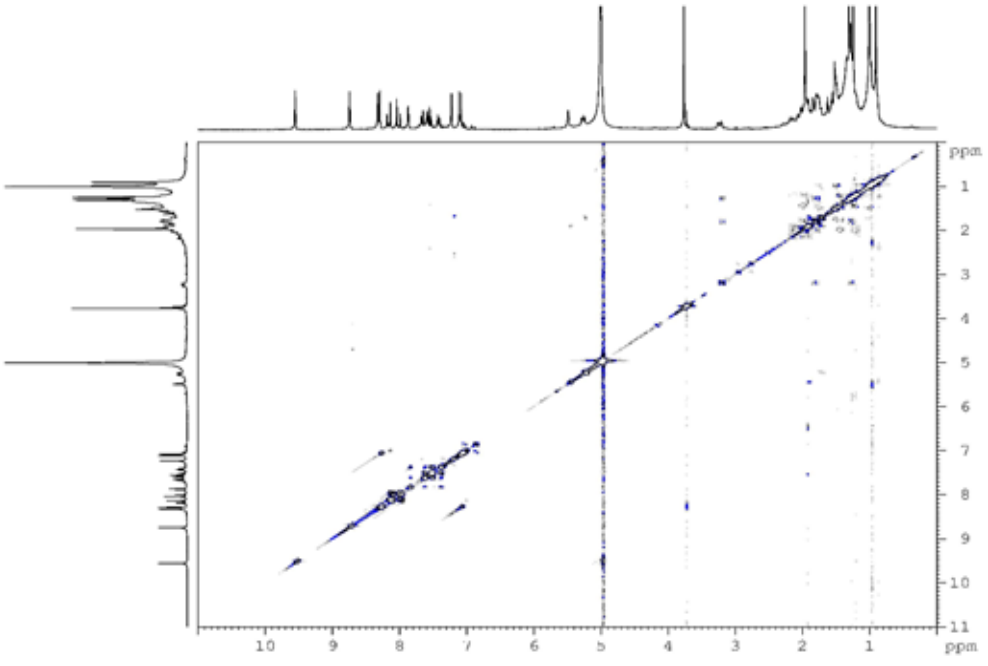


Figure 3.13. COSY Spectrum of Compound 2 (600 MHz, C_5D_5N)

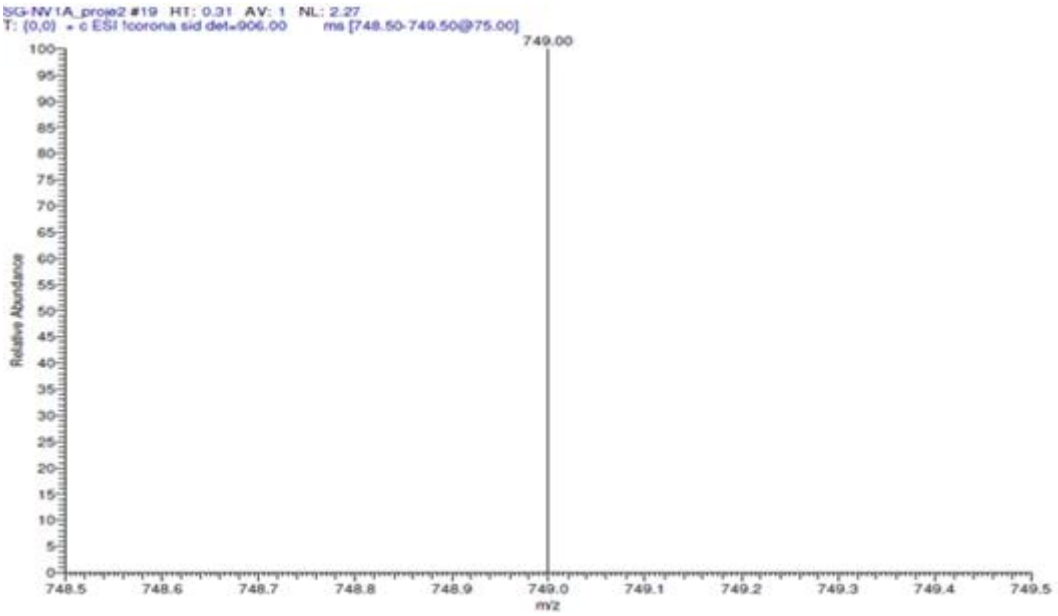


Figure 3.14. LC- Mass Spectrum of Compounds 2

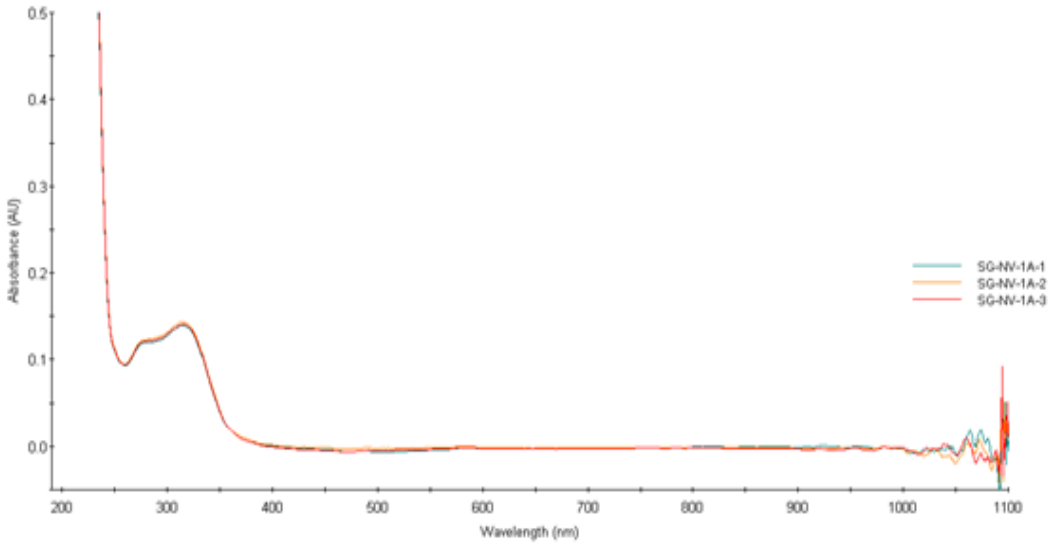


Figure 3.15. UV Spectrum of Compound 2

3.3. Structural Identification of Compound 3

Compound **3** was synthesized from Acetyl-Gypso compound and chalcone (**C3**) according to procedure given in **2.2.**

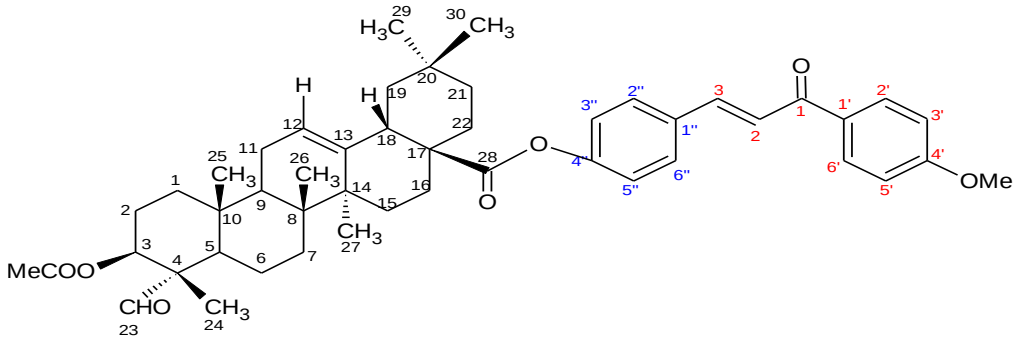
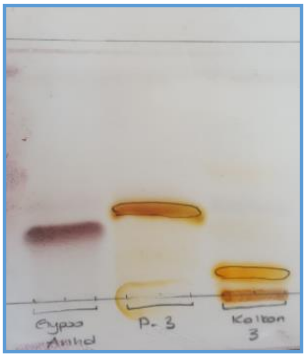


Figure 3.16. Structre of Compound 3

TLC (Thin Layer Chromatography)



R_f: 0.39 (Hexane-Ethyl acetate 8:2)

Yield(%): 23

Melting Point (°C): 217.9-219.0

FT-IR (cm^{-1}): 3040, 2927, 2849, 1731, 1624, 1571, 1443, 1309, 1240, 1162, 1087, 1026, 892, 801.

($C_{48}H_{60}O_7$) Calculated m/z (%): 748,98

LC-MS/MS m/z (%):749,00 (100) $[M]^+$

The H-4' is not observed for compound 3 at ^{1}H-NMR. It appears at 164.3 ppm (C-4′) in APT. There are no proton values for H-4" for the chalcone. The peak is 153.7 ppm (C-4") appeared in APT. Although at the C-28, which is bound to the Gypsogenin compound, which normally appears in 180.4 ppm in APT, after

the reaction it is observed in 176.4 ppm, we understand that the reaction has occurred with the ester linkage.

Table 3.3. ^{1}H- and ^{13}C-NMR Data of Compound 3(600/150 MHz, pyridine-d_5)

Part A			**Part B**		
Position	^{1}HNMR δ (ppm)	^{13}CNMR (ppm)	**Position**	^{1}HNMR δ (ppm)	^{13}CNMR (ppm)
3	4.09	73.8	1	-	188.4
4	-	55.1	2	7.76	123.5
12	5.32	123.8	3	8.04	143.2
13	-	144.0	1′	-	131.9
18	3.26	40.5	2′/6′	8.21	130.6
23	9.41	205.0	3′/5′	7.12	114.8
24	1.09	10.2	4′	-	164.3
25	0.73	15.9	-OCH_3	3.60	55.1
26	0.82	17.9	1″	-	124.2
27	1.15	26.4	2″/6"	7.68	131.9
28	-	176.4	3″/5″	6.93	123.3
29	1.15	31.2	4″	-	153.7
30	0.86	23.9			
C**H**$_3$-COO-Anh	1.81	-			
CH_3-COO-Anh	-	21.2			
CH_3-**C**OO-Anh	-	170.5			

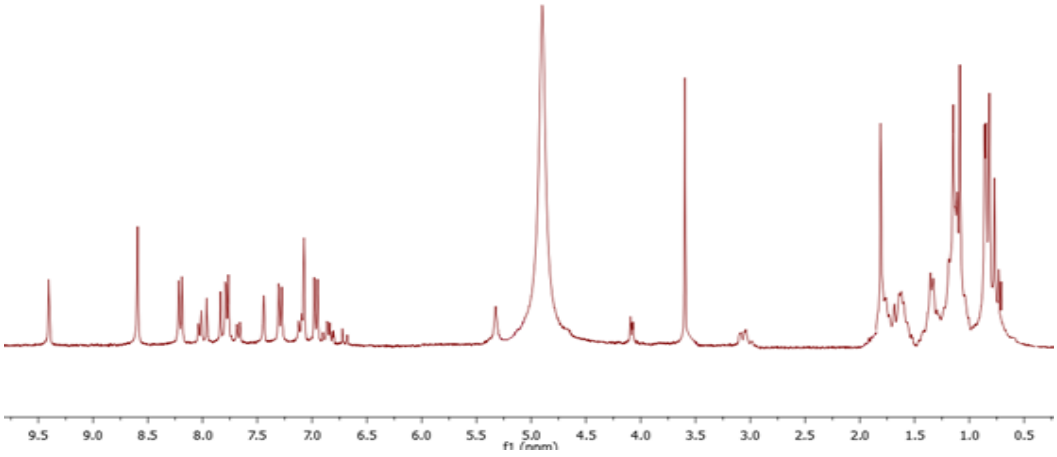


Figure 3.17. ^{1}H-NMR Spectrum of Compound 3 (600 MHz, C_5D_5N)

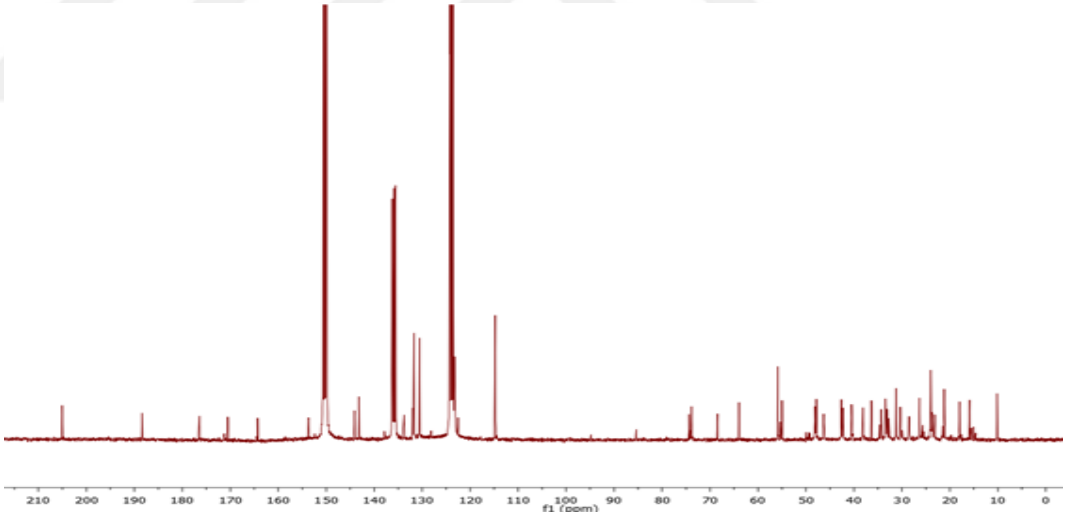


Figure 3.18. ^{13}CNMR Spectrum of Compound 3 (150 MHz, C_5D_5N)

