

**EGE UNIVERSITY GRADUATE SCHOOL OF
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(Ph. D THESIS)**

**ISOLATION AND CHARACTERIZATION
OF THE SECONDARY METABOLITES OF
ASTRAGALUS AMBLOLEPIS AND
*ASTRAGALUS WIEDEMANNIANUS***

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III

Sayın **Emre POLAT** tarafından DOKTORA TEZİ olarak sunulan **“Isolation and Characterization of The Secondary Metabolites of *Astragalus amblepis* and *Astragalus wiedemannianus*”** başlıklı bu çalışma, E. Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E. Ü. Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi’nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve 08.09.2009 tarihinde yapılan tez savunma sınavında aday oybirliği / oyçokluğu ile başarılı bulunmuştur.

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ABSTRACT

ISOLATION AND CHARACTERIZATION OF THE SECONDARY METABOLITES OF *ASTRAGALUS AMBLOLEPIS* AND *ASTRAGALUS WIEDEMANNIANUS*

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Five new cycloartane-type glycoside were isolated from the methanol extract of the roots of *Astragalus amblolepis* with a known saponin. Additionally, eleven saponins, three of which were new, were isolated from the methanol extract of *Astragalus wiedemannianus*.

Key words: Leguminosae, *Astragalus amblolepis*, *Astragalus wiedemannianus*, cycloartane, oleanane, saponin, glucuronic acid.

ÖZET

ASTRAGALUS AMBLOLEPIS VE ASTRAGALUS WIEDEMANNIANUS'UN SEKONDER METABOLİTLERİNİN İZOLASYONU VE YAPI ANALİZİ

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Astragalus amblolepis bitki köklerinin metanol ekstraktından beşi yeni olmak üzere altı sikloartan tip glikozit izole edilmiştir. Ayrıca, *Astragalus wiedemannianus* bitkisinin metanol ekstraktından 3 yeni 8 bilinen olmak üzere on bir saponin izole edilmiştir.

Anahtar Kelimeler: Leguminosae, *Astragalus amblolepis*,
Astragalus wiedemannianus, sikloartan,
oleanan, saponin, glukuronik asit.

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ABBREVIATIONS

HPLC	High Performance Liquid Chromatography
UV	Ultraviolet
LC-MS	Liquid Chromatography-Mass Spectrometry
DMSO	Dimethylsulfoxide
NMR	Nuclear Magnetic Resonance
1D-NMR	One-Dimensional Nuclear Magnetic Resonance
2D-NMR	Two-Dimensional Nuclear Magnetic Resonance
MALDITOF	Matrix-assisted Laser Desorption Ionization Time-of-flight
MPLC	Medium Pressure Liquid Chromatography
DQF-COSY	Double Quantum Filtered Correlation Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence
HMBC	Heteronuclear Multiple Bond Coherence
1D-TOCSY	One Dimensional Total Correlation Spectroscopy
CC	Column Chromatography
GC	Gas Chromatography

ABBREVIATIONS

RP-VLC	Reverse Phase Vacuum Liquid Chromatography
FT-IR	Fourier Transform Infrared
FAB-MS	Fast Atom Bombardment Mass Spectrometry
TLC	Thin Layer Chromatography
ASCC	Acyclic Side-Chain Cycloartanes
ESCC	Epoxy Side-Chain Cycloartanes
DESCC	Diepoxy Side-Chain Cycloartanes
TESCC	Triepoxy Side-Chain Cycloartanes

1. INTRODUCTION

1.1. Saponins

Saponins are generally known as non-volatile, surface active compounds that are widely distributed in nature, occurring primarily in the plant kingdom (Lasztity et al., 1998; Oleszek, 2002; Hostettmann and Marston, 2005). The name 'saponin' is derived from the Latin word *sapo*, which means 'soap', because saponin molecules form soap-like foams when shaken with water. They are structurally diverse molecules that are chemically referred to as triterpene and steroid glycosides. They consist of non-polar aglycones coupled with one or more monosaccharide moieties (Oleszek, 2002). This combination of polar and non-polar structural elements in their molecules explains their soap-like behaviour in aqueous solutions.

Saponins have a diverse range of properties, which sweetness and bitterness (Grenby, 1991; Kitagawa, 2002; Heng et al., 2006b), foaming and emulsifying properties (Price et al., 1987), pharmacological and medicinal properties (Attele et al., 1999), haemolytic properties (Oda et al., 2000; Sparg et al., 2004), as well as antimicrobial, insecticidal, and molluscicidal activities (Sparg et al., 2004). Saponins have found wide applications in beverages and confectionery, as well as in cosmetics

(Price et al., 1987; Petit et al., 1987; Uematsu et al., 2000), and pharmaceutical products (Sparg et al., 2004).

Although in former times it may have been acceptable to classify compounds based on their physicochemical or biological properties, nowadays it is no longer customary and sensible in natural product chemistry. The structural diversity of compounds showing soap-like properties in aqueous solutions is enormous. Therefore, when the term “saponin” should continue to reflect some value in natural product classification, it should be defined more precisely. This has become possible nowadays because the knowledge on chemical structures of natural products (such as saponins) and their biosynthetic pathways has grown tremendously. These advancements have stimulated the classification of natural products based on the biosynthesis of their carbon skeletons (Devon and Scott, 1972; Connolly and Hill, 1991; Xu et al., 2004). Further classification may be based on subsequent biosynthetic transformations of these main carbon skeletons, such as minor rearrangements, typical oxidation, homologation, or degradation patterns, leading to rearranged, seco, homo, or nor compounds.

Saponins are often subdivided into two main classes, the triterpenoid and the steroid saponins (Abe et al., 1993), which are both derived from the 30 carbon atoms containing precursor oxidosqualene (Haralampidis et al., 2002). The difference between the two classes lies in the fact that the steroid saponins have three methyl groups removed (i.e. they are molecules with 27 C-atoms), whereas in the triterpenoid

saponins all 30 C-atoms are retained. From a biosynthetic point of view, this differentiation is of limited use because particularly the triterpenoid saponins represent many different carbon skeletons. Moreover, the biosynthetic transformations within the two groups are neglected and under-estimated. In a recent review (Sparg et al., 2004), saponins were classified into three classes, namely, the triterpenoid saponins, the spirostanol saponins, and the furostanol saponins. However, such a classification emphasizes incidental structural elements due to secondary biotransformations and does not reflect the main biosynthetic pathways. There are some other classes of compounds that have been considered as saponins, such as the glycosteroidalkaloids (Haralampidis et al., 2002). Although these compounds have the same biosynthetic ancestor as saponins, and contain a steroid type skeleton glycosidically linked to monosaccharide moieties, they will not be considered as saponins in this thesis. Glycosteroidalkaloids contain a nitrogen atom as an intrinsic and characteristic part of their aglycone structure, which already classifies them as a separate group.

The aim of this review is to propose a classification of saponins based on the biosynthesis of the carbon skeletons of the aglycones. Subdivisions of the main classes are based on further modification of the carbon skeletons by minor rearrangement, homologation, cleavage, and degradation. Functionalization of carbon skeleton is predominantly the result of oxidation, resulting in functional groups like hydroxyl groups, carbonyl groups and carboxylic acids, which can be found at many positions in the molecules. These functional groups are further involved

in mutual chemical reactions, which may lead to additional characteristic structural elements, such as ether bridges, spiroketals or lactones. Subdivisions based on these structural elements will not be undertaken in this thesis, as they are the results of secondary type biotransformations.

When saponins are classified based on the biosynthesis of the carbon skeletons, it may be expected that similar types of saponins are found in similar plant orders. This expectation is based on the assumption that similar plant orders contain similar types of enzymes, and that these enzymes can probably catalyse similar biotransformations, ultimately leading to similar chemical structures (Vierhuis et al., 2001; Umezawa, 2003). Therefore, attempts were made to link the various carbon skeletons and their substitution patterns to plant orders.

1.2. Classification Of Saponins

1.2.1. Cyclization Of The Saponin Skeleton From Oxidosqualene

Saponin biosynthesis proceeds via the isoprenoid pathway in which 3 isoprene units (molecules containing 5 C-atoms) are first linked in a head-to-tail manner to each other, resulting in the 15 C-atom molecule farnesyl pyrophosphate. Two farnesyl pyrophosphates are subsequently linked in a tail-to-tail manner to give a compound of 30 carbon atoms, called squalene. Squalene is oxidized to oxidosqualene, which is the

common starting point for cyclization reactions in triterpenoid biosynthesis (**Figure 1.1.**) (Abe et al., 1993; Haralampidis et al., 2002). Oxidosqualene is converted to cyclic derivatives via protonation and epoxide ring opening, which creates a carbocation that can undergo several types of cyclization reactions. After these cyclizations, subsequent rearrangements can proceed in different ways by a series of hydride shifts and/or methyl migrations, which lead to the formation of new carbocations. Finally, the carbocations are neutralized by proton elimination to give a double bond or a cyclopropanyl ring, or by reaction with water to give a hydroxyl group. In **Figure 1.1.**, the main cyclization and rearrangement reactions are shown that lead to the triterpenoid and steroid skeletons that have been found to occur in the saponins in our literature search.

The type of cyclase that is involved in the cyclization reaction primarily determines the skeleton that is formed (**Figure 1.1.**). Many different kinds of cyclases (e.g. cycloartenol synthase, lanosterol synthase, β -amyrin synthase) have been described, and their mechanisms of action are well documented (Abe et al., 1993; Haralampidis et al., 2002; Wendt et al., 2000; Thoma et al., 2004; Wendt, 2005;). Cyclization of oxidosqualene to saponins can proceed in two ways, either via the 'chair- chair- chair' or via the 'chair- boat- chair' conformation. An important difference between the two resulting skeletons lies in the stereochemistry, which is most clearly shown by the configurations of the C-8 and the C-14 atoms. After cyclization of the 'chair- chair- chair' conformation, the methyl group at the C-8 atom is pointing upwards and

the one at the C-14 atom is pointing downwards, whereas the opposite is the case after cyclization of the ‘chair- boat- chair’ conformation (see the dammarenyl and protosteryl carbocations, respectively, **Figure 1.1.**).

A proton-initiated cyclization of the ‘chair- chair- chair’ conformation results in the tetracyclic dammarenyl C-20 carbocation, and all saponins derived from this carbocation are classified as ***dammarene type saponins***. A series of hydride and methyl shifts in the dammarenyl carbocation leads to the tirucallenyl C-8 carbocation, and all saponins derived from this carbocation are classified as ***tirucallane type saponins***. The 5-membered ring next to the C-20 dammarenyl carbocation can expand either by a shift of the C-16–C-17 bond, or by a shift of the C-13–C-17 bond. A shift of the C-16–C-17 bond leads to the tetracyclic C-17 baccharenyl carbocation and can be followed by a reaction with the C-24–C-25 double bond to produce the pentacyclic C-25 lupenyl carbocation. All saponins derived from this carbocation are classified as ***lupane type saponins***. The lupenyl carbocation can be rearranged further, first to the C-18 germanicenyl carbocation, and then via a series of hydride shifts to the C-13 oleanyl carbocation. All saponins derived from this oleanyl carbocation are classified as ***oleanane type saponins*** (Sparg et al., 2004). Oleanane type saponins have been isolated from a wide array of plants, and this skeleton is also referred to as the β -amyrin skeleton (Haralampidis et al., 2002). A shift of the α methyl group in the germanicenyl carbocation produces the C-20 taraxasterenyl carbocation, which can be deprotonated to yield ***taraxasterane type saponins***. A methyl shift in the germanicenyl carbocation, followed by several

hydride shifts, ultimately produces the C-13 carbocation, which can be deprotonated to *ursane type saponins*. The ursane skeleton is also called the α -amyrin skeleton. The α -amyrin and β -amyrin skeletons are cyclization products of distinct cyclases, α -amyrin synthase and β -amyrin synthase, respectively (Haralampidis et al., 2002).

A shift of the C-13–C-17 bond in the C-20 dammarenyl carbocation leads to a C-17 carbocation, which can be cyclized by a reaction with the double bond in the side chain to form the C-25 pentacyclic hopenyl carbocation. All saponins derived from this carbocation are classified as *hopane type saponins*. It has been shown that, in bacteria, hopanes are cyclized from squalene by squalene-hopane cyclase. There is no information in literature, whether hopanes in plants are formed from oxidosqualene (as indicated in **Figure 1.1.**) or from squalene. All hopane type saponins described in plants (see above) contain a hydroxyl group at the C-3 atom.

From the proton-initiated cyclization of the ‘chair- boat- chair’ conformation of oxidosqualene, a tetracyclic protosteryl C-20 carbocation is obtained, which undergoes a series of hydride and methyl shifts ultimately leading to the intermediate C-9 lanosteryl carbocation. This carbocation can undergo further shifts of a methyl group and a hydride to the C-5 cucurbitanyl carbocation. All saponins derived from this carbocation are classified as *cucurbitane type saponins*. The lanosteryl carbocation may also undergo deprotonation of the C-19 methyl group leading to formation of a cyclopropane ring as is found in

cycloartenol. All saponins derived from cycloartenol are classified as *cyclartane type saponins*.

Deprotonation of the lanosteryl carbocation gives lanosterol and all saponins derived from lanosterol are classified as *lanostane type saponins*. Lanosterol can also undergo demethylation and isomerisation of the double bond, leading to cholesterol. The saponins derived from this skeleton are classified as *steroid type saponins*. The difference in deprotonation, to a cyclopropane ring or a double bond, indicates that the cyclization is catalysed by different cyclases (cycloartenol and lanosterol synthase, respectively).

After cyclization, rearrangement and degradation, additional modifications of the different carbon skeletons follow, ultimately yielding the saponins that are found in nature. These modifications include mostly oxidation and glycosylation reactions. Very little is known about the enzymes and the biological pathways mediating these modifications (Haralampidis et al., 2002). The diversity of saponin aglycones can be seen from several reviews. Besides the carbon skeleton, the aglycones differ in their number and/or type of substituents attached (e.g. $-\text{OH}$, $=\text{O}$, CH_3 , $-\text{CH}_2\text{OH}$, $-\text{CHO}$, $-\text{COOH}$, and or glycosyl residues), the number and position of double bonds, lactones, and spirocompounds.

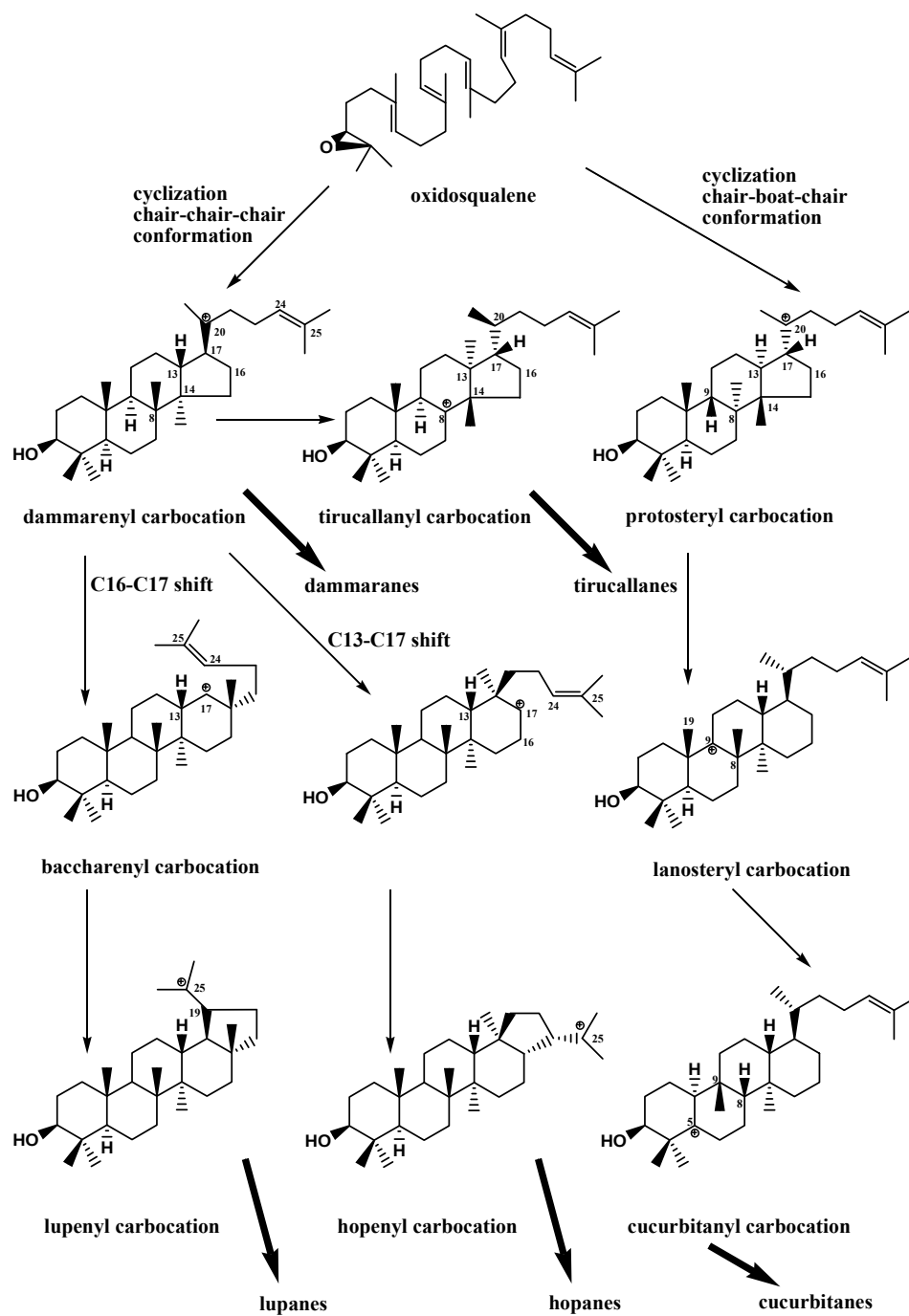


Figure 1.1. The cyclization of oxidosqualene to the various saponin skeletons

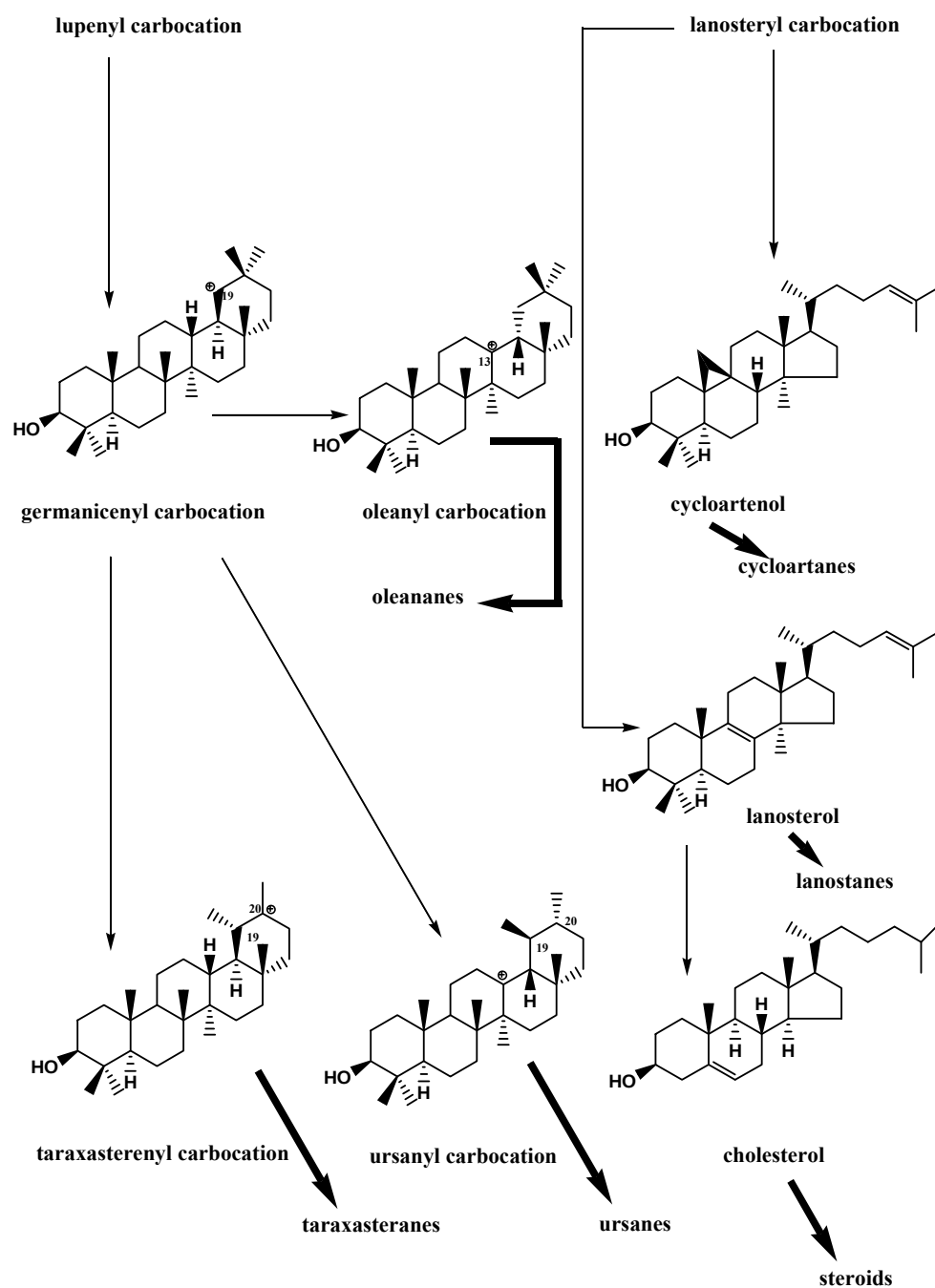


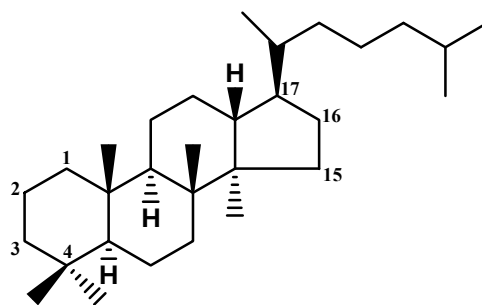
Figure 1.1. The cyclization of oxidosqualene to the various saponin skeletons (Continued)

1.2.2. Classification Of Saponins Based On Their Basic Skeletons

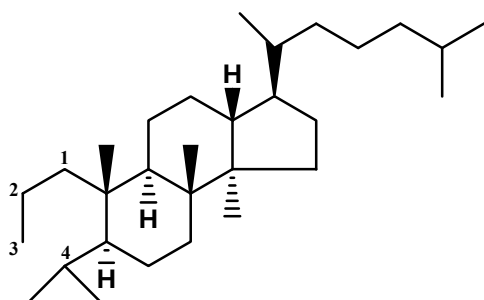
The 11 carbon skeleton shown in **Figure 1.1.**, dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, cucurbitanes, cycloartanes, lanostanes, and steroids, represent the end products of cyclization, rearrangement and degradation reactions, which cover the main saponin skeletons that have been found in our literature search. There are, however, several other biosynthetic pathways known for triterpenoids, leading to different types of triterpenoid skeletons. An example is the friedelanes (Corsino et al., 2000), which have not been included in our overview as the glycosylated form of this compound has not been described in the literature to our knowledge. Moreover, several other triterpene skeletons may occur in nature as glycosides, but have not been characterized as saponins. Although we may have overlooked some of these glycosylated triterpenoids and steroids, this does not interfere with the main principle of classification of saponins based on the biosynthesis of the carbon skeleton of their aglycones. New skeleton can be introduced easily in this system, following the biosynthesis of their carbon skeleton, as soon as sufficient examples of such new skeletons have been found in nature.

Of the 11 main saponin skeletons mentioned in this thesis, several have been found to undergo ring cleavage (leading to seco-skeletons), homologation (leading to homo or bis-homo skeletons), degradation to nor or bis-nor compounds, or minor rearrangement to related skeletons.

