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GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

(Ph.D. THESIS)

SEMI-SYNTHESIS OF ASTRAGENOL DERIVATIVES AND THEIR BIOLOGICAL ACTIVITIES

Özgür TAĞ

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Özgür TAĞ

ÖZET

ASTRAGENOL TÜREVLERİNİN YARI-SENTEZLERİ VE BUNLARIN BİYOLOJİK AKTİVİTELERİ

Özgür TAĞ

Doktora Tezi, Kimya Anabilim Dalı
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Tümör inhibitörü olmalarından dolayı bazı saponinler, son zamanlarda farmakolojik olarak anti-tümör moleküller olarak hedef durumuna gelmiştir. Saponin temelli yarı-sentetik antikanser ilaç keşfi programları ticari olarak bulunabilen triterpenler ile bir süredir yapılmaktadır. Ancak, Astragalus sikloartanlarının analoglarının hazırlanması ile ilgili çalışmalar çok sınırlıdır. Bu nedenle, sikloastragenol ve siklokantagenol'en hareketle yeni antikanser ajanların kimyasal sentez yoluyla hazırlanması konusunda çalışmalar yapılmıştır.

Bu çalışmada, *Astragalus* bitkisinden en yüksek verimle Astragaloside IV, Astragaloside VII, Siklokantagenol ve Sikloastragenol moleküllerinin eldesi amacıyla ekstraksiyon optimizasyonu yapılmıştır. Elde edilen aglikonlardan (siklokantagenol ve sikloastragenol) oksidasyon, açilasyon, alkilasyon, eliminasyon, kondenzasyon reaksiyonları ile 38 yarı sentetik türev hazırlanmıştır. Bu türevler dört farklı kanser hücre hattına karşı (insan prostat kanser hücre hatları: PC-3 and DU-145; insan kolon adenokarsinom hücre hattı: HT-29; insan meme adenokarsinom hücre hattı: MDA-MB- 231) sitotoksik etkileri yönünden taranmıştır. Kırk molekülden 16'sının başlangıç maddesi olan CG'den daha aktif olduğu belirlenmiştir. PC-3 üzerinde 3, DU-145 üzerinde 1, HT-29 üzerinde 1 ve MDA-MB-231 üzerinde 4 molekülün IC_{50} değeri 10 µM'ın altında bulunmuştur.

Anahtar Sözcükler: *Astragalus*, saponin, sikloartan, sikloastragenol, astragenol, sitotoksik aktivite, yarı-sentez.

ABSTRACT

SEMI-SYNTHESIS OF ASTRAGENOL DERIVATIVES AND THEIR BIOLOGICAL ACTIVITIES

TAĞ, Özgür

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Supervisor: Asoc. Prof. Dr. Tamer KARAYILDIRIM
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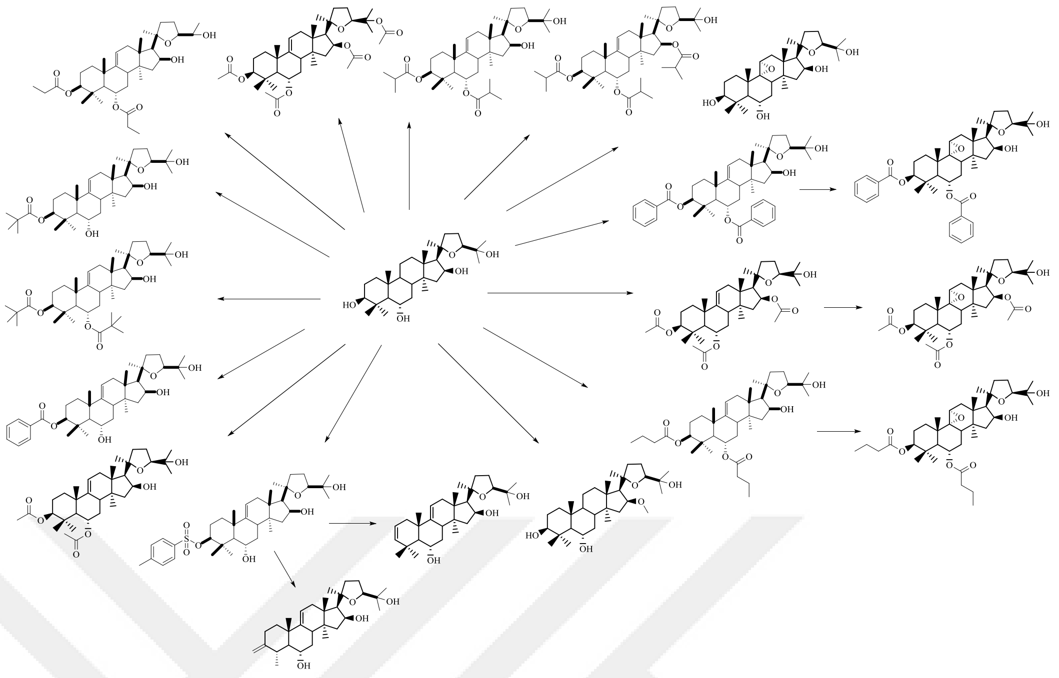
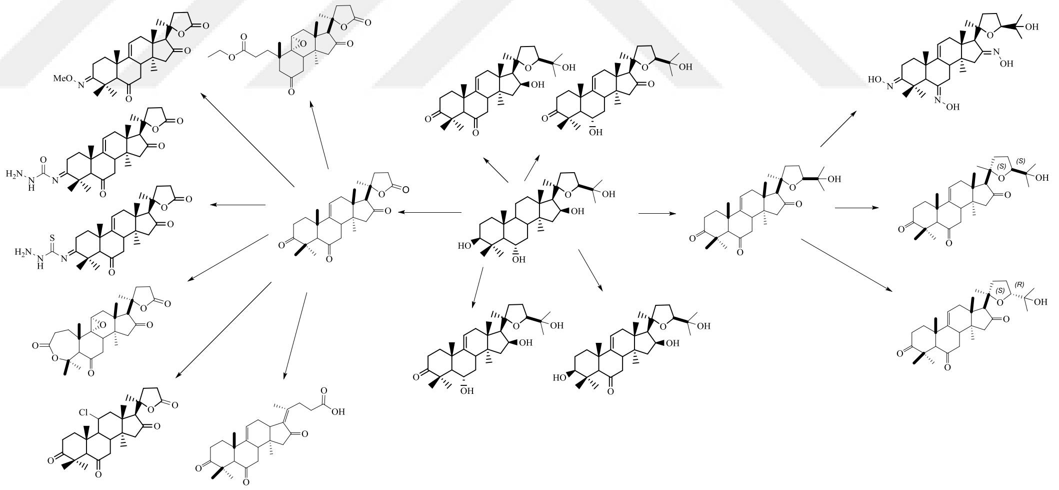
After recognizing as potent tumor inhibitors, saponins have suddenly become one of the primary targets for new pharmacologically active anti-tumor molecules. Semi-synthetic anticancer drug discovery programs focusing on saponins mainly engaged with commercially available triterpenoids. However, studies on the preparation of analogues of *Astragalus* cycloartanes have so far been limited. Therefore, the exploration of new anti-cancer agents prepared from cycloastragenol and cyclocanthagenol via semi-synthesis studies.

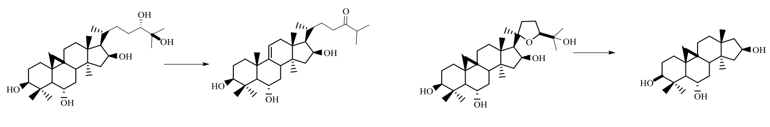
In this study, extraction conditions were optimized to achieve highest yield of Astragaloside IV, Astragaloside VII, cyclocanthagenol and cycloastragenol from *Astragalus trojanus*. Forty semi-synthetic derivatives from the isolated aglycones (cyclocanthagenol and cycloastragenol) were prepared by oxidation, esterification, alkylation, elimination and condensation reactions. The compounds were screened for their cytotoxicities against four different cancer cell lines (human prostate cancer cell lines: PC-3 and DU-145; human colon adenocarcinoma: HT-29; human breast adenocarcinoma: MDA-MB- 231). Sixteen out of 38 molecules were established to be more active than the starting compound CG. Three molecules on PC-3 cell line; 1 molecule on DU-145 cell line; 1 molecule on HT-29 cell line and 4 molecules on MDA-MB- 231 cell line exhibited IC_{50} values lower than 10 µM.

Keywords: *Astragalus*, saponin, cycloartane, cycloastragenol, astragenol, cytotoxic activity, semi-synthesis.

GRAPHICAL ABSTRACT







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ABBREVIATIONS

Ac	Acetyl
AG IV	Astragaloside IV
AG VII	Astragaloside VII
AG	Astragenol
APT	Attached-Proton-Test
n-BuOH	Butanol
$CDCl_3$	Deuterated chlorofom
CG	Cycloastragenol
CH_2Cl_2	Dichloromethane
CH_3OH	Methanol
$CHCl_3$	Chloroform
d	Doublet
DCC	*N*,*N′*-Dicyclohexylcarbodiimide
dd	Doublet of doublets
ddd	Doublet of doublet of doublets
DMAP	4-(Dimethylamino)pyridine
DMF	*N*,*N*-Dimethyl formamide
DMSO-*d6*	Deuterated dimethylsulfoxide
DQF-COSY	Double Quantum Filtered Correlation Spectroscopy
EtOAc	Ethyl acetate

EtOH	Ethanol
FAB-MS	Fast Atom Bombardment Mass Spectrometry
FT-IR	Fourier Transform Infrared
GC	Gas Chromatography
H_2O	Water
HMBC	Heteronuclear Multiple Bond Coherence
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High Performance Liquid Chromatography
HSQC	Heteronuclear Single Quantum Coherence
Hz	Hertz
IC_{50}	Half maximal inhibitory concentration
J	Spin-spin coupling
LC-MS	Liquid Chromatography-Mass Spectrometry
m	Multiple
m/*z*	Mass charge ratio
MALDITOF	Matrix-assisted Laser Desorption Ionization Time-of-flight
MeCN	Acetonitrile
MCPBA	*meta*-Chloroperoxybenzoic acid
MPLC	Medium Pressure Liquid Chromatography
NMR	Nuclear Magnetic Resonance
ppm	Parts per million

Pyridine-*d5*	Deuterated pyridine
q	Quartet
RP-VLC	Reverse Phase Vacuum Liquid Chromatography
s	Singlet
SKG	Cyclocanthogenol
t	Triplet
td	Triplet of doublets
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Trimethylsilyl
UV	Ultraviolet
^{13}C-NMR	Carbon Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance

1. INTRODUCTION

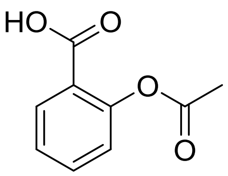
Drug Discovery from Natural Sources

Humans have been using nature for curing various illnesses starting from ancient times. Plants have been the core constituent of conventional medicine for thousands of years, and evidence of the first use of plants lead to Mesopotamia in 2600 BC. They attained more than thousand oils from plants, including *Papaver somniferum* (poppy juice), *Cydrus* (cedar), *Commiphora* species (myrrh), *Cupressus sempervirens* (cyprus), for healing today’s widespread diseases such as coughs, influenza, parasitic infection and inflammation (Newman et al., 2000).

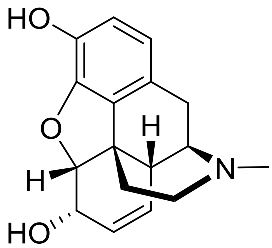
Morphine was the first recorded pharmaceutically active compound that had been isolated from *Papaver somniferum* in 1805 by Friedrich Wilhelm Adam Sertürner. The discovery of morphine, initiated a new age, hence drugs from plants began to be purified, studied, and administered in precise dosages (Buss and Butler, 2010). Recently there has been growing interest in alternative therapies and the therapeutic use of natural products, particularly in plant derived drugs and near about 25% of prescribed drug in the world originate from plants (Newman et al., 2016).

Significant amount of people around the world, excessively trust to conventional medicine, and choose as their main treatment method over modern medicine. In China, using of plants and herbs quite popular even now and this natural product sources promote new drug discoveries (Newman et al., 2000). Natural products have valuable influence on medications in developed countries. They used as drugs in their current forms or they are starting molecule for new drug discovery.

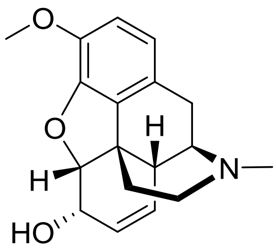
Natural products contain not only pharmaceutically or biologically active molecules, but these are also good sources for many different chemical compounds. Multiple chiral centers, heterocyclic substituents and poly-cyclic structures are the main reason of the complexity of these kind of molecules (Newman et al., 2008). However, secondary metabolites frequently have poor bioactivity, high toxicity, and poor solubility, and as a result of these, they fail to be a drug candidate. Besides, further modifications of these compounds are often necessary to improve bioactivity and physical properties for therapeutic use (Opera et al., 2001). The most well-known example, salicin isolated from the bark of *Salix alba*, was modified into aspirin then used worldwide as a potent pain killer and anti-coagulant (McRae et al., 2007). Another instance, morphine and codeine from opium poppy were model substrates for the development of the analgesics meperidine, pentazocine and dextropropoxyphene (Balandrin et al., 1985).



Aspirin



Morphine



Codeine

Figure 1.1. Aspirin, Morphine and Codeine

The β-lactam structures such as penicillin G was introduced to chemistry, which was used as antibiotic during the Second World War (Newman et al., 2012). In addition to these seemingly small natural products, there is also a wide range of larger bioactive compounds that are also useful in drug therapies, such as taxol which was originally isolated from the Pacific yew tree (*Taxus brevifolia*) (Wani et al., 1971). Taxol was shown to be the first compound containing a taxane ring to exhibit antitumor and anti-leukemic properties. Etoposide, another semi-synthetic anti neoplastic agent derived from *Podophyllum peltatum* is an approved chemotherapeutic agent used in different cancer conditions (Newman et al., 2003).

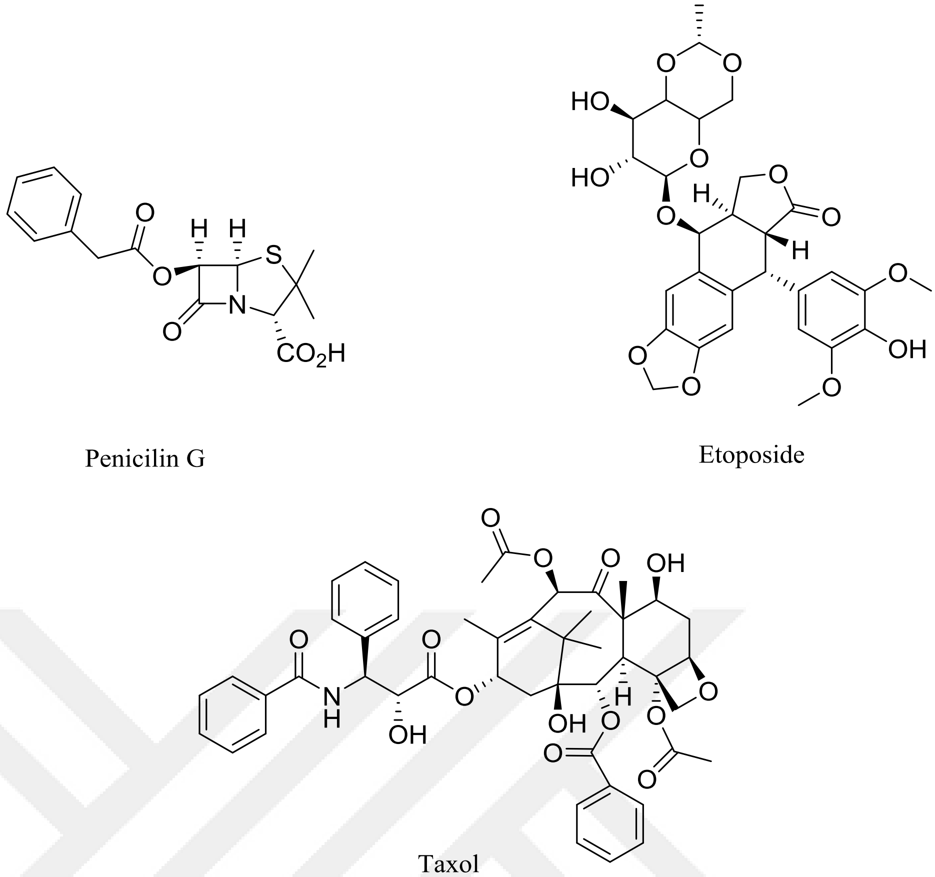


Figure 1.2. Penicillin G, Etoposide and Taxol

Penicilin G, etoposide and taxol were the first to be isolated with specific structures (Figure 1.2) giving increase to their respective biological activities and each was developed further by semi-synthesis studies to make a range of useful drug treatments.

Semisynthesis and derivatization

When a natural product is not readily available from its natural source, one way to obtain larger amounts without having to undertake a total synthesis is to once again turn to nature for another, more abundant natural product that can serve as a starting material. A good example of this is the semisynthesis of taxol (Kingston et al., 2008). This was first achieved by the isolation of 10-deacetylbaccatin III, which is abundant in the needles of many of the *Taxus* species (Figure 1.3).

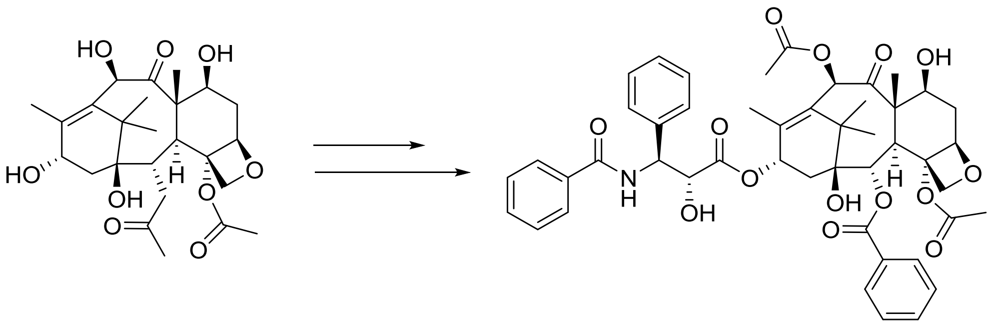


Figure 1.3. Semisynthesis of Taxol

Studies by Kingston et al. focused on the modification of taxol to learn more about its structure activity relationship. One of the first examples was the contraction of the A-ring, which was achieved by treatment of protected taxol with thionyl chloride and subsequent deprotection to yield the A-nortaxol analogue. This was found to be less cytotoxic than taxol but retained the tubulin polymerisation activity. Reductions in the C-ring gave the C-nortaxol analogue (Figure 1.4) which had reduced cytotoxicity and reduced tubulin polymerisation activity many further modifications have been made to determine the important functionality for activity (Kingston et al., 1998, 2008).

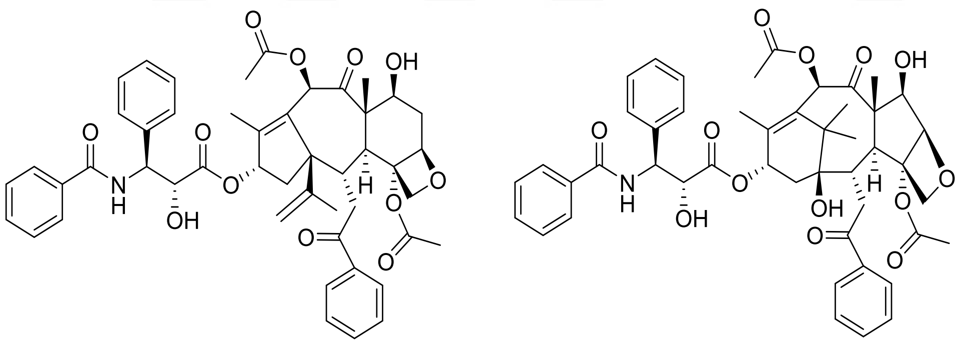


Figure 1.4. Analogues of Taxol

Natural product analogues

Biological testing of large secondary metabolite structures in vitro and in vivo often reveals that only part of the structure is required for the compounds activity. Halaven® is a microtubule dynamics inhibitor approved for use by the U.S. Food and Drug Administration for the treatment of advanced breast cancer in patients who have had two previous chemotherapeutic regimens (Shablak et al., 2013). Halaven® is an analogue of Halichondrin B that was isolated from the marine sponge *Halichondria okadai*. Halichondrin B was found to have an IC_{50} of 0.093 ng/mL against B-16 melanoma cells (Hirata et al., 1986). Through testing of synthetic halichondrin derivatives and synthetic intermediates formed during the total synthesis of halichondrin B it was discovered that the antitumor activity of the natural product resulted from the functionality in the right side of the molecule (Figure 1.6). With this information a large drug discovery effort resulted in the development of Halaven®.

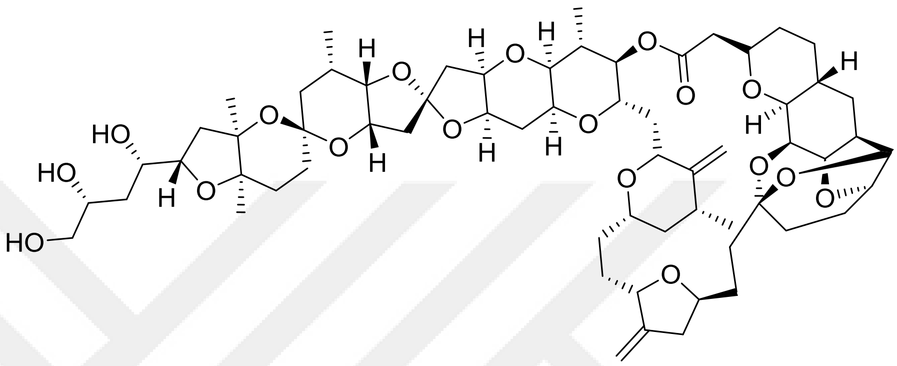


Figure 1.5. Structure of Halichondrin B

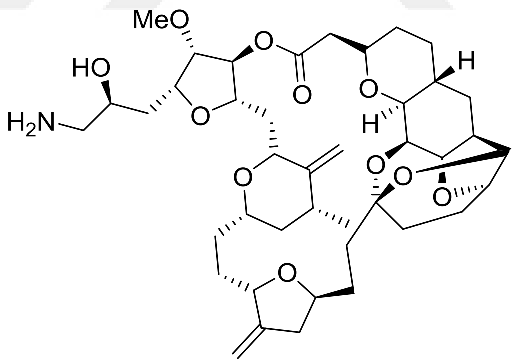


Figure 1.6. Structure of Halichondrin B's analogue Halaven®

Cancer

Cancer is the generic term for a group of diseases in which abnormal cells divide without control and are able to invade other tissues. The cells proliferate and spread beyond the normal boundaries and invade adjoining areas, eventually spreading to other organs in the body. This spread of abnormal cells is called metastasis and it is the major cause of death in cancer patients (WHO, 2016).

Cancer is a leading cause of death around the world. According to WHO 2016 statistics, “each year over 8 million people are diagnosed with and kills more people than AIDS, malaria, and TB combined”. Lung, prostate, colon, stomach, breast and liver cancer cause the most cancer related deaths each year. More than 70% of all cancer related deaths occurred in low- and middle- income countries (WHO, 2016).

Cancer develops from a single cell that has undergone a series of genetic changes. The genetic changes often arise from the interaction of a person’s genetic material with external carcinogenic agents (WHO, 2015). These changes are physical carcinogens (ionising radiation or ultraviolet light), chemical carcinogens (asbestos, components of tobacco smoke, aflatoxin) and biological carcinogens (certain viruses, bacteria and parasites can alter genetic material) (Siegel et al., 2012). Increasing age is another major risk factor of developing cancer, as over time, more risk factor exposure occurs and cellular repair mechanisms become less effective. The genetic change is a multi-stage process and two main types that lead to cancer are the inactivation of tumor suppressing genes and the activation of proto-oncogenes. Tumor suppressing genes typically code for proteins that will inhibit cellular proliferation (WHO, 2016). Five main classes of proteins are classed as tumor suppressing genes.

♦ Enzymes involved in DNA repair

♦ Proteins that promote apoptotic cell death

♦ Intracellular proteins that regulate cell cycles enzymes involved in DNA repair

♦ Receptors and signal transducers for secreted hormones and developmental signals that inhibit cellular proliferation

♦ Proteins that monitor for DNA damage and chromosomal abnormality and arrest the cell cycle if damage is found.

Loss of function in these genes allows cells that would normally be signalled for cell death to survive and multiply. Proto-oncogenes are genes that are involved in promoting cellular proliferation such as growth promoting signals and their receptors, cell survival proteins (anti-apoptotic) and transcription factors

(WHO, 2016). There are four main type of genetic mutation that can convert proto-oncogenes into their corresponding oncogenes (Ladish et al., 2015).

- Chromosomal translocation which fuses two genes together which then codes for a new chimeric protein, which has cellular proliferation abilities (and is often constantly activated).

- Point mutation in a proto-oncogene that leads to protein being constantly activated.

- Chromosomal translocation that moves a growth regulating gene under the control of a different promoter giving rise to inappropriate expression of the protein.

- Amplification of a proto-oncogene DNA segment so that multiple copies exist leading to overproduction of the protein.

The first two examples give rise to oncoproteins that are different to the normal proteins and have new cellular proliferation activities. The second two examples are where the proteins are the same proteins found in normal cells but they are produced in much higher levels or in cells where they are not normally produced.

The combination of these two types of genetic change gives rise to cancer cells that are not controlled by the normal processes that regulate cell division. It is not that they proliferate faster than normal cells but that their proliferation is uncontrolled. The cells also undergo genetic change that leads to differentiation, loss of function and they are able to be found outside of their designated tissue areas (where normal cells would be programmed for cell death if dislodged from their designated area) (DeVita and Chu, 2008).

Cancer treatment

There are three main approaches to treating cancer, surgical excisions of tumors, radiation therapy and chemotherapy. These therapies are often used in combination and different cancers require different treatment methods. Who reported that 30% of the deaths from cancer can be preventable if measures such as, no tobacco use, a healthy diet, regular exercise, limited alcohol use and protection against cancer-causing infections, are being taken (WHO, 2016).

In chemotherapy, the main challenge is to eliminate cancer cells completely however this 100% killing rate is not obtainable. In spite of relatively successful experiments with the use of cytotoxic chemotherapeutic agent, results as 99.99%, the number of alive cancer cell still would be 10 million, if there are 10 billion tumor cells in the medium. The proliferation rate decreases when the volume of the tumors increase since it is getting harder to reach blood vessels. Consequently, chemotherapeutic agents will not be able to penetrate to tumor cells (Rang et al., 2003). As an anti-proliferative molecule, the main working purpose of cytotoxic agents is to inhibit uncontrolled proliferation of cancer cells but these molecules could also attack to normal cells with the similar dividing range (e.g. bone marrow cells). In such cases; infertility, hair loss and changing in usual treatment (in terms of time and process) as well as side effects puking and nausea could be occurred.

The resistance to cancer drugs is the other difficulty. This kind of resistance may be occurred from both genetic variation of cancer cells and genetic differences among patients. The cancer cells generate more advanced resistance if the treatment overcome these variations and become more influential. Cancer cells also have ability of adjusting their cell surfaces by decreasing amount of transporter proteins to prevent drug intake (Gottesman, 2002). The drug resistance and problems regarding to this issue may be suppressed by combination of different kind of cytotoxic molecules (combinatorial chemotherapy) (Gardner et al., 2000).

These problems with drug delivery and unwanted side effects make the discovery of new anti-cancer drugs with less toxic side effects to normal cells an important goal.

Plants as a Source of Anti-cancer Compounds

Plants play an important role in the treatment of cancer and discovering of novel anti-cancer agents.

Examples of well-known anti-cancer drugs derived from plants are: vincristine and vinblastine from *Catharanthus roseus*, taxol from the bark *Taxus* *baccata*, etoposide from *Podophyllum peltatum* and camptothecin, irinotecan and topotecan from *Camptotheca acuminate* (Mans et al., 2000). There are also a number of other plant-derived agents developed that are now in various stage of clinical trials (Newman et al., 2015).

Astragalus L.

The Fabaceae is a family of flowering plants comprising about 269 genera and 5100 species and is one of the largest plant families in the world and also in Turkey (Davis, 1970; Chamberlain and Matthews, 1970). It has 68 genera and more than 900 species in Turkey. Fabaceae is one of the most important family of food plants, especially pulses (beans, gram, peas) and oil (soya, ground nut), but also tanbarks, timber, copal, gums, insecticides and cultivated ornamentals, as well as medicinal plants (Bağcı, 2006).

The genus *Astragalus* belonging to the Fabaceae family is comprising the highest number of species among the Spermatophytes. While the exact number of species in this genus has not been established recently, it is estimated to be around 3.000 (Podlech et al., 1998). Although *Astragalus* sp. are widely distributed throughout the temperate regions of the world, located principally in Europe, Asia and North America, almost all of the species distributed throughout the Old World are found in Iran, Turkey and central Asian countries (Vural et al., 2008). In Turkey, it is represented by 445 species, of which 224 are endemic. They are classified in 62 sections (Özüdoğru et al., 2011; Aytaç et al., 2000; Davis, 1970).

Astragalus root is a very old and well-known drug in traditional Chinese medicine and have been used to improve resistance to infections and to cure in immunological disorders and viral infections, and as hepatoprotective. It also stimulates the circulatory system and acts as a heart tonic. It is specifically used for symptoms of excessive sweating, deficient urination, prolapse of internal organs and rectum, and to speed the healing of bums and abscesses (Tang and Eisenbrand, 1992). "*Astragali radix*" (Huang qi in China; Japanese name, Ougi), is the dried root of *A. membranaceus* Bunge var. mongholicus Hsiao or *A. membranaceus* (Fisch.) Bunge. The Chinese name Huang qi translates as "*yellow leader*", referring to the yellow color of the root and its status as one of the most important tonic herbs. It is officially listed in the Chinese Pharmacopoeia and has been used for over 2000 years in traditional Chinese medicine prescriptions for the treatment of nephritis, diabetes, albuminuria, hypertension, cirrhosis, cancer, as well as a sedative and tonic (Li et al., 2001; Wang et al., 2004). Other traditional indications include wasting disorders, night sweats (Hong, 1986), chronic ulcerations and sores, numbness and paralysis of the limbs, and edema. (Bensky and Gamble, 1993). *Astragalus* is classically prescribed in combination with other Chinese medicinal herbs depending on the desired therapeutic effect and the exact diagnosis (Sinclair, 1998).

Astragalus species are also used in traditional medicine in Bulgaria, Russia and other European and Asiatic countries. In modern Chinese medicine, it is used in Fu Zheng therapy to improve immune system parameters and it is the most frequently used promoter for other herbal therapies. Because its properties are somewhat similar to those of the more expensive herb ginseng (Panax ginseng), it has been used as a substitute for that species (Ionkova et al., 1995). *Astragalus* is held in high esteem by the Chinese, who incorporated it into many others medicinal formulas. Herbal practitioners may suggest using this herb during treatment with chemotherapy as it stimulates the immune system. Current Western applications of *Astragalus* are primarily for restoring and strengthening the immune response, enhancing cardiovascular function, and increasing vitality (Bensky and Gamble, 1993).

In Turkey, the roots and gum of *Astragalus* species are traditionally used in treatment of jaundice, sore throat, stomach ache, diabetes and cancer (Altundağ and Öztürk, 2011). Flowering parts of some species are also used against asthma, urethritis and as an expectorant (Tuzlacı and Şenkardeş, 2011). Additionally, some parts of the genus have been used against high blood pressure, stomach inflammation and as a pain killer (Güneş and Özhatay, 2011).

Saponins from genus *Astragalus* L.

Cycloartane triterpenoids were first discovered in *Astragalus* plants. Plants of this genus drew attention due to their cycloartane production abilities. Since then, their content of cycloartane methylsteroids and glycosides came under intense investigation in many scientific centers of the world. Cycloartanes are derivatives of 9β,19-cyclolanostane and are produced exclusively by photosyntheic eukaryotes. Cycloartanes dominate the known triterpenoids in plants of this genus (Mamedova and Isaev, 2004).

Cycloastragenol, 20(*R*),24(*S*)-epoxy-3*β*,6*α*,16*β*,25-tetrahydroxycycloartane, is the most widely distributed sapogenin of *Astragalus* plant. Cycloastragenol is also known as cyclosiversigenin and astramembrangenin (Mamedova and Isaev, 2004).

Oleanane- and lanostane-type triterpenoids are not common in *Astragalus* genus as cyloartanes. The most common oleanane-type saponins which were isolated from the saponin given below.

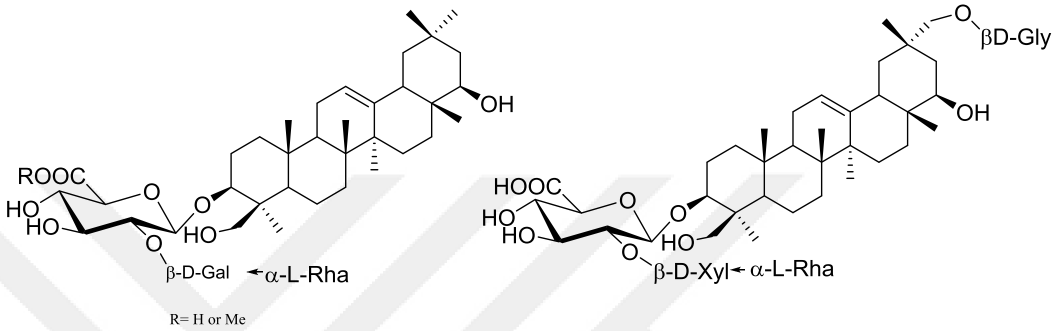
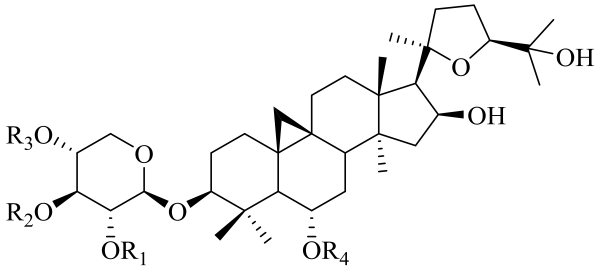


Figure 1.7. Structure of Oleanane-type Saponins

In general, triterpenoid saponins represent the major beneficial compounds responsible for the bioactivities and efficacies of Radix Astragali on human health. Especially, the four astragalosides, i.e., Astragaloside I, Astragaloside II, Astragaloside III and Astragaloside IV (AG IV), belong to cycloartane-type triterpenoid saponins. They are considered as the major bioactive constituents with better pharmacological activities and possess antioxidant, antitumor, hepatoprotective, anti-diabetic, antiviral and immunological activities. There into, Astragaloside III and AG IV represent a pair of isomeric compounds (Yan et al., 2010).



	R_1	R_2	R_3	R_4
Astragaloside I	Ac	Ac	H	Glu
Astragaloside II	Ac	H	H	Glu
Astragaloside III	Glu	H	H	H
Astragaloside IV	H	H	H	Glu

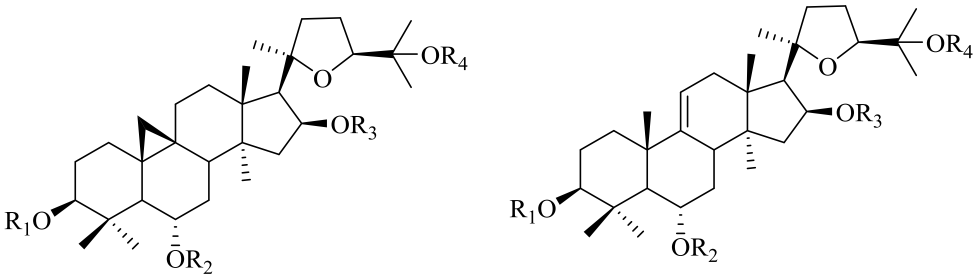
Figure 1.8. Structure of Astragalosides

Semi Synthetic Compounds from Cycloartanes

Semi-synthetic anticancer drug-discovery programs focusing on saponins mainly engaged with the commercially available triterpenoids such as oleanolic acid and ursolic acid, but less common miscellaneous aglycons such as cycloartanes, lanostanes and hopanes.

There were very limited semi-synthetic study of cycloastragenol and astragenol. The first semi-synthetic report of cycloastragenol was published in 1983. Also, the authors were reported artifact aglycone, Astragenol, obtained during hydrolysis of Astragalosides. Moreover, esterified and oxidized derivatives of Astragenol were described in this paper (Figure 1.9) (Kitagawa et al., 1983).

In 2004, Harley et al. have published a paper about semi-synthesis of cyloastragenol. They mainly focused on esterification and oxidation reactions to increase telomerase activity (Harley et al., 2004).



R_1= H	R_2= H	R_3= H	R_1= H	R_2= H	R_3= H	R_4= H
R_1= Ac	R_2= Ac	R_3= H	R_1= Ac	R_2= Ac	R_3= H	R_4= H
R_1= H	R_2= H	R_3=O	R_1= Ac	R_2= Ac	R_3= Ac	R_4= H
R_1= Ac	R_2= Ac	R_3=O	R_1= Ac	R_2= Ac	R_3= $COCF_3$	R_4= $COCF_3$

Figure 1.9. Analogues of Cycloastragenol

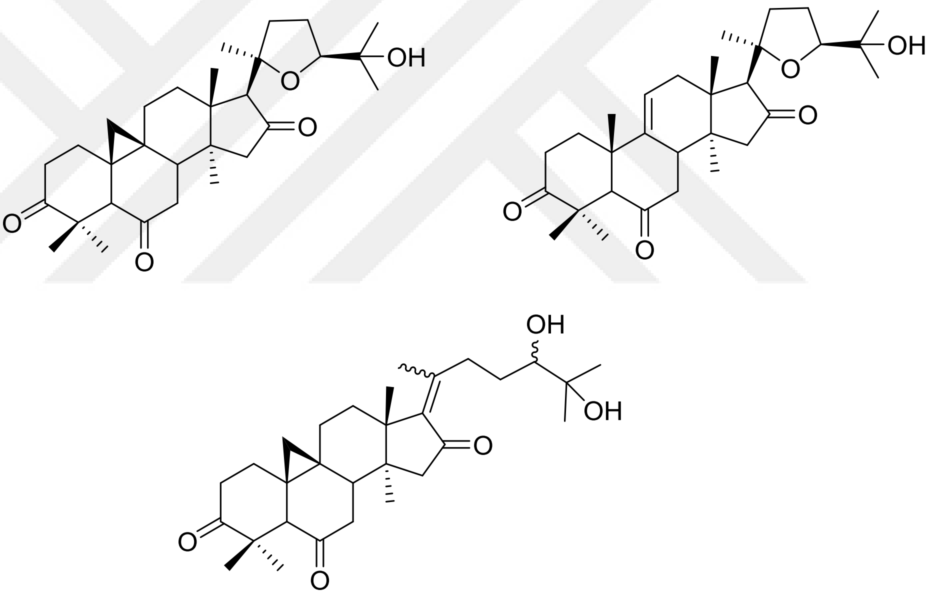


Figure 1.10. Some Semi-Synthetic Derivatives from Cycloastragenol

Recently, a few studies have been carried on to obtain Cycloastragenol derivatives by microbial transformation. Kuban et al. reported that *Cunninghamella blaksleeana* NRRL1369 could catalyse CG into a novel skeleton through cyclopropane ring cleavage (Kuban et al., 2010). Also, oxidized 3,4-*seco* derivatives of Cycloastragenol and Astragenol were obtained by microbial transformation. (Kuban et al., 2013, Bedir et al., 2015).

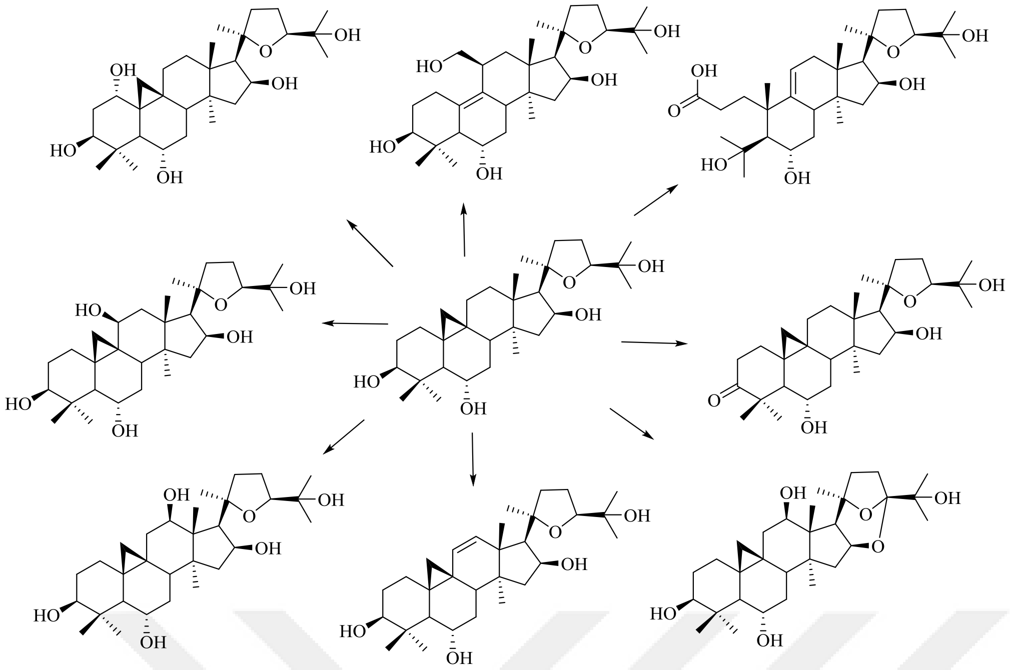


Figure 1.11. Cycloastragenol Derivatives Obtained via Microbial Transformation

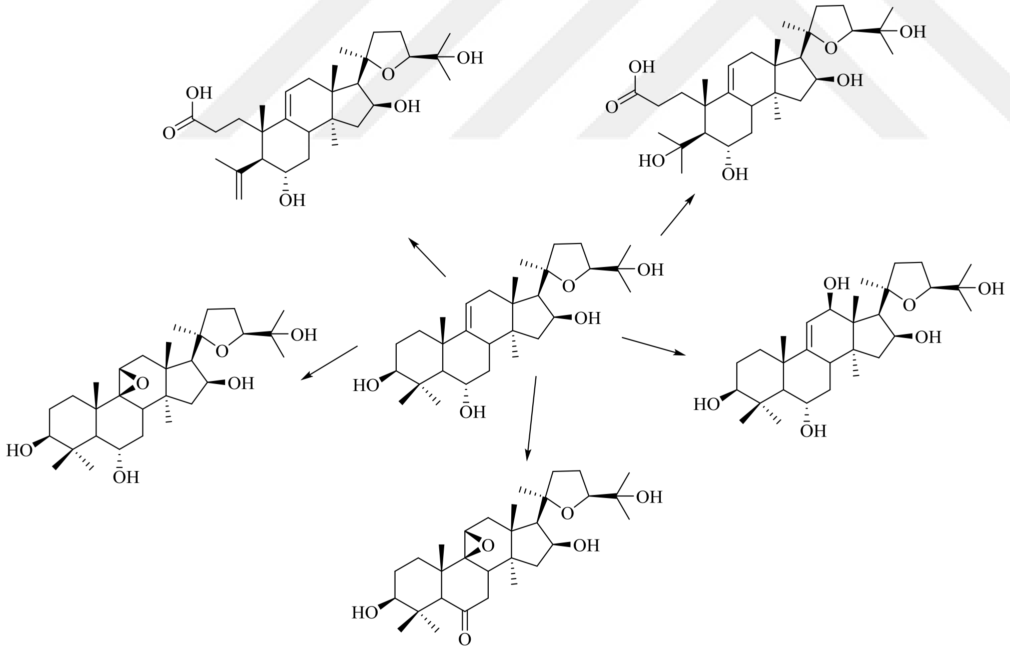


Figure 1.12. Astragenol Derivatives Obtained via Microbial Transformation

Biological Activities

Interest in the cycloartanes is due to the fact that cycloartenol, the initial compound of this series, is a key intermediate in the biosynthesis of phytosterols. However, the reason for the rising interest towards the cycloartane saponins isolated from the genus *Astragalus* is due to their wide range of biological activities.

Astragaloside IV, the primary pure saponin isolated from *Astragalus membranaceus*, displayed antifibrotic effects in hepatic stellate cells (Liu et al., 2009). It showed antiviral activity against coxsackievirus B3 (Zhang et al., 2007) as well it suppressed the expression of oncogenic VAV3.1 of human hepatocellular carcinoma cell line HepG2, implicated in tumorigenesis (Qi et al., 2010).

Cycloastragenol, was shown to delay the onset of cellular aging by increasing telomerase activity (Valenzuela et al., 2009) and enhance the antiviral function of human $CD8^+$ T lymphocytes (Fauce et al., 2008).

Moreover biological activity studies showed that *Astragalus* saponin and sapogenins have immunostimulatory (Çalış et al., 1997; Yeşilada et al., 2005; Bedir et al., 2000), anti-protozoal (Özipek et al., 2005), antiviral (Gariboldi et al., 1995), cytotoxic (Radwan et al., 2004), cardiotonic (Khushbaktova et al., 1994), wound healing (Sevimli-Gür et al., 2011), cytotoxic (Cho and Leung, 2007) and adjuvant (Nalbantsoy et al., 2011) activities too.

2. MATERIAL AND METHODS

2.1. General Experimental Procedures

Optical rotations were measured using a Perkin Elmer 341 Model Polarimeter. HR-MS spectra were obtained by micrOTOF-Q II mass spectrometer from Bruker and Agilent Series 1100 SL mass spectrometer, in positive and negative ESI modes. The 1D and 2D NMR spectra were obtained on Varian Oxford AS400 and Bruker DRX-400/500 instruments.The Proton and carbon chemical shifts are relative to TMS.

Column chromatography was carried out a silica gel 60 (Merck 7734) and Li Chroprep RP (C-18, Merck 9303). TLC was conducted on pre-coated silica gel 60 F_{254} aluminum sheets (Merck 5554) and RP-18 F_{254} (Merck 5559) plates. Most of the time the mobile phase utilized for normal phase column chromatography was chloroform: methanol or *n*-hexane: ethyl acetate, usually applied as gradients. For all reverse phase chromatographic separations, various proportions and gradients of methanol in water were used.

Extracts and pure compounds were analyzed by Dionex UltiMate 3000 HPLC-DAD system coupled with CAD (Dionex Corona) and Chromelon software was used for analysis of the HPLC data. The analytical column used was a Kinetex C18 (2.6 µm; 100mm×4.6mm), which was purchased from Phenomenex. (USA). The mobile phases were degassed using Elmasonic sonication system. The column was equilibrated and eluted under isocratic conditions at 30 °C.

Compounds were detected at 254 and 366 nm UV lamp by using CAMAG device. The spots were detected by 20% H_2SO_4 solution as a spraying reagent onto the TLC plates followed by heating the plates to 120 ^{o}C until the spots became visible.

Progress of reactions was monitored by TLC analysis. Solvents were purchased as GC or HPLC grade from Sigma-Aldrich (France) and Merck (Germany).

All reactives were purchased from Sigma-Aldrich. Jones reagent freshly prepared; 2.5 g Cr(VI)O_3 was dissolved in 2.5 mL H_2SO_4 (conc.). Solution was added very slowly to 7.5 mL H_2O that had been cooled to 0 °C and was stirred.

Standard compounds (Astragaloside IV and Cycloastragenol) were donated by Bionorm Natural Products Inc. (İzmir, Turkey).

2.2. Plant Materials

2.2.1. *Astragalus trojanus* Stev.

Astragalus trojanus Stev. (root) was collected from Turgutreis, Çanakkale (40°02′25″ N, 026°21′29″ E), from 150 m altitude, west Anatolia, Turkey and identified by Assoc. Prof. Ümit Budak. Voucher specimen has been deposited in the Herbarium of Gazi University, Ankara, Turkey.

2.3. Determination of Cycloastragenol and Astragaloside IV Content

Cycloastragenol and Astragaloside IV content of *A. trojanus* was found out by using HPLC. The mobile phase was water (A) and acetonitrile (B) mixture.The following gradient elution program was used for separation: 0-3 min, 80% (A); 3-5 min, 80-50% (A); 5-10 min, 50% (A); 10-15 min, 80 (A); the flow rate was 1.5 mL/min, the injection volume was 5 µL. After 5 min of re-equilibration, the column was ready for the next injection. Using this LC conditions, the chromatograms showed well-separated resolution, satisfactory peak shape. Typical chromatogram of system suitability test is given in Fig. 2.1 (HPLC-CAD).

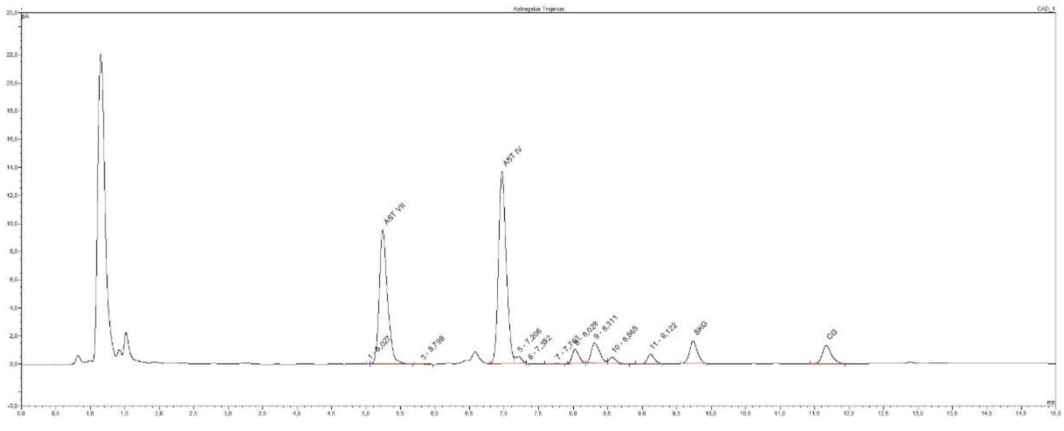


Figure 2.1. Typical Chromatogram of Astragaloside IV and Cycloastragenol

The quantifications of Astragaloside IV and Cycloastragenol were performed by standard method. The calibration curves of Astragaloside IV and Cycloastragenol showed good linearity in the range of 2.5-250 ppm.

2.4. Increasing Astragaloside IV Content

As acetylated astragalosides' amount is high in *A. trojanus*, the first study has been designed to investigate alkaline (NaOH) treatment's effects to increase Astragaloside IV content. The experimental conditions were summarized in Table 2.1. Each condition was analyzed and quantified by HPLC-CAD.

Table 2.1. Astragaloside IV Content

Astragaloside IV	**No treatment**	**pH= 10**	**pH= 11**	**pH= 12**
Room temperature (4 h)	1.0	1.2	1.1	1.0
Reflux (4 h)	1.0	1.2	1.1	1.0

2.5. Extraction and Isolation

Air-dried and powdered roots of *Astragalus trojanus* (1050 g) were extracted with *n*-BuOH. After filtration, to a solution was added NaOH (adjusted pH 11) and stirred under reflux condition for 4 hours. The mixture was quenched with HCl (10%). The crude extract (194.25 g) was added H_2O (500 mL) and partitioned with *n*-BuOH (3 x 300 mL). The *n*-BuOH extract (155.4 g) was subjected to vacuum liquid chromatography (VLC) using reversed-phase material (Lichroprep RP-18, 25-40 µm, 220g) employing H_2O:MeOH (9:1, 900 mL; 7:3, 1600 mL; 6:4, 2400 mL; 4:6, 3200 mL; 2:8, 3600 mL) and MeOH (1800 mL) to give 14 main fractions (1-14).

Fraction 10-14 (74.5 g) was submitted to silica gel (330 g) column with the solvent system EtOAc:MeOH:H_2O (100:10:5, 2000 mL; 100:20:5, 2500 mL) yielding **AST IV** (Fig. 2.2) (14.7 g), **AST VII** (Fig. 2.3) (3.23 g), and 5 subfractions. Subfraction 1 (1.52 g) was subjected to silica gel (150 g) column. Elution was carried out with *n*-hexane:EtOAc (6:4, 2500 mL) to give **CG** (Fig. 2.4) (0.874 g) and **SKG** (Fig. 2.5) (0.242 g).

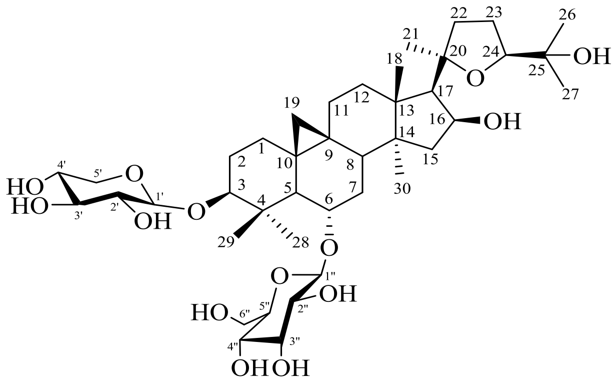


Figure 2.2. Structure of Astragaloside IV (**AST IV**)

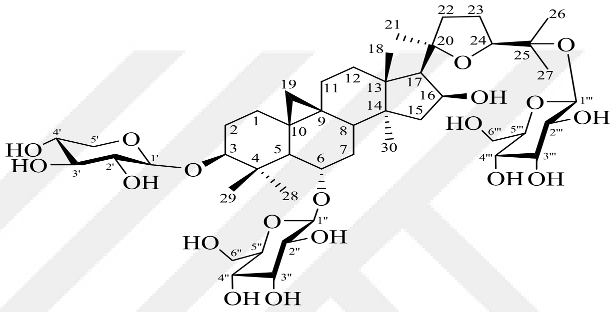


Figure 2.3. Structure of Astragaloside VII (**AST VII**)

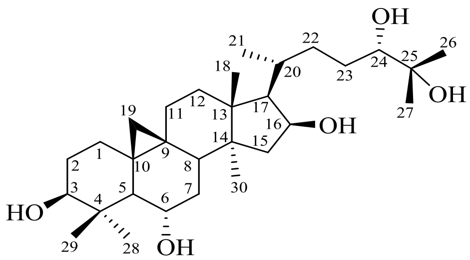


Figure 2.4. Structure of Cyclocanthogenol (**SKG**)

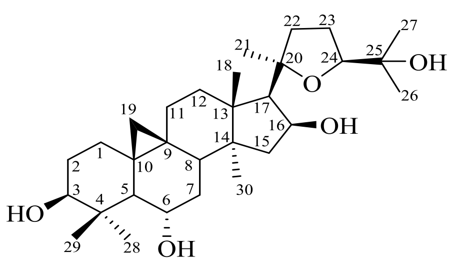


Figure 2.5. Structure of Cycloastragenol (**CG**)

Table 2.2. ^{1}H and ^{13}C NMR Assignments of **AST IV**

Position	δ_C	δ_H (***J* in Hz**)
1	32.66	1.29 m, 1.57 m
2	30.67	1.69 m, 1.96 m
3	89.77	3.21 dd (11.1, 4.5 Hz)
4	43.12	
5	52.93	1.64 m
6	79.86	3.57 ddd (10.0, 10.0, 4.5 Hz)
7	34.80	1.62 m, 1.93 m
8	46.21	1.89 m
9	22.11	
10	30.08	
11	26.63	1.37 m, 1.95 m
12	33.82	1.62 m, 1.70 m
13	46.45	
14	46.80	
15	45.87	1.42 m, 2.07 m
16	74.37	4.69 ddd (8.0, 8.0, 5.2 Hz)
17	58.82	2.40 d (8.0 Hz)
18	21.00	1.27 s
19	28.03	0.28 d, 0.62 d (4.5 Hz)
20	88.46	
21	28.19	1.23 s
22	35.19	1.66 dd (12.0, 1.5 Hz), 2.64 dd (12.0, 6.0 Hz)
23	26.44	2.06, 2.09 m
24	82.51	3.79 dd (8.0, 7.0 Hz)
25	72.50	
26	27.50	1.29 s
27	26.00	1.16 s
28	28.19	1.31 s
29	16.34	1.04 s
30	19.77	1.05 s
1′	107.00	4.31 d (7.6 Hz)
2′	75.50	3.21 dd (7.6, 8.5 Hz)
3′	77.71	3.32 t (8.5 Hz)
4′	70.96	3.49 m
5′	66.31	3.20 t (11.0 Hz), 3.85 dd (11.0, 4.0 Hz)
1′′	104.79	4.36 d (7.8 Hz)
2′′	75.32	3.26 dd (9.0, 7.8 Hz)
3′′	78.43	3.35 t (9.0 Hz)
4′′	71.50	3.29 t (9.0 Hz)
5′′	77.53	3.26 ddd (9.0, 4.5, 3.5 Hz)
6′′	62.60	3.67 dd (12.0, 4.5 Hz), 3.87 dd (12.0, 3,0 Hz)

Table 2.3. ^{1}H and ^{13}C NMR Assignments of **AST VII**

Position	δ_C	δ_H (*J* in Hz)
1	32.59	1.29 m, 1.59 m
2	30.18	1.71 m, 1.97 m
3	89.65	3.25 dd (11.1, 4.5 Hz)
4	42.80	
5	52.20	1.65 m
6	79.77	3.58 ddd (10.0, 10.0, 4.5 Hz)
7	34.74	1.66 m, 1.95 m
8	46.35	1.91 m
9	21.90	
10	29.48	
11	26.75	1.47 m, 1.91 m
12	33.79	1.66 m, 1.72 m
13	46.35	
14	47.36	
15	45.40	1.44 m, 2.08 m
16	74.43	4.70 ddd (8.0, 8.0, 5.2 Hz)
17	58.45	2.41 d (8.0 Hz)
18	21.17	1.30 s
19	29.13	0.28 d, 0.63 d (4.5 Hz)
20	87.20	
21	27.78	1.27 s
22	35.31	1.69 m, 2.57 m
23	26.50	2.03 m, 2.11 m
24	82.75	3.88 m
25	79.20	
26	25.10	1.42 s
27	22.88	1.26 s
28	28.15	1.32 s
29	15.29	1.06 s
30	19.78	1.06 s
1′	107.4	4.31 d (7.2 Hz)
2′	75.2	3.23 dd (7.5, 8.8 Hz)
3′	77.7	3.34 t (9.0 Hz)
4′	70.8	3.51 m
5′	66.5	3.22 t (11.0 Hz), 3.86 dd (11.0, 4.0 Hz)
1″	104.7	4.37 d (7.8 Hz)
2″	75.2	3.23 d (9.0, 7.8 Hz)
3″	78.3	3.37 t (9.0 Hz)
4″	71.3	3.35 t (9.0 Hz)
5″	78.0	3.39 ddd (9.0, 9.0, 4.5 Hz)
6″	62.5	3.70 dd (12.0, 4.5 Hz), 3.88 dd (12.0, 3,0 Hz)
1‴	98.5	4.56 d (7.5 Hz)
2‴	74.7	3.17 dd (9.0, 7.5 Hz)
3‴	78.3	3.37 dd (9.0, 7.5 Hz)

4‴	71.5	3.29 t (9.0 Hz)
5‴	77.6	3.34 ddd (9.0, 4.5, 2.5 Hz)
6‴	62.5	3.70 dd (11.0, 4.5 Hz), 3.88 dd (11.0, 3.5 Hz)

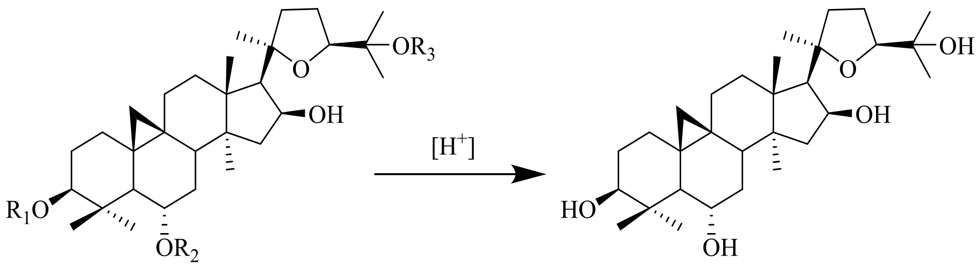
Table 2.4. ^{1}H and ^{13}C Assignments of **SKG**

Position	δ_C	δ_H **(*J* in Hz)**
1	32.2	
2	28.2	
3	78.6	3.48 dd (11.5, 4.4 Hz)
4	42.7	
5	52.5	1.89 d (8.6 Hz)
6	79.2	3.75 m
7	34.3	
8	45.6	
9	21.4	
10	28.7	
11	26.3	
12	33.2	
13	45.8	
14	46.9	
15	47.9	
16	71.9	4.66 m
17	57.2	2.32 d (7.8 Hz)
18	18.3	1.32 s
19	30.2	0.14, 0.53 d (4.0 Hz)
20	28.6	
21	18.5	1.03 d (6.4 Hz)
22	33.0	
23	27.9	
24	77.1	3.8 m
25	72.5	
26	25.8	1.44 s
27	26.5	1.42 s
28	28.6	1.35 s
29	16.7	1.98 s
30	19.8	0.94s

Table 2.5. ^{1}H and ^{13}C NMR Assignments of **CG**

Position	δ_C	δ_H (**J in Hz**)
1	33.82 t	1.68 m, 1.73 m
2	30.85 t	1.65 m, 1.75 m
3	78.96 d	3.25 dd (11.2, 4.5 Hz)
4	42.77 s	
5	54.02 d	1.37 m
6	69.39 d	3.40 ddd (10.0, 10.0, 4.5 Hz)
7	38.57 t	1.40 m, 1.50 m
8	48.35 d	1.84 m
9	22.00 s	
10	30.00 s	
11	27.80 t	1.32 m, 1.55 m
12	32.98 t	1.61 m, 1.28 m
13	46.34 s	
14	47.00 s	
15	46.58 t	1.45 m, 2.00 m)
16	74.26 d	4.69 ddd (8.0, 8.0, 5.2 Hz)
17	58.70 d	2.39 d (8.0 Hz)
18	21.57 q	1.30 s
19	31.98 t	0.42, 0.59 d (4.5Hz)
20	88.10 s	
21	28.08 q	1.25 s
22	35.17 t	1.67 m, 2.65 m
23	26.13 t	2.06 m
24	82.52 d	3.79 dd (8.0, 7.0 Hz)
25	72.90 s	
26	26.32 q	1.16s
27	27.24 q	1.26 s
28	28.37 q	1.30 s
29	15.61 q	0.93 s
30	20.36 q	1.03 s

2.6. Synthesis of Cycloastragenol (CG)



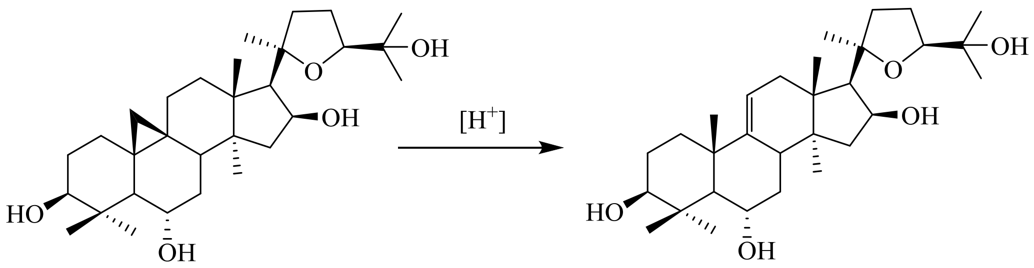
Ast IV R_1= Xyl R_2= Glu R_3= Glu
Ast VII R_1= Xyl R_2= Glu R_3= H

CG

To Astragaloside IV (1.02 g, 1.299mmol) was added HCl (1% in MeOH) and the mixture was stirred at 20 °C for 4 days. Solvent was evaporated under reduced pressure at 25 °C. After adding sat. aq. $NaHCO_3$, the aqueous mixture was extracted with CH_2Cl_2 (3 × 25mL). The combined organic layers was washed with brine solution, dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane:EtOAC (6:4) as eluent to give Cycloastragenol (**CG**), (2a*R*,3*R*,4*S*,5a*S*,7*S*,9*S*,11a*R*,12a*S*)-3-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-2a,5a,8,8-tetramethyltetradecahydro-1*H*,12*H*-cyclopenta[*a*]cyclopropa[*e*]phenanthrene-4,7,9-triol (407.4 mg, 64.0% yield) (Harley et. al., 2004).

1% HCl in MeOH was added into Astragaloside VII (500 mg, 0.529 mmol) at 20 °C stirred for 7 days. Solvent was evaporated under reduced pressure at 25 °C. After adding sat. aq. $NaHCO_3$, the aqueous mixture was extracted with CH_2Cl_2 (3 × 25mL). The combined organic layers was washed with brine solution, dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane:EtOAc (6:4) as eluent to give **CG** (101.4 mg, 48.0% yield) and Astragenol (**AG**) (36.54 mg, 14.1% yield).

2.7. Synthesis of Astragenol (AG)



CG **AG**

Astragenol (**AG**) was synthesized from three different starting compounds, **CG**, **AST IV** and **AST VII**. 2.0 mmol of Cycloastragenol (0.98 g), 0.89 mmol of Astragaloside IV (0.70 g) and 0.53 mmol of Astragaloside VII (0.50 mg) were separetly dissolved in MeOH (50 mL), then treated with conc. H_2SO_4 (5 mL). The whole mixtures were heated under reflux for 6h. The reaction mixtures were poured into water and extracted with EtOAc. The EtOAc extracts were washed successively with sat. $NaHCO_3$ aq. then water, and dried over Na_2SO_4 and filtered. The filtrates were evaporated, and chromatographic separation of the crude products on a silica gel columns were performed by using *n*-hexane:EtOAc (6:4) to give Astragenol (**AG**) (3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*] phenanthrene-3,6,16-triol as a white solid (63.89 mg, 65.2% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.80 (s, 3H), 0.92 (s, 3H), 1.05 (s, 3H), 1.07 (s, 3H), 1.14 (s, 3H), 1.23 (s, 3H), 1.30 (s, 3H), 1.33 (s, 3H), 1.37-1.80 (m, 10H), 1.81-2.16 (m, 4H), 2.35 (d, *J*=7.8 Hz, 1H), 2.45 (m, 1H), 2.58 (t, *J*=10.8 Hz, 1H), 3.19 (dd *J*=11.6, 3.7 Hz, 1H), 3.75 (ddd, *J*=8, 8, 4 Hz, 1H), 4.18 (dd, *J*=7.4, 7.4 Hz, 1H), 4.70 (ddd, *J*=7.4, 7.4, 5 Hz, 1H), 5.27 (d, *J*=6.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 146.6, 115.6, 87.2, 81.6, 79.0, 73.4, 72.0, 70.1, 57.3, 56.3, 45.2, 44.3, 44.1, 41.2, 40.9, 39.5, 37.6, 36.4, 34.6, 31.4, 31.4, 28.1, 27.9, 27.4, 26.7, 25.9, 23.4, 19.3, 18.3, 15.9; HRMS *m/z* calcd for $C_{30}H_{50}O_5$, $[M+Na]^+$ 513.3556; found 513.3575.

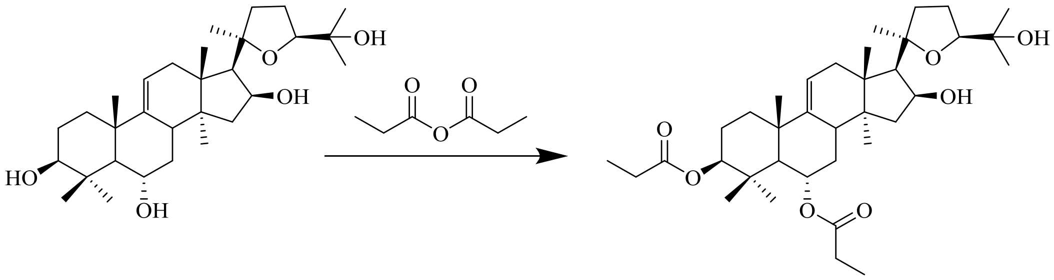
2.8. Cytotoxic Activity Assay

DU145, HT29, MDA-MB-231, PC-3 cells were obtained from American Type Culture Collection (ATCC Mannassas, VA). The cells were propagated in culture as recommended by ATCC using RPMI1640, DMEM-Ham's F12, supplemented with 10% fetal bovine serum, L-glutamine, (2 mM), penicillin (100U/mL) and streptomycin (100 µg/mL) and maintained at 37 °C with 5% CO_2 in a humidified atmosphere.