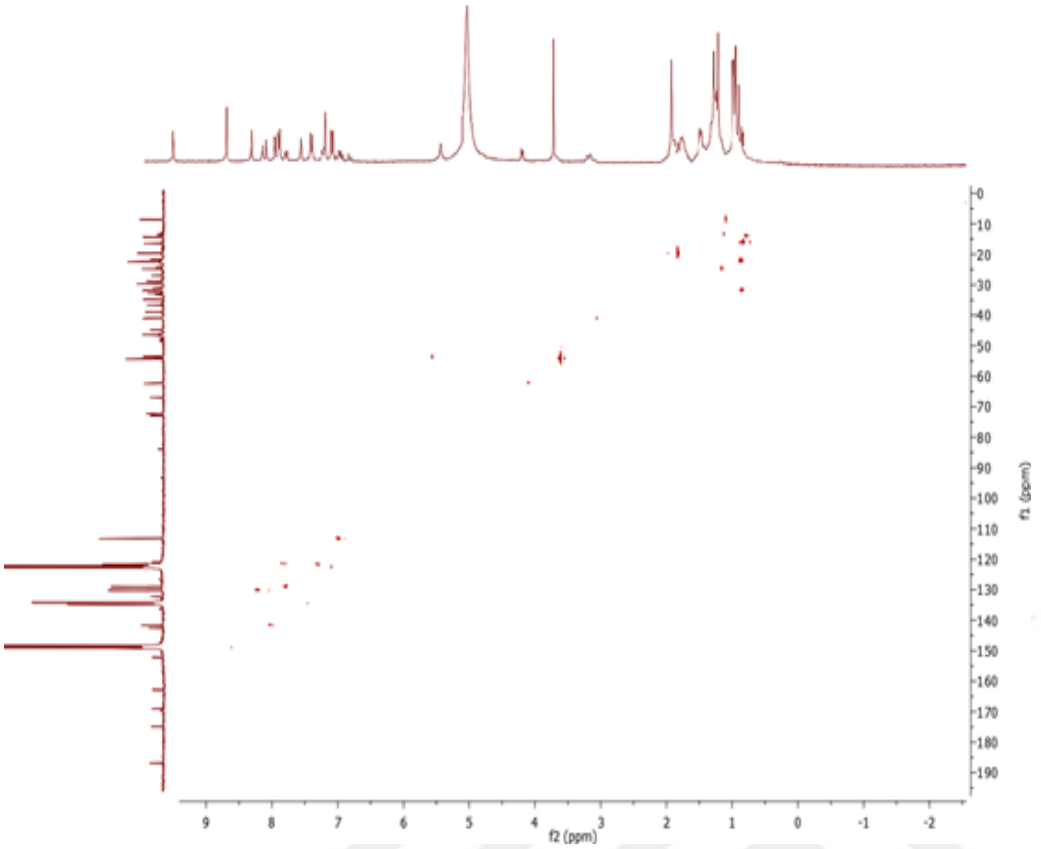


Figure 3.19. HMQC Spectrum of Compound 3 (600 MHz, C_5D_5N)

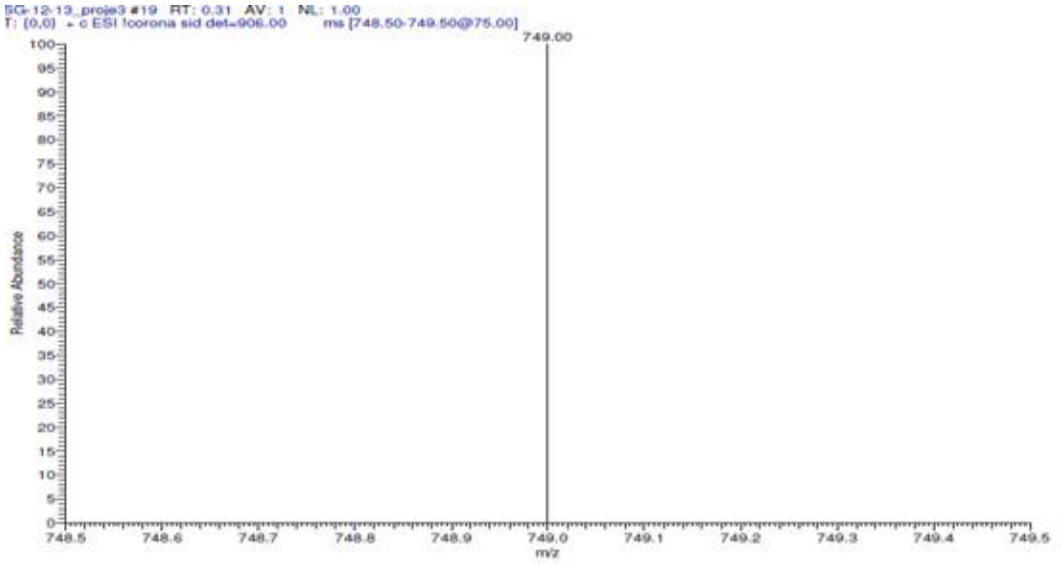


Figure 3.20. LC- Mass Spectrum of Compound 3

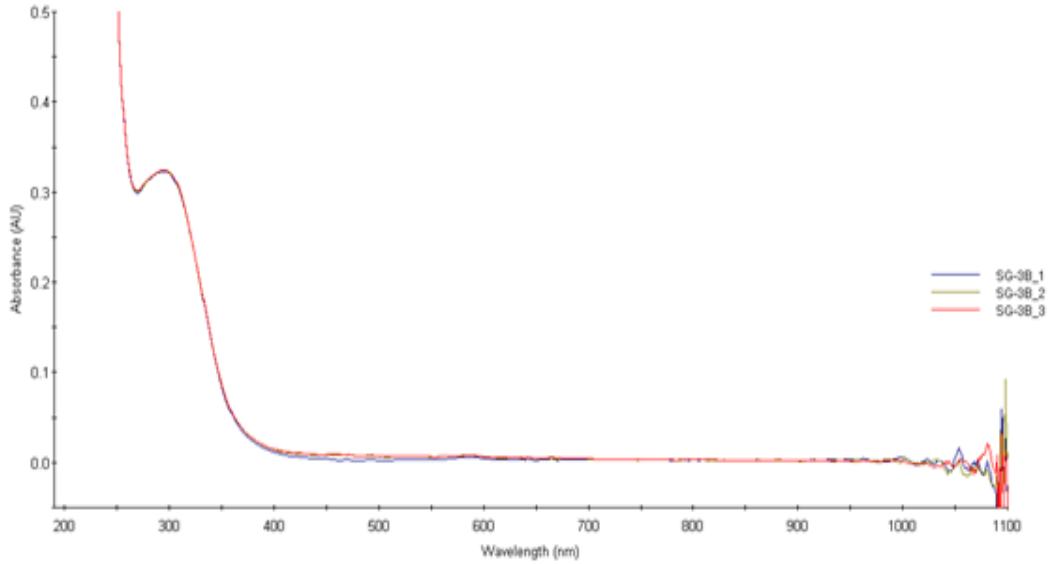


Figure 3.21. UV Spectrum of Compound 3

3.4. Structural Identification of Compound 4

Compound **4** was synthesized from Acetyl-Gypso compound and chalcone (**C4**) according to procedure given in **2.2.**

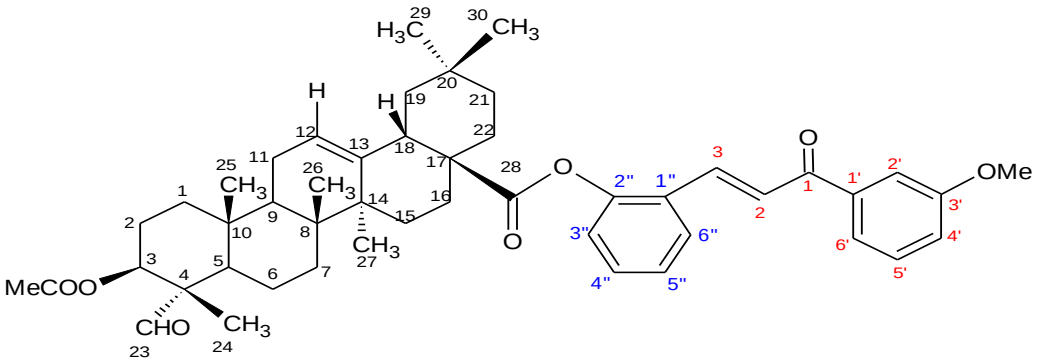
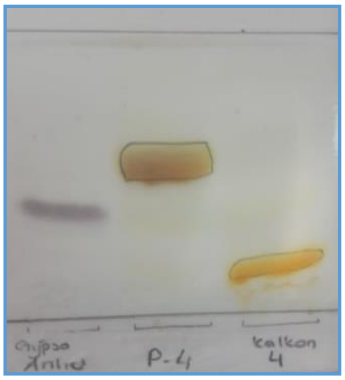


Figure 3.22. Structure of Compound 4

TLC (Thin Layer Chromatography)



Rf: 0.52 (Hexane-Ethyl acetate 8:2)

Yield(%): 13

Melting Point (ºC): 197.5-200.2;

FT-IR (cm^{-1}): 3050**,** 2923, 2850, 2340, 1744, 1699, 1652, 1553, 1457, 1380, 1336, 1308, 1237, 1181, 1002, 902, 822.

($C_{48}H_{60}O_7$) Calculated m/z (%): 748,98

LC-MS/MS m/z (%): 749,00(100) $[M]^+$

The H-3' is not observed for compound 4 at ^{1}H-NMR. It appears at 159.6 ppm in APT. There are no proton values for H-2" for the chalcone. The peak is 126.9 ppm (C-2″) appeared in APT. Although at the C-28, which is bound to the

Gypsogenin compound, which normally appears in 180.4 ppm in APT, after the reaction it is observed in 176.2 ppm, we understand that the reaction has occurred with the ester linkage.

Table 3.4. ^{1}H- and APT Data of Compound 4 (600 MHz, $CDCl_3$)

Part A			**Part B**		
Position	^{1}HNMR δ (ppm)	APT (ppm)	**Position**	^{1}HNMR δ (ppm)	APT (ppm)
3	4.97	73.4	1	-	190.6
4	-	54.2	2	7.45	122.3
12	5.37	122.7	3	8.20	142.7
13	-	143.7	1′	-	138.7
18	2.99	39.9	2′	-	115.2
23	9.28	204.9	3′	-	159.6
24	1.08	9.1	4′	7.08	119.3
25	0.98	15.3	5′	7.42	129.5
26	0.86	17.2	$-OCH_3$	3.80	55.6
27	1.22	25.5	1″	-	120.1
28	-	176.2	2″	-	126.9
29	0.88	32.7	3″	7.03	112.9
30	0.93	23.3	4″	7.32	129.6
$\underline{CH}_3$-COO-Anh	1.96	-	5″	7.02	119.3
$\underline{C}H_3$-COO-Anh	-	20.6	6″	-	129.5
CH_3-$\underline{C}$OO-Anh	-	170.7			

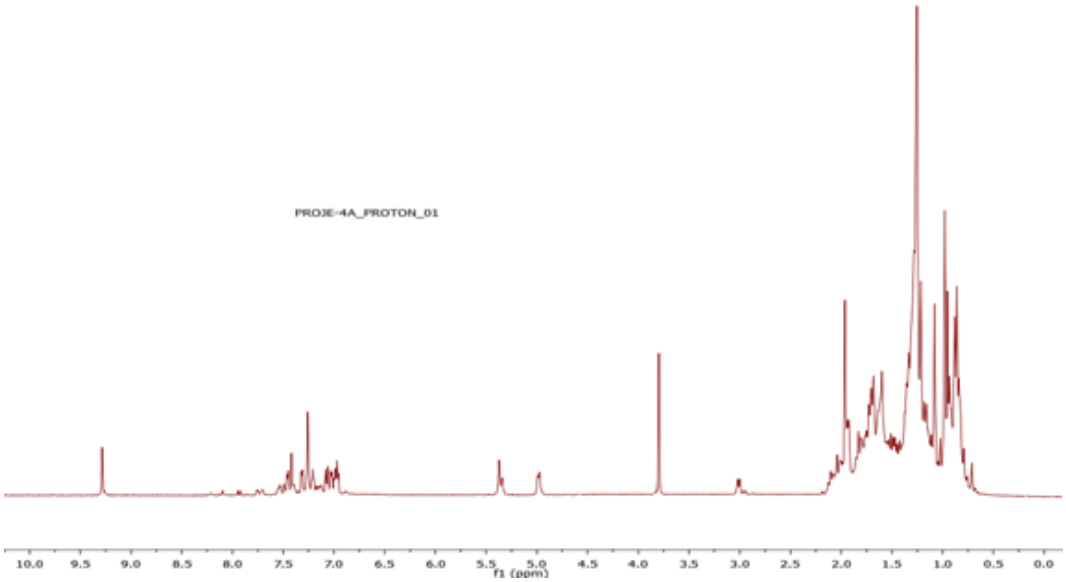


Figure 3.23. ^{1}H-NMR Spectrum of Compound 4 (600 MHz, $CDCl_3$)

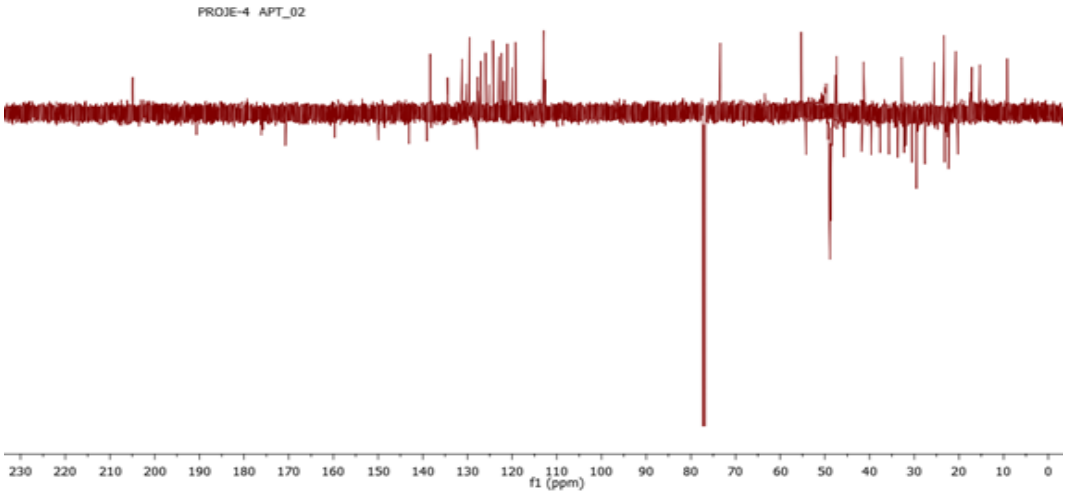


Figure 3.24. APT Spectrum of Compound 4 (150 MHz, $CDCl_3$)

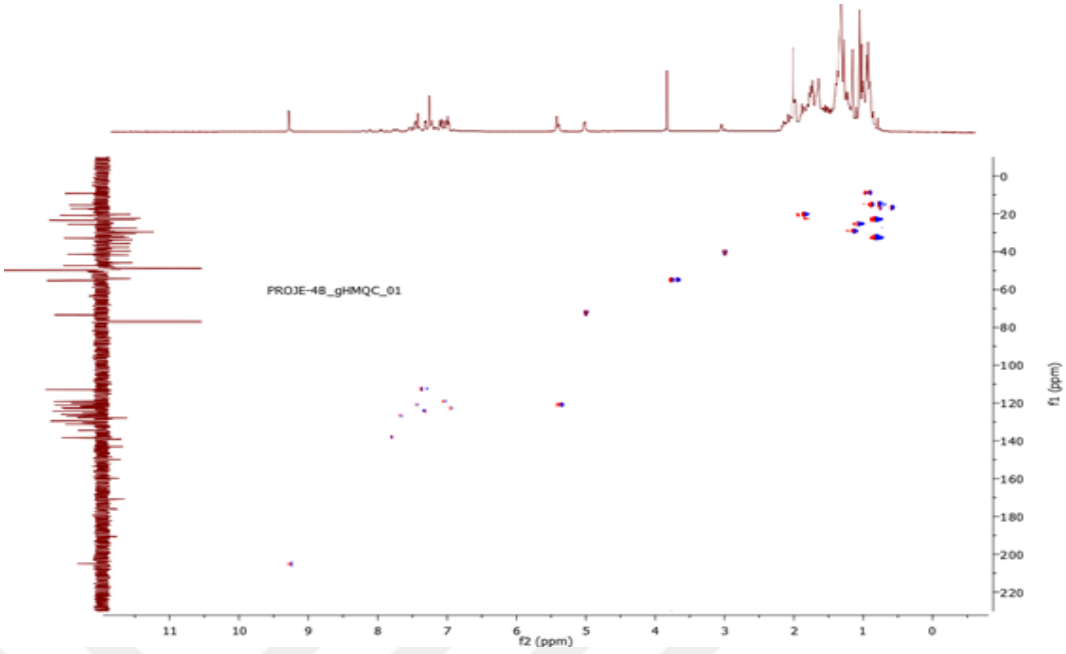


Figure 3.25. HMQC Spectrum of Compound 4 (600 MHz, $CDCl_3$)