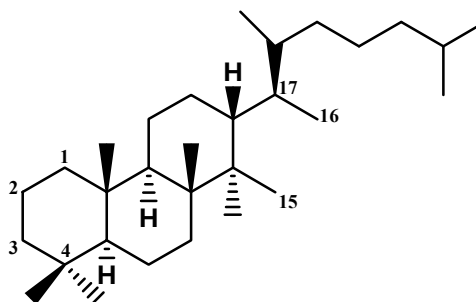
All these secondary modifications lead to skeletons, which are indicated as separate subclasses as shown in **Figure 1.2.**



Dammaranes (1)

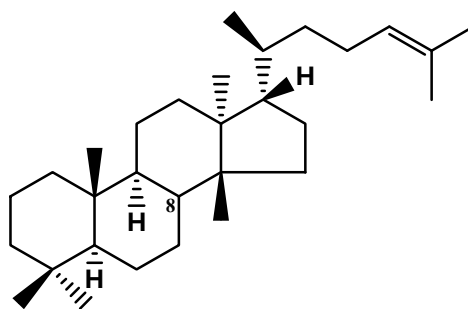


3,4-seco dammaranes (1a)

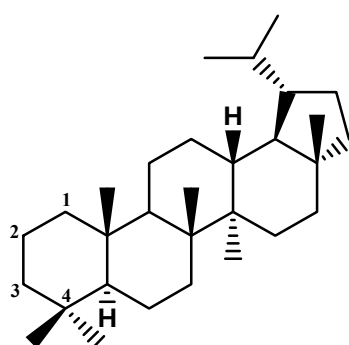


15,16-seco dammaranes (1b)

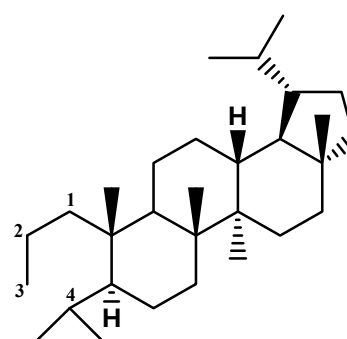
Figure 1.2. Structures of the 11 main classes of carbon skeletons of saponins after cyclization, and their various derivatives



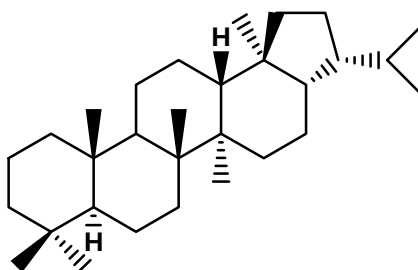
Tirucallanes (2)



Lupanes (3)

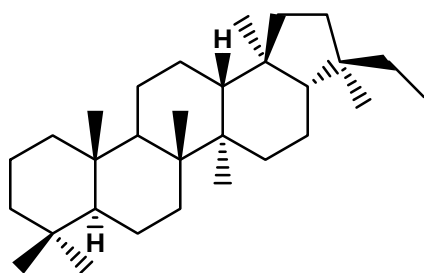


3,4-seco lupanes (3a)

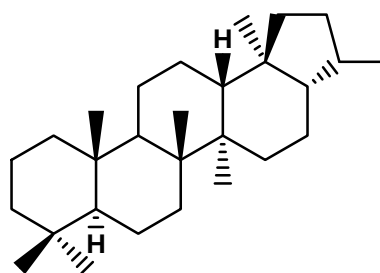


Hopanes (4)

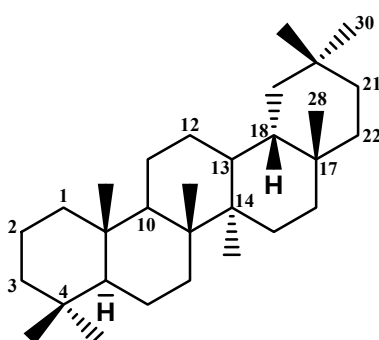
Figure 1.2. Structures of the 11 main classes of carbon skeletons of saponins after cyclization, and their various derivatives (Continued)



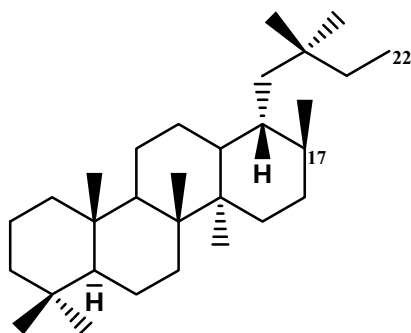
Rearranged hopanes (4a)



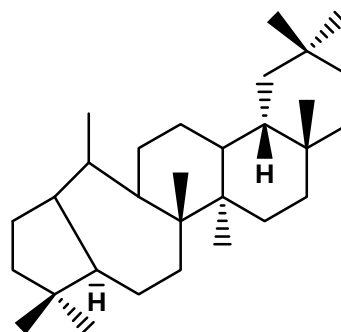
Bis-nor hopanes (4b)



Oleananes (5)

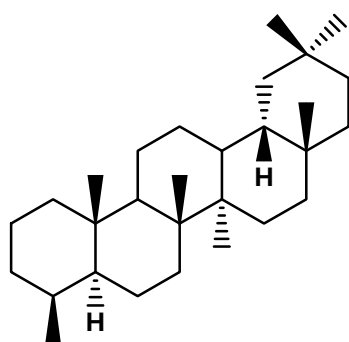


17,22-seco oleananes (5a)

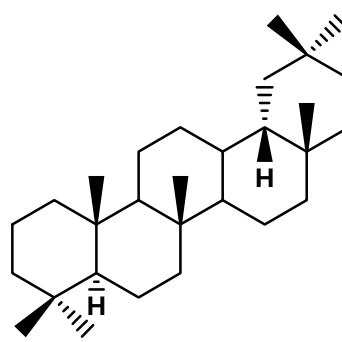


Rearranged oleananes (5b)

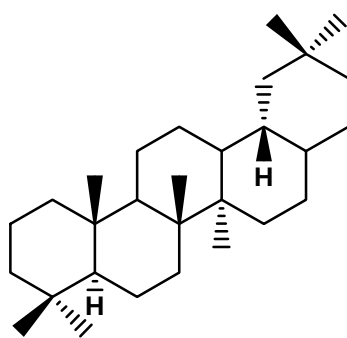
Figure 1.2. Structures of the 11 main classes of carbon skeletons of saponins after cyclization, and their various derivatives (Continued)



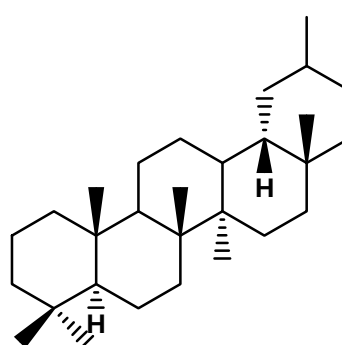
23-nor oleananes (5c)



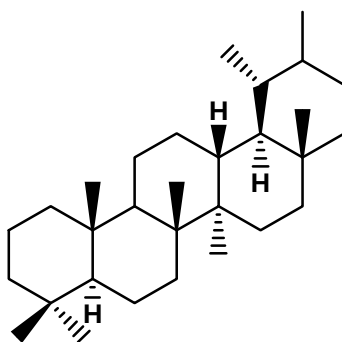
27-nor oleananes (5d)



28-nor oleananes (5e)

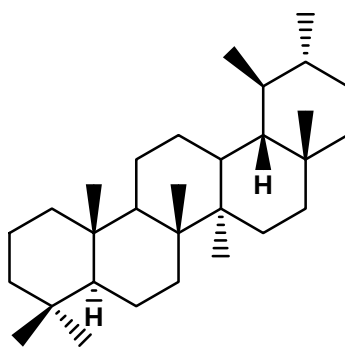


30-nor oleananes (5f)

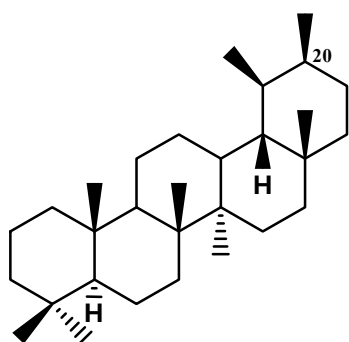


Taraxasteranes (6)

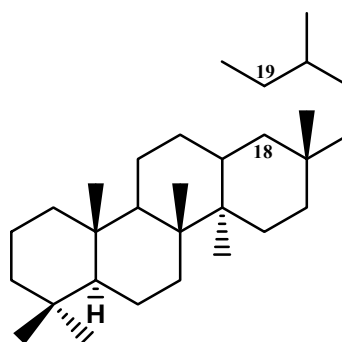
Figure 1.2. Structures of the 11 main classes of carbon skeletons of saponins after cyclization, and their various derivatives (Continued)



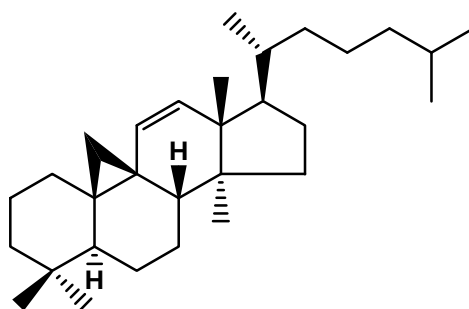
Ursanes (7)



20-epi-ursanes (7a)

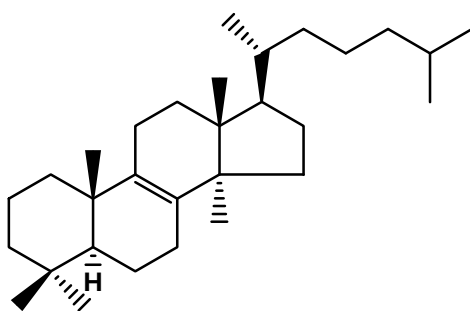


18,19-seco ursanes (7b)

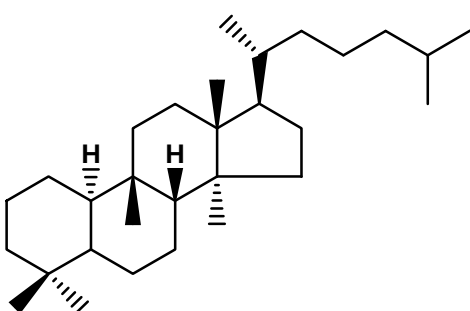


Cycloartanes (8)

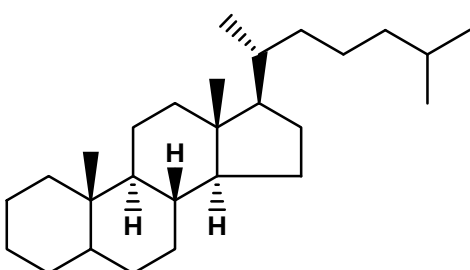
Figure 1.2. Structures of the 11 main classes of carbon skeletons of saponins after cyclization, and their various derivatives (Continued)



Lanostanes (9)



Cucurbitanes (10)



Steroids (11)

Figure 1.2. Structures of the 11 main classes of carbon skeletons of saponins after cyclization, and their various derivatives (Continued)

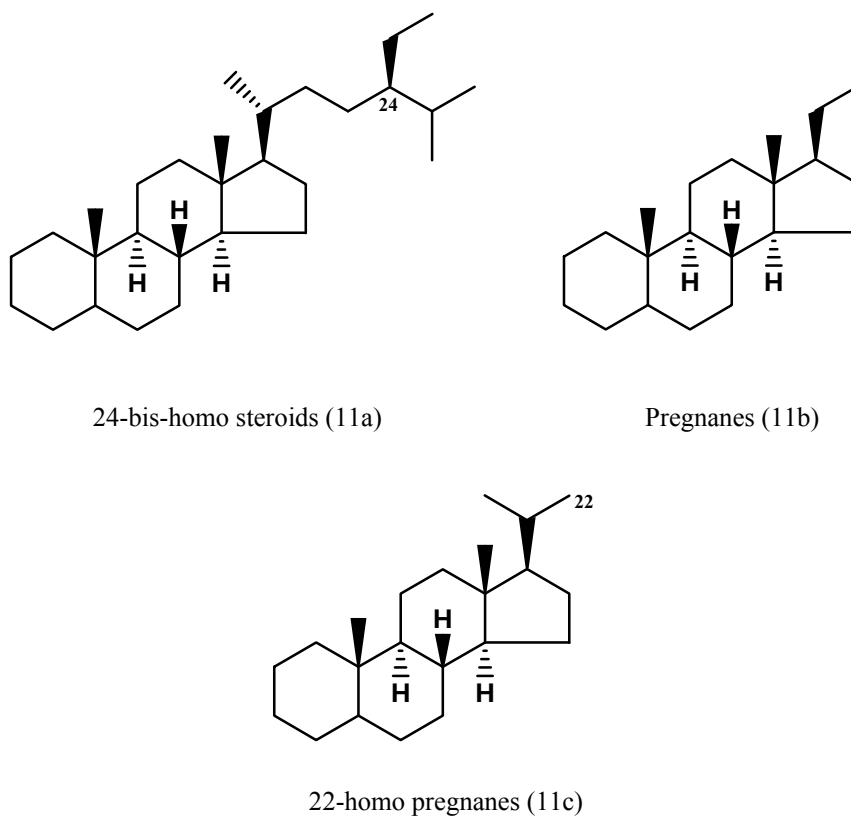


Figure 1.2. Structures of the 11 main classes of carbon skeletons of saponins after cyclization, and their various derivatives (Continued)

The skeletons in **Figure 1.2.** are numbered according to the IUPAC rules; in the seco, homo, and nor compounds the numbering of the main skeleton is maintained.

The dammarane skeleton (1) can undergo further processing to give the 3,4-seco skeleton (1a), and the 15,16-seco skeleton (1b). The lupane skeleton (3) can be cleaved to give the 3,4-seco skeleton (3a). The side chain at the five membered ring in the hopane skeleton can be rearranged to the rearranged hopanes (4a) or be degraded to the bis-nor hopanes (4b). The oleanane skeleton (5) can be fragmented to the 17,22-seco skeleton (5a), or rearranged to a ring A nor-ring B homo skeleton (5b). Degradation of the oleanane skeleton leads to the 23-nor (5c), the 27-nor (5d), the 28-nor (5e), or the 30-nor skeletons (5f). The ursane skeleton can be rearranged to the 20-epi skeleton (7a), or cleaved to the 18,19-seco skeleton (7b). The steroid skeleton (11) can be homologated to the 24-bis-homo skeleton (11a). Furthermore, the steroid skeleton can be degraded in the side-chain to the nor-skeleton (11b), which belongs to the pregnane type steroids, and to the skeleton (11c), which has been indicated as a 22-homo pregnane skeleton.

1.3. Astragalus Genus

There are two groups of *Astragalus* species that are closely related to health (Rios and Waterman, 1997).

1. The Toxic Species
2. The Medicinal Plants

1.3.1. The Toxic Species

The poisonous *Astragalus* species can be classified into three general groups, according to the toxins and their effects on animals:

1.3.1.A. Species That Synthesize Aliphatic Nitro Compounds

Some species can synthesize aliphatic nitro compounds and they are transformed in the rumen to the toxic principles 3-nitro-1-propanol (3-NPOH) (**Figure 1.3.**) and 3-nitropropionic acid (3-NPA) (**Figure 1.4.**). It seems that an individual species can synthesize 3-NPOH or 3-NPA derivatives, but not both (Williams, 1992). The 3-NPOH derivatives occur as β -glucosides (miserotoxin) and the 3-NPA compounds can be mono, -di, or -tri glucose esters (Infante et al., 1991). Species with this kind of principle include *A. miser*, *A. falcatus*, *A. glycyphyllos*, and *A. hamosus* (Williams and Davis, 1982). *A. lusitanicus*, a species widely distributed in Southwestern Spain, Portugal and Northern Africa, causes livestock poisoning. The results obtained from studies on the toxins of this species are controversial, but Tarazona and Sanz (1987) reported the presence of aliphatic nitro compounds and they hypothesize that these compounds are responsible for the toxicity. Although nitro derivatives liberated in the toxin metabolism produce methaemoglobinaemia, it is not the cause of death in intoxication with *Astragalus*.

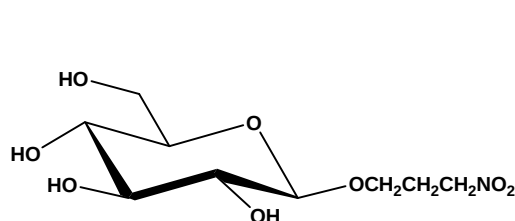


Figure 1.3. Miserotoxin (3-nitro-1-propyl-β-D-glucopyranoside)

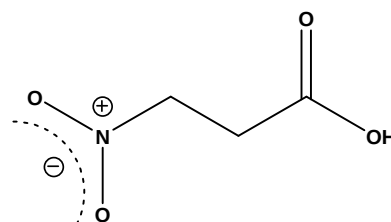
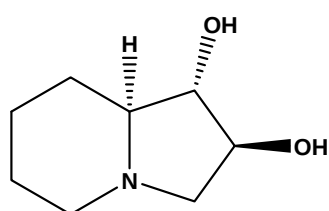


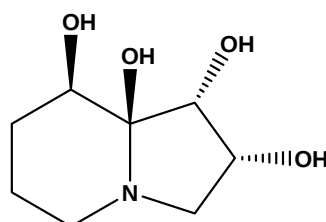
Figure 1.4. 3-nitropropionic acid (3-NPA)

1.3.1.B. Species Causing Locoweed Poisoning

The plants that cause locoweed poisoning (‘loco’ is a Spanish word that means crazy) contain swainsonine, an indolizidine alkaloid (**Figure 1.5**).



Lentiginosine



Swainsonine

Figure 1.5. Indolizidine alkaloids glycosidase inhibitors occurring in *Astragalus* species

All, to date, are species of sections *Astragalus* and *Oxytropis*. Livestock must graze these plants for a long period of time before signs of intoxication appear. Intoxication with these plants produces neurological disturbances, emaciation, habituation, reproductive alterations, and congestive right-heart failure. Swainsonine is now being studied for its antimetastatic properties, and castanospermine, a related alkaloid, as an anti-HIV agent (James et al., 1990). *Astragalus lentiginosus*, *A. pubentissimus*, *A. mollissimus*, *A. wootoni*, *A. thuseri*, and *A. nothrosys* are species included in this group.

Swainsonine is eliminated partially in milk and it can be fed to young calves, and induces high mountain disease when the animals graze at high elevation (James et al., 1990). The alkaloid completely inhibits acidic α -mannosidases at different concentrations in various tissues, but it is less effective against β -mannosidase and some other enzymes. This effect alters the synthesis of glycoproteins, but does not appear to produce clinical effects, although the associated accumulation of lysosomal storage products produces the same symptoms as locoweed poisoning (Abraham et al., 1983). The accumulation of mannose-rich oligosaccharides in testis and epididymis induces biochemical abnormalities in the reproductive tissues and impacts on male reproduction (Tulsiani et al., 1990).

The ingestion of locoweed by pregnant livestock may result in fetal malformations, delayed placentation, reduced placental and uterine vascular development, hydrops amnii, hydrops allantois, abnormal

cotyledonary development, interruption of fetal fluid balance and abortion (Bunch et al., 1992).

1.3.1.C. Species That Can Accumulate Selenium

The third group includes plants growing on seleniferous soils that are able to accumulate several thousand ppm of selenium. Chronic poisoning is characterized by rough hair coat, loss of hair, hoof lesions, lameness, decreased vitality, loss of appetite, emaciation, and a decrease in reproductive efficiency. In livestock, reduced reproductive performance is the most significant effect of chronic intoxication (James et al., 1990). The selenium compounds are toxic because of their pro-oxidant catalytic activity to produce superoxide, hydrogen peroxide, and probably other cascading oxy-radicals, but they can also have a carcinostatic effect (Spallholz, 1994). Among the known seleniferous species are *A. bisulcatus*, *A. saurinus*, *A. praelongus*, *A. flavus*, and *A. tenellus* (Lowgill and Landenberger, 1992).

1.3.2. The Medicinal Plants

Several *Astragalus* species are used as medicinal plants, especially *A. membranaceus*, which is the one most widely used and studied. It is officially listed in the Chinese Pharmacopoeia, as the species *A. complanatus* and *A. mongholicus* (*A. membranaceus* var. *mongholicus*).

Some pharmaceutical preparations are made from extracts of these species. The pharmacologically active constituents of *Astragalus* belong to two different kinds of chemical compounds, polysaccharides and saponins, and the most interesting pharmacological properties are hepatoprotective, immunostimulant and antiviral (Rios and Waterman, 1997).

1.3.2.A. Polysaccharides

Pectins, resins, and mucilages are the major polysaccharides (**Figure 1.6.**) used in medical practice. Pectins serve as emulsifiers and stabilizers, constitute the main components of ointments with salicylic and boric acids, and enter the composition of drugs decreasing blood cholesterol content and modulating bile acid metabolism. Resins are used for the production of oil emulsions, mucilaginous solutions, and blood substitute liquids. Aqueous and mucilaginous extracts prepared from mucilage-containing plants, flax and marsh mallow, are used to treat catarrhs and gastrointestinal tract lesions and to counteract the irritant effects of other drugs (Lovkova et al., 2001).

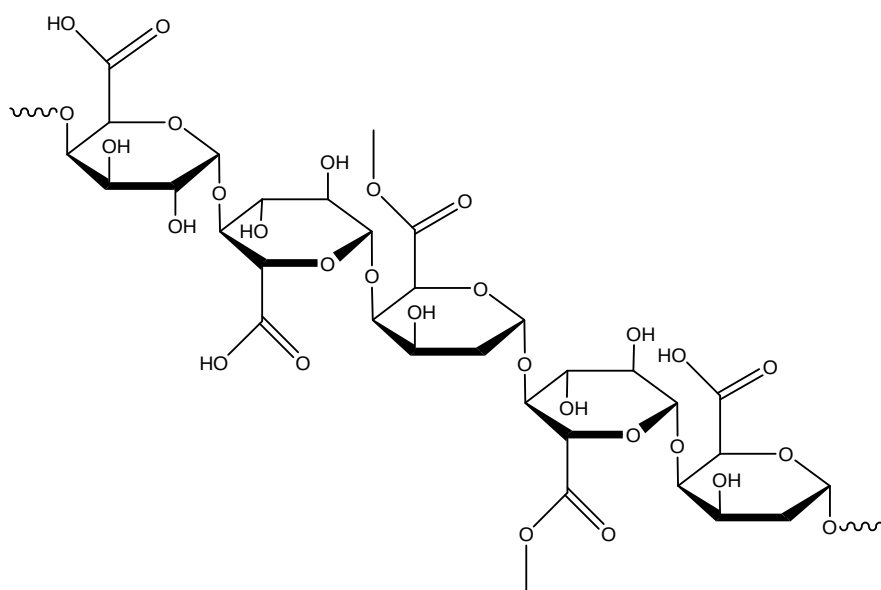


Figure 1.6. Polysaccharide chain

1.3.2.B. Cycloartane Saponins

Triterpenoids common secondary plant metabolites possessing different frameworks such as cycloartanes, lanostanes, and oleananes. Cycloartenols occupy a special place among the bioregulators having low molecular weight. Because they are only produced by the organisms carrying photosynthesis, and they serve as a key intermediate in the biosynthesis of different phytosterols. The biosynthesis of the phyto and zoosterols are divided into branches during cyclization step of squalene-2-3-epoxide. All steroidal metabolites originate biogenetically from this single precursor. While lanosterol is biosynthesized from squalene-2-3-

epoxide in animal organisms, fungi and yeasts in the pathway resulting cholesterol and other steroids; in higher plants and algal, cycloartenol takes place as key intermediate (**Figure 1.7.**).

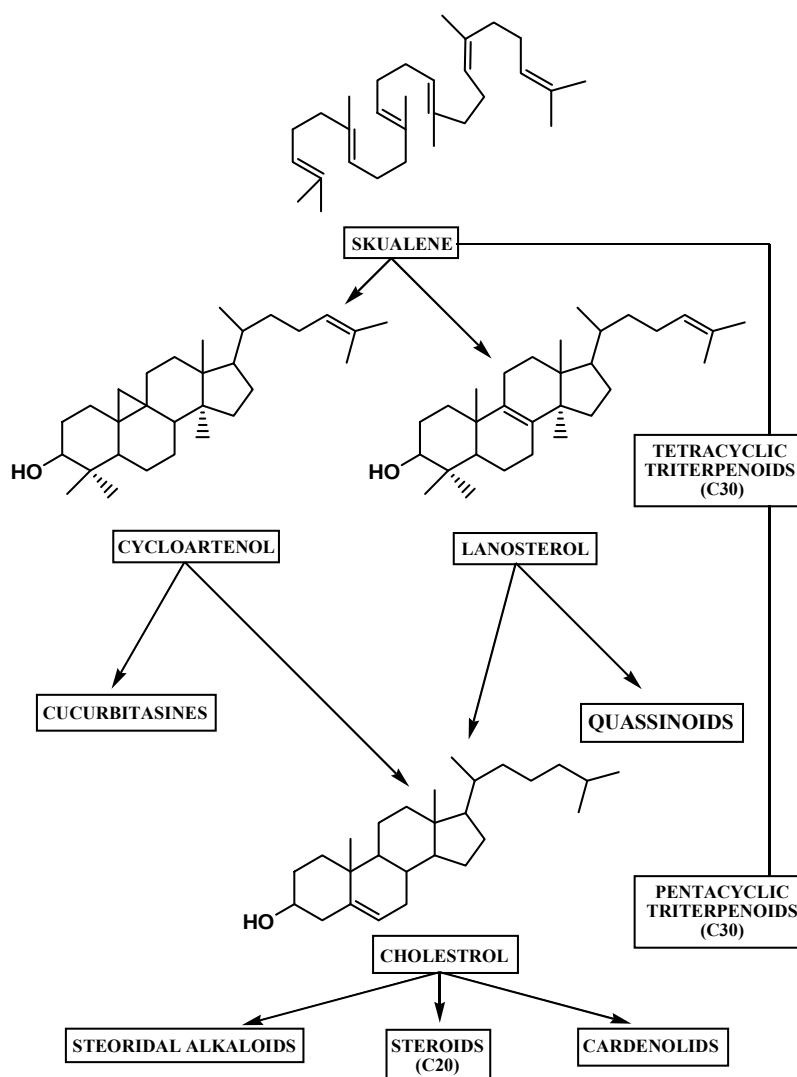


Figure 1.7. Biosynthesis of Triterpenes and Steroids

Enlightening of this step caused an interest for cycloartenols. However, the reason of attention is not only discovery of this step, but also spring from these compounds having a large biological activity spectrum.

Nevertheless, whereas cycloartanes and lanostanes are biogenetically related, oleananes (**Figure 1.10.**) have no biogenetic link to them. A peculiarity of phytosteroid biosynthesis is the generation of an additional 9,19-three-membered ring during cyclization of squalen-2,3-oxide with subsequent opening of this ring. This is responsible for the biogenetic relationship of cycloartane (**Figure 1.9.**) and lanostane (**Figure 1.8.**) triterpenoids.

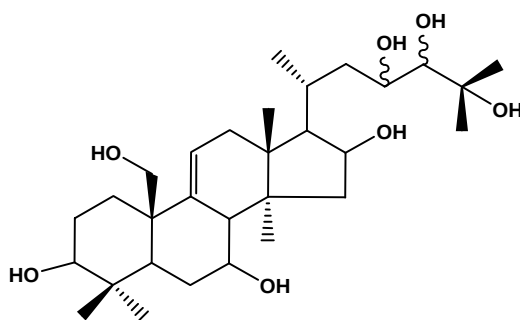


Figure 1.8. Orbigenin (Lanostane Type Glycoside)

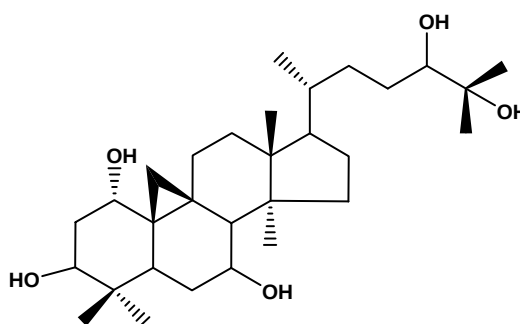


Figure 1.9. Cyclomacrogenin B (Cycloartane Type Glycoside)

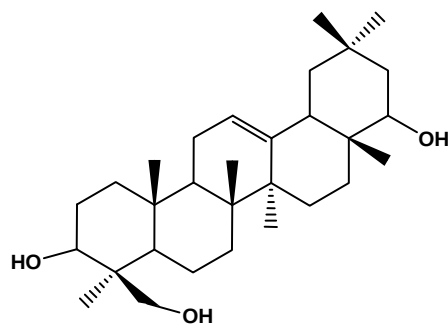


Figure 1.10. Sapogenol B (Oleanane Type Glycoside)

Just two groups of natural compounds carry the 9,19-three-membered ring (cyclopropane) and steroidal nucleus together. These are the alkaloids of the plants of *Buxus* and cycloartenols (**Figure 1.11.**) (Cerny and Sorn, 1967).