The cytotoxicity of the pure compounds was measured using MTT assay at certain doses and time intervals. Cells in exponential growth phase were plated into 96-wells to make 4000 cells/well. After 24 hours of resting, compounds were added in different concentrations ranging from 0.1 to 10 µg/mL and cells were incubated for 4, 8, 16, 32, 60 and 96 hours. Cells grown in DMEM-HAM's/F-12 used as negative control and all calculations were performed according to DMSO added mock control. Proliferation was determined by adding MTT reagent (0.5 mg/mL), prepared as a sterile stock-solution of 5 mg/mL in DMEM (Gibco, USA), diluted 1:10 using medium prior to use. Medium was removed 4 h later and blue formazan crystals were dissolved by adding 200 µL of DMSO to each well. Absorbance was read by multi-well spectrometry and the amount of MTT formazan product was quantitated. The data obtained was from three independent experiments, using duplicates of wells for each assay.

3. SEMI-SYNTHETIC DERIVATIVES

3.1. Synthesis of A2

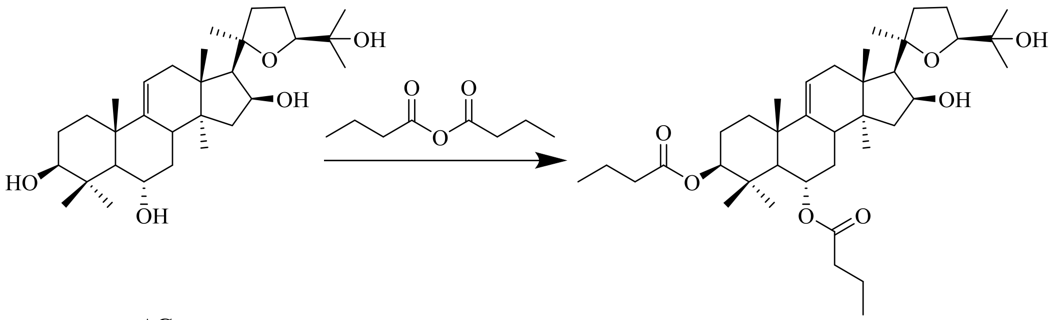


AG **A2**

To a solution of **AG** (0.204 mmol) in pyridine was added propionic anhydride (1.0 mmol) and catalytic amount of DMAP. The reaction mixture was stirred at reflux for 4h and then cooled to room temperature. After removing the solvent in vacuo, the residue was partitioned with EtOAc and saturated with aqueous $NaHCO_3$ solution (3 × 25 mL); then, the organic layer was washed with saturated brine and dried over Na_2SO_4 and the solvent was removed in vacuo. Chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane:EtOAc (8:2) to give **A2** (3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-16-hydroxy-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl) -2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,6-diyl dipropionate (48.8% yield) as white solid.

^{1}H NMR (**A2**) (400 MHz, $CDCl_3$) δ (ppm): 0.79 (s, 3H), 0.92 (s, 3H), 0.97 (s, 3H), 1.04 (s, 3H), 1.16 (m, 6H), 1.25 (s, 3H), 1.25 (s, 3H), 1.26 (s, 3H), 1.31 (s, 3H), 1.35-2.15 (m, 14H), 2.26-2.30 (m, 5H), 2.48 (m, 1H), 2.59 (q, *J*=10.6 Hz, 1H), 3.76 (dd, *J*=7.4, 7.4 Hz, 1H), 4.47 (dd, *J*=12, 4 Hz, 1H), 4.68 (ddd, *J*=7.4, 7.4, 5 Hz, 1H), 5.31 (d, *J*=4Hz, 1H), 5.41 (ddd, *J*=8, 8, 4 Hz, 1H); ^{13}C NMR (100 MHz, Pyridine-*d*5) δ (ppm): 173.7, 173.4, 145.5, 116.3, 86.9, 81.5, 80.0, 73.0, 71.6, 71.0, 56.6, 54.8, 44.7, 43.9, 43.7, 40.9, 40.9, 40.3, 37.9, 37.9, 37.3, 35.7, 34.7, 30.6, 28.3, 27.9, 27.9, 26.9, 26.2, 23.8, 22.7, 19.0, 18.1, 17.0, 9.3, 9.0; HRMS *m/z* calcd for $C_{36}H_{58}O_7$, $[M+Na]^+$ 625.4080; found 625.4103.

3.2. Synthesis of A3



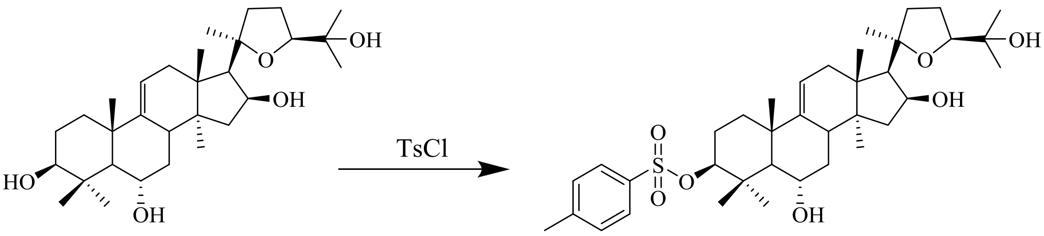
AG

A3

0.05 mmol DMAP was added into 50 mg **AG** and 1.0 mmol butyric anhydride solution in 4 mL pyridine. The reaction mixture was stirred for 4h under reflux, and then quenched with HCl (%5). The mixture was poured into a separatory funnel containing EtOAc (50 mL) and partitioned with H_2O. The aqueous layer was extracted again with EtOAc (3 × 50 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated. The residue was subjected to chromatography (*n*-hexane:EtOAc; 8:2) to afford pure **A3** (3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-16-hydroxy-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-2, 3, 4, 5, 6, 7, 8, 10, 12, 13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,6-diyl dibutyrate. (63.4 % yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.78 (s, 3H), 0.92 (s, 3H), 0.96 (o, 9H), 1.04 (s, 3H), 1.15 (s, 3H), 1.15 (s, 3H), 1.24 (s, 3H), 1.30 (s, 3H), 1.35-2.15 (m, 14H), 2.26-2.30 (m, 9H), 2.48 (m, 1H), 2.57 (q, *J*=10.6 Hz, 1H), 3.75 (dd, *J*=7.4, 7.4 Hz, 1H), 4.45 (dd *J*=12.0, 4.0 Hz, 1H), 4.67 (ddd, *J*=7.4, 7.4, 5.0 Hz, 1H), 5.30 (d *J*=4.0 Hz, 1H), 5.40 (ddd, *J*=8.0, 8.0, 4.0 Hz, 1H); ^{13}C NMR (125 MHz, Pyridine-*d5*) δ (ppm): 173.0, 172.7, 145.5, 116.3, 86.9, 81.5, 80.0, 73.0, 71.6, 71.1, 56.6, 54.8, 44.6, 43.9, 43.7, 40.9, 40.3, 37.9, 37.3, 36.9, 36.5, 35.7, 34.7, 30.7, 28.3, 27.9, 26.9, 26.2, 23.8, 22.8, 19.0, 18.7, 18.4, 18.1, 17.1, 13.6; HRMS *m/z* calcd for $C_{38}H_{58}O_7$, $[M+Na]^+$ 653.4393; found 653.4435.

3.3. Synthesis of A8

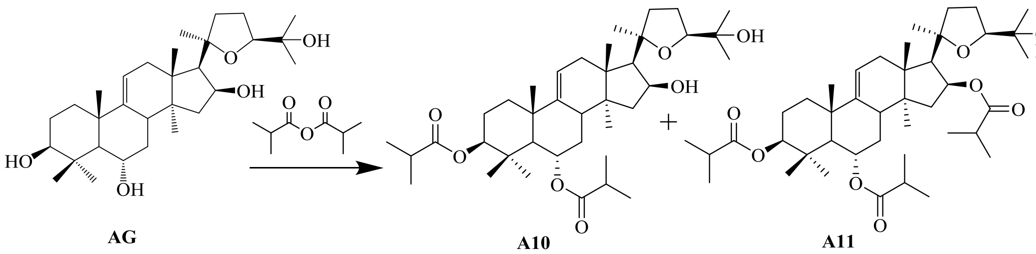


AG **A8**

To a solution of **AG** (50 mg, 0.102mmol) and DMAP (1 mg) in pyridine (2 mL) at 25 ^{o}C was added excess amount TsCl (250 mg). After stirring for 4h at room temperature, the reaction mixture was quenched with water (20 mL) and extracted with EtOAc (3 × 50 mL). The organic layers were dried over Na_2SO_4 and concentrated. The residue was then purified through column chromatography (*n*-hexane:EtOAc; 8:2) to produce **A8** (3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-6,16-dihydroxy-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta [*a*]phenanthren-3-yl 4-methylbenzenesulfonate (35.3% yield).

^{1}H NMR (**A8**) (400 MHz, $CDCl_3$) δ (ppm): 0.66 (s, 3H), 0.81 (s, 3H), 0.86 (s, 3H), 0.98 (s, 3H), 1.00 (s, 3H), 1.16 (s, 3H), 1.31 (s, 3H), 1.43 (s, 3H), 1.50-2.14 (m, 15H), 2.22 (d, *J*=8 Hz, 1H), 2.44 (s, 3H), 2.57 (q, *J*=10.6 Hz, 1H), 2.85 (m, 1H), 3.75 (t-like, *J*=7.4 Hz, 1H), 4.22 (dd, *J*=10.0, 4.4 Hz, 1H), 4.70 (ddd, *J*=7.4, 7.4, 5 Hz, 1H), 5.15 (d, *J*=4.0 Hz, 1H), 5.61 (dd *J*=12.0, 4.0 Hz, 1H), 7.32 (d, *J*=8.3 Hz, 1H), 7.80 (d, *J*=8.3 Hz, 1H); HRMS *m/z* calcd for $C_{37}H_{56}O_7S$, $[2M+H]^+$ 1311.7391; found 1311.8171.

3.4. Synthesis of A10 and A11

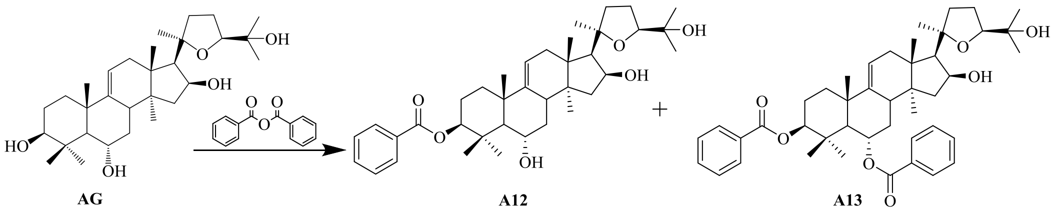


To a solution of **AG** (50 mg, 0.102 mmol) and DMAP (0.099 mmol) in pyridine (5 mL) at 25 ^{o}C was added isobutiric anhydride (1.0 mmol). The reaction mixture was stirred under reflux for 4h, quenched with water (50 mL) and extracted with EtOAc (3 × 50 mL). The organic layers were dried over Na_2SO_4 and concentrated. The residue was then purified through silica gel column (*n*-hexane:EtOAc; 8:2) to produce **A10** (3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-16-hydroxy-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4, 4, 10, 13, 14-pentamethyl-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta [*a*]phenanthrene-3,6-diyl bis(2-methylpropanoate) (46.6% yield) and **A11** (3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,6,16-triyl tris(2-methylpropanoate) (9.2% yield)

^{1}H NMR (**A10**) (400 MHz, $CDCl_3$) δ (ppm): 0.79 (s, 3H), 0.82 (s, 3H), 0.92 (s, 3H), 1.09 (s, 3H), 1.14 (s, 3H), 1.15-1.20 (m, 12H) 1.26 (s, 3H), 1.31 (s, 3H), 1.33 (s, 3H), 1.37-2.26 (m, 14H), 2.40-2.56 (m, 5H), 3.73 (ddd, *J*=8.0, 8.0, 4.0 Hz, 1H), 4.44 (m, 1H), 4.67 (m, 1H), 5.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 176.9, 176.8, 145.7, 116.6, 85.7, 82.5, 80.2, 75.5, 71.8, 71.1, 56.3, 54.9, 45.2, 44.6, 44.5, 41.3, 40.4, 38.1, 37.3, 35.9, 34.7, 34.5, 34.4, 30.8, 27.8, 27.6, 26.1, 24.7, 23.7, 23.3, 19.6, 19.4, 19.3, 19.1, 18.7, 18.6, 17.8, 17.2; HRMS *m/z* calcd for $C_{38}H_{62}O_7$, $[M+Na]^+$ 653.4393; found 653.4360

^{1}H NMR (**A11**) (400 MHz, $CDCl_3$) δ (ppm): 0.83 (s, 3H), 0.96 (s, 3H), 1.06 (s, 3H), 1.08 (s, 3H), 1.16 (s, 3H), 1.16-1.20 (m, 20H), 1.25 (s, 3H), 1.32 (s, 3H), 1.37-1.85 (m, 17H), 2.00 (dd, *J*=17.4, 5.4 Hz, 1H) 2.10-2.30 (m, 3H), 3.69 (m, *J*=7.6 Hz, 1H), 4.43 (m, 1H), 5.35 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 176.9, 176.8, 176.7, 145.7, 116.6, 87.2, 80.2, 75.5, 72.0, 71.1, 56.3, 54.9, 45.2, 44.5, 44.3, 41.2, 40.4, 38.1, 37.3, 35.9, 34.7, 34.6, 34.6, 34.4 (2), 30.8, 29.9, 27.8, 27.6, 26.7, 26.1, 25.7, 24.7, 23.7, 23.3, 19.6, 19.5, 19.3, 19.1, 18.7, 18.6, 17.2; HRMS *m/z* calcd for $C_{42}H_{68}O_8$, $[M+Na]^+$ 723.4914; found 723.4912.

3.5. Synthesis of A12 and A13



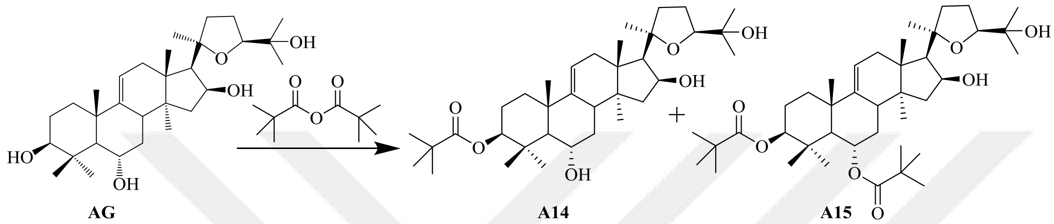
To a solution of **AG** (50 mg, 0.102mmol) and DMAP (0.99 mmol) in pyridine (5 mL) at 25 °C was added benzoic anhydride (1.0 mmol). The reaction mixture was stirred under reflux for 4h, quenched with water (50 mL) and extracted with EtOAc (3 × 50 mL). The organic layers were dried over Na_2SO_4 and concentrated. The residue was then purified through column chromatography (*n*-hexane:EtOAc; 8:2) to produce **A12** (3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-6,16-dihydroxy-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl benzoate (13.0% yield) and **A13** (3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-16-hydroxy-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,8,10,12,13,14, 15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,6-diyl dibenzoate (38.9% yield)

^{1}H NMR (**A12**) (400 MHz, $CDCl_3$) δ (ppm): 0.82 (s, 3H), 0.94 (s, 3H), 1.15 (s, 3H), 1.16 (s, 3H), 1.28 (s, 3H), 1.30 (s, 3H), 1.31 (s, 3H), 1.33 (s, 3H), 1.5-2.0 (m, 15H), 2.35 (d, J=7.9 Hz, 1H), 2.55-2.63 (m, 2H), 2.87 (m, 1H), 3.77 (t, *J*=7.4 Hz, 1H), 4.07 (t, *J*=6.8 Hz, 1H), 4.23 (m, 1H), 4.71 (dd, *J*=7Hz, 1H), 5.32 (d, *J*=6.3 Hz, 1H), 7.45 (t, *J*=7.6 Hz, 2H), 7.55 (d, *J*=7.5 Hz, 1H), 8.06 (d, *J*=7.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.5, 146.2, 136.1, 132.9, 131.2, 129.8, 128.5, 125.0, 115.9, 87.2, 81.6, 73.5, 72.0, 70.0, 57.5, 56.3, 44.3, 44.2, 41.0, 40.9, 38.8, 37.7, 31.3, 30.5, 29.6, 28.0, 26.7, 26.1, 24.0, 23.4, 22.9, 19.4, 18.3, 17.4, 14.3; HRMS *m/z* calcd for $C_{37}H_{54}O_6$, $[M+Na]^+$ 617.3818; found 617.3765.

^{1}H NMR (**A13**) (400 MHz, $CDCl_3$) δ (ppm): 0.83 (s, 3H), 0.95 (s, 3H), 1.16 (s, 3H), 1.17 (s, 3H), 1.25 (s, 3H), 1.26 (s, 3H), 1.27 (s, 3H), 1.30 (s, 3H), 1.4-2.0 (m, 13H), 2.17 (d, *J*=17.3 Hz, 1H), 2.35 (d, *J*=7.9 Hz, 1H), 2.61 (m, 2H), 3.75 (t,

J=7.3 Hz, 1H), 4.68 (dd, J=14.4 Hz, 1H), 4.75 (dd, J=7.5 Hz, 1H), 5.38 (d, J=5.7 Hz, 1H), 5.78 (td J=10.9, 4.3 Hz, 1H), 7.41 (dd J=15.8, 7.9 Hz, 4H), 7.53 (dd J=13.4, 7.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.5, 165.9, 145.7, 133.1, 133.0, 131.0, 130.9, 130.0, 129.7, 128.6, 128.5, 116.4, 87.2, 81.6, 81.3, 73.4, 72.6, 71.9, 56.3, 55.4, 44.7, 44.3, 44.1, 41.3, 40.3, 38.4, 37.7, 36.1, 34.6, 31.3, 30.5, 29.9, 29.6, 28.1, 27.9, 26.8, 25.9, 23.9, 23.3, 22.8, 19.3, 18.3, 17.5, 14.3 ; HRMS *m/z* calcd for $C_{44}H_{58}O_7$, $[M+Na]^+$ 721.4080; found 721.4178.

3.6. Synthesis of A14 and A15

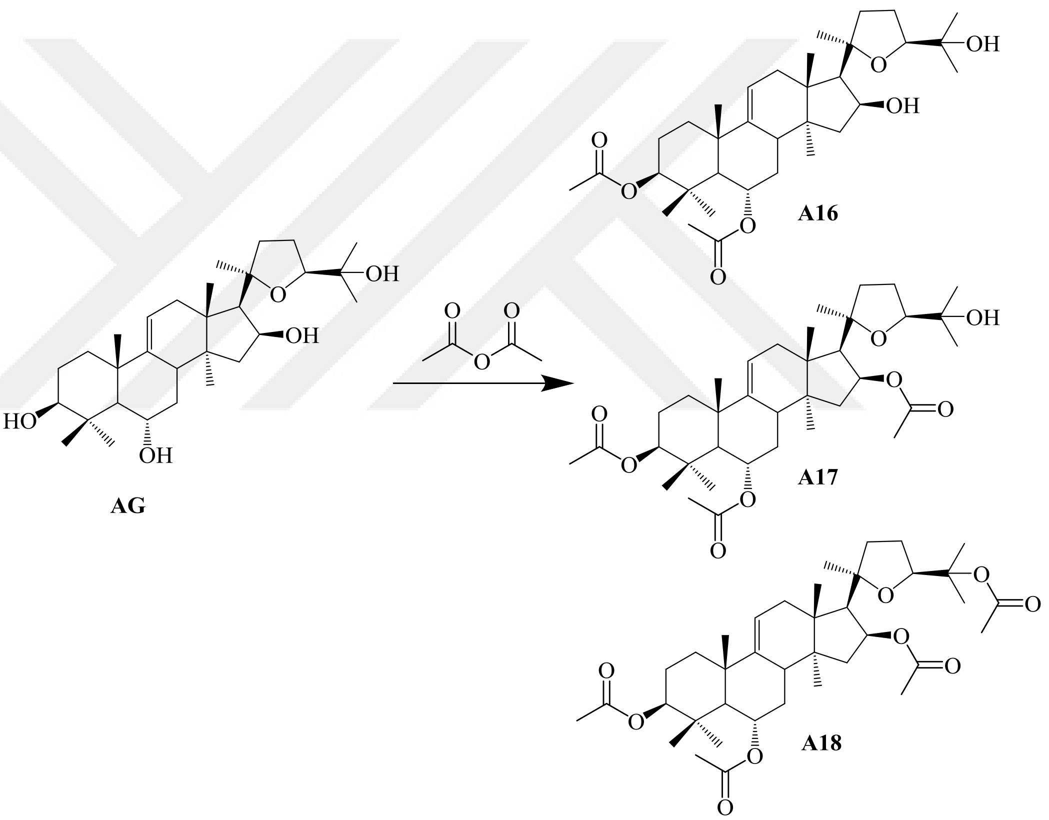


To a solution of **AG** (50 mg, 0.102mmol) and DMAP (0.099 mmol) in pyridine (5 mL) at 25 ^{o}C was added pivalic anhydride (1.0 mmol). The reaction mixture was stirred under reflux for 4h, quenched with water (50 mL) and extracted with EtOAc (3 × 50 mL). The organic layers were dried over Na_2SO_4 and concentrated. The residue was then purified through column chromatography (*n*-hexane: EtOAc; 8:2) to produce **A14**, (3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-6,16-dihydroxy-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl pivalate, (29.8% yield) and **A15**, (3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-16-hydroxy-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-2, 3, 4, 5, 6, 7, 8, 10, 12, 13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,6-diyl bis(2,2-dimethylpropanoate), (11.5% yield).

^{1}H NMR (**A14**) (400 MHz, $CDCl_3$) δ (ppm): 0.80 (s, 3H), 0.92 (s, 3H), 1.10 (s, 3H), 1.15 (s, 3H), 1.18 (s, 3H), 1.21 (s, 9H), 1.24 (s, 3H), 1.26 (s, 3H), 1.30 (s, 3H), 1.39-2.06 (m, 13H), 2.13 (d, J=16.8 Hz, 1H), 2.34 (d, J=7.8 Hz, 1H), 2.57 (dd, J=21.5, 10.4 Hz, 1H), 3.75 (t, J=7.3 Hz, 1H), 4.19 (m, 1H), 4.41 (dd, J=11.1, 4.1 Hz, 1H), 4.70 (dd, J=14.4, 7.9 Hz, 1H), 5.28 (d, J=5.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 178.2, 146.0, 115.7, 87.0, 81.4, 80.3, 73.3, 71.8, 69.7, 57.2, 56.1, 44.9, 44.1, 43.9, 40.8, 40.7, 39.2, 39.0, 38.4, 37.4, 35.8, 34.5, 31.0, 29.7, 27.8, 27.2 (×3), 26.5, 26.6, 23.5, 23.2, 19.1, 18.1, 17.0; HRMS *m/z* calcd for $C_{35}H_{58}O_6$, $[M+Na]^+$ 597.4131; found 597.4086.

^{1}H NMR (**A15**) (400 MHz, $CDCl_3$) δ (ppm): 0.79 (s, 3H), 0.92 (s, 3H), 0.99 (s, 3H), 1.08 (s, 3H), 1.16 (s, 3H), 1.21 (s, 18H), 1.24 (s, 3H), 1.26 (s, 3H), 1.31 (s, 3H), 1.35-2.00 (m, 13H), 2.13 (d, *J*=17.6 Hz, 1H), 2.33 (d, *J*=7.7 Hz, 1H), 2.56 (m, 2H), 3.76 (t, *J*=7.4 Hz, 1H), 4.41 (m, 1H), 4.68 (dd, *J*=14.8, 7.5 Hz, 1H), 5.31 (d, *J*=5.6, Hz, 1H), 5.43 (td *J*=10.8, 4.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 178.1, 178.0, 145.6, 115.9, 87.0, 81.4, 80.2, 73.3, 72.1, 71.8, 56.1, 54.7, 44.7, 44.1, 44.0, 40.0, 39.0, 38.8, 37.9, 37.4, 35.8, 34.5, 34.3, 30.9, 29.7, 27.8, 27.2 (×6) 26.5, 25.6, 23.4, 23.4, 23.1, 19.1, 18.1, 17.0; HRMS *m/z* calcd for $C_{40}H_{66}O_7$, $[M+Na]^+$ 681.4706; found 681.4666.

3.7. Synthesis of A16, A17 and A18



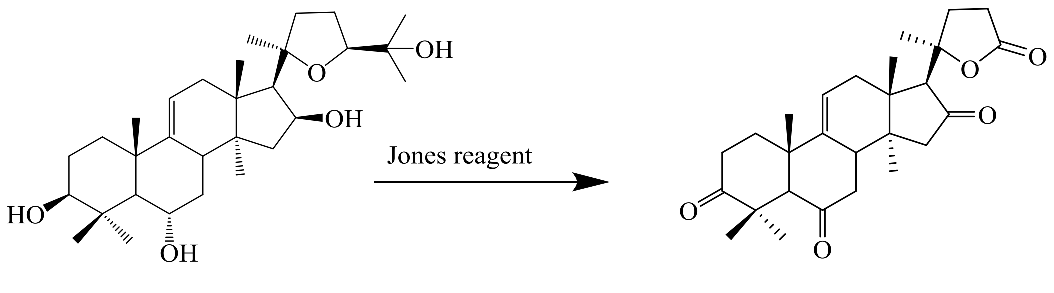
To a solution of **AG** (50 mg, 0.102mmol) in pyridine (5 mL) at 25 ^{o}C was added acetic anhydride (250 µL). The reaction mixture was stirred at room temperature for 12h, quenched with water (50 mL) and extracted with EtOAc (3 × 50 mL). The organic layers were dried over Na_2SO_4 and concentrated. The residue was then purified through column chromatography (*n*-hexane: EtOAc; 8:2) to produce **A16**, (3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-16-hydroxy-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta [*a*]phenanthrene-3,6-diyl diacetate (11.4% yield), **A17**, (3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,6,16-triyl triacetate (65% yield) and **A18**, (3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-17-((2*R*,5*S*)-5-(2-acetoxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta [*a*]phenanthrene- 3,6,16-triyl triacetate, (9.2% yield).

^{1}H NMR (**A16**) (400 MHz, $CDCl_3$) δ (ppm): 0.78 (s, 3H), 0.92 (s, 3H), 0.97 (s, 3H), 1.16 (s, 3H), 1.24 (s, 3H), 1.29 (s, 3H), 1.31 (s, 3H), 1.33 (s, 3H), 1.4-2.0 (m, 12H), 2.06 (m, 6H), 2.33 (d, *J*=7.7 Hz, 1H), 2.60 (m, 1H), 3.76 (t, *J*=7, 4 Hz, 1H), 4.45 (dd, *J*=11.0, 4.0 Hz, 1H), 4.68 (q, *J*=7.1 Hz, 1H), 5.37 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 170.9, 170.2 145.5, 116.0, 87.0, 81.4, 80.5, 77.2, 73.3, 71.8, 56.0, 54.8, 44.7, 44.1, 44.0, 40.9, 40.0, 37.7, 34.5, 34.3, 31.9, 30.5, 30.3, 27.8, 26.5, 25.6, 23.6, 23.0, 22.7, 21.9, 21.3, 19.1, 18.1, 17.0; HRMS *m/z* calcd for $C_{34}H_{54}O_7$, $[M+Na]^+$ 597.3768; found .597.3528

^{1}H NMR (**A17**) (400 MHz, $CDCl_3$) δ (ppm): 0.82 (s, 3H), 0.96 (s, 3H), 1.05 (s, 3H), 1.09 (s, 3H), 1.15 (s, 3H), 1.21 (s, 3H), 1.26 (s, 3H), 1.30 (s, 3H), 1.54-2.00 (m, 13H), 2.03 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.20 (m, 2H), 2.34 (td, *J*=11.4, 5.1 Hz, 1H), 2.48 (d, *J*=8.2 Hz, 1H), 3.69 (dd *J*=8.6, 7.1 Hz, 1H), 4.46 (dd, *J*=11.2, 3.8 Hz, 1H), 5.34 (m, 2H), 5.42 (td, *J*=8.0, 5.4 Hz, 1H); HRMS *m/z* calcd for $C_{36}H_{56}O_8$, $[M+Na]^+$ 639.3872; found 639.7107.

^{1}H NMR (**A18**) (400 MHz, $CDCl_3$) δ (ppm): 0.80 (s, 3H), 0.95 (s, 3H), 0.98 (s, 3H), 1.05 (s, 3H), 1.15 (s, 3H), 1.26 (s, 3H), 1.32 (s, 3H), 1.44 (s, 3H), 1.46-1.94 (m, 12H), 1.96 (s, 3H), 2.01 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.1-2.2 (m, 3H), 2.42 (d, *J*=8.0 Hz, 1H), 2.49 (m, 1H), 4.02 (dd, *J*=14.1, 6.7 Hz, 1H), 4.45 (m, 1H), 5.34 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 171.1, 171.1, 170.6, 170.4, 145.5, 116.8, 85.6, 83.2, 81.4, 80.6, 75.8, 72.0, 56.6, 55.0, 45.4, 44.6, 41.2, 40.4, 37.9, 37.6, 37.2, 36.0, 34.6, 30.8, 30.5, 29.9, 27.2, 26.5, 23.8, 23.3, 23.0, 22.7, 22.1, 21.8, 21.5, 19.5, 17.8, 17.2; HRMS *m/z* calcd for $C_{38}H_{58}O_9$, $[M+Na]^+$ 681.3979; found 681.3206.

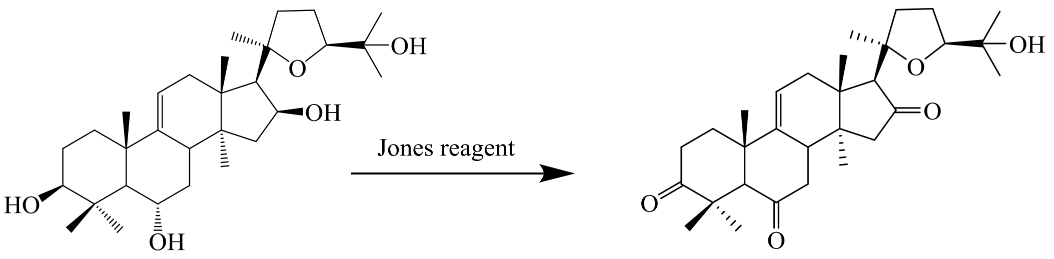
3.8. Synthesis of A4



To a stirred solution of **AG** (500 mg, 1.14 mmol) in acetone (150 mL), the Jones reagent (0.4 mL, 3.3 mmol) was added slowly at room temperature and stirred overnight. After adding sat. aq. $NaHCO_3$, the aqueous mixture was extracted with CH_2Cl_2 (3 × 25mL). The combined organic layers was washed with brine solution, dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using hexanes: ethyl acetate (8:2) as eluent to give **A4**, (10*S*,13*R*,14*S*,17*S*)-4,4,10,13,14-pentamethyl-17-((*R*)-2-methyl-5-oxotetrahydrofuran-2-yl)-4,5,7,8,10,12,13,14,15,17-decahydro-1*H*-cyclopenta[*a*]phenanthrene-3,6,16(2*H*)-trione as a white solid (72.0% yield).

^{1}H NMR (**A4**) (400 MHz, $CDCl_3$) δ (ppm): 1.01 (s, 3H), 1.04 (s, 3H), 1.12 (s, 3H), 1.35 (s, 3H), 1.50 (s, 3H), 1.53 (s, 3H), 2.0-2.40 (m, 10H), 2.45 (t, *J*=12.8 Hz, H), 2.50-2.63 (m, 3H), 2.87 (m, 3H), 5.63 (d *J*=2.7Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 214.6, 213.7, 209.6, 176.3, 144.3, 118.7, 86.9, 63.6, 62.1, 48.4, 47.0, 44.5, 44.4, 43.6, 42.6, 41.2, 37.1, 35.8, 35.1, 34.2, 29.9, 28.9, 26.2, 24.7, 23.2, 22.3, 18.6, 17.3, HRMS *m/z* calcd for $C_{27}H_{36}O_5$, $[M+NH_4]^+$ 458.2963; found 458.2924.

3.9. Synthesis of A5

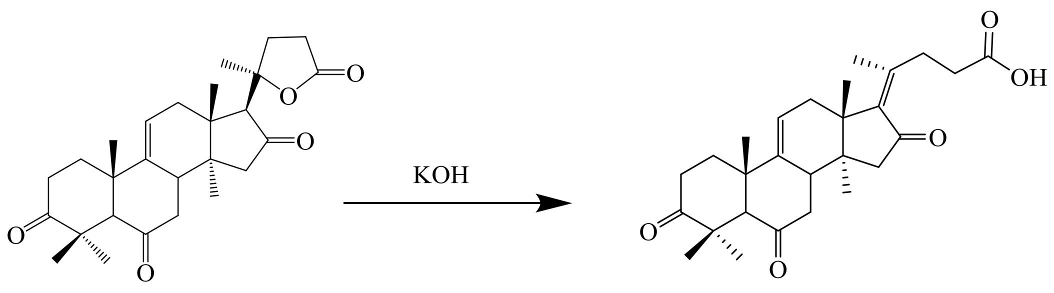


AG **A5**

To a stirred solution of **AG** (100 mg, 0.02 mmol) in acetone (100 mL), the Jones reagent (0.4 mL, 3.3 mmol) was added slowly at ice bath and stirred till completion of the reaction. After adding sat. $NaHCO_3$ aq., the aqueous mixture was extracted with CH_2Cl_2 (3 × 25mL). The combined organic layers was washed with brine solution, dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using hexanes: ethyl acetate (8:2) as eluent to give **A5**, (10*S*,13*R*,14*S*,17*S*)-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)- 4,4,10,13,14-pentamethyl-4,5,7,8,10,12,13,14,15,17-decahydro-1*H*-cyclopenta[*a*]phenanthrene-3,6,16(2*H*)-trione as a white solid (77.2% yield).

^{1}H NMR (**A5**) (400 MHz, $CDCl_3$) δ (ppm): 0.91 (s, 3H), 1.04 (s, 3H), 1.12 (s, 3H), 1.21 (s, 3H), 1.25 (s, 3H), 1.35 (s, 3H), 1.53 (s, 3H), 1.62 (s, 3H), 1.40-2.40 (m, 11H), 2.41-2.58 (m, 3H), 2.88 (m, 3H), 3.77 (dd, *J*=8.2, 5.9 Hz, 1H), 5.62 (d, *J*=6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 216.9, 213.9, 209.8, 144.3, 118.7, 84.5, 82.4, 71.0, 64.1, 62.2, 49.0, 47.0, 44.3, 43.7, 42.7, 41.0, 37.1, 36.2, 34.2, 31.4, 29.9, 28.1, 26.5, 25.4, 24.6, 23.2, 22.3, 18.6, 17.2, 14.3; HRMS *m/z* calcd for $C_{30}H_{44}O_5$, $[M+Na]^+$ 507.3086; found 507.3117.

3.10. Synthesis of A9

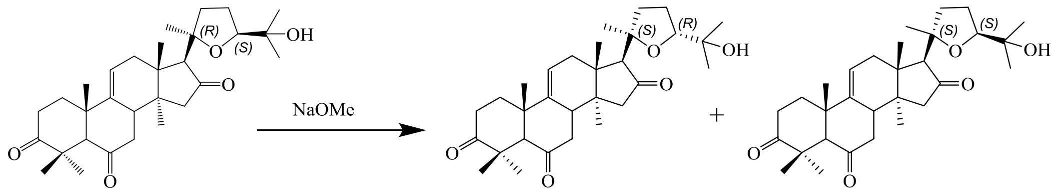


A4 **A9**

A4 (20 mg) was dissolved in 4mL EtOH and added KOH (100 mg) and stirred at room temperature for overnight. The reaction mixture quenched with HCl (10%), and the solvent was evaporated. After adding water, the aqueous mixture was extracted with CH_2Cl_2 (3 × 25mL). The combined organic layers was washed with brine solution, dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane: ethyl acetate (6:4) as eluent to give **A9**, (*Z*)-4-((10*S*,14*S*)-4,4,10,14-tetramethyl-3,6,16-trioxo-1,2,3,4,5,6,7,8,10,12,13,14,15,16-tetradecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)pentanoic acid as a white solid (64.0% yield).

^{1}H NMR (**A9**) (400 MHz, $CDCl_3$) δ (ppm): 0.96 (s, 3H), 1.03 (s, 3H), 1.04 (s, 3H), 1.15 (s, 3H), 1.16 (s, 3H), 1.25 (s, 3H), 1.40-2.00 (m, 3H), 2.1-2.6 (m, 5H), 2.73 (m, 3H), 2.8-3.0 (m, 3H), 5.80 (d, *J*=6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 213.9, 209.8, 205.6, 173.6, 149.1, 139.8, 138.9, 120.3, 66.7, 51.8, 48.6, 47.5, 46.0, 41.8, 40.9, 39.9, 35.8, 34.5, 32.7, 30.9, 29.8, 24.9, 24.3, 20.8, 20.6, 19.6; HRMS *m/z* calcd for $C_{26}H_{34}O_5$, $[M+K]^+$ 465.2043; found 465.2377

3.11. Synthesis of A27 and 29



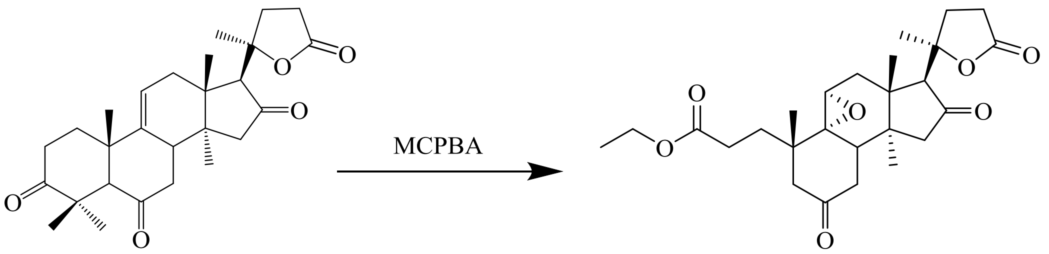
A5 A27 A29

A5 (120 mg) was dissolved in 8 mL THF and was added NaOMe (135 mg) at room temperature. The reaction mixture stirred overnight and adding sat. aq. $NaHCO_3$, the aqueous mixture was extracted with CH_2Cl_2 (3 × 25mL). The combined organic layers was washed with brine solution, dried over Na_2SO_4 and filtered. The filtrate was evaporated, and a chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane:EtOAc (9:1) as eluent to give **A27**, (10*S*,13*R*,14*S*,17*S*)-17-((2*S*,5*R*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-4,5,7,8,10,12,13,14,15, 17-decahydro-1*H*-cyclopenta[*a*]phenanthrene-3,6,16(2*H*)-trione, as a white solid (24.9% yield) and **A29**, (10*S*,13*R*,14*S*,17*S*)-17-((2*S*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-4,5,7,8,10,12,13,14,15,17-decahydro-1*H*-cyclopenta[*a*]phenanthrene-3,6,16(2*H*)-trione as a white solid.(16.0% yield).

^{1}H NMR (**A27**) (400 MHz, $CDCl_3$) δ (ppm): 1.01 (s, 3H), 1.05 (s, 3H), 1.09 (s, 3H), 1.14 (s, 3H), 1.16 (s, 3H), 1.28 (s, 3H), 1.36 (s, 3H), 1.54 (s, 3H), 1.60-2.50 (m, 15H), 2.56 (s, 2H), 2.73 (m, 3H), 2.90 (m, 3H), 3.88 (dd, *J*=10.8, 5.7 Hz, 1H), 5.63 (d, *J*=5.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 215.6, 214.0, 210.0, 144.3, 119.0, 88.4, 83.8, 70.4, 63.5, 62.3, 48.9, 47.0, 45.1, 44.4, 43.8, 42.7, 40.7, 37.2, 36.4, 34.3, 29.9, 29.5, 28.2, 25.4, 24.7, 24.2, 23.2, 22.3, 18.6, 17.1; HRMS *m/z* calcd for $C_{30}H_{44}O_5$, $[M+Na]^+$ 507.3086; found 507.3139

^{1}H NMR(**A29**) (400 MHz, $CDCl_3$) δ (ppm): 1.05 (s, 3H), 1.09 (s, 3H), 1.12 (s, 3H), 1.16 (s, 3H), 1.19 (s, 3H), 1.24 (s, 3H), 1.27 (s, 3H), 1.43 (s, 3H), 1.50-2.40 (m, 15H), 2.41-2.65 (m, 2H), 2.75 (m, 3H), 2.8-3.0 (m, 3H), 3.76 (dd, *J*=8.3, 5.9 Hz, 1H), 5.81 (d, *J*=6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 216.6, 213.9, 209.8, 139.3, 120.5, 84.5, 82.5, 70.9, 66.7, 64.0, 48.8, 46.0, 44.0, 42.0 (×2), 41.2, 41.1, 36.7, 34.7, 34.5, 32.9, 30.5, 29.9, 28.2, 26.5, 25.4, 25.0, 24.3, 18.2, 17.3; HRMS *m/z* calcd for $C_{30}H_{44}O_5$, $[M+Na]^+$ 507.3086; found 507.3056

3.12. Synthesis of A25

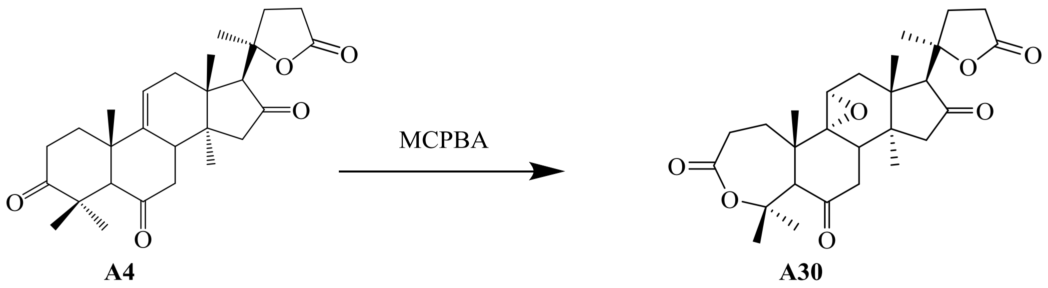


A4 **A25**

To a stirred solution of **A4** (200 mg, 0.454 mmol) in chloroform (8 mL), five equivalent of MCPBA (0.509 g, 2.27 mmol) was added portion wise at room temperature. The mixture was refluxed by stirring for 8h. The mixture was allowed to cool down to room temperature followed by evaporation in vacuo. The residue was dissolved in minute amount of EtOH, and then saturated $NaHCO_3$ solution was added, which is followed by EtOAc extraction (3 × 25mL). The combined organic layers was washed with water (3 × 25mL), brine (3 × 25mL), dried over anhyd. Na_2SO_4, and filtered. The resulting filtrate was evaporated in vacuo to give a solid. The solid residue was subjected to column chromatography [*n*-hexane:EtOAc (7:3)] to give **A25** as a white solid (64% yield).

^{1}H NMR (**A25**) (400 MHz, Pyridine-*d*5) δ (ppm): 1.04 (s, 3H), 1.07 (s, 3H), 1.11 (s, 3H), 1.16 (t, *J*=7.2 Hz, 3H), 1.47 (s, 3H), 1.50-2.00 (m, 5H), 2.12 (dd, *J*=14.8, 5.7 Hz, 1H), 2.22-2.70 (m, 11H), 3.07 (s, 1H), 3.36 (d, *J*=5.6 Hz, 1H), 4.15 (q, *J*=6.8 Hz, 2H); ^{13}C NMR (100 MHz, Pyridine-d5) δ (ppm): 215.9, 209.6 177.2, 174.5, 87.5, 66.5, 64.5, 61.4, 55.8, 50.9, 50.8, 45.9, 43.0, 41.9, 41.6, 38.9, 34.0, 31.3, 30.9, 30.2, 29.9, 27.2, 23.6, 19.6, 19.5, 15.2; HRMS *m/z* calcd for $C_{26}H_{36}O_7$, $[M+H]^+$ 461.2556; found 461.2549.

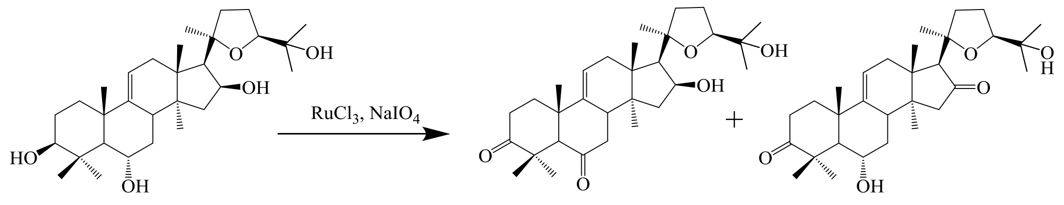
3.13. Synthesis of A30



To a stirred solution of **A4** (200 mg, 0.454 mmol) in chloroform (10 mL), two equivalent of MCPBA was added portion wise at room temperature. The mixture was refluxed by stirring for 6h. The mixture was allowed to cool down to room temperature followed by evaporation in vacuo. The residue was dissolved in minute amount of CH_2Cl_2, and then saturated $NaHCO_3$ solution was added, which is followed by CH_2Cl_2 extraction (3 × 50mL). The combined organic layers was washed with water (3 × 25mL), brine (3 × 25mL), dried over anhyd. Na_2SO_4, and filtered. The resulting filtrate was evaporated in vacuo to give a solid. The solid residue was subjected to column chromatography [*n*-hexane:EtOAc (7:3)] to give **A30**, (5a*S*,5b*R*,6a*R*,7a*R*,8*S*,10a*S*)-1,1,5a,7a,10a-pentamethyl-8-((*R*)-2-methyl-5-oxotetrahydrofuran-2-yl) dodecahydrocyclopenta [5,6]oxireno [2',3':8,8a]naphtha [2,1-*c*]oxepine-3,9,12(1*H*)-trione as a white solid (14.1% yield).

^{1}H NMR (**A30**) (400 MHz, DMSO-*d*6) δ (ppm): 0.99 (s, 3H), 1.05 (s, 3H), 1.10 (s, 3H), 1.31 (s, 3H), 1.37 (s, 3H), 1.66 (s, 3H), 1.80-2.25 (m, 5H), 2.30-3.00 (m, 11H), 3.22 (s, 1H), 3.56 (d, *J*= 6.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 216.0, 210.4, 176.4, 174.2, 87.8, 82.4, 67.7, 63.8, 63.2, 56.3, 50.0, 46.9, 44.9, 44.5, 41.2, 39.0, 33.1, 32.9, 32.0, 31.1, 29.6, 29.0, 27.8, 24.9, 19.7, 19.2, 18.6; HRMS *m/z* calcd for $C_{27}H_{36}O_7$, $[M+H]^+$ 473.2461; found 473.2460.

3.14. Synthesis of A34 and A35



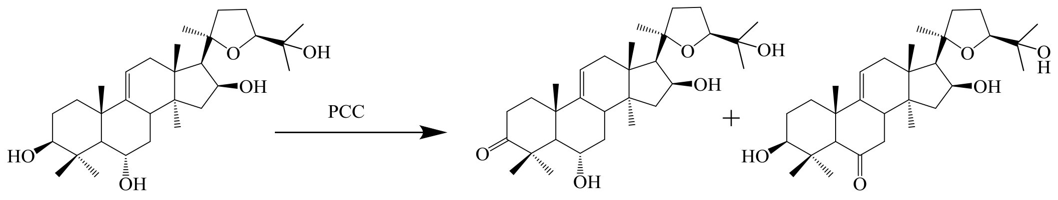
AG A34 A35

To a solution of **AG** (100 mg) in 6 mL chloroform was added $NaIO_4$ (150 mg) in 8 mL water: acetonitrile and 2% $RuCl_3$. After being stirred at 25 ^{o}C for overnight. The residue was dissolved in minute amount of chloroform, and then saturated $NaHCO_3$ solution was added, followed by chloroform extraction (3 × 50mL). The combined organic layers was washed with water (3 × 25mL), brine (3 × 25mL), dried over anhyd. Na_2SO_4 and filtered. The resulting filtrate was evaporated in vacuo to give a solid. The solid residue was subjected to column chromatography [*n*-hexane:EtOAc (9:1)] to give **A34**, (10*S*,13*R*,14*S*,16*S*,17*R*)-16-hydroxy-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-1,4,5,7,8,10,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[*a*]phenanthrene-3,6(2*H*)-dione, as a white solid (44.2% yield) and **A35**, (6*S*,10*S*,13*R*,14*S*,17*S*)-6-hydroxy-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-1,4,5,6,7,8,10,12,13,14,15,17-dodecahydro-3*H*-cyclopenta[*a*]phenanthrene-3,16(2*H*)-dione, as a white solid (16.8% yield).

^{1}H NMR (**A34**) (400 MHz, $CDCl_3$) δ (ppm): 0.86 (s, 3H), 0.98 (s, 3H), 1.11 (s, 3H), 1.16 (s, 3H), 1.25 (s, 3H), 1.31 (s, 3H), 1.32 (s, 3H), 1.53 (s, 3H), 1.8-2.08 (m, 6H), 2.1-2.6 (m, 12H), 2.87 (td, *J*=14.4, 5.6 Hz, 1H), 3.76 (t, *J*=7.4 Hz, 1H), 4.69 (ddd, *J*=8.0, 8.0, 7.8 Hz, 1H), 5.50 (d *J*=6 Hz, 1H); HRMS *m/z* calcd for $C_{30}H_{46}O_5$, $[M+H]^+$ 487.3345; found.487.2817

^{1}H NMR (**A35**) (400 MHz, $CDCl_3$) δ (ppm): 0.80 (s, 3H), 0.96 (s, 3H), 0.97 (s, 3H), 1.09 (s, 3H), 1.18 (s, 3H), 1.24 (s, 3H), 1.35 (s, 3H), 1.36 (s, 3H), 1.44-2.24 (m, 14H), 2.41 (m, 3H), 2.72 (ddd, *J*=16.1, 10.7, 7.1 Hz, 1H), 2.92 (bs, 1H), 3.72 (dd, *J*=8.4, 5.7 Hz, 1H), 3.99 (dd, *J*=10.7, 7.1 Hz, 1H), 5.45 (d *J*=6 Hz, 1H); HRMS *m/z* calcd. for $C_{30}H_{46}O_5$, $[M+H]^+$ 487.3345; found.487.2817.

3.15. Synthesis of A38 and A36



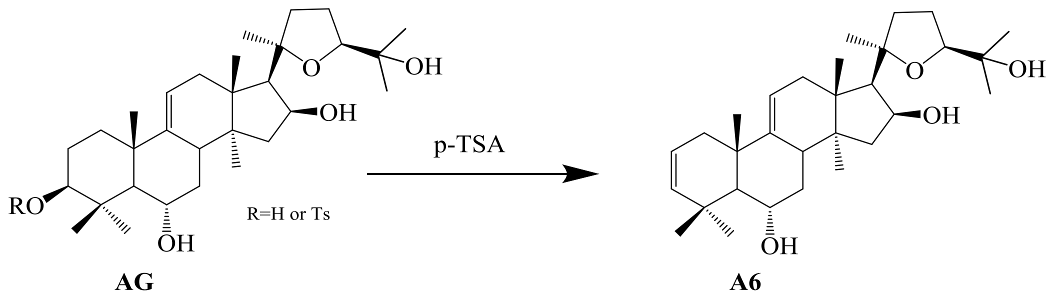
AG **A38** **A36**

To a solution of **AG** (100 mg) in acetone was added three equivalent of PCC and stirred at 4 ^{o}C for 8h. The reaction mixture quenched with water. Then saturated $NaHCO_3$ solution was added, followed by chloroform extraction (3 × 50mL). The combined organic layers was washed with water (3×), brine (3×), dried over anhyd. Na_2SO_4 and filtered. The resulting filtrate was evaporated in vacuo to give a solid. The solid residue was subjected to column chromatography [hexanes-EtOAc (6:4)] to give **A38**, (6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-6,16-dihydroxy-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-1, 2,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-3*H*-cyclopenta[*a*]phenanthren-3-one, as a white solid (6% yield) and **A36**, (3*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-3,16-dihydroxy-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-1,2,3,4,5,7,8,10,12,13,14,15,16,17-tetradecahydro-6*H*-cyclopenta [*a*]phenanthren-6-one as a white solid (16% yield).

^{1}H NMR (**A38**) (400 MHz, $CDCl_3$) δ (ppm): 0.76 (s, 3H), 0.88 (s, 3H), 0.93 (s, 3H), 1.09 (s, 3H), 1.17 (s, 3H), 1.26 (s, 3H), 1.31 (s, 3H), 1.32 (s, 3H), 1.38-1.59 (m, 5H), 1.68 (d, *J*=10.5 Hz, 1H), 1.75-2.50 (m, 12H), 2.50-2.75 (m, 2H), 3.70 (dd, *J*=8.6, 5.1 Hz, 1H), 3.95 (m, 1H), 4.69 (ddd, *J*=8.0, 8.0, 7.4 Hz, 1H), 5.33 (d, *J*=5.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 218.9, 145.3, 117.7, 86.5, 81.2, 73.2, 72.0, 69.1, 56.9, 56.1, 47.5, 45.0, 44.0, 43.5, 40.6, 40.1, 37.9, 37.6, 35.2, 34.3, 33.3, 31.4, 28.0, 27.6, 26.5, 25.9, 24.1, 20.5, 18.9, 18.0; HRMS *m/z* calcd for $C_{30}H_{48}O_5$, $[M+Na]^+$ 511.3399; found 511.3388

^{1}H NMR (**A36**) (400 MHz, $CDCl_3$) δ (ppm): 0.86 (s, 3H), 0.93 (s, 3H), 1.05 (s, 3H), 1.06 (s, 3H), 1.15 (s, 3H), 1.24 (s, 3H), 1.25 (s, 3H), 1.30 (s, 3H), 1.39-2.04 (m, 12H), 2.12-2.47 (m, 6H), 2.57 (m, 1H), 2.72 (m, 1H), 3.12 (dd, *J*=10.8, 4.0 Hz, H), 3.75 (t, *J*=7.3 Hz, 1H), 4.69 (ddd, *J*=8.0, 8.0, 7.8 Hz, 1H), 5.47 (d, *J*=5.8 Hz, 1H); HRMS *m/z* calcd for $C_{30}H_{48}O_5$, $[M+Na]^+$ 511.3399; found 511.3388

3.16. Synthesis of A6

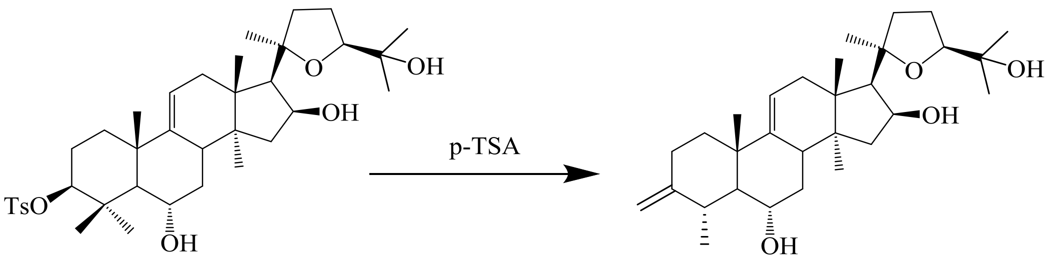


100 mg of the **A8** was dissolved in 4 mL pyridine. p-Toluenesulfonic acid (150 mg) was added and the reaction mixture was stirred under reflux for 6h. The reaction mixture was transferred into a separatory funnel and washed with H_2O (2 × 25mL), 1% aq. HCl (2 × 25mL), 0.1N aq. NaOH (2 × 25mL), sat. $NaHCO_3$ (3 × 25mL), H_2O (1 × 25mL) and brine (1 × 25mL), the organic layer was separated, dried over Na_2SO_4, filtered and the solvent was evaporated under vacuum. The residue was purified using column chromatography with hexanes:EtOAc (85:15) to furnish 20 mg of the product **A6**, (6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-4,5,6,7,8,10,12,13,14,15,16,17-dodecahydro-1*H*-cyclopenta[*a*]phenanthrene-6,16-diol.

Also, this compound was obtained by acidic hydrolysis from **CG** as a side product (6% yield)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.80 (s, 3H), 0.95 (s, 3H), 1.05 (s, 3H), 1.14 (s, 3H), 1.18 (s, 3H), 1.20 (s, 3H), 1.24 (s, 3H), 1.29 (s, 3H), 1.37-1.70 (m, 6H), 1.70-2.20 (m, 9H), 2.40 (m, 2H), 2.58 (m, 1H), 3.75 (t, *J*=7.2 Hz, 1H), 4.06 (td, *J*=10.7, 3.9 Hz, 1H), 4.71 (q, *J*=7.7 Hz, 1H), 5.31 (m, 2H), 5.50 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 145.9, 139.6, 120.3, 116.3, 87.2, 81.6, 73.5, 72.0, 70.3, 56.3, 55.5, 45.2, 44.3, 44.0, 41.0, 40.4, 38.7, 38.0, 36.1, 35.0, 34.7, 29.9, 28.1, 27.9, 26.7, 25.9, 23.8, 23.1, 19.3, 18.2; HRMS *m/z* calcd for $C_{30}H_{48}O_4$,$[M+H]^+$ 495.3553; found 495.3450.

3.17. Synthesis of A7

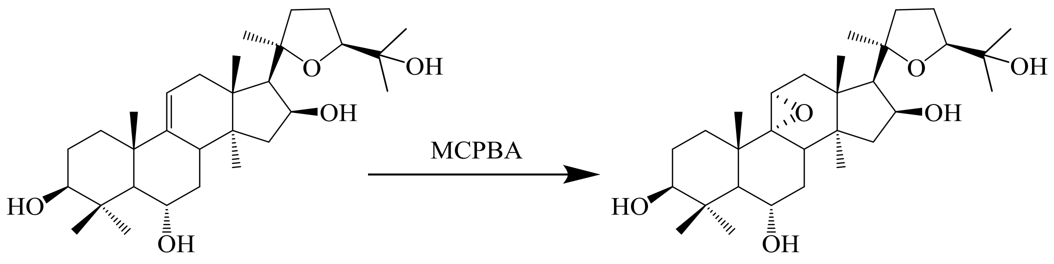


A8 **A7**

50 mg of the **A8** was dissolved in 4 mL pyridine. p-Toluenesulfonic acid (150 mg) was added and the reaction mixture was stirred under reflux for 6h. The reaction mixture was transferred into a separatory funnel and washed with H_2O (2 × 25mL), 1% aq. HCl (2 × 25mL), 0.1N aq. NaOH (2 × 25mL), sat. $NaHCO_3$ (3 × 25mL), H_2O (1 × 25mL) and brine (1 × 25mL), the organic layer was separated, dried over Na_2SO_4, filtered and the solvent was evaporated under vacuum. To the residue was purified using column chromatography with *n*-hexane:EtOAc (85:15) to furnish 20 mg of the product **A7**, (3*R*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-3,10,13,14-tetramethyl-4-methylene-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthrene-6,16-diol.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.84 (s, 3H), 0.88 (s, 3H), 0.94 (s, 3H), 1.07 (s, 3H), 1.08 (d, J=7.2 Hz, 3H), 1.13 (s, 3H), 1.29 (s, 3H), 1.40-1.75 (m, 6H), 1.80-2.20 (m, 8H), 2.36 (m, 2H), 2.58 (m, 2H), 3.75 (dd, *J*=8, 6.4 Hz, 1H), 4.04 (td, *J*=10.6, 4.1 Hz, 1H), 4.70 (m, 2H), 5.06 (bs, 1H), 5.41 (d, *J*=5.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 151.9, 144.7, 117.3, 107,5, 87.2, 81.6, 73.3, 71.9, 66.1, 56.3, 52.1, 48.5, 45.2, 44.5, 44.0, 39.9, 39.5, 37.8, 37.1, 34.9, 34.7, 29.6, 28.1, 27.8, 26.6, 25.9, 21.1, 19.3, 18.7, 18.1; HRMS *m/z* calcd for $C_{30}H_{48}O_4$, $[M+H]^+$ 495.3553; found 495.3450

3.18. Synthesis of A19

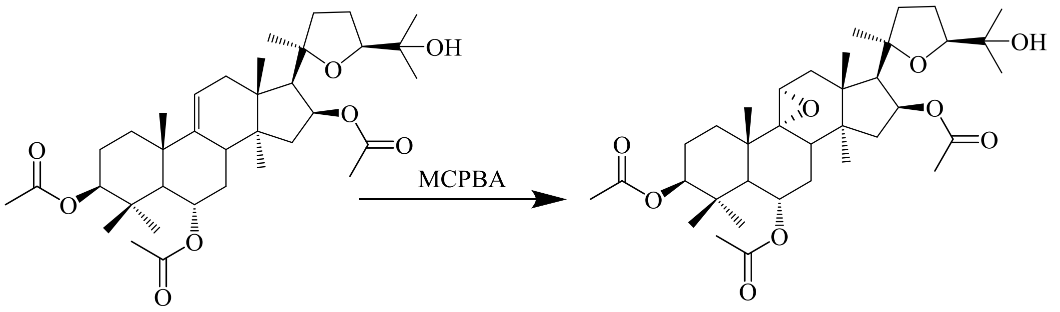


AG **A19**

To a solution of **AG** (50 mg) was dissolved in 2 mL chloroform and added MCPBA (350 mg). The reaction mixture was stirred at room temperature for 12h. After adding sat. aq. $NaHCO_3$, the aqueous mixture was extracted with CH_2Cl_2 (3 × 25mL). The combined organic layers was washed with brine solution, dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane:EtOAc (85:15) as eluent to give **A19**, (2*S*,4a*S*,4b*R*,5a*R*,6a*R*,7*R*,8*S*,9a*S*,11*S*)-7-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-1,1,4a,6a,9a-pentamethylhexadecahydrocyclopenta [1,2]phenanthro[4,4a-b]oxirene-2,8,11-triol, as a white solid (50.0% yield)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.89 (s, 3H), 1.02 (s, 3H), 1.11 (s, 3H), 1.16 (s, 3H), 1.23 (s, 3H), 1.26 (s, 3H), 1.31 (s, 3H), 1.33 (s, 3H), 1.40-1.80 (m, 9H), 1.80-2.10 (m, 5H), 2.27 (d, *J*=7.5 Hz, 1H), 2.34 (dd, *J*=13.4, 4.1 Hz, 1H), 2.54 (m, 1H),3.18 (d, *J*=6.1 Hz, 1H), 3.76 (m, 1H), 4.19 (t-like, *J*=9.2, 4 Hz, 1H), 4.70 (dd, *J*=13.7, 7.6 Hz, 1H); ^{13}C NMR (100 MHz, Pyridine-*d*5) δ (ppm): 87.4, 82.2, 73.9, 71.8, 70.4, 69.5, 57.5, 56.1, 55.5, 46.8, 45.0, 44.6, 40.8, 40.7, 39.6, 37.7, 36.0, 35.6, 35.5, 32.1, 30.5, 29.0, 28.6, 28.3, 27.6, 26.9, 21.1, 20.5, 20.2, 17.0; HRMS *m/z* calcd for $C_{30}H_{50}O_6$, $[M+H]^+$ 507.3686; found 507.3081.

3.19. Synthesis of A20

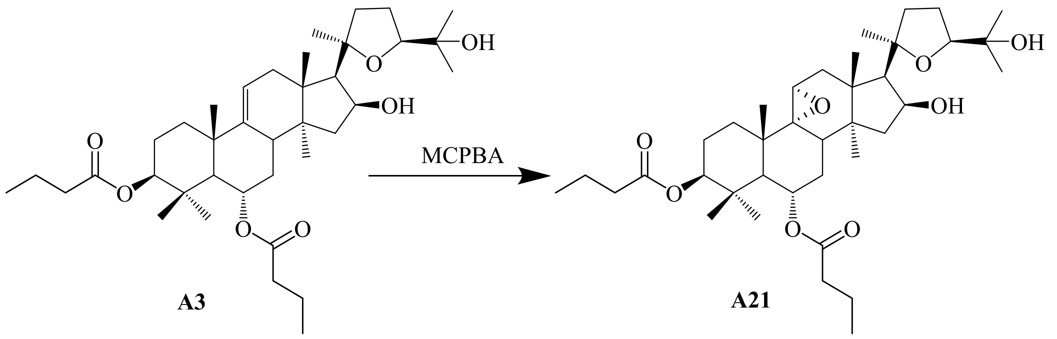


A17 **A20**

To a solution of **A17** (50 mg) was dissolved in 4 mL chloroform and added MCPBA (200 mg). The reaction mixture was stirred at room temperature for 12h. After adding sat. aq. $NaHCO_3$, the aqueous mixture was extracted with CH_2Cl_2 (3 × 25mL). The combined organic layers was washed with brine solution, dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane:EtOAc (9:1) as eluent to give **A20**, (2*S*,4a*S*,4b*R*,5a*R*,6a*R*,7*R*,8*S*,9a*S*,11*S*)-7-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-1,1,4a,6a,9a-pentamethylhexadecahydrocyclopenta [1,2]phenanthro[4,4a-b]oxirene-2,8,11-triyl triacetate, as a white solid (47.7% yield).

^{1}H NMR (A20) (400 MHz, $CDCl_3$) δ (ppm): 0.82 (s, 3H), 0.98 (s, 3H), 1.07 (s, 3H), 1.09 (s, 3H), 1.16 (s, 3H), 1.21 (s, 3H), 1.28 (s, 3H), 1.30 (s, 3H), 1.54-2.00 (m, 12H), 2.03 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.20-2.50 (m, 5H), 3.38 (d, *J*=6.2 Hz, 1H), 3.69 (dd *J*=8.6, 7.1 Hz, 1H), 4.46 (dd, *J*=11.2, 3.8 Hz, 1H), 5.34 (m, 2H); HRMS *m/z* calcd for $C_{36}H_{56}O_9$, $[M+H]^+$ 655.3822; found 655.3535.