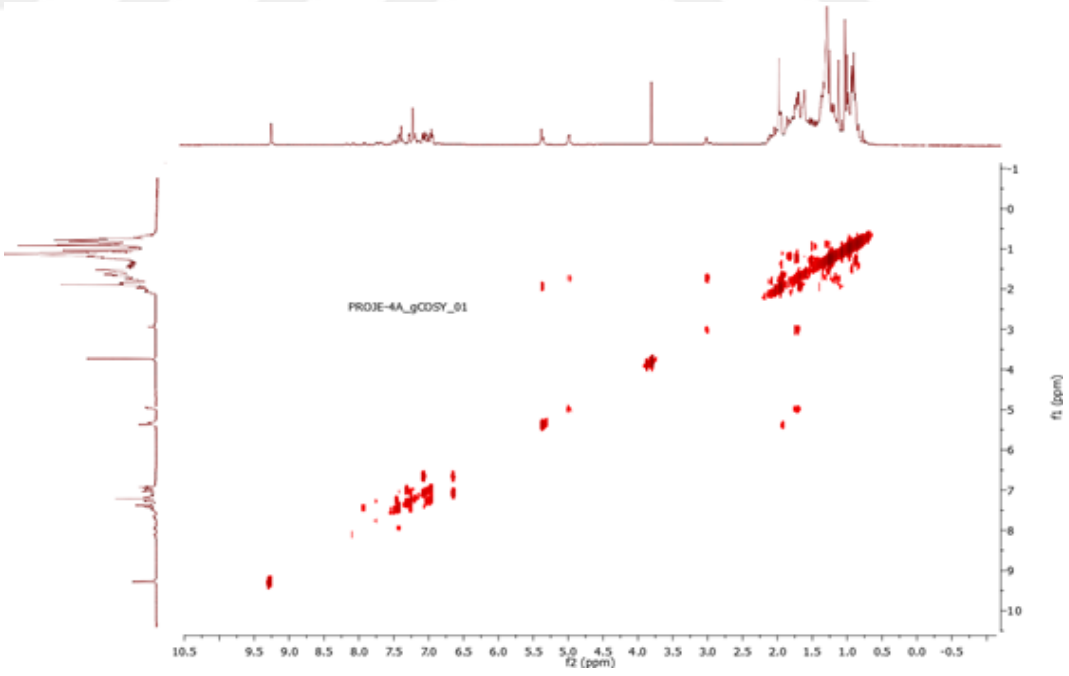


Figure 3.26. COSY Spectrum of Compound 4 (600 MHz, $CDCl_3$)

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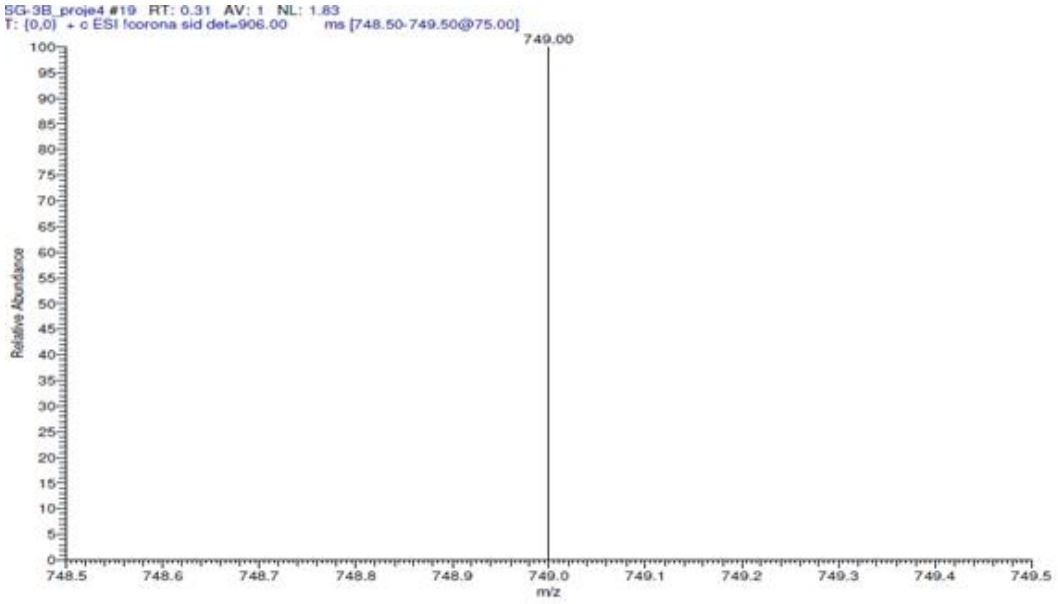


Figure 3.27. LC- Mass Spectrum of Compound 4

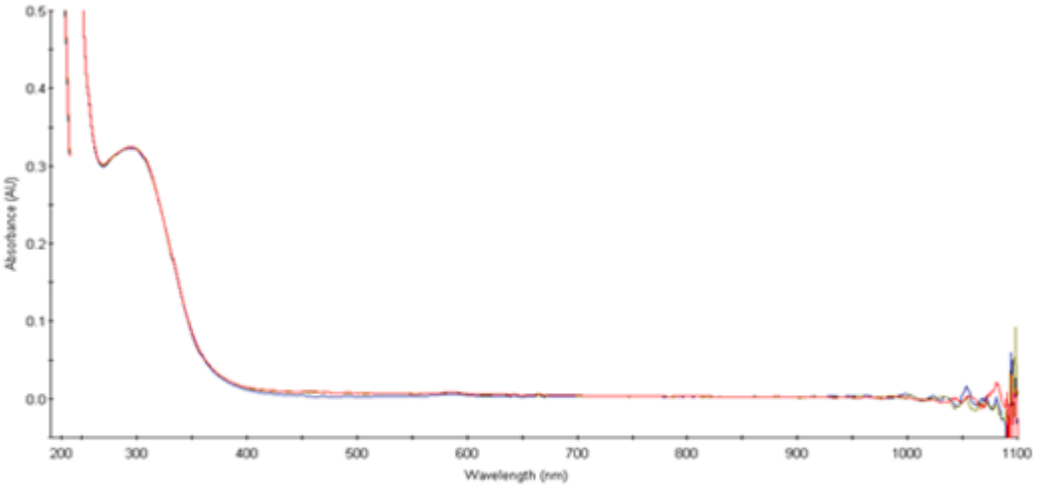


Figure 3.28. UV Spectrum of Compound 4

3.5. Structural Identification of Compound 5

Compound **5** was synthesized from Acetyl-Gypso compound and chalcone (**C5**) according to procedure given in **2.2.**

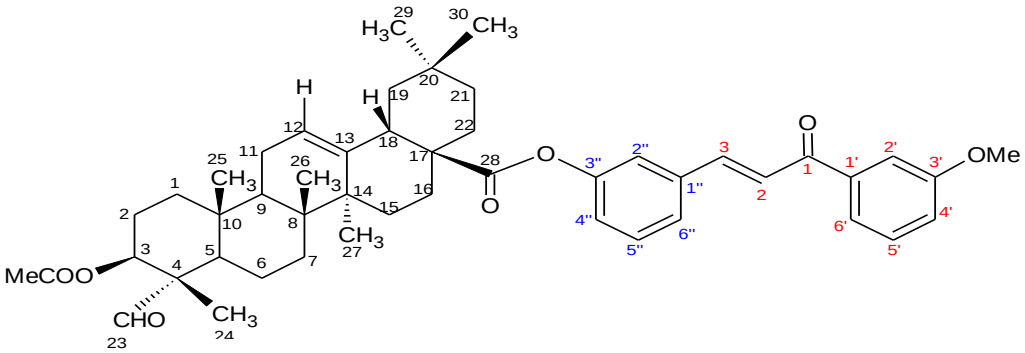


Figure 3.29. Structure of Compounds 5

TLC (Thin Layer Chromatography)

R_f: 0.48 (Hekzan-Etilasetat: 8:2)

Yield (%): 51

Melting Point (°C): 152-153

FT-IR (cm^{-1}): 3045, 2924, 2891, 2683, 2361, 1785, 1718, 1646, 1560, 1354, 1335, 1261, 1175, 1078, 1048, 961, 877,744.

($C_{48}H_{60}O_7$)Calculated m/z (%): 748,98

Positive LC-MS/MS m/z (%):749,00 (100) $[M]^+$

The H-3' is not observed for compound 5 at ^{1}H-NMR. It appears at 159.9 ppm (C-3') in APT. There are no proton values for H-3" for the chalcone. The peak is 159.8 ppm (C-3")appeared in APT. Although at the C-28, which is bound to the Gypsogenin compound, which normally appears in 180.4 ppm in APT, after the reaction it is observed in 176.2 ppm, we understand that the reaction has occurred with the ester linkage.

Table 3.5. ^{1}H- and APT Data of Compound 5 (600/150 MHz, $CDCl_3$)

Part A			**Part B**		
Position	^{1}HNMR δ (ppm)	APT (ppm)	**Position**	^{1}HNMR δ (ppm)	APT (ppm)
3	4.97	73.5	1	-	190.1
12	5.37	122.0	2	7.51	122.0
13	-	143.4	3	7.75	143.9
18	2.96	41.0	1’	-	139.4
23	9.28	204.5	2’	7.46	112.9
24	1.07	9.3	3’	-	159.9
25	0.95	15.4	4’	7.05	119.9
26	0.88	17.2	5’	7.41	129.8
27	1.18	25.9	6’	7.59	121.1
28	-	176.2	$-OCH_3$	3.88	55.7
29	0.93	32.9	1”	-	136.4
30	0.97	23.4	2”	-	112.7
$\underline{CH}_3$-COO-Anh	1.96		3”	-	159.8
$\underline{C}H_3$-COO-Anh	-	20.7	5″	7.29	129.5
CH_3-$\underline{C}$OO-Anh	-	170.1	6″	7.13	119.3

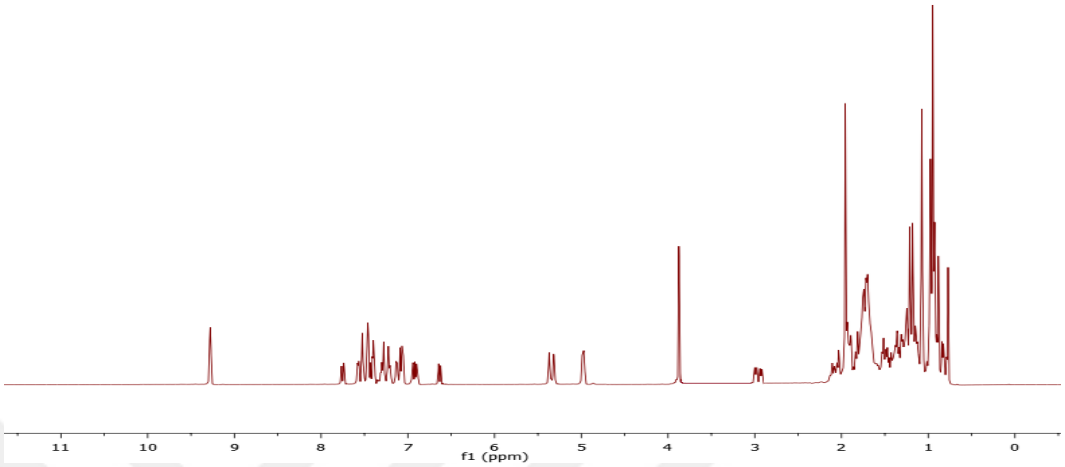


Figure 3.30. ^{1}H-NMR Spectrum of Compound 5 (600 MHz, $CDCl_3$)

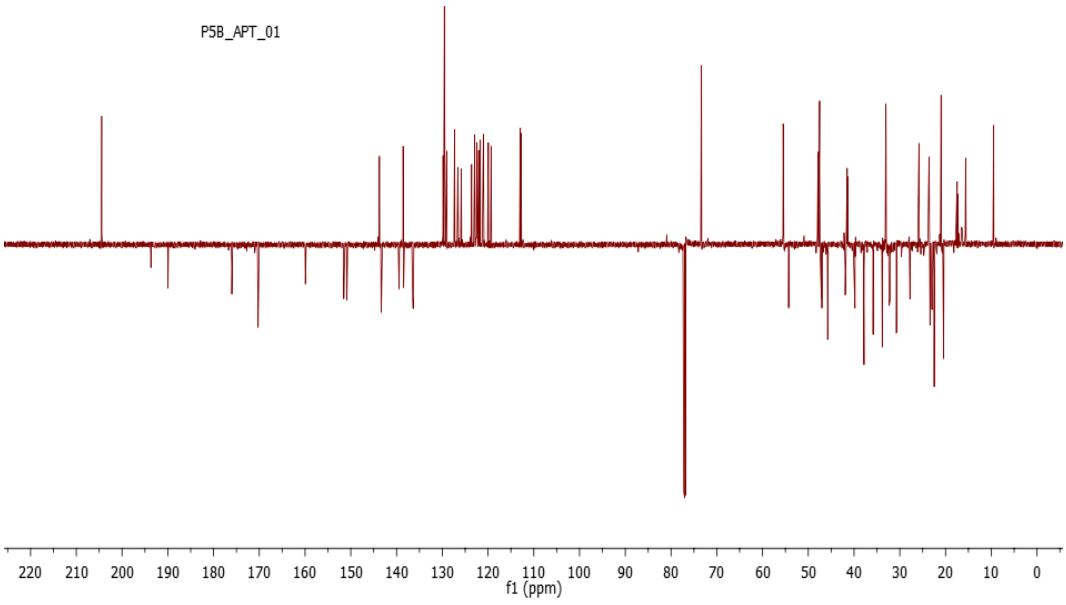


Figure 3.31. APT Spectrum of Compound 5 (150 MHz, $CDCl_3$)