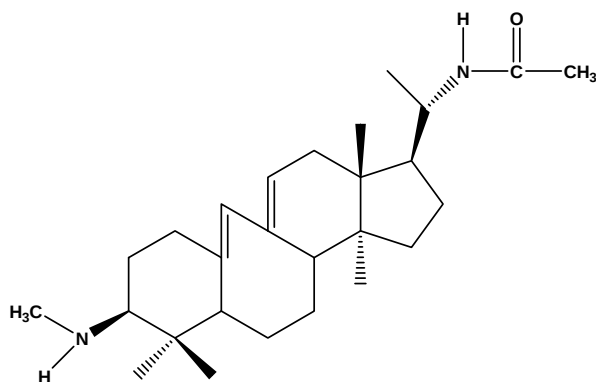


Figure 1.11. N₂₀-acetylbuxamine (Isolated from *Buxus sempervirens*)

Cycloartane triterpenoids were first discovered in *Astragalus* plants. Plants of this genus drew attention to themselves after it was established that they produce cycloartane triterpenoids. These studies turned a new page in the study of *Astragalus* plants. Since then, their content of cycloartane methylsteroids and glycosides have come under intense scrutiny in many scientific centers of the world. Cycloartanes are derivatives of 9 β ,19-cyclolanostane and are produced exclusively by photosynthetic eukaryotes. Cycloartanes dominate the known triterpenoids in plants of this genus.

As a result, while in the 1970's, that is, after the enlightening of the first cycloartenol structure, only 45 variations were known; this number reached 200 in the middle of 1980's and now it is over 500.

It is observed that these compounds are commonly found especially in the following 8 families.

Leguminosae	(<i>Astragalus</i>)
Ranunculaceae	(<i>Thalictrum</i> , <i>Beesia</i> , <i>Souliea</i> , <i>Cimicifuga</i>)
Meliaceae	(<i>Heynea</i> , <i>Aglaia</i>)
Orchidaceae	(<i>Cirrhopetalum</i> , <i>Pholidata</i> , <i>Otochilus</i>)
Passifloraceae	(<i>Passiflora</i>)
Combretaceae	(<i>Combretum</i>)
Asteraceae	(<i>Balsamorhiza</i>)
Anacardiaceae	(<i>Mangifera</i>)

Regarding the fact that the nearly 172 of the cycloartanes, whose number have exceeded 500 today, are originated from *Astragalus*, it can be understood how rich this genus in terms of cycloartanes is.

A total of 44 structurally distinct genins have been found in the plants under discussion (**1-44**). Some of these (**6, 7, 11, 29, 37**) have not been characterized as pure compounds but as the corresponding glycosides. Methylsteroids **1, 2, 19**, and **21** have been found only in the free state.

The structures of **1-44** show that the side chains of the compounds differ substantially. Therefore, our classification of these substances is based on structural features of the side-chain.

1. Cycloartanes which have acyclic side-chain
2. Cycloartanes which have 20,24-epoxy side-chain
3. Cycloartanes which have 20,25-epoxy side-chain
4. Cycloartanes which have 16 β ,23;16 α ,24-diepoxy side-chain
5. Cycloartanes which have 16 β ,24;20,24-diepoxy side chain
6. Cycloartanes which have 24-nor-16 β ,23-epoxy side-chain
7. Cycloartanes which have 16 β ,23;22 β ,25-diepoxy side-chain
8. Cycloartanes which have 16 β ,23;23,26;24,25-triepoxy side-chain
9. Cycloartanes Which Have 16 β ,23-Epoxy Side-Chain

1.3.2.B.1. Cycloartanes Which Have Acyclic Side-Chain

The common feature of the compounds in this group, as it can be understood from the title, is an acyclic side-chain on the C-17 carbon atom. This group compounds constitutes the largest group after the 20,24-epoxy cycloartanes. As a result of the conducted literature research, 16 different aglycone types have been determined belonging to this group. Although the first 5 of these groups were obtained in both the genin and the glycoside forms; only the glycoside forms of the other 11 groups could be isolated from *Astragalus* species. These compounds are presented below in their aglycone forms (**Figure 1.12.**).

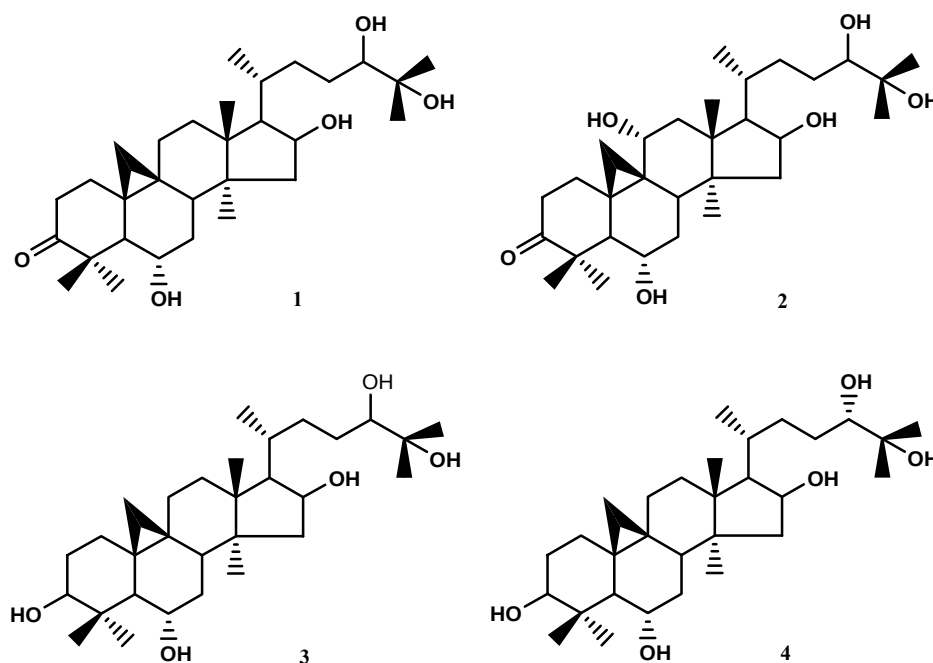


Figure 1.12. Cycloartanes Which Have Acyclic Side-Chain

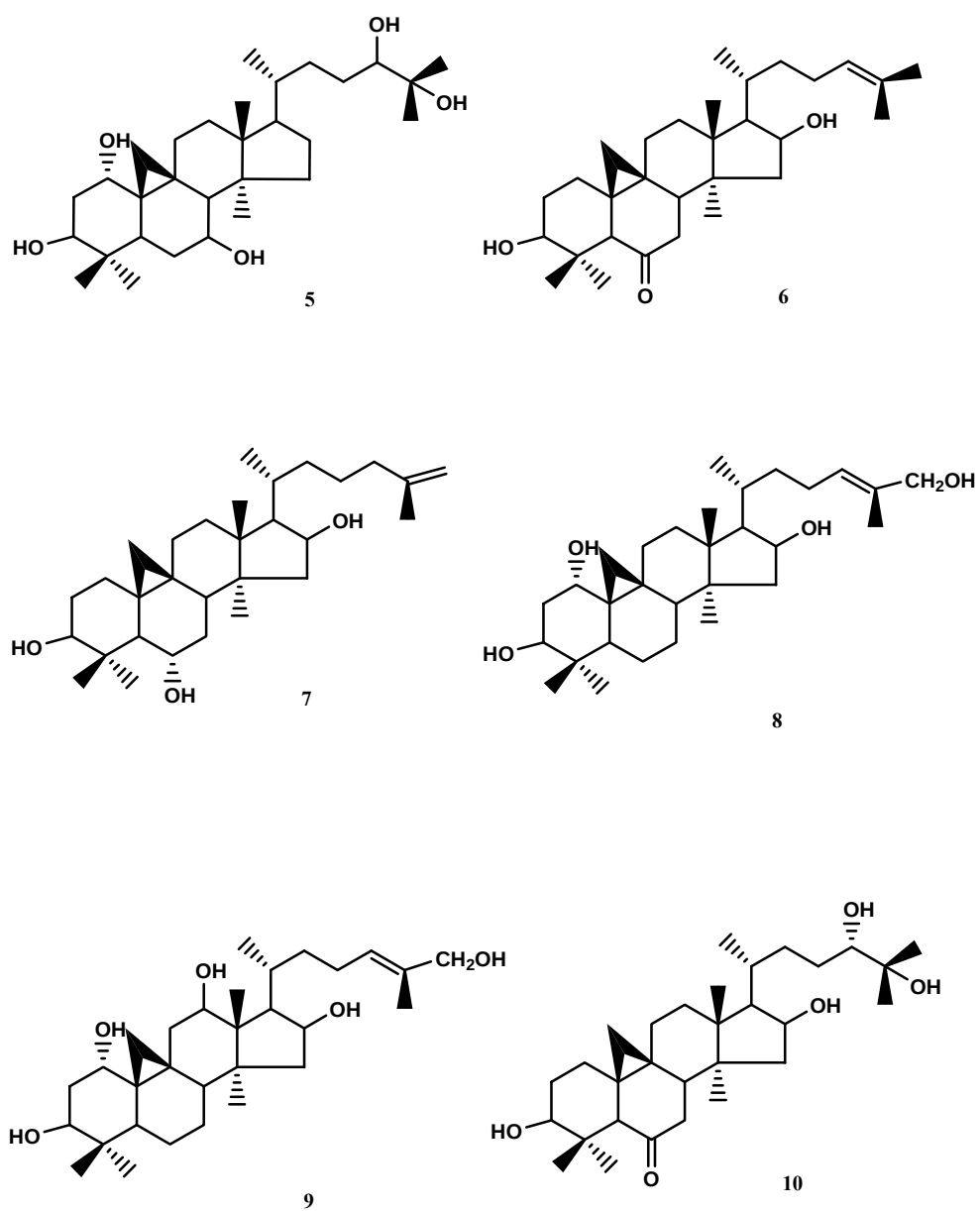


Figure 1.12. Cycloartanes Which Have Acyclic Side-Chain (Continued)

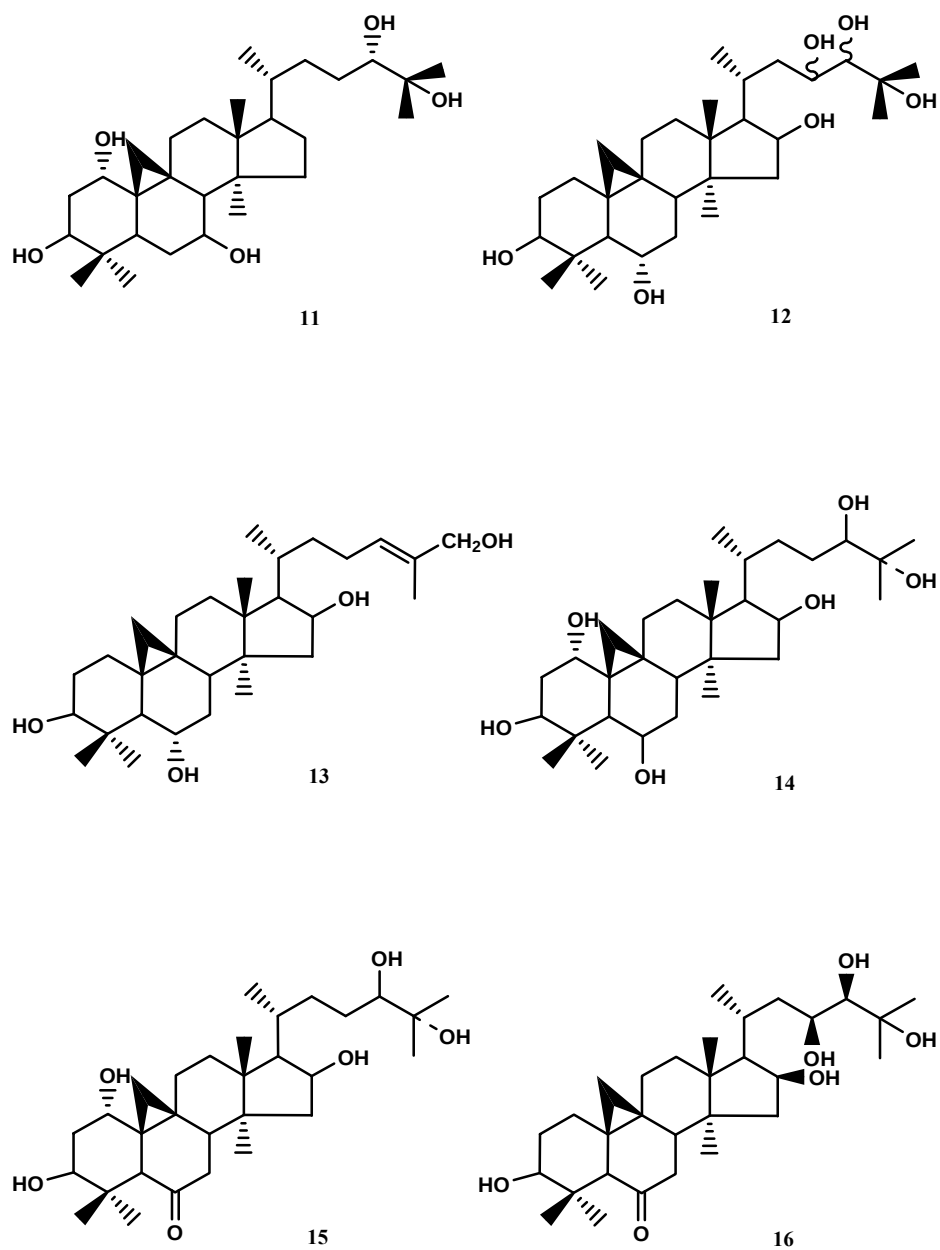


Figure 1.12. Cycloartanes Which Have Acyclic Side-Chain (Continued)

Among these aglycones, cyclocanthogenin and its glycosides constitute the largest group. The difference between the cyclocanthogenin and cycloasgenin B is the configuration of C-24 a chiral center. While the configuration of this stereocenter is $24S$ in cyclocanthogenin, it is $24R$ in cycloasgenin B (**Figure 1.13.**).

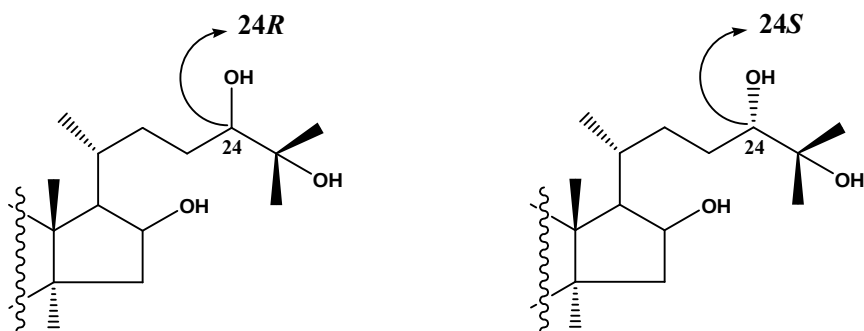


Figure 1.13. *R* and *S* Configurations of C-24

Triterpenoids of *Astragalus* plants are polyhydroxycompounds. Only genin **6** retains the intact side chain of cycloartenol. The side chain of the remaining compounds is oxidized to one degree or another. Most of the compounds contain an α -diol on C-24–C-25 in the acyclic side chain, and a 16β -hydroxyl.

In the cycloartanes possessing acyclic side-chain, sites of the oxygen substitution are 3, 6, 16, 24, and 25^{th} carbon atoms. Moreover,

acyclic side-chain cycloartanes having oxygen substitution at C-1, C-7, C-11, C-12, C-23, and C-26 positions were encountered (**Figure 1.14.**).

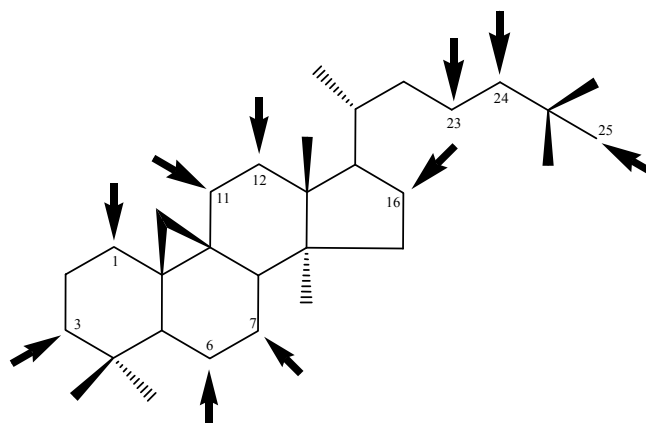


Figure 1.14. Hydroxyl Substitution Sites on Acyclic Side-Chain Aglycon

On one hand the C-16 secondary hydroxyl is sterically hindered by a *cis*-oriented side chain; on the other, it creates an intramolecular H-bond (IHB) with the oxygenated functional groups of the side chain. Therefore, the reactivity of the 16 β -hydroxyl toward alkylation and acylation is much less than the 1 α -, 3 β -, 6 α -, 7 β -, and 11 α -hydroxyls. Only 12 α - and 12 β -hydroxyls and the C-25 tertiary hydroxyl are less reactive than the 16 β -hydroxyl (Agzamova and Isaev, 1994, 1995). The difference in the reactivities of the hydroxyls enables derivatives with a free C-16 hydroxyl to be prepared.

Until recent years, except the saponins carrying double bond, this group of compounds have been evaluated as compounds which certainly contain 16,24,25-triolic part. In a study on *Astragalus oleifolius*, the macrophyllsaponins missing C-16 OH group were characterized (Çalış et al., 1996): Therefore, the generalization lost its validity.

At the same time as these compounds from *Astragalus oleifolius* carry secondary hydroxyl group on 1, 3, and 7 positions, they are the second sapogenol moiety in nature having three hydroxyl groups on the A-B rings. On the other hand, the first aglycone including the same framework was cycloorbigenin B isolated from *Astragalus orbiculatus* (Agzamova et al., 1989).

Configurations of the hydroxyl groups located at C-1, C-3, C-6, and C-16 are: α , β , α , and β , respectively.

Acylation can be seen on acyclic side-chain cycloartanes. The first acylated derivative of acyclic side-chain cycloartane (**Figure 1.15.**) was reported by Zhu et al. (1992) from *Astragalus mongholicus* Bunge. Moreover, acylations were found on C-3, C-6, and C-16 hydroxyl groups (Zhu et al., 1992; Kucherbaev et al., 2002).

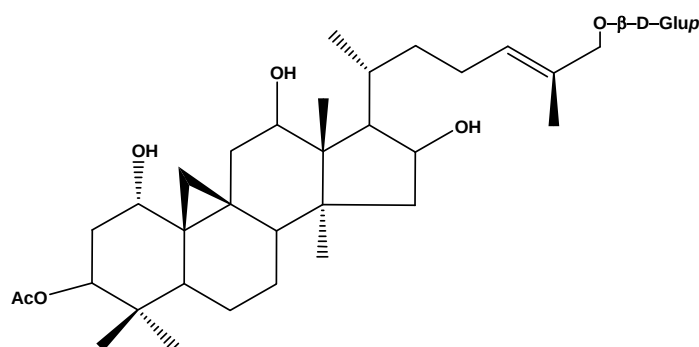


Figure 1.15. Mongholicoside II

Secondary hydroxyl groups at C-3 and C-6 are generally glycosylation sites on acyclic side-chain cycloartanes' (Isaev et al., 1992; Orsini et al., 1994; Hirotani et al., 1994a,b).

Besides glycosylation at 16, 24, 25, and 27 positions, although rare, might occur (Fadeev et al., 1988; Çalış et al., 1996; Zhu et al., 1992). Generally these compounds have monodesmosidic or bidesmosidic structure. The first compound with tridesmosidic structure cephalotoside A isolated from *Astragalus cephalotes* (Çalış et al., 1999). This compound is the only example of tridesmoside ASCC's missing a sugar unit at C-6 (O) position.

On the other hand, tetradesmosidic cycloartane glycosides are very unusual. The first tetradesmosidic cycloartane glycoside with an acyclic side-chain is cyclostipuloside E isolated from *Tragacantha stipulosa*

(Leguminosae). In *Astragalus* species, the first and only tetra-desmosidic cycloartane glycoside was isolated from *Astragalus eremophilus* and named as eremophiloside A (Perrone et al., 2008). Cyclostipuloside E was characterized with the same aglycon of eremophiloside A, but different sugar units and glycosylation sites (**Figure 1.16.**).

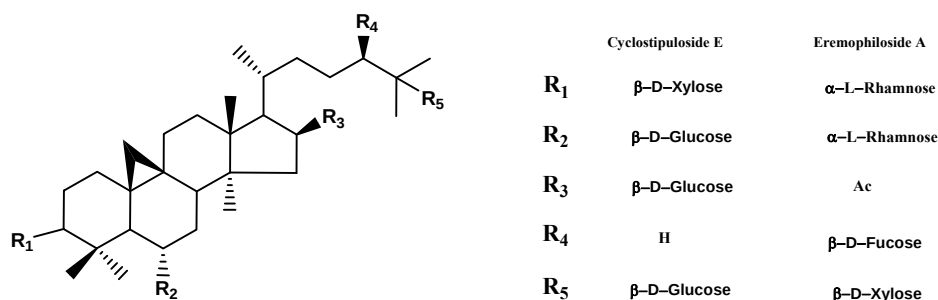


Figure 1.16. Structures of Cyclostipuloside E and Eremophiloside A

ASCC's have generally monoglycosidic, diglycosidic, and triglycosidic structures. No ASCC including tetraglycosidic structure has been found in *Astragalus* genus till 1999. The first two tetraglycosidic examples of cycloartanes were obtained from *Astragalus trojanus* and named as trojanoside E and trojanoside F (**Figure 1.17.**) (Bedir et al., 1999).

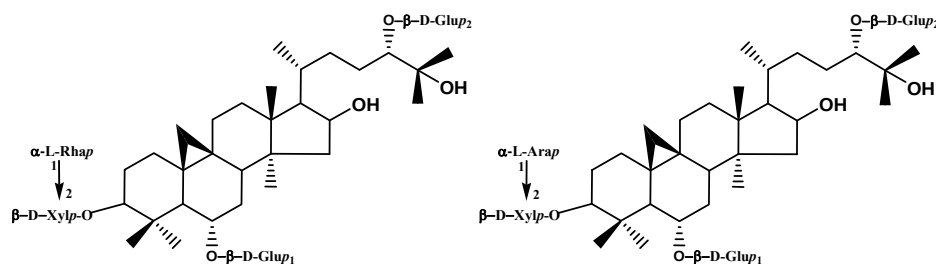


Figure 1.17. Structures of Trojanoside E and F

The most common sugar forms are β -D-xylose and β -D-glucose. In addition, even it is quite rare, ASCC's possessing α -L-arabinose and α -L-rhamnose were also identified (Polat et al., 2009; Isaev, 1995).

The cycloartanes derived from *Astragalus* genus are called as xylosaponins because of the xylose moiety mostly attached to C-3 (O). *Astragalus* cycloartanes rarely carry glucose unit at C-3 position. Macrophyllsaponins (**Figure 1.18.**) are the first cycloartanes having rhamnose at C-3 position in the nature (Çalış et al., 1996).

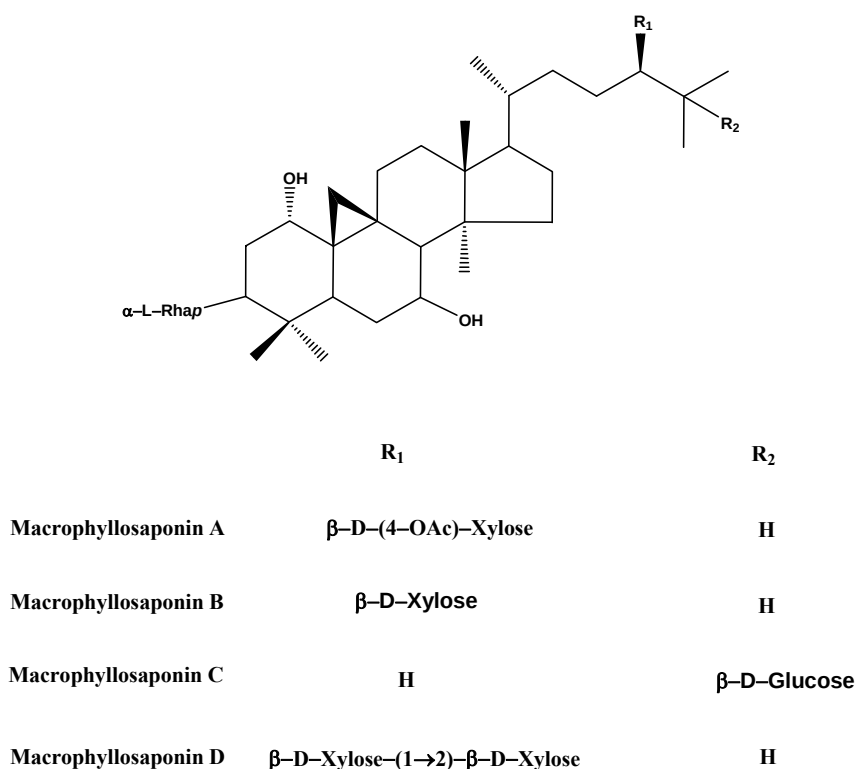


Figure 1.18. Structures of Macrophyllsaponins A-D

The monosaccharide D-apiose was found only in two glycosides. These are cycloaralosides C and F, which were isolated from *Astragalus amarus* and *Astragalus villosissimus*, respectively (Isaev and Abubakirov, 1990; Isaev and Imomnazarov, 1991). Moreover, cycloaraloside C was also obtained from *Astragalus iliensis* by Chen et al. (1990) and named as astrailienine A.