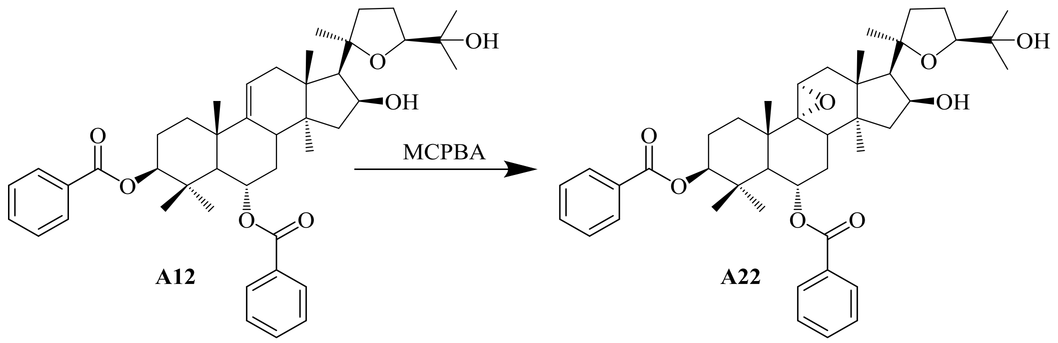
3.20. Synthesis of A21



To a solution of **A3** (50 mg) was dissolved in 2 mL chloroform and added MCPBA (350 mg). The reaction mixture was stirred at room temperature for 12h. After adding sat. aq. $NaHCO_3$, the aqueous mixture was extracted with CH_2Cl_2 (3 × 25mL). The combined organic layers was washed with brine solution, dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane:EtOAc (85:15) as eluent to give **A21**, (2*S*,4a*S*,4b*R*,5a*R*,6a*R*,7*R*,8*S*,9a*S*,11*S*)-8,11-dihydroxy-7-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-1,1,4a,6a,9a-pentamethyl hexadecahydrocyclopenta[1,2]phenanthro[4,4a-b]oxiren-2-yl butyrate as a white solid (50.2% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.92 (s, 3H), 0.94 (s, 3H), 0.96 (s, 3H), 1.02 (s, 3H), 1.10 (s, 3H), 1.15 (s, 3H), 1.22 (s, 3H), 1.30 (s, 6H), 1.40-2.05 (m, 19H), 2.15-2.40 (m, 5H), 2.53 (m, 1H), 3.18 (d, *J*=6.1 Hz, 1H), 3.74 (t, *J*=7.3 Hz, 1H), 4.41 (dd, *J*=11.3, 4.5 Hz, 1H), 4.67 (m, 1H), 5.40 (td, *J*=10.9, 4.3 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 173.5, 173.0, 86.9, 81.5, 79.5, 73.5, 72.1, 71.8, 68.2, 56.2, 55.3, 52.4, 45.8, 44.8, 44.1, 40.1, 38.0, 37.3, 36.9, 36.3, 35.4, 34.8, 33.8, 30.1, 29.9, 29.4, 28.1, 27.9, 26.8, 25.8, 23.2, 20.5, 20.0, 19.5, 18.8, 18.5, 17.1, 13.9; HRMS calcd for $C_{38}H_{62}O_8$, $[M+H]^+$ 647.4523; found 647.4691.

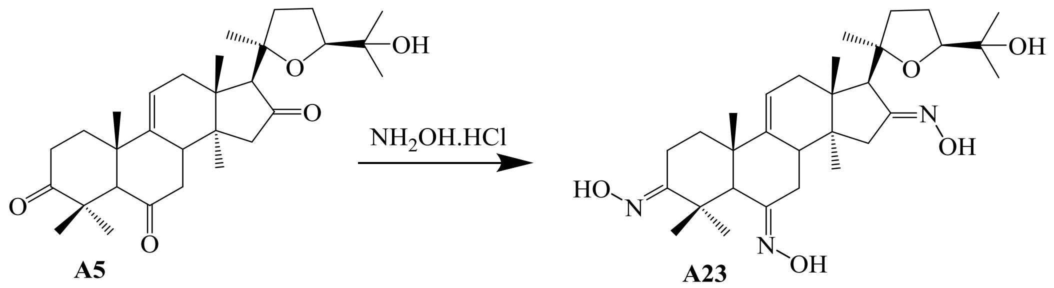
3.21. Synthesis of A22



To a solution of **A12** (25 mg) was dissolved in 4 mL chloroform and added MCPBA (200 mg). The reaction mixture was stirred at room temperature for 12h. After adding sat. aq. $NaHCO_3$, the aqueous mixture was extracted with CH_2Cl_2 (3×25mL). The combined organic layers was washed with brine solution, dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane:EtOAc (85:15) as eluent to give **A22**, (2*S*,4a*S*,4b*R*,5a*R*,6a*R*,7*R*,8*S*,9a*S*,11*S*)-8-hydroxy-7-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-1,1,4a,6a,9a-pentamethylhexadecahydro cyclopenta [1,2] phenanthro[4,4a-*b*]oxirene-2,11-diyl dibenzoate as a white solid (52.1% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.95 (s, 3H), 1.13 (s, 3H), 1.15 (s, 3H), 1.16 (s, 3H), 1.22 (s, 3H), 1.28 (s, 3H), 1.30 (s, 3H), 1.33 (s, 3H), 1.50-2.12 (m, 12H), 2.27 (d, *J*=7.7 Hz, 1H), 2.48 (dd, *J*= 13.2, 3.6 Hz, 1H), 2.58 (m, 1H), 3.26 (d, *J*=6.1 Hz, 1H), 3.75 (t, *J*=7.2 Hz, 1H), 4.69 (m, 2H), 5.77 (m, 1H), 7.40 (m, 4H), 7.53 (m, 2H), 7.98 (m, 2H), 8.05 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.1, 165.6, 133.0, 132.8, 130.7, 130.6, 129.9, 129.9, 129.5, 129.5, 128.4, 128.4, 128.3, 128.3, 86.7, 81.3, 80.2, 73.3, 72.3, 71.9, 68.1, 56.0, 55.3, 52.5, 45.7, 44.6, 44.0, 40.0, 38.3, 36.2, 35.2, 34.6, 33.8, 31.5, 27.9, 27.7, 26.6, 25.6, 23.0, 22.7, 20.4, 19.9, 19.3, 17.2; HRMS *m/z* calcd for $C_{44}H_{58}O_8$, $[M+Na]^+$ 737.4029; found 737.4078.

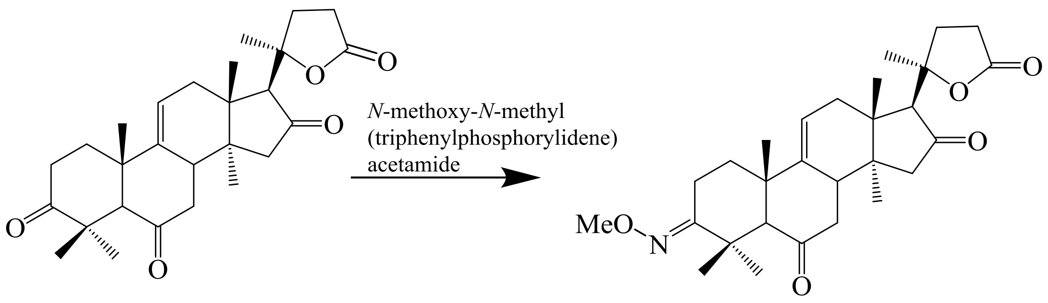
3.22. Synthesis of A23



To a solution of **A5** (150 mg) in pyridine (3 mL) was added $NH_2OH.HCl$ (147 mg) and stirred at room temperature for overnight. The reaction mixture was quenched by HCl (10%). Then saturated $NaHCO_3$ solution was added, which is followed by chloroform extraction (3 × 50mL). The combined organic layers was washed with water (3 × 25mL), brine (3 × 25mL), dried over anhyd. Na_2SO_4 and filtered. The resulting filtrate was evaporated in vacuo to give a solid. The solid residue was subjected to column chromatography [*n*-hexane:EtOAc (9:1)] to give **A23**, (3*E*,6*E*,10*S*,13*R*,14*S*,17*S*)-3,6-bis(hydroxyimino)-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,10,13,14-pentamethyl-1,2,3,4,5,6,7,8,10,12,13,14,15,17-tetradecahydro-16*H*-cyclopenta[*a*]phenanthren-16-one, as a white solid (36.0% yield).

^{1}H NMR (400 MHz, Pyridine-*d*5) δ (ppm): 0.83 (s, 3H), 1.24 (s, 3H), 1.26 (s, 3H), 1.38 (s, 3H), 1.59 (s, 3H), 1.80 (s, 3H), 1.83 (s, 3H), 1.89 (s, 3H), 1.40-2.05 (m, 12H), 2.15-2.40 (m, 5H), 3.90 (m, 1H), 5.40 (d, *J*=6.0 Hz, 1H); ^{13}C NMR (100 MHz, Pyridine-*d*5) δ (ppm): 164.3, 162.9, 157.7, 146.6, 122.7, 85.6, 81.9, 70.5, 62.5, 58.4, 44.5, 43.3, 42.8, 42.2, 42.0, 41.6, 41.3, 39.8, 38.8, 36.7, 31.6, 26.5, 26.2, 26.1, 26.1, 24.9, 23.5, 22.9, 22.7, 18.1.

3.23. Synthesis of A24

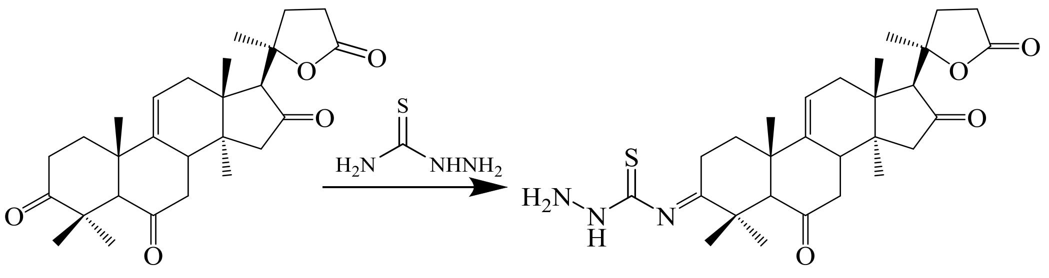


A4 **A24**

To a stirred solution of **A4** (100 mg, 0.202 mmol) in THF (15 mL) were added *N*-methoxy-*N*-methyl(triphenylphosphorylidene)acetamide (245 mg). The mixture was stirred for 6h under reflux. Then, the mixture was added water and extracted with CH_2Cl_2 (3 × 25mL). The combined organic layers was washed with brine solution, dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane:EtOAc (8:2) as eluent to give **A24**, (10*S*,13*R*,14*S*,17*S*,*E*)-3-(hydroxyimino)-4,4,10,13,14-pentamethyl-17-((*R*)-2-methyl-5-oxotetrahydrofuran-2-yl)-2,3,4,5,7,8,10,12,13,14,15,17-dodecahydro-1*H*-cyclopenta[*a*]phenanthrene-6,16-dione, as a white solid (26.4% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.92 (s, 3H), 1.03 (s, 3H), 1.12 (s, 3H), 1.35 (s, 3H), 1.54 (s, 3H), 1.95 (s, 3H), 2.00-2.54 (m, 12H), 2.86-2.96 (m, 5H), 3.67 (s, 3H), 5.61 (d, *J*=6.0 Hz, 1H); LCMS *m/z* calcd for $C_{28}H_{39}NO_5$, $[M+Na]^+$ 470.2906; found .470.29.

3.24. Synthesis of A31

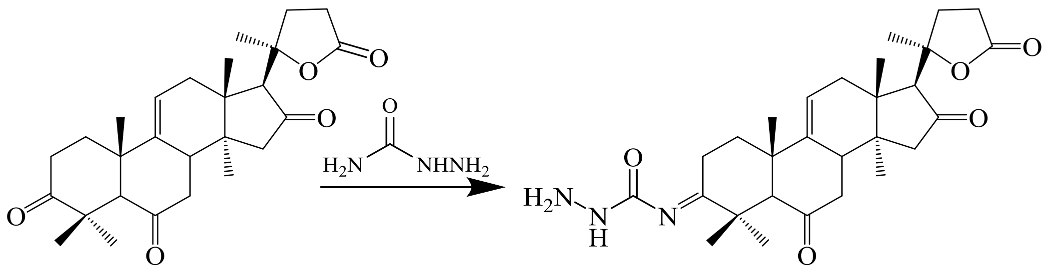


A4 **A31**

To a stirred solution of **A4** (100 mg, 0.202 mmol) in ethanol (7 mL) were added thiosemicarbazide (200 mg) and conc. HCl (1 mL). The mixture was stirred for 6h at 50 ^{o}C. Then the crude was evaporated in vacuo. After adding sat. aq. $NaHCO_3$, the aqueous mixture was extracted with CH_2Cl_2 (3 × 25mL). The combined organic layers was washed with brine solution, dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane:EtOAc (7:3) as eluent to give **A31**, *N*-((10*S*,13*R*,14*S*,17*S*,*E*)-4,4,10,13,14-pentamethyl-17-((*R*)-2-methyl-5-oxotetrahydrofuran-2-yl)-6,16-dioxo-1,2,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-3*H*-cyclopenta[*a*]phenanthren-3-ylidene) hydrazine carbothioamide as a white solid (29.8% yield)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.98 (s, 3H) 1.03 (s, 3H), 1.22 (s, 3H), 1.28 (s, 3H), 1.50 (s, 3H), 1.52 (s, 3H), 2.00-2.52 (m, 17H), 2.62 (m, 3H), 2.86 (m, 1H), 2.92 (s, 1H), 5.58 (d, *J*=4.8 Hz, 1H), 7.92 (bs, 2H); LCMS *m/z* calcd for $C_{28}H_{39}N_3O_4S$, $[M+Na]^+$ 536.2661; found 536.32.

3.25. Synthesis of A32

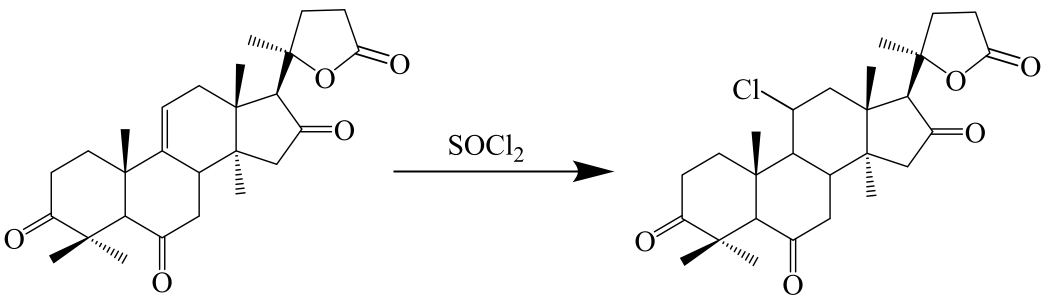


A4 **A32**

To a stirred solution of **A4** (100 mg, 0.202 mmol) in ethanol (7 mL) were added semicarbazide.HCl (200 mg). The mixture was stirred for 4h at 60 ^{o}C. Then the crude was evaporated in vacuo. After adding sat. aq. $NaHCO_3$, the aqueous mixture was extracted with CH_2Cl_2 (3 × 25mL). The combined organic layers was washed with brine solution, dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane:EtOAc (6:4) as eluent to give **A32**, *N*-((10*S*,13*R*,14*S*,17*S*,*E*)-4,4,10,13,14-pentamethyl-17-((*R*)-2-methyl-5-oxotetrahydrofuran-2-yl)-6,16-dioxo-1,2,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-3*H*- cyclopenta[*a*]phenanthren-3-ylidene)hydrazinecarboxamide as a white solid (24.3% yield)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.03 (s, 3H), 1.21 (s, 3H), 1.25 (s, 3H), 1.27 (s, 3H), 1.50 (s, 3H), 1.51 (s, 3H), 1.94-2.41 (m, 10H), 2.42-2.70 (m, 4H), 2.78 (m, 1H), 2.85 (m, 1H), 2.92 (s, 1H), 5.59 (d, *J*=5.4 Hz, 1H), 8.66 (bs, 2H); LCMS *m/z* calcd for $C_{28}H_{39}N_3O_5$, $[M+H]^+$ 498.2890; found 498.26.

3.26. Synthesis of A28

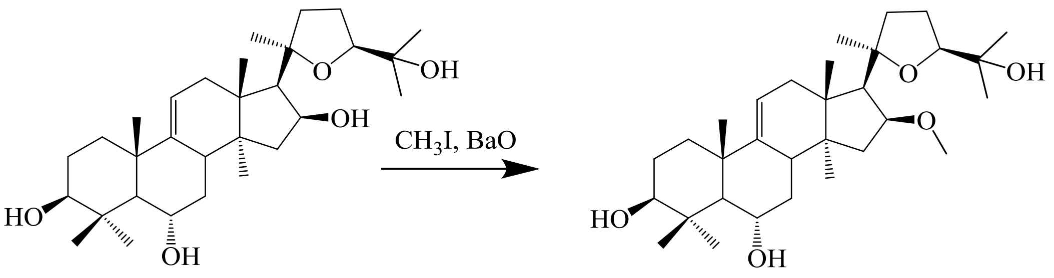


A4 A28

To a solution of **A4** (50 mg) in THF (2 mL) were added thionyl chloride (1 μL) at 0 °C under nitrogen, and the mixture was stirred at 0 °C for 4h. After water addition, the mixture was extracted with ethyl acetate. The organic layer was washed with water and brine and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane:EtOAc 8:2) to afford compound **A28**, (10*S*,13*R*,14*S*,17*S*)-11-chloro-4,4,10,13,14-pentamethyl-17-((*R*)-2-methyl-5-oxotetrahydrofuran-2-yl)dodecahydro-1*H*-cyclopenta[*a*]phenanthrene-3,6,16(2*H*)-trione as a white solid (43.9% yield)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.14 (s, 3H), 1.15 (s, 3H), 1.30 (s, 3H), 1.45 (s, 3H), 1.48 (s, 3H), 1.78 (s, 3H), 1.88-2.37 (m, 11H), 2.54-2.88 (m, 8H), 3.33 (bs, 1H), 3.50 (m, 1H); LCMS *m/z* calcd for $C_{27}H_{37}ClO_5$, $[M+H]^+$ 477.2330; found.477.20

3.27. Synthesis of A37

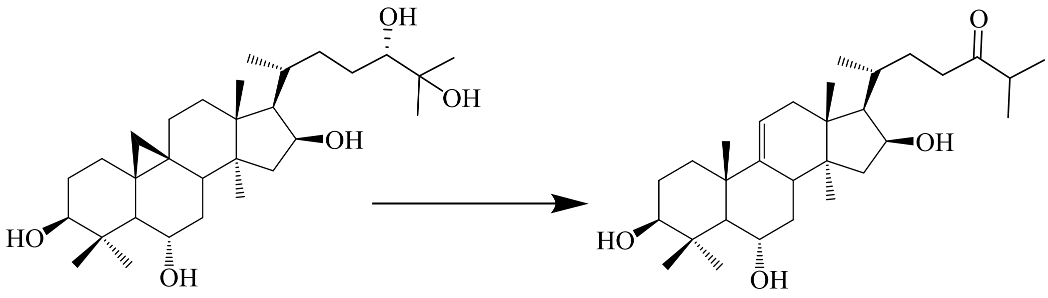


AG **A37**

To a solution of **AG** (100 mg) in DMF (8 mL) were added iodomethane (2 mL) and barium oxide (0.5 mg) at room temperature under nitrogen, and the mixture was stirred for overnight. Water was added and the mixture was extracted with ethyl acetate. The organic layer was washed with water and brine and dried on anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (9:1 *n*-hexane:EtOAc) to afford compound **A37**, (3*S*,6*S*,10*R*,13*R*,14*S*,16*S*,17*R*)-17-((2*R*,5*S*)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-16-methoxy-4,4,10,13,14-pentamethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthrene -3,6-diol, as a white solid (57.0% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.81 (s, 3H), 0.88 (s, 3H), 1.06 (s, 3H), 1.07 (s, 3H), 1.10 (s, 3H), 1.22 (s, 3H), 1.24 (s, 3H), 1.32 (s, 3H), 1.40-1.70 (m, 9H), 1.80-2.20 (m, 6H), 2.43 (m, 1H), 2.53 (d, *J*=8.2 Hz, 1H), 3.16 (s, 3H), 3.19 (m, 1H), 3.75 (m, 1H), 4.20 (m, 2H), 5.29 (d, *J*=5.8 Hz, 1H).

3.28. Synthesis of AHSKG

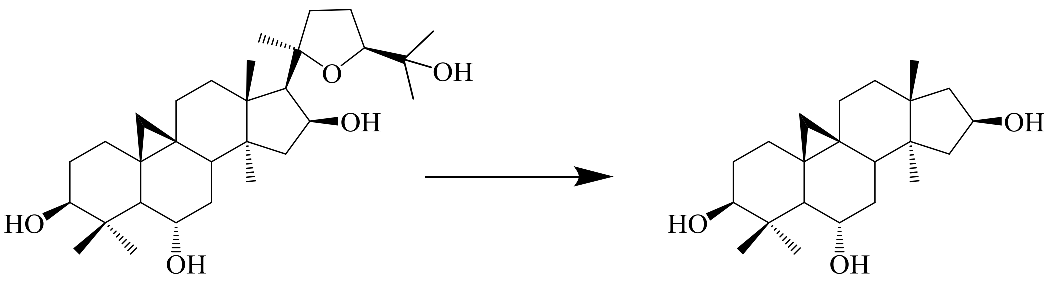


SKG **AHSKG**

A solution of **SKG** (100 mg) in MeOH (15 mL) was treated with conc. H_2SO_4 (1 mL) and the whole mixture was heated under reflux for 6h. The reaction mixture was poured into water and extracted with EtOAc. The EtOAc extract was washed successively with aq. sat. $NaHCO_3$ and water, and dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane:EtOAc (6:4) to give **AHSKG**, (6*R*)-2-methyl-6-((3*S*,6*S*,10*S*,13*R*,14*S*,16*S*,17*R*)-3,6,16-trihydroxy-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,8,10,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)heptan-3-one (57 mg) as a white solid (55% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 0.76 (s, 3H), 0.81 (s, 3H), 1.05 (s, 3H), 1.07 (s, 3H), 1.09 (d, *J*= 0.8 Hz, 3H), 1.11 (d, *J*= 0.8 Hz, 3H), 1.26 (s, 3H), 1.32 (s, 3H), 1.37-2.12 (m, 23H), 2.38-2.73 (m, 4H), 3.19 (dd, *J*= 11.6, 4.0 Hz, 1H), 4.18 (td, *J*=10.7, 4.1 Hz, 1H), 4.56 (m, 1H), 5.23 (d *J*= 5.7 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 217.0, 146.8, 115.6, 79.1, 72.3, 70.2, 57.3, 56.2, 45.2, 44.7, 44.4, 41.1, 41.1, 39.5, 39.4, 37.2, 36.9, 36.5, 31.3, 29.9 (×2), 29.6, 27.5, 23.4, 19.3, 18.6, 18.5, 18.1, 15.9, 15.5.

3.29. Synthesis of SCG



CG **SCG**

A solution of **CG** (100 mg) in DMF (20 mL) was treated with NaOH (1 mg) and the whole mixture was heated under reflux for 12h. The reaction mixture was poured into water and extracted with EtOAc. The EtOAc extract was washed successively with aq. sat. $NaHCO_3$ and water, and dried over Na_2SO_4 and filtered. The filtrate was evaporated, and chromatographic separation of the crude product on a silica gel column was performed by using *n*-hexane:EtOAc (8:2) to give **SCG**, (2a*R*,4*R*,5a*S*,7*S*,9*S*,11a*R*,12a*S*)-2a,5a,8,8-tetramethyltetradecahydro-1*H*,12*H*-cyclopenta[*a*]cyclopropa[*e*]phenanthrene-4,7,9-triol (7 mg) as a white solid (5% yield).

^{1}H NMR (400 MHz, Pyridine-*d*5) δ (ppm): 0.27 (d, *J*= 4.2 Hz, 1H), 0.62 (d, *J*= 3.8 Hz, 1H) 1.13 (s, 3H), 1.22-1.36 (m, 2H), 1.38 (s, 3H), 1.45 (s, 3H), 1.46-1.54 (m, 2H) 1.62-1.88 (m, 6H), 1.90 (s, 3H), 1.94-2.15 (m, 6H), 3.68 (dd, *J*= 11.4, 4.5 Hz, 1H), 3.86 (m, 1H), 4.86 (dd, *J*= 14.4, 7.2 Hz, 1H); ^{13}C NMR (100 MHz, Pyridine-*d*5) δ (ppm): 78.6, 71.5, 68.2, 54.0, 49.9, 48.4, 46.9, 46.7, 45.3, 42.7, 38.9, 32.9, 31.6 (×2), 30.1, 29.9, 29.5, 26.9, 25.7, 21.4, 20.9, 16.4; LCMS *m/z* calcd for $C_{22}H_{36}O_3$, $[M+H]^+$ 349.2743; found 349.27

4. RESULTS AND DISCUSSION

4.1 Chemical Remarks

Optimum extraction conditions were developed for Astragaloside IV, Astragaloside VII and Cycloastragenol from the roots of *Astragalus trojanus*. Different extraction solvents used for the extraction of Astragaloside IV, Astragaloside VII and Cycloastragenol were water, methanol, ethanol and *n*-butanol. Table 4.1 shows that all the compounds could be extracted with polar solvents except the water/CG case. The highest amounts of the compounds were provided by *n*-butanol. As most of the cycloartane glycosides of *A. trojanus* were found in acetylated forms (mainly sugar residues), an attempt was made to increase the content of astragaloside IV and astragaloside VII via alkaline (NaOH) treatment (Table 2.1). Each condition was analyzed and quantified by HPLC-CAD.

Table 4.1. Astragaloside VII, Astragaloside IV and Cycloastragenol Content of Different Exctracts

Compounds	Water	Methanol	Ethanol	*n*-Butanol
Astragaloside IV	0.60	1.10	1.10	1.20
Astragaloside VII	0.12	0.16	0.14	0.25
Cycloastragenol	0	0.25	0.35	0.40

The main starting material astragenol was prepared from astragaloside VII, astragaloside IV and cycloastragenol.

The compound **A2** was obtained from an esterification reaction of Astragenol using propionic anhydride and DMAP in pyridine. HR-ESI-MS spectrum showing a major ion peak at *m/z* 625.4103 ($[M+Na]^+$) indicated molecular formula of $C_{36}H_{58}O_7$. In the ^1H NMR the characteristic signals belonging to H-3 and H-6 were shifted to the downfield implying acylation positions. Also, two new methyl signals observed in the upfield region were assigned to the propionyl groups' terminal methyl groups. In addition, in the ^{13}C NMR spectrum, two carbons at 173.3 and 173.7 ppm were readily attributed to the ester carbonyls. Thus, the structure of **A2** was established as **3,6,dipropionyl-AG.**

The molecular formula of **A3** ($C_{38}H_{62}O_7$) was established by HRMS analysis (*m/z* 653.4435, calcd. for $[M+Na]^+$: 653.4393). The molecular weight of **A3** was 140 amu more than the starting molecule, AG, suggesting two additional butyryl group. Moreover, two ester carbonyl carbons were determined at δ 173.02 and 172.65 in the ^{13}C-NMR spectrum. In the ^{1}H-NMR spectrum, the low-field characteristic signals belonging to H-3 and H-6 were shifted to the downfield region about (δ_{H-3} 4.45 and δ_{H-6} 5.40) ppm, respectively, confirming esterification positions. Based on the above deductions, compound **A3** was identified as **3,6-dibutyryl-AG**.

Compounds **A10** and **A11** were obtained from esterifications reaction of astragenol using isobutiric anhydride and DMAP in pyridine. Based on the major ion peak at *m/z* 653.4435 ($[M+Na]^+$), together with ^{13}C-NMR spectrum, molecular formula of **A10** was found to be $C_{38}H_{62}O_7$. In the ^{1}H-NMR spectrum, the characteristic signals belonging to H-3 and H-6 were shifted to the downfield region suggesting acylation positions (δ_{H-3} 4.44 and δ_{H-6} 5.35). Moreover, 4 secondary methyl groups were observed at δ 1.15 and 1.20 (each 6H). In the carbon NMR spectrum, 8 additional resonances were observed compared to the starting compound AG, indicating diesterification. Two of these extra signals were consistent with ester-type carbonyl carbons (δ 176.82 and 176.92). As a result, the structure of **A10** was determined to be **3,6-diisopropionate-AG**. The HRMS spectrum of **A11** provided a major ion peak at *m/z* 723.4912 ($[M+Na]^+$) indicating a molecular formula of $C_{42}H_{68}O_8$. An additional ester carbonyl carbon was observed in the carbon NMR spectrum when compared to **A10**. Also, the characteristic signal belonging to H-16 oxymethine proton was shifted downfield and resonated at δ 5.35. Thus, compound **A11** was identified as **3,6,16-triisopropionate-AG.**

The reaction of **AG** with benzoic anhydride and DMAP in pyridine, gave **A12** and **A13**. The proton NMR spectra of **A12** and **A13** differed in the signals H-3 and H-6. In the ^{1}H NMR spectrum of **A12**, the signal of H-3 appeared at δ 4.07 and that of H-6 at δ 4.23, whereas for **A13**, the signals of these protons were observed at δ 4.68 and 5.78, respectively, indicating acylation positions. In the carbon NMR spectrum of **A12**, the signal of the ester carbonyl carbon showed at δ 166.5, whereas for **A13**, the signals were detected at δ 166.5 and 165.9. Also, characteristic aromatic signals of benzoyl residues were readily deduced from ^{1}H and ^{13}C NMR spectra. Based on the above findings and ESI-MS data (*m/z* 617.3765 $[M+Na]^+$), the structure of **A12** was identified C-3(O)-benzoylated analogue of AG. Compound **A13** was confirmed by ESIMS analysis (*m/z* 721.4178 $[M+Na]^+$ ion) and identified as C-3,C6-di-(O)-benzoylated analogue of **AG**.

A14 and **A15** were obtained from an esterification reaction of astragenol using pivalic anhydride and DMAP in pyridine. Compound **A14** gave a major ion peak at *m/z* 597.4082 ($C_{35}H_{58}O_6$ $[M+Na]^+$). The major differences in the ^1H NMR spectrum of **A14** and **A15** compared to **AG** were the chemical shifts of H-3 and H-6. The down-field shifted proton signals for **A14** were resonated at δ 4.41 (m, H-3) and δ 5.43 (td, *J*=10.8, 4.1 H-6) whereas the same deshielding effect was observed for H-3 signal of **A15** at δ 4.41 (dd, *J*=11.1, 4.1, H-3). Also, carbonyl carbons were resonated at δ 178.1 and δ 178.0 for **A14**, and at δ 178.2 for **A15** in the ^{13}C NMR spectra. Therefore, **A14** was identified as **3,6-dipivaloyl-AG**. Also, based on above finding and MS data (*m/z* 681.4666 $[M+Na]^+$), the structure of **A15** was established as **3-pivaloyl-AG**.

Compounds **A16**, **A17** and **A18** were obtained from the reaction of astragenol with acetic anhydride and DMAP. Compound **A16** had the molecular formula of $C_{34}H_{54}O_7$ based on its MS data (*m/z* 597.3528). The molecular formula of **A17** was established as $C_{36}H_{56}O_8$ by MS analysis (*m/z* 639.7107). **A18** was deduced to possess the molecular formula $C_{38}H_{58}O_9$ on the basis of its MS data (*m/z* 681.3206). In the ^1H NMR spectra, the characteristic signals belonging to H-3, H-6, H-16 and H-24 were shifted down-field and summarized in Table 4.2. Furthermore, the acetyl methyls showed ^1H NMR signals at 2.06 (6H) for compound **A16** whereas for **A17**, the signals were detected at 2.03, 2.05 and 2.06, as well methyls of **A18** were observed at 1.96, 2.01, 2.05, 2.06. Also, ester carbonyl carbons (δ = ~ 170) were detected in the ^{13}C-NMR spectra. Therefore, compounds **A16**, **A17** and **A18** were identified as **3,6-diacetyl-AG**, **3,6,16-triacetyl-AG** and **3,6,16,24-tetraacetyl-AG**.

Table 4.2. Chemical shifts of **A16**, **A17** and **A18**

δ_H	A16	A17	A18
H-3	4.45 (dd, *J*=11.0, 4.0 Hz)	4.46 (dd, *J*=11.2, 3.8 Hz)	4.45 (m)
H-6	5.37 (m)	5.34 (m)	5.34 (m)
H-16	4.68 (q, *J*=7.4 Hz)	5.42 (dd, *J*=11.1, 4.1 Hz)	5.34 (m)
H-24	3.76 (t, *J*=7.4 Hz)	3.69 (dd, *J*=8.6, 7.1 Hz)	4.02 (dd, *J*=14.1, 6.7 Hz)

Compounds **A4** and **A5** were obtained from an oxidation reaction of Astragenol. **A4** and **A5** were isolated as major products depending on the reaction temperature. At 0 °C Jones oxidation resulted in **A5** in 64% yield. When compared to Astragenol, the absence of low-field oxymethine signals due to H-3, H-6 and H-16 in the ^{1}H NMR, and the observation of three carbonyl carbons (216.9, 213.9, and 209.8) instead of the corresponding oxymethine carbons in the ^{13}C NMR spectrum confirmed the formation of compound **A5**. The accurate mass measurement also substantiated these findings (HRESIMS: *m/z* 507.3115 $[M+Na]^+$: Calcd: 507.3086 for $C_{30}H_{44}O_5$). On the other hand, when the oxidation was performed at room temperature, an alternative major product, **A4**, was observed. The HRESIMS spectrum of **A4** showed a major ion at *m/z* 458.2924 that was assigned to $[M+NH_4]^+$. Together with the ^{13}C NMR data, accurate mass data supported a molecular formula of $C_{27}H_{36}O_5$ (Calcd.: 458.2963). The NMR spectra of **A4** and **A5** were identical except for the 20,24-lactone ring. The C-25*(O)* resonance and its attached methyl groups (Me-26 and Me-27) were missing in the ^{1}H and ^{13}C NMR spectra of **A4**, whereas two low-field carbon signals at δ 86.9 and 176.3 were attributed to C-20 and C-24, respectively, indicating a 20,24-olide ring system (γ-lactone).

Potassium hydroxide catalyzed 20,24-lactone ring-opening of **A4** yielded **A9** as a white solid. When compared to starting molecule (**A4**), the observation of low field quaternary olefinic signals, and carboxylic acid carbon resonance (δ 173.7) in the ^{13}C NMR spectrum confirmed the formation of **A9**. The accurate mass measurement also substantiated these findings (HRESIMS: *m/z* 465.2377 $[M+K]^+$: Calcd: 465.2406 for $C_{26}H_{34}O_5$).

Treatment of **A5** with sodium methoxide in THF provided two compounds (**A27** and **A29**). The HRESIMS data of **A27** and **A29** displayed no change (m/z 467.3280 $[M+Na]^+$) compared to the starting molecule **A5**. Inspection of the spectral data (1D- and 2D-NMR) revealed that **A27** and **A29** were formed through rearrangement of the E ring (20,24-epoxy side chain or tetrahydrofurane ring). The carbon data of **A27** and **A29** provided evidence for stereochemical alteration at C-20 and C-24 positions (Table 5.2). A plausible mechanism for the transformation of **A5** into the compounds **A27** and **A29** was illustrated below (Figure 5.1).

Table 4.3. ^{13}C-NMR Data of **A27** and **A29**

Compound	$\delta_{\mathbf{C\text{-}20}}$	$\delta_{\mathbf{C\text{-}24}}$
AG	87.2	82.6
A-27	83.8	88.4
A-29	84.5	82.5

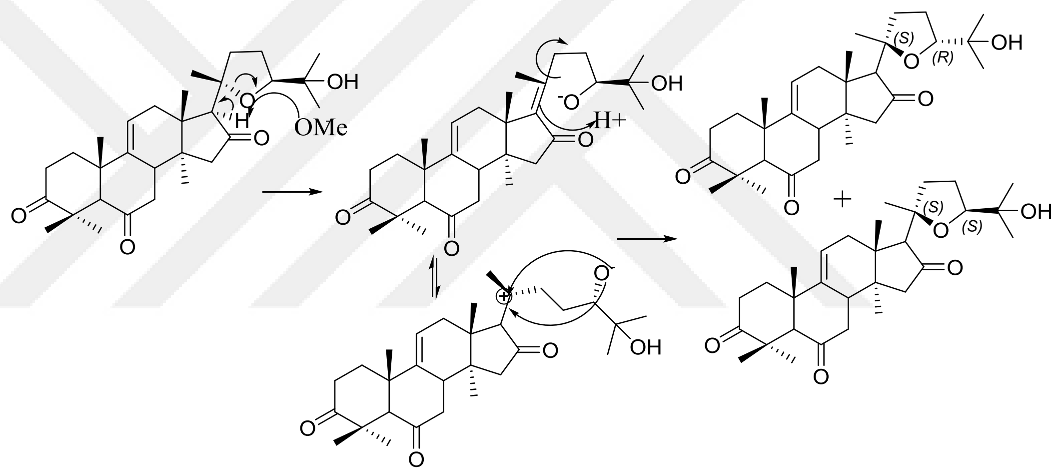


Figure 4.1. A plausible mechanism fort the transformation of **A5** into **A27** and **A29**

Compound **A30** gave a major ion peak at m/z 473.2460 ($C_{27}H_{36}O_7$) (m/z 473.2461 calc.). Comparison of the NMR data for compound **A30** with those of **A4** showed the presence of the same partial structures except A and C ring. On the other hand, the characteristic olefinic signals of C-9 and C-11 were replaced with at δ 67.7 and δ 63.2. With MS data in mind, it was suggested that the double bond had been oxidized to C-9-C-11 epoxide. Also, the characteristic signal of C-3 was replaced with at *δ* 174.2. On the basis of these results, the A ring was oxidation into seven membered lactone.

The HRESIMS of compound **A25** provided $[M+H]^+$ at m/z 461.2549 implying the molecular formula $C_{26}H_{36}O_7$. In the ^{1}H NMR spectrum of **A25** showed noteworthy discrepancies compared to strating molecule **A4**. Firstly, instead of five tertiary methyls, a primary (δ 1.16, t, 6.8 Hz) and four tertiary methyl groups were observed together with two down field signals at δ 4.15 (q, J = 16.8 Hz) and δ 3.36 (d, J = 6.0 Hz). Second, the absence of $\Delta^{9(11)}$ olefinic protons was apparent from the ^{1}H NMR. The ^{13}C and DEPT spectra showed that the resonances assigned to the product comprised 5 methyl, 9 methylene, 3 methine, and 9 quaternary carbons, four of which were carbonyl carbons as ester/lactone (δ 174.5 and 177.3) or ketone carbons (δ 209.6 and 215.9). The relative stereochemistry of the new chiral center at C-11 was assigned by a combination of coupling constant analysis and molecular modeling studies performed on **A25**. The minimum energy conformations of a- and b-oriented epoxy rings were calculated with a 3-D computer-generated model. Since the H-11 resonance was observed as a doublet with a 6.0 Hz coupling constant (δ 3.36), it must be equatorial, while the dihedral angle between H-11 equiv and H-12 ax is required to be about 90^{o}. Only in the case of an α-oriented epoxy ring is this dihedral angle possible, rulingout the β-orientation of the epoxy ring.

BV oxidation reaction of **A4** can yield ester **A25** or **A30** depending on the added alcohol in work-up steps. Although the formation of **A30** was expected from BV oxidation of **A4**, carbonyls can be further protonated by excess *m*-chloroperbenzoic acid to form activated carbonyls as in compound **A30**. After the addition of an alcohol, equilibrium will be formed between compounds **A30** and A4-c as shown in Figure 4.2. This equilibrium can be disturbed toward the formation of an enol product (A4-d) by a retro-aldol reaction with elimination of an acetone. Such enol (A-4d) rearranges its keto form (A4-e) in alcoholic solution. So the addition of ethanol in work-up step of Baeyer-Villiger reaction mixture of steroid **A4** yields the corresponding **A25.**

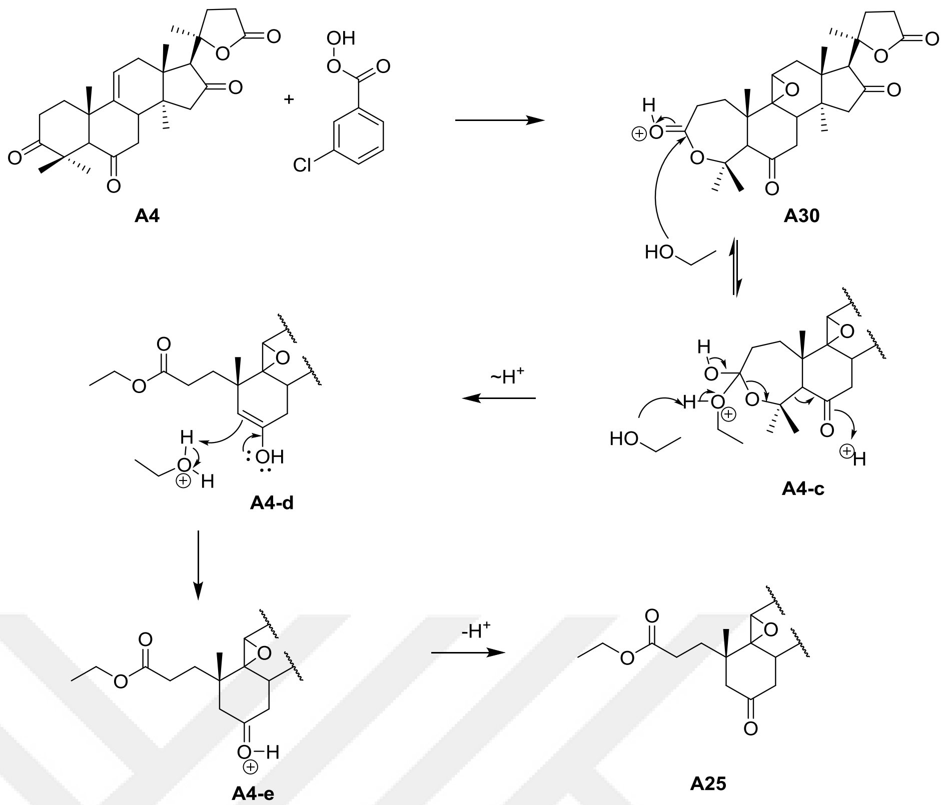


Figure 4.2. A plausible mechanism fort the transformation of **A4** into **A25**

Compounds **34** and **35** were obtained from oxidation reaction with $RuCl_3$ and $NaIO_4$ from Astragenol. LC-MS spectrum of **A34** showed a major ion peak at *m/z* 487.2817. $[M+H]^+$ indicated a molecular formula of $C_{30}H_{46}O_5$. The down-field characteristic signals belonging H-11, H-16 and H-24 were observed unchanged. However, the hydroxymethine protons at H-3 and H-6 were lost in the ^1H NMR spectrum. It is concluded that the hydroxyl groups at C-3 and C-6 were oxidized to ketone. The LC-MS pectrum of **A35** provided a major ion peak at *m/z* 487.2817. $[M+H]^+$ ($C_{30}H_{46}O_5$). In the case of **A35**, the hydroxymethine protons H-3 and H-16 were absent in the ^1H NMR spectrum. Thus it was concluded that the hydroxyl groups at C-3 and C-16 were oxidized to ketone.

Compounds **A38** and **A36** were obtained from oxidation reaction with PCC. Initial inspection of the ^1H NMR spectrum of **A38** implied an oxidation of C-3 secondary alcohol to a ketone. The accurate mass data from the LC-MS showed a peak at *m/z* 511.3388 ($C_{30}H_{48}O_5Na$) (*m/z* 511.3388 calcd. 511.3399). The LC-MS spectrum of **A36** [(+) mode] showed a major peak at *m/z* 511.3388 $[M+Na]^+$ ($C_{30}H_{48}O_5Na$) (*m/z* 511.3388 calcd. 511.3399). The down-field characteristic signal of H-6 was disappeared in the ^{1}H NMR spectrum revealing a C-6 keto structure of **AG**.

A8 was obtained from an esterification reaction of Astragenol using *p*-Toluenesulfonyl chloride and DMAP in pyridine. The HRESIMS data of **A8** indicated a molecular formula of $C_{37}H_{56}O_7S$ (*m/z* $[2M+H]^+$ 1311.7391 calcd.) that was deduced from the ion peak at *m/z* $[2M+H]^+$ 1311.8171. The most prominent changes in the ^{1}H NMR spectrum of **A8** were two new proton resonances observed in the aromatic region at δ 7.80 (2H, d, *J*=8.0 Hz) and 7.32 (2H, d, *J*=8.0 Hz). Also, the characteristic signal of H-3 was downfield shifted (δ 5.60). These data was consistent with C-3(*O*)-tosylated analog of AG.

Compounds **A6** and **A7** were obtain from mild acidic hydrolysis of Astragenol. The MS spectrum gave an $[M+H]^+$ ion at *m/z* 495.3450 (calcd. 495.3553), corresponding to the molecular formula $C_{30}H_{48}O_4$. The ^{1}H NMR spectrum of **A6** was very similar to that starting compound astragenol, except for disappearance of the characteristic signal H-3 and appearance of a new olefinic signals at δ 5.50 and δ 5.31. Furthermore, in the ^{13}C NMR observed that disubstituted olefinic signals at δ 120.3 (C-2) and δ 139.6 (C-3). These deductions established the double bond at $\Delta^{2,3}$.

The structure of **A7** were characterized by MS and NMR analyses. The MS spectrum showed a major ion peak at m/z 495.3450 ($C_{30}H_{48}O_4Na$) that were assigned to $[M+Na]^+$ Together with the ^{13}C NMR data, accurate mass data supported a molecular formula of $C_{30}H_{48}O_4$. Firstly, instead of eight tertiary methyls, a secondary (δ_H 1.08, d, 7.2 Hz) and six tertiary methyl groups (δ_H 0.84, 0.88, 0.94, 1.07, 1.13, 1.29, each s) were observed together with six low-field signals at δ_H 4.71 (q, *J* = 6.8 Hz), 4.04 (ddd, *J* = 4.0, 10.4, 10.4 Hz), 3.75 (dd, *J* = 8.0, 8.6 Hz), 5.40 (d, *J* = 5.9 Hz), 5.06 (bs) and (4.70 bs). Second, the absence of characteristic H-3 oxymethyne proton was apparent from the ^{1}H NMR spectrum. However, the characteristic signals belonging H-6, H-16 and H-24 oxymethyne protons in the lowfield showed no alteration compared to Astragenol. The ^{13}C and APT spectra showed that the resonances assigned to the product comprised 7 methyl, 8 methylene, 8 methine, and 7 quaternary carbons, two of which were quaternary olefinic carbons (δ 144.6 and δ 151.9). Furthermore, the ^{13}C spectrum, two new olefinic carbon signals (δ 107.5, 151.9) were observed in the lowfield. In the HMQC spectrum, correlations of the double bond carbon with two protons indicated a disubstituted olefinic system. This indicated that dehydration of C-3 was followed by a methyl migration yielding an exo-olefinic system. Thereby, one of the two methyl groups at C-4 was migrated to C-3 position and the methyl protons gave an upfield doublet, and the other methyl located at C-3 became an exo-olefin. In the HMBC spectrum, correlation of C-3 and H-29a and H-29b (δ 5.06 and 4.70) proved this founding. The configuration at C-4 was deduced based on the 1D-NOESY data.

Compounds **A19**, **A20**, **A21** and **A22** were obtained from epoxidation reaction of **AG**, **A17**, **A3** and **A12**, respectively.

The HRESIMS spectrum of **A19** gave an ion peak at *m/z* 507.3081 $[M+Na]^+$ ($C_{30}H_{50}O_6Na$). The characteristic olefinic signals of C-11 and C-9 were replaced with two resonances at δ 69.5 and δ 57.5 in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum of **A19** showed that the characteristic H-11 signal was disappeared, and a new doublet signal showed up at δ 3.18. This evidence revealed that the double bond was oxidized to epoxide. As in **A19,** the ^{1}H NMR spectra of **A20**, **A21** and **A22** were inspected, and it was found out that the characteristic H-11 was replaced with a new doublet signal at δ 3.18, 3.20 and 3.26, respectively. There was no other significant chemical shift for above mentioned molecules compared to the starting molecules astragenol, **A17**, **A3** and **A12**, respectively.

The reaction of compound **A5** with hydroxylamine hydrochloride ($NH_2OH.HCl$) in pyridine afforded compound **A23**. In the ^{1}H-NMR spectrum of **A23**, the HC=N- hydrogen was observed as a triplet at 8.15 ppm**.** In the ^{13}C NMR spectrum of this compound the C=N carbon signal appeared at δ_C 164.3, 162.9 and 157.7 ppm, supporting a structure of 3,6,16-trioxime analog of **AG**.

The reaction of **A4** and semicarbazide afforded compound **A32**. Its structure was confirmed by ^{1}H and ^{13}C NMR spectra. The ^{1}H-NMR spectrum showed a resonance derived from an NH group at δ 8.66. The ^{13}C NMR spectrum of **A32** showed that a carbonyl carbon (C-3) was shifted at δ 158.99. The LC-MS of **A32** provided evidence for the moleculer formula of $C_{28}H_{39}O_5N_3$ (*m/z*= 498.26, calcd. for $[M+H]^+$: 498.2890). Thus, **A32** was identified as 3(*O*)-semicarbazide analogue of **AG**.

The reaction of compound **A4** and thiosemicarbazide in MeOH afforded compound **A31.** The ^{13}C NMR spectrum showed a thiocarbonyl carben at δ 157.7. The LC-MS pectrum of **A31** showing a major ion peak at *m/z* 536.32. $[M+H]^+$

($C_{28}H_{39}N_3O_4S$). Thus, **A31** was identified as C-3(*O*)-thiosemicarbazide analogue of **AG**.

The LC MS of compound **A28** provided evidence for the moleculer formula of $C_{27}H_{37}O_5Cl$ (*m/z*= 477.20, calcd. for $[M+H]^+$: 477.2330). In the ^{1}H NMR spectrum, the characteristic H-11 signal was absent, and a new signal was observed at δ 3.33. Therefore, **A28** was identified as C-11-chloride analogue of **AG**.

The ^{1}H NMR spectrum of **A37** displayed a new methoxy signal at δ 3.16. Also, the characteristic signal belonging to H-16 was down-field shifted compared to Astragenol. Thus, **A37** was identified as C-16(*O*)-methoxy derivative of **AG**.

Acid catalyzed cyclopropane ring-opening of Cyclocanthogenol yielded “Canthogenol” (**AHSKG**). The structure of **AHSKG** was readily deduced based on the following observations: 1) The disappearance of the characteristic 9,19-cyclopropane protons and 24-H proton. 2) The appearance of a trisubstituted olefinic system due to the $\Delta^{9(11)}$ double bond (δ_H 5.23; δ_C 146.8 and 115.6) in the ^{1}H and ^{13}C NMR.

In the ^{13}C NMR, 217.0 ppm resonance suggested the existence of a ketone, which was interpreted as an indication of a pinacol rearrangement occurring at the C-24 and C-25 positions. As a result of pinacol rearrangment, two methyl protons at 1.09 and 1.11 was converted to doublet from singlet. Based on the above evidence, **AHSKG** was identified as “Canthogenol”, (20 *R*, 24 *S*).-3β,6α,16β-trihydroxy-lanost-9(11)-en-24-one.

The reaction of **CG** and NaOH in DMF afforded compound **SCG**. The LC-MS spectrum indicated a major ion peak at *m/z*= 349.27 that were assigned to $[M+H]^+$. The MS data and ^{13}C NMR data supported a molecular formula of $C_{22}H_{36}O_3$. The ^{1}H NMR spectrum of **SCG** showed resonances for four methyl groups (δ 1.13, 1.38, 1.45 and 1.90) and three oxygenated methines (δ 3.68, 3.86 and 4.86). The ^{1}H and ^{13}C NMR spectrum of **SCG** showed noteworthy discrepancies compared to **CG**. The characteristic C-25(*O*) resonance and its attached methyl groups (C-26 and C-27) were missing in the NMR spectra of **SCG**. Also, two downfield characteristic signals of C-20 and C-24 and upfield methyl signal of C-21 were dissappered in the ^{13}C NMR spectrum. Moreover, the characteristic doublet signal of H-17 was shifted upfield and changed to multiplet.

Since no other alterations were observed on the NMR data of the A, B and C rings, an elimination of the E ring was suggested. Therefore, compound **SCG** was confirmed (2a*R*,4*R*,5a*S*,7*S*,9*S*,11a*R*,12a*S*)-2a,5a,8,8-tetramethyltetradecahydro-1*H*,12*H*-cyclopenta[*a*]cyclopropa[*e*]phenanthrene-4,7,9-triol.

4.2. Cytotoxic Evaluation

Forty compounds were tested against 4 different cell lines *in vitro* using MTT assay method. The cell lines used were two human prostate cancer cell lines (DU-145 and PC-3), a human colon adenocarcinoma (HT-29), together with a human breast adenocarcinoma (MDA-MB-231) cell lines. Table 4.1 demonstrates cell viability and IC_{50} values of the compounds against PC-3 cell line while Table 4.2 provides the data against DU-145 cell line. Table 4.3 illustrates cell viability and IC_{50} values of the compounds against HT-29 whereas Table 4.4 gives the results for MDA-MB-231cell line.

Compounds which exhibited high cytotoxic activity on PC-3 (human prostate cancer) cell line were **A22** (3.6 μM), **A8** (6.5 μM), **A20** (6.7 μM), **A7** (9.6 μM), **A2** (14 μM) and **A16** (30.1 μM), whereas the high cytotoxic activities on DU-145 (human prostate cancer) cell line were as follows: **A8** (4.8 μM), **A22** (11.5 μM), **A20** (12.4 μM), **A14** (12.6 μM), **A12** (13.5 μM), **A2** (16.3 μM) and **A7** (23.3 μM). Moreover, **A22** (6.3 μM), **A20** (11.2 μM), **A7** (13.2 μM), **A15** (14.6 μM), **A31** (15.5 μM), **A8** (19.1 μM), **A2** (19.6 μM) and **A3** (21.4 μM) showed significant cytotoxic activity on HT-29 (human colorectal adenocarcinoma) cell line.

Compounds that indicated high cytotoxic activity on MDA-MB-231 (human breast adenocarcinoma) cell line and their IC_{50} values were **A22** (5.1 μM), **A8** (7.2 μM), **A20** (9.1 μM), **A7** (9.2 μM), **A21** (13.2 μM), **A15** (15.3 μM), **A6** (18.9 μM), **A2** (19.3 μM), **A10** (24.6 μM), **A12** (26.1 μM) and **A13** (26.3 μM).

Table 4.4. Cell Viability and IC_{50} Values of the Compounds Against PC-3 Cell Line

	PC3						
	1	**5**	**10**	**25**	**50**	**100**	**IC_{50}**
CG			78.9	61.8	59.3		>100
AG			65.3	60.8	46.6		46.7
A2			85.1	17.0	7.2		14.0
A3			85.1	57.7	43.6		39.2
A4			>100	>100	99.6		>100
A5			97.8	99.7	96.1		>100
A6	>100	>100	92.2	69.5	60.7	56.2	>100
A7	102.9	93.6	64.6	9.4	1.9	0.4	9.6
A8			26.5	13.2	8.9		6.5
A9			>100	63.5	65.5		>100
A10			75.6	66.9	61.3		>100
A11			68.1	55.7	53.8		87.1
A12			>100	60.8	48.2		52.1
A13			>100	91.3	83.1		>100
A14			76.0	65.7	62.8		98.2
A15			71.0	70.1	65.0		74.0
A16			68.1	52.6	45.3		30.1
A17			94.2	63.3	42.3		46.1
A18			>100	59.6	41.4		44.6
A19			99.3	96.6	92.9		>100
A20	>100	62.3	42.8	12.1	6.8	2.7	6.7
A21			85.9	60.1	52.5		64.7
A22			25.3	14.1	8.9		3.6
A23			99.3	82.1	68.8		72.6
A24			>100	>100	86.1		>100
A25			>100	>100	99.5		>100
A27			>100	>100	>100		>100
A28			>100	85.6	32.2		43.6
A29	>100	>100	>100	>100	97.2	90.6	>100
A30	>100	90.9	86.3	82.8	74.8	56.1	>100
A31	96.5	92.7	88.5	74.7	75.2	31.6	80.7
A32	98.9	100.1	81.1	81.5	79.1	67.3	>100
A34	94.8	94.3	85.7	76.0	74.5	71.6	>100
A35	92.0	86.3	72.7	65.3	52.5	36.0	59.0
A36			>100	84.8	82.0		>100
A37	>100	>100	99.6	92.3	84.9	79.3	>100
A38			>100	>100	99.6		>100
AHSK	>100	>100	>100	93.7	78.6	71.1	>100
SCG			>100	>100	99.6		>100

Table 4.5. Cell Viability and IC_{50} Values of the Compounds Against DU-145 Cell Line

	DU-145						
	1	**5**	**10**	**25**	**50**	**100**	**IC_{50}**
CG			65.1	61.8	29.7		36.6
AG			70.1	56.7	41.0		39.5
A2			>100	31.3	5.6		16.3
A3			>100	49.1	39.4		21.5
A4			89.4	83.5	73.0		>100
A5			94.4	83.3	77.7		>100
A6	>100	>100	99.3	87.5	79.2	71.1	>100
A7	>100	>100	72.1	59.3	38.2	30.8	23.3
A8			19.8	12.3	9.4		4.8
A9			96.2	65.5	48.4		48.8
A10			78.4	60.4	44.7		51.0
A11			>100	96.7	77.8		>100
A12			52.8	46.4	47.9		13.5
A13			58.9	53.6	41.6		34.3
A14			53.9	45.4	45.0		12.6
A15			77.8	73.9	67.8		>100
A16			>100	87.4	82.2		>100
A17			>100	91.0	57.5		85.8
A18			>100	91.6	63.8		>100
A19			>100	>100	>100		>100
A20	>100	83.7	57.9	40.21	28.9	21.5	12.4
A21			95.3	63.5	49.8		49.0
A22			48.0	33.5	24.9		11.5
A23			>100	93.3	87.1		>100
A24			>100	>100	81.6		>100
A25			>100	>100	77.3		>100
A27			>100	>100	>100		>100
A28			>100	>100	67.5		>100
A29	>100	>100	>100	>100	96.1	92.4	>100
A30	>100	>100	>100	>100	>100	70.6	>100
A31	>100	>100	95.4	87.8	54.3	48.9	56.6
A32	>100	>100	>100	95.6	83.7	86.4	>100
A34	>100	>100	>100	>100	>100	89.2	>100
A35	>100	>100	92.4	88.9	77.2	71.1	>100
A36			>100	>100	>100		>100
A37	>100	>100	>100	97.1	92.6	87.3	>100
A38			>100	>100	>100		>100
AHSK	>100	>100	91.4	86.3	82.9	75.7	>100
SCG			>100	>100	95.4		>100

Table 4.6. Cell Viability and IC_{50} Values of the Compounds Against HT-29 Cell Line

	HT-29						
	1	5	10	25	50	100	IC_{50}
CG			83.0	47.0	40.2		30.5
AG			71.5	50.7	42.1		41.6
A2			78.3	38.1	22.0		19.6
A3			59.1	41.2	45.1		21.4
A4			>100	>100	>100		>100
A5			80.5	81.2	63.3		84.2
A6	92.3	85.3	77.9	71.8	66.3	59.2	>100
A7	94.6	90.1	74.4	3.6	1.8	0.5	13.2
A8			51.1	47.7	38.7		19.1
A9			>100	68.8	39.6		39.4
A10			92.0	91.2	69.0		>100
A11			88.5	47.7	38.7		31.6
A12			96.4	91.2	62.5		87.4
A13			>100	>100	>100		>100
A14			66.9	62.8	59.6		>100
A15			47.8	48.3	43.3		14.6
A16			68.8	60.1	42.4		33.0
A17			66.9	43.5	45.4		24.3
A18			>100	58.4	44.4		47.0
A19			95.6	97.2	97.4		>100
A20	75.7	72.9	54.3	27.5	18.3	1.6	11.2
A21			78.9	74.9	71.7		>100
A22			28.2	19.5	19.3		6.3
A23			>100	96.1	72.7		>100
A24			>100	>100	>100		>100
A25			>100	>100	90.8		>100
A27			>100	>100	>100		>100
A28			>100	50.6	40.6		44.3
A29	>100	>100	>100	>100	>100	91.6	>100
A30	>100	97.0	84.9	77.5	71.2	61.7	>100
A31	87.7	78.3	66.5	36.3	32.0	18.3	15.5
A32	98.3	92.6	91.2	88.1	88.1	37.3	92.0
A34	96.8	91.3	86.3	76.3	77.3	54.5	>100
A35	>100	>100	98.3	92.6	91.6	88.1	>100
A36			>100	>100	>100		>100
A37	>100	>100	91.7	87.5	78.3	73.2	>100
A38			>100	>100	99.8		>100
AHSK	>100	85.3	51.1	33.1	26.5	20.5	11.2
SCG			>100	>100	>100		>100

Table 4.7. Cell Viability and IC_{50} Values of the Compounds Against MDA-MB-231 Cell Line

	MDA-MB-231						
	1	**5**	**10**	**25**	**50**	**100**	**IC_{50}**
CG			98.1	84.9	63.9		>100
AG			94.2	93.7	50.7		70.0
A2			93.1	31.1	12.5		19.3
A3			86.8	56.1	39.8		36.1
A4			85.7	75.3	70.2		>100
A5			>100	88.3	72.5		>100
A6	>100	78.7	79.5	44.9	27.7	19.8	18.9
A7	>100	>100	>100	12.0	3.5	1.9	9.2
A8			18.9	10.0	10.4		7.2
A9			78.2	53.4	50.6		62.5
A10			72.2	50.6	30.6		24.6
A11			84.9	58.0	56.9		60.1
A12			69.4	45.2	35.1		26.1
A13			85.5	38.5	36.8		26.3
A14			60.4	56.6	56.0		>100
A15			48.4	41.2	37.8		15.3
A16			>100	95.1	89.4		>100
A17			93.8	61.7	47.6		49.1
A18			>100	81.4	60.7		99.6
A19			69.5	59.7	57.9		>100
A20	>100	80.5	49.1	40.4	32.6	24.7	9.1
A21			47.0	43.2	29.6		13.2
A22			32.2	13.6	9.5		5.1
A23			89.8	90.6	67.0		>100
A24			>100	>100	>100		>100
A25			>100	>100	>100		>100
A27			>100	>100	>100		>100
A28			>100	>100	90.6		>100
A29	>100	>100	>100	>100	90.6	80.4	>100
A30	>100	>100	>100	>100	>100	>100	>100
A31	97.6	91.5	73.0	63.6	53.0	48.9	49.5
A32	>100	97.4	98.9	81.6	70.9	68.2	>100
A34	>100	>100	>100	>100	>100	>100	>100
A35	86.2	64.4	61.9	59.7	46.3	38.5	40.7
A36			>100	>100	>100		>100
A37	>100	>100	>100	>100	96.3	88.7	>100
A38			>100	>100	98.6		>100
AHSKG	>100	88.6	73.9	71.4	56.6	54.3	>100
SCG			>100	>100	>100		>100

5. CONCLUSION

Cancer is the generic term for a group of diseases in which abnormal cells divide without control and are able to invade other tissues. The cells proliferate and spread beyond the normal boundaries and invade adjoining areas, eventually spreading to other organs in the body. This spread of abnormal cells is called metastasis and it is the major cause of death in cancer (WHO, 2016).

Carcinogenesis is based on basis of gathering of mutations phase by phase that affecting biological incidents such as; cell's life cycle, control of growth and differentiation. During cancer development process, cancer cells gain many phenotypic properties. These alterations cause fast and unlimited proliferation and spreading to surrounding tissues. Also, these cells have ability of living independently from genuine micro-environment and metastasis. Serial mutations of proto-oncogenes and tumor suppressive genes contribute formation of malign phenotype by different mechanisms (Doğan et. al., 2006). Variation of cancer cells according to individual's genetic and epigenetic characteristics while progressions of cancer in this multi stage process; make it impossible to treat cancer only with one tumor agent.

The beneficial effects of natural products on health are known since ancient times and their use in traditional medicine is very wide. While western countries are indigenizing modern food and medication production instead of traditional medicine, natural products had been developed over centuries, experiences had been gained in this field and epidemiological observations that are providing preliminary data for cancer treatments had been made in traditional Chinese and Japanese medicine (Signorelli et. al., 2005, Baker et. al., 2007).

Although, we know chemical productions are mainly used in the history of pharmaceutical industry, fast and sharply return to natural and biotechnological pharmaceutics products had been observed lately. Such that, more than 85% of over 400 biotechnological drugs and vaccines which are currently available on the market had been approved and manufactured on 2000s (Kayser, S.R., 2005). Green therapy, using of molecules obtained from bioactive plants for treatment, has an important place among natural pharmaceutical products. The results of anticancer drugs Taxol and Etoposide, corroborated studies in this aspect and made them more sense.

Saponins are forming one of the most widespread secondary metabolites group in the plant kingdom. Saponins are polar glycosides consist of one or more sugar groups that bound to triterpene/steroid aglikon. Biological activities of saponins from high structured plants spread to wide spectrum. The major ones can be enumerable as hemolytic, molusisidal, anti-inflammtory, antifungal, antimicrobial, antiparasitic, antiviral, cytotoxic and anti-tumor activities (Sparg et. al., 2004; Osbourn et. al., 2011).

The anticancer potential of saponins is partially low in most cases. For that reason, scientists start to design and manufacture bioactivity-guided drugs to discover new structures with high potential. Recently, the results from many studies performed saponins anti-tumor activity have been interest.

Semi-synthetic anticancer drug-discovery programs focusing on saponins mainly engaged with the commercially available triterpenoids such as oleanolic acid and ursolic acid, but less common miscellaneous aglycons such as cycloartanes, lanostanes and hopanes. There were very limited semi-synthetic study of Cycloastragenol and Astragenol. Thus, the examination of new anti-cancer agents prepared from Cycloastragenol and Cyclocanthagenol via semi-synthesis studies.

Firstly, the extraction conditions of *Astragalus* was investigated and optimized. After isolation and prufication process, 4 natural products, Astragaloside VII, Astragaloside IV, Cyclocanthogenol and Cycloastagenol, were obtained as starting molecules. The artifact aglycon astragenol was obtained from astragaloside VII, astragaloside IV and cycloastragenol for semi synthetic studies.

Thirty eight semi-synthetic compounds were synthesized via oxidation, alkylation, acylation, condensation, and elimination reactions. Also, some unexpected products were obtained.

Of the 38 compounds, 4 compounds (**A25**, **A7**, **AHSKG** and **SCG**) were identified as novel skeleton natural product derivatives and 29 compounds were synthesized for the first time.

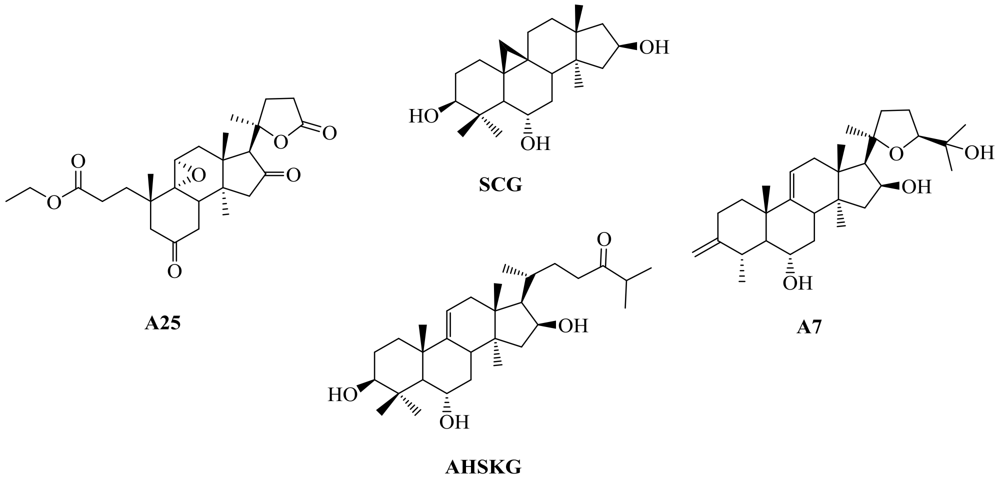


Figure 5.1. Structure of **SCG**, **A7**, **A25** and **AHSKG**

The semi synthetic derivatives were tested for their cytotoxic potantiel against four human cancer cell lines including two human prostate (DU-145 and PC-3), one human colon adenocarcinoma (HT-29) and one human breast adenocarcinoma (MDA-MB-231) cell lines, using the MTT assay. The cytotoxicity assays of semi synthetic compounds were performed at different concentrations (100, 50, 25, 10, 5, 1 μM) for a 48 h treatment period to assess their cytotoxic effects on these cells. The IC_{50} values (above 50 μM) of derivatives of astragenol were summarized in Table 5.1.