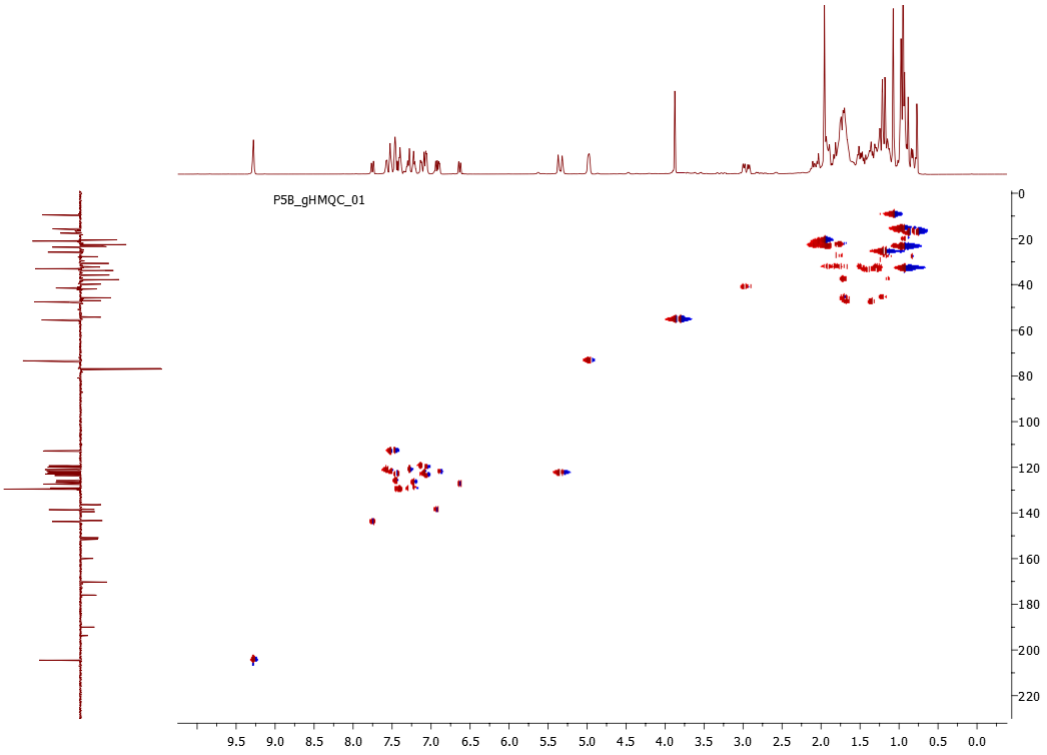


Figure 3.32. HMQC Spectrum of Compound 5 (600 MHz, $CDCl_3$)

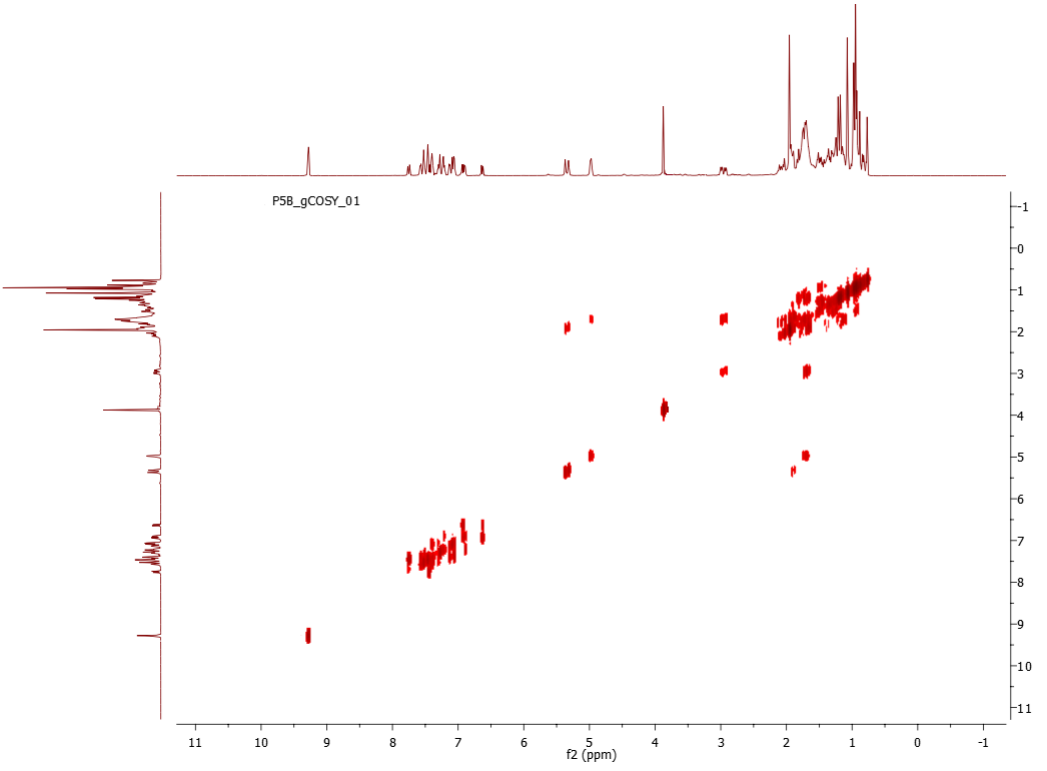


Figure 3.33. COSY Spectrum of Compound 5 (600 MHz, $CDCl_3$)

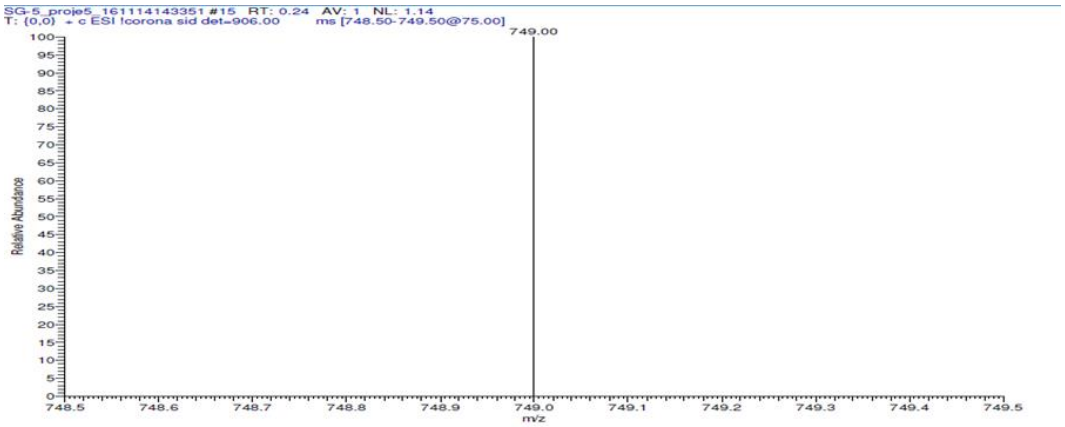


Figure 3.34. LC- Mass Spectrum of Compound 5

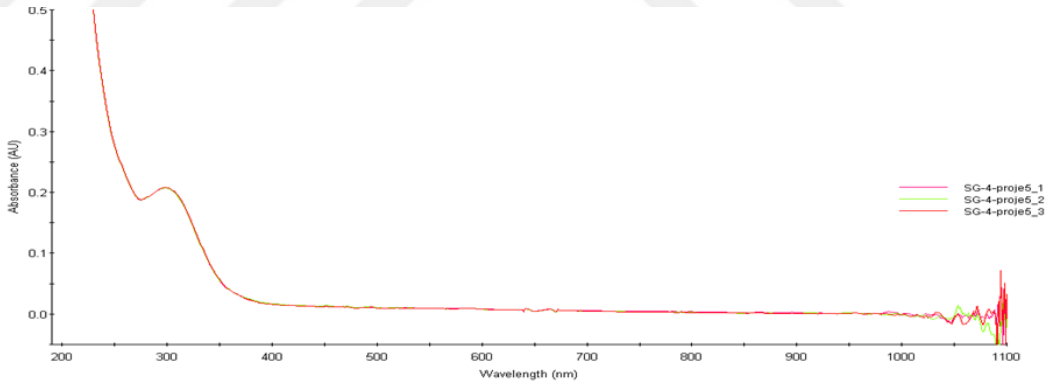


Figure 3.35. UV Spectrum of Compound 5

3.6. Structural Identification of Compound 6

Compound **6** was synthesized from Acetyl-Gypso compound and chalcone (**C6**) according to procedure given in **2.2.**

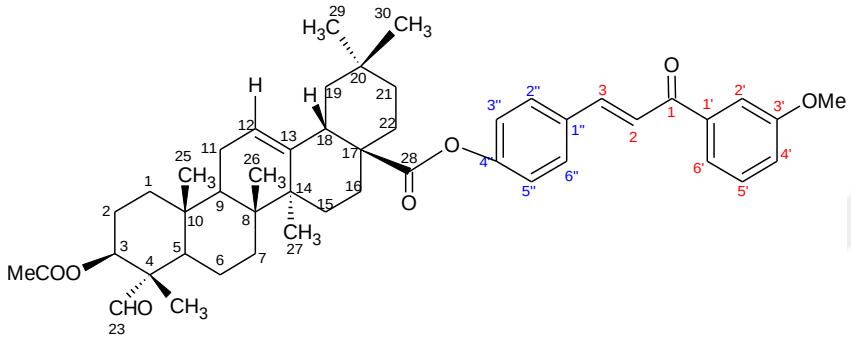
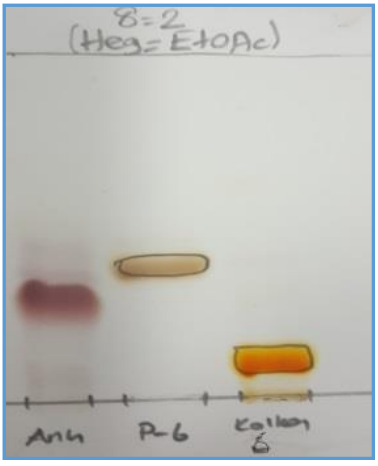


Figure 3.36. Structure of Compounds 6

TLC (Thin Layer Chromatography)



R_f: 0.4 (Hexane-Ethyl acetate 8:2)

Yield(%): 42

Melting Point (°C): 101.2- 102.4

FT-IR (cm^{-1}): 3043, 2928, 2854, 1733, 1698, 1663, 1505, 1451, 1369, 1239, 1164, 1150, 1028, 894.

($C_{48}H_{60}O_7$) Calculated m/z (%): 748,98

Positive LC-MS/MS m/z (%): 749.91 (100) $[M+1]^+$

The H-3' is not observed for compound 6 at ^{1}H-NMR. It appears at 161.5 ppm (C-3') in APT. There are no proton values for H-4'' for the chalcone. The peak is 161.5 ppm (C-4'') appeared in APT. Although at the C-28, which is bound to the Gypsogenin compound, which normally appears in 180.4 ppm in APT, after the reaction it is observed in 177.4 ppm, we understand that the reaction has occurred with the ester linkage.

Table 3.6. ^{1}H- and APT Data of Compound 6 (600/150 MHz, $CDCl_3$)

Part A			**Part B**		
Position	^{1}HNMR δ (ppm)	APT (ppm)	**Position**	^{1}HNMR δ (ppm)	APT (ppm)
3	5.00	74.1	1	-	189.2
12	5.36	123.1	2	7.32	119.4
13	-	143.9	3	7.78	143.4
18	2.96	42.7	1’	-	138.7
23	9.28	204.3	2’	7.46	112.7
24	1.06	9.5	3’	-	161.5
25	0.98	15.6	4’	7.08	119.4
26	0.81	17.2	5’	7.41	129.5
27	1.12	25.8	6’	7.53	120.1
28	-	177.4	-OCH_3	3.89	55.5
29	0.90	33.0	2”/6″	7.50	132.5
30	0.95	23.5	3″/5″	6.91	-
C**H**$_3$-COO-Anh	1.96	-	4″	-	161.5
CH_3-COO-Anh	-	20.9			
CH_3-**C**OO-Anh	-	170.1			

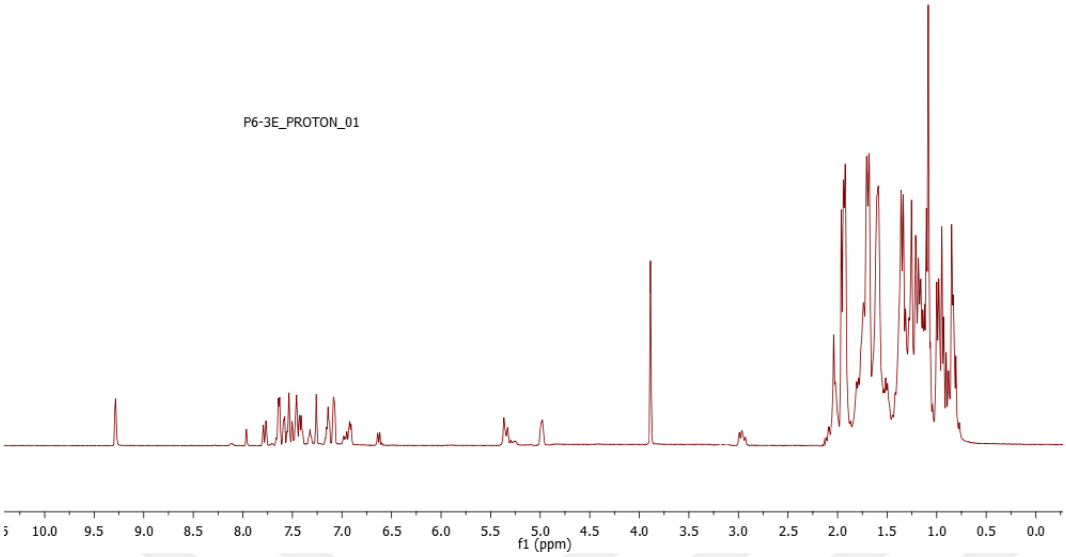


Figure 3.37. ^{1}H-NMR Spectrum of Compound 6 (600 MHz, $CDCl_3$)