Furthermore, eremophiloside A contains a rare sugar unit β -D-fucose at C-24 position (**Figure 1.16.**).

3-*O*-[β -D-glucuronopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl]-25-*O*- β -D-glucopyranosyl-3 β ,6 α ,16 β ,24(*S*),25-pentahydroxy-cycloartane (**Figure 3.5.**) obtained from *Astragalus amblolapis* represents the first report of a cycloartane-type triterpen glycoside possessing glucuronic acid residue in plant kingdom (Polat et al., 2009) (Elaborate knowledge is given in the chapter of Results&Discussion of this thesis).

Compounds of 6-*O*- α -L-rhamnopyranosyl-16,24-di-*O*- β -D-glucopyranosyl-3 β ,6 α ,16 β ,24(*S*),25-pentahydroxy-cycloartane and 6-*O*- α -L-rhamnopyranosyl-16,25-di-*O*- β -D-glucopyranosyl-3 β ,6 α ,16 β ,24(*S*),25-pentahydroxy-cycloartane (**Figure 3.9.**, **Figure 3.11.**) (Polat et al., 2009) isolated from *Astragalus amblolapis* are the first cycloartane glycosides possessing rhamnosyl unit at C-6 position on cycloanthogenol skeleton which is one of the most common aglycons in *Astragalus* genus together with cycloastragenol.

Additionally, these metabolites contain no sugar residue at C-3 position and so they are rather unusual in nature.

Although acylation is quite rare on aglycones, the existence of acylated sugar moieties are characteristics of cycloartane-type glycosides from *Astragalus* genus. The acetyl groups are generally found on the xylopyranosyl moiety engaged to C-3 (OH). However, acetyl groups on rhamnopyranosyl and glucopyranosyl residues were also observed (Imomnazarova and Isaev, 1992; Pistelli et al., 1997). More than one acylation on same xylose unit was also a common feature for *Astragalus* cycloartanes (**Figure 1.19.**).

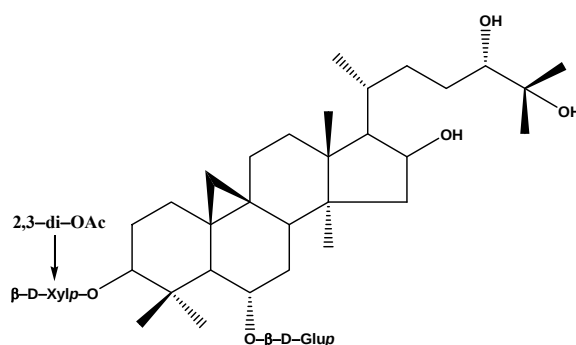


Figure 1.19. Xylose Residue With 2 Acetyl Groups

1.3.2.B.2. Cycloartanes Which Have 20,24-Epoxy Side-Chain

In the cycloartanes having 20,24-epoxy side-chain, which consist the largest group among the cycloartanes isolated from *Astragalus* species, 12 different sapogenol types were elucidated (**Figure 1.21.**). Of the four possible side-chain stereoisomers [two pairs of enantiomers: 20*R*,24*S* and 20*S*,24*R* (1) and 20*R*,24*R* and 20*S*,24*S* (2)], at present only the first pair has been found in *Astragalus* plants. Side chains in 11 genins have identical stereochemistry (20*R*,24*S*) whereas that of **27** is enantiomeric.

Cyclosieversigenin is the most widely distributed genin of *Astragalus* plant glycosides. For this reason, it is not surprising that it was discovered first. Cyclosieversigenin is also known as cycloastragenol and astramembrangenin. In addition, cycloastragenol was isolated from *Astragalus wiedemannianus* in this study (**Figure 3.14.**).

There are 2 chiral centers in 20,24-epoxy cycloartanes and the only difference between the cycloastragenol and cyclogalegigenin sapogenols is the configurations on these stereo centers. Thus, chemical and spectral investigations of cycloastragenol and cyclogalegigenin led convincingly to the conclusion that the side chains of these aglycones are enantiomers and have the alternate stereochemistry 20*R*,24*S* and 20*S*,24*R*. On the other hand, the erroneous selection of the stereochemistry of cycloastragenol led to the incorrect configuration for cyclogalegigenin.

X-ray structural analysis verified the 20*S*,24*R* configuration for cyclogalegigenin, which, in turn, determined the 20*R*,24*S* configuration for cycloastragenol. X-ray studies of nona-O-methyl ether of astragaloside IV (cyclosieversioside F, which is cyclosieversigenin 3-O- β -D-xylopyranoside, 6-O- β -D-glucopyranoside) and astramembrangenin 3,6-diacetate (cyclosiversigenin 3,6-diacetate) justify the stereochemistry of cycloastragenol (**Figure 1.20.**).

Table 1.1. gives the values of the chemical shifts of the C-20 and C-24 chiral atoms of the known 20,24-ESCC's. As it can be seen from the **Table 1.1.**, the chemical shifts of the C-24 atoms differ substantially according to their configuration, a 24*R* atom resonating in a field approximately 3.5 ppm weaker than a 24*S* atom. The signal of the 20*R* atom is likewise found in a weaker field than of the 20*S* atom but the difference in this case is not so great (0.4 ppm). The magnitude of the difference in the chemical shifts ($\Delta\delta = \gamma_{C-20} - \delta_{C-24}$) is also characteristic for each enantiomer with respect to the side chain (Isaev et al., 1989).

Table 1.1. ^{13}C NMR Values for C-20 and C-24 Chiral Atoms

References	δ C-20	δ C-24	$\Delta\delta$ (δ C-20– δ C24)	Configuration
Abubakirov et al., 1986 (1)	87.0	81.7	5.3	20 <i>R</i> ,24 <i>S</i>
Kholzineva et al., 1987 (2)	87.14	81.56	5.58	20 <i>R</i> ,24 <i>S</i>
Kravtsov et al., 1988 (3)	87.1	81.6	5.5	20 <i>R</i> ,24 <i>S</i>
Alaniya et al., 1983 (4)	86.7	85.0	1.7	20 <i>S</i> ,24 <i>R</i>
Sakurai et al., 1986 (5)	86.2	85.2	1.0	20 <i>S</i> ,24 <i>R</i>
Sakurai et al., 1986 (7)	84.4	83.5	0.9	20 <i>S</i> ,24 <i>R</i>

The presence and nature of substituents in the region of rings C and D are reflected in the values of the chemical shifts of the asymmetric atoms of the side chain that are under consideration. The acetylation of hydroxyl groups at C-16 and C-18 leads to upfield shifts of the C-20 and C-24 signals by 1.8 and 1.7 ppm, respectively. The small deviations in the values of the C-20 and C-24 chemical shifts are obviously the consequence of the influence of the C-12 β hydroxyl group and the C-15 α acetoxy group (Isaev et al., 1989).

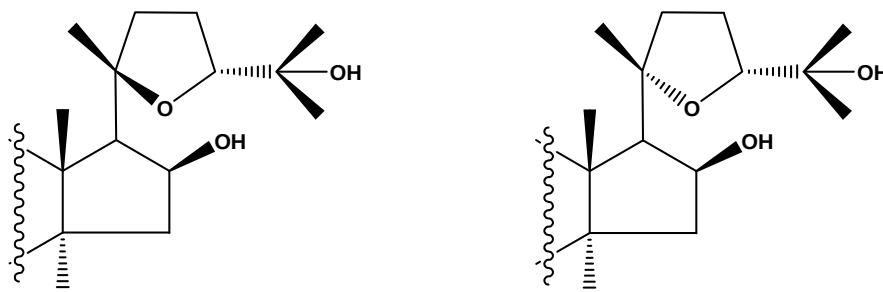


Figure 1.20. Chiral centers in 20,24-epoxy cycloartanes

As it can be understood from **Table 1.1.**, there is a oxygen containing substituent (generally hydroxyl, rarely ketone) at 3, 16, and 25th carbon atoms in the substances of this group.

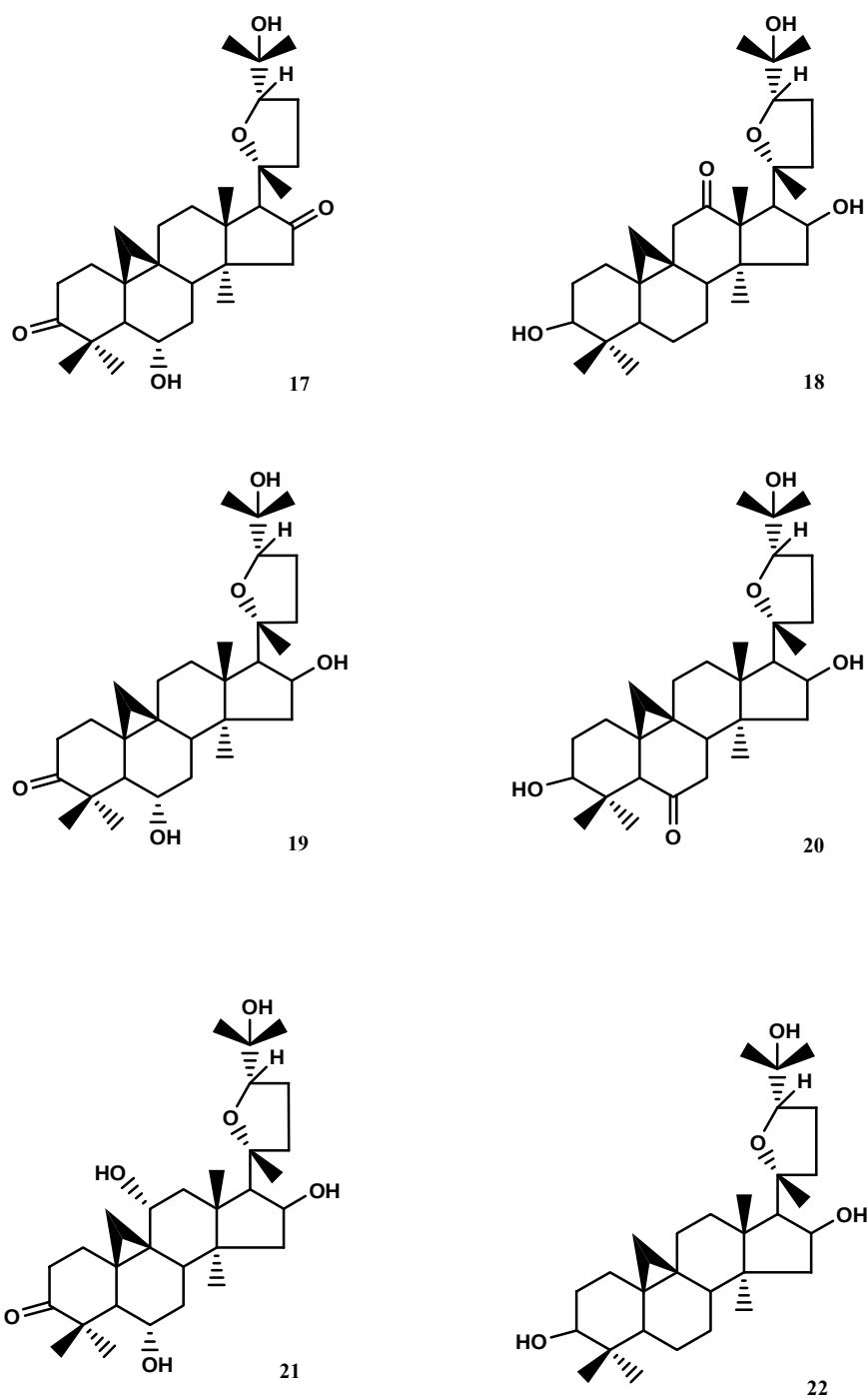


Figure 1.21. Cycloartanes Which Have 20,24-Epoxy Side-Chain

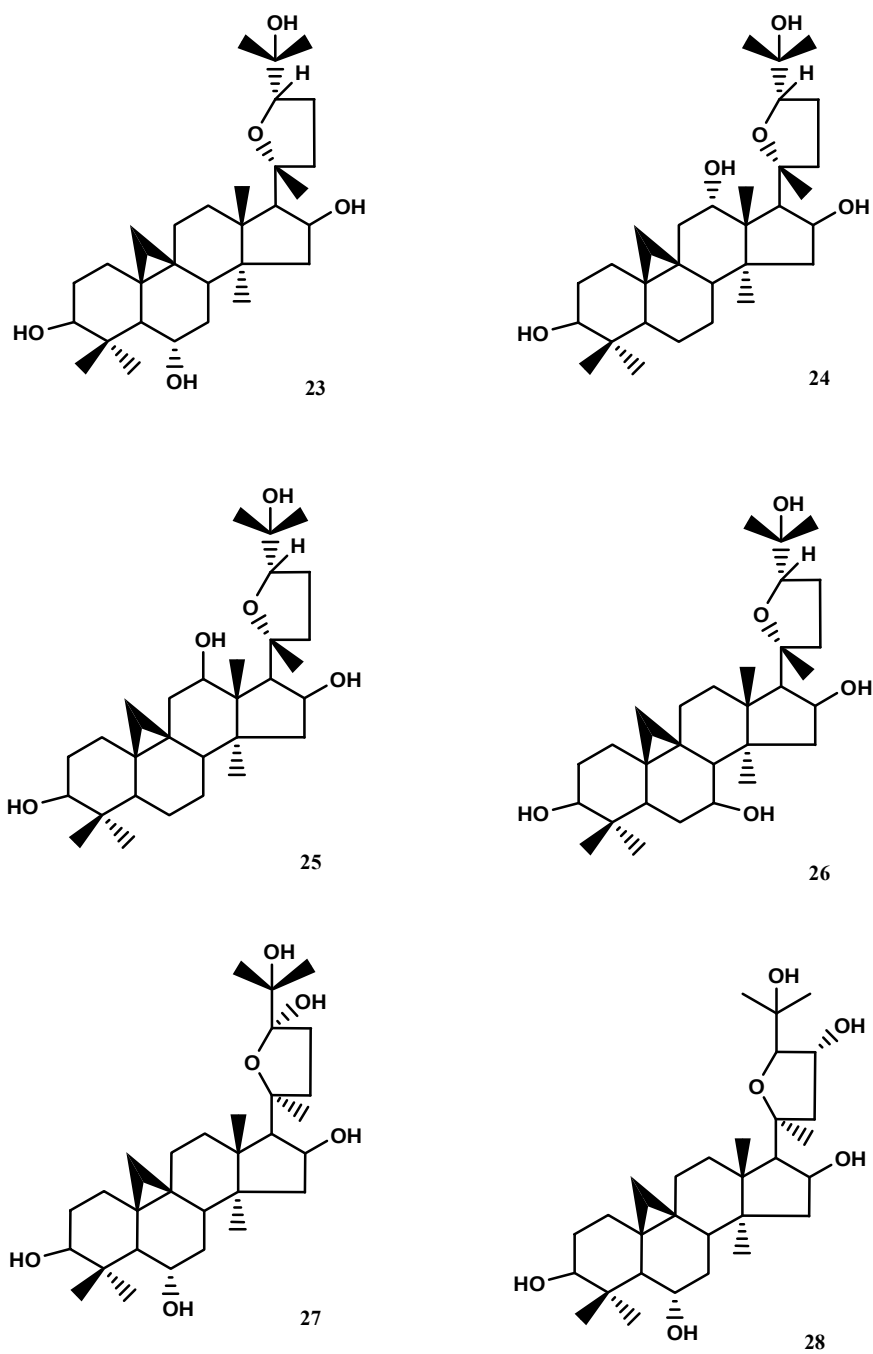


Figure 1.21. Cycloartanes Which Have 20,24-Epoxy Side-Chain (Continued)

Besides molecules having hydroxyl or ketone groups were encountered at 7, 11, and 12th carbon atoms. In a study on *Astragalus campylosema* Boiss. ssp. *campylosema*, three unconventional 20,24-epoxy cycloartane having oxygen substituent on 23rd carbon atom were isolated and their genins were placed as a new genin among 20,24-epoxy cycloartanes (Çalış et al., 2008). These compounds represent a small group of 20,24-ESCC.

The most common sapogenol moiety found in *Astragalus* cycloartanes is cycloastragenol (=cyclosieversigenin) (**Figure 3.14.**).

In this group, there were a few reports of ketone functionality. The ketone group were found to be located at C-3 (**19**), C-6 (**20**), C-12 (**18**), and C-16 (**17**) positions (**Figure 1.22.**).

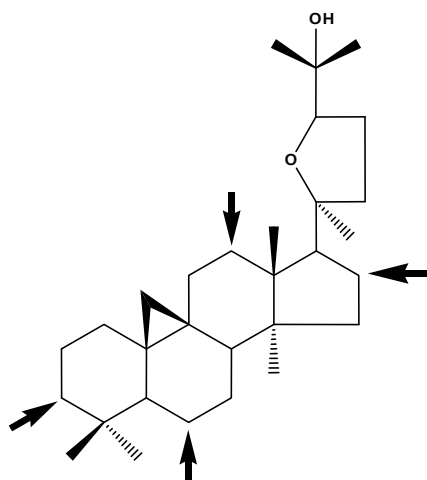


Figure 1.22. Ketone Functional Groups on Sapogenol Moiety

Acylation is not a common feature on 20,24-ESCC's. Up to now only four compounds have been reported: Huangqiyein D at C-6 (Kuang et al., 1998), trojanosides A and I (Bedir et al., 1999, 2001) at C-16, and 3-*O*-[α -L-arabinopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl]-16-*O*-hydroxyacetoxy-23-*O*-acetoxy-3 β ,6 α ,16 β ,23 α ,25-pentahydroxy-20(*R*),24(*S*)-epoxycycloartane at C-23 (Çalış et al., 2008).

In these compounds, glycosylation takes place especially secondary hydroxyl groups located at C-3, C-6, and C-16, and on tertiary hydroxyl group at C-25. Even though there are hydroxyl groups on 7, 11, and 12th carbon atoms, no secondary metabolite glycosylated at these points have been found. Cycloartanes having C-3 ketone functionality (cycloasgenin A, cycloasgenin B, and 3-dehydrocycloasgenin C) were only isolated in non-glycosidic forms. Based on that, the researchers state that the C-3 (O) position is the key point where the first glycosylation happens, and after that biosynthesis of bidesmosidic and tridesmosidic cycloartanes occur.

It was pointed out that the monodesmosidic glycosides had only C-3 (O) glycosylation while all bidesmosidic and tridesmosidic metabolites were found to include a sugar unit at C-3 (O) position justifying the aforementioned conclusion. However, trigonoside I (**Figure 1.23.**) (cycloastragenol-6-*O*- β -D-xylopyranosyl) isolated during a study conducted on *Astragalus trigonus* (Garibaldi et al., 1995), and cephalotoside B (cyclocanthogenol-6-*O*- β -D-xylopyranosyl) isolated

from *Astragalus cephalotes* (Yusufoğlu, 1996) have refuted this hypothesis with a single sugar unit at C-6 position.

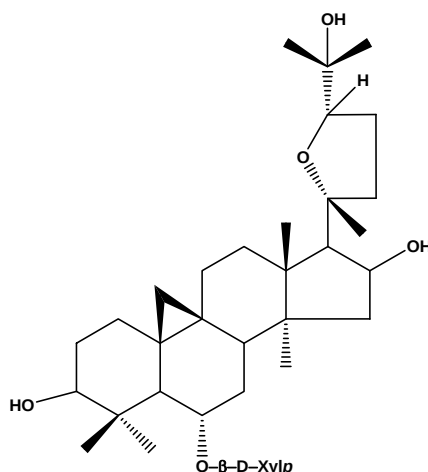


Figure 1.23. Structure of Trigonoside I

Mono- and bidesmosidic glycosides of 20,24-ESCC's were frequently come across; however, tridesmosidic structures were also reported. In fact the first tridesmosidic molecule in plant kingdom was a 20,24-ESCC known as astragaloside VII. As well five other tridesmosides were also obtained from *Astragalus* genus known as agroastragaloside IV (Hirotani, 1999), trojanoside B, trojanoside E, trojanoside F, and trojanoside K (Bedir et al., 1999).

Though the monoglycosidic, diglycosidic, and triglycosidic structures are common in this group, no tetraglycosidic secondary metabolite has been characterized so far. In addition, glucose and xylose are observed as the most widespread sugar forms in 20,24-epoxy

cycloartanes. On the other hand, apiose, arabinose, and rhamnose were also identified as part of 20,24-ESCS glycosides.

As in ASCC's, acetyl groups were mostly found to be on xylose units attached to C-3 (O) position of 20,24-ESCC.

1.3.2.B.3. Cycloartanes Which Have 20,25-Epoxy Side-Chain

20,25-epoxy side-chain a rare feature in saponins is encountered in dammaran type saponins of *Panax* genus. In the plant kingdom, the first cycloartane with 20,25-ESCC was fruticin B obtained from *Parthenium fruticosum* (Asteraceae) (**Figure 1.25.**).

During phytochemical studies on Turkish *Astragalus* species, Bedir et al. (1998) were characterized a cycloartane with 20,25-ESCC and named the compound as cyclocephaloside I.

After identification of the first 20,25-ESCC (cyclocephaloside I) in *Astragalus*, five more compounds were elucidated (Alaniya et al., 2008; Sukhina et al., 2007).

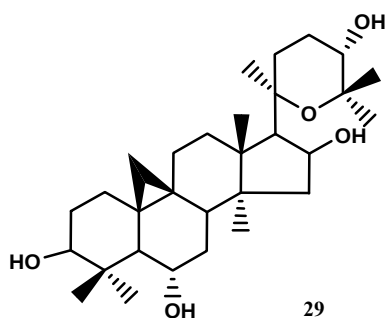


Figure 1.24. Cycloartanes Which Have 20,25-Epoxy Side-Chain

The structural difference between cyclocephaloside I's aglycon (cyclocephagenol) and fruticin B is C-6 hydroxyl group missing in fruticin B. Since there is no sugar unit on fruticin B, cyclocephaloside I is the first glycoside in *Astragalus* genus possessing 20,25-ESCC.

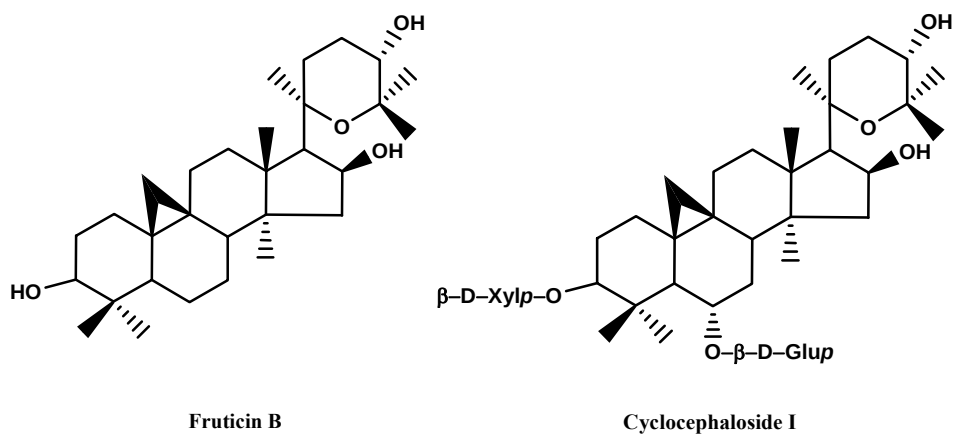


Figure 1.25. Structures of Fruticin B and Cyclocephaloside I

In 20,25-epoxy cycloartanes, three kinds of sugar units were characterized: xylose, glucose, and rhamnose. Sugar units were found to be linked to the secondary hydroxyl groups at C-3, C-6, and C-24 positions.

The compound obtained from *Astragalus peregrinus* is only acylated 20,25-ESCC isolated from *Astragalus* genus (Verotta et al., 2001).

1.3.2.B.4. Cycloartanes Which Have $16\beta,23;16\alpha,24$ -Diepoxy Side-Chain

$16\beta,23;16\alpha,24$ -diepoxy cycloartanes are unusual secondary metabolites in *Astragalus* species. In plant kingdom, *Cimicifuga* species (Ranunculaceae) are rich in this group of compounds. Except *Cimicifuga* genus, $16\beta,23;16\alpha,24$ -DESCC's were only provided by *Astragalus orbiculatus* (Agzamova et al., 1986a).

Four different sapogenols (**30-33**) derived from *Astragalus orbiculatus* are shown in **Figure 1.26**.

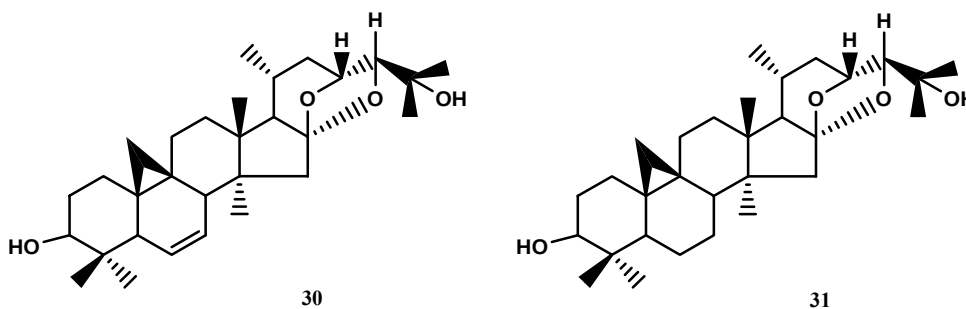


Figure 1.26. Cycloartanes Which Have $16\beta,23;16\alpha,24$ -Diepoxy Side-Chain

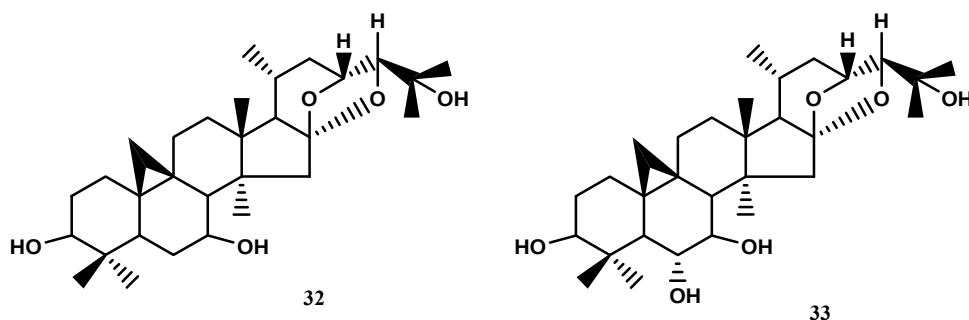


Figure 1.26. Cycloartanes Which Have $16\beta,23;16\alpha,24$ -Diepoxy Side-Chain (Continued)

Genins **30-33** have identical absolute stereochemistry $16\beta,23R;16\alpha,24S$.