Table 5.1. IC_{50} Values of Astragenol Derivatives

	PC-3	DU-145	HT-29	MDA-MB-231
CG	>100	36.6	30.5	>100
AG	46.7	39.5	41.6	70
A2	14.0	16.3	19.6	19.3
A3	39.2	21.5	21.4	36.1
A8	6.5	4.8	19.1	7.2
A10	>100	51.0	>100	24.6
A11	87.1	>100	31.6	60.1
A12	52.1	13.5	87.4	26.1
A13	>100	34.3	>100	26.3
A14	98.2	12.6	>100	>100
A15	74.0	>100	14.6	15.3
A16	30.1	>100	33.0	>100
A17	46.1	85.8	24.3	49.1
A18	44.6	>100	47.0	99.6
A35	59.0	>100	>100	40.7
A20	6.7	12.4	11.2	9.1
A21	64.7	49.0	>100	13.2
A22	3.6	11.5	6.3	5.1
A6	>100	>100	>100	18.9
A7	9.6	23.3	13.2	9.2
A31	80.7	56.6	15.5	49.5

Compounds **A2**, **A8**, **A20**, **A22** and **A7** showed the most potent cytotoxicity against all tested cell lines. Three molecules (**A7**, **A8** and **A22**) on PC-3 cell line; **A8** on DU-145 cell line; 2 molecules on HT-29 cell line and 4 molecules (**A7**, **A8, A20** and **A22**) on MDA-MB- 231 cell line exhibited IC_{50} values lower than 10 μM.

The common point in these molecules implied that the presence of high electron density and H-bond acceptors at C-3 position (acyl substitutions especially sulfonyl esters), an epoxy ring between C-9 and C-11, and a double bond in A ring were important. However, inactive molecules with similar groups in the same area was detected. These indicated that structure activity relationship is considerably complicated.

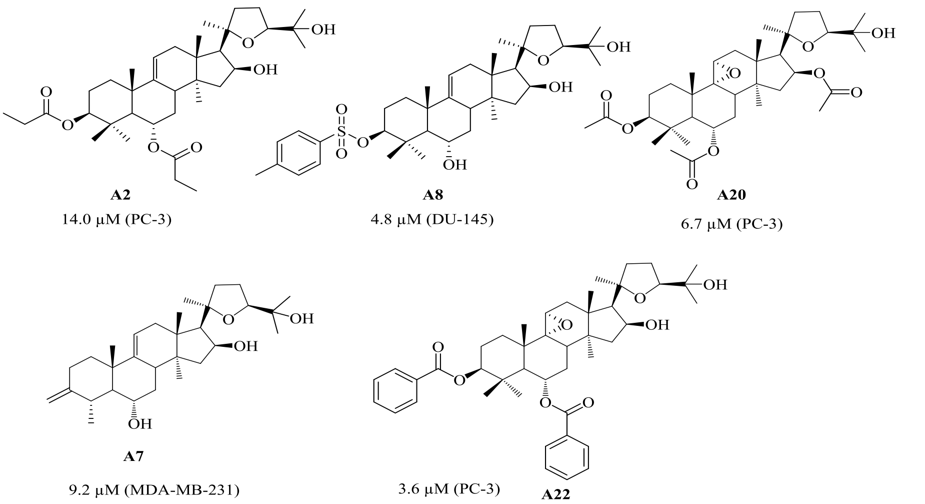


Figure 5.2. Structures and IC_{50} Values of **A7**, **A20** and **A22**

A31 showed promising selective cytotoxicity against HT-29. Compound A14 showed interesting selectivite cytotoxicity against DU-145. Also, endo elimination product (A6) indicated promising selective cytotoxicity against MDA-MB-231. All oxidized derivatives of astragenol (A4, A5, A9, A27, A29, A25, A30, A34, A38, A35 and A36) showed no cytotoxic activity against all tested cell lines.

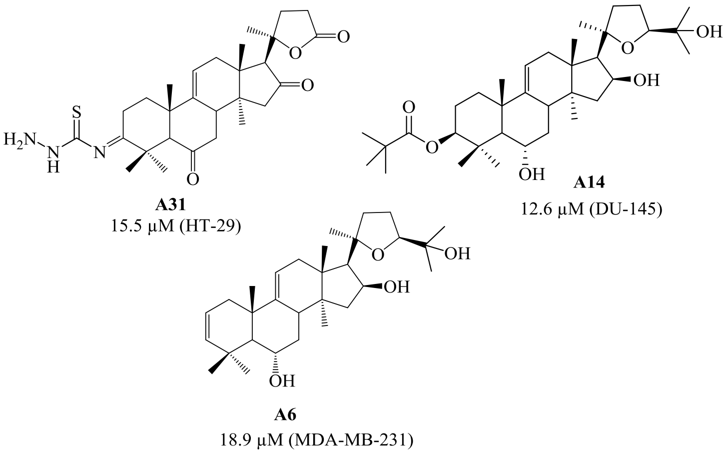
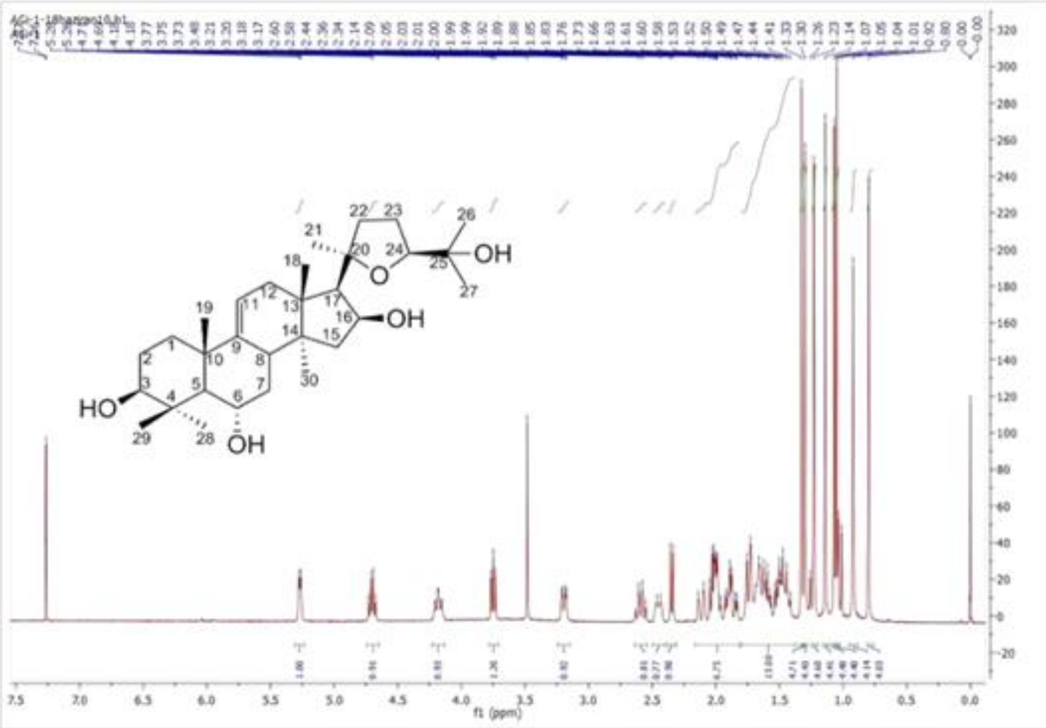


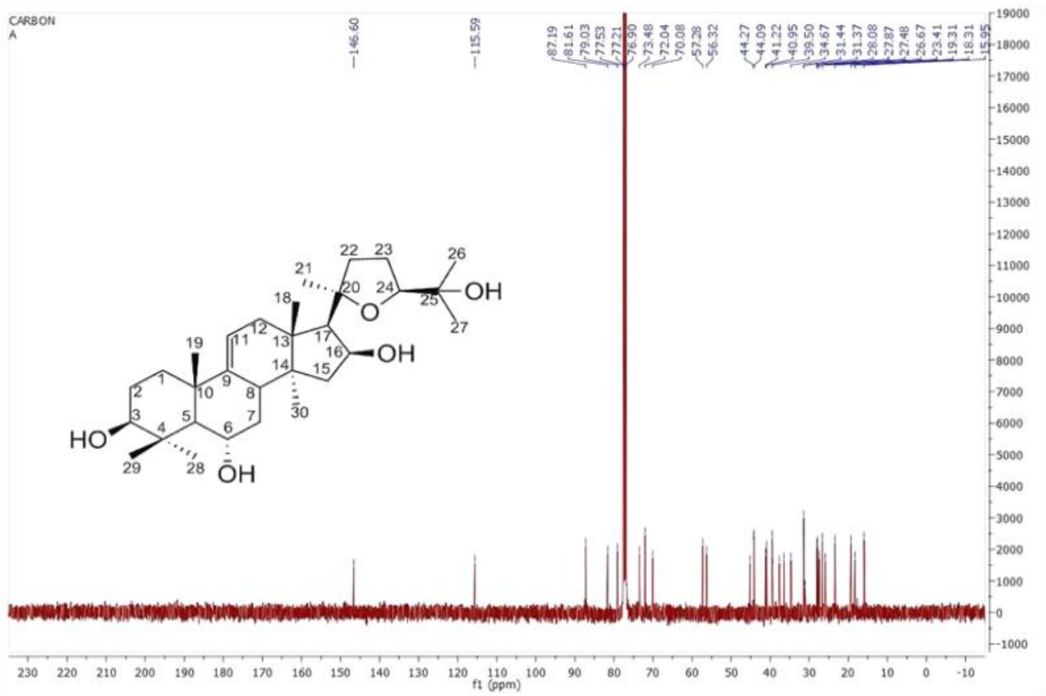
Figure 5.3. Structure of **A6**, **A14** and **A31**

This Ph.D. thesis was focused on advancing the understanding and knowledge about chemistry of phytochemicals and derivatives in the field of drug discovery for cancer through the extraction optimization, isolation and semi synthetic analogues from *Astragalus* plants. The synthezied of 38 compounds, including four novel structures, with 16 of these showed potent cytotoxicity against different human cancer cells has made a contribution to the discovery of natural product-derived compounds with anti-cancer potential.

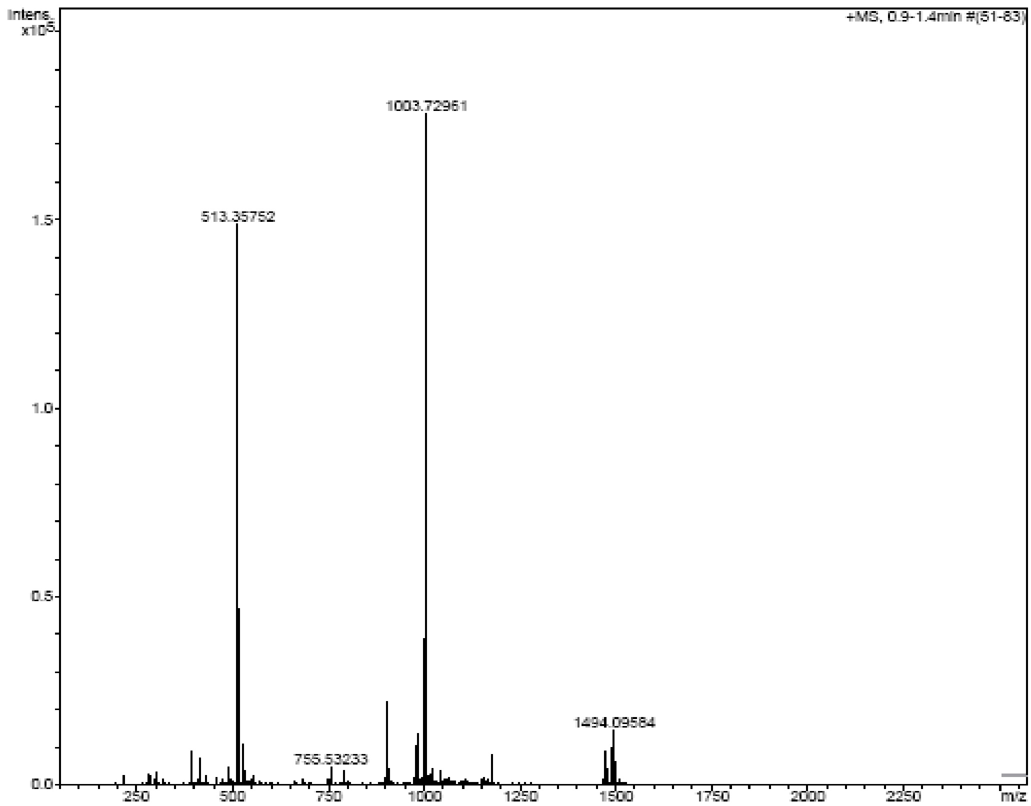
SPECTRA



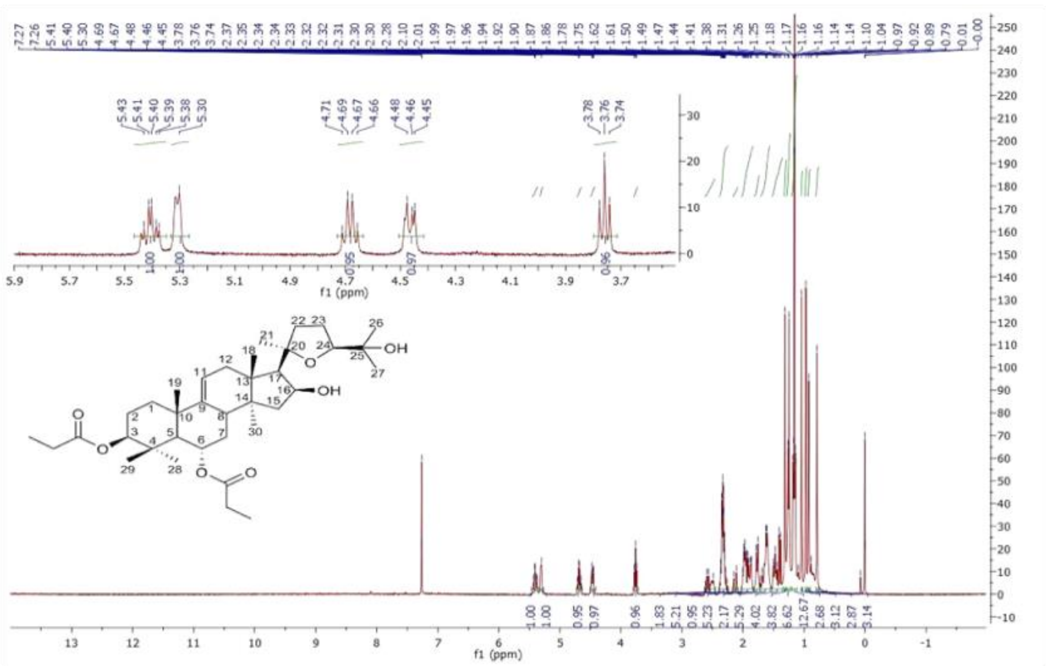
Spectrum 1. ^{1}H-NMR Spectrum of **Astragenol**



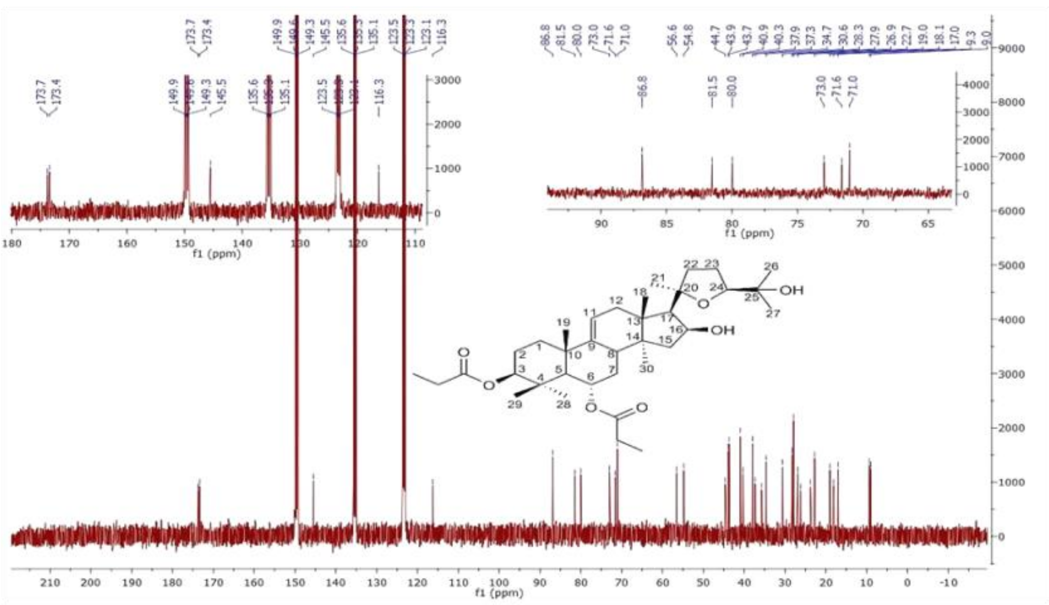
Spectrum 2. ^{13}C-NMR Spectrum of **Astragenol**



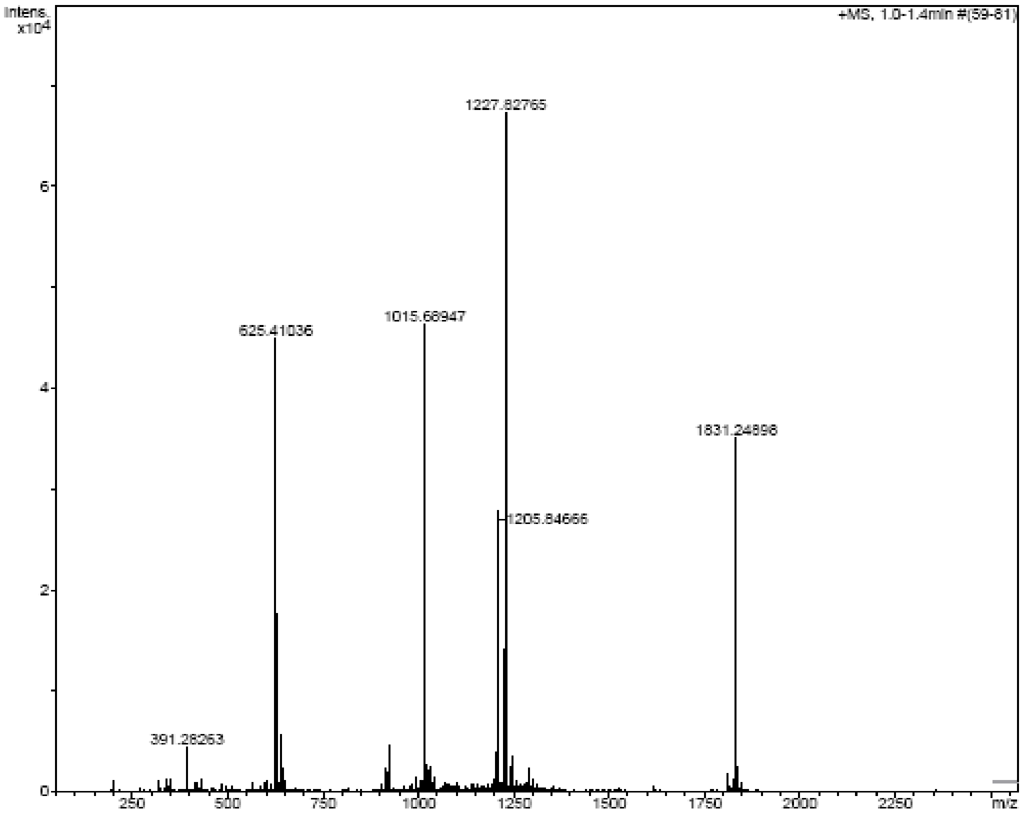
Spectrum 3. Mass spectrum of **Astragenol**



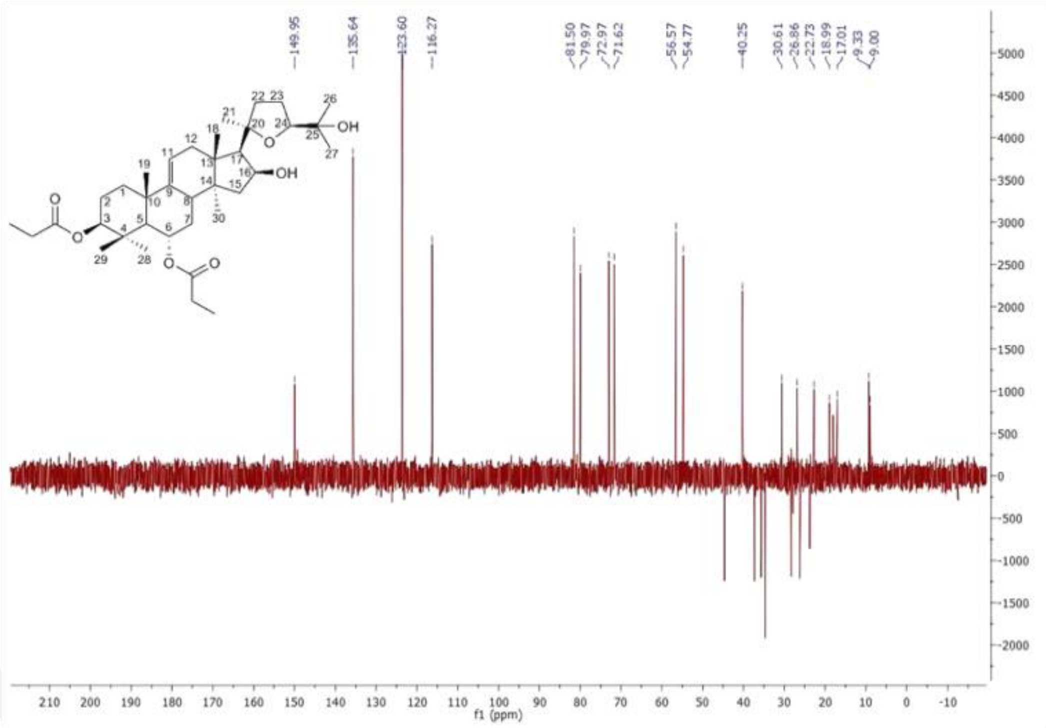
Spectrum 4. [1]H-NMR Spectrum of **A2**



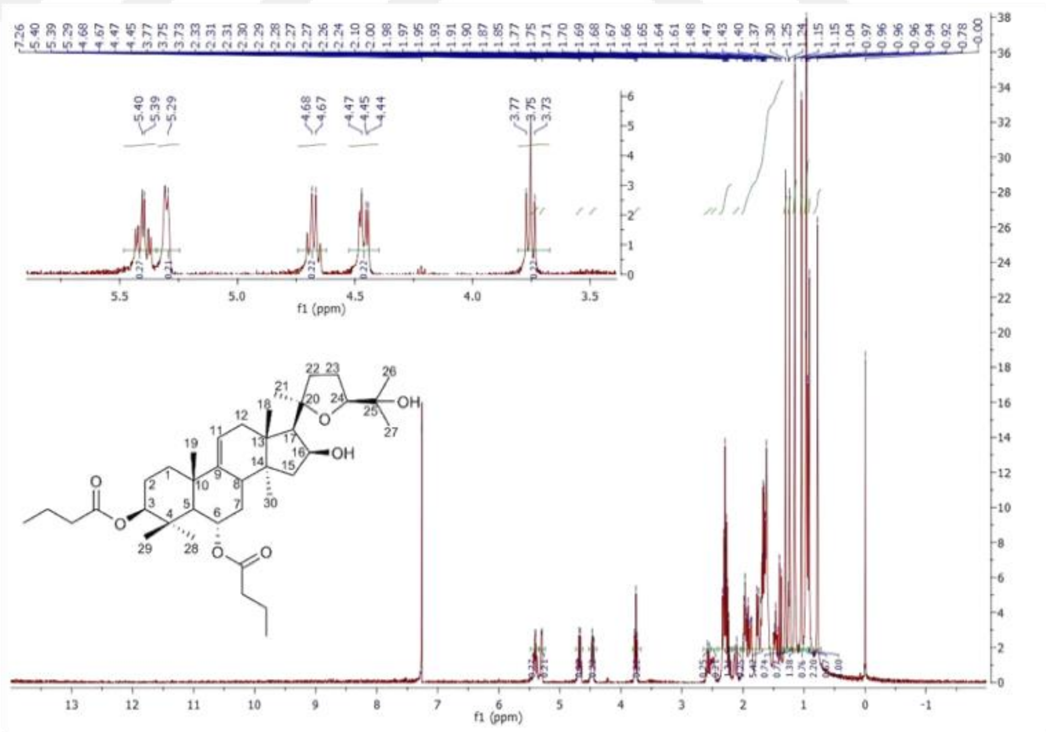
Spectrum 5. ^{13}C-NMR Spectrum of **A2**



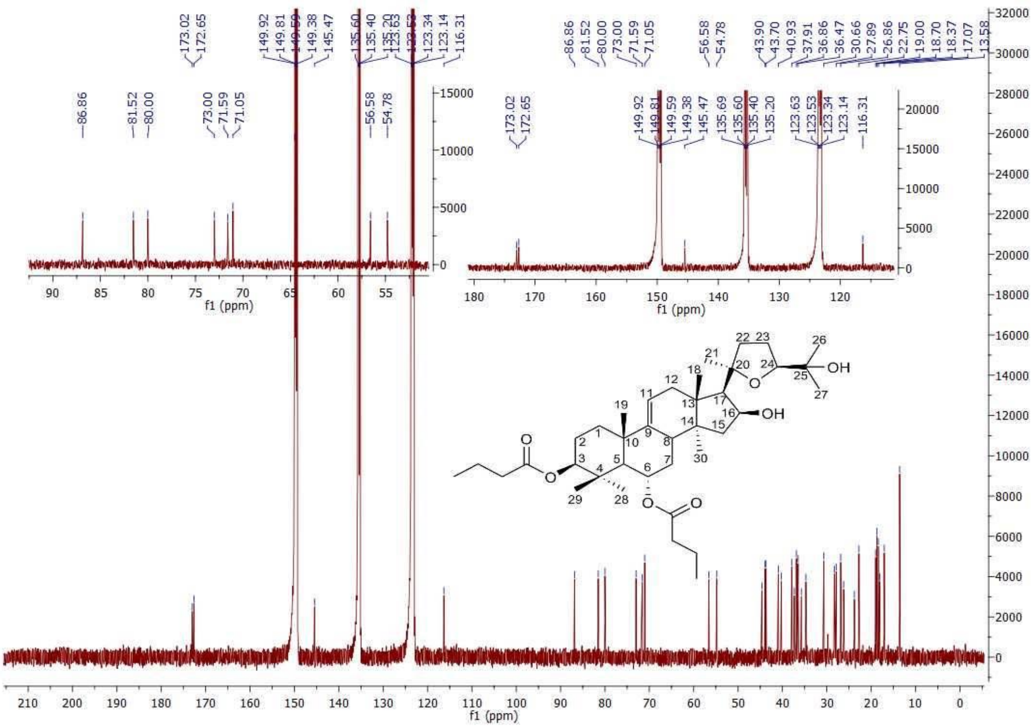
Spectrum 6. Mass Spectrum of **A2**



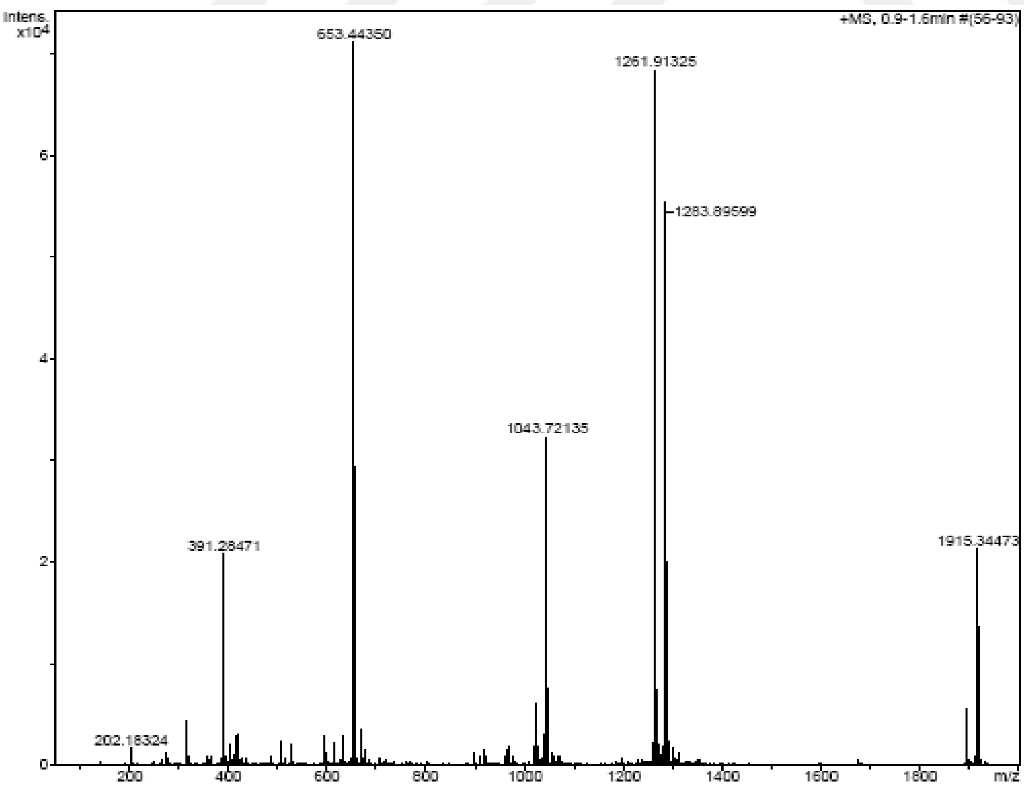
Spectrum 7. DEPT135 NMR Spectrum of **A2**



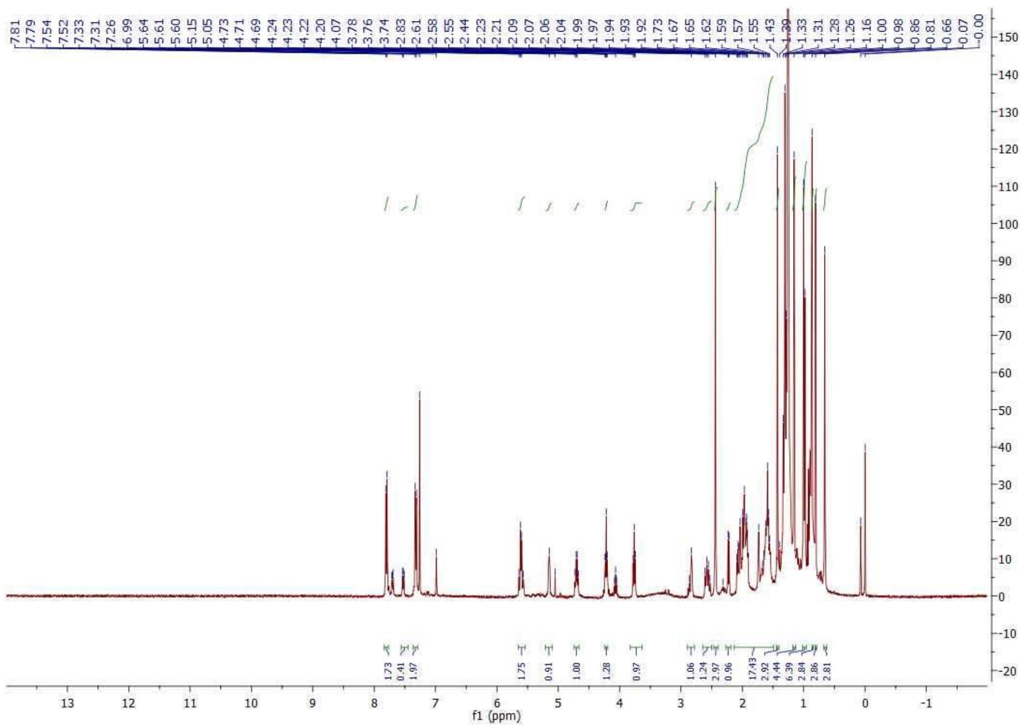
Spectrum 8. ^{1}H-NMR Spectrum of **A3**



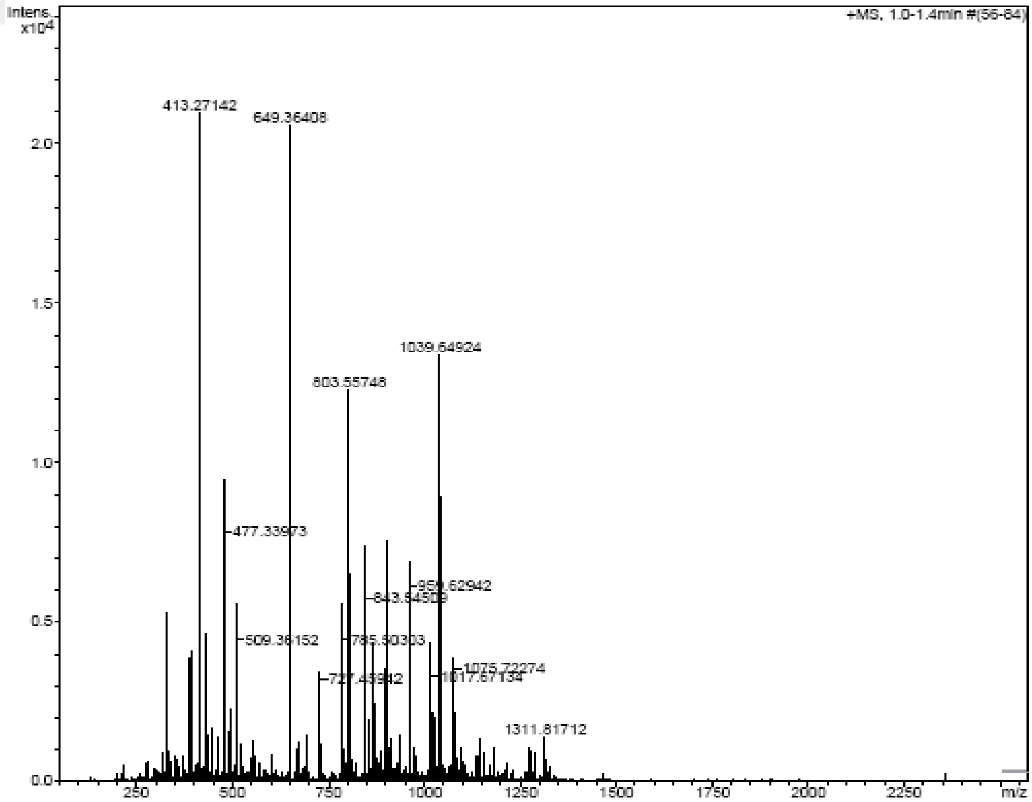
Spectrum 9. ^{13}C-NMR Spectrum of **A3**



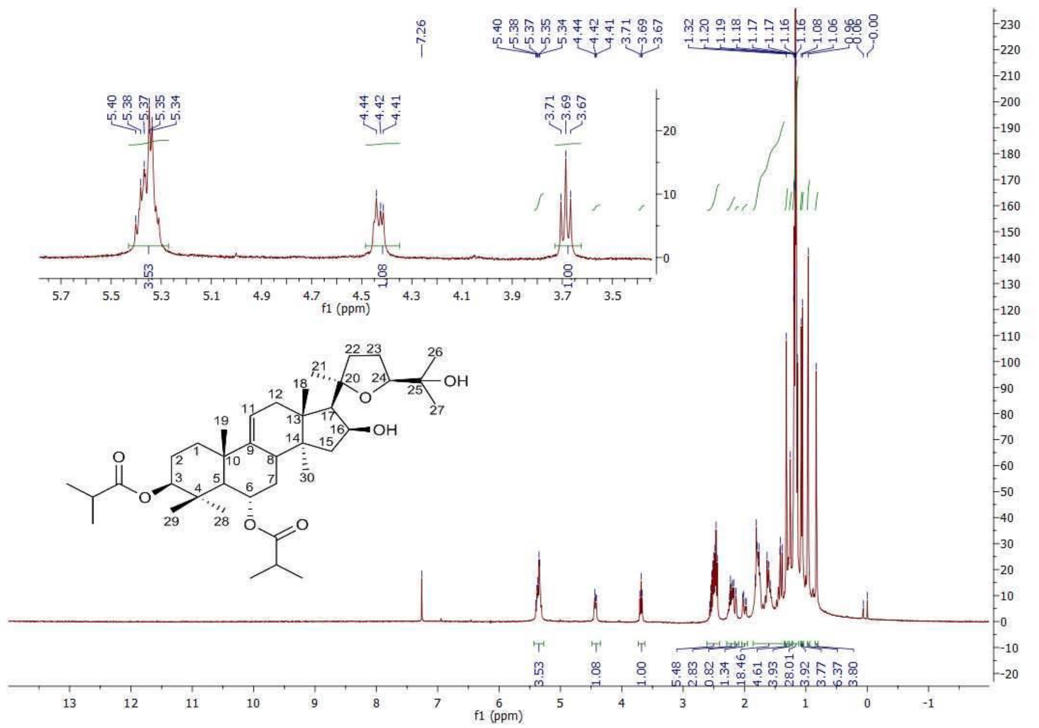
Spectrum 10. Mass Spectrum of **A3**



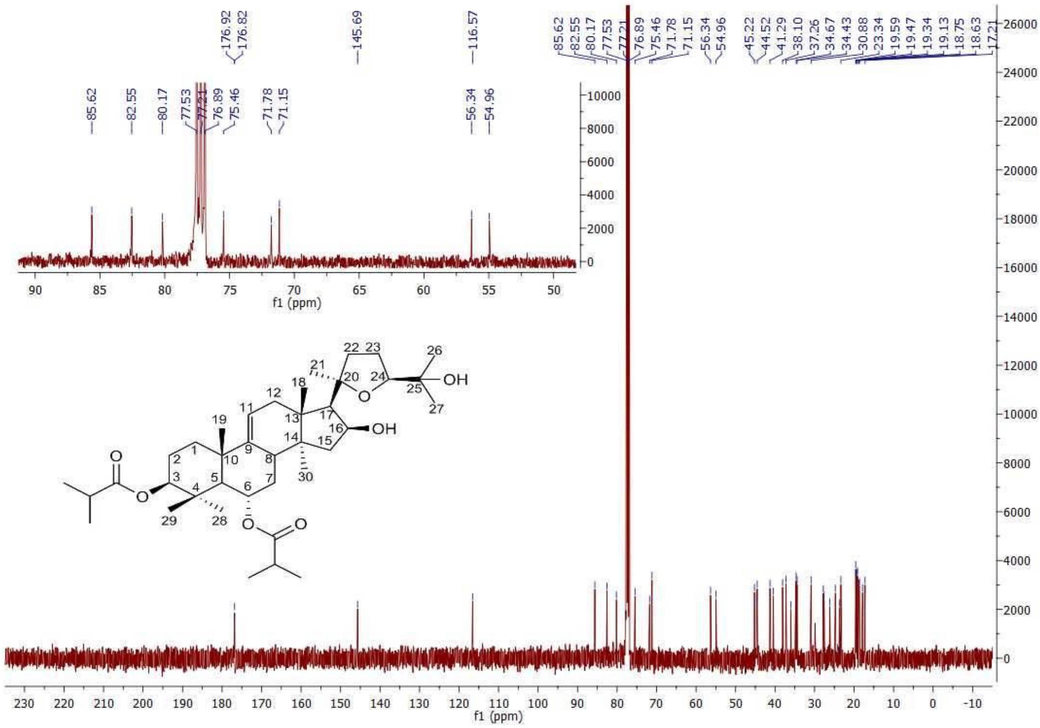
Spectrum 11. ^{1}H-NMR Spectrum of **A8**



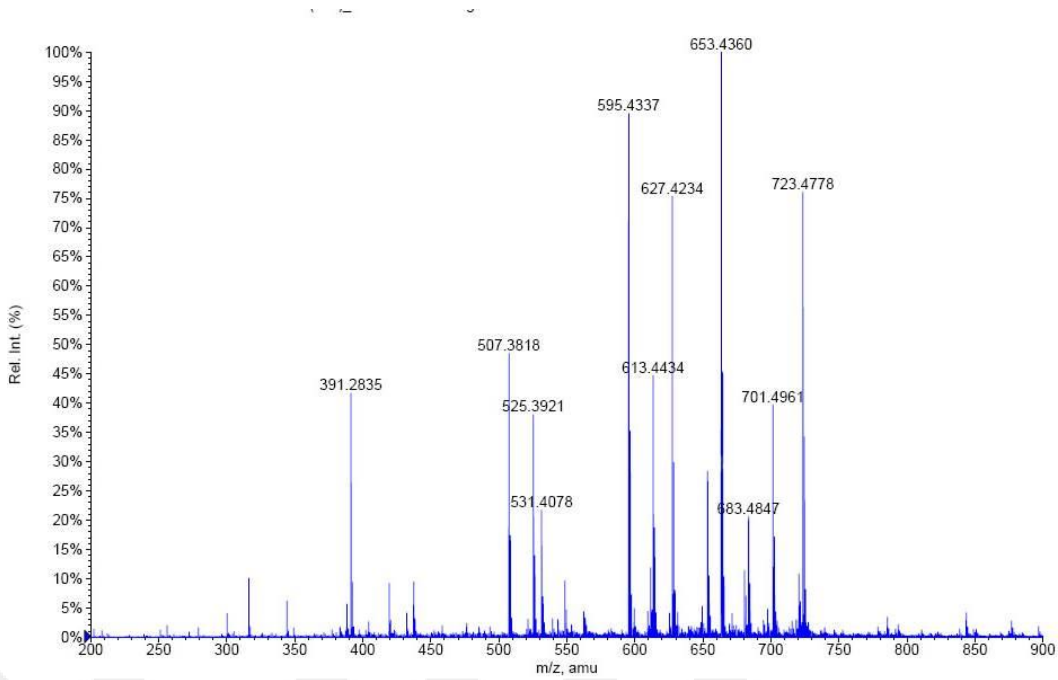
Spectrum 12. Mass Spectrum of **A8**



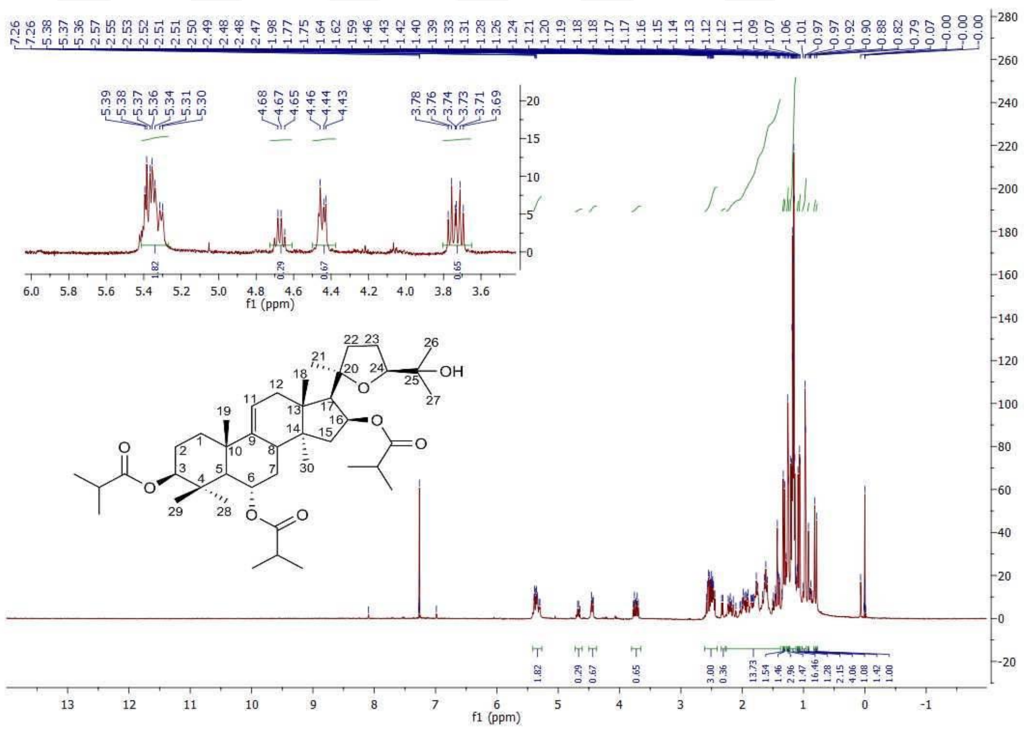
Spectrum 13. ^{1}H-NMR Spectrum of **A10**



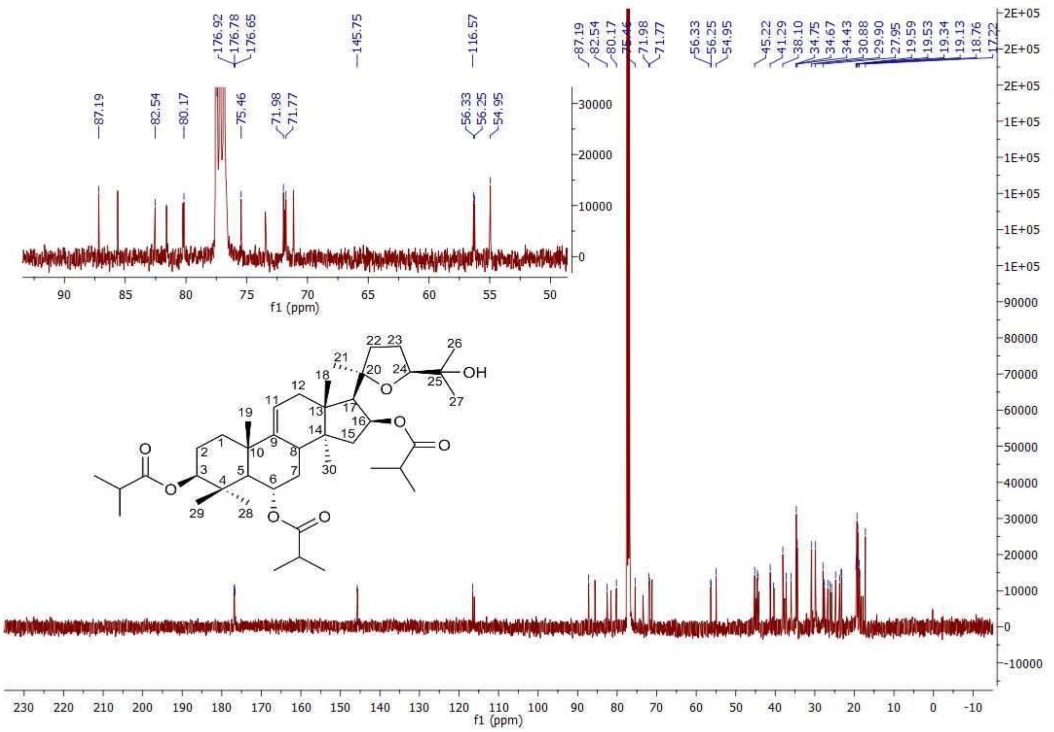
Spectrum 14. ^{13}C-NMR Spectrum of **A10**



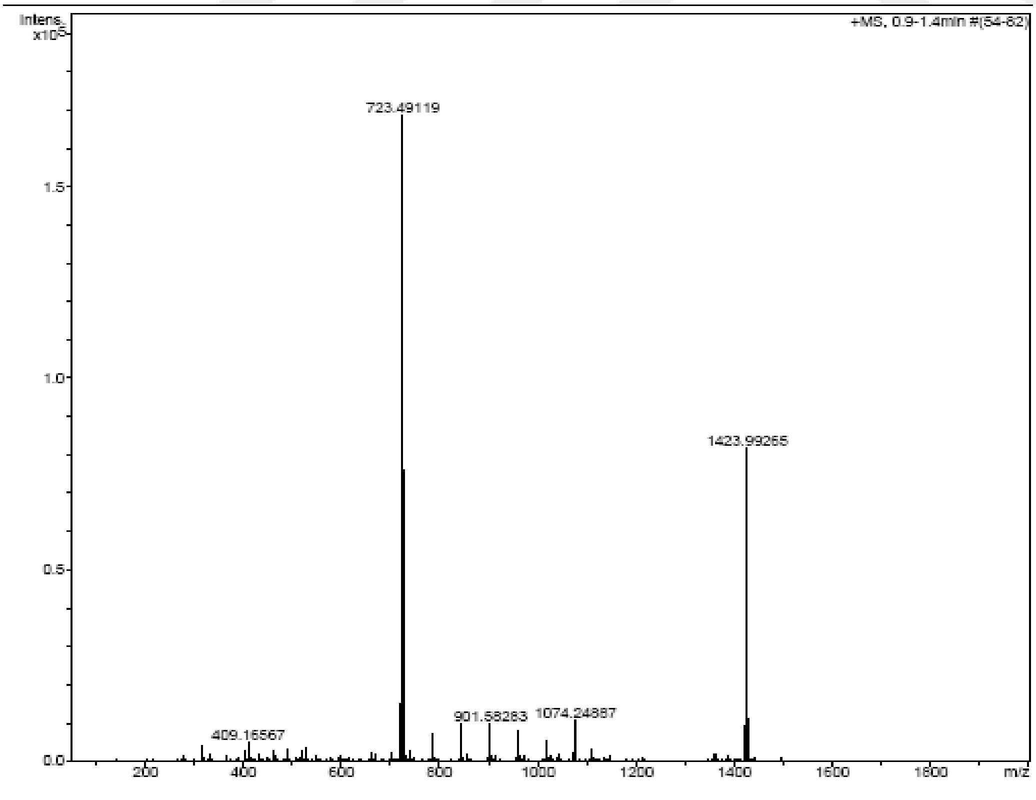
Spectrum 15. Mass Spectrum of **A10**



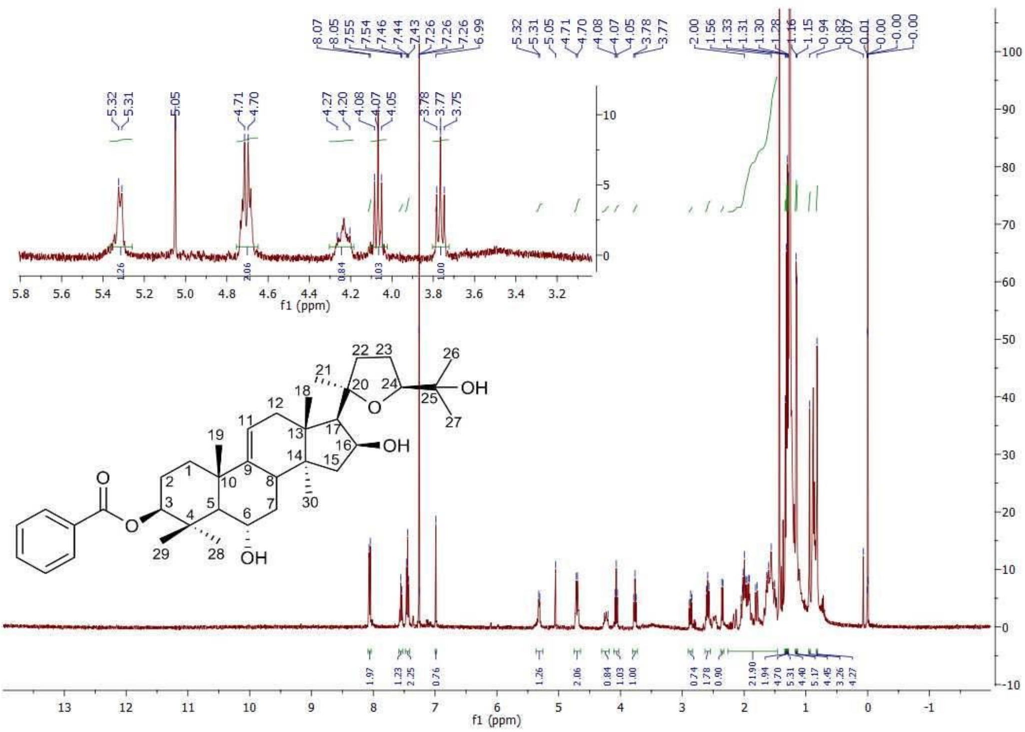
Spectrum 16. [1]H-NMR Spectrum of **A11**



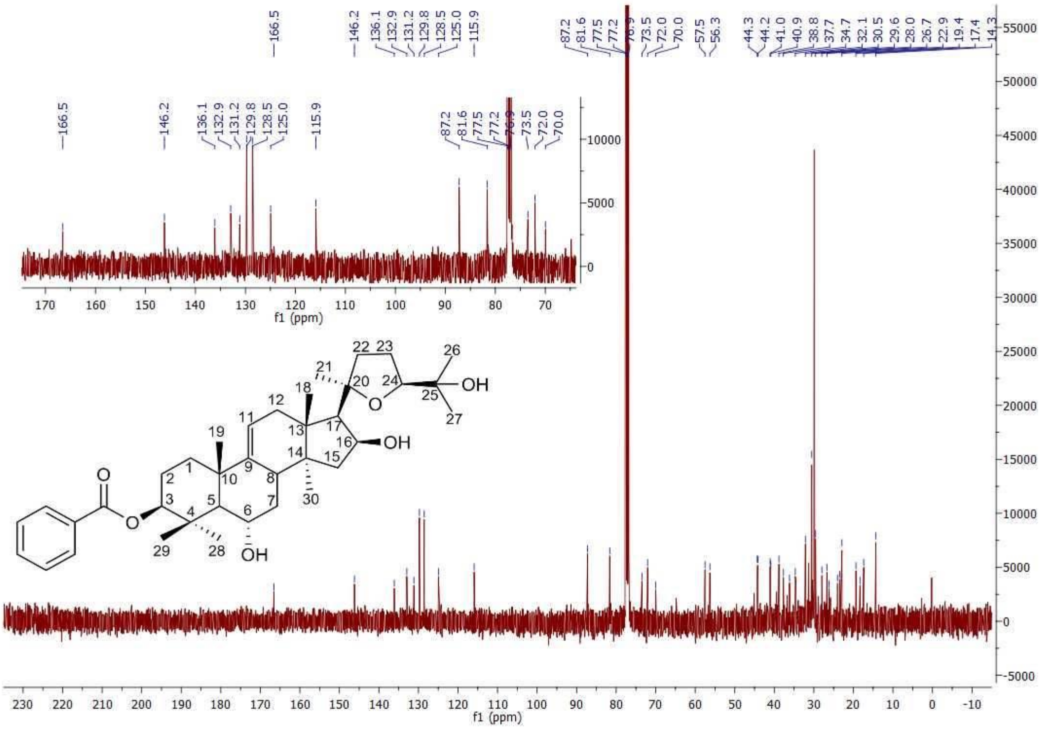
Spectrum 17. ^{13}C-NMR Spectrum of **A11**



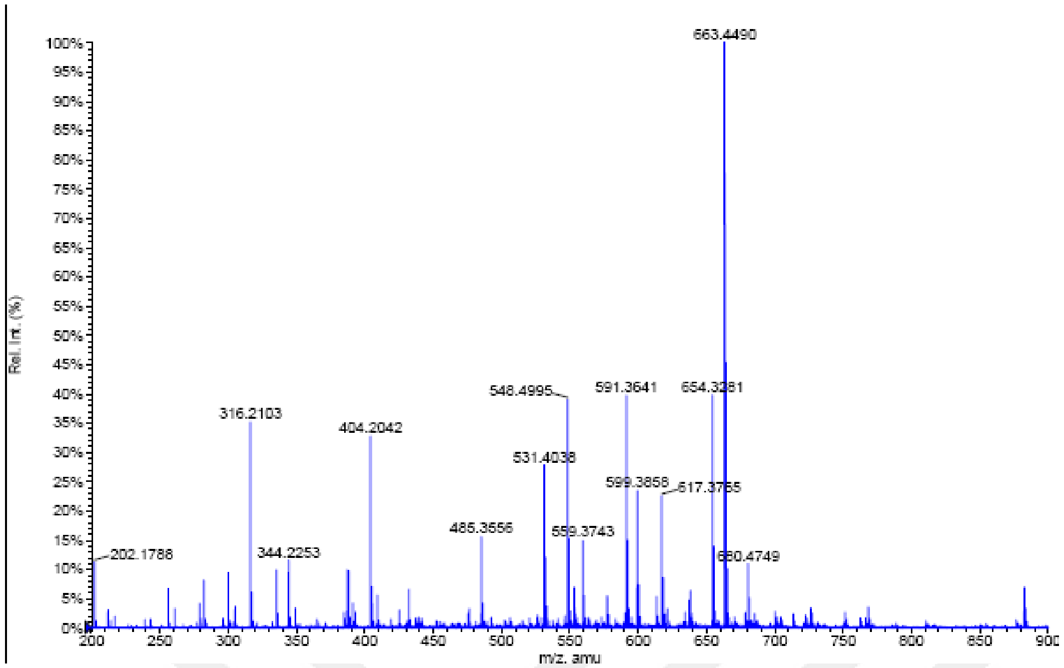
Spectrum 18. Mass Spectrum of **A11**



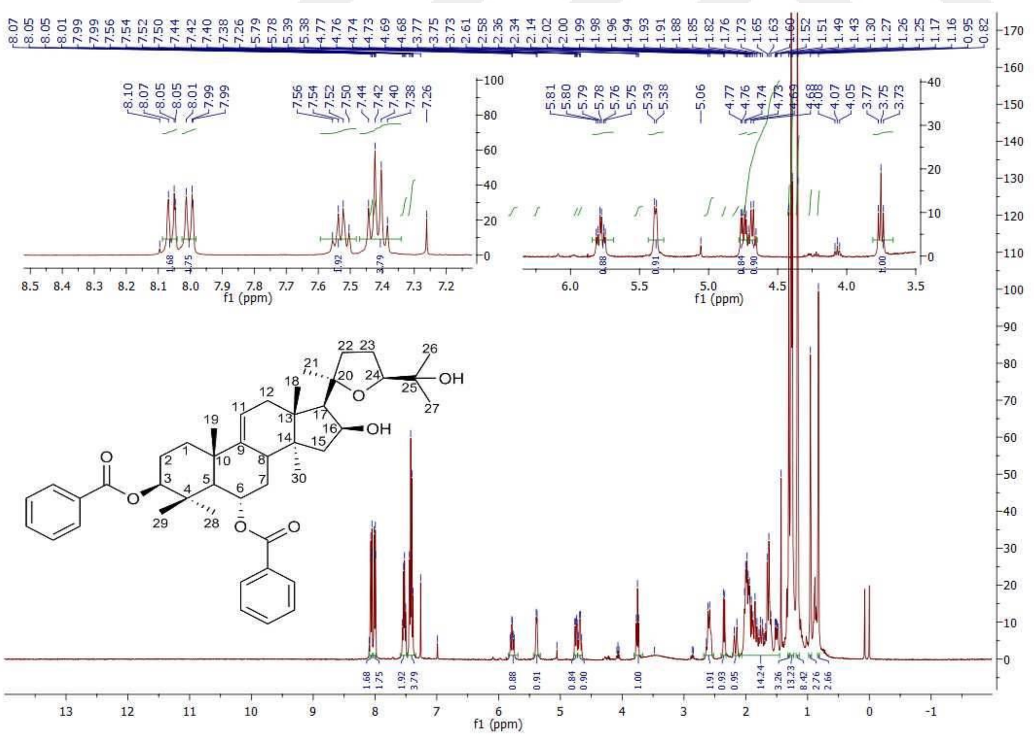
Spectrum 19. ^{1}H-NMR Spectrum of **A12**



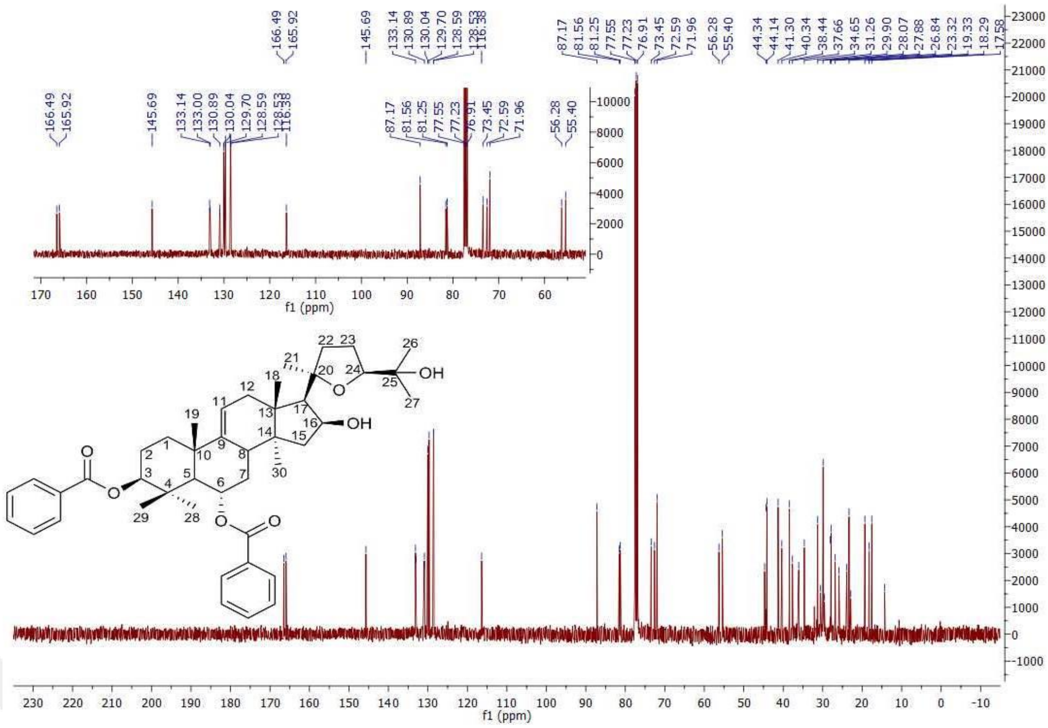
Spectrum 20. ^{13}C-NMR Spectrum of **A12**



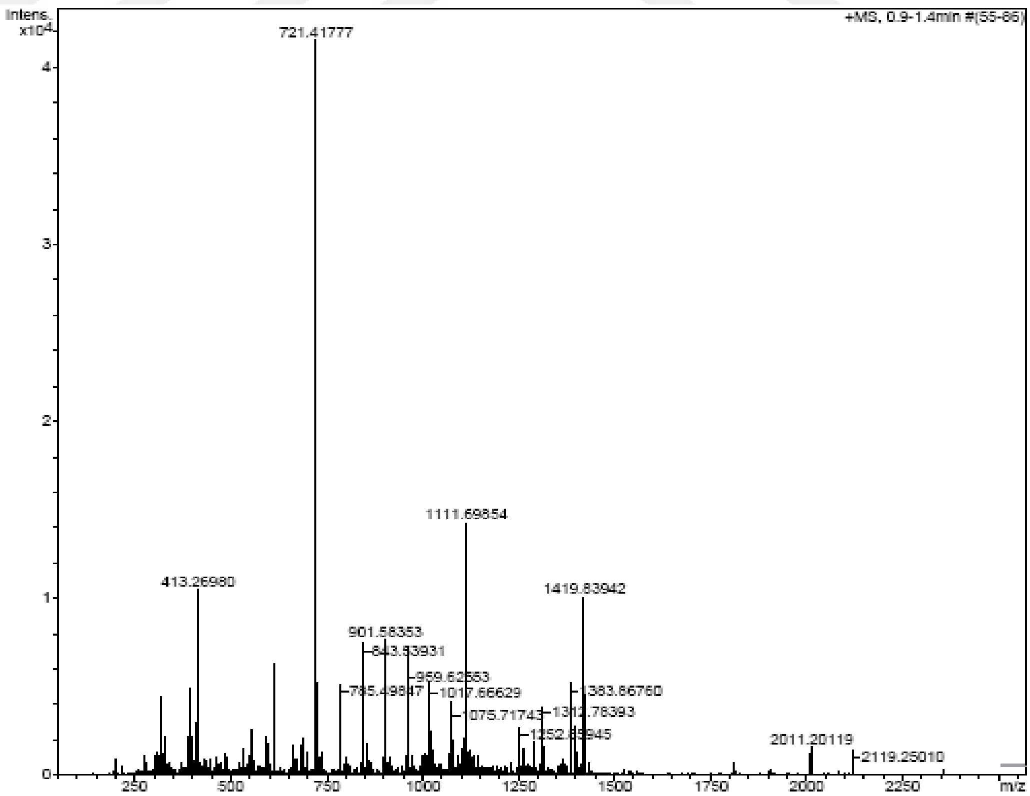
Spectrum 21. Mass Spectrum of **A12**



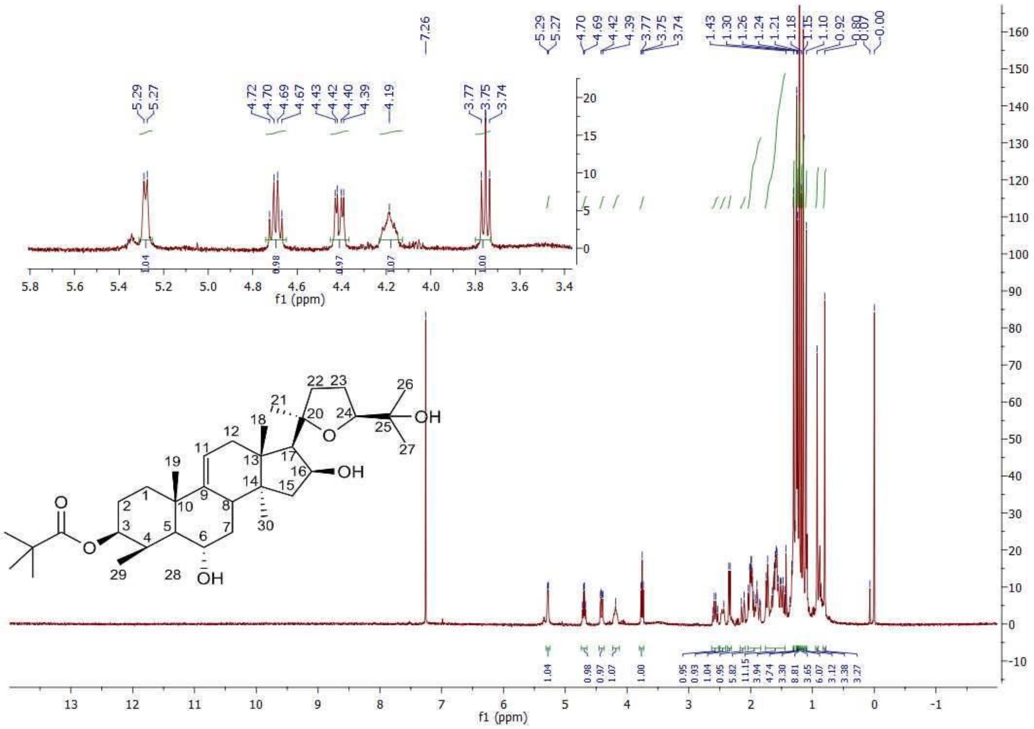
Spectrum 22. [1]H-NMR Spectrum of **A13**



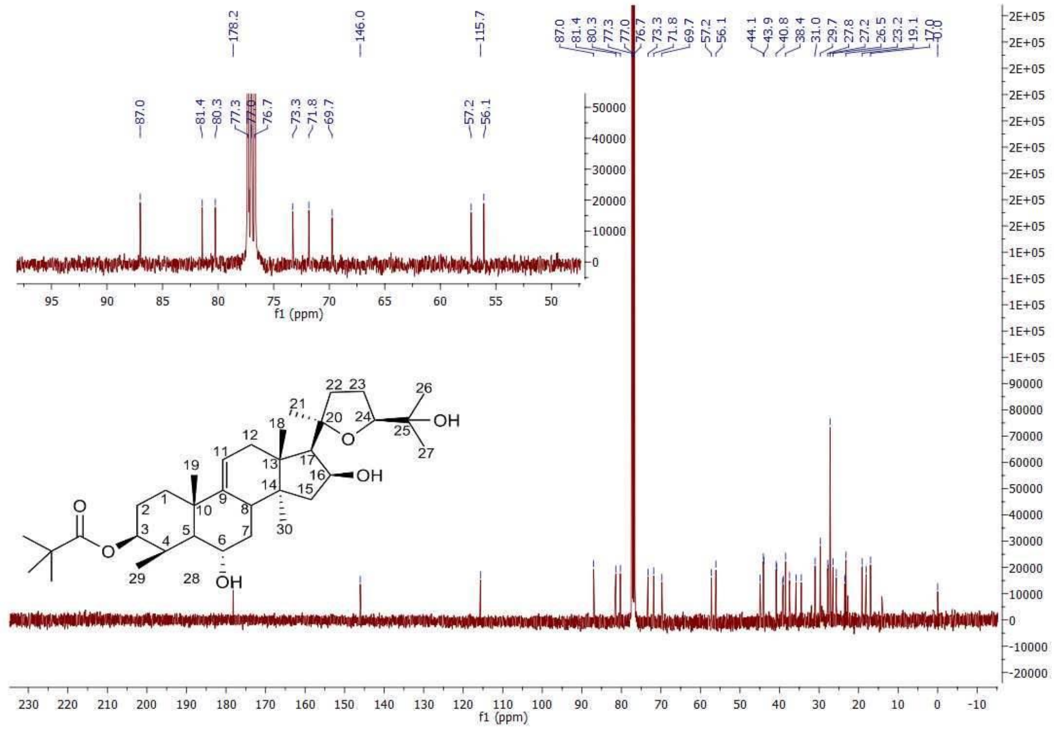
Spectrum 23. ^{13}C-NMR Spectrum of **A13**