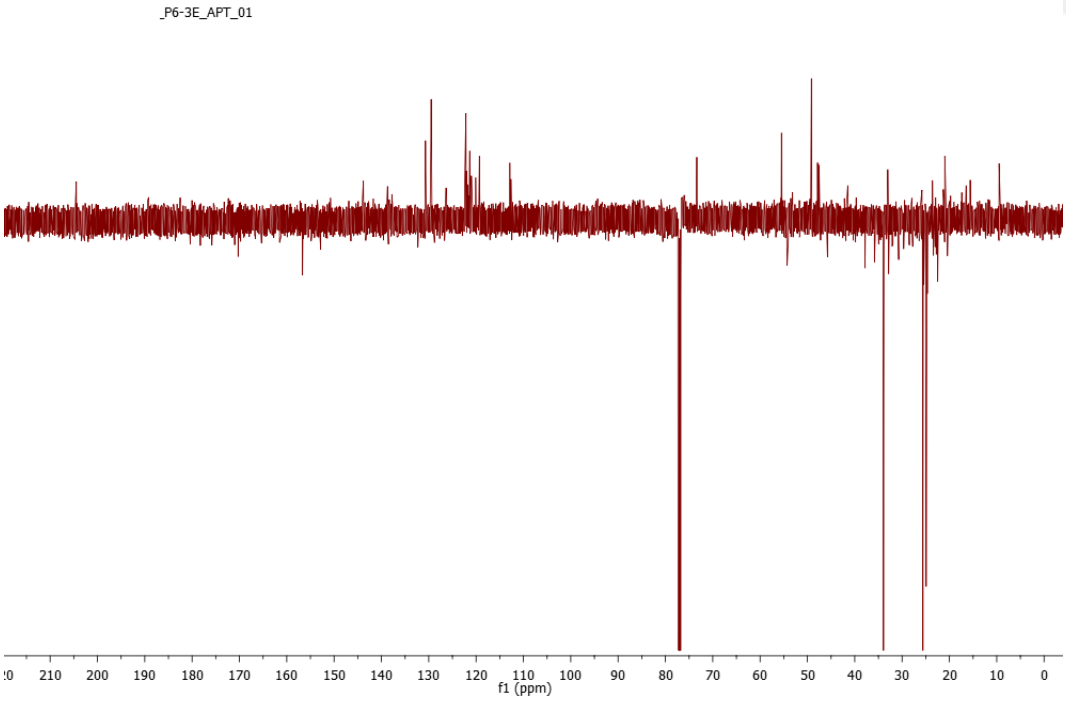


Figure 3.38. APT Spectrum of Compound 6 (150 MHz, $CDCl_3$)

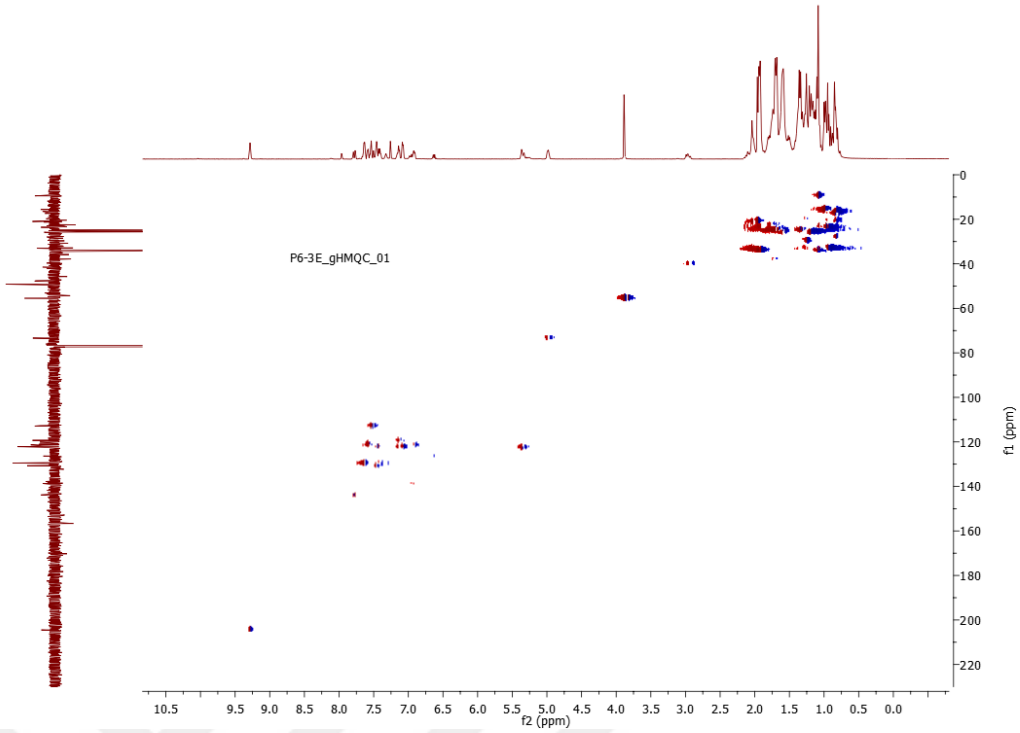


Figure 3.39. HMQC Spectrum of Compound 6 (600 MHz, $CDCl_3$)

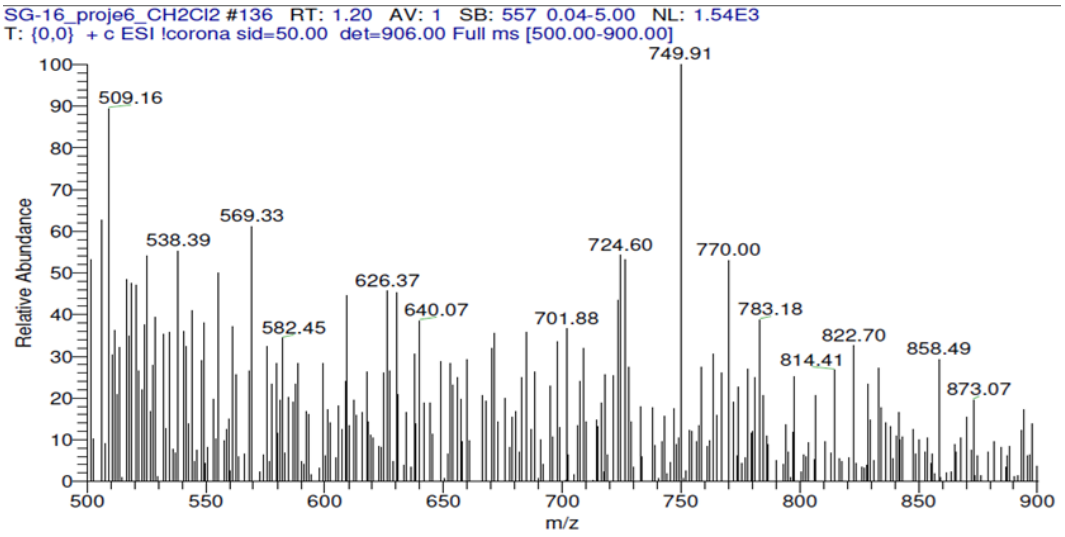


Figure 3.40. LC- Mass Spectrum of Compound 6

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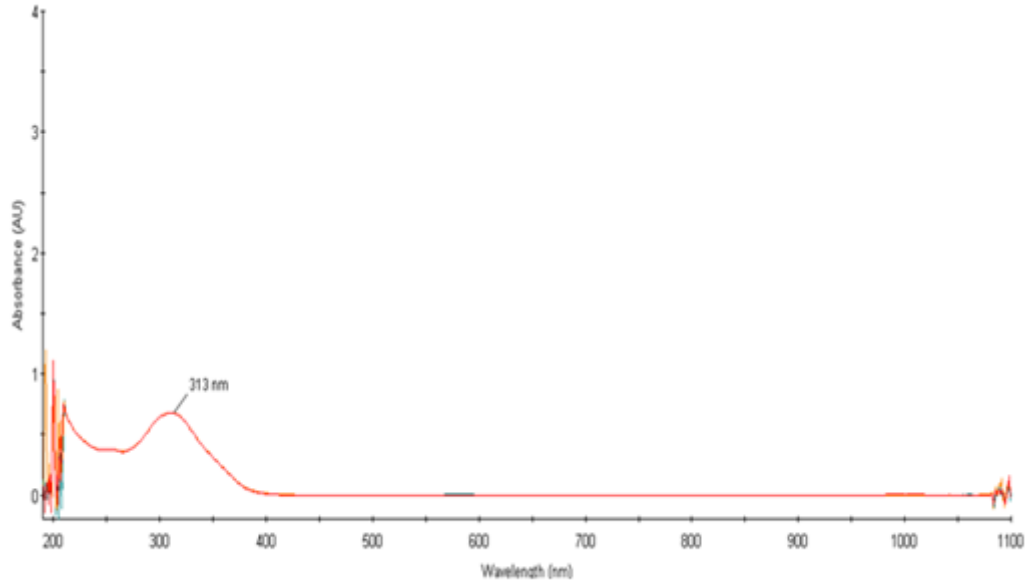


Figure 3.41. UV Spectrum of Compound 6

3.6. Structural Identification of Compound 7

Compound **7** was synthesized from Acetyl-Gypso compound and chalcone (**C6**) according to procedure given in **2.2.**

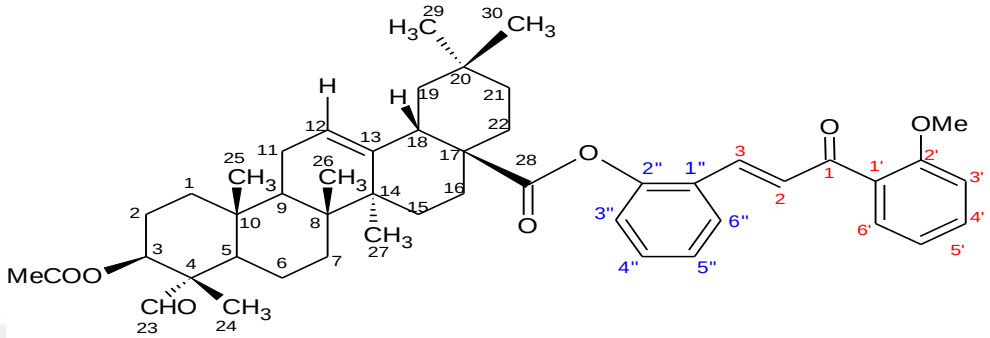
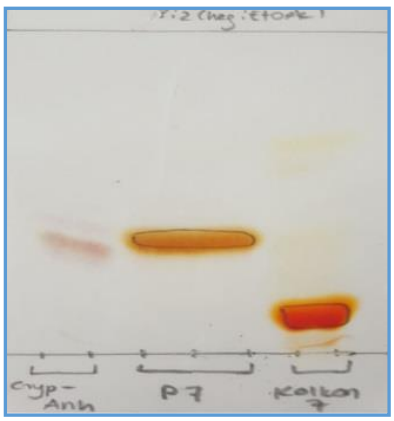


Figure 3.42. Structure of Compound 7

TLC (Thin Layer Chromatography)



R_f: 0.38 (Hexane-Ethyl acetate 8:2)

Yield(%): 20

Melting Point (ºC): 181.6 -182.4

FT-IR (cm^{-1}): 3039, 2928, 2118, 1734,1604, 1554, 1483, 1450, 1240, 1150, 1027, 891.

($C_{48}H_{60}O_7$) Calculated m/z (%): 748,98

Pozitif LC-MS/MS m/z (%): 743 (100) $[M-OCH_3+Na+2]^+$

The H-2' is not observed for compound 7 at 1H-NMR. It appears at 158.6 ppm (C-2') in APT. There are no proton values for H-2" for the chalcone. The

peak (C-2") is 158.1 ppm appeared in APT. Although at the C-28, which is bound to the Gypsogenin compound, which normally appears in 180.4 ppm in APT, after the reaction it is observed in 178.2 ppm, we understand that the reaction has occurred with the ester linkage.

Table 3.7. ^{1}H- and APT Data of Compound 7 (600/150 MHz, $CDCl_3$)

Part A			**Part B**		
Position	^{1}HNMR δ (ppm)	APT (ppm)	**Position**	^{1}HNMR δ (ppm)	APT (ppm)
3	4.98	73.3	1	-	193.4
4	-	54.3	2	6.90	121.3
12	5.35	122.6	3	7.90	143.5
13	-	143.4	1′	-	128.9
18	2.98	41.5	2′	-	158.6
23	9.28	204.4	3′	7.03	111.8
24	1.08	9.6	4′	6.84	136.4
25	0.98	14.1	5′	6.92	121.4
26	0.84	15.6	6′	7.54	129.0
27	1.20	25.6	$-OCH_3$	3.84	55.5
28	-	178.2	1″	-	120.1
29	0.88	32.9	2″	-	158.1
30	0.94	23.6	3″	6.93	116.3
$\underline{CH}_3$-COO-Anh	1.96	-	4″	7.61	131.1
$\underline{C}H_3$-COO-Anh	-	20.9	5″	7.00	125.4
CH_3-$\underline{C}$OO-Anh	-	170.2	6″	7.46	129.0

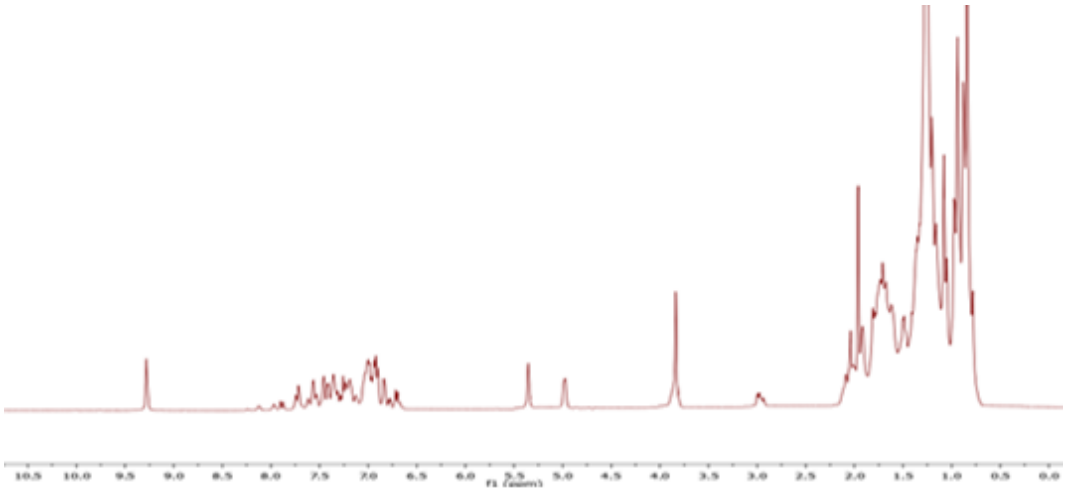


Figure 3.43. ^{1}H-NMR Spectrum of Compound 7 (600 MHz, $CDCl_3$)

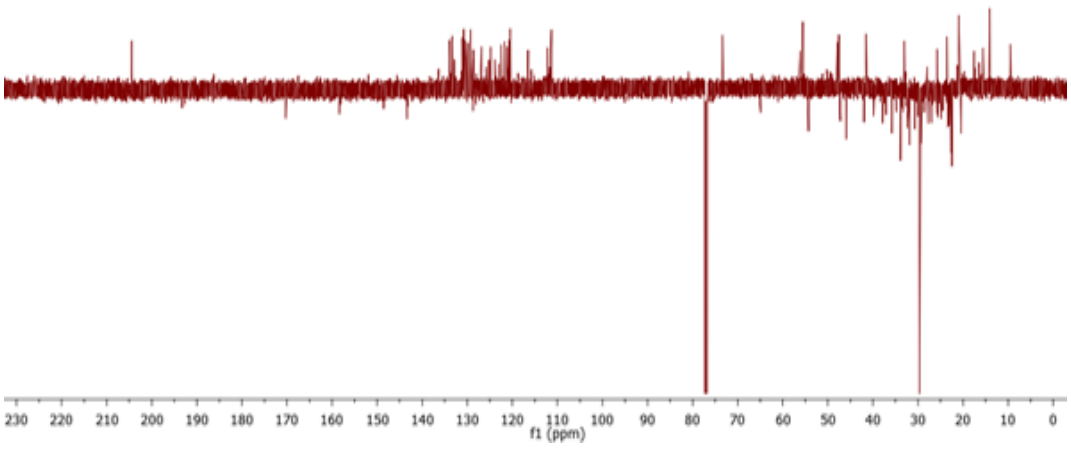


Figure 3.44. APT Spectrum of Compound 7 (150 MHz, $CDCl_3$)