An examination of molecular models indicates that the heterocycles can be fused in two ways: $16\beta,23R;16\alpha,24$ and $16\beta,23S;16\alpha,24$. Depending on the stereochemistry of C-24, each type of fusion can give two stereoisomers (**Figure 1.27.**). As a result, four isomers are possible: $16\beta,23R;16\alpha,24S$, $16\beta,23R;16\alpha,24R$, $16\beta,23S;16\alpha,24R$, $16\beta,23S;16\alpha,24S$ (Mamedova and Isaev, 2004).

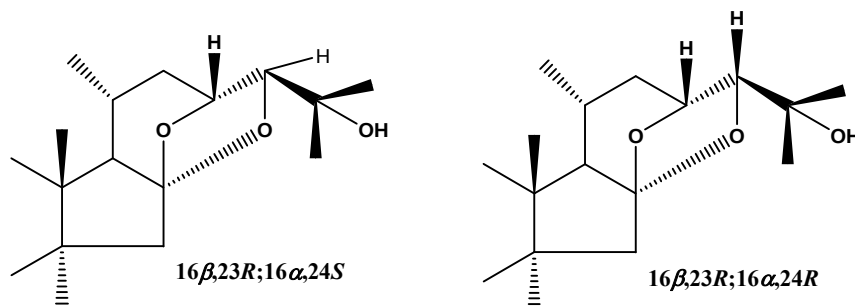


Figure 1.27. Stereochemistry of $16\beta,23;16\alpha,24$ -Diepoxy Cycloartanes

1.3.2.B.5. Cycloartanes Which Have 16 β ,24;20,24-Diepoxycycloartane Side Chain

Up to now, only two compounds belonging to 16 β ,24;20,24-diepoxycycloartanes have been identified. The first was cycloalpioside isolated from *Astragalus alopecurus* Pall (Agzamova and Isaev, 1995). The latter was isolated from *Astragalus campylosema* Boiss. ssp. *campylosema* and established as 3-*O*-[α -L-arabinoprinosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl]-3 β ,6 α ,23 α ,25-tetrahydroxy-20(*R*),24(*R*)-16 β ,24;20,24-diepoxycycloartane (Çalış et al., 2008).

Glycosylation sites on the genins are the same for both compounds at C-3 (O) position. Cycloalpioside had three hydroxyl groups at C-3, C-7, and C-25 positions, whereas the compound from *Astragalus campylosema* Boiss. ssp. *campylosema* possessed four hydroxyl groups located at C-3, C-6, C-23, and C-25 positions. As a result, there are two sapogenins in the class of 16 β ,24;20,24-DESCC (**34** and **35**).

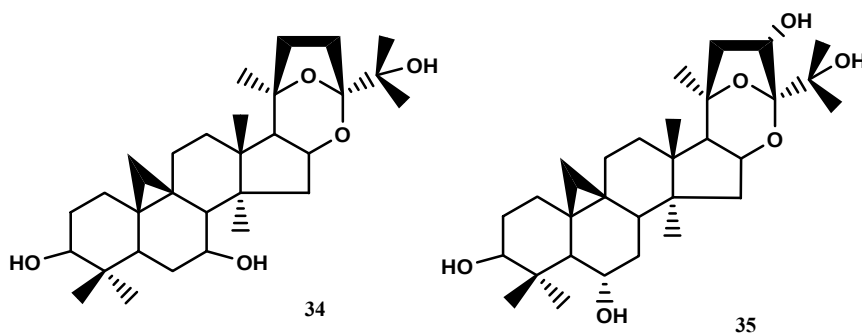


Figure 1.28. Cycloartanes Which Have 16 β ,24;20,24-Diepoxycycloartane Side Chain

On the other hand, configuration of the chiral atoms C-20 and C-24 of **34** deserves attention. An examination of molecular models indicates that the heterocycles can be fused as 20*R*,24*R*- or 20*S*,24*S*-configurations. Therefore, establishing the stereochemistry of one of these asymmetric centers defines the configuration of the other chiral atom.

Cycloalpigenin was converted by acid hydrolysis with subsequent periodate oxidation into lactone **1** in order to determine the configuration of C-20. The analogous lactone **2** was prepared from the known glycoside cyclocarposide, possessing 20*R*-configuration. C-20 in ¹³C NMR spectra of **1** and **2** resonated at δ 90.43 and 90.23, respectively. The good agreement of these values indicated that the configuration of C-20 is identical in **1** and **2** suggesting that **34** had 20*R*,24*R* stereochemistry. This conclusion was confirmed by chemical modification of the structures of cycloalpigenin and cycloalpigenin D (**26**) by converting them in three steps into **1** (Mamedova and Isaev, 2004).

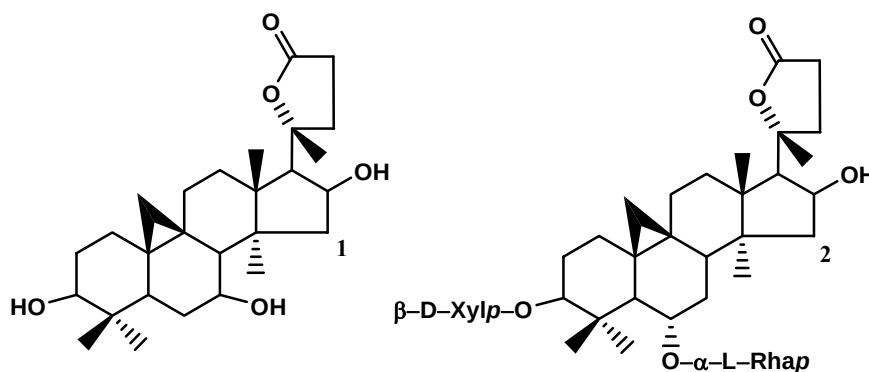


Figure 1.29. Lactones

1.3.2.B.6. Cycloartanes Which Have 24-Nor-16 β ,23-Epoxy Side-Chain

The sixth structural type, 24-nor-16 β ,23-epoxy cycloartane, includes four different genins shown in **Figure 1.30**. These compounds are rare in plant kingdom because of their unstable nature on the side chain.

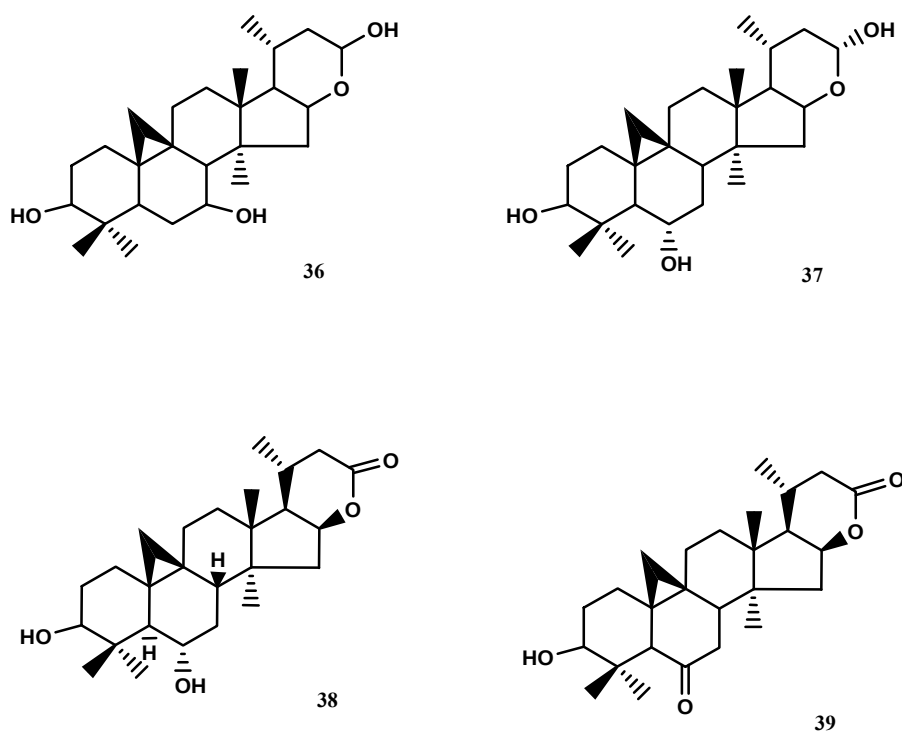


Figure 1.30. Cycloartanes Which Have 24-Nor-16 β ,23-Epoxy Side Chain

Up to now, 24-nor-16 β ,23-ESCC's have only been determined in four *Astragalus* species: *Astragalus dasyanthus* Pall., *Astragalus tomentosus* Lam., *Astragalus bicuspis*, and *Astragalus eremophilus*. Eremophilosides isolated from *Astragalus eremophilus* have a sugar sequence of 3-*O*- α -L-arabinopranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl. Only tomentoside IV contains glucose as a sugar moiety.

As a rule, all of these compounds carry xylose moiety at C-3, so they can be called xylocycloartanes. Except glycosilation at C-3 (O) position, no different glycosilation sites have been obtained on sapogenol of 24-nor-16 β ,23-ESCC up to present. They are monodesmosidic glycosides and only two 24-nor-16 β ,23-ESCC (tomentoside III and IV) contain acylation at C-6 position on sapogenol moiety (Radwan et al., 2004).

Compounds of this structural type are hemiacetals. Therefore, native genins can not be prepared by methods such as acid catalysis. Under acid catalysis conditions, hemiacetals are readily alkylated to form acetals. Conditions favorable for forming acetals arise often during isolation and separation of plant substances. During isolation of acetals of type **36-39** genins, the question of the authenticity of the isolated substance must be resolved (Mamedova and Isaev, 2004).

1.3.2.B.7. Cycloartanes Which Have $16\beta,23;22\beta,25$ -Diepoxy Side-Chain

Up till now, two $16\beta,23;22\beta,25$ -diepoxy side-chain cycloartane glycosides have been determined; eremophiloside J and K. Each of the secondary metabolites were isolated from the same plant known as *Astragalus eremophilus* (Perrone et al., 2008). Only difference between J and K is generated on C-6 atom. Eremophiloside J includes a hydroxyl group at C-6 while eremophiloside K has a ketone function at the same position. Thus, two different aglycones arise in this class (**40 and 41**).

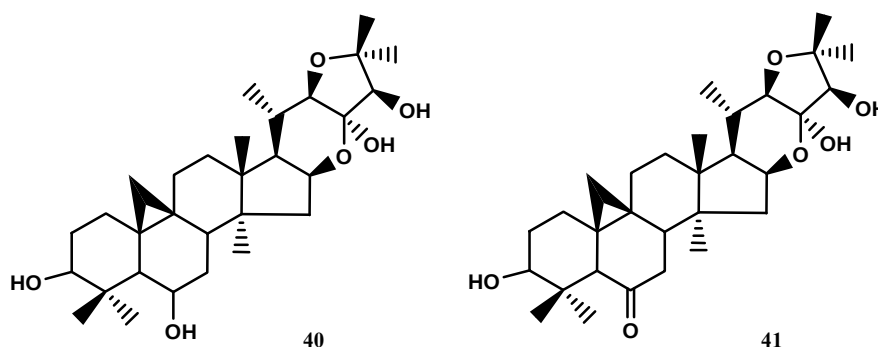


Figure 1.31. Cycloartanes Which Have $16\beta,23;22\beta,25$ -Diepoxy Side-Chain

These compounds are monodesmosidic glycosides and their sugar link consist of arabinose and xylose. No acylation have been observed on the aglycon and sugar skeleton of $16\beta,23;22\beta,25$ -DESCC's.

1.3.2.B.8. Cycloartanes Which Have $16\beta,23;23,26;24,25$ -Triepoxy Side-Chain

Finally, the last structural type, $16\beta,23;23,26;24,25$ -triepoxy cycloartane, is quite rare and includes only bicusposide C. The compound was isolated from *Astragalus bicuspis* (Choudhary et al., 2008). Now it is clear that the side-chain of bicusposide C consist of three epoxy groups. This group is only represented by **42** in **Figure 1.32**.

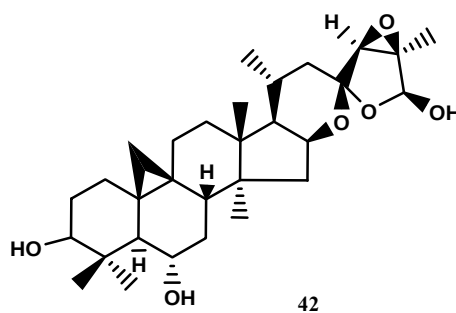


Figure 1.32. Cycloartanes Which Have $16\beta,23;23,26;24,25$ -Triepoxy Side-Chain

Bicusposide C is a monodesmosidic glycoside including xylose moiety at C-3 (O) position.

1.3.2.B.9. Cycloartanes Which Have $16\beta,23$ -Epoxy Side-Chain

In nature, three $16\beta,23$ -epoxy side-chain cycloartane glycosides have been determined; eremophiloside G, H, and I. Each of the

secondary metabolites were isolated from the same plant known as *Astragalus eremophilus* (Perrone et al., 2008). Eremophiloside H and I have the same aglycone. Thus, two different aglycones arise in this class (**43** and **44**). Only difference between **43** and **44** is generated on C-22 and C-23 positions. Genin **44** differed from **43** only the absence of the double bond between C-22 and C-23 and the presence of a methoxy (in eremophiloside H) and ethoxy (in eremophiloside I) of a ketalic function at C-23.

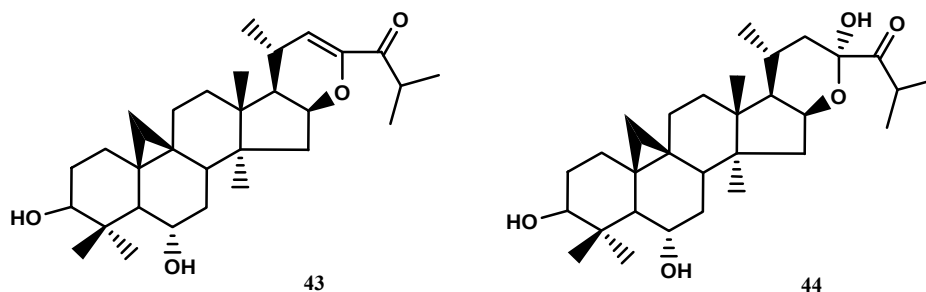


Figure 1.33. Cycloartanes Which Have 16 β ,23-Epoxy Side-Chain

These compounds are monodesmosidic glycosides and their sugar link consist of arabinose and xylose. No acylation have been observed on the aglycon and sugar skeleton of 16 β ,23-ESCC's.

Astragalus species that have been more or less thoroughly studied for secondary metabolites content are listed below.

1. *Astragalus taschkendicus* Bunge
2. *Astragalus adsugens* Pall
3. *Astragalus aitosensis* MB
4. *Astragalus alexandrinus* Boiss
5. *Astragalus alopecurus* Pall
6. *Astragalus amarus* Pall
7. *Astragalus babatagi* M. Pop
8. *Astragalus basineri* Trautv
9. *Astragalus brachypterus* Fischer
10. *Astragalus cephalotes* var. *Brevicalyx*
11. *Astragalus chrysopterus* Bunge
12. *Astragalus coluteocarpus* Boiss
13. *Astragalus complanatus* R. Br
14. *Astragalus dasyanthus* Pall
15. *Astragalus dissectus* B. Fedtsch. Et N. Ivanova
16. *Astragalus aphemerotorum* Gontsch
17. *Astragalus ernestii* Comb
18. *Astragalus exilis* A. Kor
19. *Astragalus falcatus* Lam
20. *Astragalus galegiformis* L
21. *Astragalus glycyphyllos* L
22. *Astragalus iliensis*
23. *Astragalus kuhitangi* (Nevski) Sirj

24. *Astragalus kulabensis* Lipsky
25. *Astragalus melanophrurius* Boiss
26. *Astragalus membranaceus* Bunge
27. *Astragalus microcephalus* Willd
28. *Astragalus mongholicus* Bunge
29. *Astragalus oleifolius* DC
30. *Astragalus orbiculatus* Ledeb
31. *Astragalus pamirensis* Ovcz. Et Rassulova
32. *Astragalus peregrinus*
33. *Astragalus prusianus* DC
34. *Astragalus pterocephalus* Bunge
35. *Astragalus pycnanthus* Boriss
36. *Astragalus qusqualis* Bunge
37. *Astragalus schahrudensis* Bunge
38. *Astragalus siculus* Biv
39. *Astragalus sieberi*
40. *Astragalus sieversianus* Pall
41. *Astragalus spinosus* Vahl
42. *Astragalus stipulosus* Boriss
43. *Astragalus tomentosus* Lam
44. *Astragalus tragacantha* Habl
45. *Astragalus trigonus* DC
46. *Astragalus trojanus* Stev
47. *Astragalus unifoliolatus* Bunge
48. *Astragalus uninodus* M. Pop. Et Vved
49. *Astragalus verrucosus* Moris

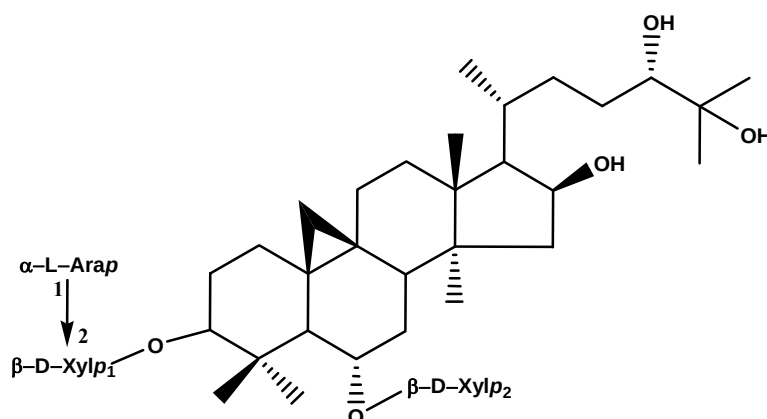
50. *Astragalus villosissimus* Bunge
51. *Astragalus danicus* Retz
52. *Astragalus inopinatus* Boriss
53. *Astragalus kahiricus* DC
54. *Astragalus gilvus* Boiss
55. *Astragalus caucasicus*
56. *Astragalus membranaceus* var. *mongholicus*
57. *Astragalus chivensis*
58. *Astragalus bicuspis*
59. *Astragalus eremophilus* Boiss
60. *Astragalus campylosema* Boiss. Subsp. *campylosema*
61. *Astragalus macropus* Bunge
62. *Astragalus glycyphyllos*
63. *Astragalus sempervirens*
64. *Astragalus caprinus* Maire
65. *Astragalus corniculatus* Bieb
66. *Astragalus flavescens*
67. *Astragalus suberi* L.
68. *Astragalus bungeanus* Boriss
69. *Astragalus aspera*
70. *Astragalus flexus* Fisch
71. *Astragalus vogelii*
72. *Astragalus miser*
73. *Astragalus zahlbruckneri*
74. *Astragalus elongatus*

Astragalus cycloartanes were reviewed by Isaev and his coworkers till 2004 (Mamedova and Isaev, 2004). In these papers, chemical and biological properties of cycloartanes were reviewed together with their spectral data.

Therefore, we have decided to make a literature search for the cycloartanes appeared after 2004. The following part covers the cycloartanes isolated from *Astragalus* species in between July 2004 and June 2009.

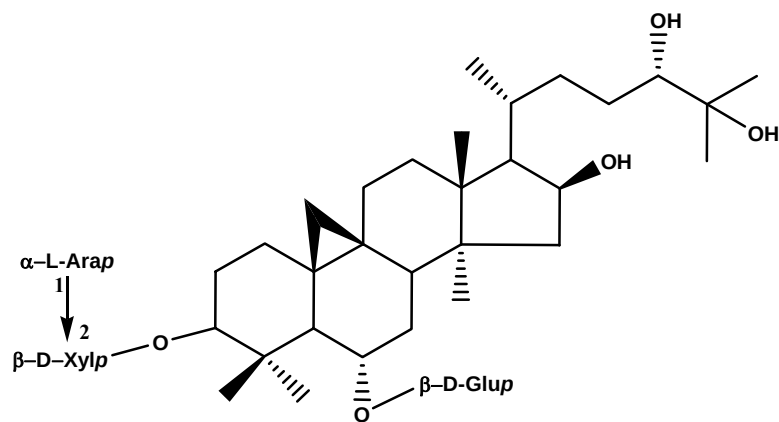
1.4. New Cycloartanes Appearing After 2004

Oleifolioside A (Özipek et al., 2005)



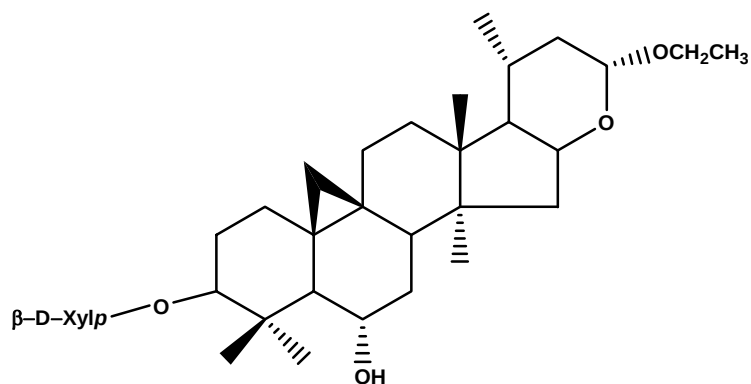
Plant Material	: <i>Astragalus oleifolius</i>
Molecular Formula	: C ₄₅ H ₇₆ O ₁₇
Molecular Weight	: 911
Optical Rotation	: [α] _D ²⁷ +18.9 (c 0.1, MeOH)
¹³ C-NMR Solvent	: C ₅ D ₅ N
Name	: 3-O-[β -xylopyranosyl-(1 $\rightarrow$ 2)- α -arabinopyranosyl]-6-O- β -xylopyranosyl-3 β ,6 α ,16 β ,24(<i>S</i>),25-pentahydroxycycloartane

C-1	31.8	C-13	45.9	C-25	72.5	β -D-Xylp ₁ -1	105.3
2	30.1	14	46.8	26	26.5	2	83.6
3	87.7	15	47.2	27	25.8	3	77.5
4	42.7	16	72.0	28	27.5	4	71.0
5	51.9	17	56.9	29	16.4	5	66.0
6	77.2	18	17.5	30	19.5	β -D-Xylp ₂ -1	105.8
7	33.0	19	25.0	α -L-Arap-1	106.8	2	75.5
8	43.2	20	28.7	2	73.7	3	78.4
9	21.5	21	18.4	3	74.3	4	71.1
10	27.9	22	32.8	4	69.1	5	66.9
11	26.4	23	27.8	5	67.1		
12	33.2	24	77.1				

Oleifolioside B (Özipek et al., 2005)

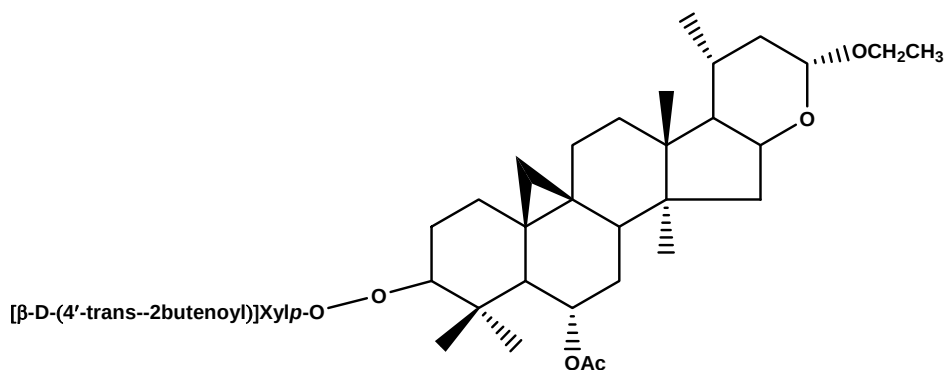
Plant Material : *Astragalus oleifolius*
Molecular Formula : $C_{46}H_{78}O_{18}$
Molecular Weight : 941
Optical Rotation : $[\alpha]_D^{27} +21.9$ (c 0.1, MeOH)
 ^{13}C -NMR Solvent : C_5D_5N
Name : 3-*O*-[β -xylopyranosyl-(1-2)- α -arabinopyranosyl]-
6-*O*- β -glucopyranosyl-3 β ,6 α ,16 β ,24(*S*),25-
pentahydroxycycloartane

C-1	32.1	C-13	45.8	C-25	72.5	β -D-Xylp-1	105.4
2	30.2	14	46.9	26	26.5	2	83.6
3	87.9	15	47.6	27	25.8	3	77.5
4	42.7	16	72.0	28	27.9	4	71.0
5	52.3	17	57.1	29	16.4	5	66.6
6	78.4	18	18.1	30	19.7	β -D-Glup-1	105.2
7	33.8	19	26.9	α -L-Arap-1	106.7	2	75.7
8	44.5	20	28.6	2	73.7	3	79.0
9	21.5	21	18.4	3	74.3	4	72.0
10	28.3	22	32.9	4	69.2	5	78.1
11	26.3	23	27.8	5	67.1	6	63.3
12	33.2	24	77.1				