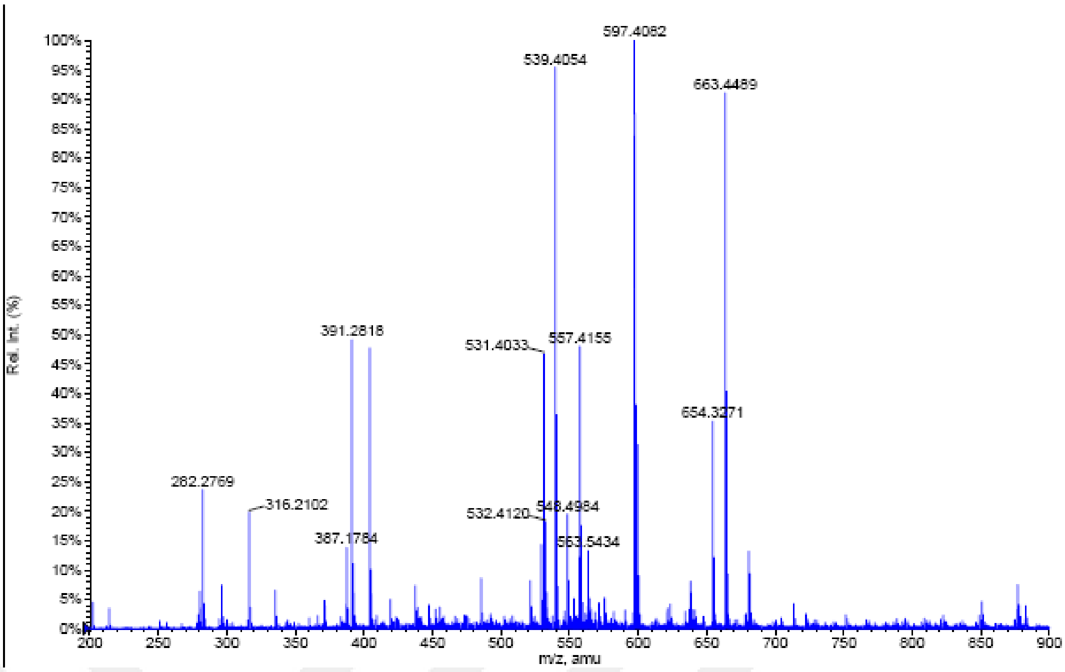
Spectrum 24. Mass Spectrum of **A13**



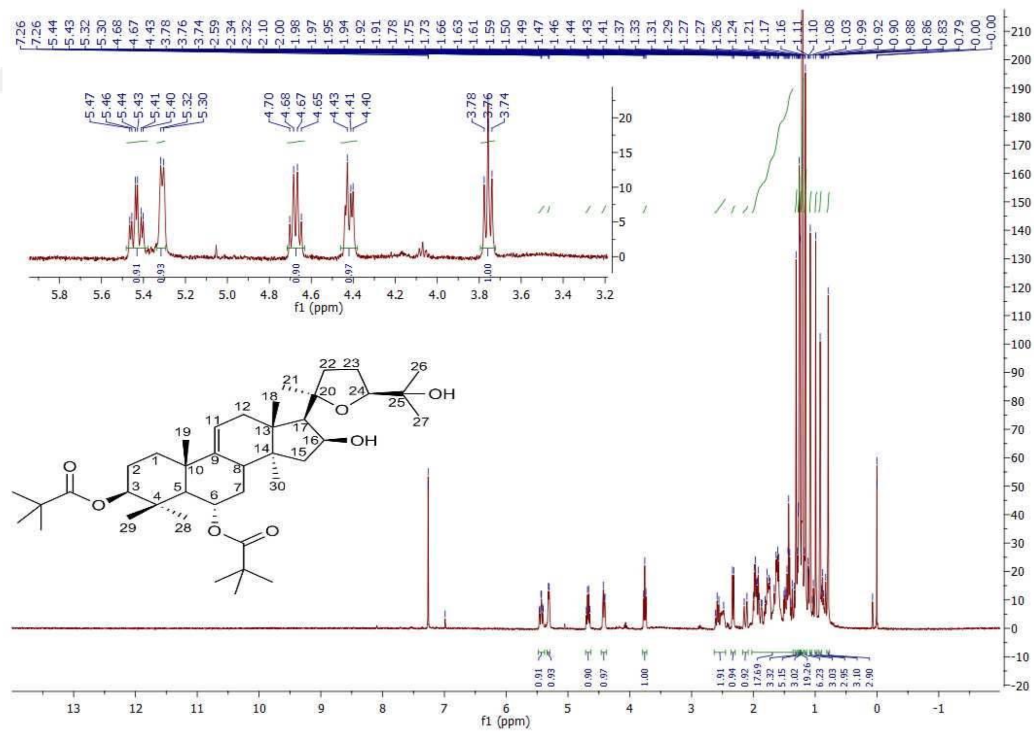
Spectrum 25. ^{1}H-NMR Spectrum of **A14**



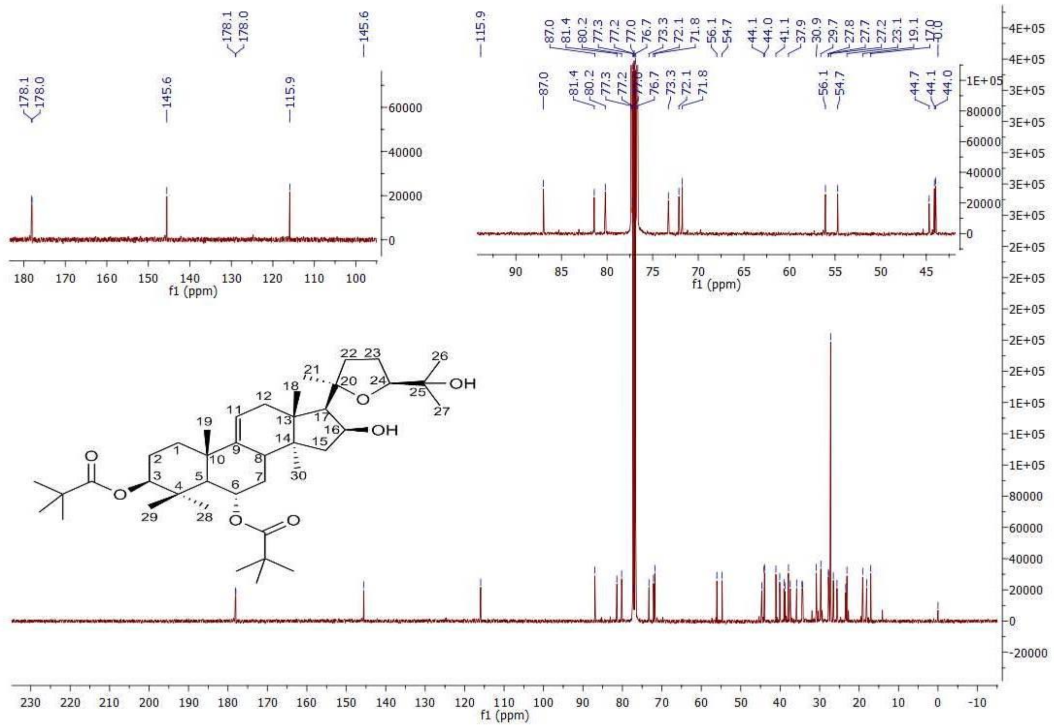
Spectrum 26. ^{13}C-NMR Spectrum of **A14**



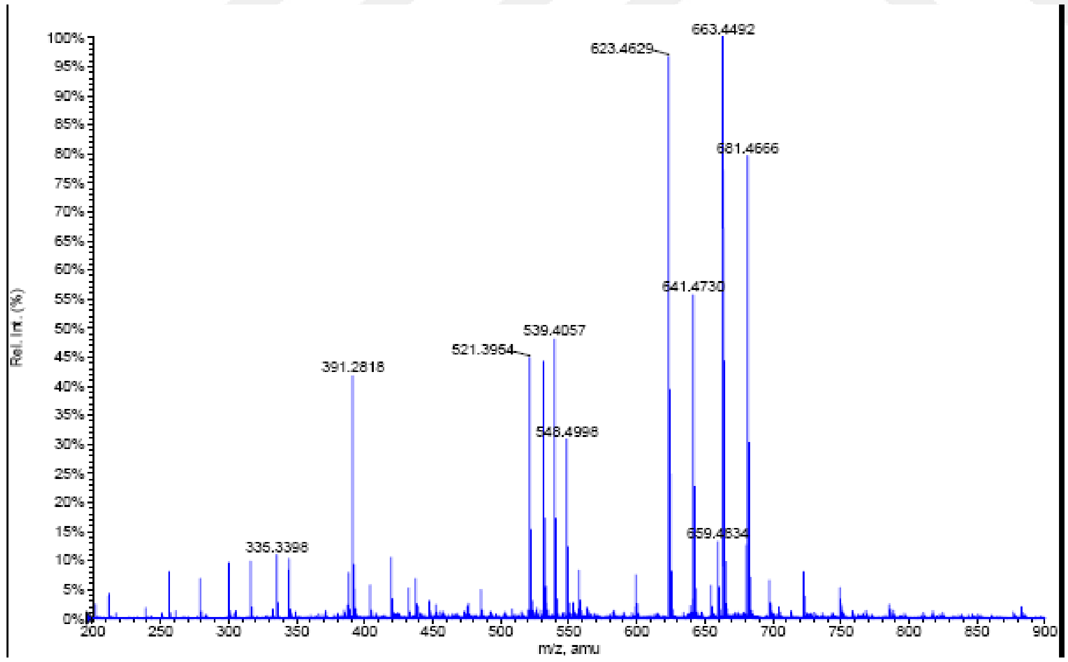
Spectrum 27. Mass Spectrum of **A14**



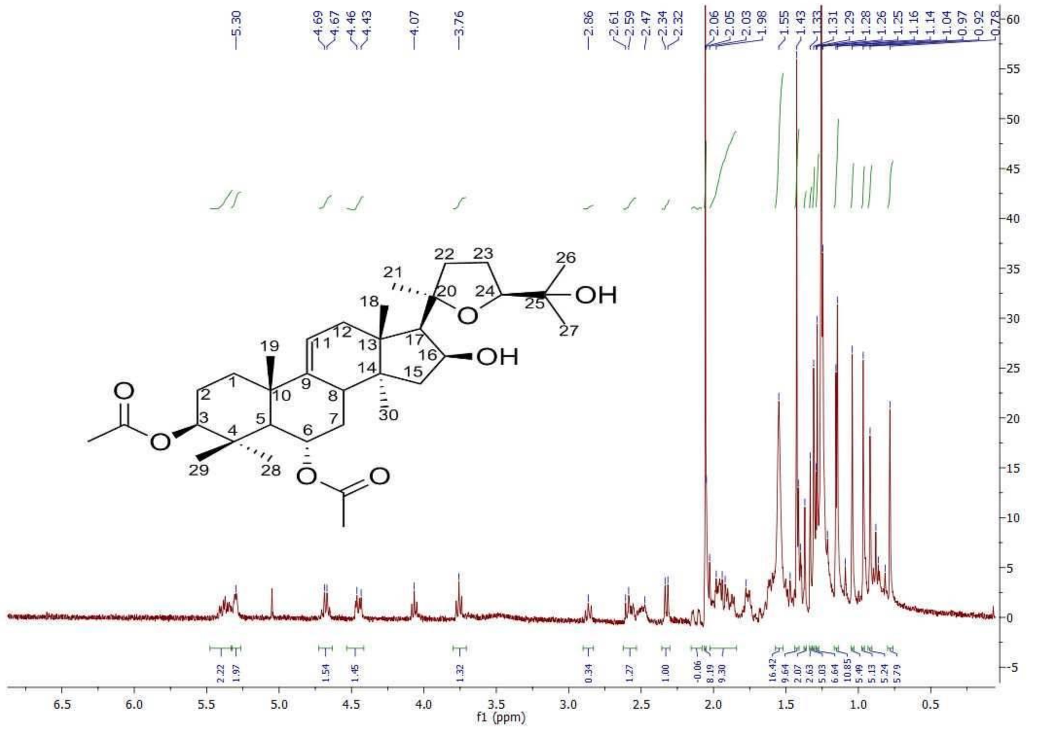
Spectrum 28. ¹H-NMR Spectrum of **A15**



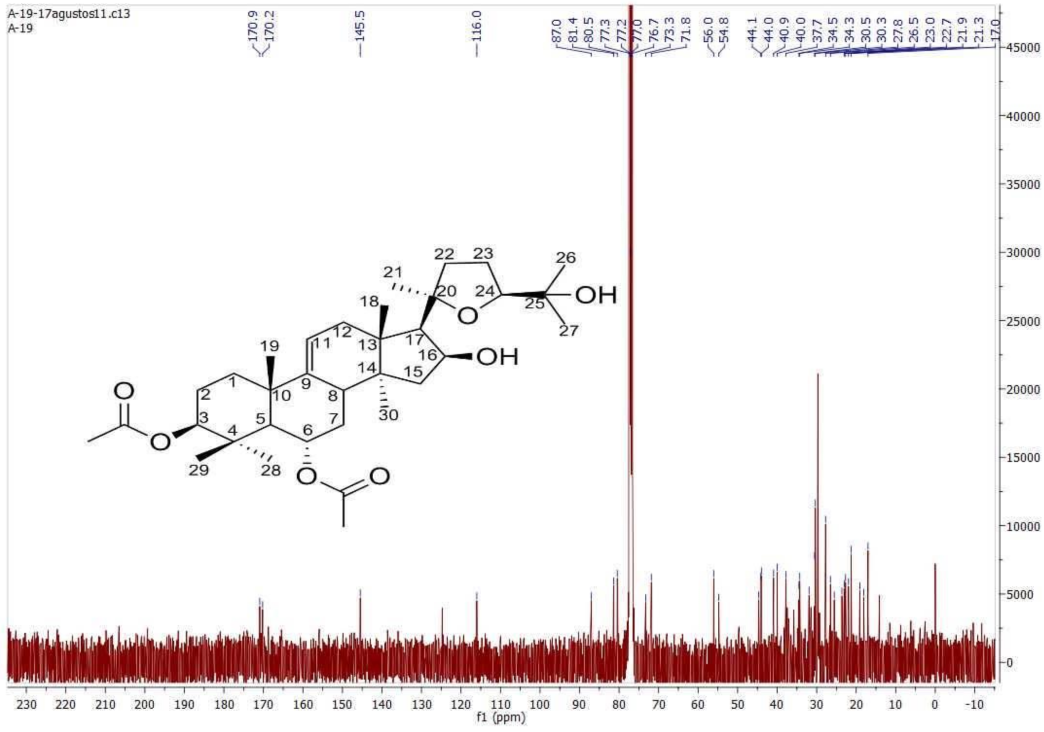
Spectrum 29. ^{13}C-NMR Spectrum of **A15**



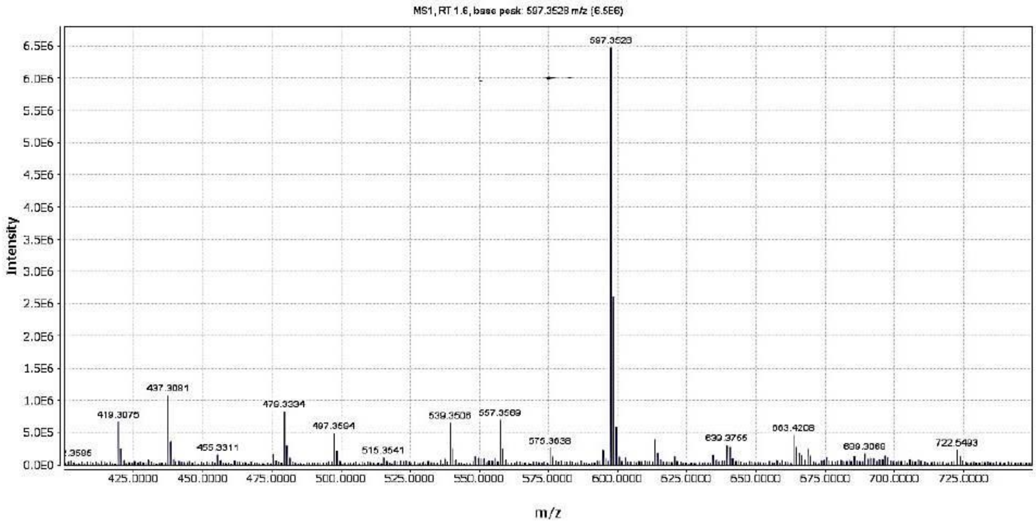
Spectrum 30. Mass Spectrum of **A15**



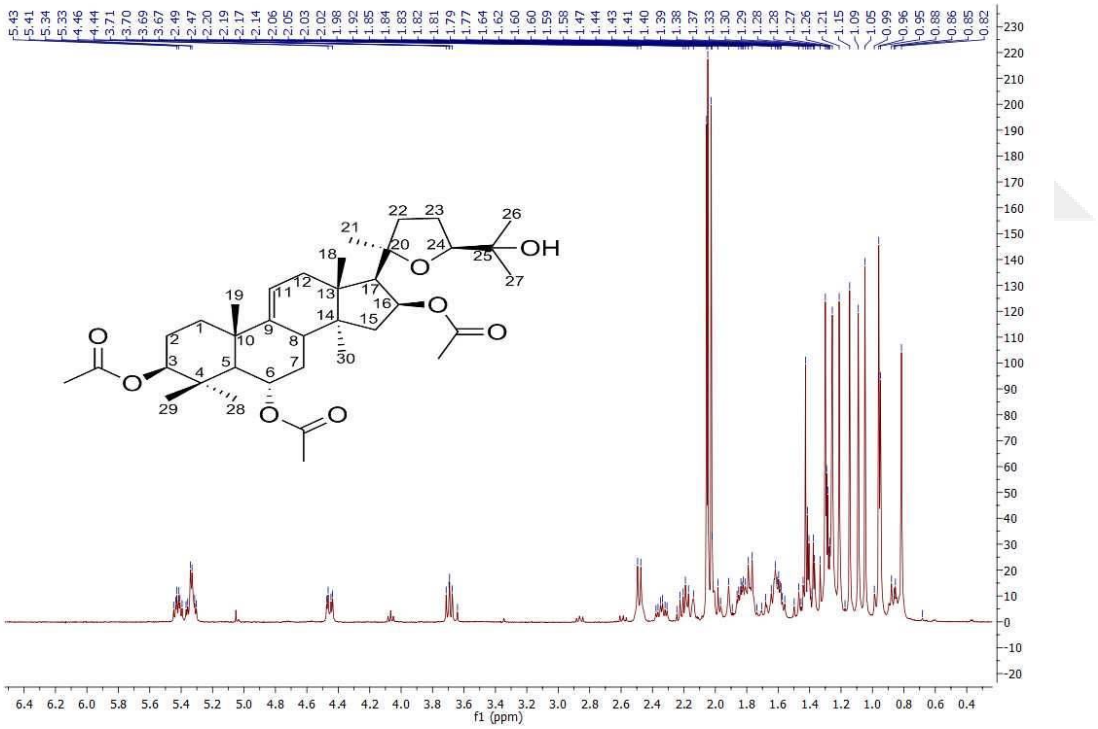
Spectrum 31. ^{1}H-NMR Spectrum of **A16**



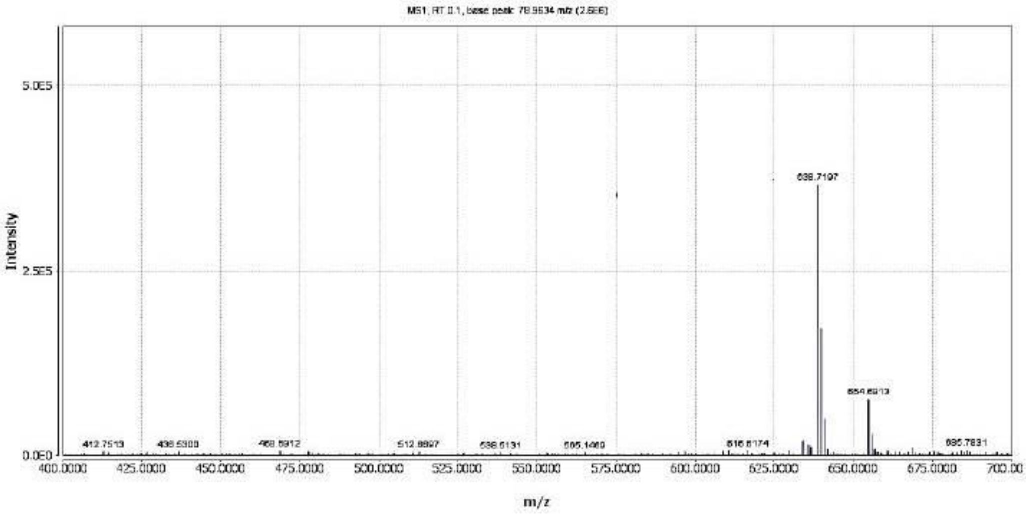
Spectrum 32. ^{13}C-NMR Spectrum of **A16**



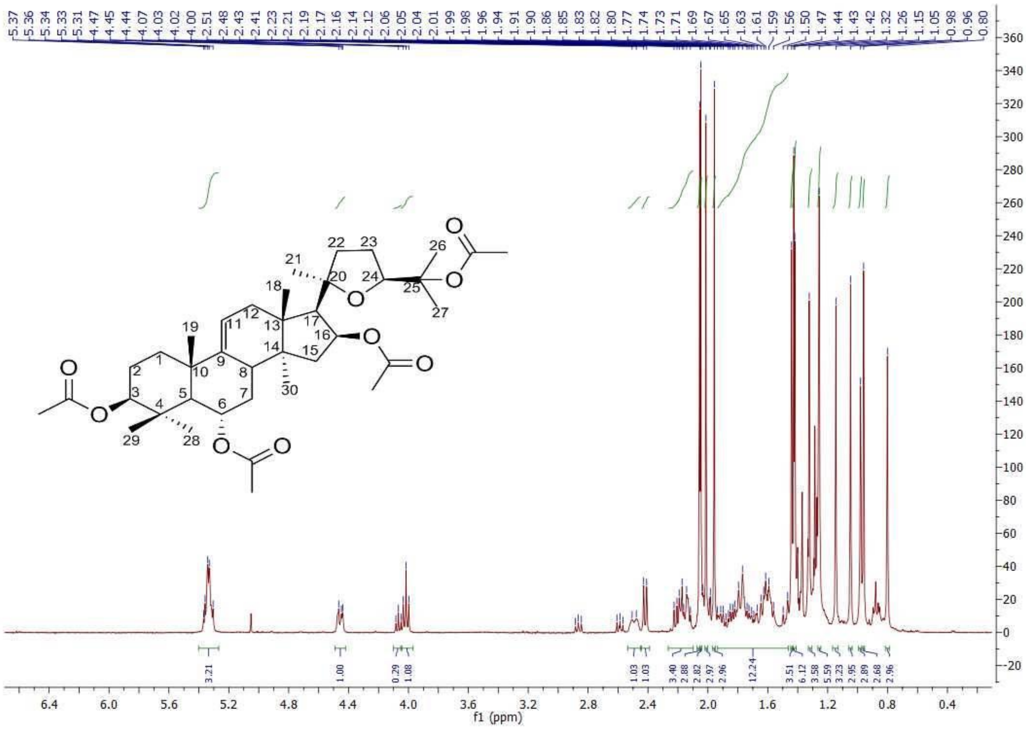
Spectrum 33. Mass Spectrum of **A16**



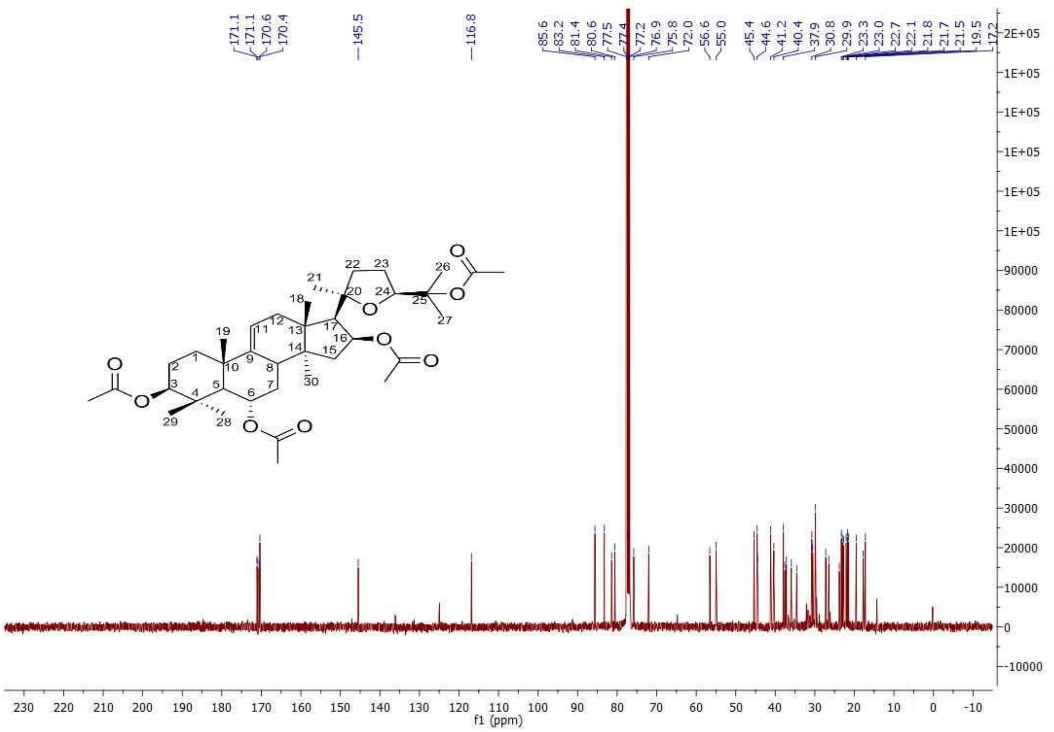
Spectrum 34. ^{1}H-NMR Spectrum of **A17**



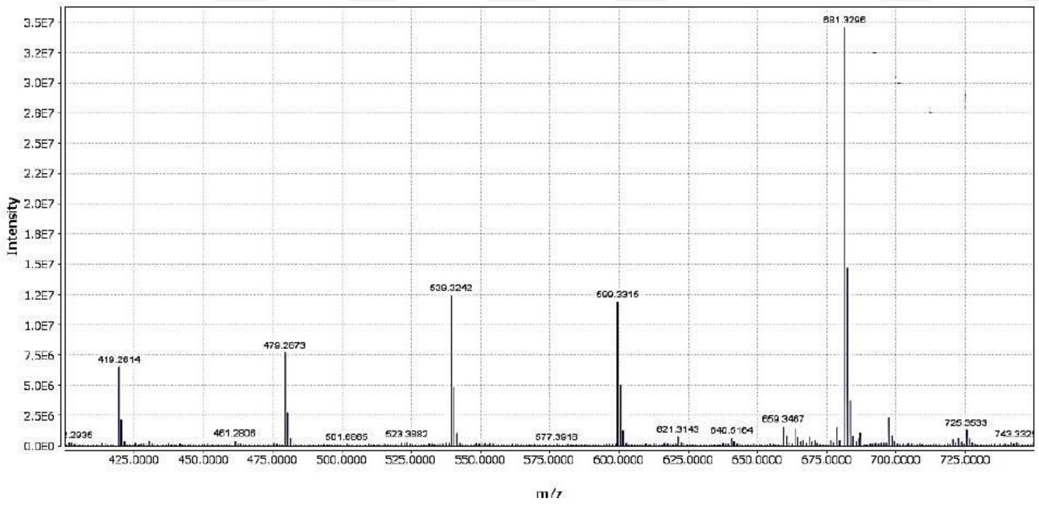
Spectrum 35. Mass Spectrum of **A17**



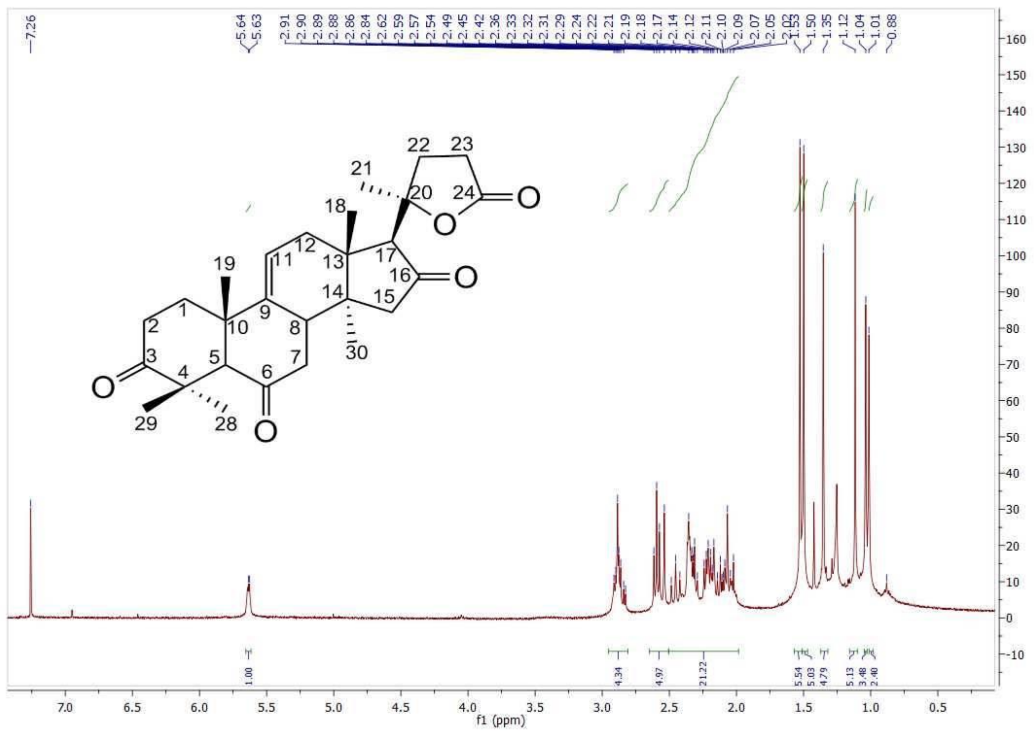
Spectrum 36. ^{1}H-NMR Spectrum of **A18**



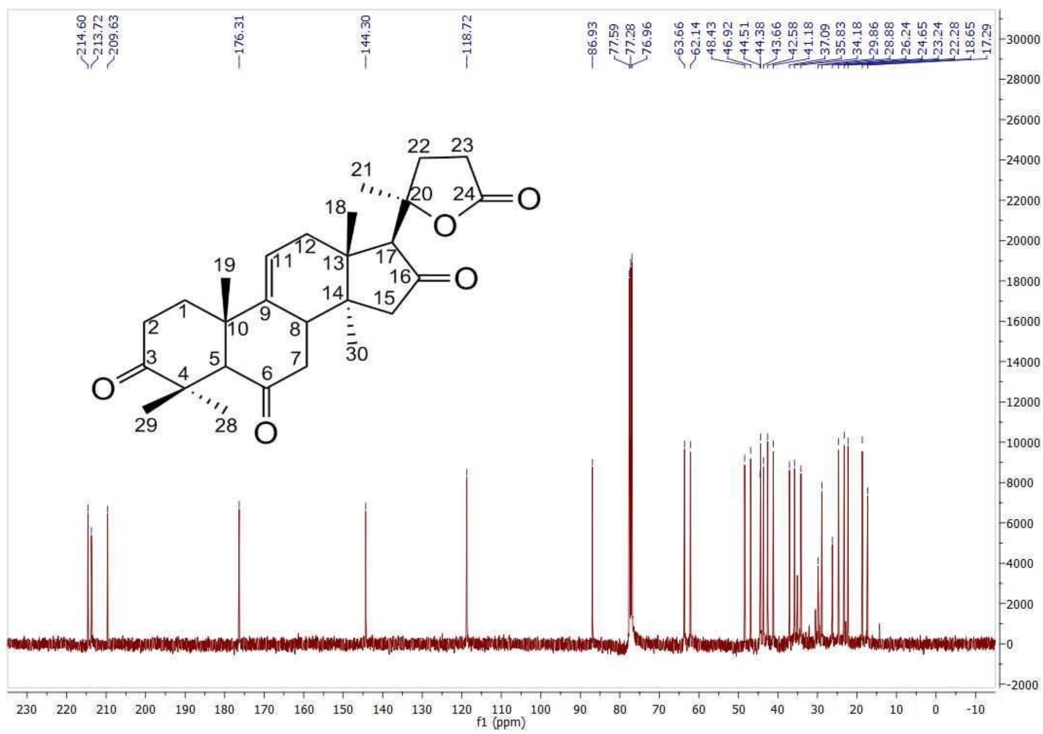
Spectrum 37. ^{13}C-NMR Spectrum of **A18**



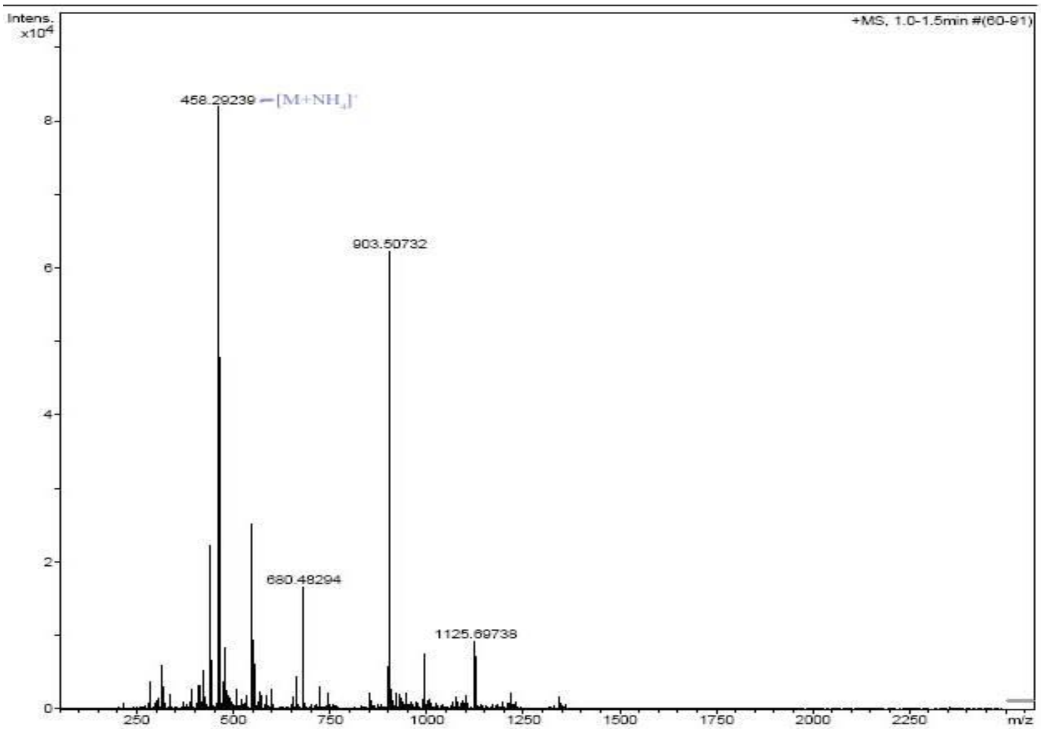
Spectrum 38. Mass Spectrum of **A18**



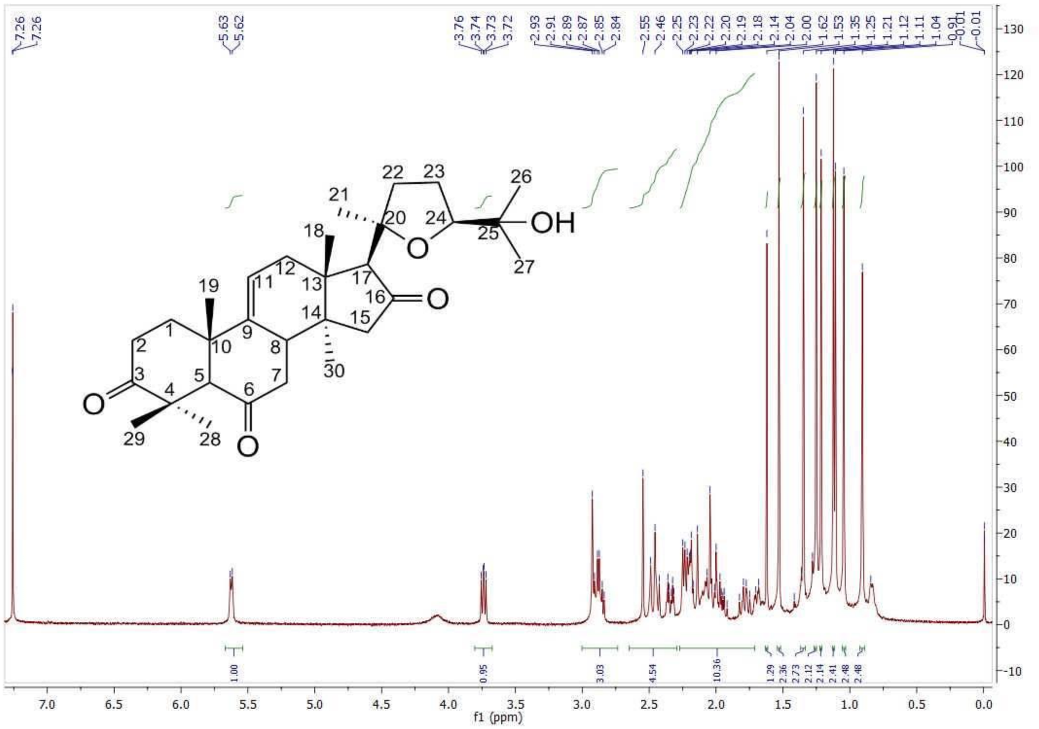
Spectrum 39. ^{1}H-NMR Spectrum of **A4**



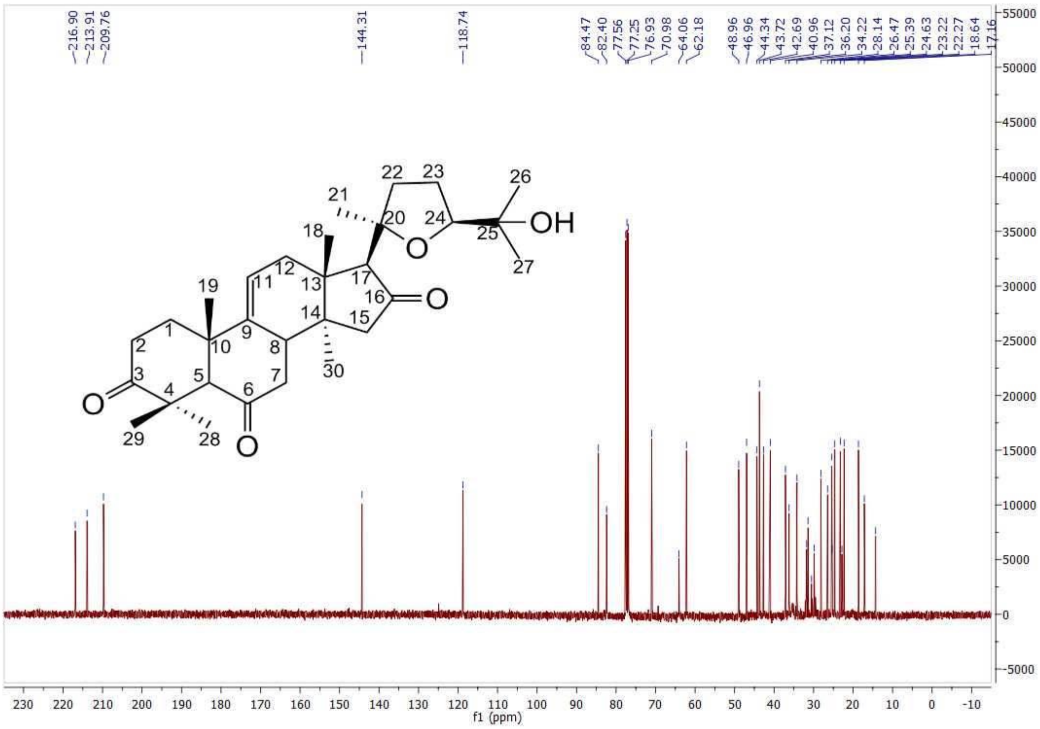
Spectrum 40. ^{13}C-NMR Spectrum of **A4**



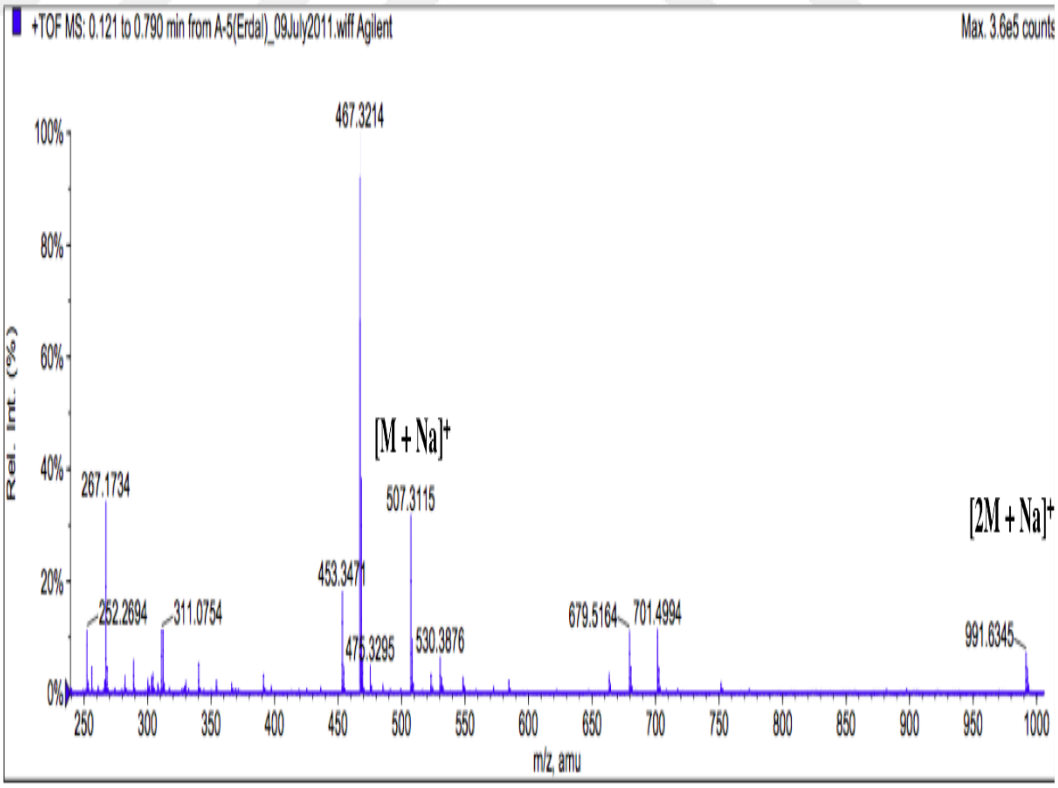
Spectrum 41. Mass Spectrum of **A4**



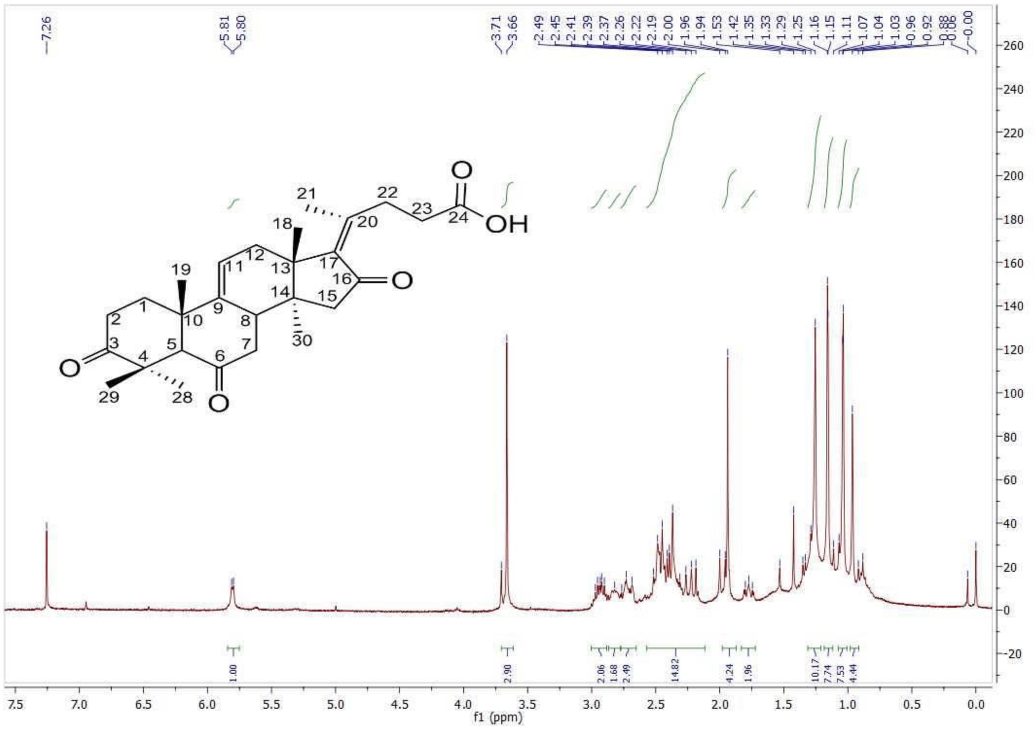
Spectrum 42. ¹H-NMR Spectrum of **A5**



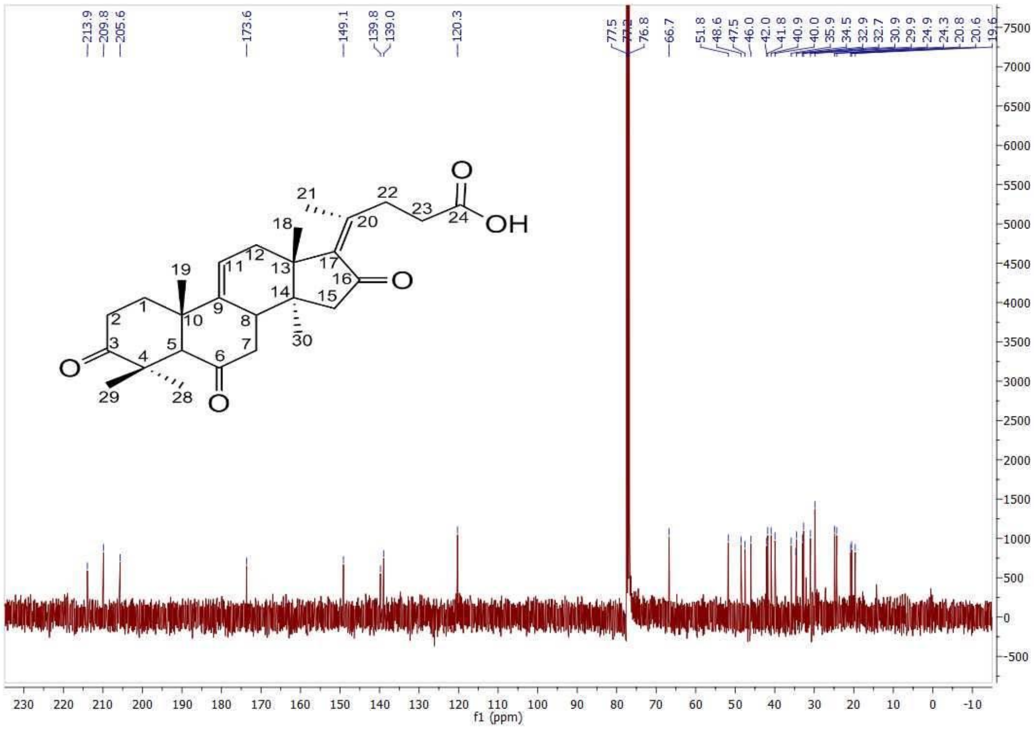
Spectrum 43. ^{13}C-NMR Spectrum of **A5**



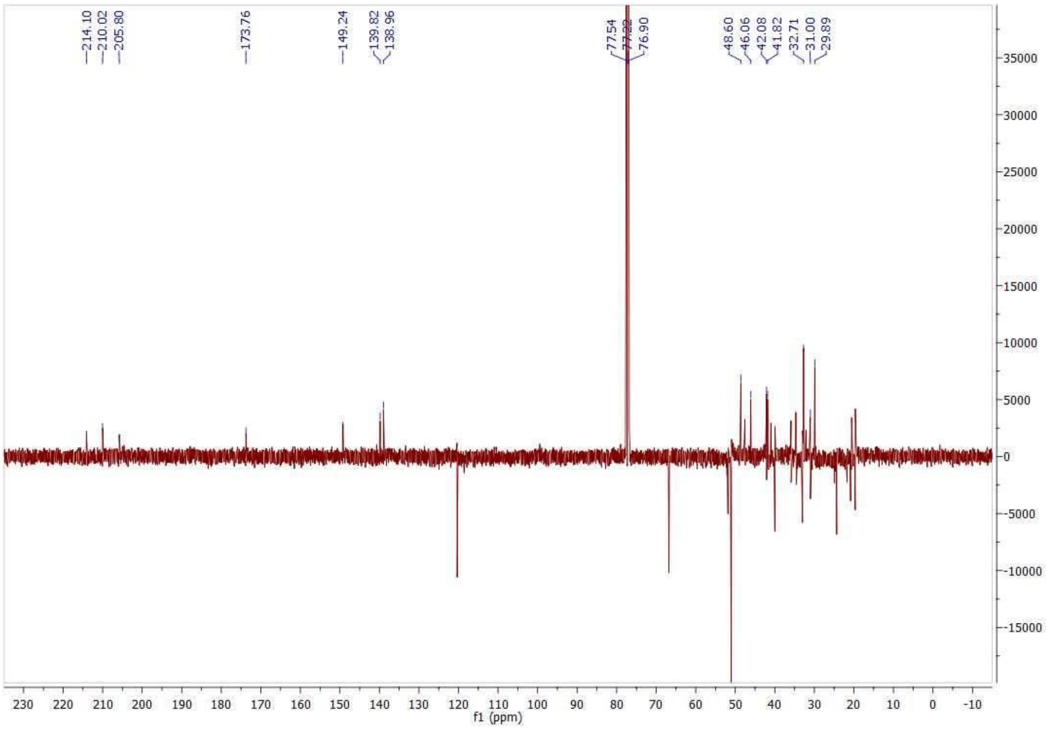
Spectrum 44. Mass Spectrum of **A5**



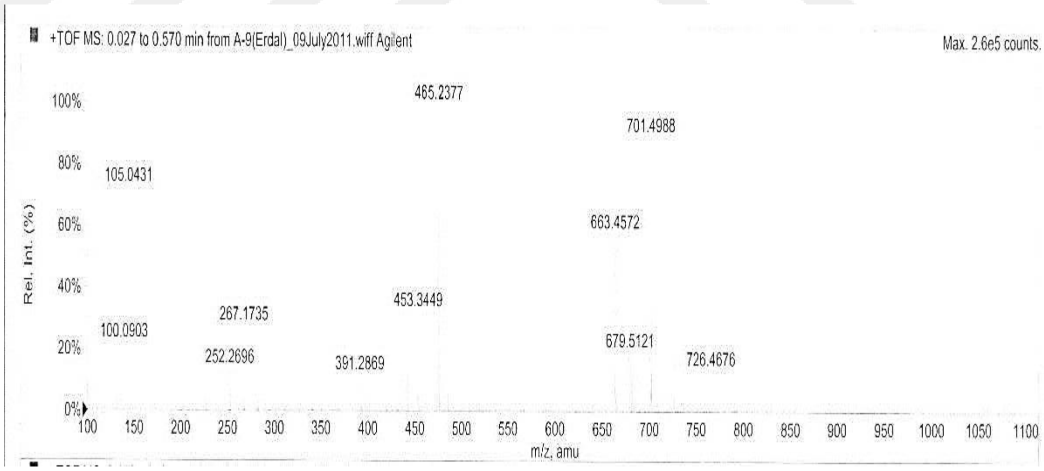
Spectrum 45. ^{1}H-NMR Spectrum of **A9**



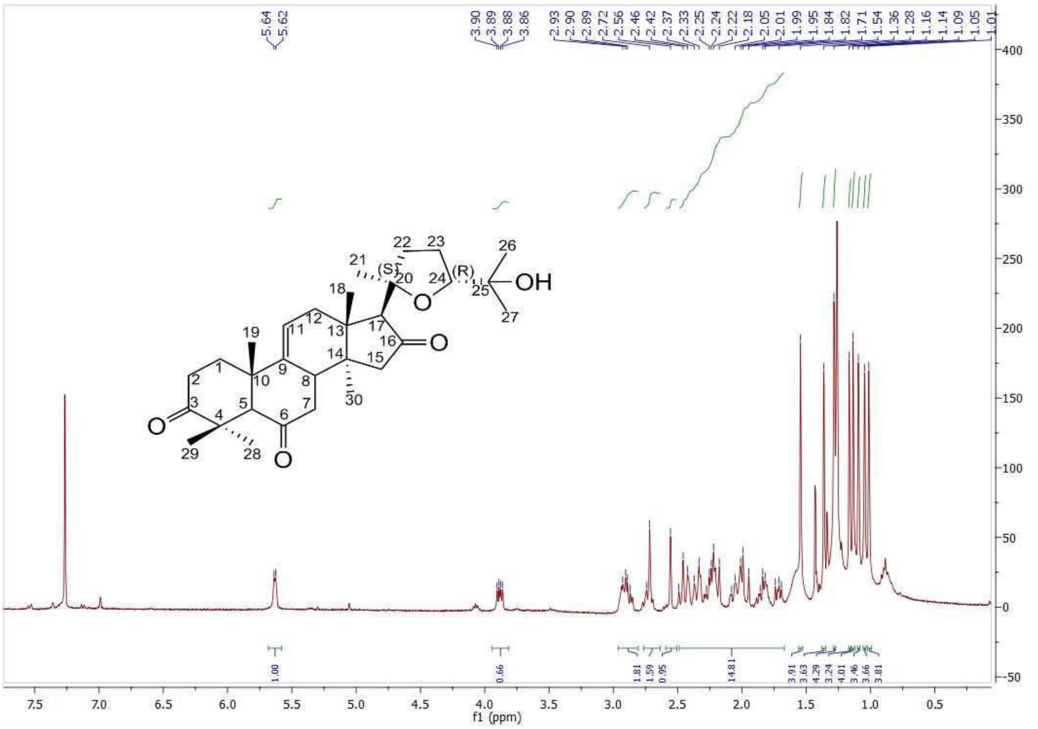
Spectrum 46. ^{13}C-NMR Spectrum of **A9**



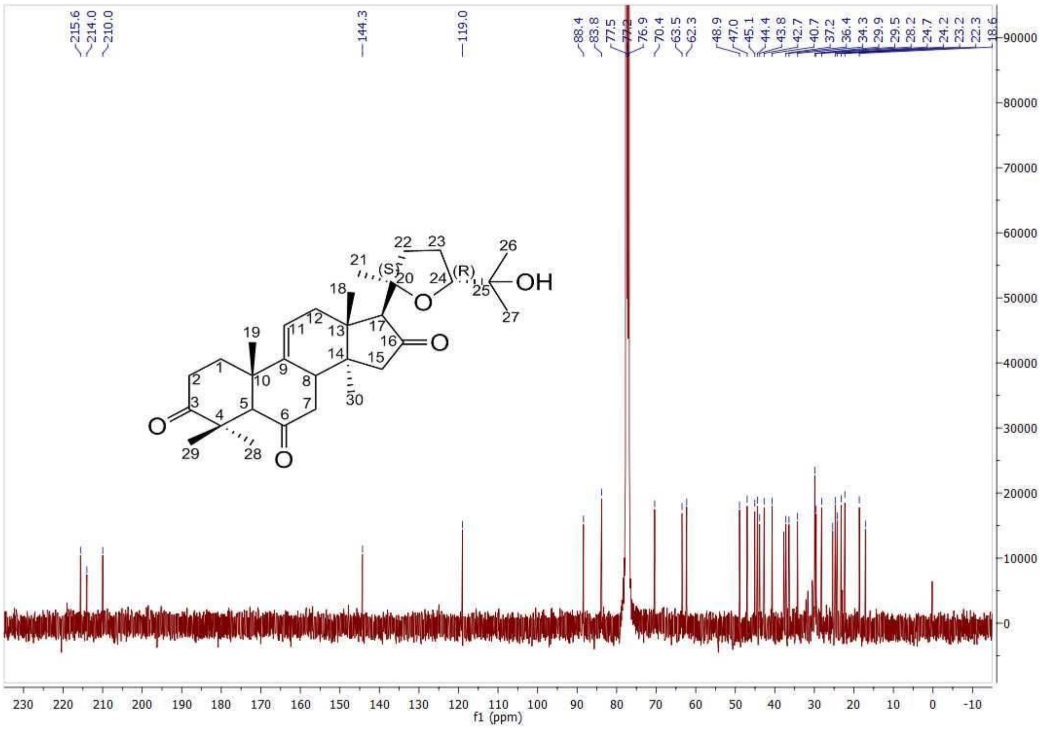
Spectrum 47. APT-NMR Spectrum of **A9**



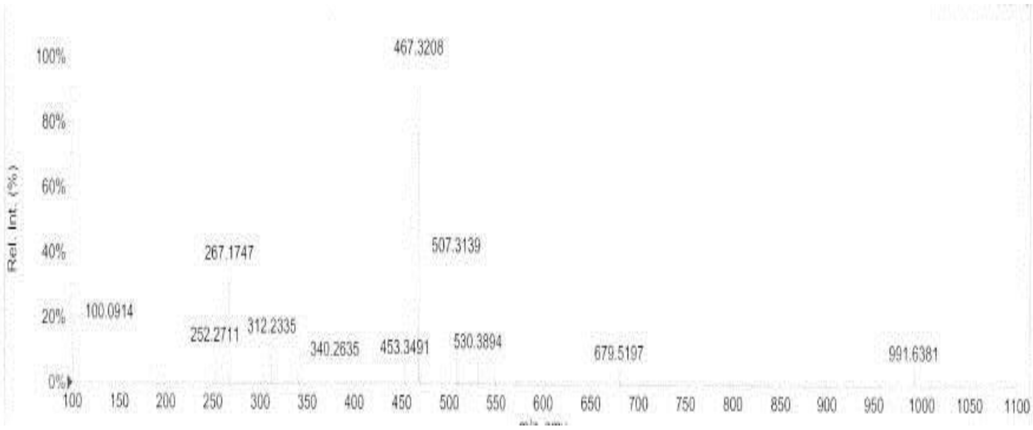
Spectrum 48. Mass Spectrum of **A9**



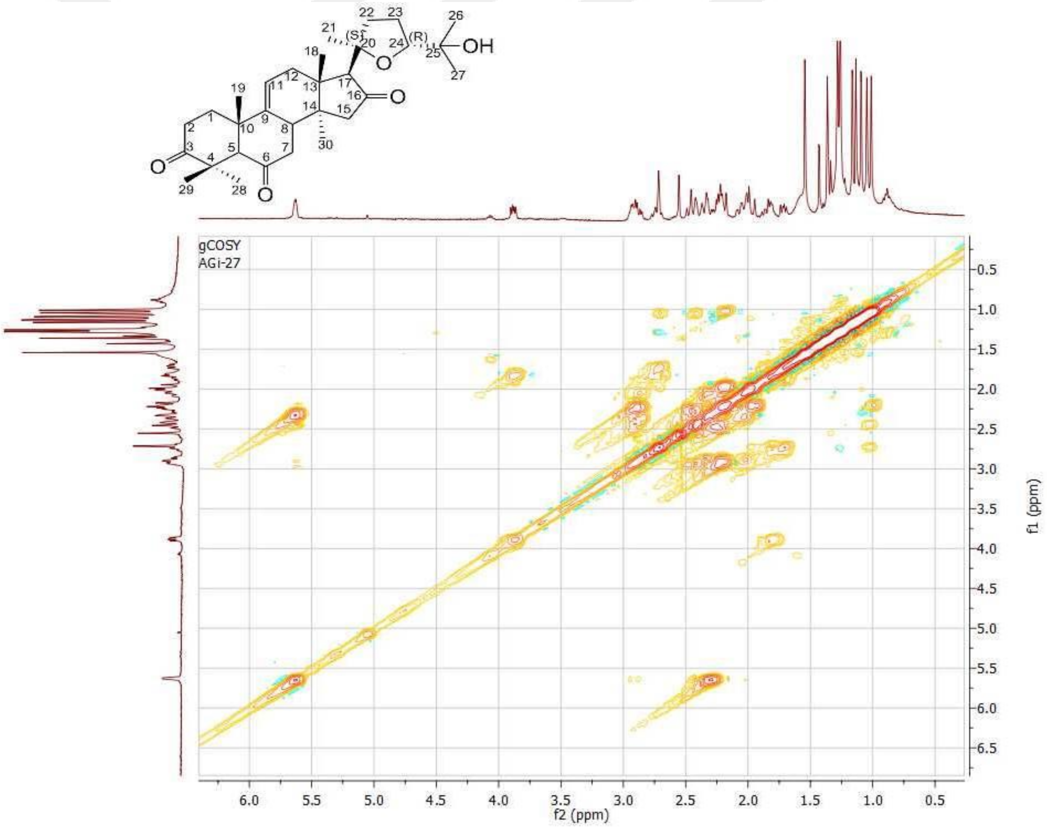
Spectrum 49. ^{1}H-NMR Spectrum of **A27**



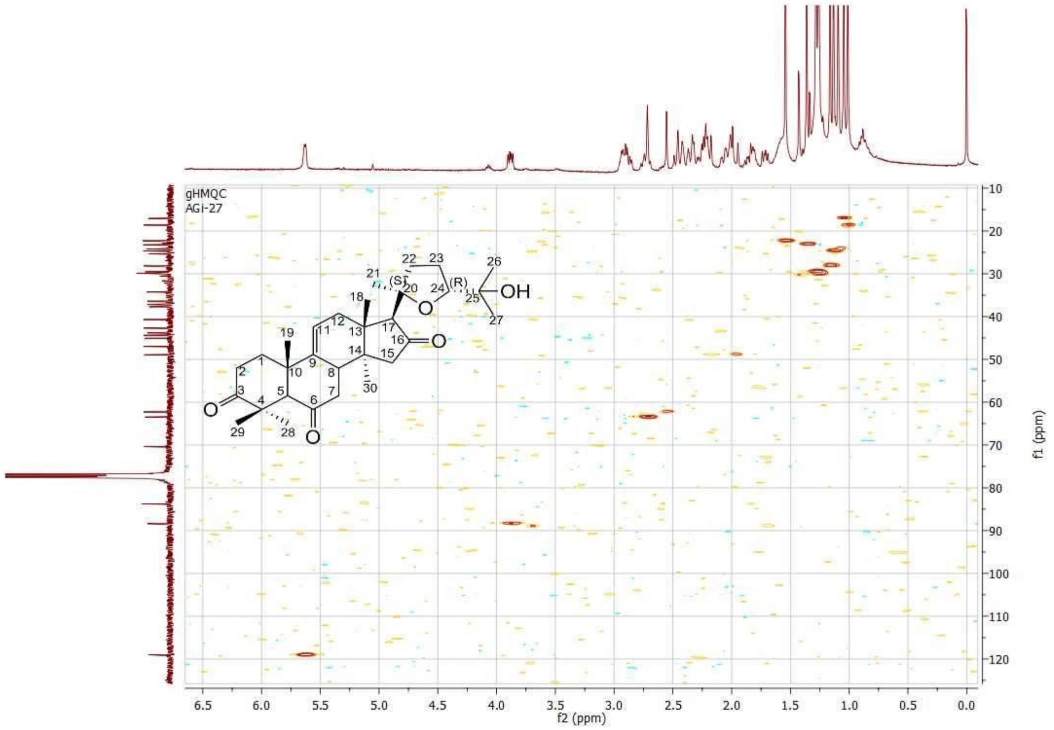
Spectrum 50. ^{13}C-NMR Spectrum of **A27**