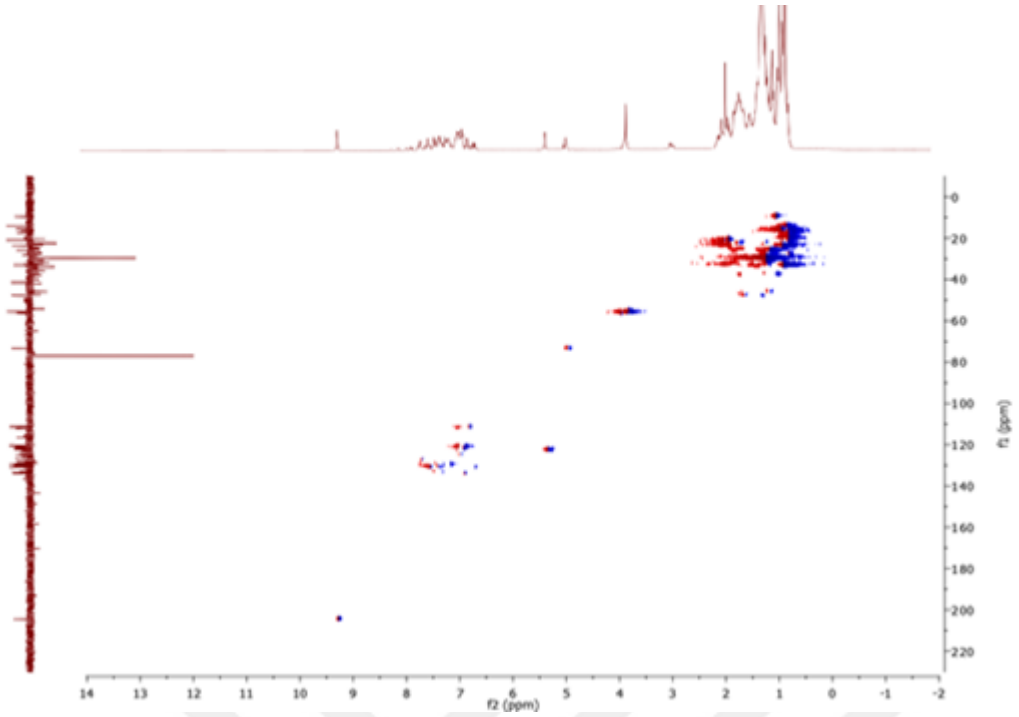


Figure 3.45. HMQC Spectrum of Compound 7 (600 MHz, $CDCl_3$)

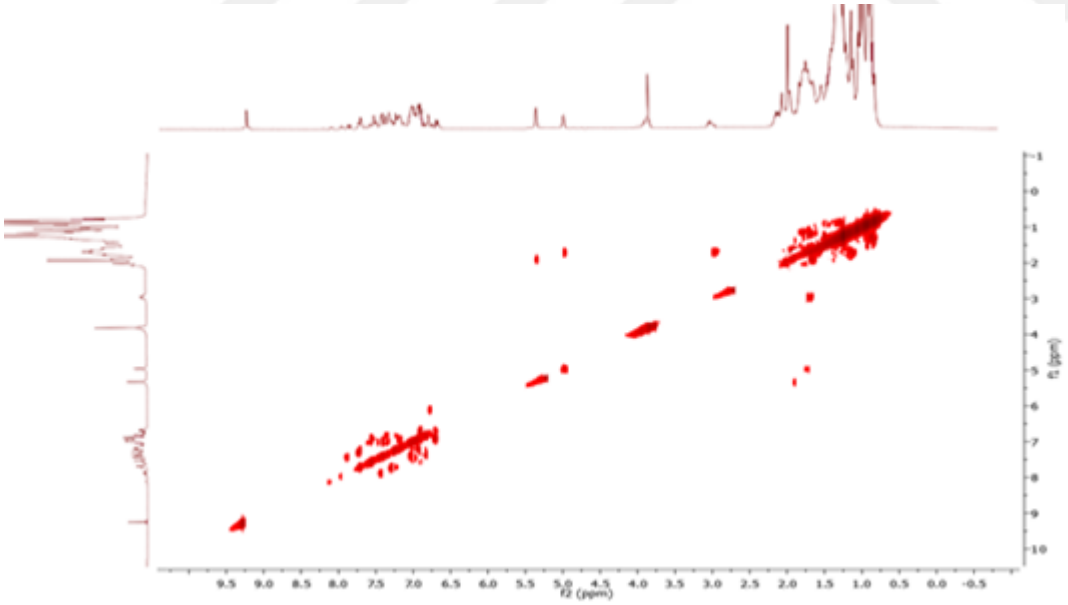


Figure 3.46. COSY Spectrum of Compound 7 (600 MHz, $CDCl_3$)

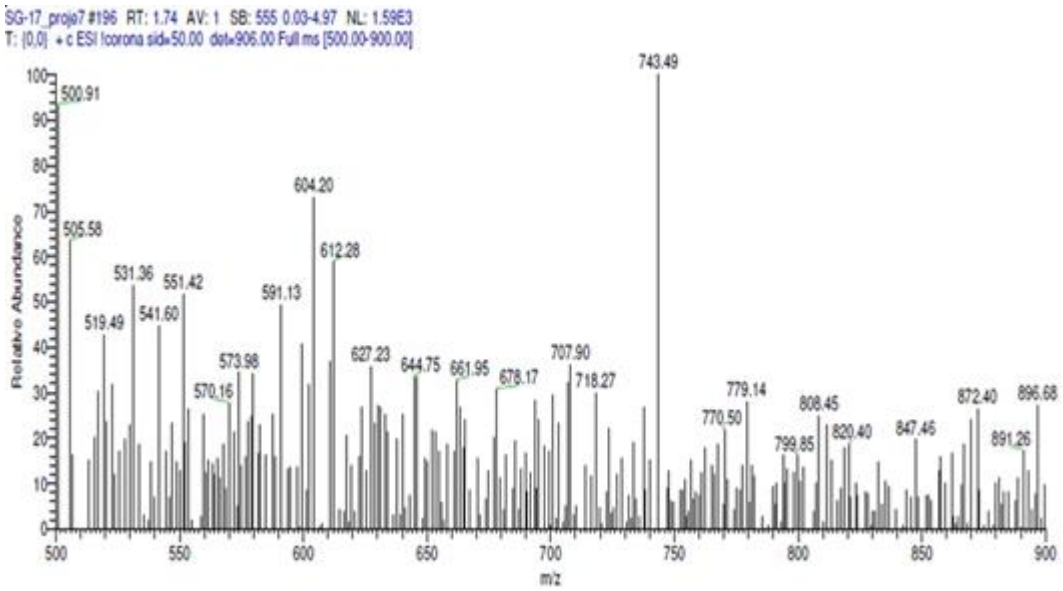


Figure 3.47. LC- Mass Spectrum of Compound 7

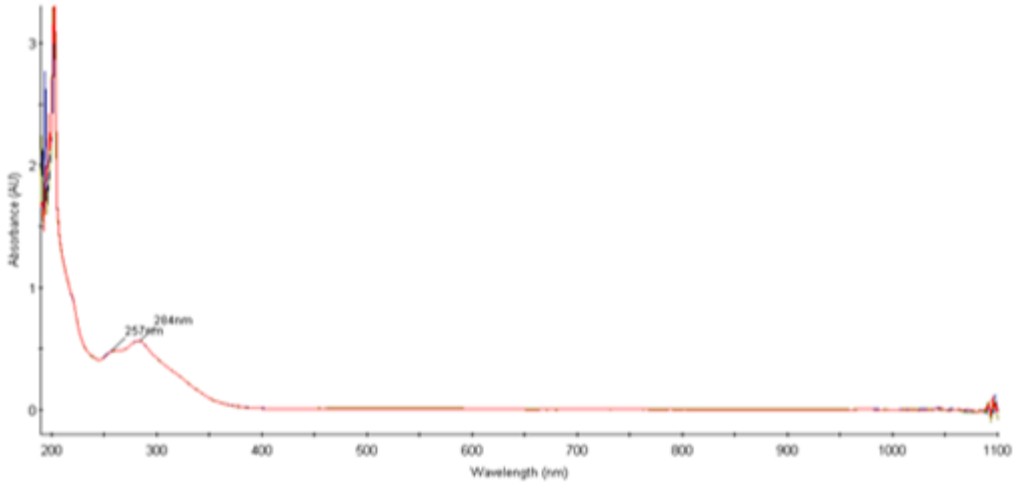


Figure 3.48. UV Spectrum of Compound 7

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3.7. Structural Identification of Compound 8

Compound **8** was synthesized from Acetyl-Gypso compound and chalcone (**C8**) according to procedure given in **2.2.**

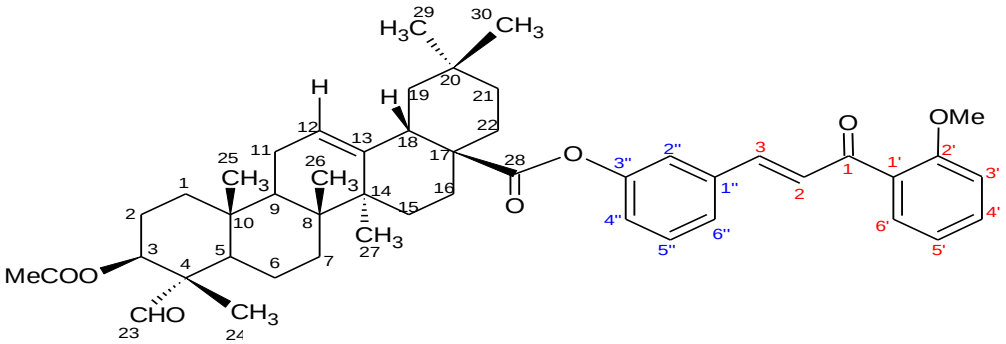
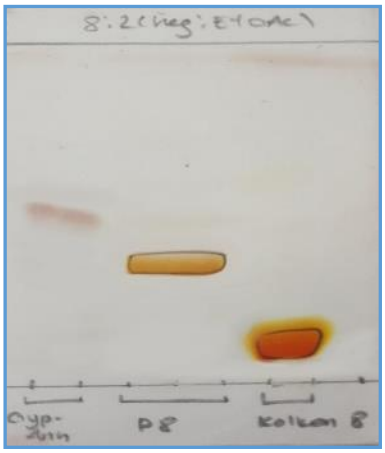


Figure 3.49. Structure of Compounds 8

TLC (Thin Layer Chromatography)



R_f: 0.38 (Hexane-Ethyl acetate 8:2)

Yield(%): 68

Melting Point (°C): 211.6-213.2

FT-IR (cm^{-1}): 3043, 2929, 1733, 1629, 1563, 1450, 1370, 1318, 1242, 1149, 1028, 891.

($C_{48}H_{60}O_7$) Calculated m/z (%): 748,98

Pozitif LC-MS/MS m/z (%): 716 (100) $[M-(CH_3CO+OMe)+K+2]^+$

The H-2' is not observed for compound 8 at ^{1}H-NMR. It appears at 158.1 ppm (C-2′) in APT. There are no proton values for H-3" for the chalcone. The

peak is 156.8 ppm (C-3'') appeared in APT. Although at the C-28, which is bound to the Gypsogenin compound, which normally appears in 180.4 ppm in APT, after the reaction it is observed in 176.1 ppm, we understand that the reaction has occurred with the ester linkage.

Table 3.8. ^{1}H- and APT Data of Compound 8 (600/150 MHz, $CDCl_3$)

Part A			**Part B**		
Position	^{1}HNMR δ (ppm)	APT (ppm)	**Position**	^{1}HNMR δ (ppm)	APT (ppm)
3	4.98	73.4	1	-	192.8
4	-	54.3	2	7.69	125.6
12	5.35	122.4	3	7.53	142.1
13	-	143.3	1′	-	129.2
18	2.97	41.5	2′	-	158.1
23	9.26	204.5	3′	6.98	111.7
24	1.07	9.5	-OCH_3	3.88	55.8
25	0.94	15.6	1″	-	136.7
26	0.84	17.4	3″	-	156.8
27	1.20	25.6	5″	7.02	-
28	-	176.1	6″	7.46	-
29	0.91	33.0			
30	0.96	23.6			
C**H**$_3$-COO-Anh	1.95	-			
CH_3-COO-Anh	-	20.4			
CH_3-**C**OO-Anh	-	170.3			

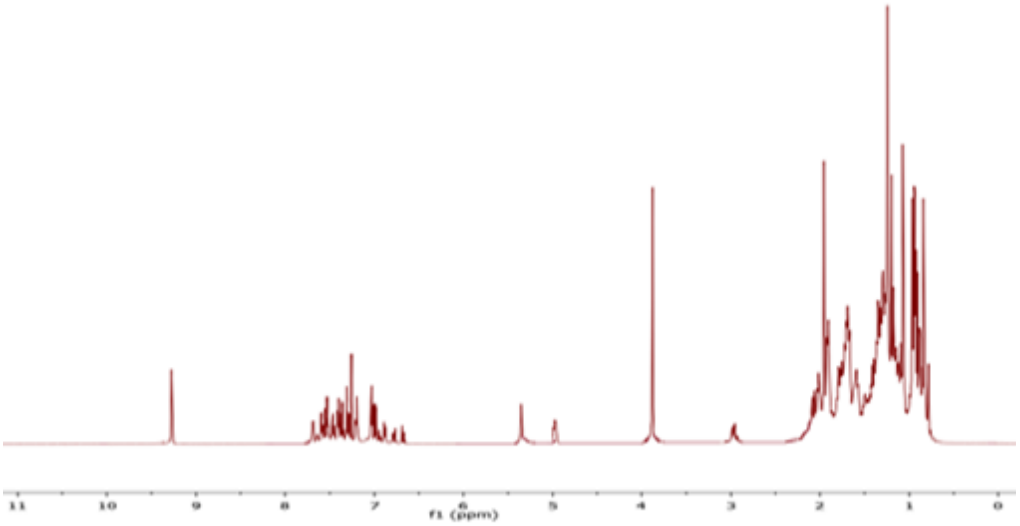


Figure 3.50. ^{1}H-NMR Spectrum of Compound 8 (600 MHz, $CDCl_3$)