Deacetyltomentoside I (Radwan et al., 2004)

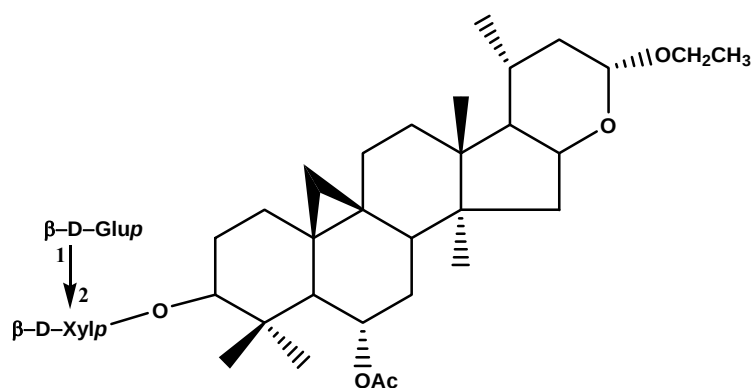
Plant Material	: <i>Astragalus tomentosus</i> Lam.
Molecular Formula	: C ₃₃ H ₅₄ O ₈
Molecular Weight	: 578
Optical Rotation	: [α] _D ²⁵ -22.7 (c 0.092, MeOH)
¹³ C-NMR Solvent	: C ₅ D ₅ N
Name	: 6α-hydroxy-23α-ethoxy 16β,23-(<i>R</i>)-epoxy-24.25,26,27-tetranor-9-19cyclostanosta-3- <i>O</i> -β-D-xylopyranoside
MP	: 283 °C

C-1	32.4	C-13	44.8	C-29	28.7
2	30.3	14	46.1	30	16.6
3	88.6	15	43.5	CH ₂	62.7
4	42.7	16	70.6	CH ₃	15.7
5	56.7	17	46.2	β-D-Xylp-1	107.7
6	67.1	18	20.2	2	75.7
7	38.2	19	29.6	3	78.6
8	53.9	20	25.6	4	71.3
9	21.2	21	20.6	5	67.4
10	29.2	22	33.3		
11	26.2	23	99.1		
12	33.3	28	29.5		

Tomentoside III (Radwan et al., 2004)

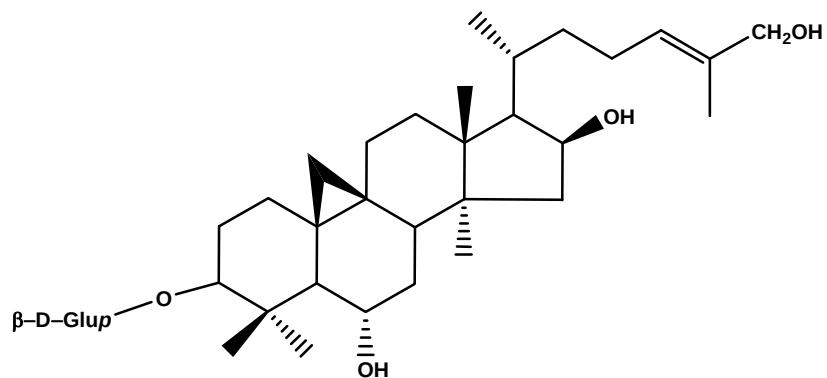
Plant Material	: <i>Astragalus tomentosus</i> Lam.
Molecular Formula	: C ₃₉ H ₅₉ O ₁₀
Molecular Weight	: 687
Optical Rotation	: [α] _D ²⁵ -29.7 (c 0.30, MeOH)
¹³ C-NMR Solvent	: C ₅ D ₅ N
Name	: 6α-acetoxy-23α-ethoxy-16β,23R-epoxy-24,25,26,27-tetranor-9,19-cyclolanosta-3-O-[β-D-(4'-trans-2-butenoyl)]xylopyranoside
MP	: 190-200 °C

C-1	31.9	C-13	44.3	C-29	26.7	β-D-Xylp-1	107.4
2	29.9	14	46.0	30	16.4	2	75.1
3	87.6	15	43.3	CH ₂	62.7	3	75.7
4	42.2	16	27.5	CH ₃	15.7	4	72.9
5	56.6	17	44.5	OCH ₃ CO	21.7	5	63.4
6	70.3	18	19.8	OCH ₃ CO	170.2		
7	38.1	19	27.8	OCOCH=CHCH ₃	17.6		
8	49.9	20	25.6	OCOCH=CHCH ₃	145.2		
9	21.0	21	20.6	OCOCH=CHCH ₃	122.9		
10	28.1	22	33.2	OCOCH=CHCH ₃	166.1		
11	25.9	23	99.1				
12	33.0	28	19.3				

Tomentoside IV (Radwan et al., 2004)

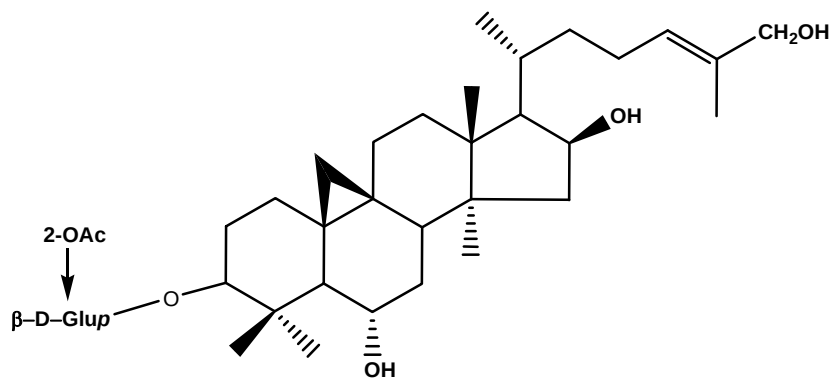
Plant Material	: <i>Astragalus tomentosus</i> Lam.
Molecular Formula	: C ₄₁ H ₆₆ O ₁₄
Molecular Weight	: 782
Optical Rotation	: [α] _D ²⁵ -17.5 (c 0.29, MeOH)
¹³ C-NMR Solvent	: C ₅ D ₅ N
Name	: 6α-acetoxy-23α-ethoxy 16β,23(<i>S</i>)-epoxy-24,25,26,27-tetranor-9-19cycloolanosta-3- <i>O</i> -[β-D-glucopyranosyl(1→2)]-β-D-xylopyranoside
MP	: 178-180 °C

C-1	31.8	C-13	44.2	C-29	26.8	β-D-Glup-1	105.6
2	30.0	14	46.0	30	16.3	2	77.1
3	87.4	15	43.2	CH ₂	62.7	3	78.4
4	42.3	16	70.5	CH ₃	15.7	4	71.7
5	56.5	17	44.8	OCH ₃ CO	21.7	5	78.1
6	70.2	18	19.8	OCH ₃ CO	170.3	6	70.2
7	38.1	19	27.5	β-D-Xylp-1	106.3		
8	49.8	20	25.6	2	83.6		
9	21.0	21	20.6	3	78.1		
10	28.0	22	33.2	4	71.0		
11	26.0	23	99.1	5	66.8		
12	32.9	28	19.3				

Kahiricoside II (Radwan et al., 2004)

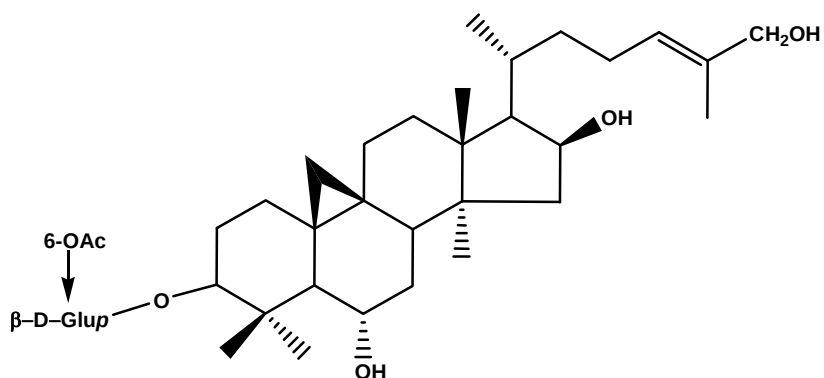
Plant Material	: <i>Astragalus kahiricus</i>
Molecular Formula	: $C_{36}H_{60}O_9$
Molecular Weight	: 636
Optical Rotation	: $[\alpha]_D^{25} +12.3$ (c 0.06, MeOH)
^{13}C -NMR Solvent	: C_5D_5N
Name	: 9 β ,19-cyclolanost-24E-ene-3 β ,6 α ,16 β ,27-tetraol-3-O- β -D-glucopyranoside
MP	: 118 $^{\circ}C$

C-1	32.4	C-13	45.8	C-25	135.3
2	30.2	14	47.0	26	14.0
3	89.1	15	49.2	27	68.3
4	42.6	16	71.3	28	29.0
5	54.1	17	57.0	29	16.7
6	68.1	18	19.0	30	20.2
7	38.5	19	29.9	β -D-Glup-1	107.0
8	46.9	20	30.8	2	76.0
9	21.3	21	18.2	3	78.8
10	29.2	22	36.8	4	71.9
11	26.4	23	25.6	5	78.2
12	33.3	24	125.9	6	63.1

Kahiricoside III (Radwan et al., 2004)

Plant Material	: <i>Astragalus kahiricus</i>
Molecular Formula	: C ₃₈ H ₆₂ O ₉
Molecular Weight	: 679
Optical Rotation	: $[\alpha]_D^{25} +49$ (c 0.065, MeOH)
¹³ C-NMR Solvent	: C ₅ D ₅ N
Name	: 9 β ,19-cyclolanost-24E-ene-3 β ,6 α ,16 β ,27-tetraol-3-O-(2'-O-acetyl)- β -D-glucopyranoside
MP	: 140 °C

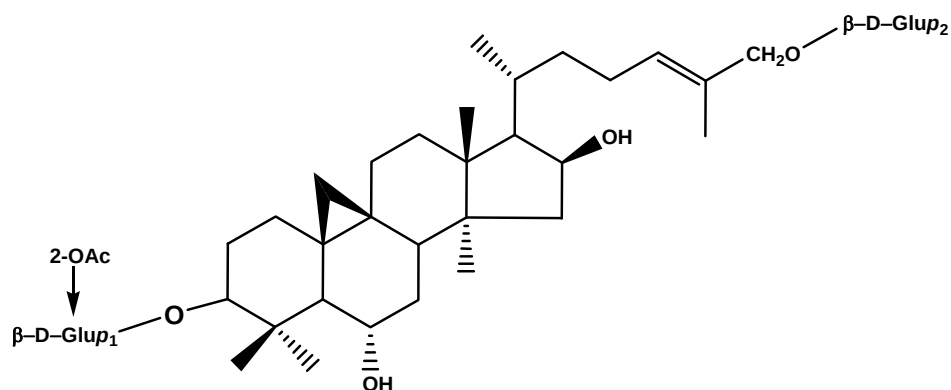
C-1	32.3	C-13	45.8	C-25	135.3	β -D-Glup-1	104.0
2	30.1	14	47.1	26	14.0	2	75.9
3	89.4	15	49.3	27	68.3	3	76.3
4	42.3	16	71.3	28	28.7	4	71.9
5	54.0	17	57.0	29	16.6	5	78.4
6	68.0	18	19.1	30	20.2	6	62.7
7	38.6	19	30.0	OCH ₃ CO	21.3		
8	46.9	20	30.8	OCH ₃ CO	170.1		
9	21.3	21	18.2				
10	29.2	22	36.9				
11	26.3	23	25.6				
12	33.3	24	125.8				

Kahiricoside IV (Radwan et al., 2004)

Plant Material	: <i>Astragalus kahiricus</i>
Molecular Formula	: $C_{38}H_{62}O_9$
Molecular Weight	: 679
Optical Rotation	: $[\alpha]_D^{25} +175$ (c 0.02, MeOH)
^{13}C -NMR Solvent	: C_5D_5N
Name	: 9 β ,19-cyclolanost-24E-ene-3 β ,6 α ,16 β ,27-tetraol-3-O-(6'-O-acetyl)- β -D-glucopyranoside
MP	: 165 $^{\circ}C$

C-1	32.5	C-13	45.8	C-25	135.3	β -D-Glup-1	107.0
2	30.2	14	47.0	26	14.0	2	75.8
3	89.3	15	49.2	27	68.3	3	78.5
4	42.6	16	71.3	28	28.9	4	71.7
5	54.2	17	57.0	29	16.7	5	74.9
6	67.9	18	19.0	30	20.2	6	64.8
7	38.5	19	29.9	OCH ₃ CO	21.4		
8	46.9	20	30.8	OCH ₃ CO	170.8		
9	21.4	21	18.3				
10	29.3	22	36.9				
11	26.4	23	25.6				
12	33.3	24	125.8				

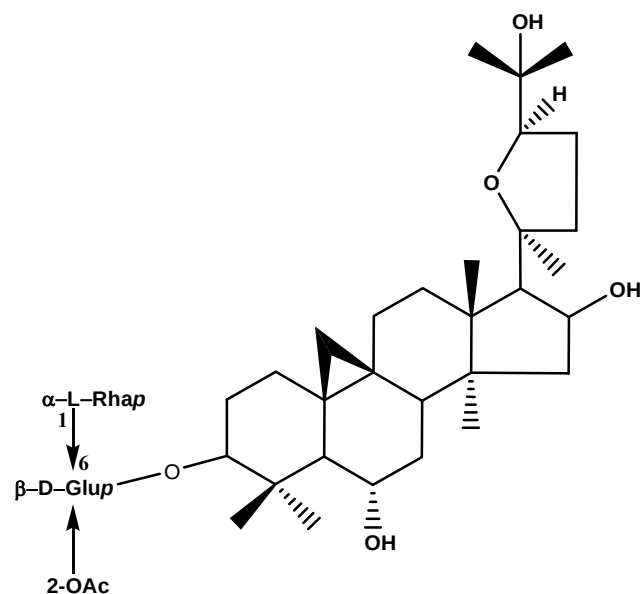
Kahiricoside V (Radwan et al., 2004)



Plant Material	: <i>Astragalus kahiricus</i>
Molecular Formula	: C ₄₂ H ₇₀ O ₁₄
Molecular Weight	: 821
Optical Rotation	: $[\alpha]_D^{25}$ +86.7 (c 0.015, MeOH)
¹³ C-NMR Solvent	: C ₅ D ₅ N
Name	: 9 β ,19-cyclolanost-24E-ene-3 β ,6 α ,16 β ,27-tetraol-3-O- β -D-glucopyranosyl-27-O- β -D-glucopyranoside
MP	: 157-158 °C

C-1	32.5	C-13	45.8	C-25	131.8	β -D-Glup ₂ -1	103.5
2	30.2	14	47.1	26	14.3	2	75.3
3	89.1	15	49.7	27	75.3	3	78.7
4	42.7	16	71.4	28	29.0	4	71.8
5	54.1	17	57.0	29	16.8	5	78.5
6	68.0	18	19.1	30	20.3	6	63.0
7	38.5	19	30.0	β -D-Glup ₁ -1	107.0		
8	46.9	20	30.8	2	76.0		
9	21.3	21	18.3	3	78.8		
10	29.3	22	36.6	4	71.9		
11	26.4	23	25.6	5	78.2		
12	33.3	24	129.6	6	62.9		

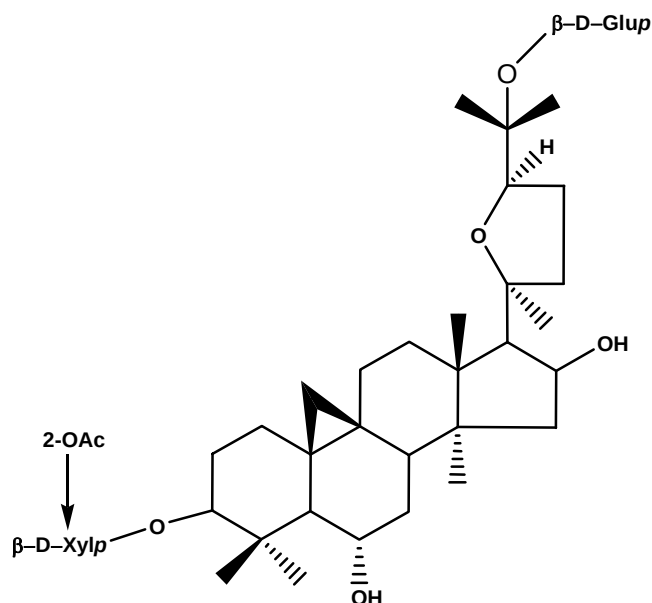
Cycloascauloside A (Alaniya et al., 2006)



Plant Material	: <i>Astragalus caucasicus</i>
Molecular Formula	: C ₄₄ H ₇₂ O ₁₆
Molecular Weight	: 856
Optical Rotation	: [α] _D ²⁰ -4 (c 0.1, EtOH)
¹³ C-NMR Solvent	: C ₅ D ₅ N
Name	: 20 <i>S</i> ,24 <i>R</i> -epoxycycloartan-3β,6α,16β,25-tetraol-3- O-[α-L-Rhamnopyranosyl(1→6)]-β-D-(2'-O- acetyl)-glucopyranoside
MP	: 210-215 °C

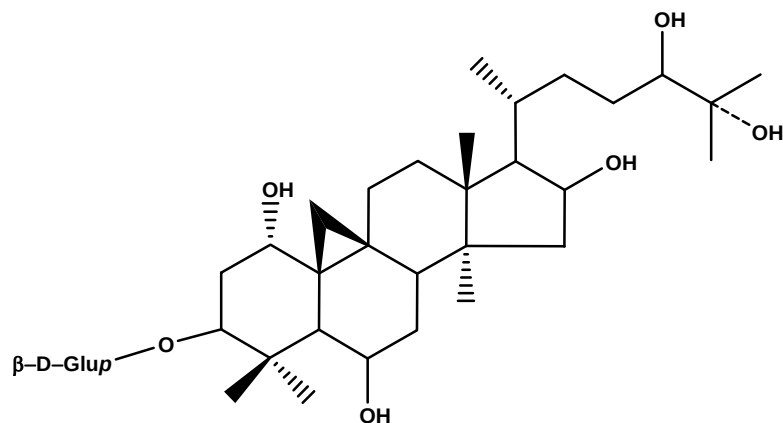
C-1	32.7	C-13	45.5	C-25	70.1	β-D-Glup-1	104.6
2	29.6	14	46.3	26	27.5	2	79.0
3	88.1	15	47.4	27	28.1	3	78.5
4	42.7	16	73.3	28	20.3	4	72.0
5	53.8	17	58.8	29	29.1	5	77.8
6	68.4	18	21.6	30	16.0	6	67.6
7	37.8	19	30.2	COCH ₃	170.0	α-L-Rhap-1	101.0
8	46.3	20	86.9	COCH ₃	19.7	2	72.0
9	20.3	21	28.8			3	71.7
10	29.6	22	34.7			4	74.8
11	26.2	23	26.1			5	68.9
12	33.7	24	85.0			6	17.8

Cyclogaleginoside D (Alaniya et al., 2006)



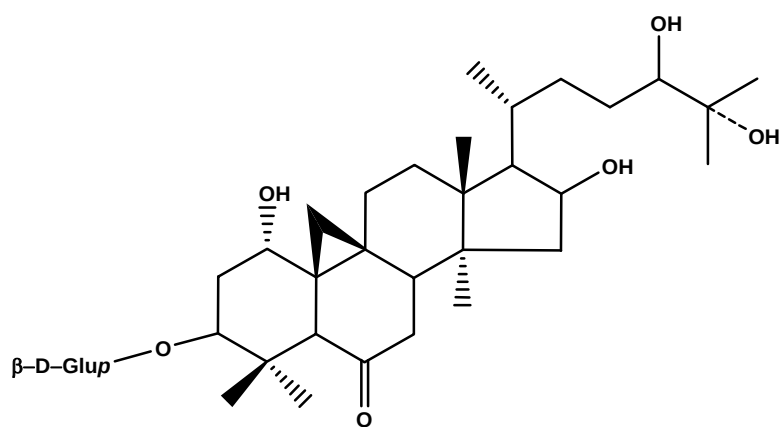
Plant Material	: <i>Astragalus galegiformis</i>
Molecular Formula	: C ₄₃ H ₇₀ O ₁₅
Molecular Weight	: 826
¹³ C-NMR Solvent	: CD ₃ OD
Name	: 25- <i>O</i> -β-D-glucopyranoside-20 <i>S</i> ,24 <i>R</i> -epoxycycloartan-3β,6α,16β,25-tetraol-3- <i>O</i> -β-D-(2'- <i>O</i> -acetyl)xylopyranoside
MP	: 215-217 °C

C-1	33.0	C-13	47.7	C-25	78.8	β-D-Xylp-1	104.8
2	30.3	14	47.3	26	24.8	2	76.0
3	91.6	15	49.1	27	23.4	3	75.0
4	42.7	16	74.4	28	20.8	4	71.3
5	54.3	17	56.2	29	28.6	5	66.6
6	69.6	18	21.4	30	16.2	β-D-Glup-1	99.1
7	38.8	19	32.2	COCH ₃	169.5	2	75.0
8	48.5	20	88.7	COCH ₃	21.1	3	77.6
9	21.8	21	24.0			4	71.8
10	30.4	22	27.9			5	77.6
11	27.0	23	24.2			6	62.8
12	34.1	24	86.3				

Mongholicoside A (Yu et al., 2007)

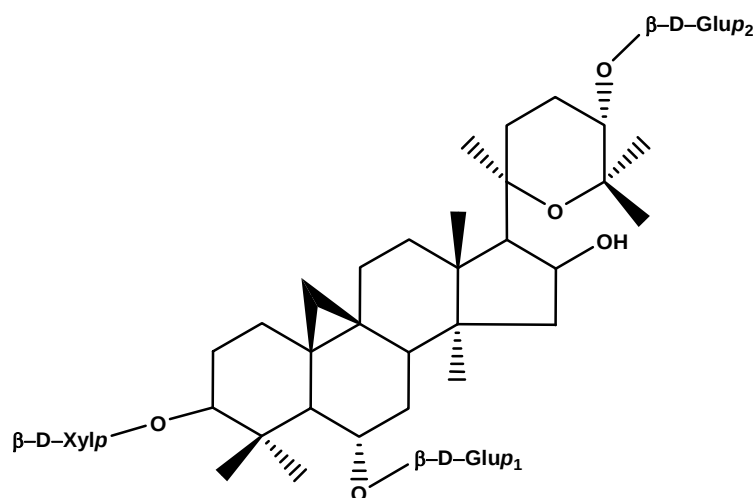
Plant Material	: <i>Astragalus membranaceus</i> var. <i>mongholicus</i>
Molecular Formula	: C ₃₆ H ₆₂ O ₁₁
Molecular Weight	: 693
Optical Rotation	: $[\alpha]_D^{25}$ +57.23 (c 0.1, MeOH)
¹³ C-NMR Solvent	: C ₅ D ₅ N
Name	: 3-O-β-D-glucopyranosyl-9β,19-cyclolanost-1α,3β,6α,16β,24,25pentaol
MP	: 275-278 °C

C-1	72.3	C-13	45.8	C-25	72.5
2	37.8	14	46.9	26	25.7
3	85.3	15	48.7	27	26.5
4	42.7	16	72.0	28	29.1
5	47.1	17	57.4	29	16.1
6	68.5	18	19.2	30	20.1
7	38.7	19	30.3	β-D-Glup-1	107.0
8	47.5	20	28.6	2	76.0
9	22.0	21	18.3	3	78.7
10	33.6	22	33.3	4	71.7
11	25.6	23	27.9	5	77.8
12	33.0	24	77.1	6	62.9

Mongholicoside B (Yu et al., 2007)

Plant Material	: <i>Astragalus membranaceus</i> var. <i>mongholicus</i>
Molecular Formula	: C ₃₆ H ₆₀ O ₁₁
Molecular Weight	: 691
Optical Rotation	: $[\alpha]_D^{25}$ +32.15 (c 0.1, MeOH)
¹³ C-NMR Solvent	: C ₅ D ₅ N
Name	: 3-O-β-D-glucopyranosyl-9β,19-cyclolanost-1α,3β,16β,24,25pentaol-6-one
MP	: 281-283 °C

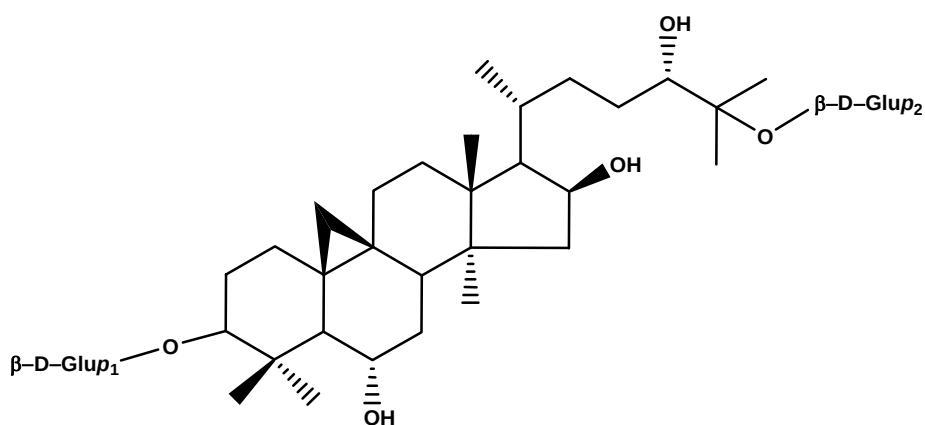
C-1	71.6	C-13	46.3	C-25	72.5
2	37.6	14	48.0	26	25.7
3	84.0	15	46.5	27	26.4
4	42.0	16	71.6	28	27.4
5	52.7	17	56.4	29	15.5
6	212.5	18	18.4	30	18.6
7	41.9	19	21.0	β-D-Glup-1	106.9
8	42.7	20	28.8	2	75.9
9	22.3	21	15.9	3	78.7
10	33.6	22	33.1	4	71.7
11	26.5	23	27.9	5	78.0
12	33.2	24	77.2	6	63.0

Cyclotrisectoside (Sukhina et al, 2007)