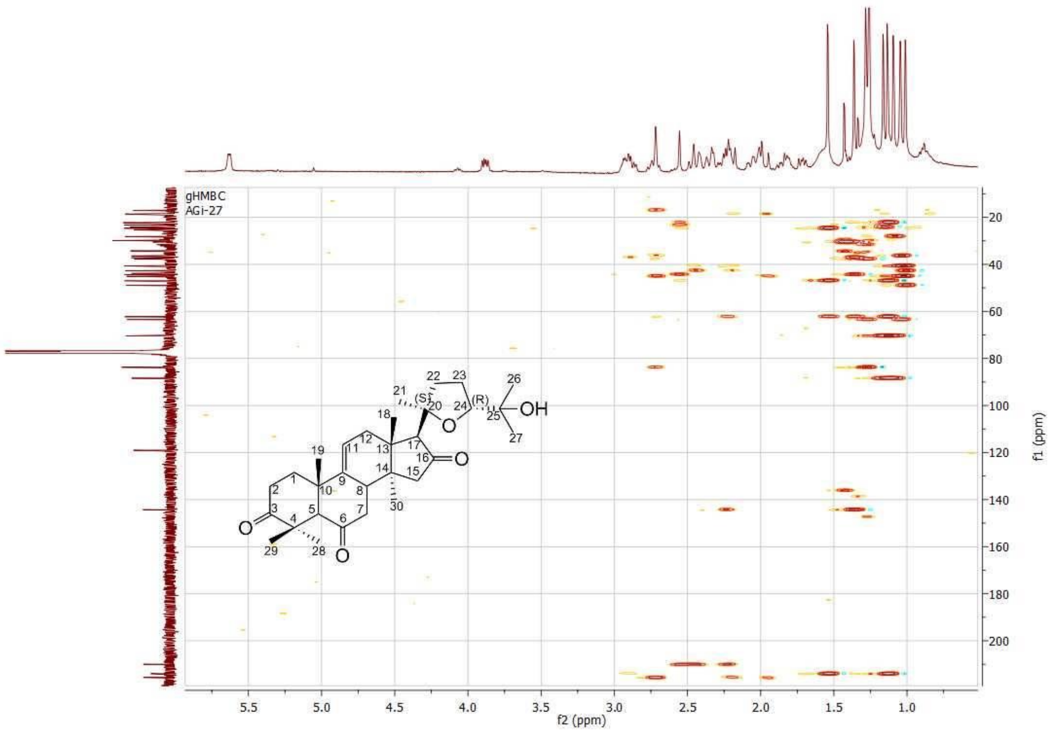
Spectrum 51. Mass Spectrum of **A27**



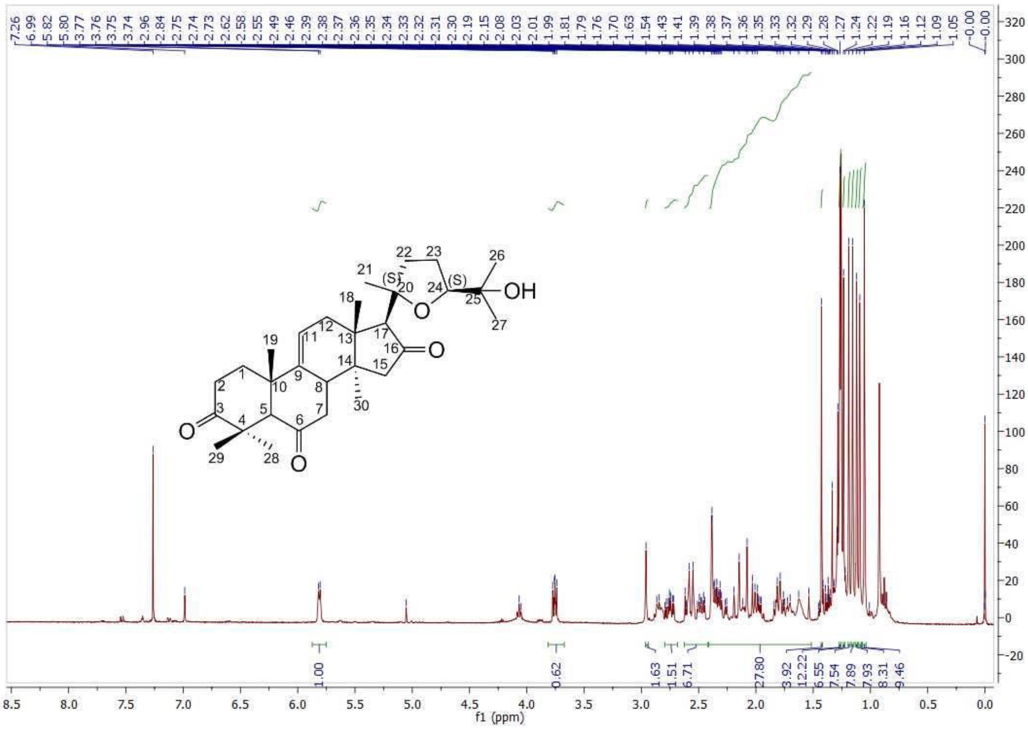
Spectrum 52. COSY Spectrum of **A27**



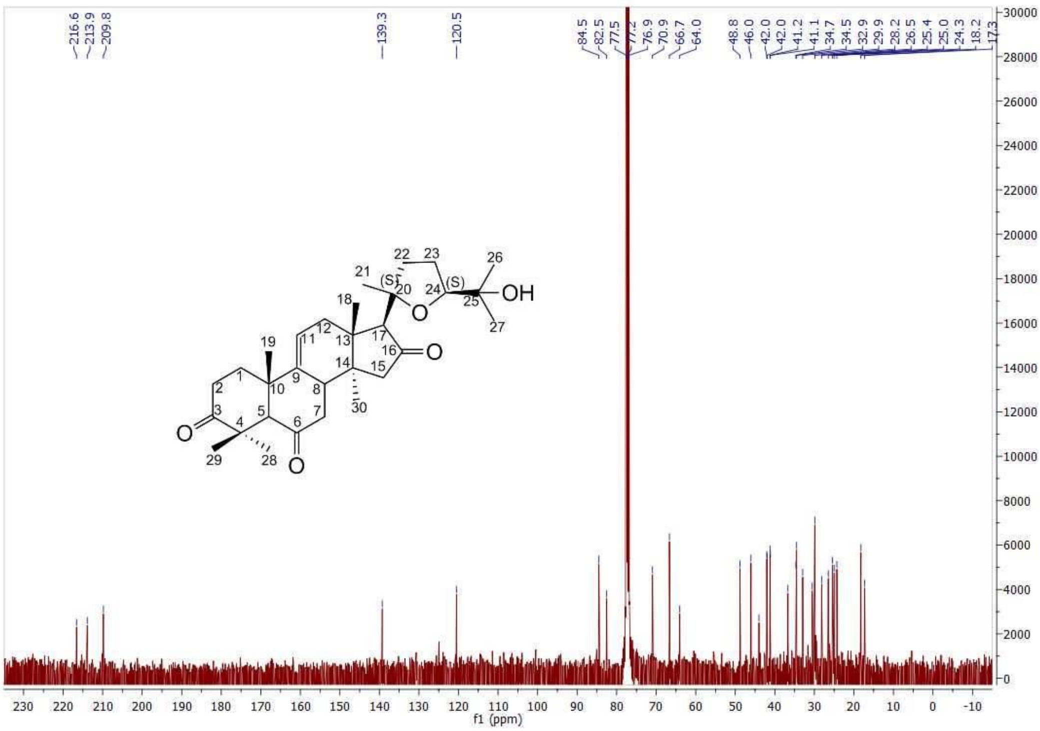
Spectrum 53. HMQC Spectrum of **A27**



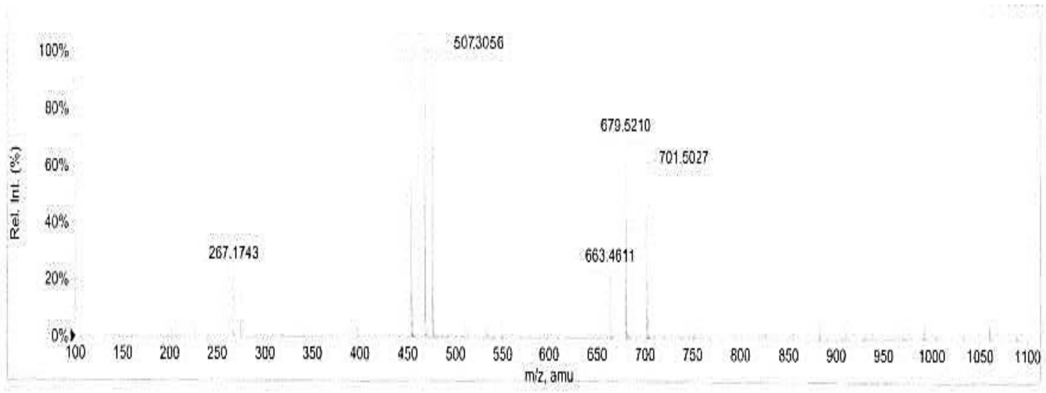
Spectrum 54. HMBC Spectrum of **A27**



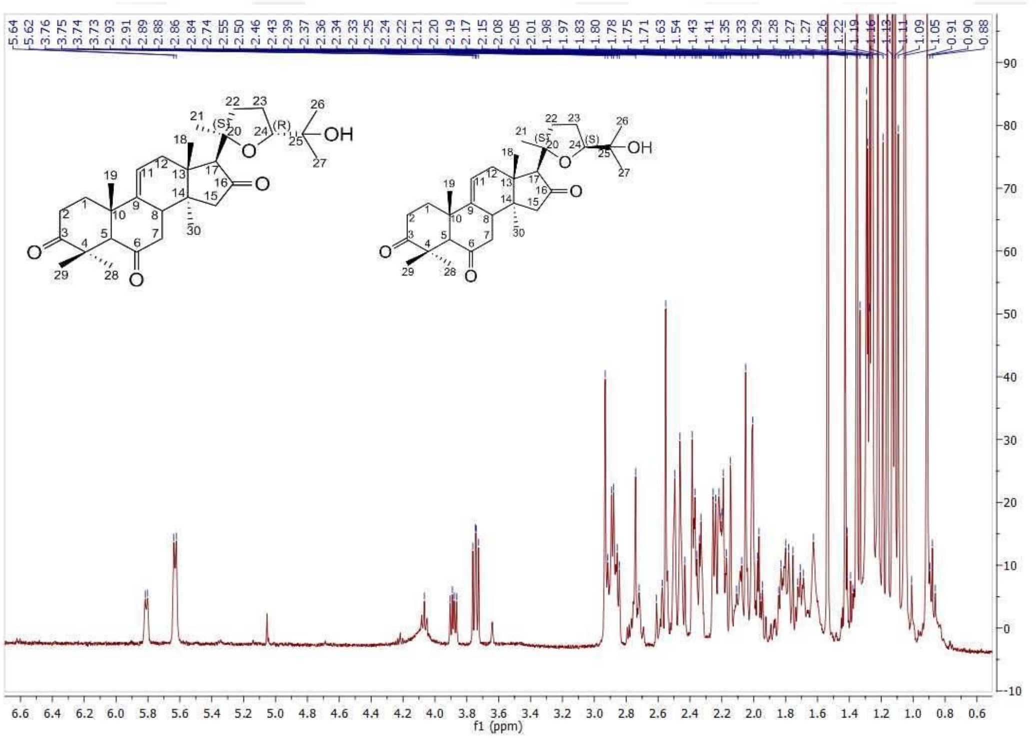
Spectrum 55. [1]H-NMR Spectrum of **A29**



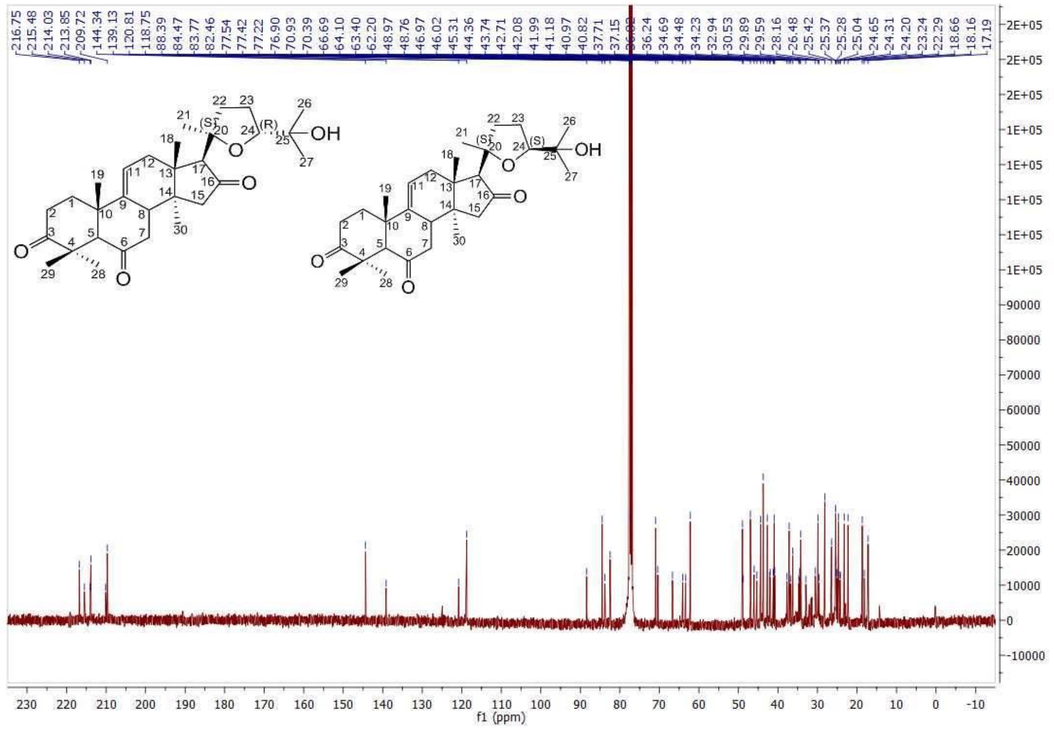
Spectrum 56. [13]C-NMR Spectrum of **A29**



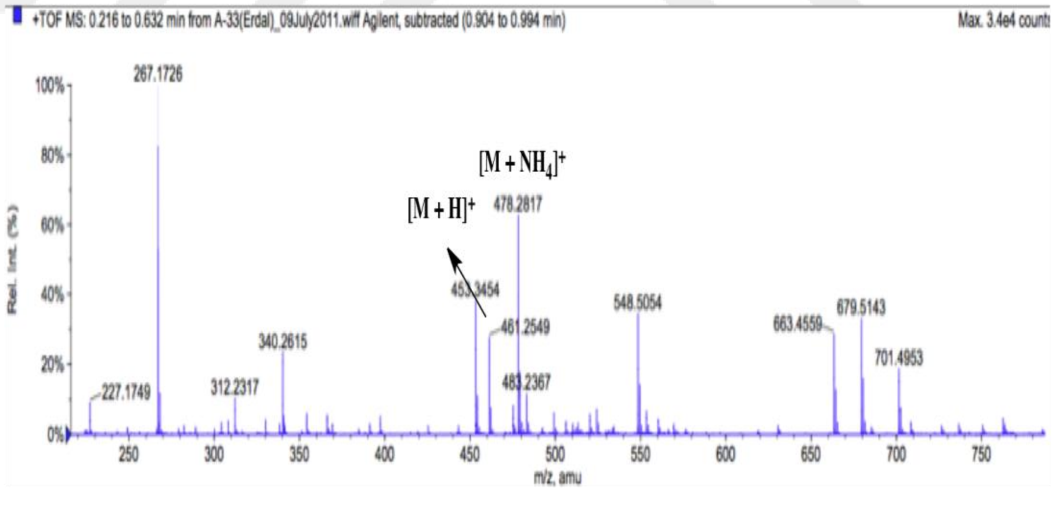
Spectrum 57. Mass Spectrum of **A29**



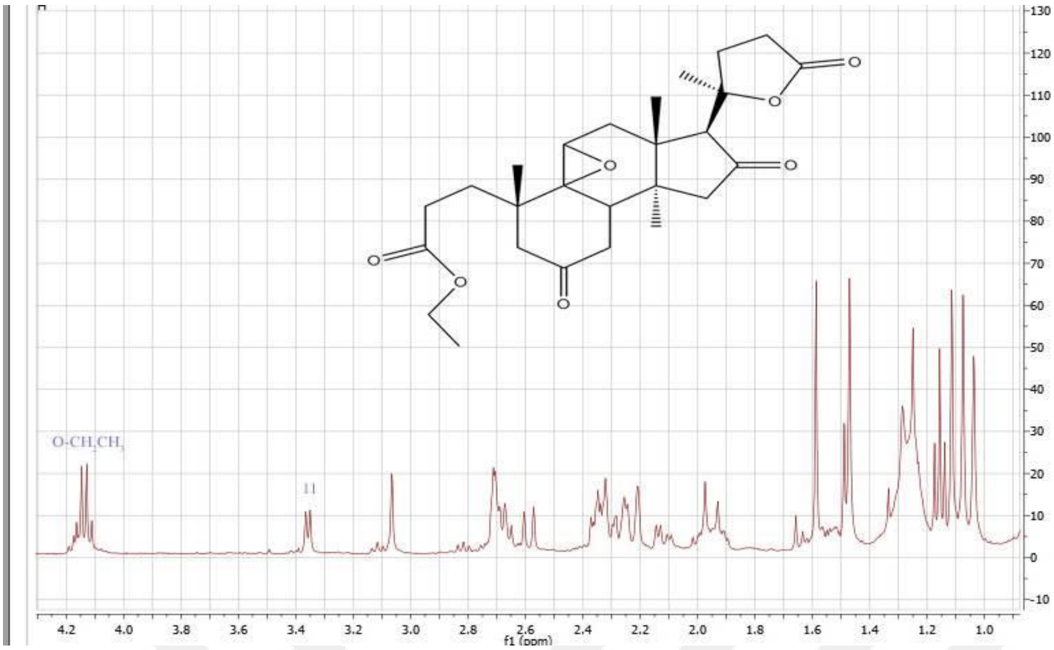
Spectrum 58. ^{1}H-NMR Spectrum of **A27** and **A29**



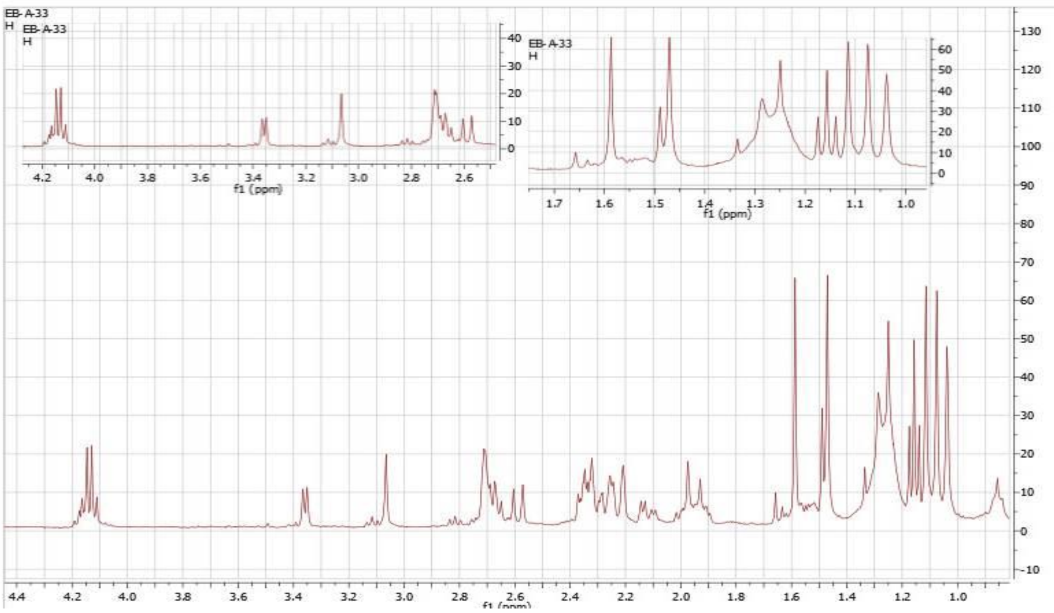
Spectrum 59. ^{13}C-NMR Spectrum of **A27** and **A29**



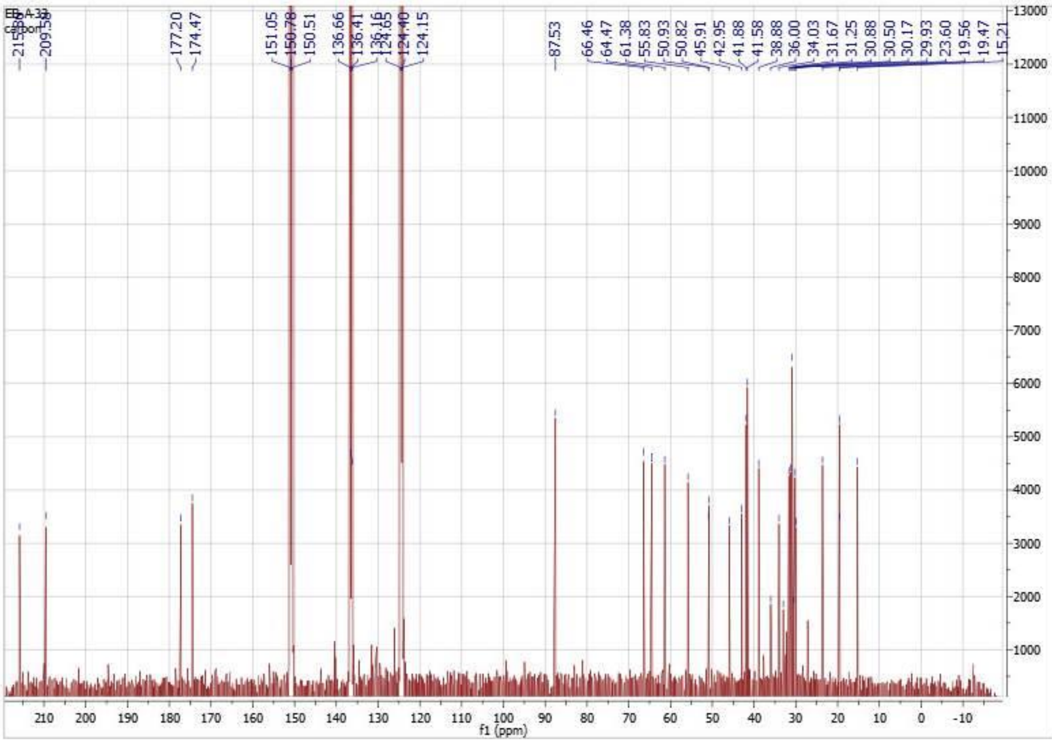
Spectrum 60. Mass Spectrum of **A25**



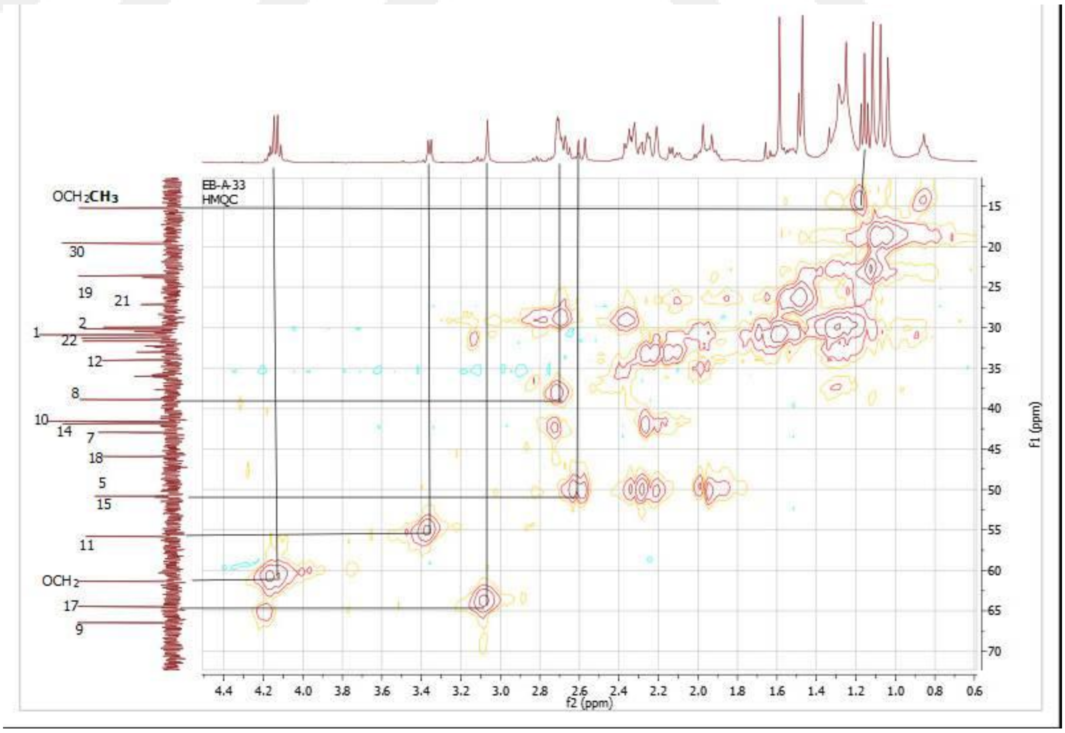
Spectrum 61. ^{1}H-NMR Spectrum of **A25**



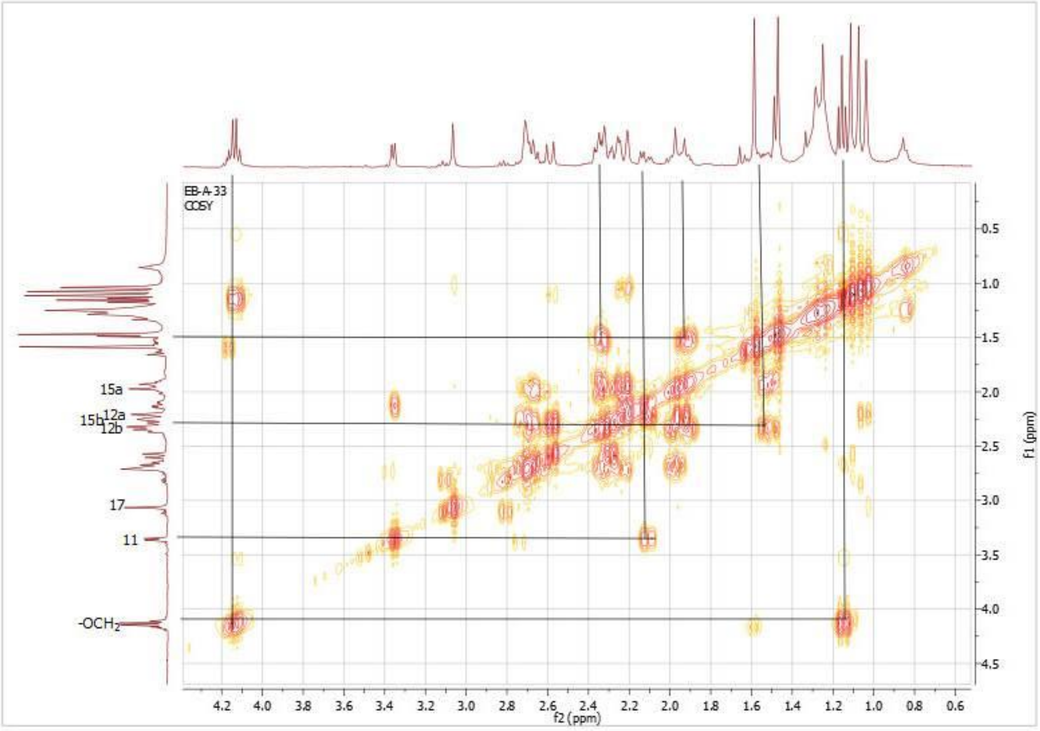
Spectrum 62. ^{1}H-NMR Spectrum of **A25**



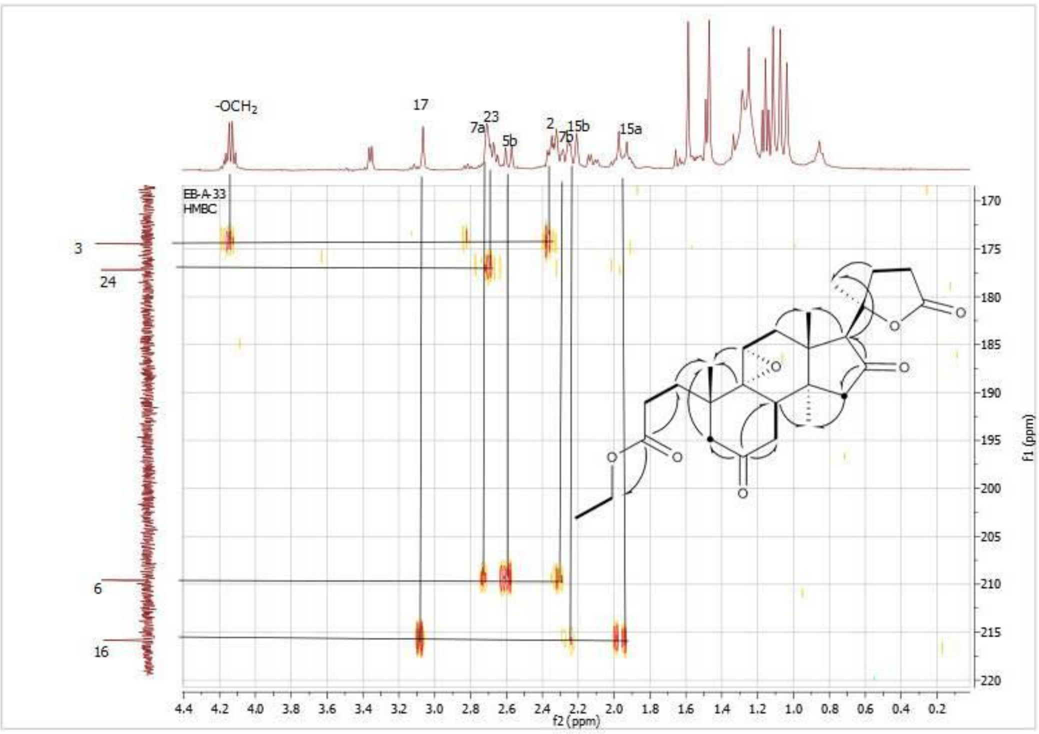
Spectrum 63. ^{13}C-NMR Spectrum of **A25**



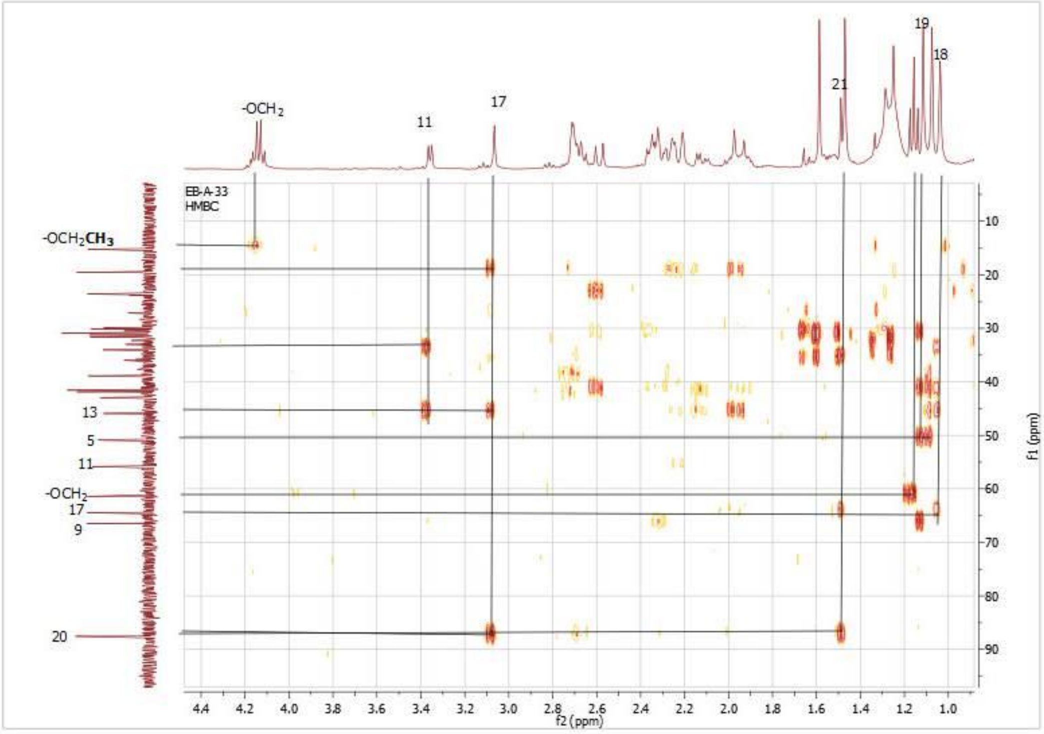
Spectrum 64. HMQC Spectrum of **A25**



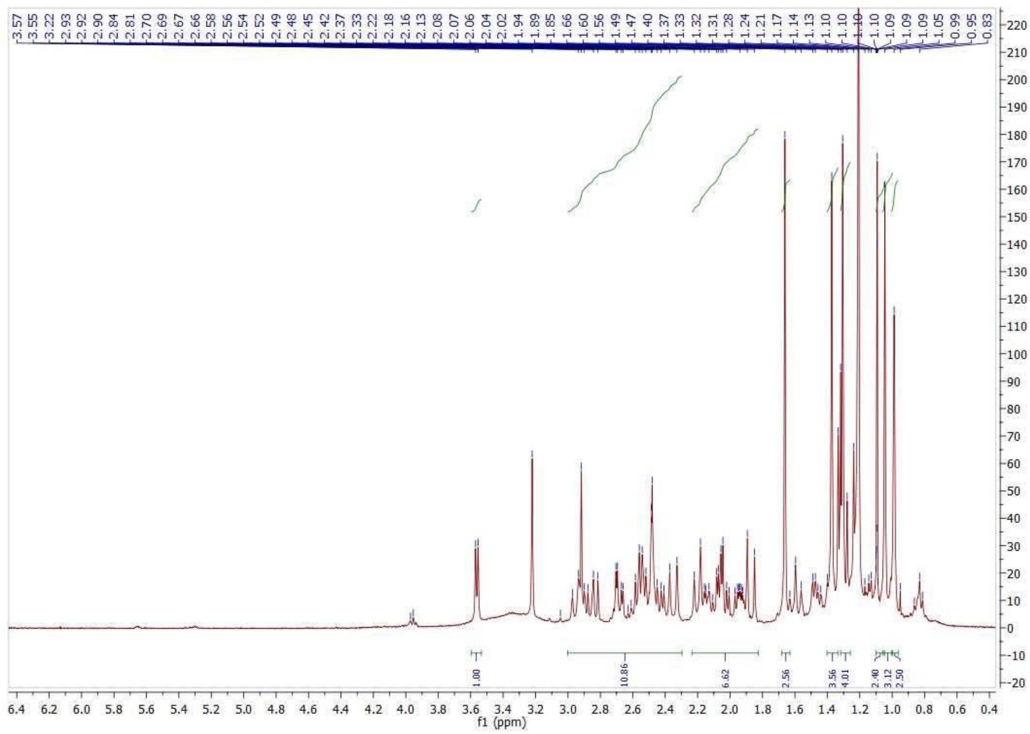
Spectrum 65. COSY Spectrum of **A25**



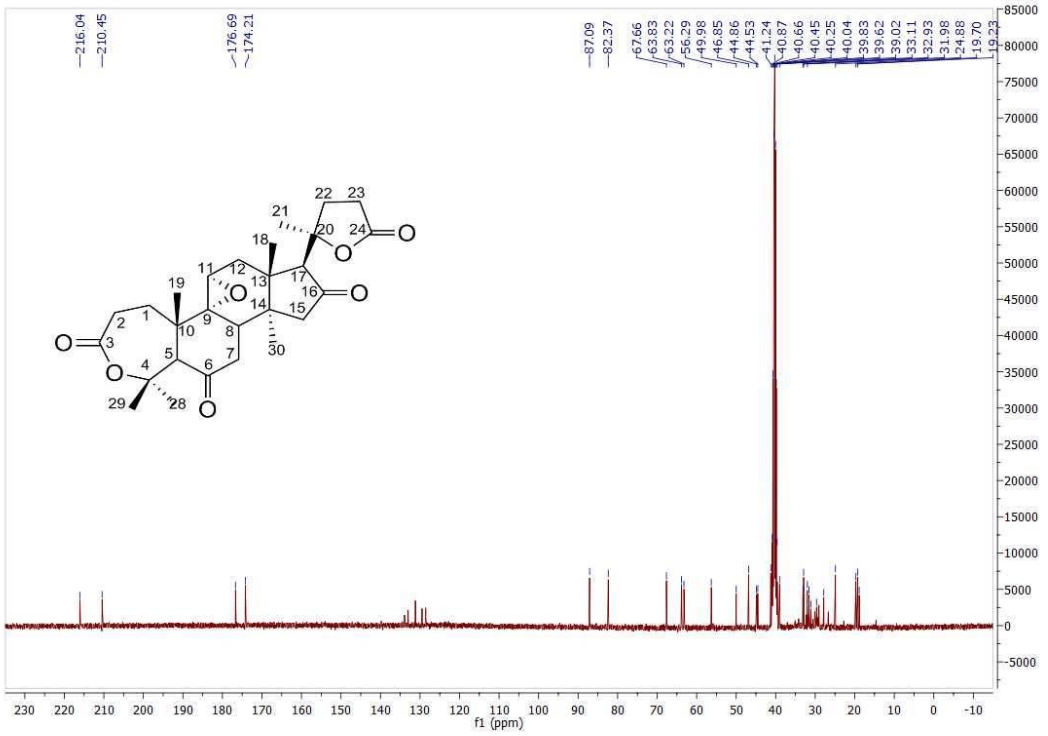
Spectrum 66. HMBC Spectrum of **A25**



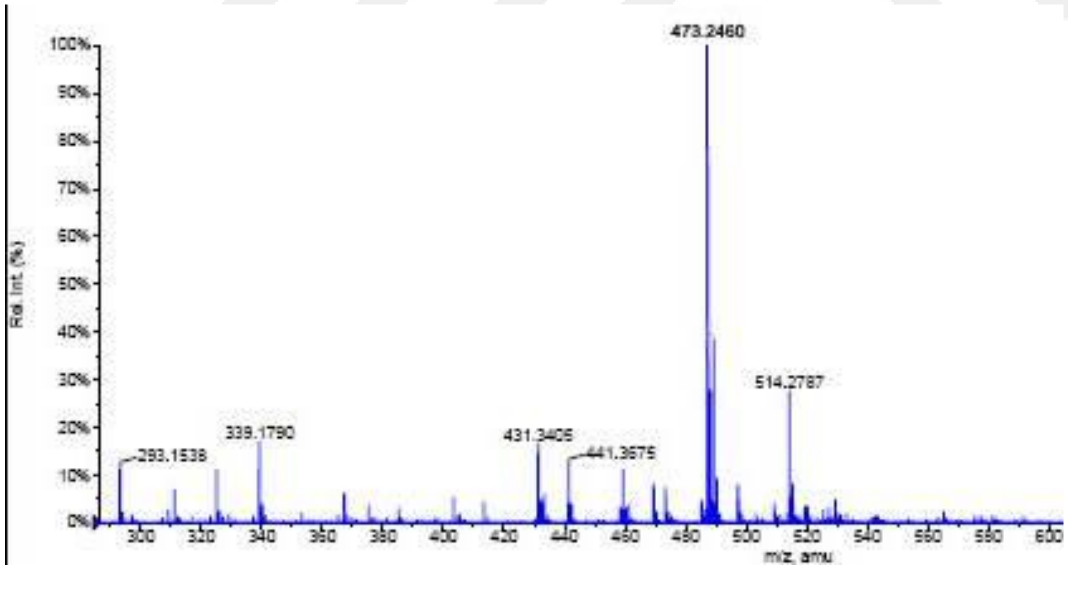
Spectrum 67. HMBC Spectrum of **A25**



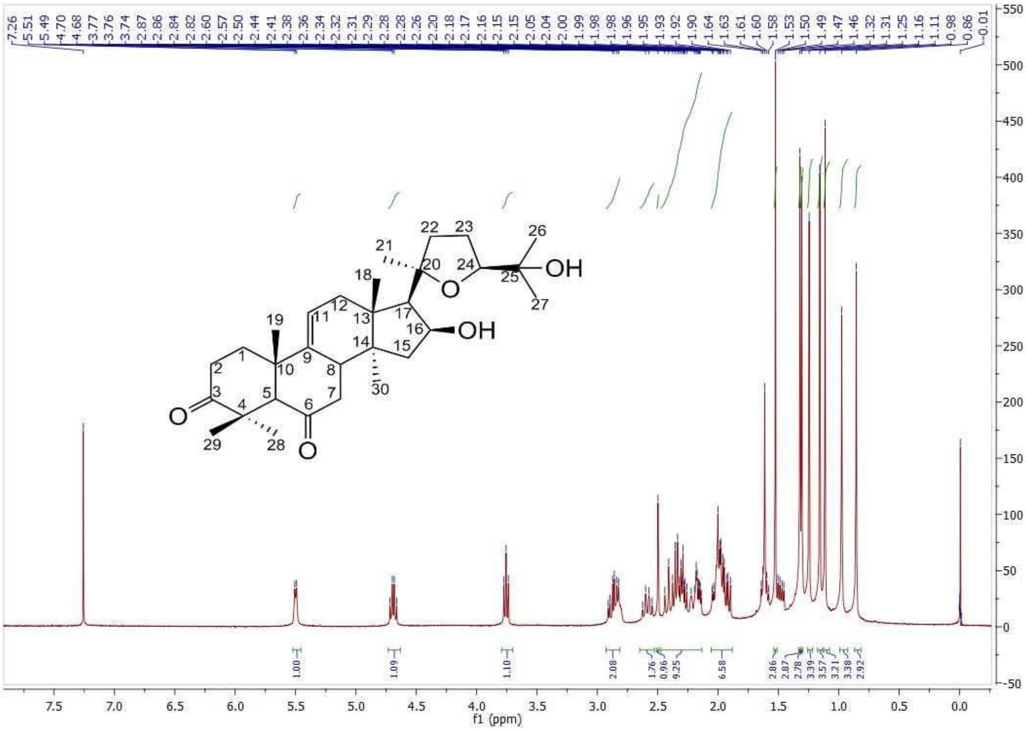
Spectrum 68. ^{1}H-NMR Spectrum of **A30**



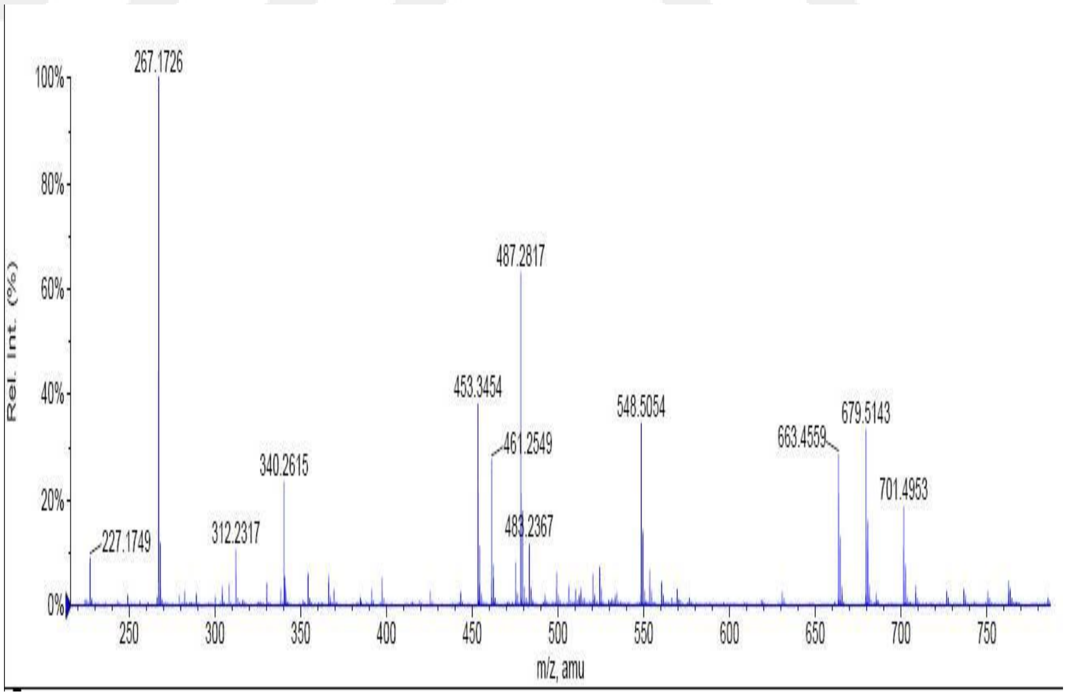
Spectrum 69. ^{13}C-NMR Spectrum of **A30**



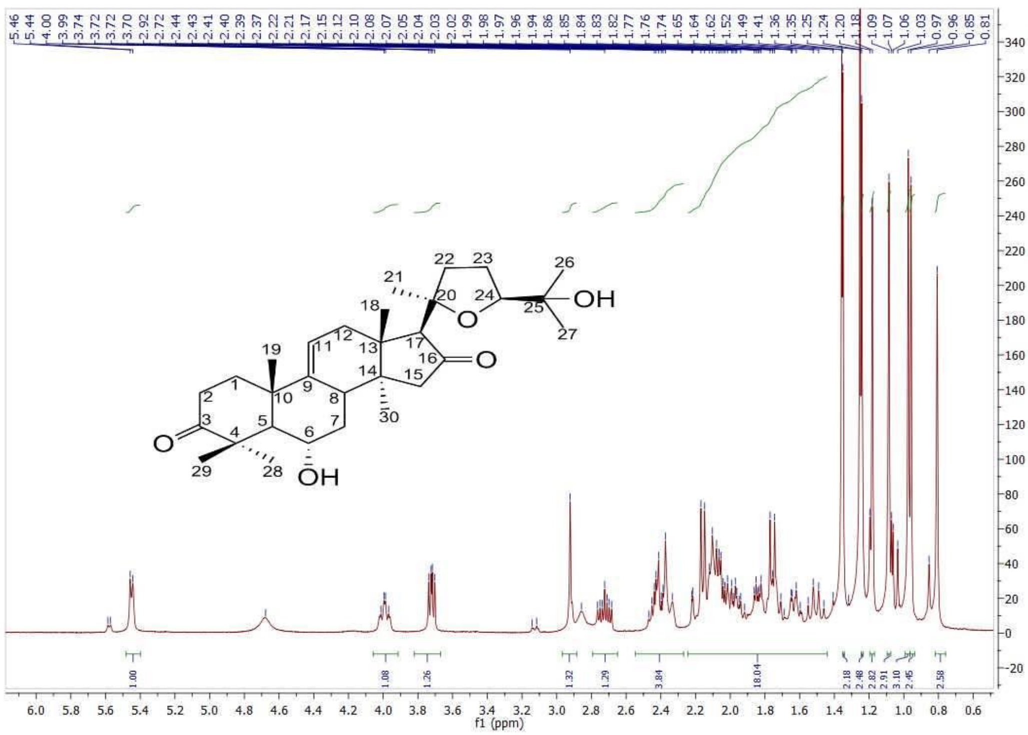
Spectrum 70. Mass Spectrum of **A30**



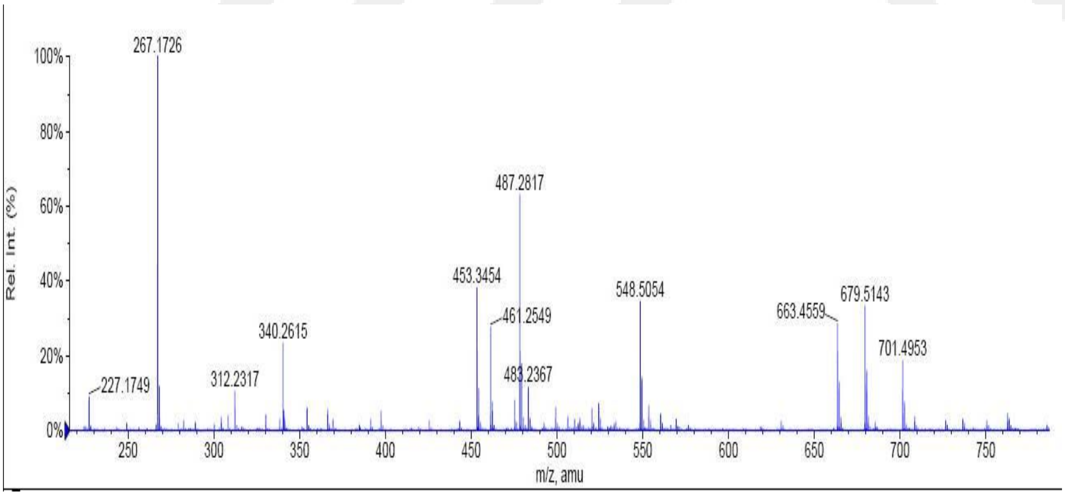
Spectrum 71. ^{1}H-NMR Spectrum of **A34**



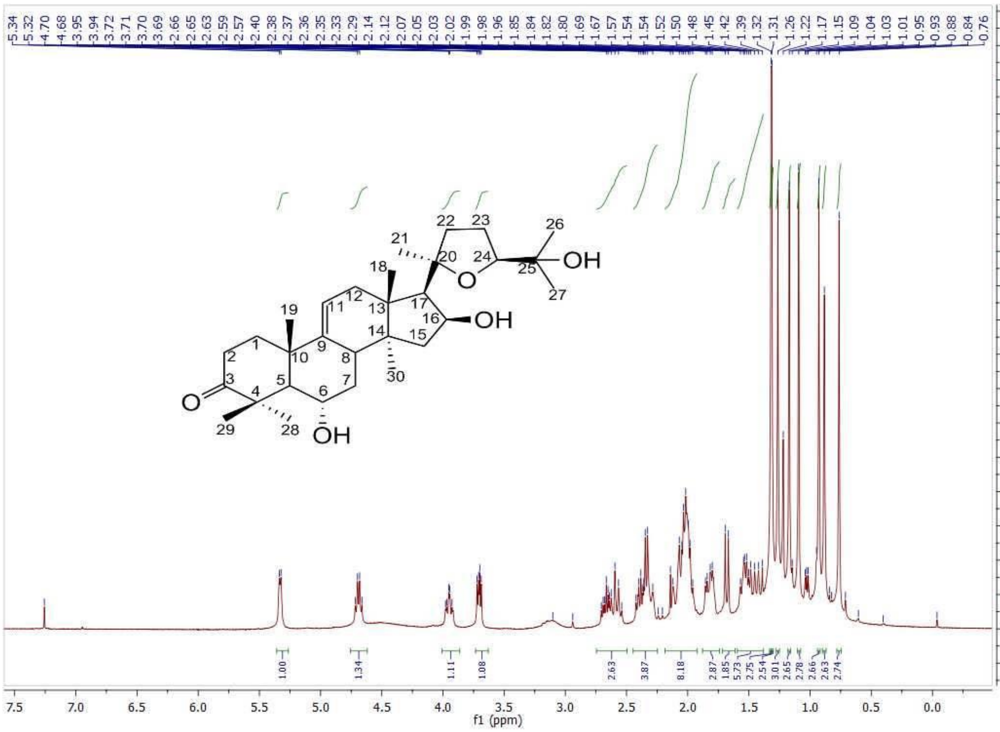
Spectrum 72. Mass Spectrum of **A34**



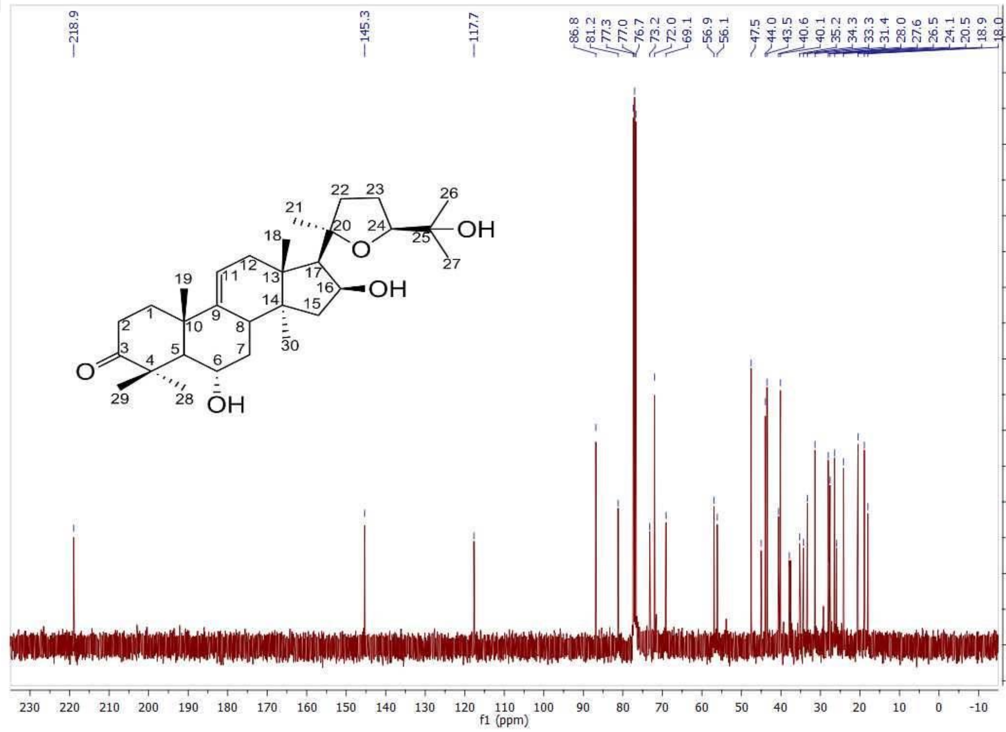
Spectrum 73. ^{1}H-NMR Spectrum of **A35**



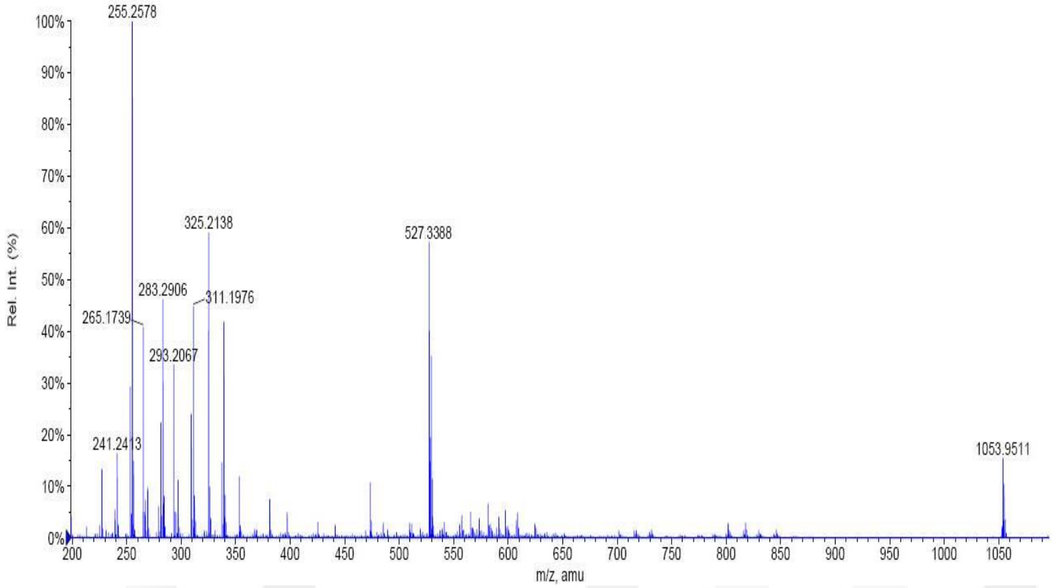
Spectrum 74. Mass Spectrum of **A35**



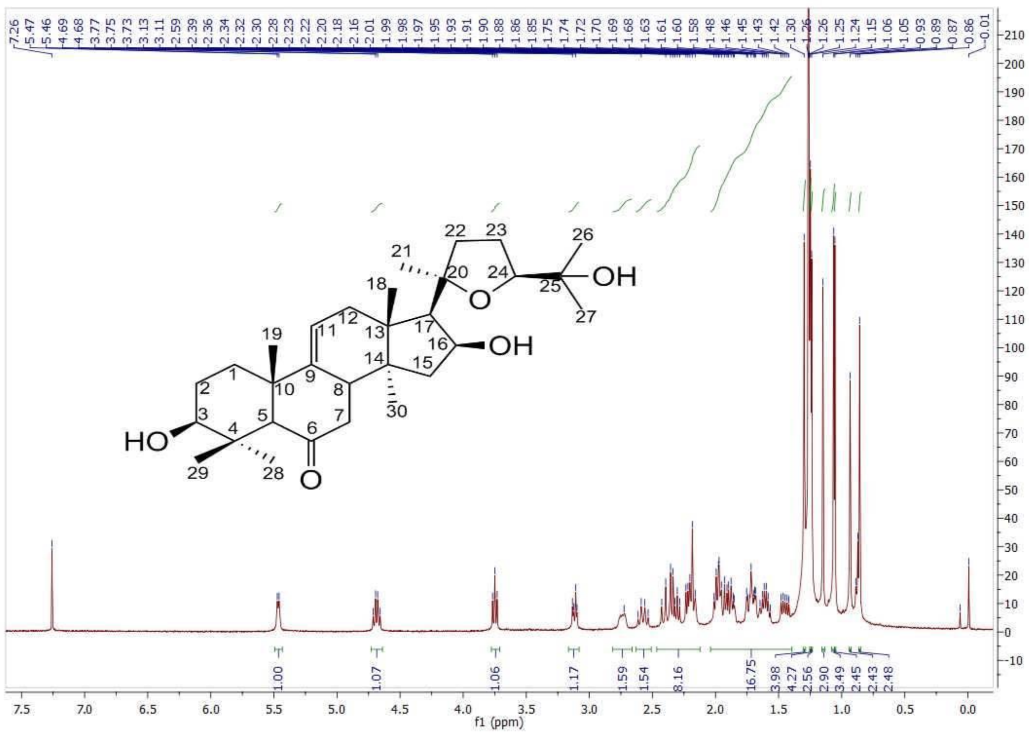
Spectrum 75. ^{1}H-NMR Spectrum of **A38**



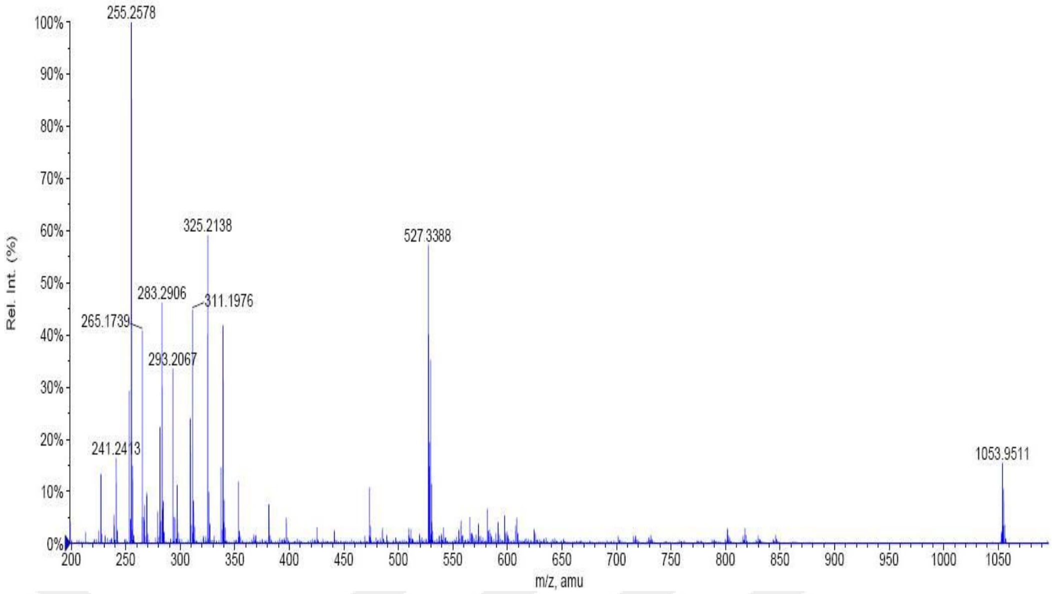
Spectrum 76. ^{13}C-NMR Spectrum of **A38**



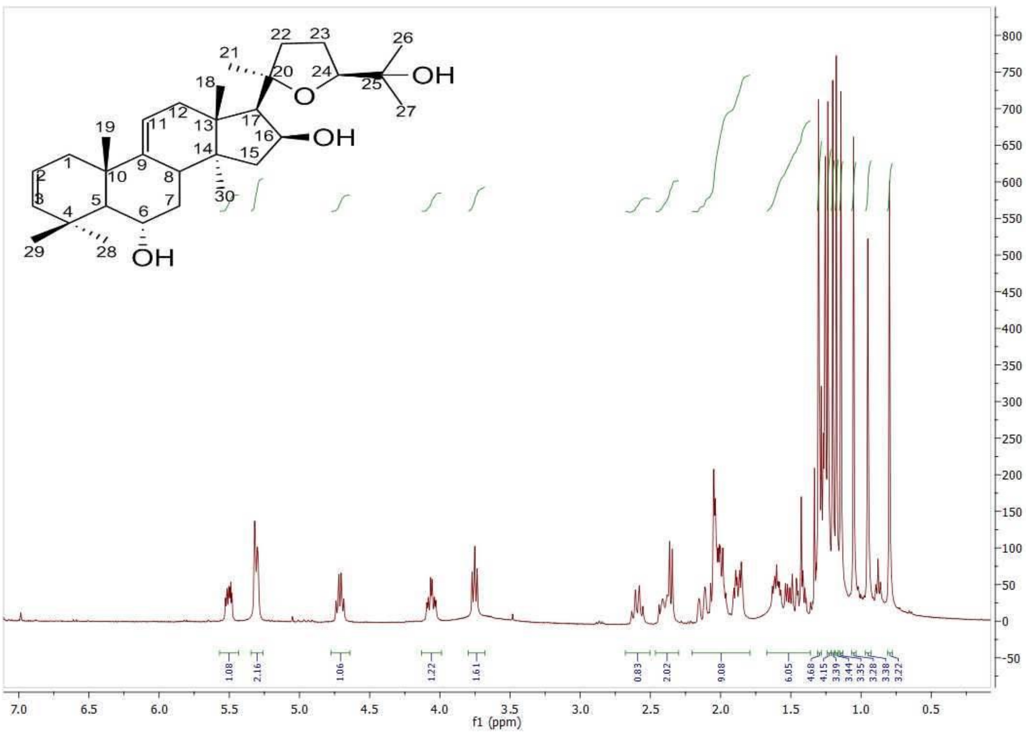
Spectrum 77. Mass Spectrum of **A38**



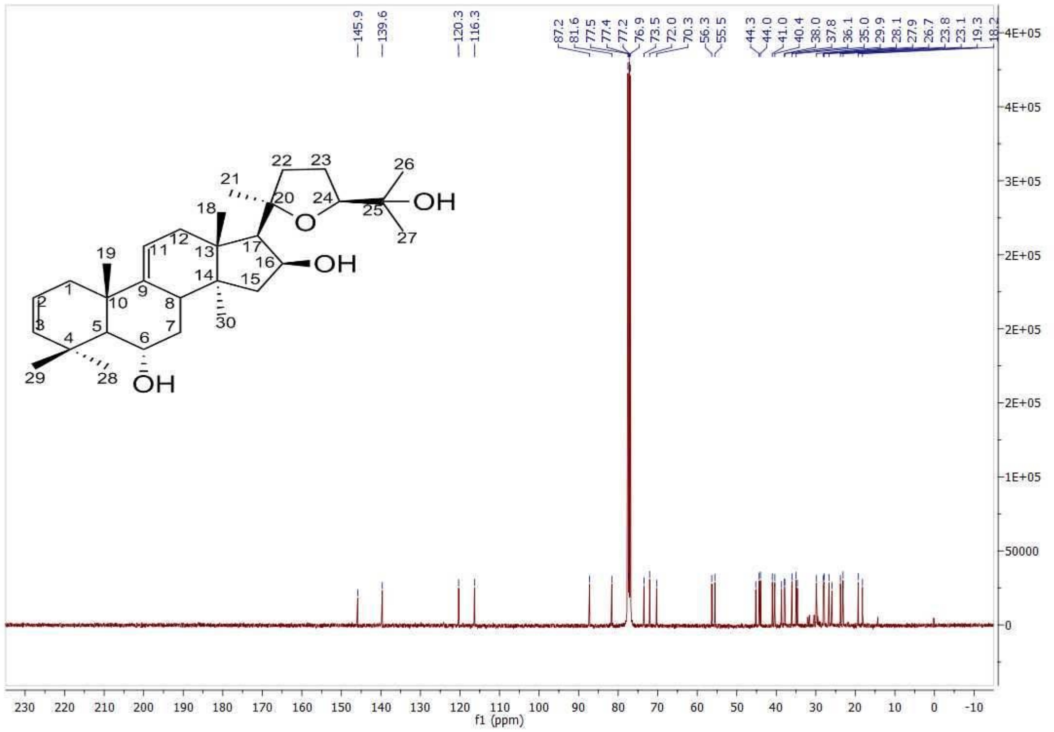
Spectrum 78. ^{1}H-NMR Spectrum of **A36**



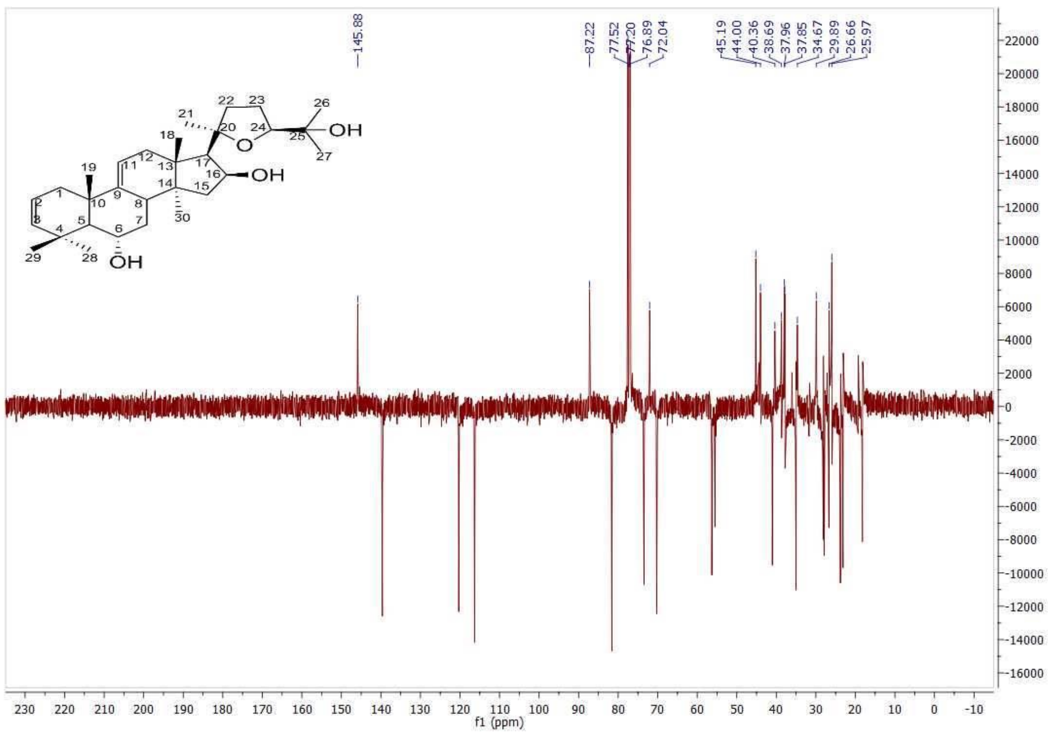
Spectrum 79. Mass Spectrum of **A36**



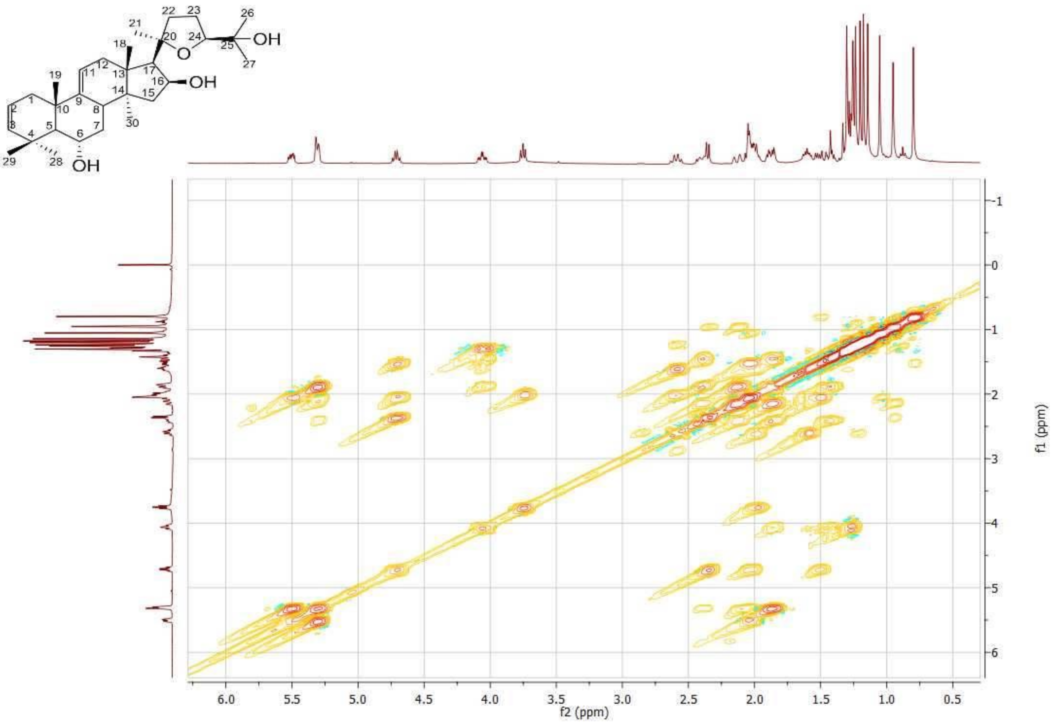
Spectrum 80. ^{1}H-NMR Spectrum of **A6**