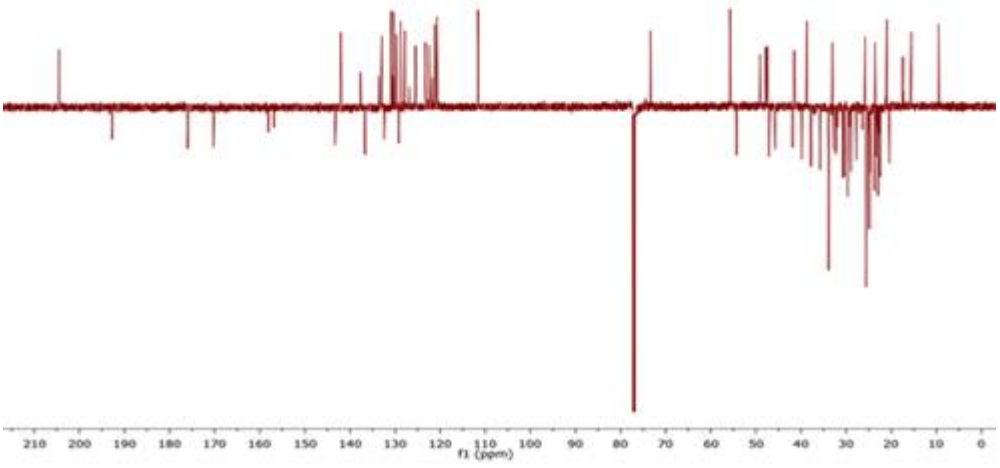


Figure 3.51. APT Spectrum of Compound 8 (150 MHz, $CDCl_3$)

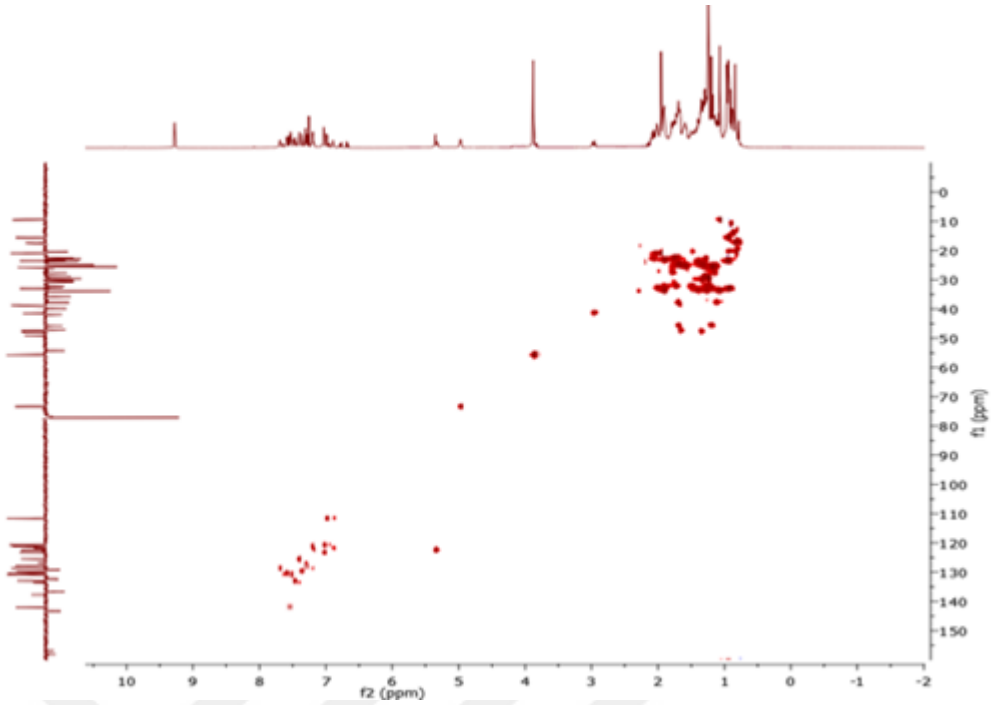


Figure 3.52. HMQC Spectrum of Compound 8 (600 MHz, $CDCl_3$)

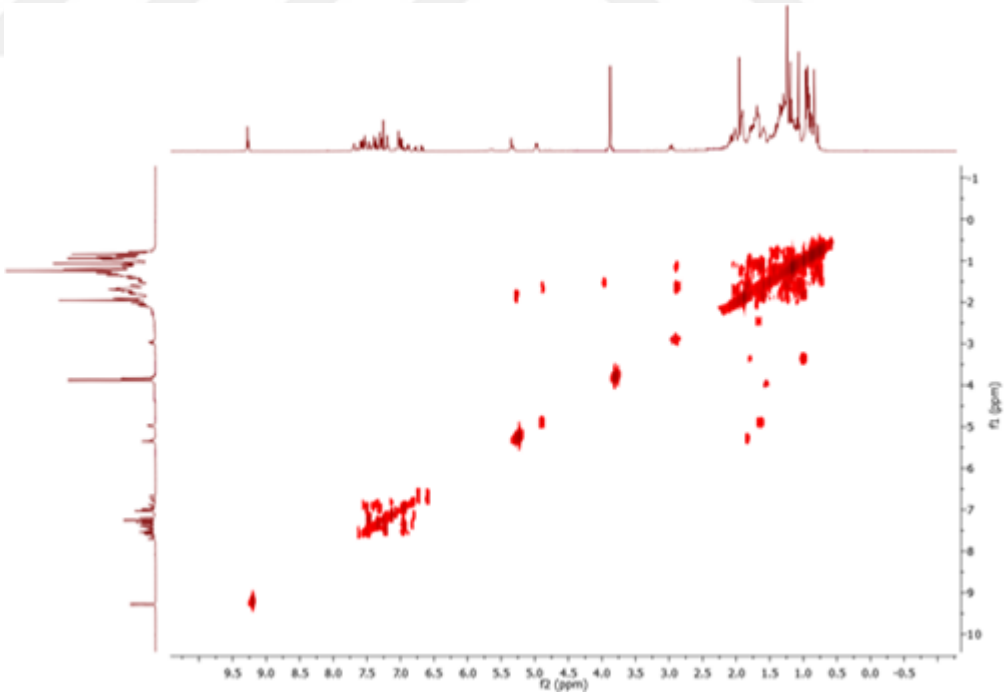


Figure 3.53. COSY Spectrum of Compound 8 (600 MHz, $CDCl_3$)

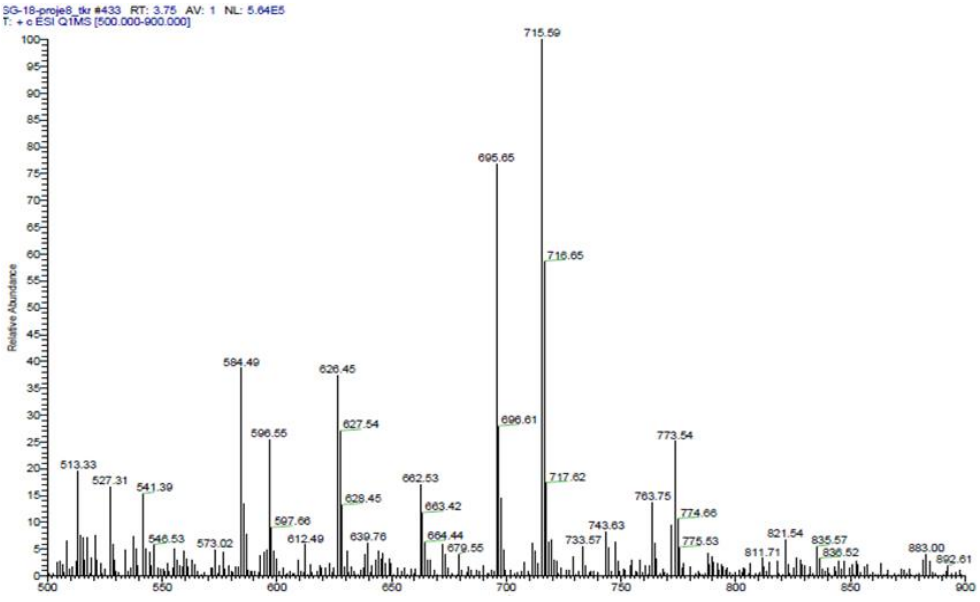


Figure 3.54. LC- Mass Spectrum of Compound 8

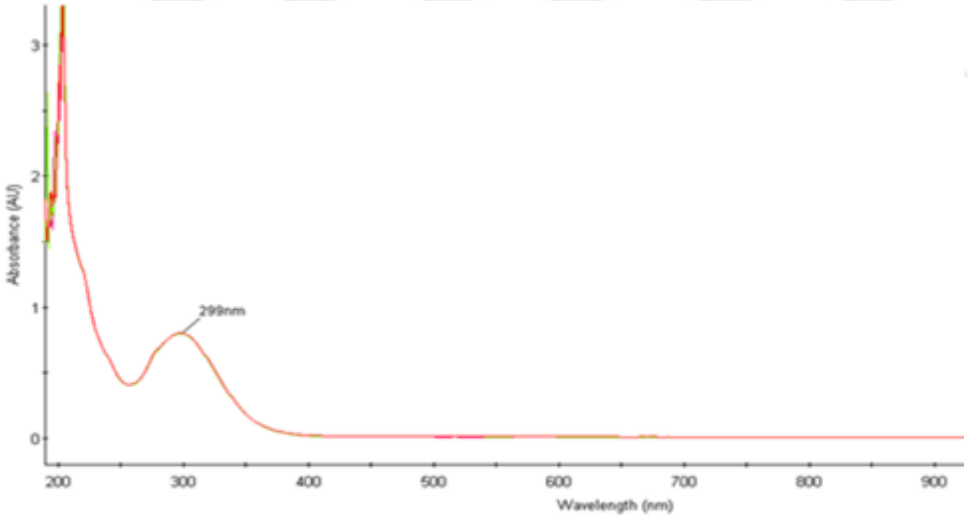


Figure 3.55. UV Spectrum of Compound 8

3.8. Structural Identification of Compound 9

Compound **9** was synthesized from Acetyl-Gypso compound and chalcone (**C9**) according to procedure given in **2.2.**

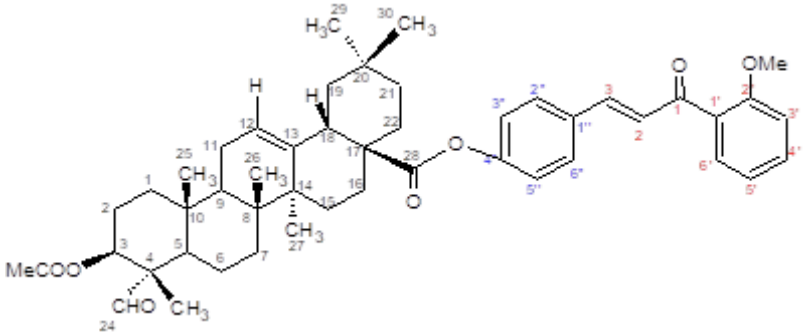
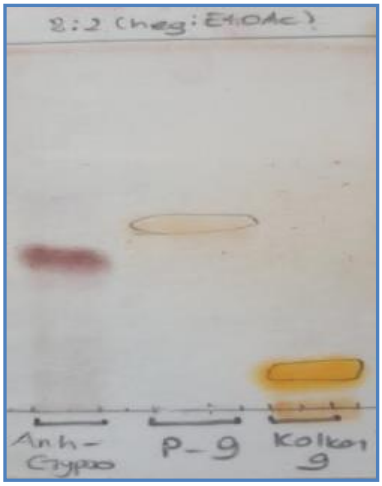


Figure 3.56. Structure of Compound 9

TLC (Thin Layer Chromatography)



R_f: 0.5 (Hexane-Ethyl acetate 8:2)

Yield(%): 32

Melting Point (ºC): 202.0 - 204.3

FT-IR (cm^{-1}): 3045, 2926, 2883, 1967, 1719,1603, 1466, 1341, 1279, 1095, 960, 841.

($C_{48}H_{60}O_7$) Calculated m/z (%): 748,98

LC-MS/MS m/z (%):749,00 (100) $[M]^+$

The H-2' is not observed for compound 9 at ^{1}H-NMR. It appears at 158.2 ppm (C-2') in APT. There are no proton values for H-4" for the chalcone. The peak is 161.6 ppm (C-4") appeared in APT. Although at the C-28, which is bound to the Gypsogenin compound, which normally appears in 180.4 ppm in APT, after the reaction it is observed in 176.2 ppm, we understand that the reaction has occurred with the ester linkage.

Table 3.9. ^{1}H- and APT Data of Compound 9 (600 /150 MHz, $CDCl_3$)

Part A			**Part B**		
Position	^{1}HNMR δ (ppm)	APT (ppm)	**Position**	^{1}HNMR δ (ppm)	APT (ppm)
3	4.98	73.3	1	-	196.2
4	-	54.2	2	7.05	-
12	5.34	122.2	3	7.58	142.3
13	-	143.4	1′	-	129.2
18	2.97	41.5	2′	-	158.2
23	9.28	205.2	3′	6.98	111.6
24	1.09	9.3	4′	7.68	133.7
25	0.99	15.4	5′	6.92	120.9
26	0.85	17.2	6′	7.70	130.7
27	1.20	25.7	-OCH_3	3.84	55.6
28	-	176.2	2″/6″	7.62	129.4
29	0.94	32.8	3″/5″	6.75	-
30	0.96	23.4	4″	-	161.6
C**H**$_3$-COO-Anh	1.96	-			
CH_3-COO-Anh	-	20.7			
CH_3-**C**OO-Anh	-	170.4			

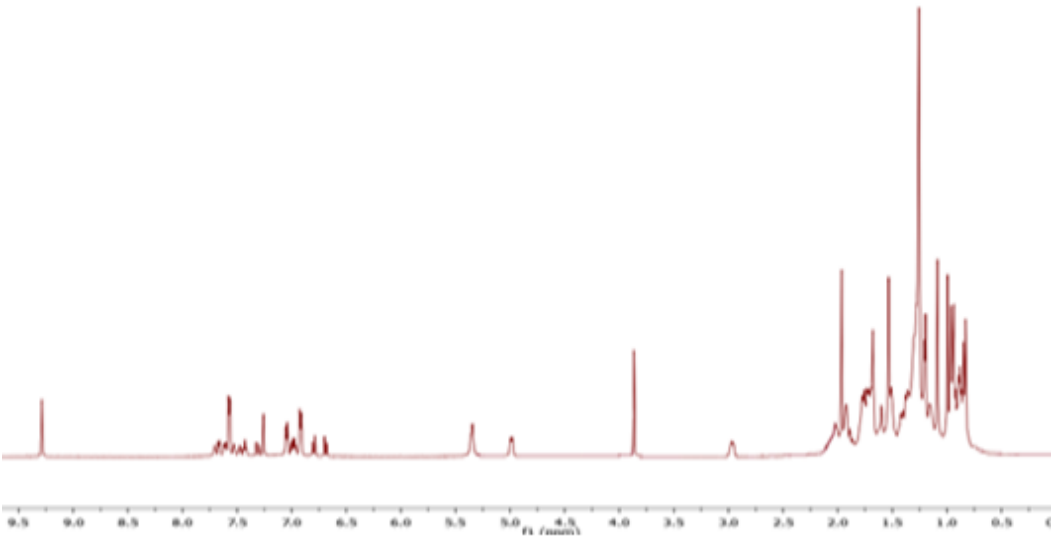


Figure 3.57. ^{1}H-NMR Spectrum of Compound 9 (600 MHz, $CDCl_3$)