Plant Material : *Astragalus dissectus* B. Fedtsch et Ivanova
 Molecular Formula : $C_{47}H_{78}O_{19}$
 Molecular Weight : 946
 ^{13}C -NMR Solvent : C_5D_5N
 Name : 20*R*,25-epoxy-24*S*-cycloartan-3 β ,6 α ,16 β ,24-tetraol-3-*O*- β -D-xylopyranoside-6,24-di-*O*- β -D-glucopyranoside

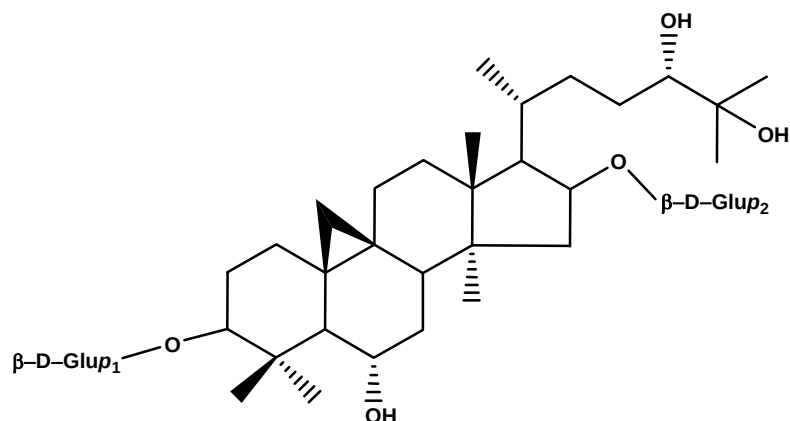
C-1	32.2	C-13	45.9	C-25	74.6	β -D-Glup ₁ -1	105.0
2	29.3	14	46.5	26	28.7	2	75.1
3	88.5	15	46.6	27	27.9	3	78.5
4	42.6	16	74.1	28	19.9	4	71.6
5	52.6	17	60.7	29	28.7	5	78.1
6	79.2	18	21.0	30	16.5	6	63.0
7	34.0	19	28.9	β -D-Xylp-1	107.6	β -D-Glup ₂ -1	100.6
8	45.6	20	78.6	2	75.5	2	74.2
9	21.0	21	28.7	3	77.9	3	78.5
10	30.1	22	26.6	4	71.2	4	71.3
11	26.2	23	18.8	5	67.0	5	79.0
12	33.9	24	79.5			6	63.1

Cyclochivinoside B (Naubeev et al., 2007)



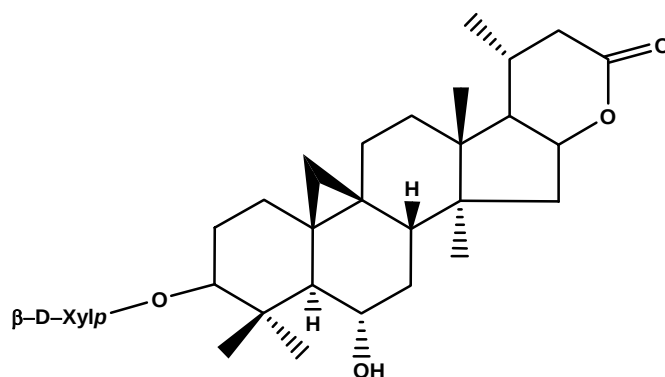
Plant Material	: <i>Astragalus chivensis</i> Bunge
Molecular Formula	: C ₄₂ H ₇₂ O ₁₅
Molecular Weight	: 816
¹³ C-NMR Solvent	: C ₅ D ₅ N
Name	: 24 <i>S</i> -cycloartan-3β,6α,16β,24,25-pentaol-3,25-di- O-β-D-glucopyranoside
MP	: 254-256 °C

C-1	32.4	C-13	45.7	C-25	80.9	β-D-Glup ₂ -1	98.7
2	30.2	14	46.9	26	24.2	2	75.4
3	89.1	15	48.6	27	21.5	3	78.7
4	42.6	16	71.7	28	20.2	4	71.8
5	54.1	17	57.3	29	28.9	5	78.2
6	68.0	18	18.8	30	16.7	6	62.8
7	38.4	19	30.0	β-D-Glup ₁ -1	106.9		
8	47.0	20	31.5	2	75.9		
9	21.3	21	18.8	3	78.7		
10	29.2	22	35.0	4	71.9		
11	26.3	23	29.3	5	78.2		
12	33.2	24	78.9	6	63.1		

Cyclochivinoside C (Naubeev and Uteniyazov, 2007)

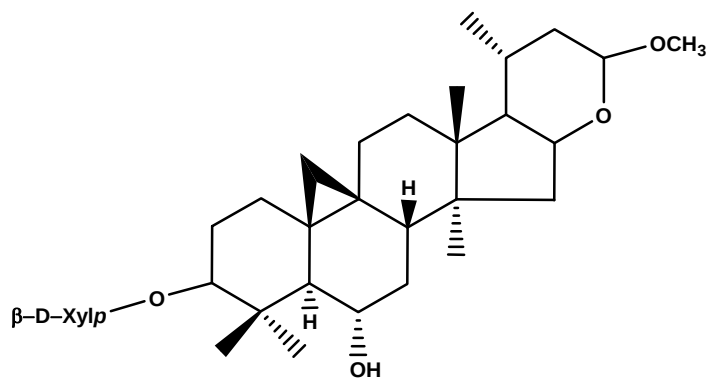
Plant Material	: <i>Astragalus chivensis</i> Bunge
Molecular Formula	: C ₄₂ H ₇₂ O ₁₅
Molecular Weight	: 816
¹³ C-NMR Solvent	: C ₅ D ₅ N
Name	: 24 <i>S</i> -cycloartan-3β,6α,16β,24,25-pentaol-3,16-di- O-β-D-glucopyranoside
MP	: 258-260 °C

C-1	32.5	C-13	45.7	C-25	72.5	β-D-Glup ₂ -1	106.7
2	30.1	14	46.1	26	25.7	2	75.6
3	88.9	15	47.5	27	26.5	3	78.2
4	42.7	16	82.1	28	20.2	4	71.6
5	54.1	17	57.3	29	28.5	5	77.9
6	68.1	18	18.2	30	16.4	6	62.8
7	38.4	19	29.6	β-D-Glup ₁ -1	106.9		
8	46.3	20	31.1	2	75.8		
9	21.2	21	18.9	3	78.6		
10	29.3	22	33.9	4	71.7		
11	26.2	23	29.7	5	78.0		
12	33.1	24	77.2	6	63.1		

Bicusposide A (Choudhary et al., 2008)

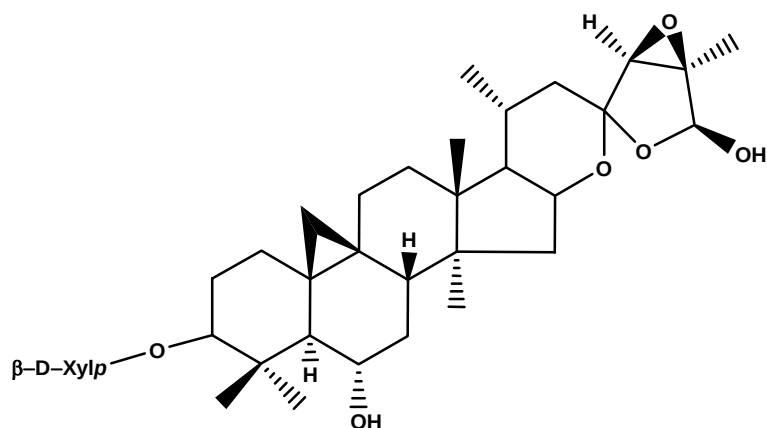
Plant Material	: <i>Astragalus bicuspis</i>
Molecular Formula	: $C_{31}H_{49}O_8$
Molecular Weight	: 549
Optical Rotation	: $[\alpha]_D^{25} -4.0$ (c 0.5, MeOH)
^{13}C -NMR Solvent	: C_5D_5N
Name	: 6 α -hydroxy-3-O- β -xylopyranosyloxy-24,25,26,27-tetranor-9,19-cyclolanosta-16,23-lactone

C-1	32.3	C-13	46.6	29	16.6
2	30.2	14	44.8	30	19.9
3	88.5	15	43.4	β -D-Xylp-1	107.6
4	42.6	16	80.6	2	75.6
5	53.7	17	54.1	3	78.6
6	67.0	18	19.4	4	71.3
7	37.8	19	29.0	5	67.1
8	45.7	20	27.3		
9	21.1	21	21.2		
10	29.1	22	38.8		
11	26.0	23	173.9		
12	32.3	28	28.6		

Bicusposide B (Choudhary et al., 2008)

Plant Material : *Astragalus bicuspis*
 Molecular Formula : $C_{32}H_{51}O_8$
 Molecular Weight : 563
 Optical Rotation : $[\alpha]_D^{25} -4.4$ (c 0.5, MeOH)
 ^{13}C -NMR Solvent : C_5D_5N
 Name : 6 α -hydroxy-23-methoxy-16 β ,23(*R*)-epoxy-24,25,26,27-tetranor-9,19-cyclolanosta-3-*O*- β -xyloside

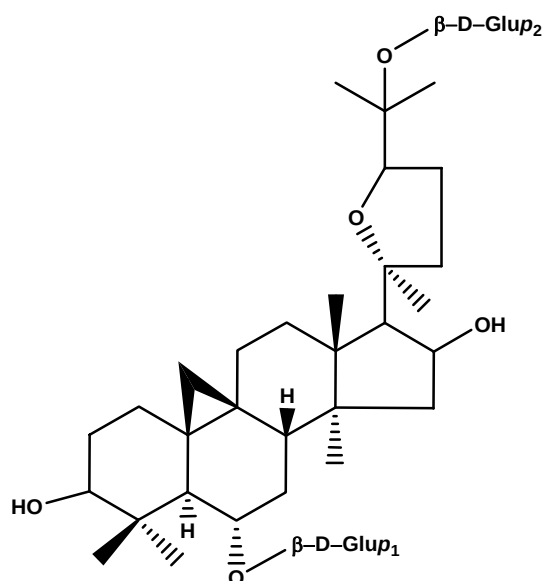
C-1	32.4	C-13	46.1	29	16.6
2	30.3	14	44.8	30	20.2
3	88.6	15	43.5	OCH ₃	54.6
4	42.7	16	70.7	β -D-Xylp-1	107.6
5	53.9	17	56.6	2	75.6
6	67.4	18	19.5	3	78.5
7	37.9	19	29.6	4	71.3
8	46.2	20	25.6	5	67.1
9	21.2	21	20.5		
10	29.2	22	38.5		
11	26.2	23	100.4		
12	33.3	28	28.7		

Bicusposide C (Choudhary et al., 2008)

Plant Material : *Astragalus bicuspis*
 Molecular Formula : $C_{35}H_{53}O_{10}$
 Molecular Weight : 633
 Optical Rotation : $[\alpha]_D^{25} -4.8$ (c 0.5, MeOH)
 ^{13}C -NMR Solvent : C_5D_5N
 Name : 23(*R*),24(*S*),25(*R*),26(*S*)-16 β /23,23/26,24/25-triepoxo-6 α ,26-dihydroxy,9,19-cyclolanosta-3-*O*- β -xyloside

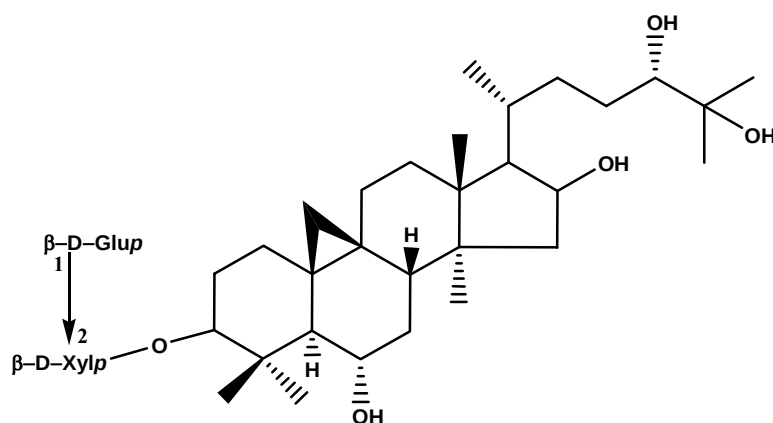
C-1	32.4	C-13	46.2	25	63.7
2	30.3	14	44.7	26	97.7
3	88.6	15	43.7	27	13.2
4	42.6	16	74.5	28	28.7
5	53.7	17	56.4	29	16.6
6	67.4	18	19.5	30	20.2
7	38.2	19	29.7	β -D-Xylp-1	107.6
8	46.1	20	26.2	2	75.6
9	21.1	21	20.6	3	78.5
10	29.2	22	42.5	4	71.3
11	26.1	23	105.9	5	67.0
12	33.2	24	64.1		

Astramembranoside A (Kim et al., 2008)



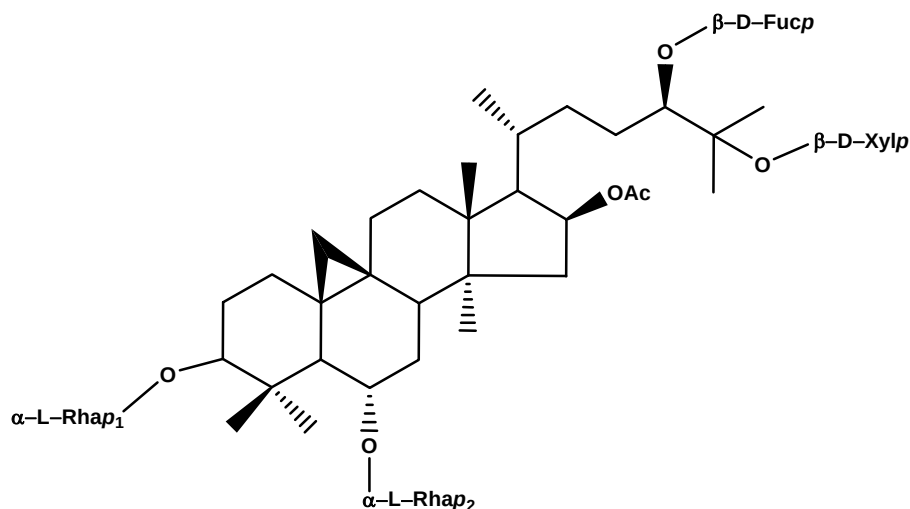
Plant Material : *Astragalus membranaceus*
 Molecular Formula : $C_{42}H_{69}O_{15}$
 Molecular Weight : 813
 Optical Rotation : $[\alpha]_D^{18} +23.5$ (c 0.11, MeOH)
 ^{13}C -NMR Solvent : C_5D_5N
 Name : Cycloastragenol 6,25-di-*O*- β -D-glucopyranoside

C-1	32.6	C-13	45.3	25	78.7	β -D-Glup ₂ -1	99.0
2	31.3	14	46.2	26	22.9	2	75.2
3	78.3	15	45.8	27	25.7	3	78.6
4	42.5	16	73.6	28	29.1	4	71.4
5	52.5	17	58.1	29	16.1	5	78.1
6	79.6	18	21.3	30	19.9	6	62.8
7	34.5	19	29.4	β -D-Glup ₁ -1	105.0		
8	46.0	20	87.2	2	75.6		
9	21.1	21	27.8	3	79.4		
10	29.5	22	35.1	4	71.9		
11	26.3	23	26.1	5	78.2		
12	33.5	24	82.1	6	63.0		

Astramembranoside B (Kim et al., 2008)

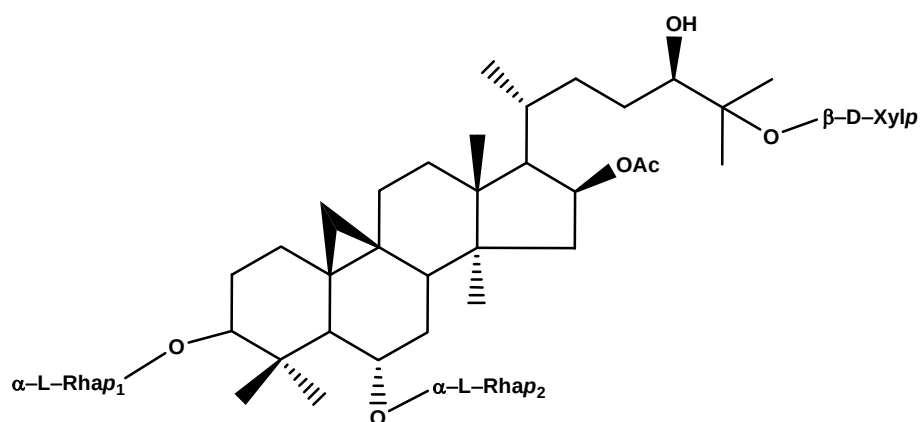
Plant Material : *Astragalus membranaceus*
Molecular Formula : $C_{41}H_{69}O_{14}$
Molecular Weight : 785
Optical Rotation : $[\alpha]_D^{25} +32.1$ (c 0.15, MeOH)
 ^{13}C -NMR Solvent : C_5D_5N
Name : Cyclocanthogenin 3-*O*- β -D-glucopranosyl(1 $\rightarrow$ 2)- β -D-xylopranoside

C-1	32.5	C-13	45.7	25	72.5	β -D-Glup-1	106.2
2	30.4	14	46.8	26	25.8	2	77.1
3	88.6	15	48.3	27	26.5	3	78.3
4	42.8	16	72.0	28	28.8	4	71.7
5	54.0	17	57.3	29	16.6	5	78.0
6	67.7	18	18.8	30	20.1	6	62.8
7	38.3	19	29.6	β -D-Xylp-1	105.7		
8	46.7	20	28.6	2	83.4		
9	21.4	21	18.3	3	77.9		
10	29.1	22	33.0	4	71.0		
11	26.3	23	27.9	5	66.7		
12	33.2	24	77.2				

Eremophiloside A (Perrone et al., 2008)

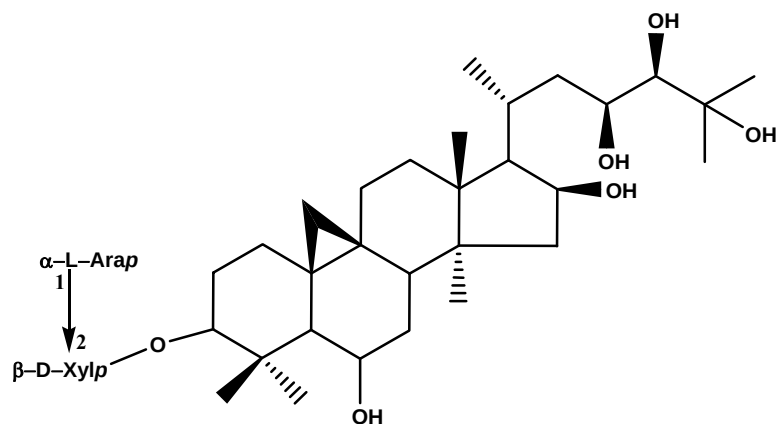
Plant Material : *Astragalus eremophilus*
 Molecular Formula : C₅₅H₉₂O₂₂
 Molecular Weight : 1127
 Optical Rotation : $[\alpha]_D^{25} +8.7$ (c 0.2, MeOH)
¹³C-NMR Solvent : CD₃OD
 Name : 3-*O*- α -L-rhamnoprinosyl-6-*O*- α -L-rhamnoprinosyl-24-*O*- β -D-fucopyranosyl-25-*O*- β -D-xylopyranosyl-16-*O*-acetox-3 β ,6 α ,16 β ,24(*R*),25-pentahydroxycycloartane
 MP : 264-266 °C

C-1	32.5	C-16	76.8	OCOCH ₃	173.2		
2	29.5	17	56.8	OCOCH ₃	21.8		
3	89.7	18	18.3				
4	42.6	19	30.2	α -L-Rhap ₁ -1	104.0	β -D-Fucp-1	104.9
5	53.0	20	32.0	2	72.0	2	73.3
6	80.6	21	18.2	3	72.3	3	75.7
7	34.8	22	34.4	4	73.0	4	73.7
8	47.5	23	30.2	5	69.8	5	71.2
9	21.4	24	86.8	6	17.6	6	16.4
10	29.4	25	83.4	α -L-Rhap ₂ -1	103.9	β -D-Xylp-1	98.7
11	26.6	26	19.5	2	72.4	2	74.8
12	33.2	27	24.3	3	72.2	3	77.4
13	46.4	28	28.9	4	73.5	4	71.0
14	47.8	29	16.4	5	69.5	5	66.4
15	46.1	30	20.2	6	17.6		

Eremophiloside B (Perrone et al., 2008)

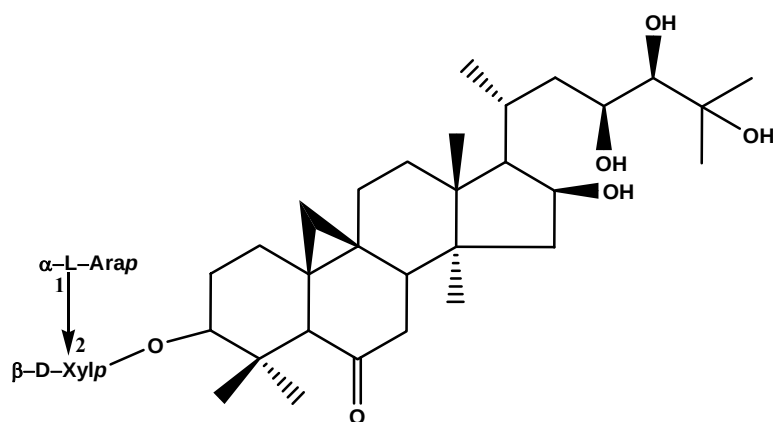
Plant Material : *Astragalus eremophilus*
 Molecular Formula : $C_{49}H_{82}O_{18}$
 Molecular Weight : 981
 Optical Rotation : $[\alpha]_D^{25} -2.5$ (c 0.2, MeOH)
 ^{13}C -NMR Solvent : CD_3OD
 Name : 3-*O*- α -L-rhamnoprinosyl-6-*O*- α -L-rhamnoprinosyl-25-*O*- β -D-xylopyranosyl-16-*O*-acetox-3 β ,6 α ,16 β ,24(*R*),25-pentahydroxy cycloartane
 MP : 270-272 °C

C-1	32.5	C-16	76.8	OCOCH ₃	173.2		
2	29.5	17	56.8	OCOCH ₃	21.8		
3	89.7	18	18.3				
4	42.6	19	30.2	α -L-Rhap ₁ -1	104.0	β -D-Xylp-1	98.7
5	53.0	20	32.0	2	72.0	2	74.8
6	80.6	21	18.2	3	72.3	3	77.4
7	34.8	22	34.4	4	73.0	4	71.0
8	47.5	23	30.2	5	69.8	5	66.4
9	21.4	24	78.9	6	17.6		
10	29.4	25	80.9	α -L-Rhap ₂ -1	103.9		
11	26.6	26	21.0	2	72.4		
12	33.2	27	23.3	3	72.2		
13	46.4	28	28.9	4	73.5		
14	47.8	29	16.4	5	69.5		
15	46.1	30	20.2	6	17.6		

Eremophiloside C (Perrone et al., 2008)

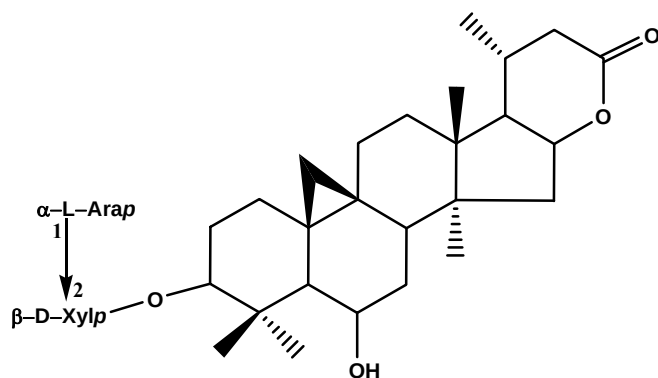
Plant Material	: <i>Astragalus eremophilus</i>
Molecular Formula	: C ₄₀ H ₆₈ O ₁₄
Molecular Weight	: 795
Optical Rotation	: $[\alpha]_D^{25} +23.7$ (c 0.2, MeOH)
¹³ C-NMR Solvent	: CD ₃ OD
Name	: 3-O-[α-L-arabinopranosyl-(1→2)-β-D-xylopyranosyl]-3β,6α,16β,23(<i>R</i>),24(<i>R</i>),25-hexahydroxycycloartane
MP	: 278-280 °C

C-1	33.0	C-13	46.5	25	74.5		
2	30.2	14	47.8	26	23.7		
3	89.4	15	47.9	27	28.0		
4	42.9	16	73.0	28	28.3		
5	54.6	17	58.2	29	16.0		
6	69.1	18	18.3	30	20.1		
7	38.6	19	31.2	β-D-Xylp-1	105.1	α-L-Arap-1	106.2
8	48.5	20	27.4	2	83.1	2	73.2
9	21.7	21	19.9	3	76.6	3	73.8
10	29.9	22	42.6	4	70.7	4	69.2
11	26.6	23	72.8	5	65.8	5	66.8
12	33.4	24	79.3				

Eremophiloside D (Perrone et al., 2008)

Plant Material : *Astragalus eremophilus*
Molecular Formula : C₄₀H₆₆O₁₄
Molecular Weight : 793
Optical Rotation : $[\alpha]_D^{25} +10.3$ (c 0.1, MeOH)
¹³C-NMR Solvent : CD₃OD
Name : 3-O-[α-L-arabinopyranosyl-(1→2)-β-D-xylopyranosyl]-3β,6α,16β,23(R),24(R),25-pentahydroxycycloartan-6-one
MP : 289-291 °C