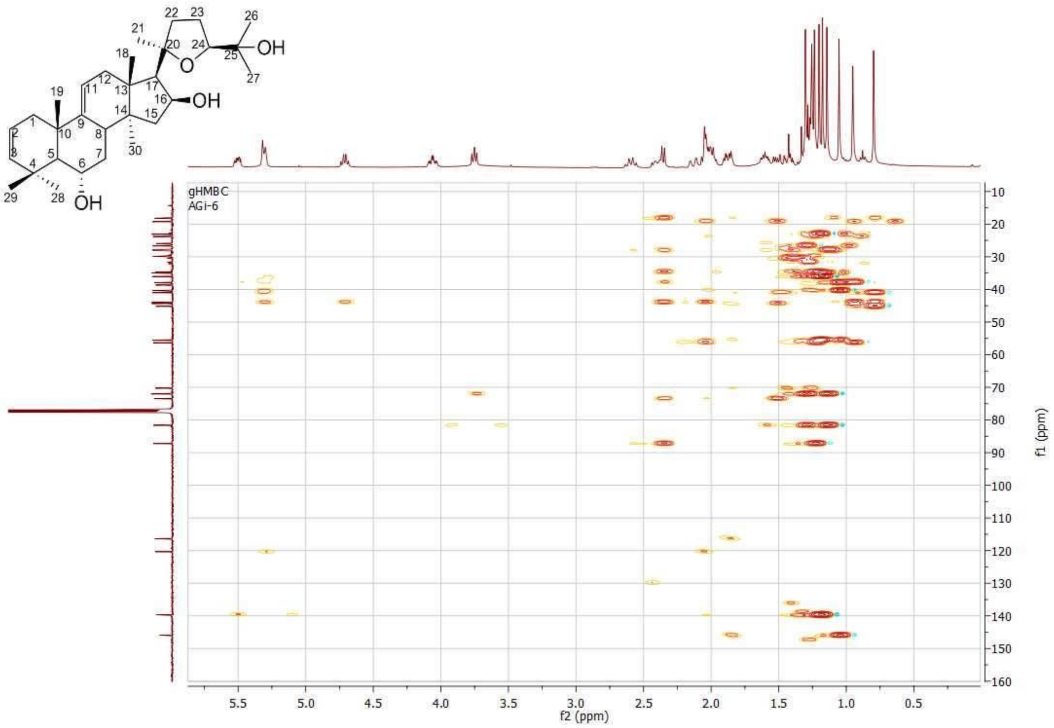
Spectrum 81. ^{13}C-NMR Spectrum of **A6**



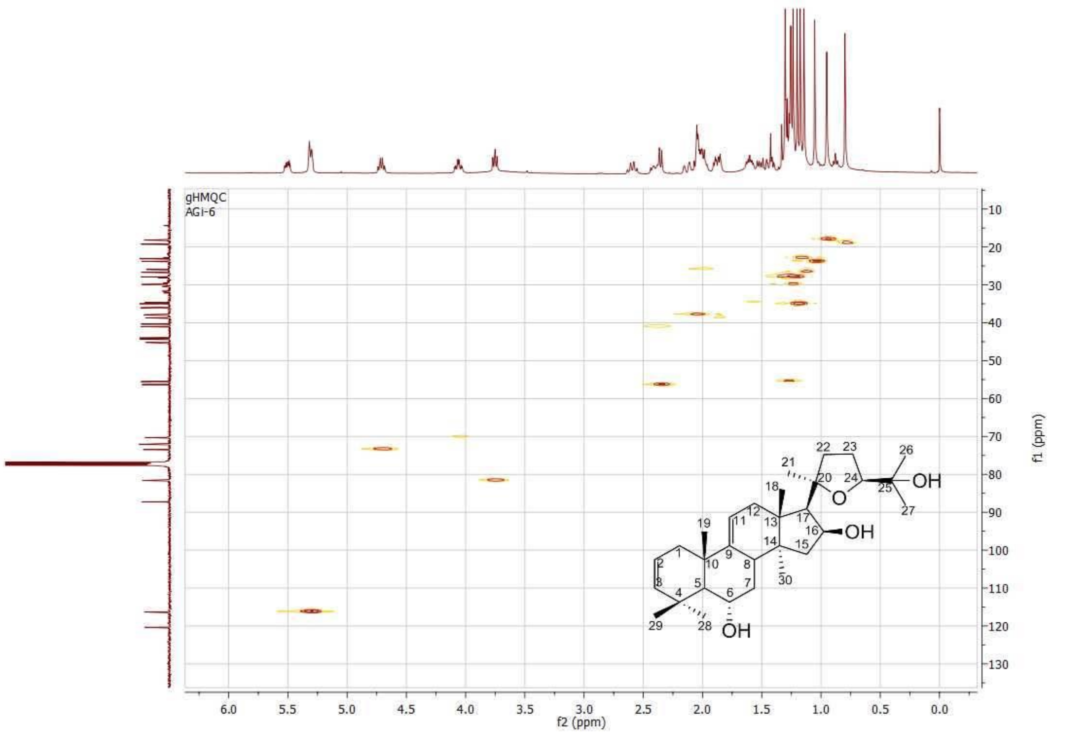
Spectrum 82. APT Spectrum of **A6**



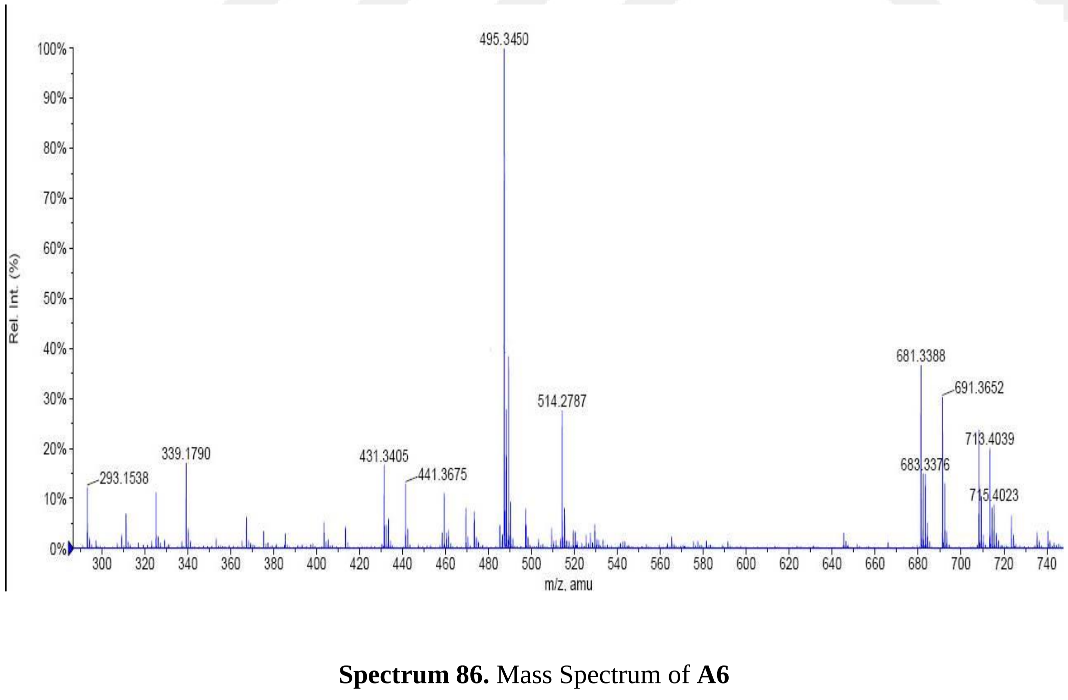
Spectrum 83. COSY Spectrum of **A6**



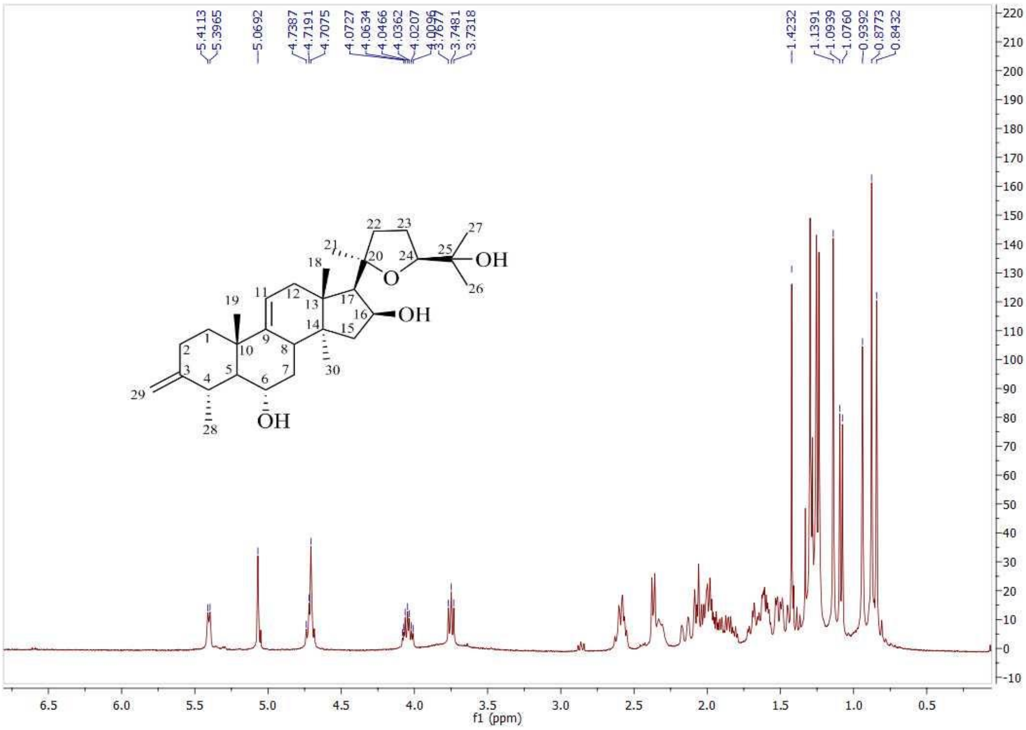
Spectrum 84. HMBC Spectrum of **A6**



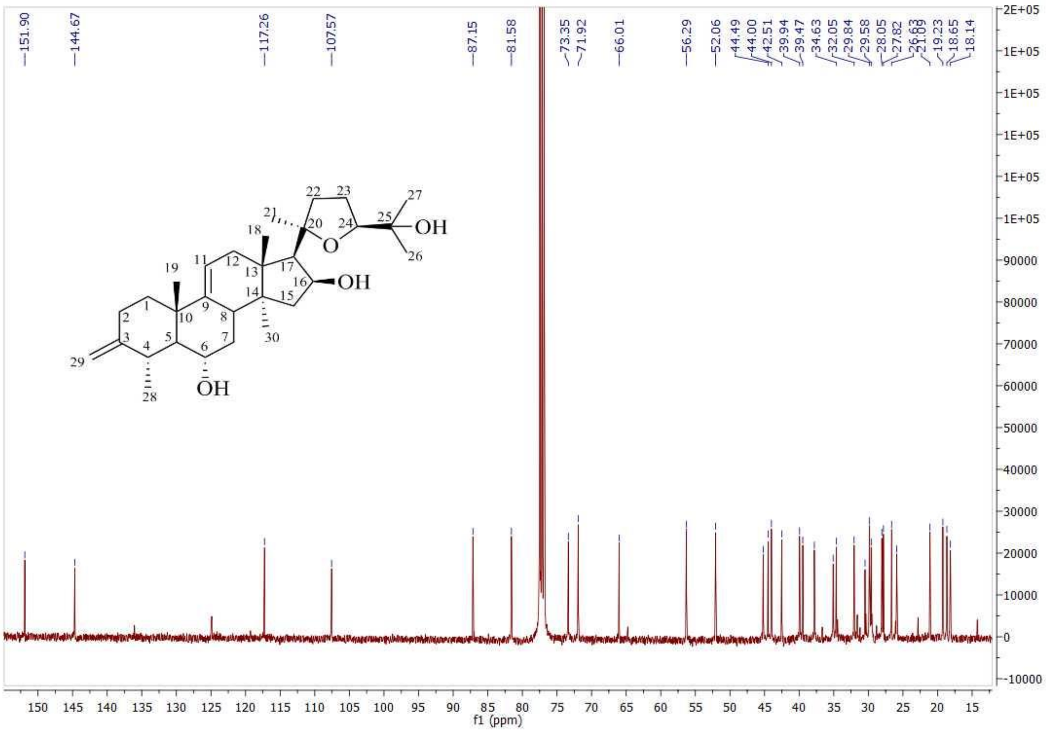
Spectrum 85. HMQC Spectrum of **A6**



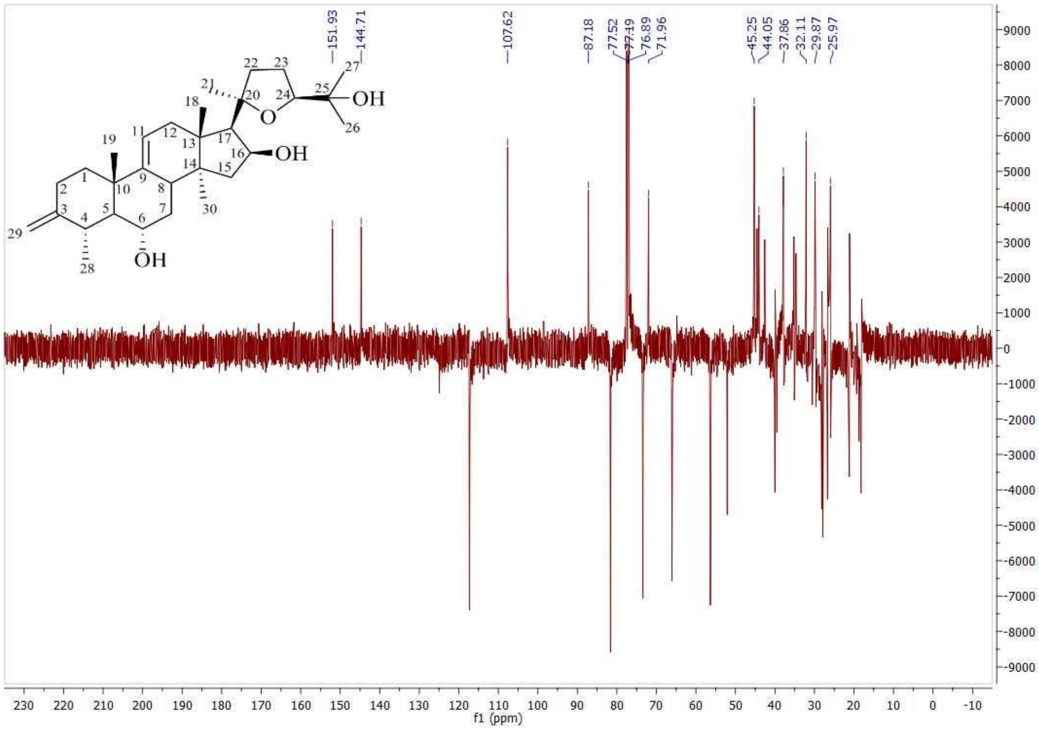
Spectrum 86. Mass Spectrum of **A6**



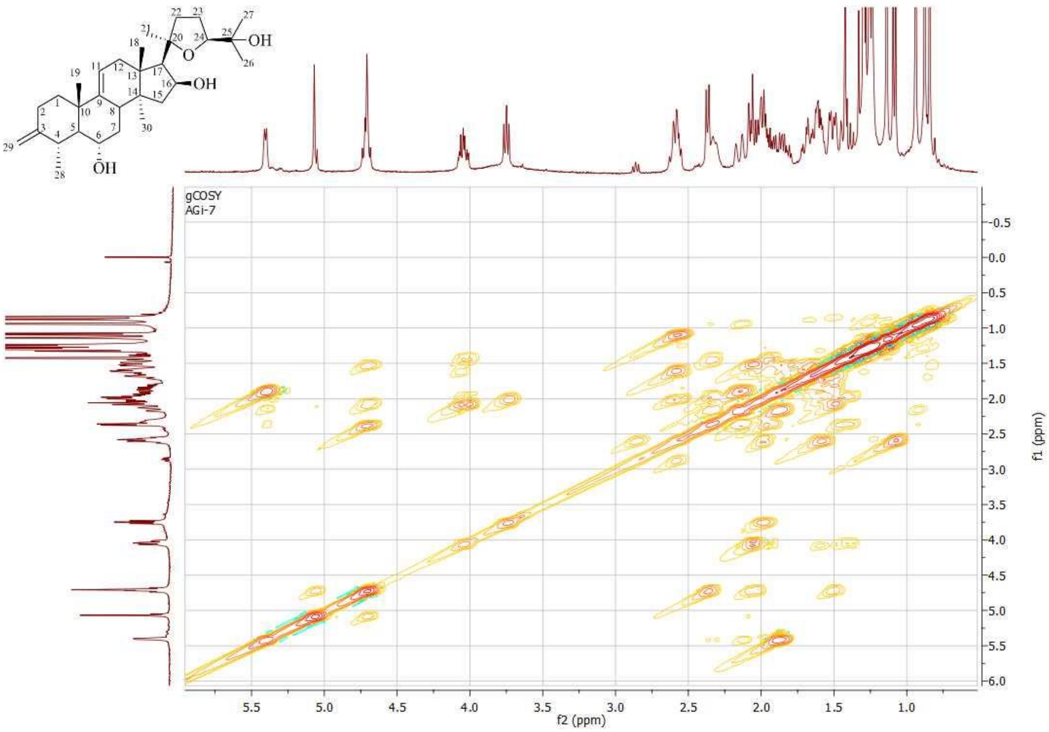
Spectrum 87. ^{1}H-NMR Spectrum of **A7**



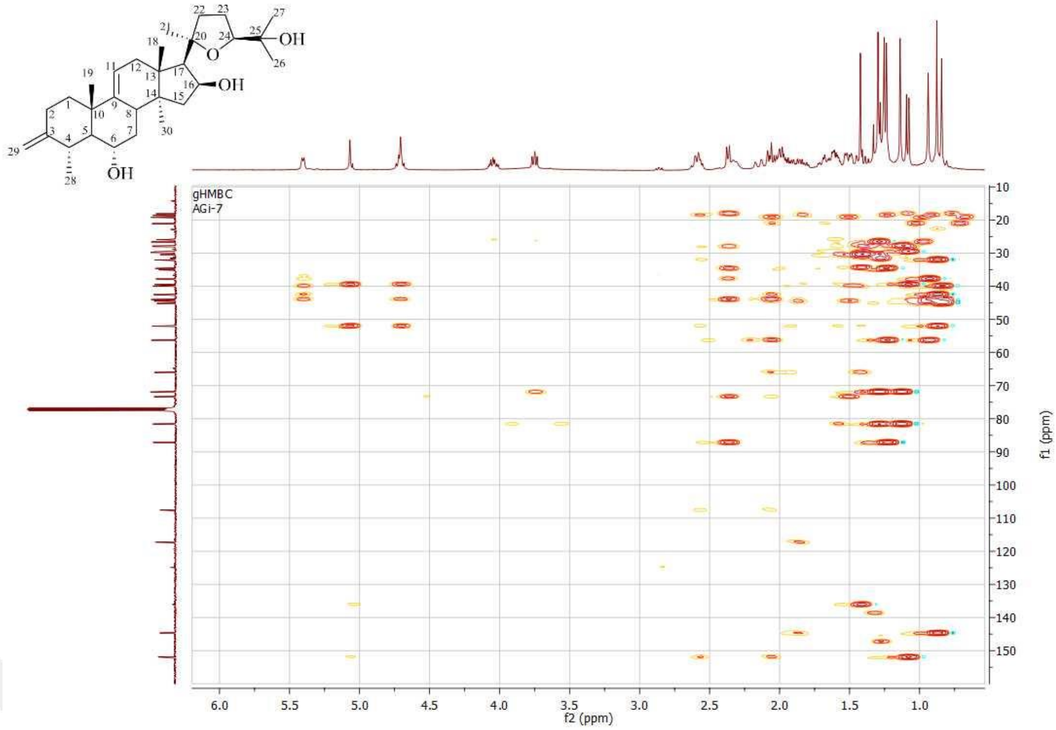
Spectrum 88. ^{13}C-NMR Spectrum of **A7**



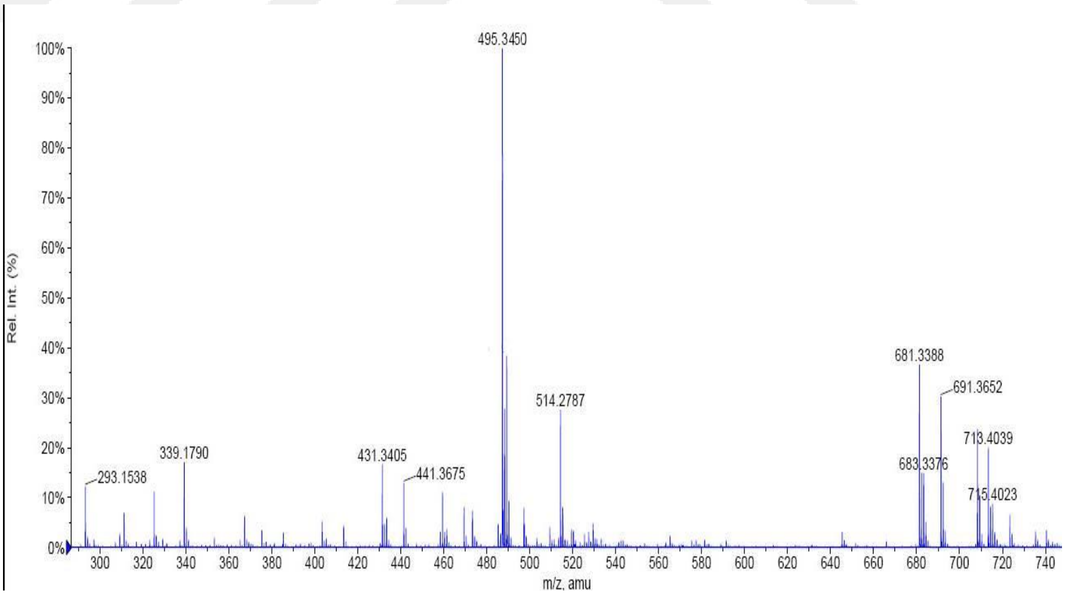
Spectrum 89. APT Spectrum of **A7**



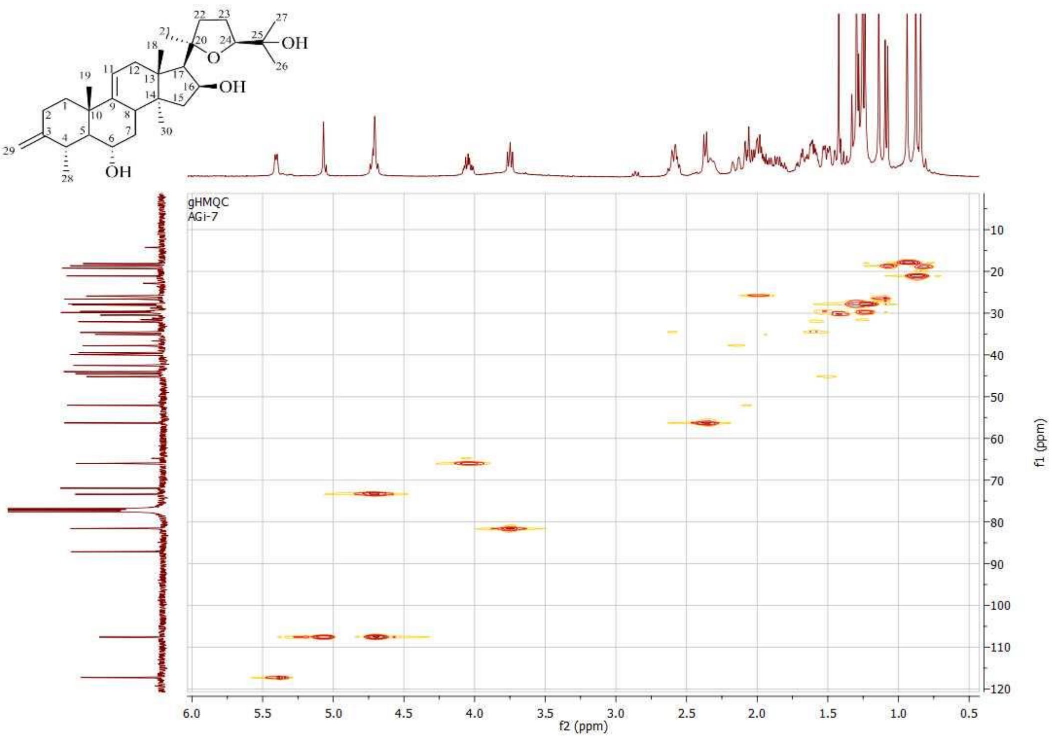
Spectrum 90. COSY Spectrum of **A7**



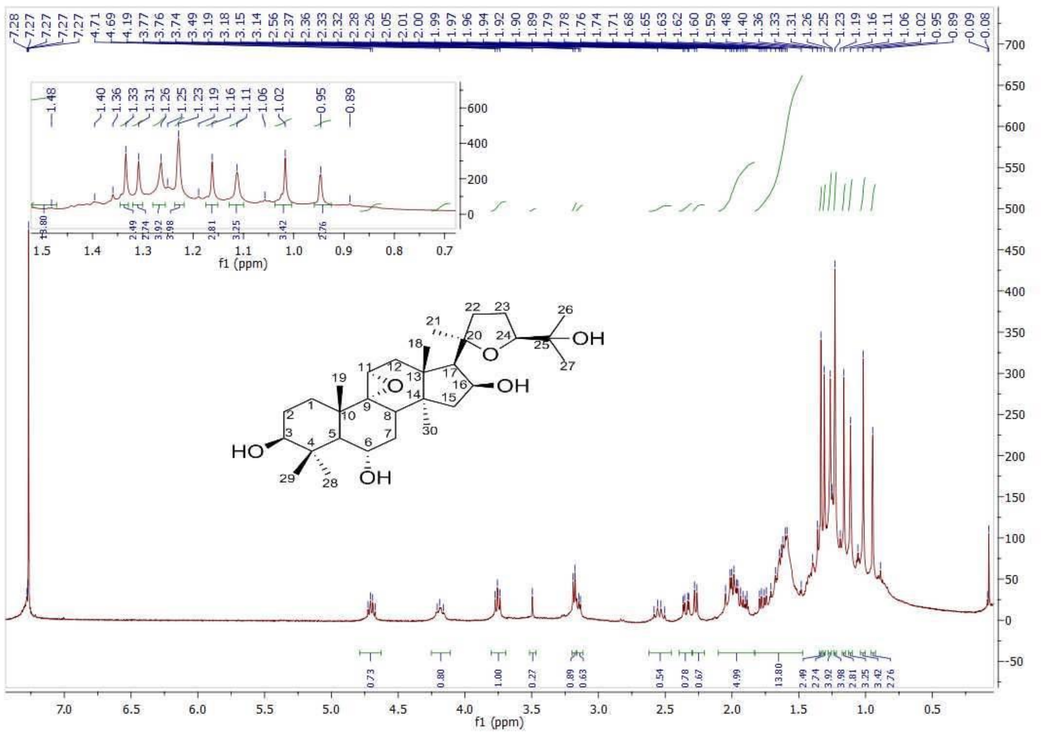
Spectrum 91. HMBC Spectrum of **A7**



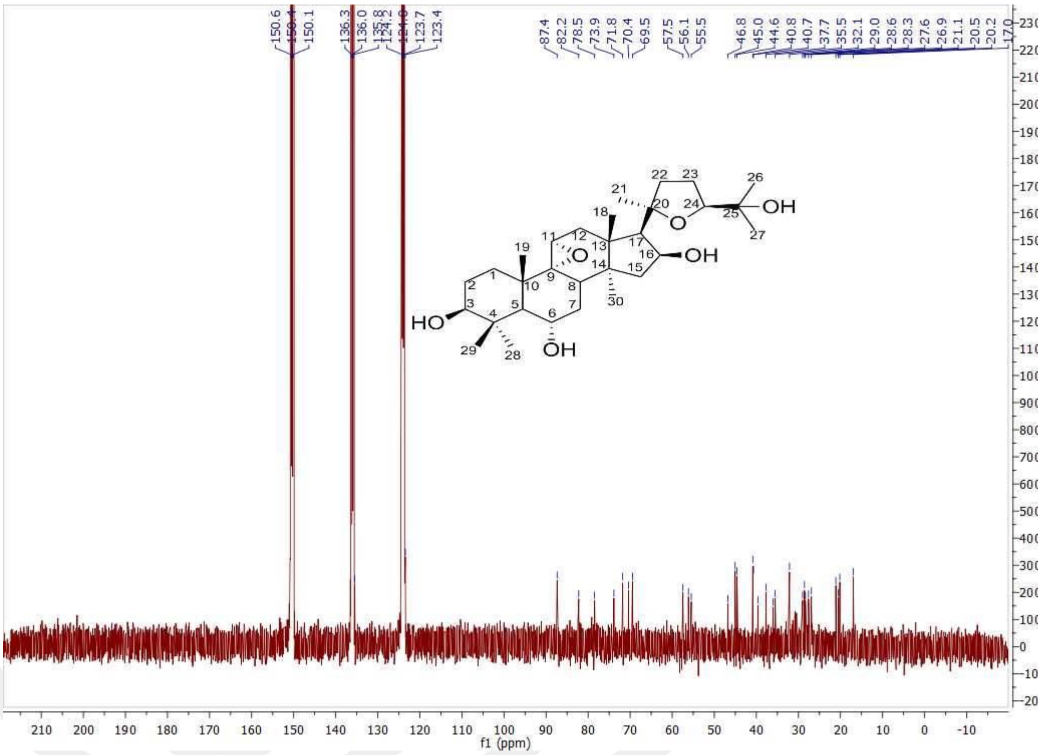
Spectrum 92. Mass Spectrum of **A7**



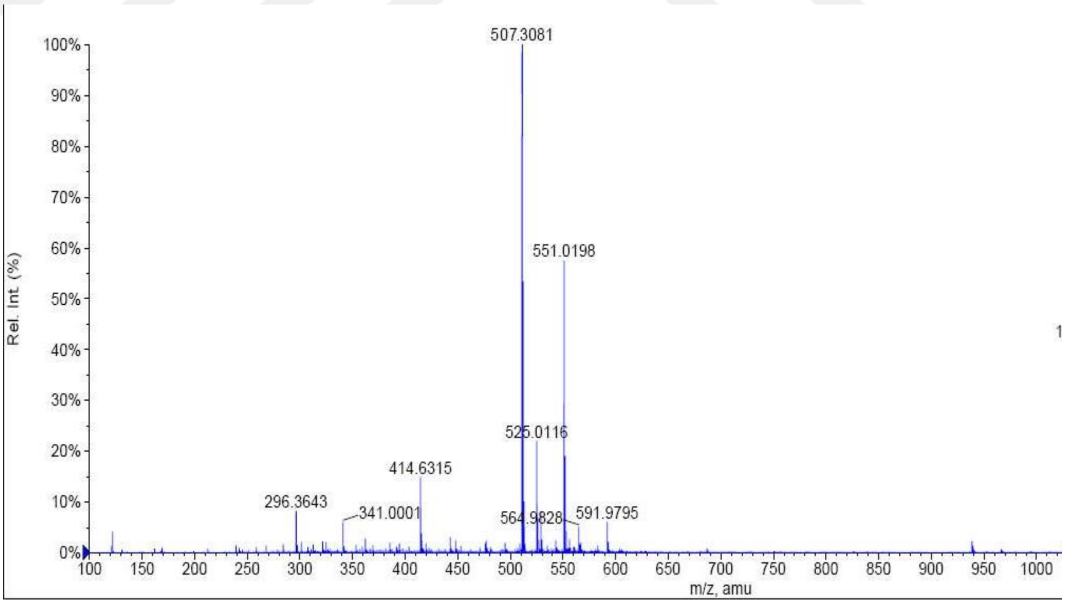
Spectrum 93. HMQC Spectrum of **A7**



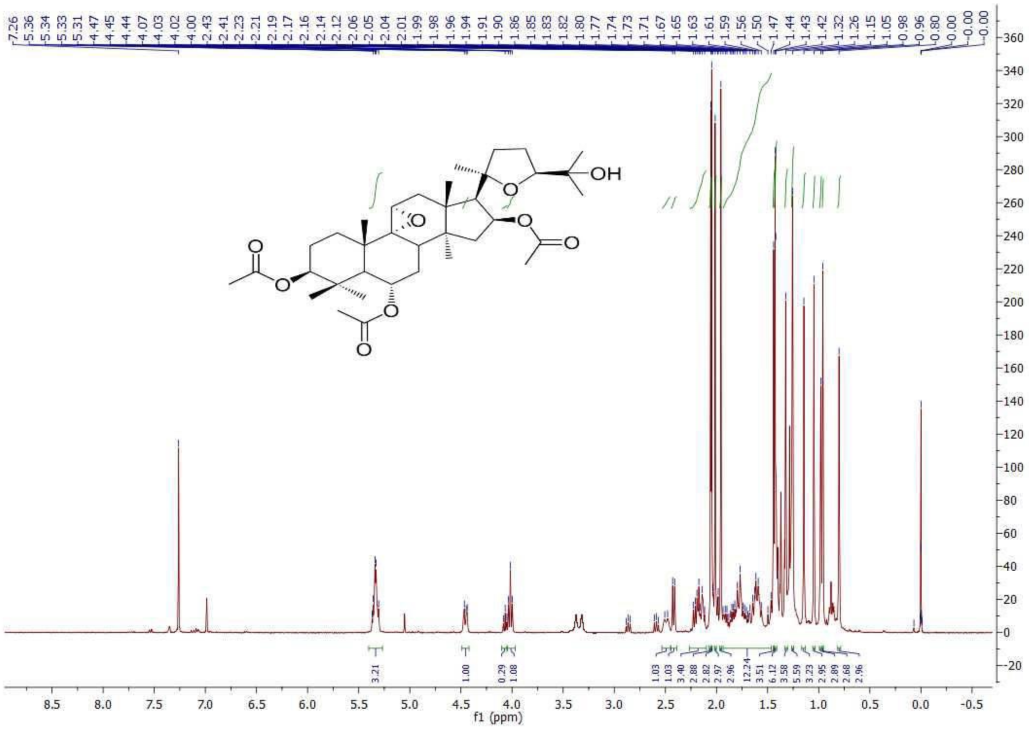
Spectrum 94. ^{1}H-NMR Spectrum of **A19**



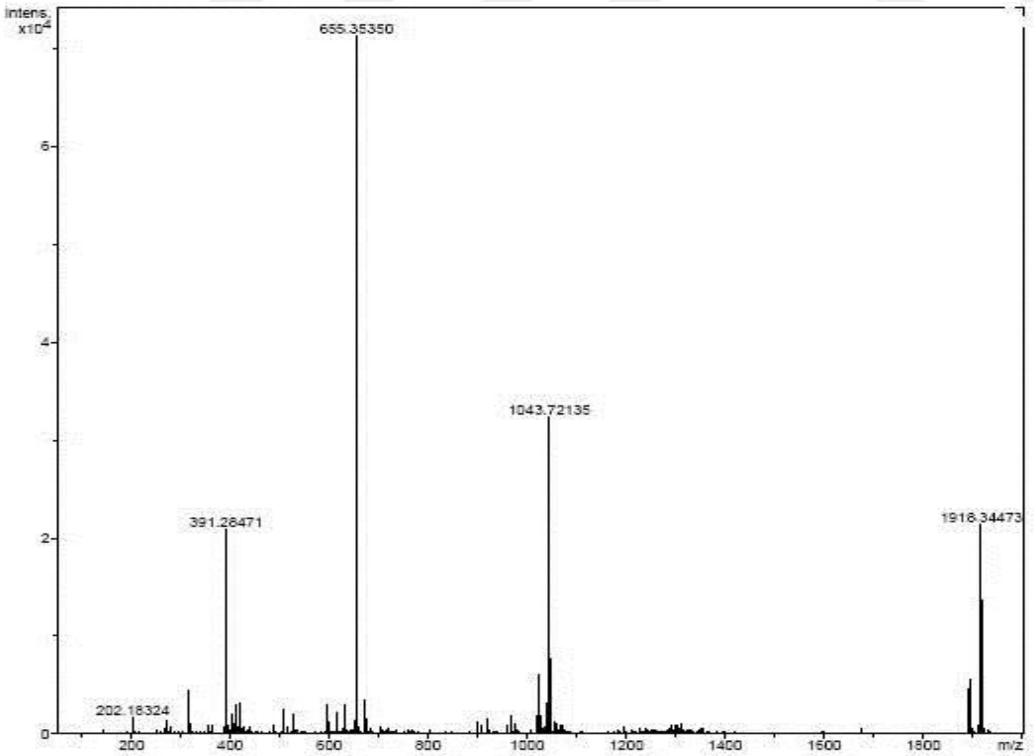
Spectrum 95. ^{13}C-NMR Spectrum of **A19**



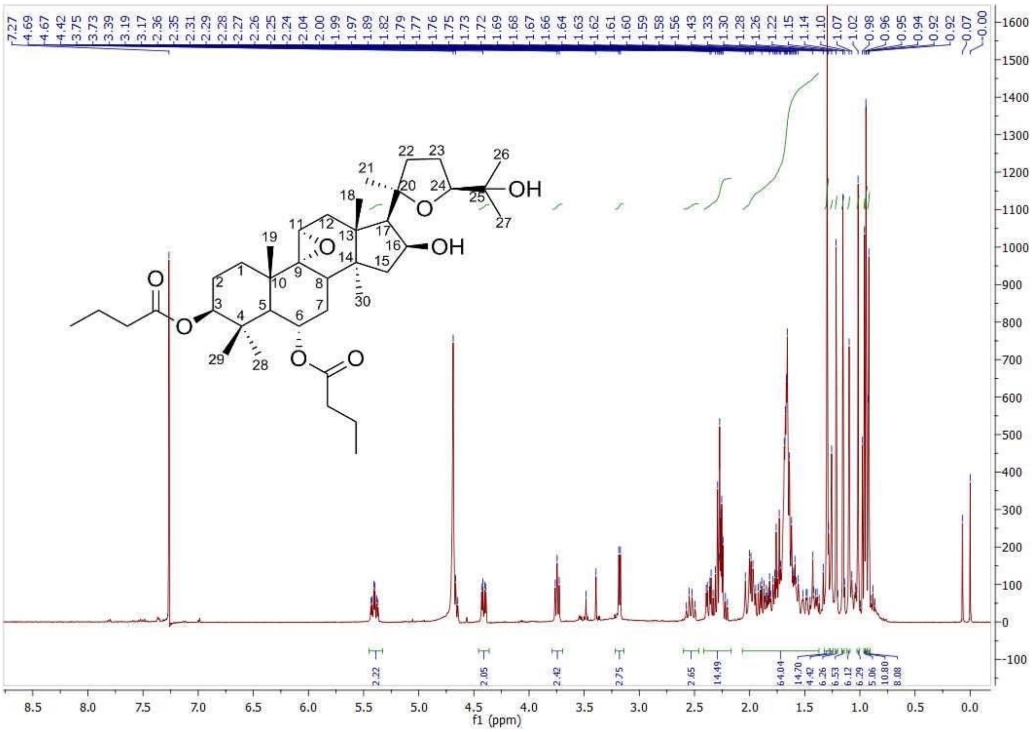
Spectrum 96. Mass Spectrum of **A19**



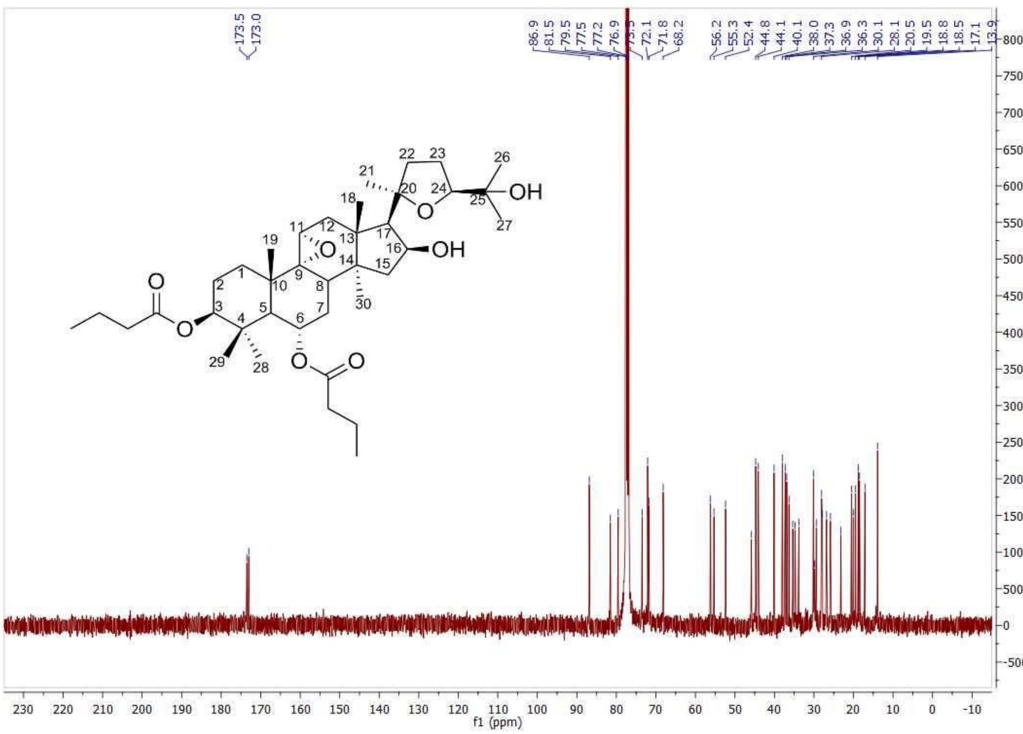
Spectrum 97. ^{1}H-NMR Spectrum of **A20**



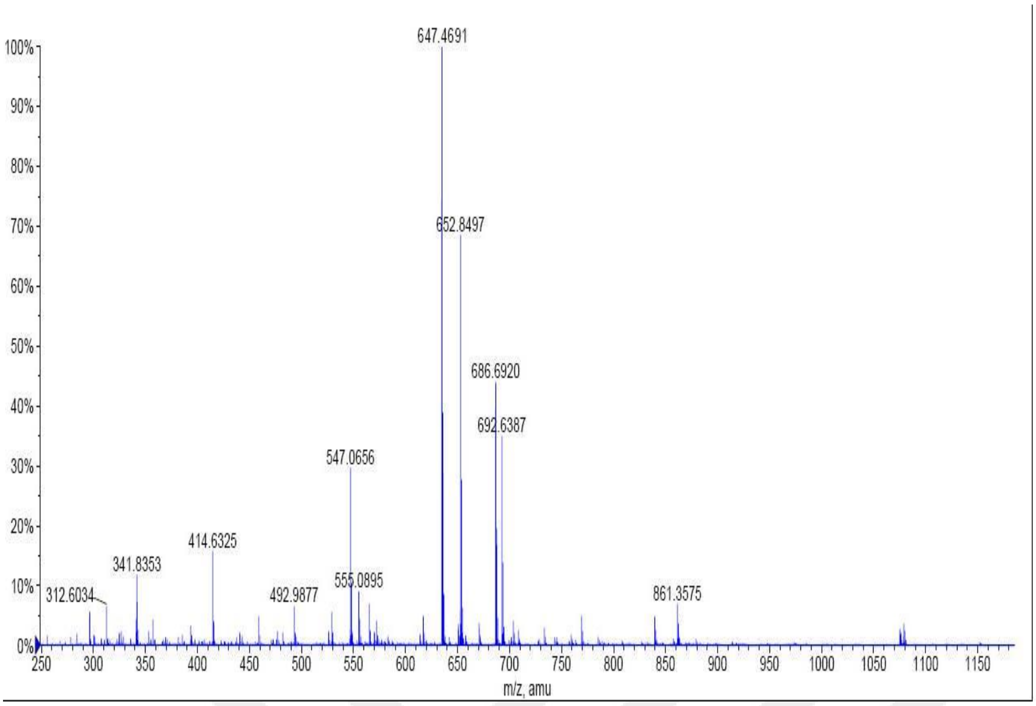
Spectrum 98. Mass Spectrum of **A20**



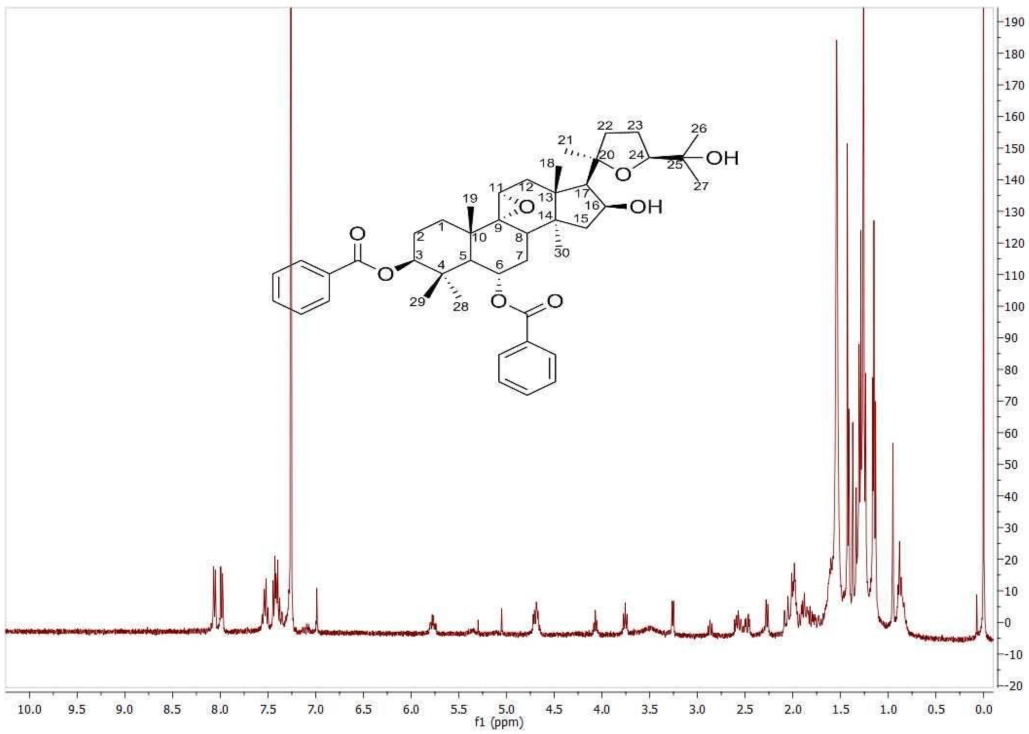
Spectrum 99. ^{1}H-NMR Spectrum of **A21**



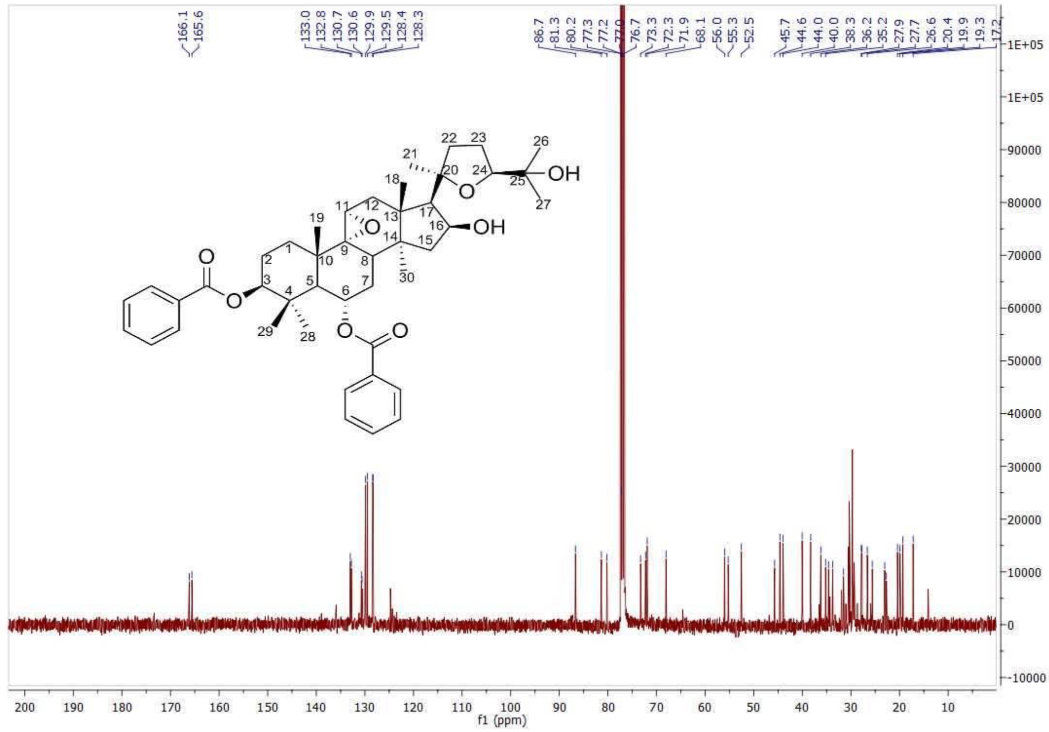
Spectrum 100. ^{13}C-NMR Spectrum of **A21**



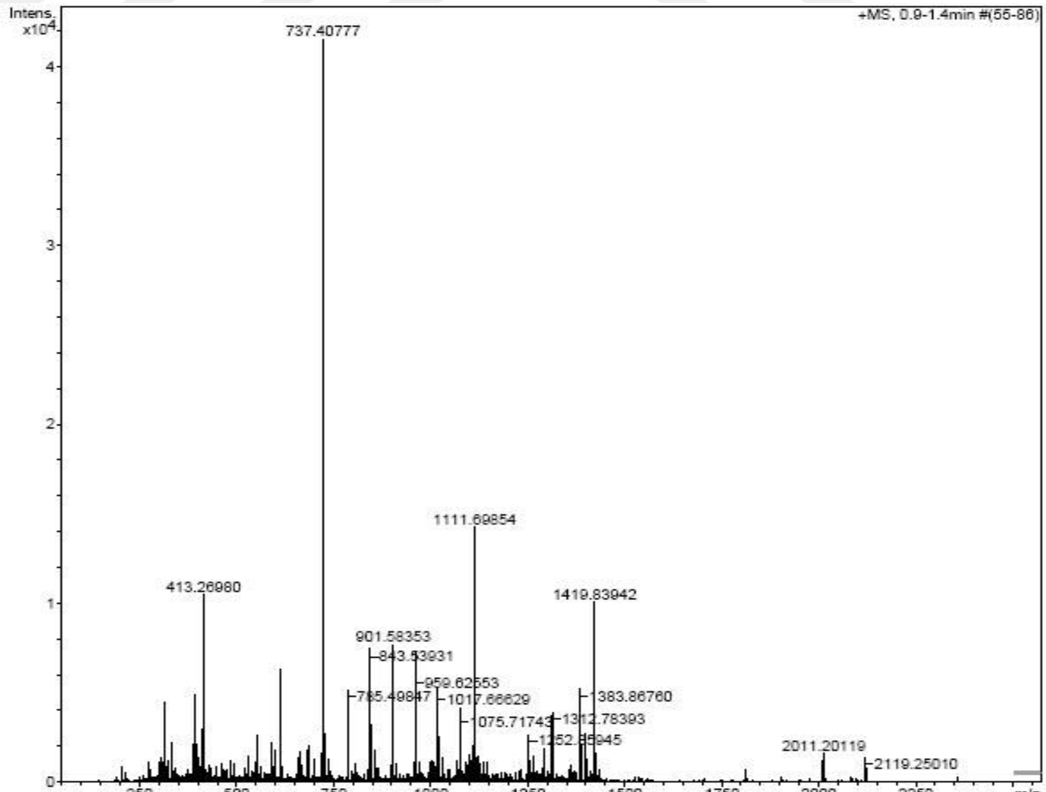
Spectrum 101. Mas Spectrum of **A21**



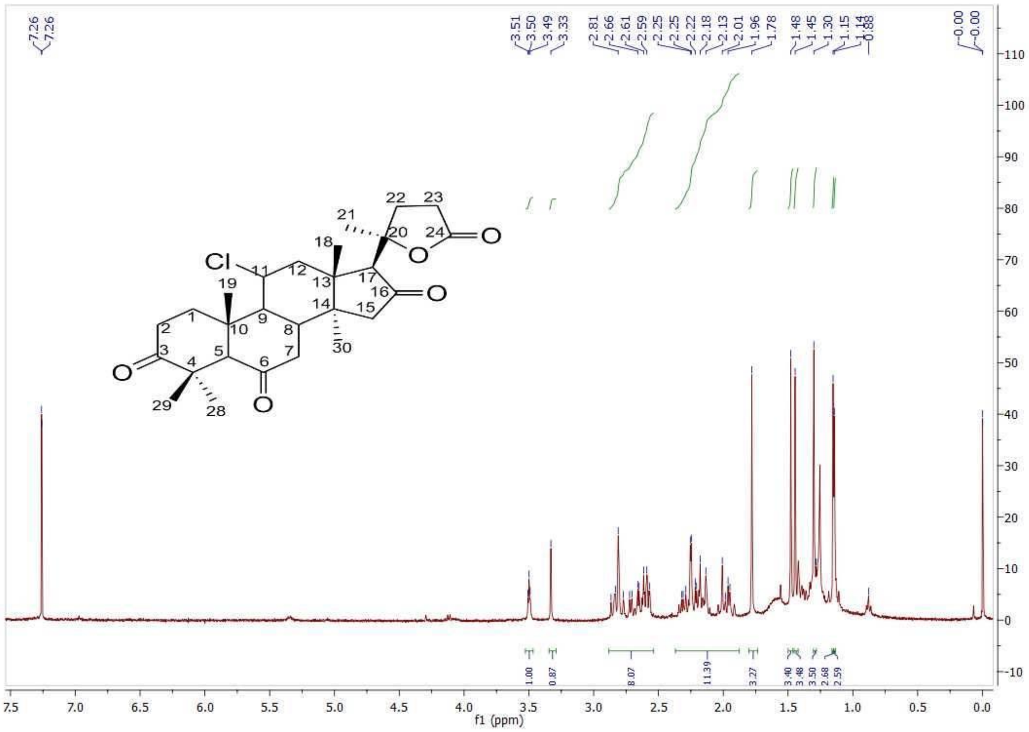
Spectrum 102. ^{1}H-NMR Spectrum of **A22**



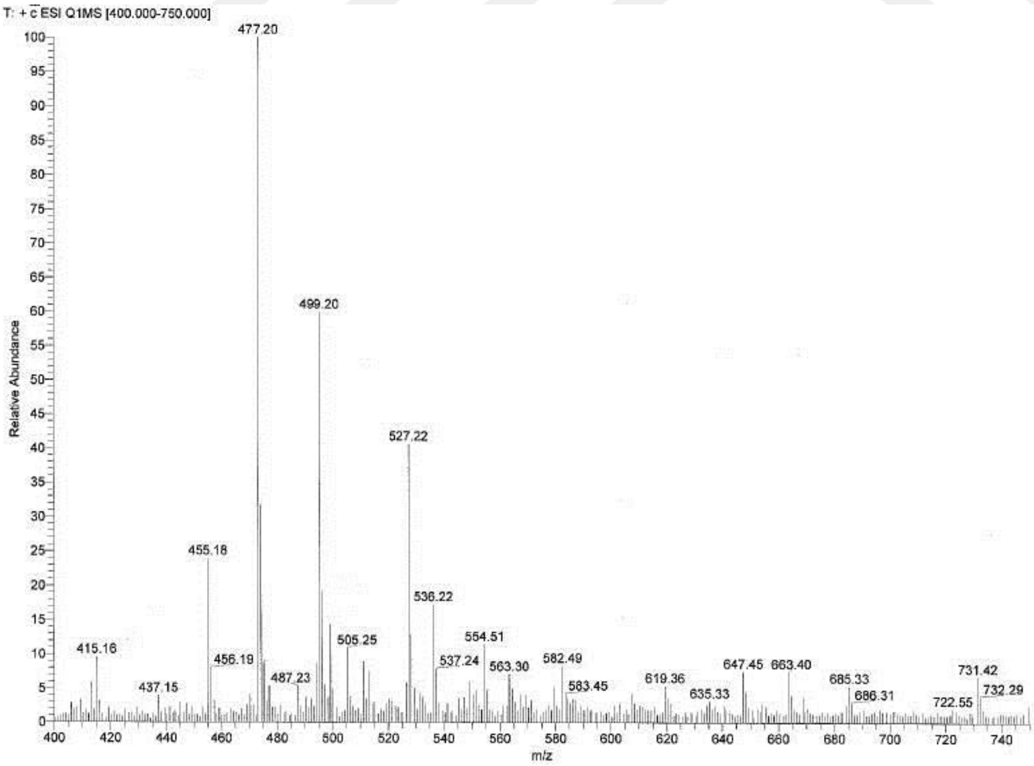
Spectrum 103. ^{13}C-NMR Spectrum of **A22**



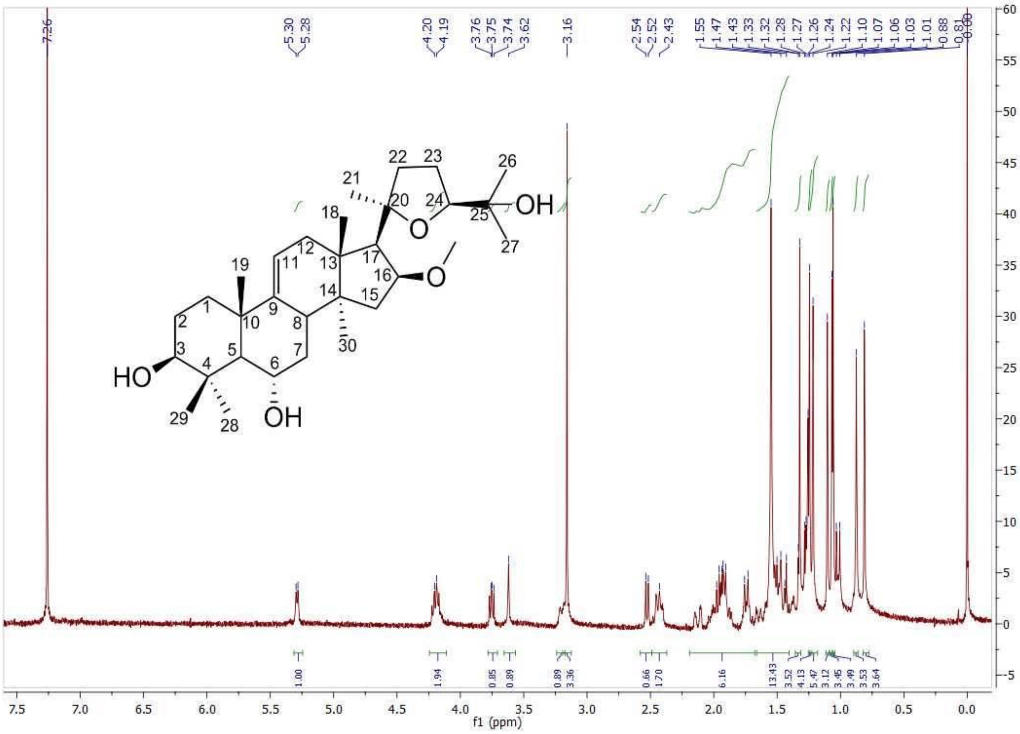
Spectrum 104. Mass Spectrum of **A22**



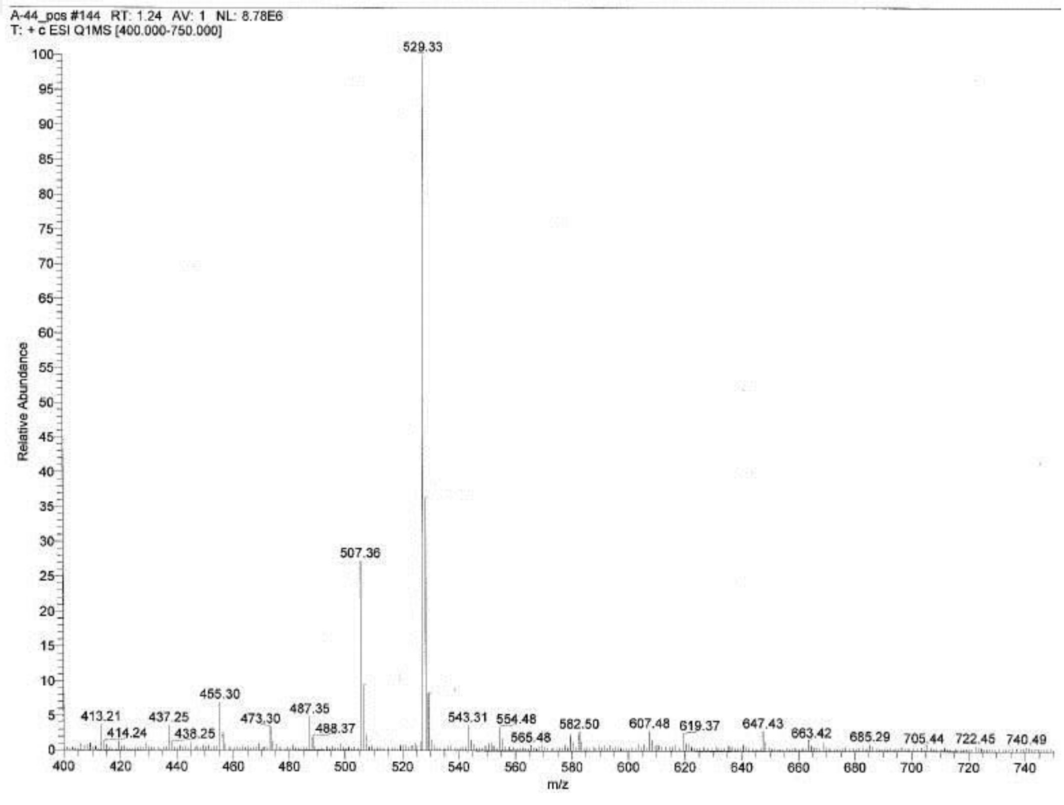
Spectrum 105. ^{1}H-NMR Spectrum of **A28**



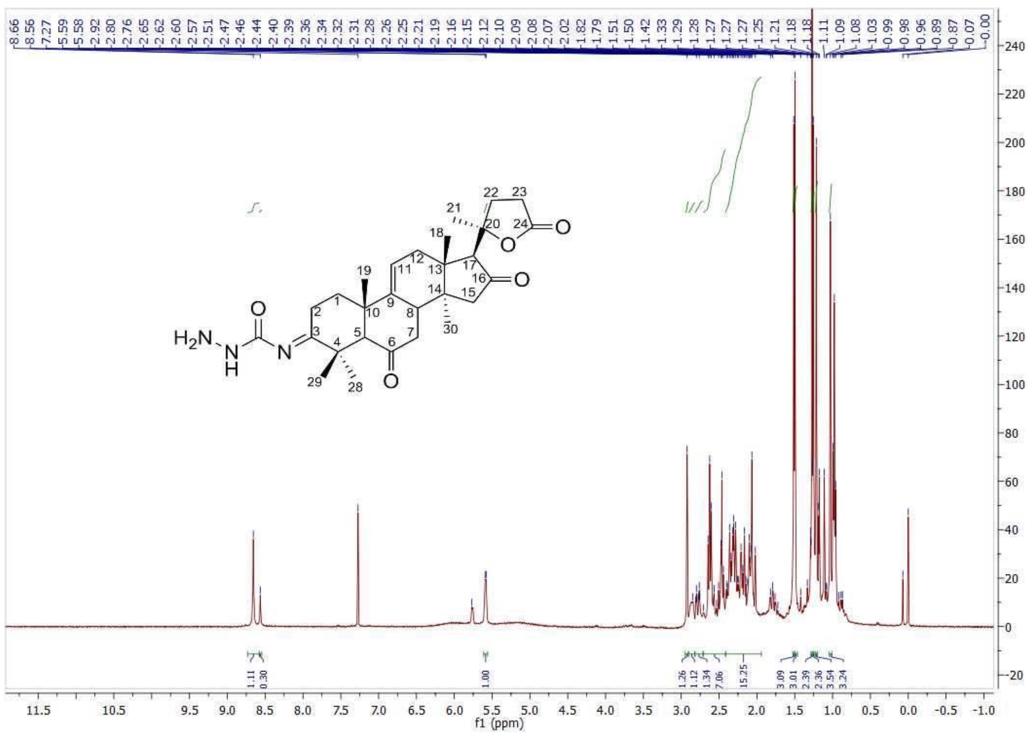
Spectrum 106. Mass Spectrum of **A28**



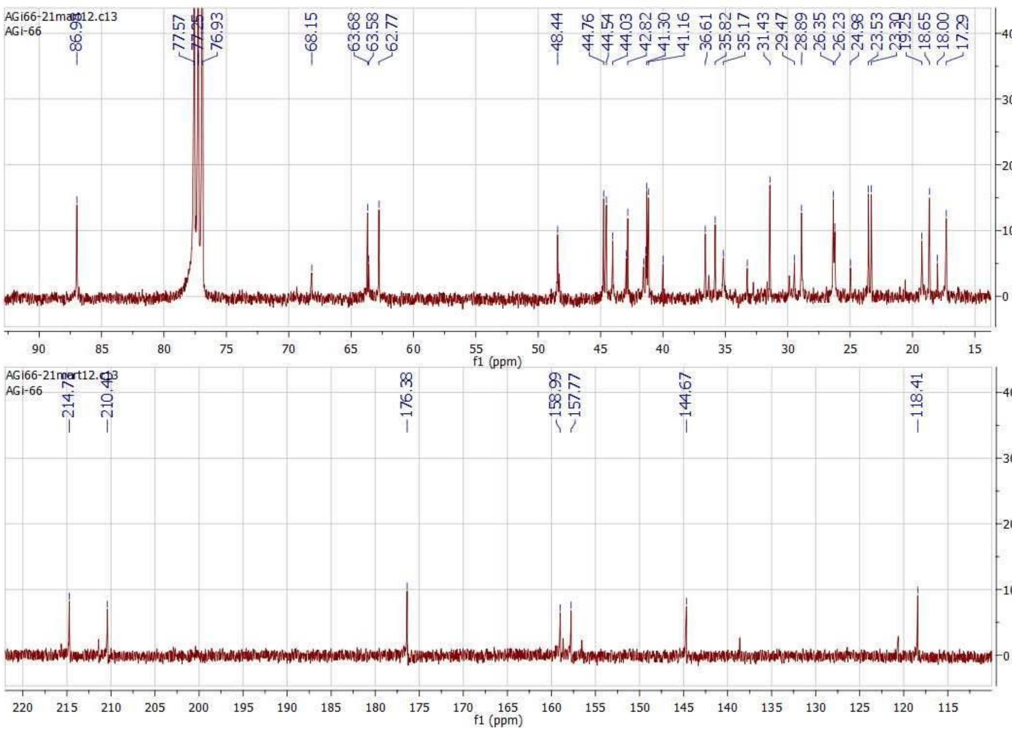
Spectrum 107. ^{1}H-NMR Spectrum of **A37**