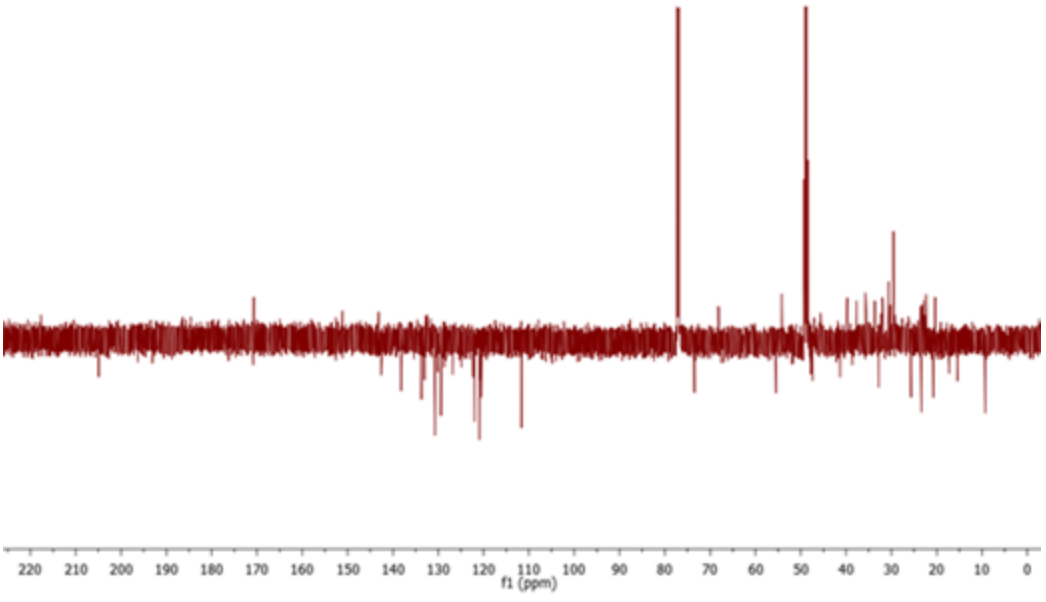


Figure 3.58. APT Spectrum of Compound 9 (150 MHz, $CDCl_3$)

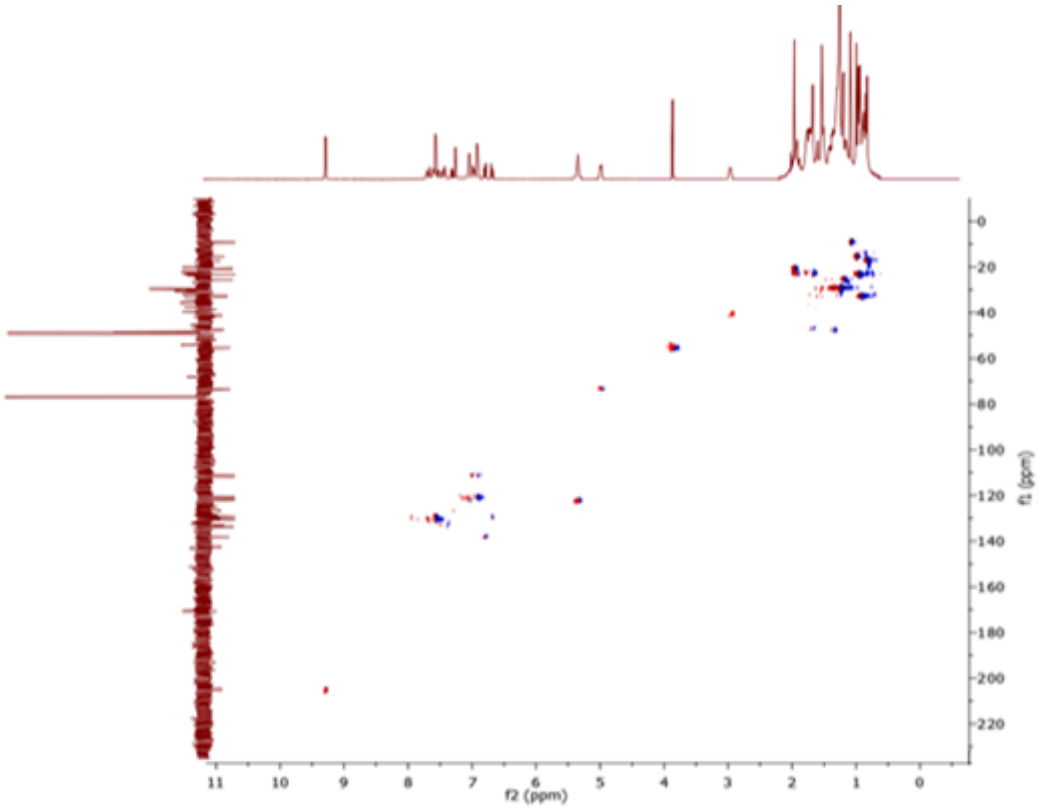


Figure 3.59. HMQC Spectrum of Compound 9 (600 MHz, $CDCl_3$)

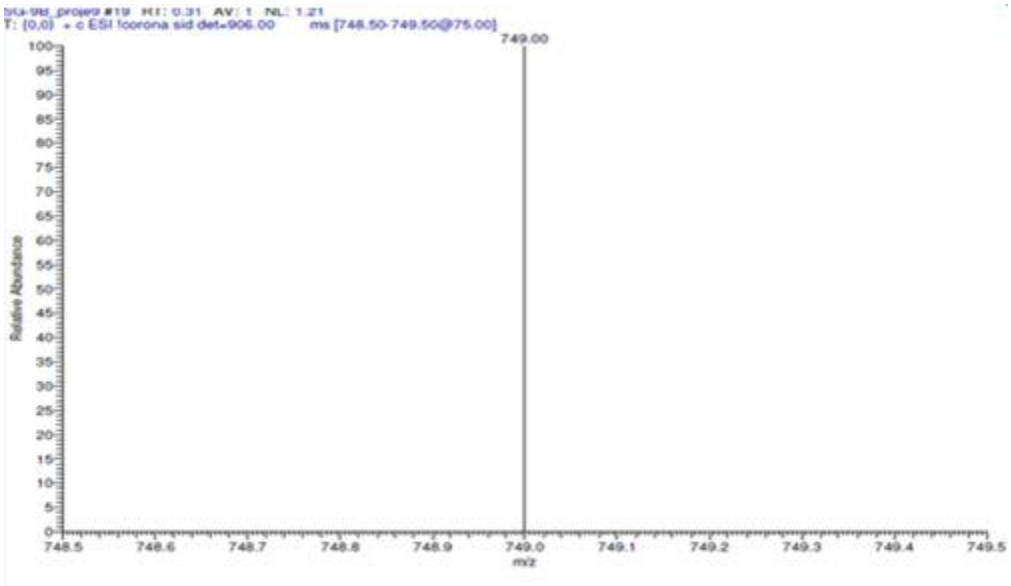


Figure 3.60. LC- Mass Spectrum of Compound 9

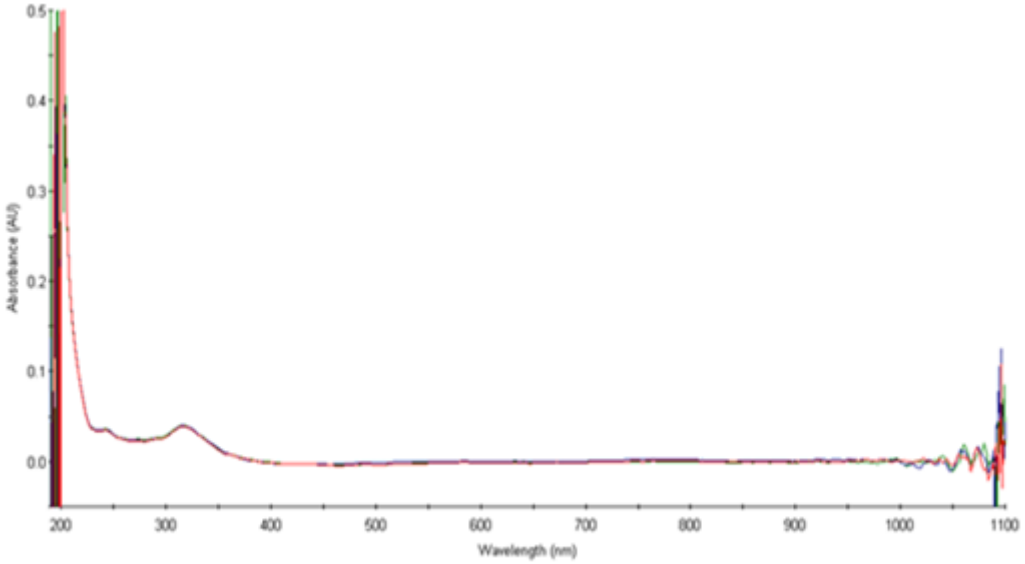


Figure 3.61. UV Spectrum of Compound 9

3.9. Antimicrobial Activity of gypsogenin-COO-chalcone hybrid compounds

Antimicrobial activity tests are those tests conducted to determine the in-vitro efficacy of an antimicrobial agent against a particular bacterial challenge. The lowest concentration of antibiotics (µg / ml) that prevents visible reproduction of a microorganism within a defined period is defined as minimal inhibitory concentration (MIC) which is a guide to the susceptibility of microorganisms and helps when deciding on treatment.

The antimicrobial activities of these compounds were evaluated by applying to different strains. The antimicrobial activity 3 different gram positive of (*Staphylococcus aureus ATCC 29213, Bacillus subtilis RSKK 02021 and Enterococcus faecalis ATCC 29212*) and 2 different gram-negative bacteria of (*Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853*) and 1 yeast (*Candida albicans ATCC 90028*) was determined by microdilution method. The results are given in **Table 3.10.**

*:

Table 3.10. Antimicrobial Activity Results of Compounds 1-9

Microorganism and minimal inhibitory concentration (MIC) determination (μg/mL)						
	Gram Positive			**Gram Negative**		**Yeast**
Compound No	***B.subtilis***	***E. faecalis***	***S.aureus***	***E. coli***	***P.aeruginosai***	***C. albicans***
1	512	256	128	>1024	512	>1024
2	256	256	512	>1024	512	>1024
3	64	32	128	512	>1024	>1024
4	256	64	128	>1024	512	>1024
5	256	512	128	1024	512	>1024
6	128	32	256	1024	1024	>1024
7	256	64	>1024	1024	1024	>1024
8	512	64	>1024	>1024	>1024	>1024
9	256	256	512	1024	512	>1024
Gypsogenin	256	64	64	>1024	1024	>1024
Acetyl-Gypso	128	64	128	>1024	1024	>1024
Siprofloksasin	0.06	0.25 (0.25-1)*	0.25 (0.12-0.5)*	0.015	0.25 (0.25-1)*	-
Flukonazol	-	-	-	-	-	1 (0.25-1)+
DMSO	ED	ED	ED	ED	ED	ED

* Acceptable quality control of ciprofloxacin for reference bacteria MIC limit values

+ Reference for Candida Acceptable quality control of fluconazole MIC limit values

Note: DMSO, which we used as a solvent with the ingredients, was also included in the study, no inhibition was observed.

4. CONCLUSION

In this study, we are synthesized between the natural Acetyl-Gypso compound and chalcones, both with high-active, the synthesise 9 new compounds from acetyl-gypso and chalcones at the first time.

All the compounds were isolated and purified by chromatographic methods thin layer chromatography (TLC) and colon chromatography (CC) and identified using spectroscopic techniques (^{1}H, ^{13}C- NMR, HMQC, APT, COSY, LC-MS, FT-IR and UV). When the compounds were examined, the chalcone part (B) and acetyl-gypso compound part (A), the bonding at C28 and the different structures for each compound product has ensured that we were able to interpret that the reaction took place by showing different spectral values in the illumination of the spectroscopic structure. Gypsogenin, Acetyl-Gypso compound and synthesized derivatives were evaluated for their antimicrobial activities (against five bacteria and one yeast) using the MIC method.

When the results of antimicrobial activity on the compounds in **Table 3.10.** are examined, compounds **2, 3, 4, 5, 6, 7,8** and **9** inhibits the growth of gram-positive bacteria used at concentrations of 16-256 μg / mL. The compounds **1**, **2, 3, 4, 5** and 9 are inhibited the growth of gram-negative bacteria used at concentrations of 256-512 μg / mL. Thus, these compounds have useful capacities with a distinct bactericidal effect in gram-positive bacterial infections. The activity of synthesized compounds was found to be effective in the gram-positive bacterial selectivity of compounds **3** and **6** and gram-negative bacterial selectivity of compounds **1, 2, 4, 5** and **9** which are compared to the main starting compound is Gypsogenin. However, no compound showed an antifungal effect.

As can be understood from the activities described above, it has been proven that of each compound shows high activity, besides, the new semisynthesis reactions formed which is showed high activity by combining two different compounds.

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CURRICULUM VITAE

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EĞİTİM VE SEMİNERLER

- 1st International Eurasian Conference on Biological and Chemical Sciences (EurasiaBioChem'18) - Nisan 2018
- 1st International Congress on Medicinal and Aromatic Plants (TABKON'17) - Mayıs 2017
- The 3rd International Mediterranean Symposium on Medicinal and Aromatic Plants (MESMAP3) - Nisan 2017
- Uluslararası Katılımlı 5. İlaç Kimyası: İlaç Etken Maddesi Tasarımı, Sentezi, Üretimi ve Standardizasyonu Kongresi - Nisan 2017
- The 2nd Japan-Turkey International Symposium on Pharmaceutical and Biomedical Sciences (JATUSPAB-2) – Eylül 2017
- Bahçeşehir Üniversitesi- 4th International BAU Drug Design Congress-Ekim 2016
- Ege Üniversitesi - 6. Ulusal Kimya Öğrenci Kongresi- Mayıs 2015
- Elginkan Vakfı-TS-EN ISO 17025 Kalibrasyon ve Laboratuvar Akreditasyon Yönetim Sistemi- Haziran 2014
- Elginkan Vakfı- TS-EN ISO 22000 Gıda Güvenliği Yönetim Sistemi- Haziran 2014
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