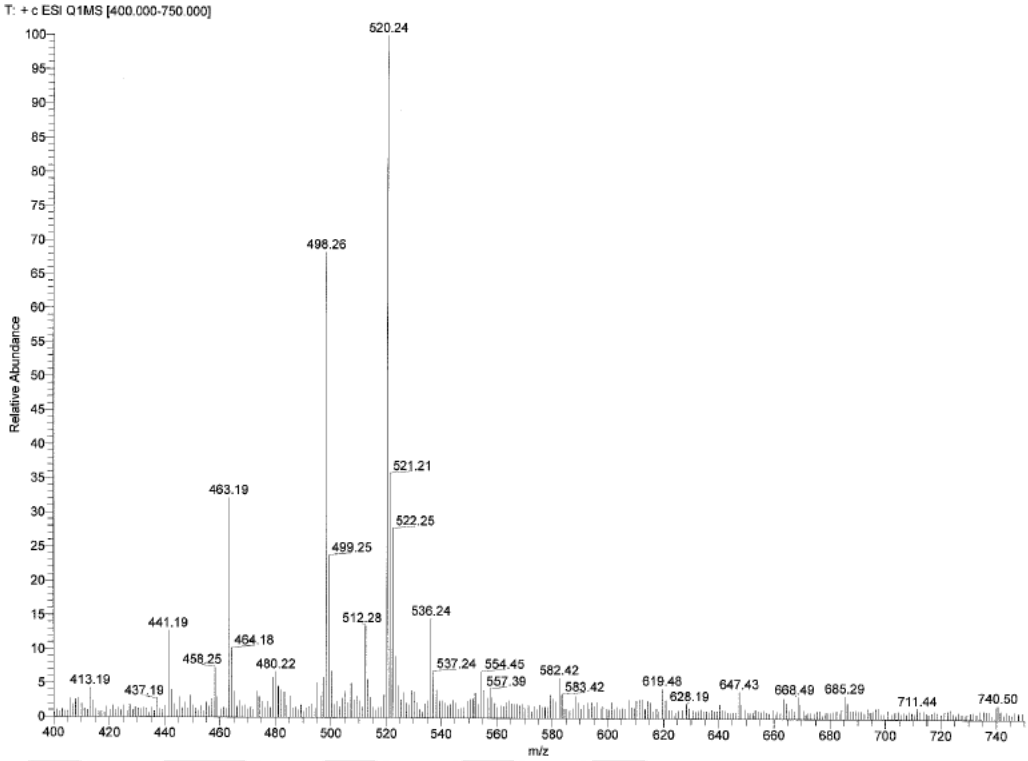
Spectrum 108. Mass Spectrum of **A37**



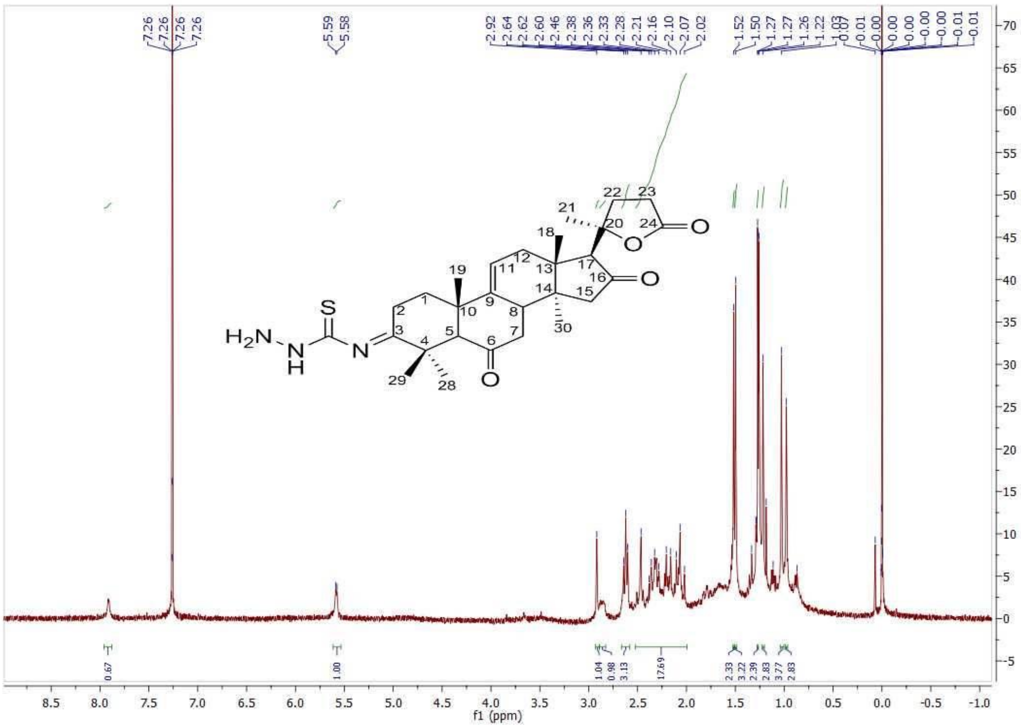
Spectrum 109. ^{1}H-NMR Spectrum of **A32**



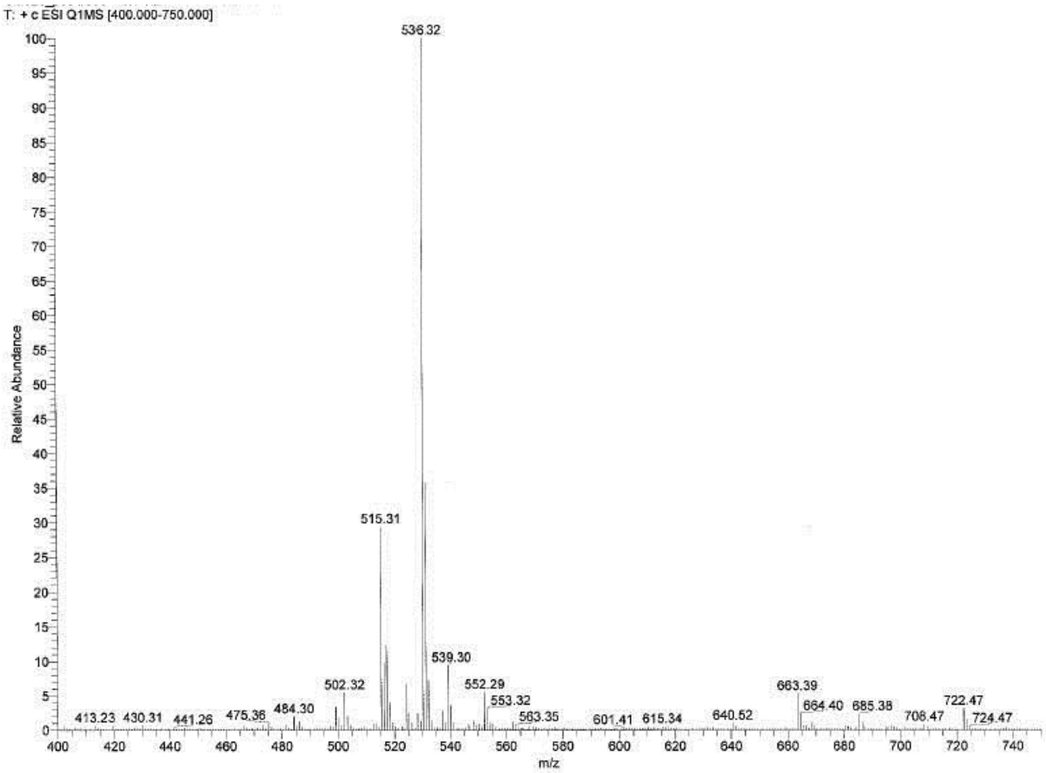
Spectrum 110. ^{13}C-NMR Spectrum of **A32**



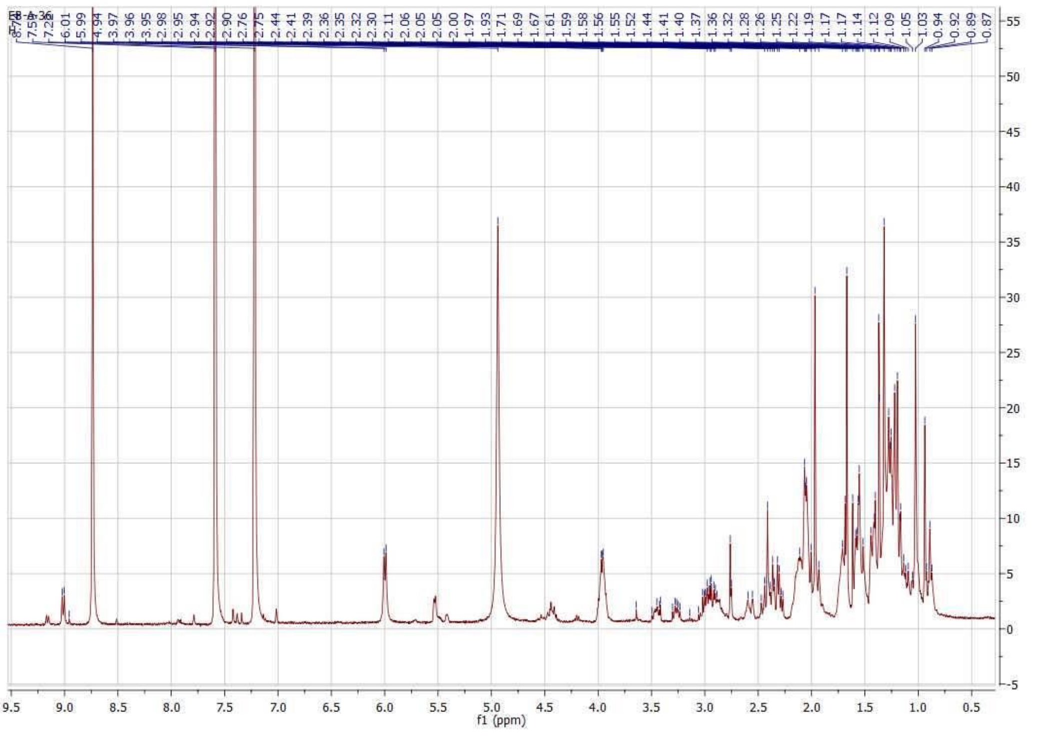
Spectrum 111. Mass Spectrum of **A32**



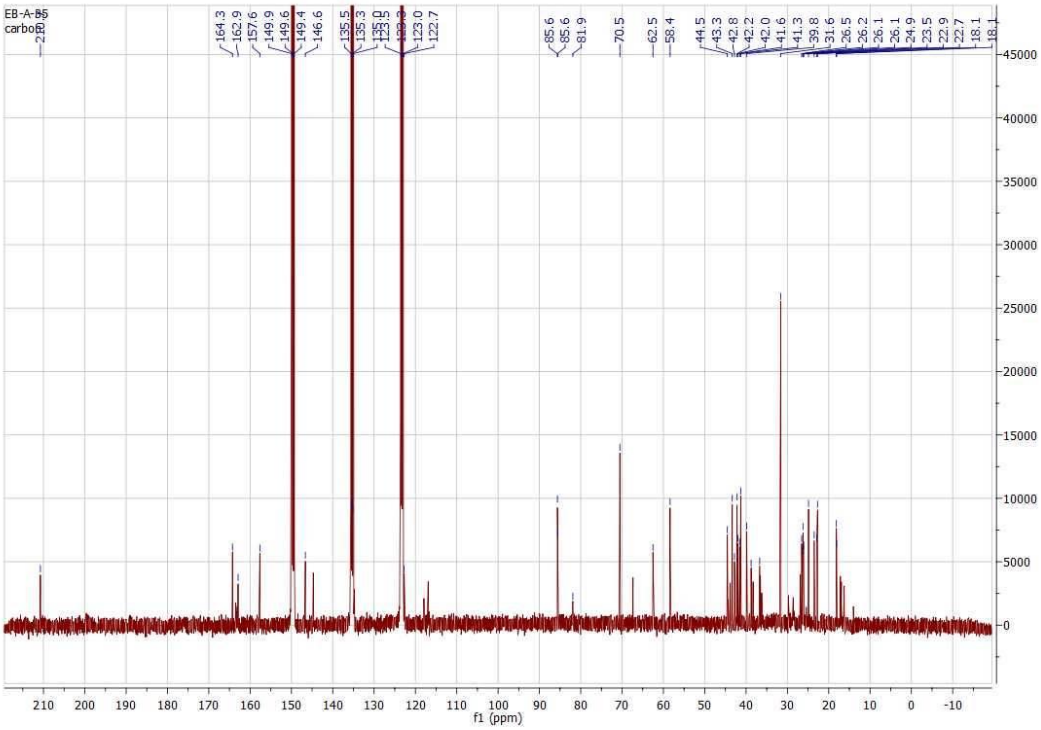
Spectrum 112. [1]H-NMR Spectrum of **A31**



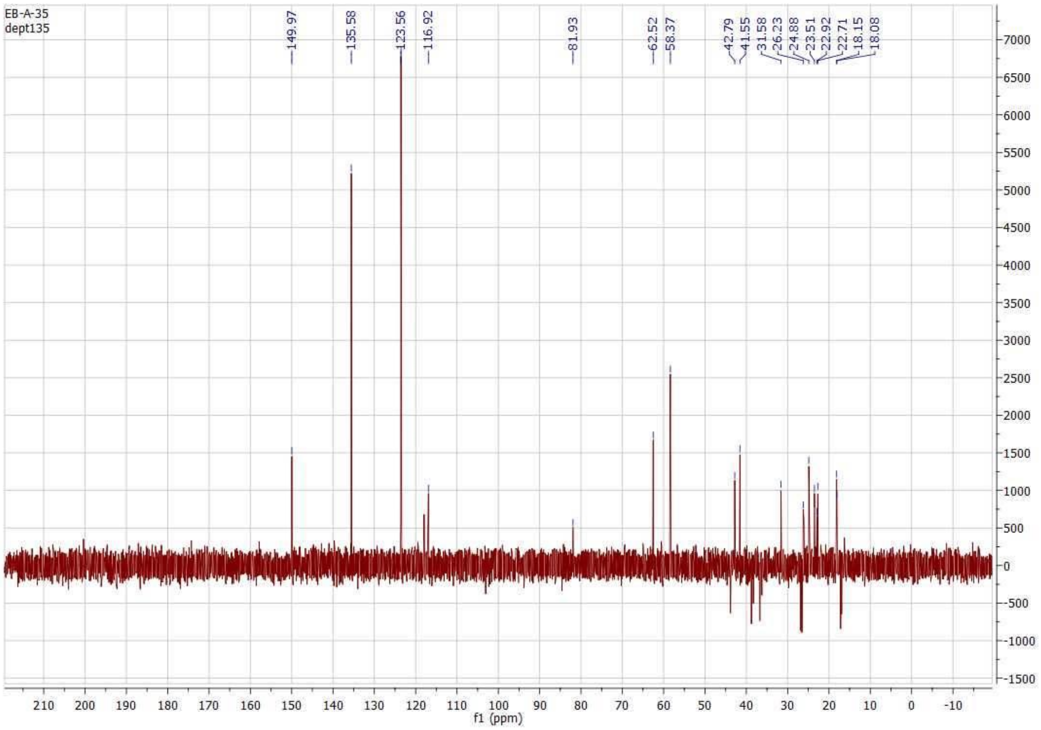
Spectrum 113. Mass Spectrum of **A31**



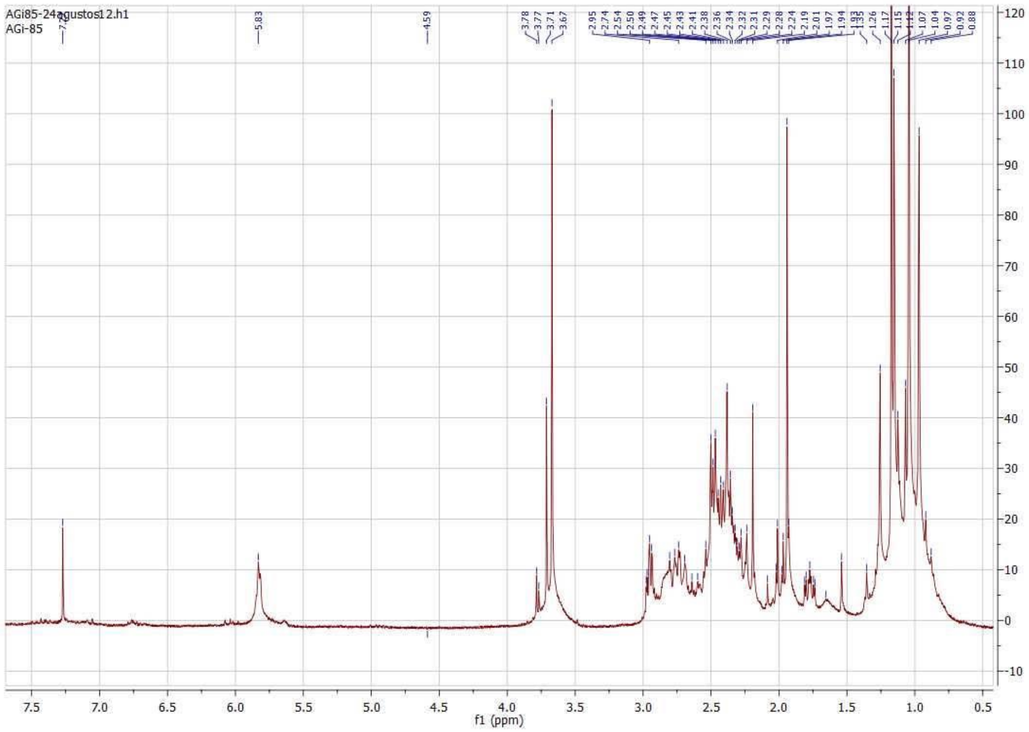
Spectrum 114. ^{1}H-NMR Spectrum of **A23**



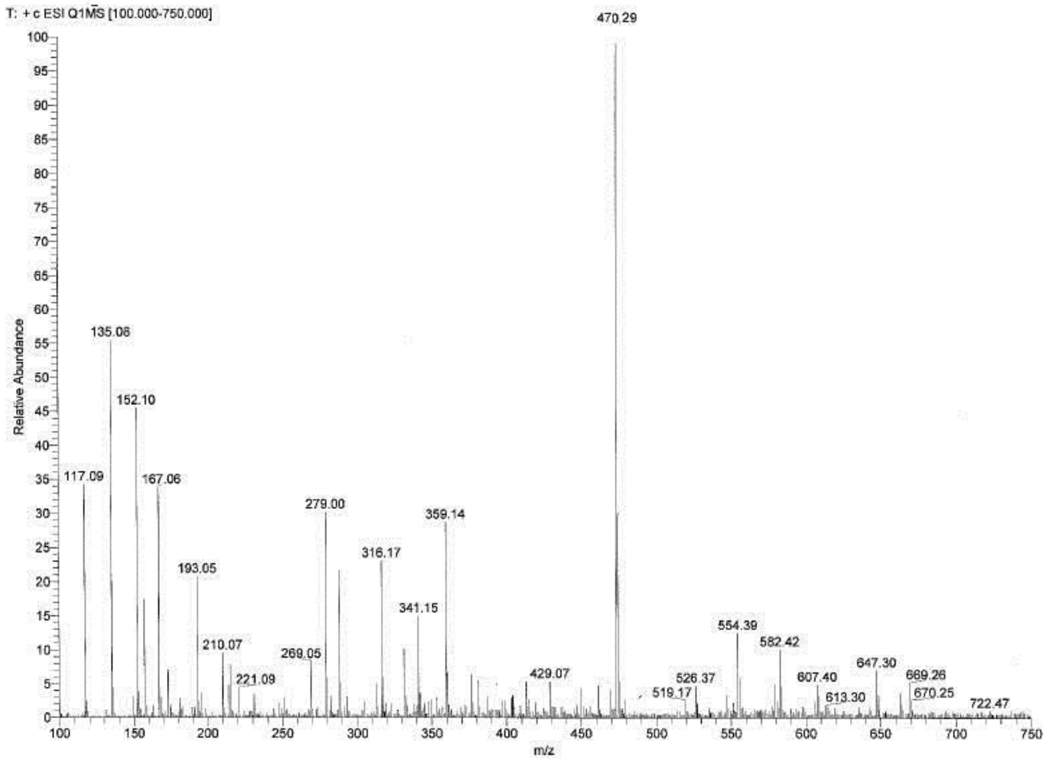
Spectrum 115. ^{13}C-NMR Spectrum of **A23**



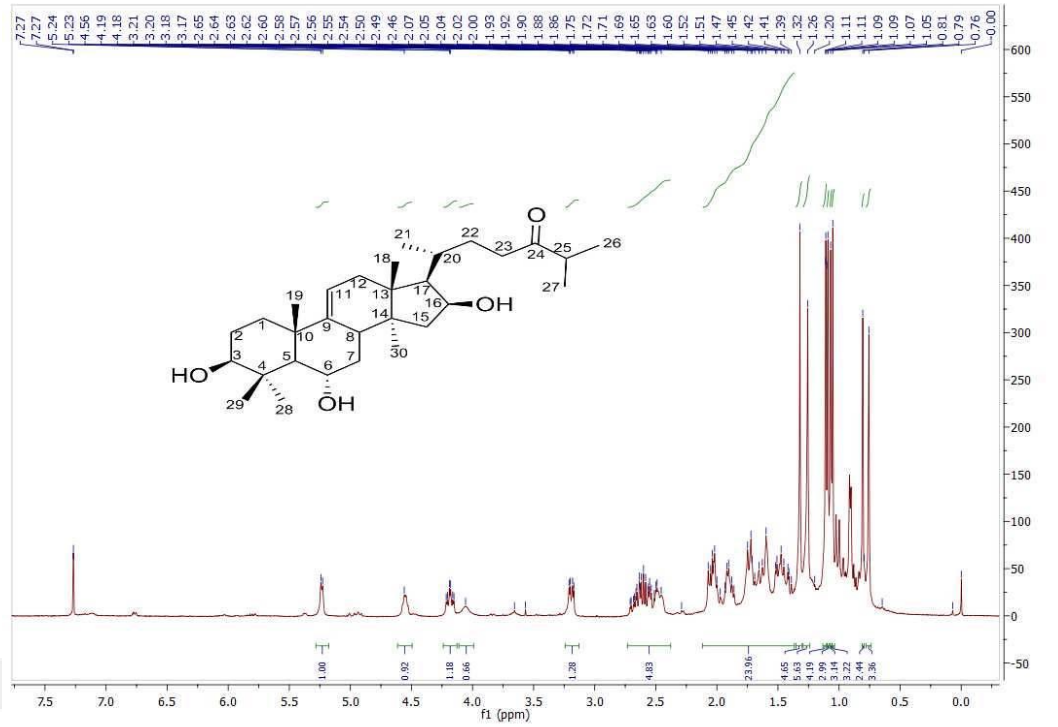
Spectrum 116. DEPT-NMR Spectrum of **A23**



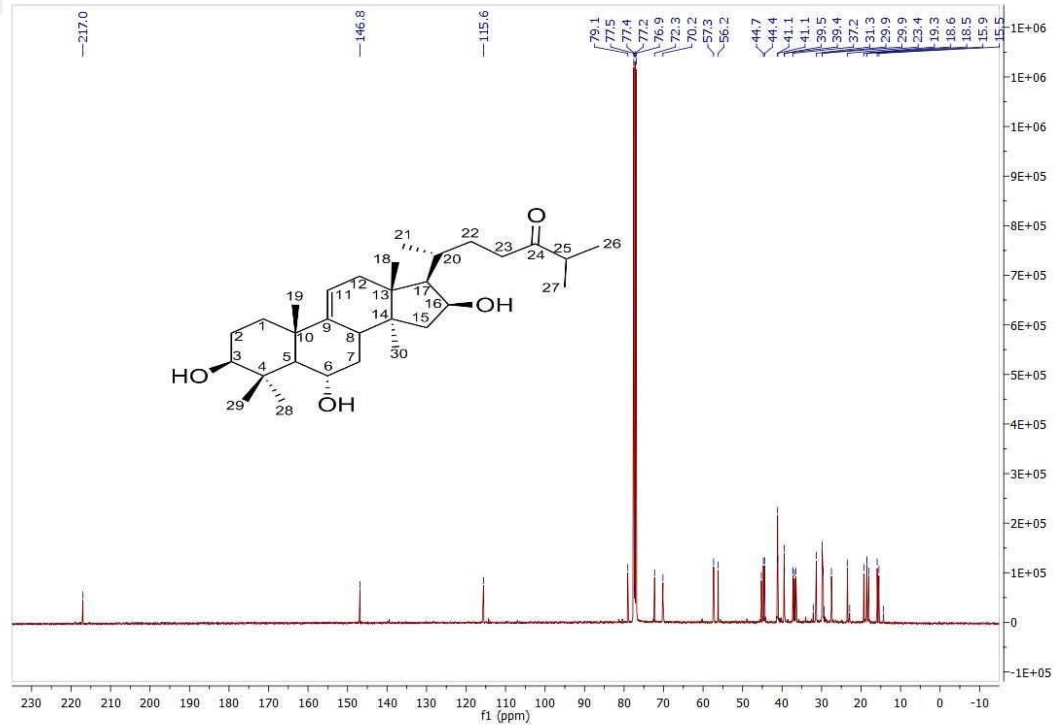
Spectrum 117. ^{1}H-NMR Spectrum of **A24**



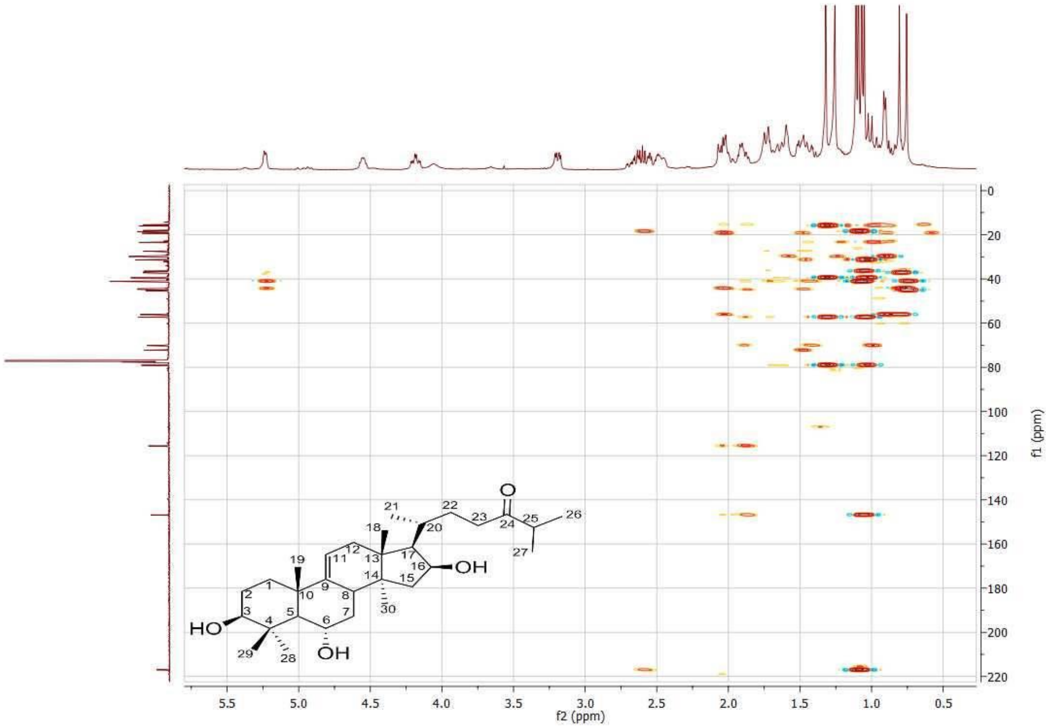
Spectrum 118. Mass Spectrum of **A24**



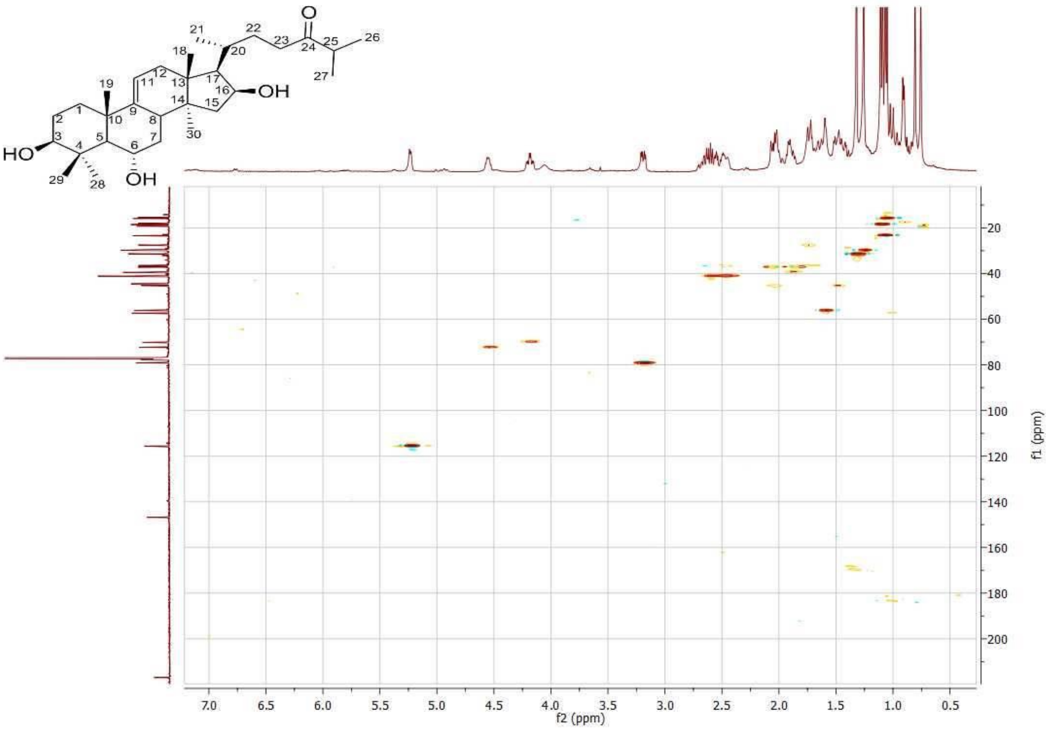
Spectrum 119. ^{1}H-NMR Spectrum of **AHSKG**



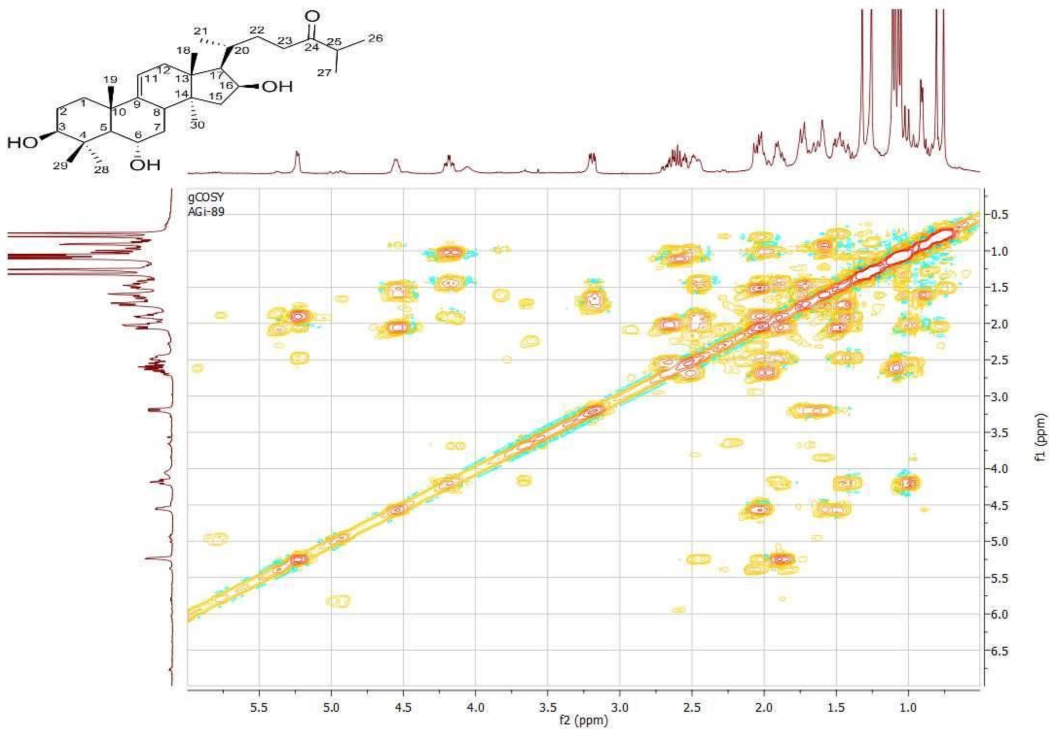
Spectrum 120. ^{13}C-NMR Spectrum of **AHSKG**



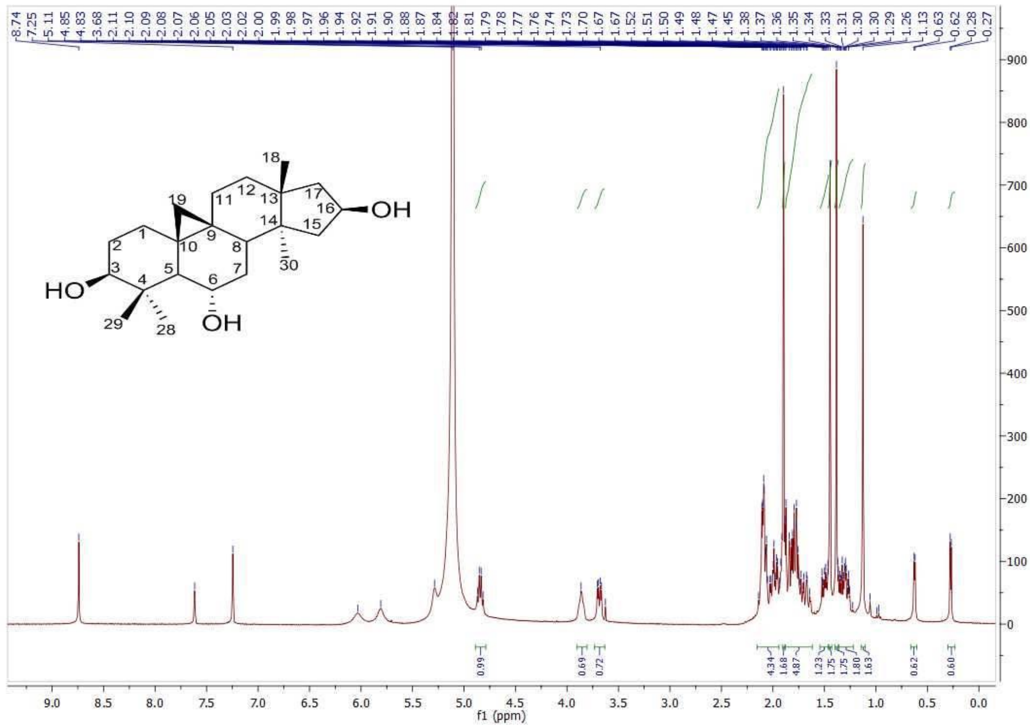
Spectrum 121. HMBC Spectrum of **AHSKG**



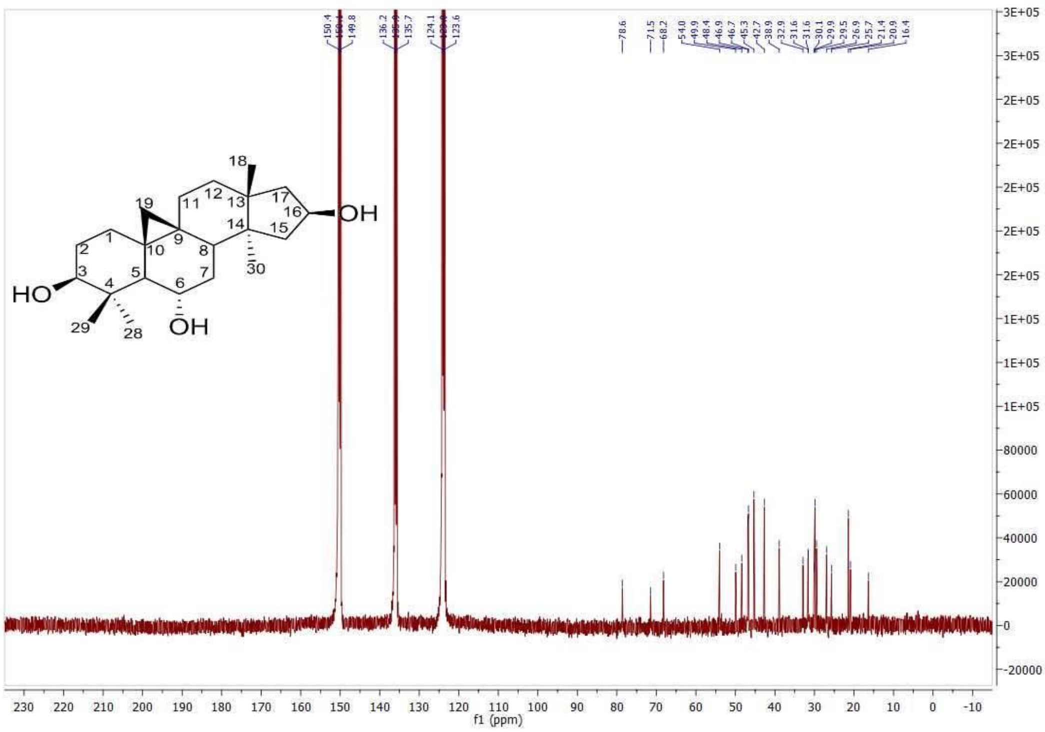
Spectrum 122. HMQC Spectrum of **AHSKG**



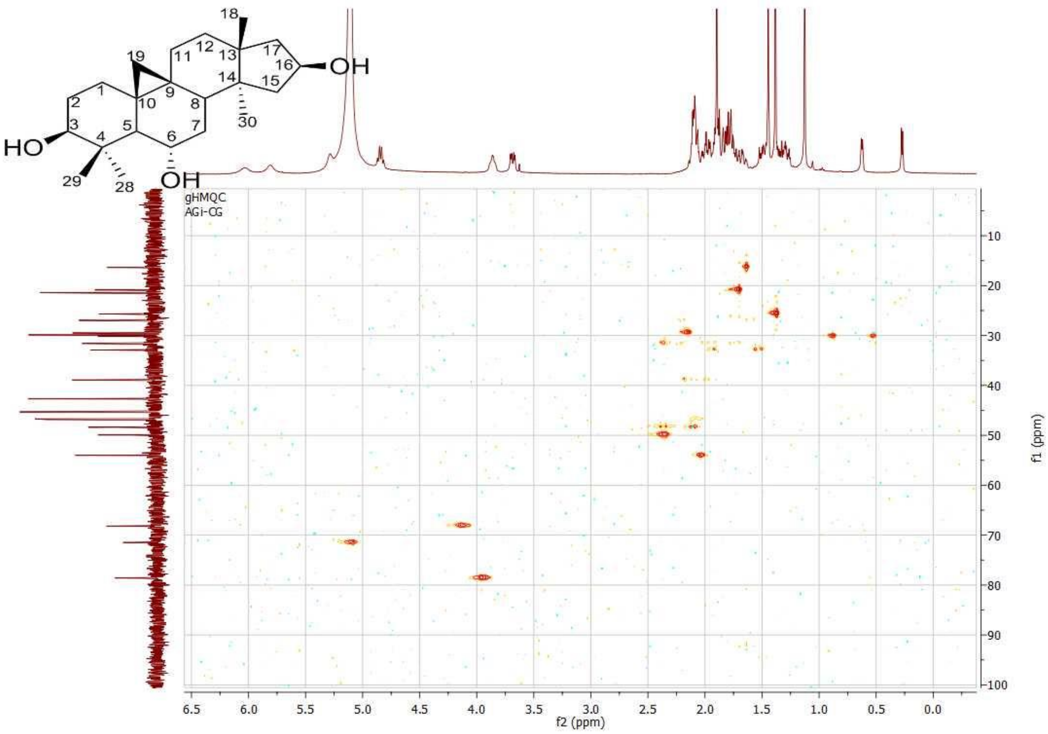
Spectrum 123. COSY Spectrum of **AHSKG**



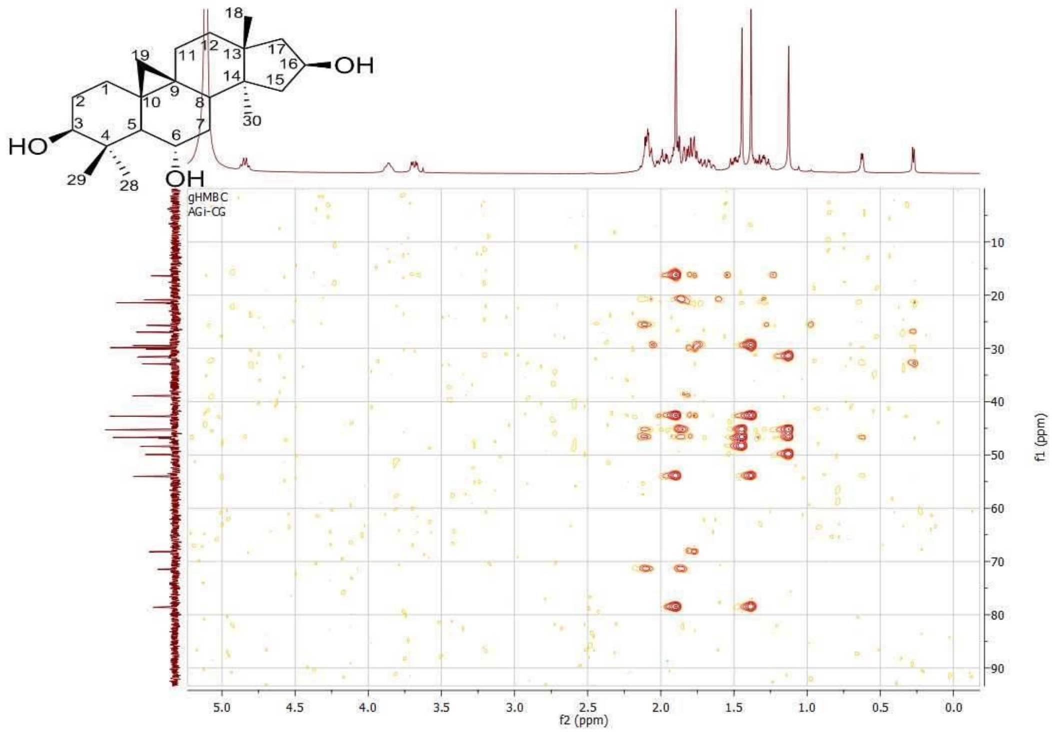
Spectrum 124. [1]H-NMR Spectrum of **SCG**



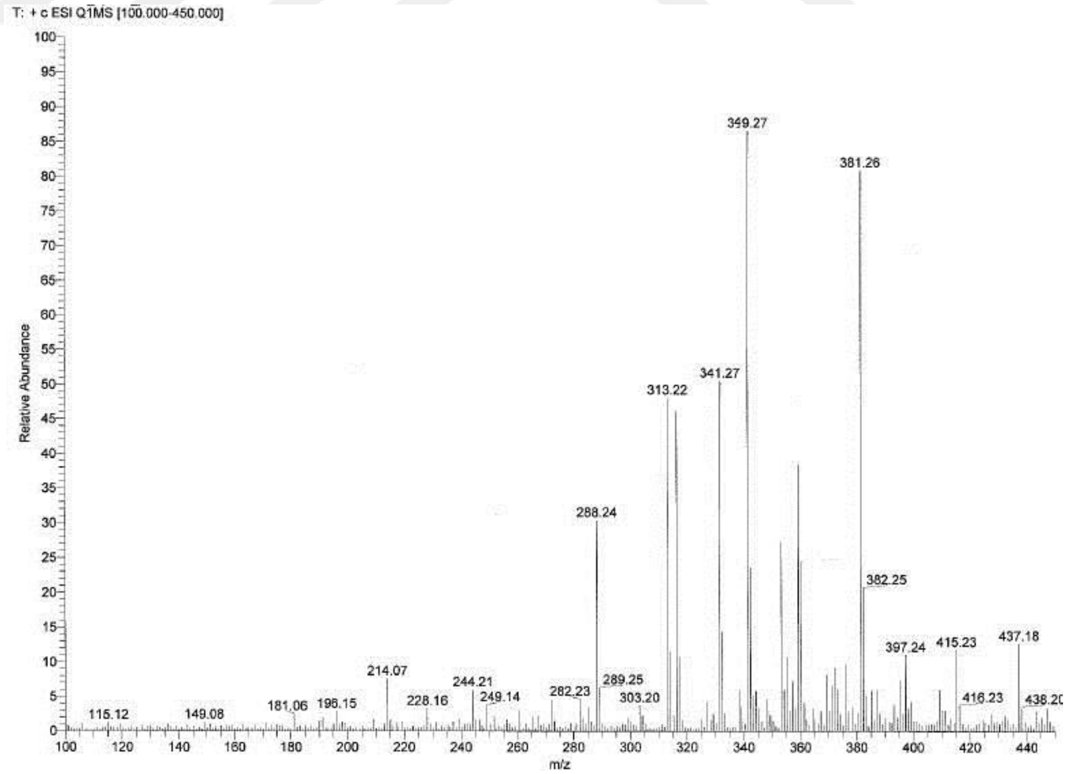
Spectrum 125. ^{13}C-NMR Spectrum of **SCG**



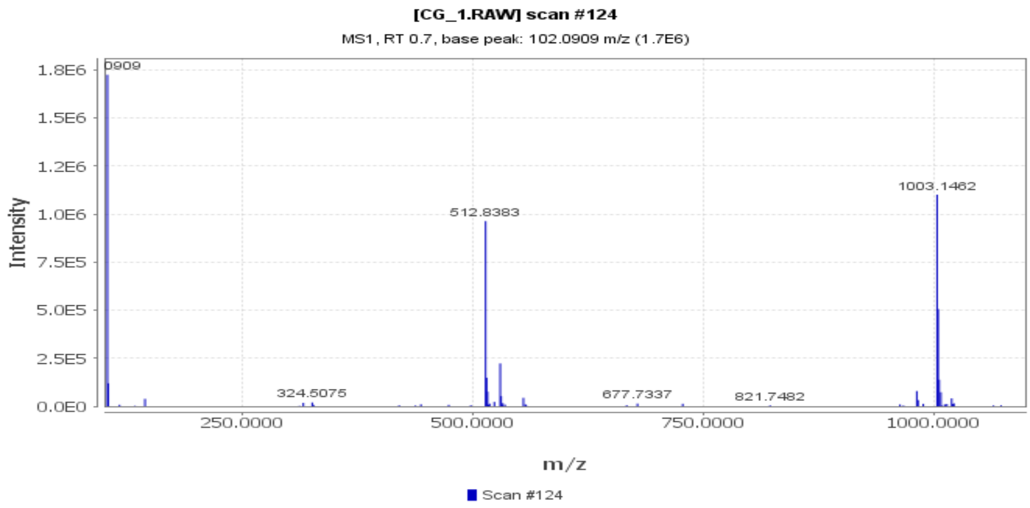
Spectrum 126. HMQC Spectrum of **SCG**



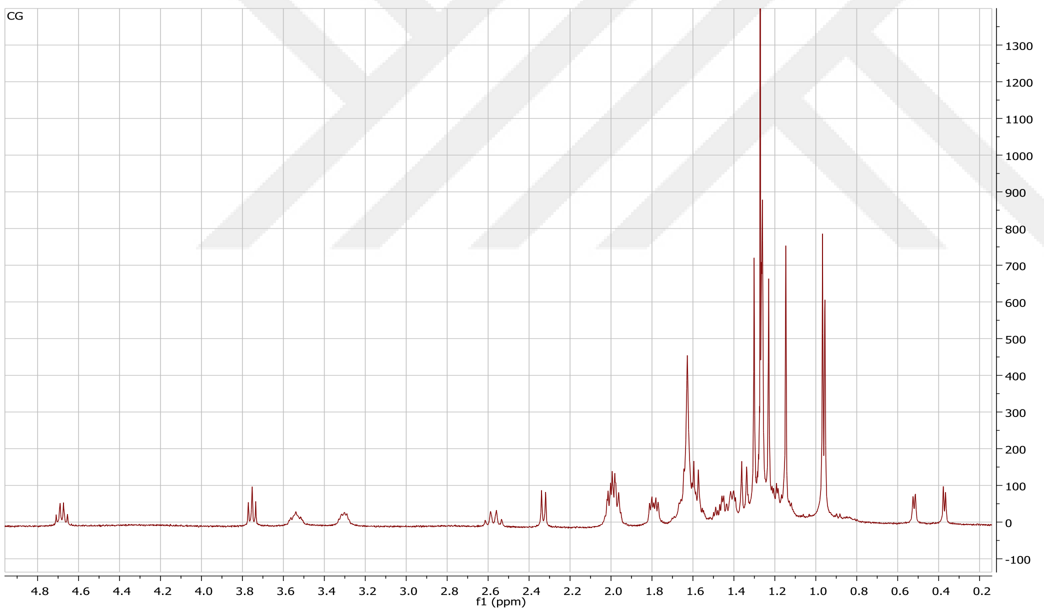
Spectrum 127. HMBC Spectrum of **SCG**



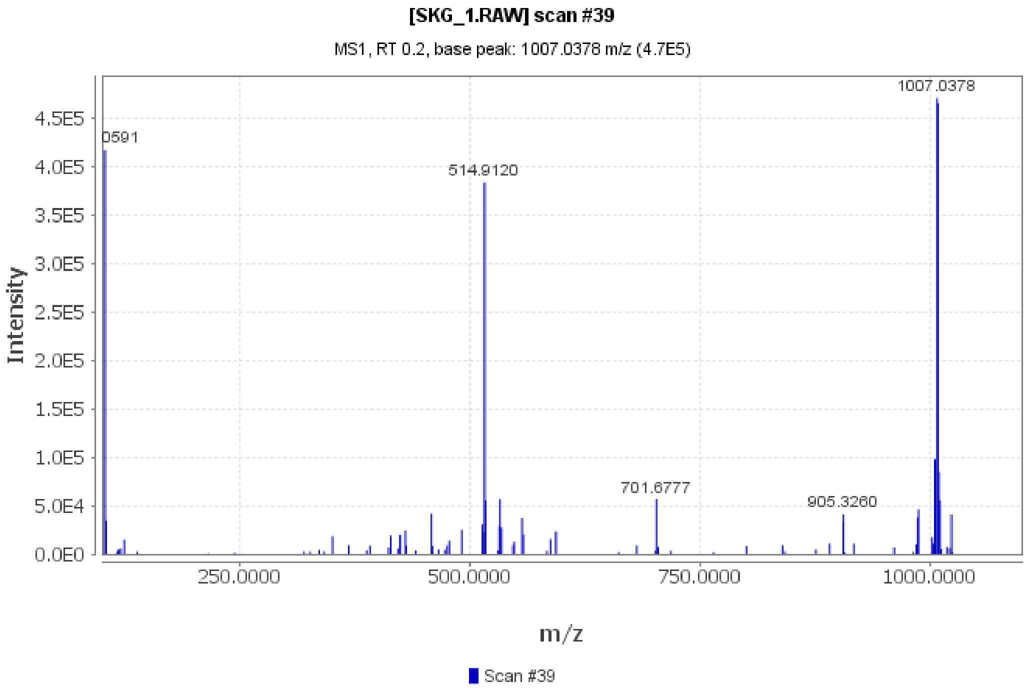
Spectrum 128. Mass Spectrum of **SCG**



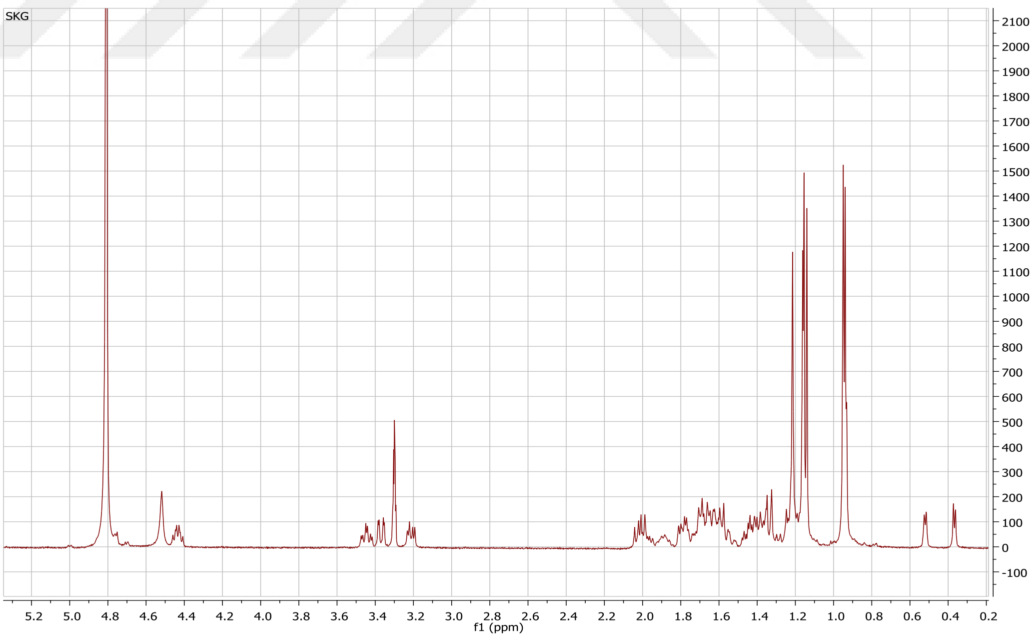
Spectrum 129. Mass Spectrum of **CG**



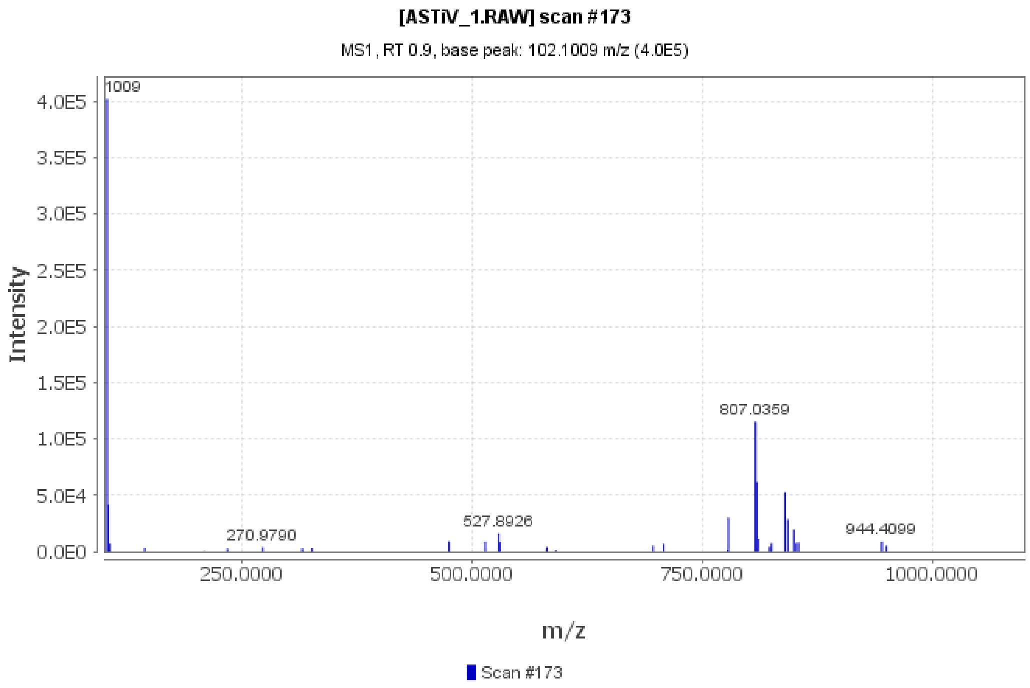
Spectrum 130. ^{1}H-NMR Spectrum of **CG**



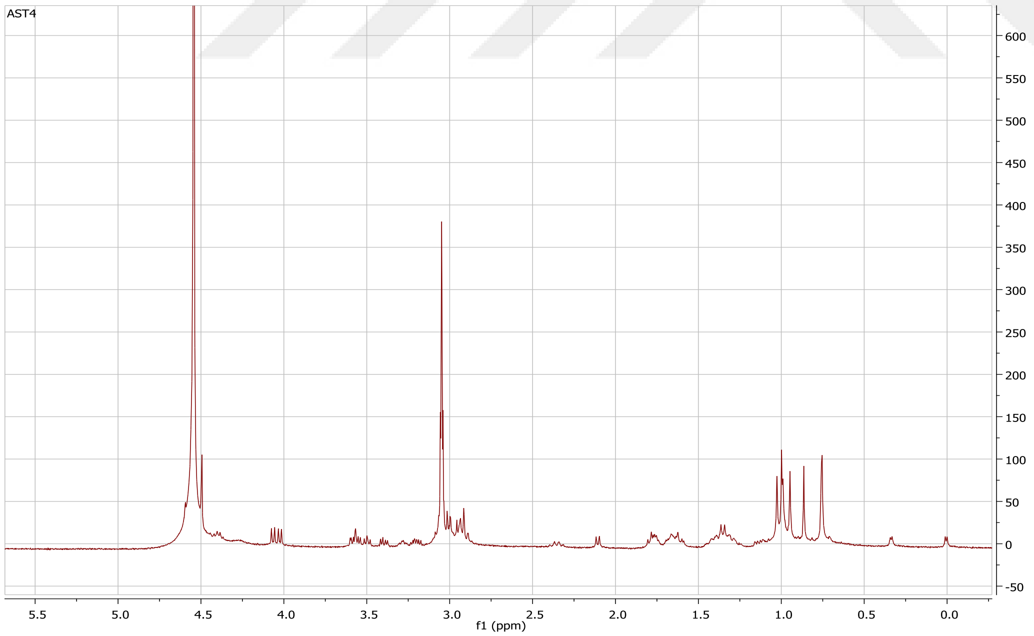
Spectrum 131. Mass Spectrum of **SKG**



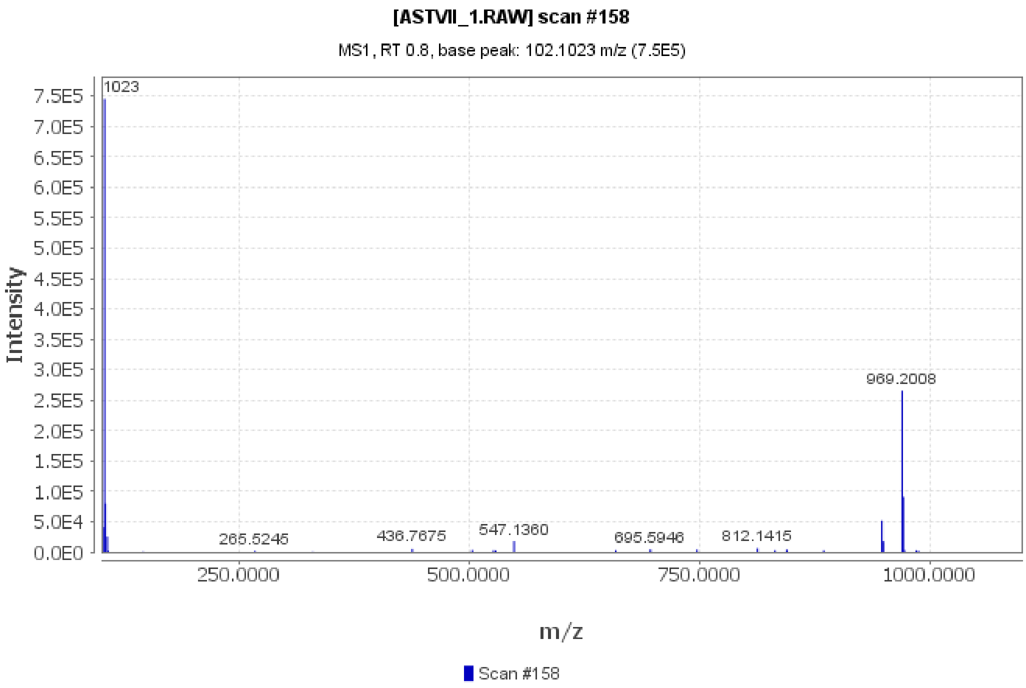
Spectrum 132. [1]H-NMR Spectrum of **SKG**



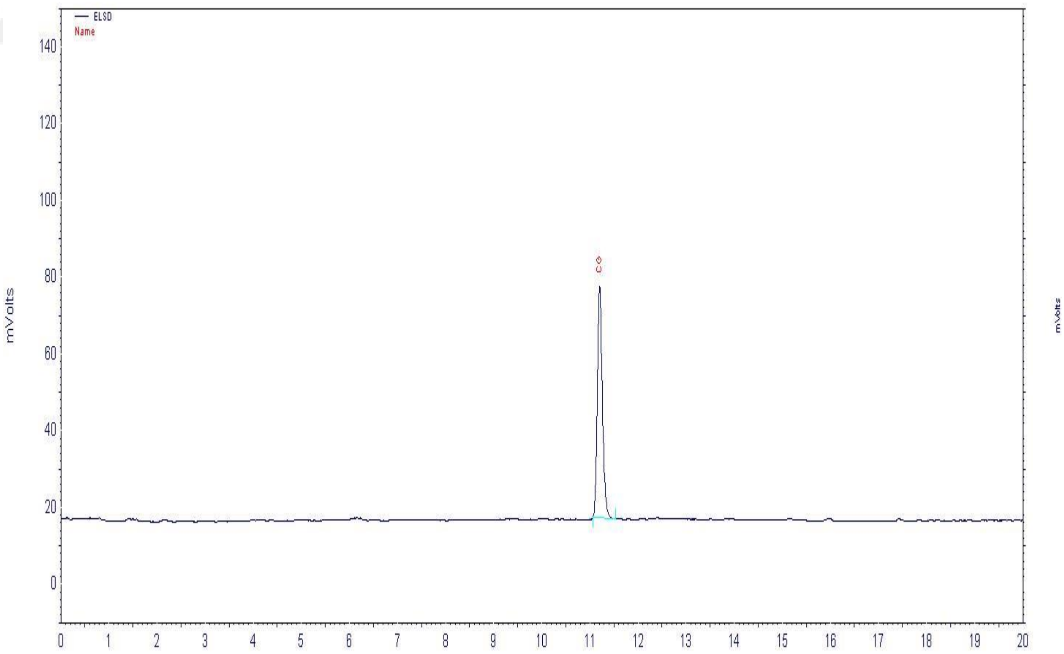
Spectrum 133. Mass Spectrum of **AST4**



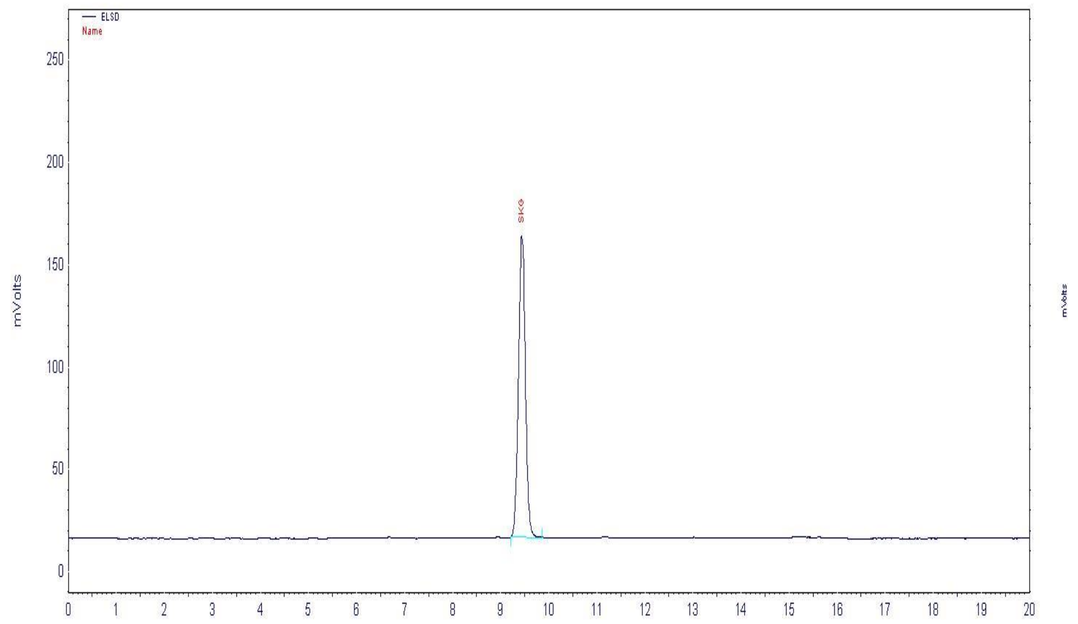
Spectrum 134. ^{1}H-NMR Spectrum of **AST4**



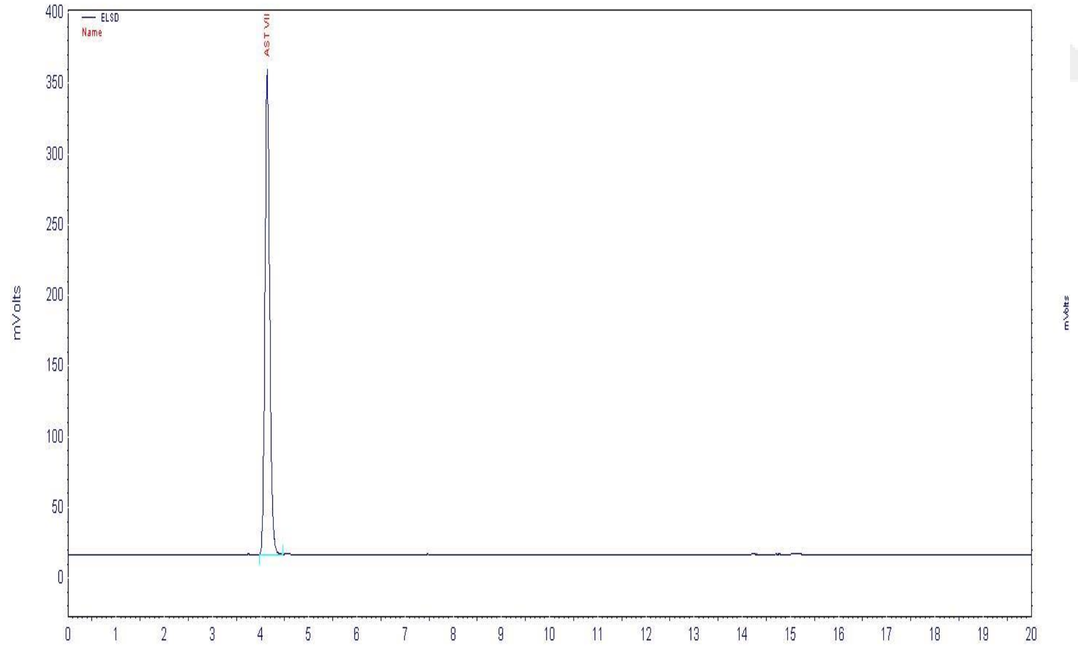
Spectrum 135. Mass Spectrum of **AST7**



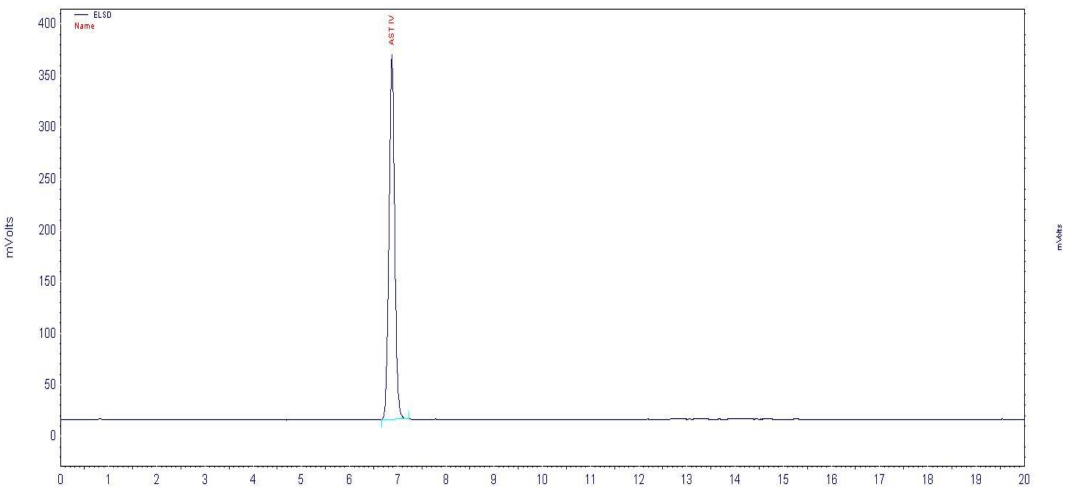
Spectrum 136. HPLC Chromatogram of **CG**



Spectrum 137. HPLC Chromatogram of **SKG**



Spectrum 138. HPLC Chromatogram of **AST7**



Spectrum 139. HPLC Chromatogram of **AST4**

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