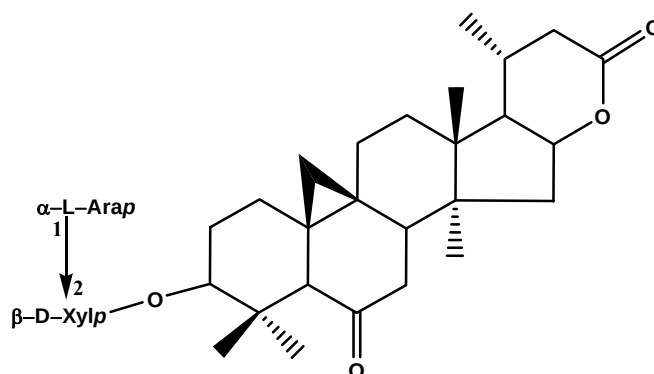
C-1	31.2	C-13	46.4	25	74.5		
2	29.4	14	48.2	26	23.8		
3	89.4	15	45.3	27	27.7		
4	42.2	16	72.7	28	26.7		
5	58.8	17	57.2	29	14.9		
6	214.6	18	15.4	30	19.2		
7	42.2	19	22.3	β-D-Xylp-1	105.1	α-L-Arap-1	106.2
8	44.1	20	27.8	2	83.1	2	73.2
9	22.4	21	19.9	3	76.6	3	73.8
10	30.5	22	42.8	4	70.7	4	69.2
11	27.0	23	72.8	5	65.8	5	66.8
12	33.4	24	79.4				

Eremophiloside E (Perrone et al., 2008)

Plant Material	: <i>Astragalus eremophilus</i>
Molecular Formula	: C ₃₆ H ₅₆ O ₁₂
Molecular Weight	: 703
Optical Rotation	: $[\alpha]_D^{25}$ -9.9 (c 0.15, MeOH)
¹³ C-NMR Solvent	: CD ₃ OD
Name	: 3- <i>O</i> -[α -L-arabinopranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl]-3 β , 6 α ,16 β -trihydroxy-24,25,26,27-tetranorcycloartan- 23,16 β -olide
MP	: 275-278 °C

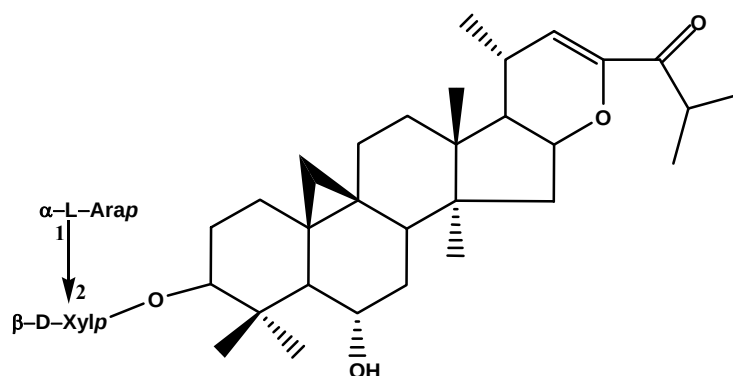
C-1	33.0	C-13	45.6	29	15.9
2	30.3	14	47.2	30	19.6
3	89.5	15	44.4	β -D-Xylp-1	105.1
4	42.8	16	82.3	2	83.1
5	54.4	17	55.2	3	76.6
6	68.8	18	20.3	4	70.7
7	38.3	19	31.0	5	65.8
8	47.8	20	28.0	α -L-Arap-1	106.2
9	21.8	21	21.3	2	73.2
10	30.6	22	39.0	3	73.8
11	26.6	23	177.1	4	69.2
12	33.1	28	28.2	5	66.8

Eremophiloside F (Perrone et al., 2008)



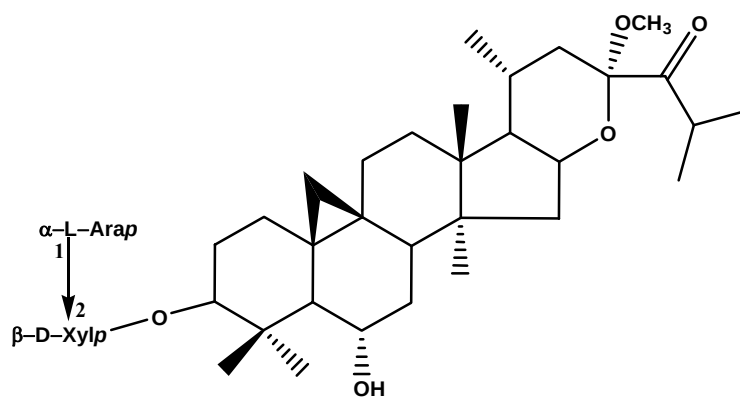
Plant Material	: <i>Astragalus eremophilus</i>
Molecular Formula	: C ₃₆ H ₅₄ O ₁₂
Molecular Weight	: 701
Optical Rotation	: [α] _D ²⁵ +11.1 (c 0.1, MeOH)
¹³ C-NMR Solvent	: CD ₃ OD
Name	: 3- <i>O</i> -[α-L-arabinopranosyl-(1→2)-β-D-xylopyranosyl]-3β,16β-dihydroxy-24,25,26,27-tetranorcytartin-6-one-23,16β-olide
MP	: 285-288 °C

C-1	31.1	C-13	45.8	29	15.0
2	29.4	14	48.3	30	18.6
3	88.4	15	42.1	β-D-Xylp-1	105.1
4	41.8	16	81.6	2	83.1
5	58.8	17	54.3	3	76.6
6	215.0	18	17.9	4	70.7
7	42.0	19	21.9	5	65.8
8	43.5	20	27.7	α-L-Arap-1	106.2
9	22.4	21	21.8	2	73.2
10	30.9	22	38.6	3	73.8
11	26.9	23	177.3	4	69.2
12	32.6	28	26.7	5	66.8

Eremophiloside G (Perrone et al., 2008)

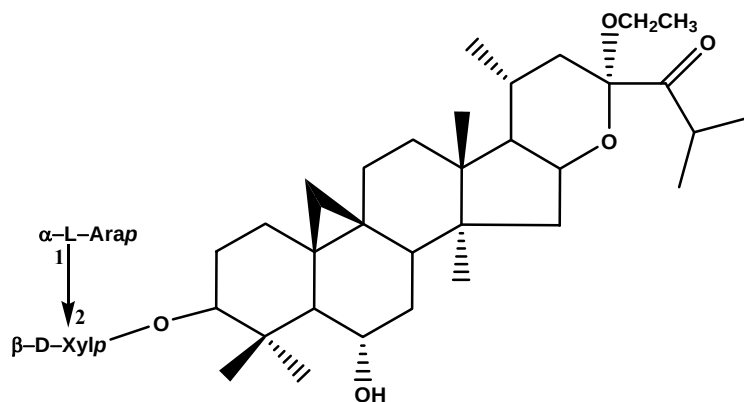
Plant Material	: <i>Astragalus eremophilus</i>
Molecular Formula	: C ₄₀ H ₆₂ O ₁₂
Molecular Weight	: 757
Optical Rotation	: $[\alpha]_D^{25}$ -30.8 (c 0.1, MeOH)
¹³ C-NMR Solvent	: CD ₃ OD
Name	: 3-O-[α-L-arabinopranosyl-(1→2)-β-D-xylopyranosyl]-3β,6α-dihydroxy-16β,23-epoxy-22,23-didehydro-25-hydrocycloartan-24-one
MP	: 160-162 °C

C-1	33.1	C-13	46.6	25	35.5		
2	30.4	14	47.9	26	18.8		
3	89.6	15	46.8	27	18.9		
4	43.2	16	77.0	28	28.6		
5	54.7	17	53.1	29	16.1		
6	69.3	18	19.1	30	21.2		
7	38.8	19	32.2	β-D-Xylp-1	105.1	α-L-Arap-1	106.2
8	48.8	20	25.5	2	83.1	2	73.2
9	22.0	21	25.1	3	76.6	3	73.8
10	30.5	22	118.5	4	70.7	4	69.2
11	26.8	23	150.3	5	65.8	5	66.8
12	30.8	24	203.6				

Eremophiloside H (Perrone et al., 2008)

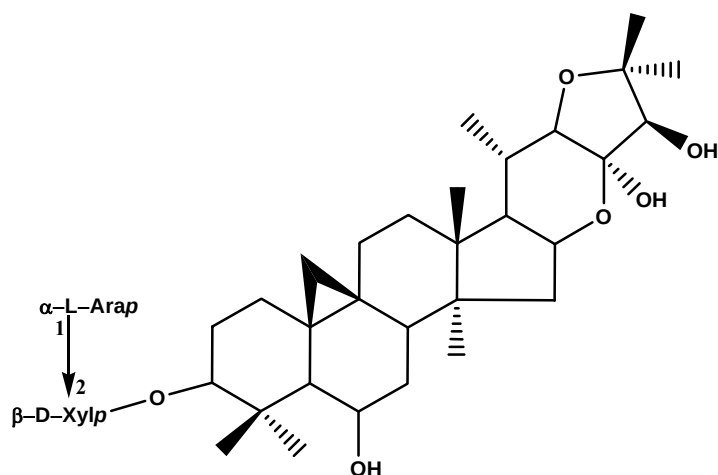
Plant Material	: <i>Astragalus eremophilus</i>
Molecular Formula	: C ₄₁ H ₆₆ O ₁₃
Molecular Weight	: 789
Optical Rotation	: [α] _D ²⁵ -10.7 (c 0.1, MeOH)
¹³ C-NMR Solvent	: CD ₃ OD
Name	: 3-O-[α -L-arabinopranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl]-3 β ,6 α -dihydroxy-23 α -methoxy-16 β ,23-epoxy-25-hydroxycycloartan-24-one
MP	: 169-171 °C

C-1	32.9	C-13	45.5	25	36.0		
2	30.2	14	47.6	26	19.2		
3	89.4	15	43.8	27	19.3		
4	43.0	16	73.5	28	28.2		
5	54.4	17	57.2	29	15.8		
6	69.0	18	20.7	30	19.7		
7	38.5	19	31.2	OCH ₃	51.0		
8	47.9	20	26.5	β -D-Xylp-1	105.1	α -L-Arap-1	106.2
9	21.8	21	20.5	2	83.1	2	73.2
10	30.4	22	39.9	3	76.6	3	73.8
11	26.6	23	104.3	4	70.7	4	69.2
12	33.6	24	215.7	5	65.8	5	66.8

Eremophiloside I (Perrone et al., 2008)

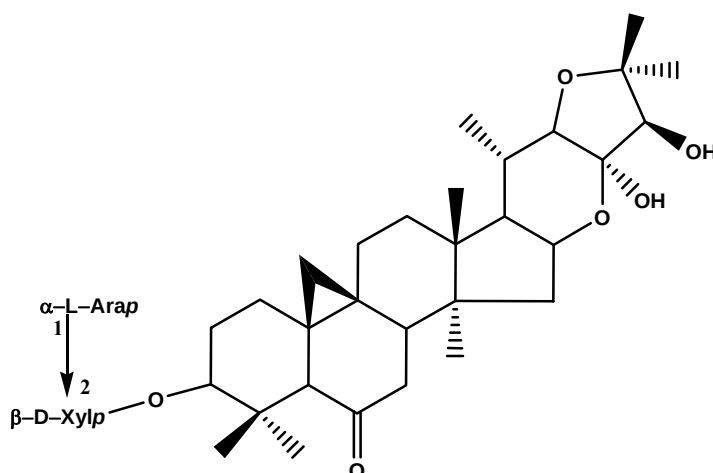
Plant Material	: <i>Astragalus eremophilus</i>
Molecular Formula	: C ₄₂ H ₆₈ O ₁₃
Molecular Weight	: 803
Optical Rotation	: $[\alpha]_D^{25}$ -12.0 (c 0.1, MeOH)
¹³ C-NMR Solvent	: CD ₃ OD
Name	: 3-O-[α-L-arabinopranosyl-(1→2)-β-D-xylopyranosyl]-3β, 6α-dihydroxy-23α-ethoxy-16β,23epoxy-25-hydroxycycloartan-24-one
MP	: 170-173 °C

C-1	33.2	C-13	45.4	25	36.0	β-D-Xylp-1	105.1
2	30.2	14	47.3	26	19.2	2	83.1
3	89.4	15	44.1	27	19.3	3	76.6
4	42.8	16	73.6	28	28.4	4	70.7
5	54.4	17	57.4	29	16.0	5	65.8
6	68.9	18	20.7	30	19.7	α-L-Arap-1	106.2
7	38.6	19	31.5	OCH ₂ CH ₃	59.6	2	73.2
8	48.0	20	26.5	OCH ₂ CH ₃	15.6	3	73.8
9	21.9	21	20.5			4	69.2
10	30.3	22	40.3			5	66.8
11	26.7	23	104.0				
12	33.8	24	215.4				

Eremophiloside J (Perrone et al., 2008)

Plant Material	: <i>Astragalus eremophilus</i>
Molecular Formula	: C ₄₀ H ₆₄ O ₁₄
Molecular Weight	: 791
Optical Rotation	: $[\alpha]_D^{25} +13.9$ (c 0.1, MeOH)
¹³ C-NMR Solvent	: CD ₃ OD
Name	: 3-O-[α-L-arabinopranosyl-(1→2)-β-D-xylopyranosyl]-3β,6α,23α,24β-tetrahydroxy-16β,23;22β,25-diepoxy-cycloartane
MP	: 260-262 °C

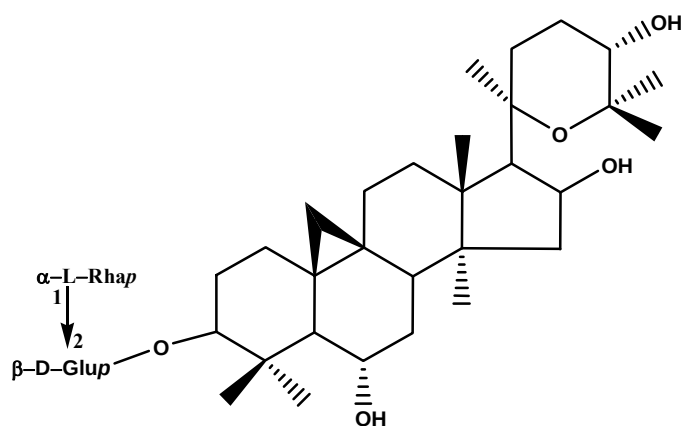
C-1	33.1	C-13	45.3	25	80.8		
2	30.4	14	47.2	26	28.8		
3	89.4	15	43.7	27	21.1		
4	42.7	16	72.8	28	28.3		
5	54.5	17	52.5	29	15.9		
6	69.1	18	20.6	30	19.7		
7	38.7	19	31.9	β-D-Xylp-1	105.1	α-L-Arap-1	106.2
8	48.2	20	36.5	2	83.1	2	73.2
9	21.3	21	16.8	3	76.6	3	73.8
10	29.8	22	85.4	4	70.7	4	69.2
11	26.6	23	103.5	5	65.8	5	66.8
12	34.1	24	81.9				

Eremophiloside K (Perrone et al., 2008)

Plant Material	: <i>Astragalus eremophilus</i>
Molecular Formula	: C ₄₀ H ₆₂ O ₁₄
Molecular Weight	: 789
Optical Rotation	: $[\alpha]_D^{25} +7.5$ (c 0.1, MeOH)
¹³ C-NMR Solvent	: CD ₃ OD
Name	: 3-O-[α-L-arabinopranosyl-(1→2)-β-D-xylopyranosyl]-3β,23α,24β-trihydroxy-16β,23;22β,25-diepoxy-cycloartan-6-one
MP	: 269-271 °C

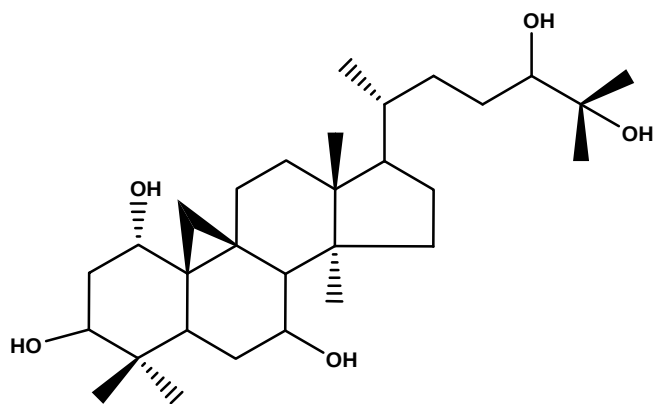
C-1	31.1	C-13	45.8	25	80.6		
2	29.5	14	48.3	26	29.1		
3	88.7	15	41.7	27	21.4		
4	41.8	16	72.6	28	26.8		
5	58.8	17	52.0	29	15.1		
6	215.0	18	17.8	30	18.9		
7	42.4	19	22.6	β-D-Xylp-1	105.1	α-L-Arap-1	106.2
8	44.3	20	36.5	2	83.1	2	73.2
9	22.4	21	17.2	3	76.6	3	73.8
10	30.9	22	85.3	4	70.7	4	69.2
11	27.2	23	103.3	5	65.8	5	66.8
12	34.0	24	82.0				

Cycloascauloside B (Alaniya et al., 2008)



Plant Material : *Astragalus caucasicus*
 Molecular Formula : $C_{42}H_{70}O_{14}$
 Molecular Weight : 798
 ^{13}C -NMR Solvent : C_5D_5N
 Name : 20*R*,25-epoxy-24*S*-cycloartan-3 β ,6 α ,16 β ,24-tetraol-3-*O*-[α -L-rhamnopyranosyl(1 $\rightarrow$ 2)]- β -D-glucopyranoside

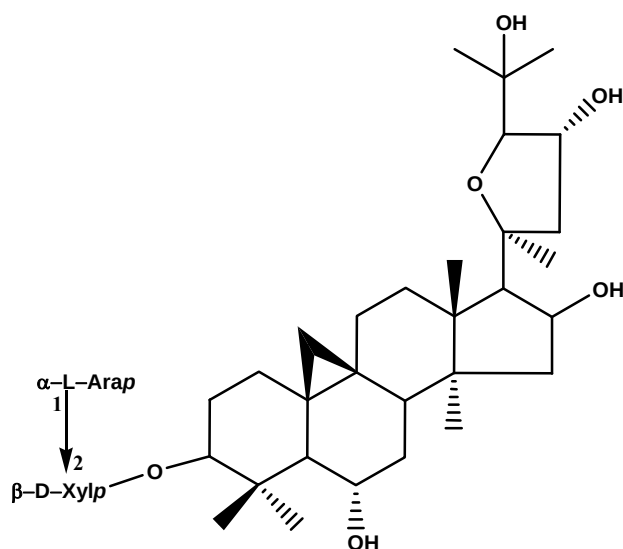
C-1	32.6	C-13	45.9	C-25	75.3		
2	30.5	14	46.9	26	28.6		
3	88.8	15	47.8	27	28.7		
4	42.6	16	74.1	28	19.1		
5	54.2	17	60.8	29	20.3		
6	68.7	18	20.7	30	16.7		
7	38.5	19	30.3	β -D-Glup-1	105.9	α -L-Rhap-1	101.8
8	46.7	20	79.9	2	79.0	2	72.1
9	21.0	21	28.0	3	78.0	3	72.5
10	28.7	22	26.6	4	72.5	4	73.9
11	26.3	23	24.0	5	78.1	5	69.6
12	34.2	24	68.0	6	62.8	6	18.7

Cyclomacrogin B (Iskenderov et al., 2008)

Plant Material : *Astragalus macropus* Bunge
Molecular Formula : C₃₀H₅₂O₅
Molecular Weight : 492
¹³C-NMR Solvent : C₅D₅N
Name : 24*R*-cycloartan-1α,3β,7β,24,25-pentaol
MP : 209-211 °C

C-1	72.6	C-13	45.9	C-25	72.7
2	38.9	14	49.1	26	25.9
3	72.9	15	37.8	27	26.1
4	40.9	16	28.9	28	19.0
5	39.3	17	52.3	29	26.0
6	32.3	18	17.9	30	13.9
7	70.1	19	28.9		
8	55.2	20	36.4		
9	21.0	21	18.6		
10	31.3	22	34.2		
11	26.4	23	28.2		
12	33.3	24	79.0		

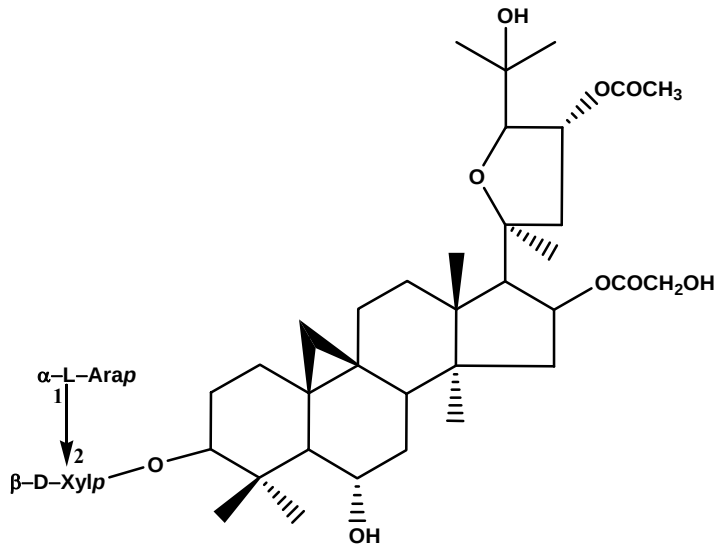
No Name (Çalış et al., 2008)



Plant Material : *Astragalus campylosema* Boiss. ssp. *campylosema*
Molecular Formula : $C_{40}H_{66}O_{14}$
Molecular Weight : 793
Optical Rotation : $[\alpha]_D^{35} +40$ (c 0.1, MeOH)
 ^{13}C -NMR Solvent : CD_3OD
Name : 3-*O*-[α -L-arabinopranosyl-(1→2)- β -D-xylopyranosyl]-3 β ,6 α ,16 β ,23 α ,25-pentahydroxy-20(*R*),24(*S*)-epoxycycloartane

C-1	33.1	C-13	45.3	25	71.5		
2	30.5	14	46.4	26	26.1		
3	89.5	15	46.5	27	27.7		
4	42.9	16	74.3	28	28.4		
5	54.6	17	59.4	29	15.9		
6	69.2	18	21.9	30	20.0		
7	38.8	19	31.9	β -D-Xylp-1	105.8	α -L-Arap-1	106.4
8	48.5	20	87.9	2	83.2	2	73.4
9	21.5	21	29.7	3	76.7	3	73.9
10	30.2	22	46.6	4	70.8	4	69.3
11	26.6	23	74.2	5	65.8	5	67.0
12	33.7	24	90.4				

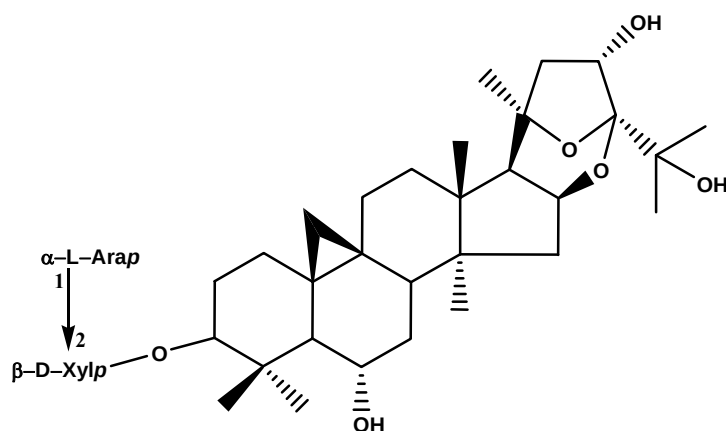
No Name (Çalış et al., 2008)



Plant Material : *Astragalus campylosema* Boiss. ssp. *campylosema*
Molecular Formula : C₄₄H₇₀O₁₇
Molecular Weight : 893
Optical Rotation : [α]_D²⁵ +67 (c 0.1, MeOH)
¹³C-NMR Solvent : CD₃OD
Name : 3-*O*-[α-*L*-arabinopranosyl-(1→2)-β-*D*-xylopyranosyl]-16-*O*-hydroxyacetox-23-*O*-acetox-3β,6α,16β,23α,25-pentahydroxy-20(*R*), 24(*S*)-epoxycycloartane

C-1	33.2	C-13	46.3	25	71.0	β-D-Xylp-1	105.8
2	30.3	14	46.7	26	26.0	2	83.2
3	89.4	15	46.8	27	27.0	3	76.7
4	43.0	16	77.6	28	28.3	4	70.8
5	54.3	17	58.5	29	16.0	5	65.8
6	69.1	18	21.1	30	20.5	α-L-Arap-1	106.4
7	38.5	19	31.7	OCOCH ₃	172.1	2	73.4
8	48.3	20	86.0	OCOCH ₃	20.7	3	73.9
9	21.2	21	28.3	OCOCH ₂ OH	173.8	4	69.3
10	30.1	22	44.8	OCOCH ₂ OH	61.3	5	67.0
11	26.7	23	76.0				
12	33.3	24	85.6				

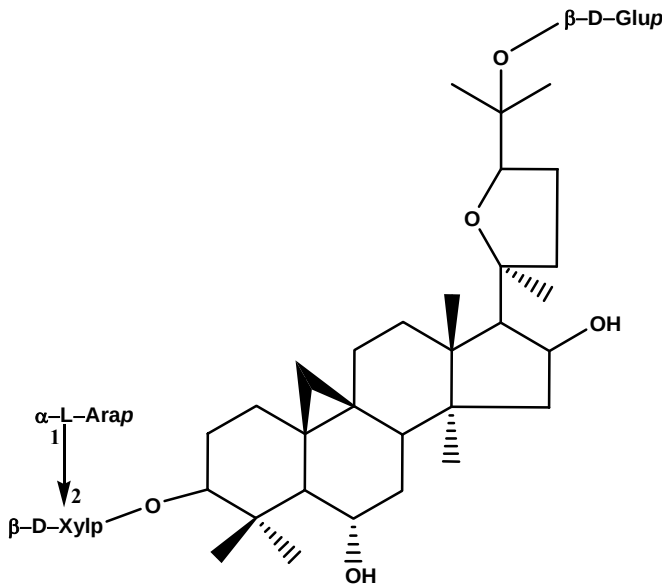
No Name (Çalış et al., 2008)



Plant Material : *Astragalus campylosema* Boiss. ssp. *campylosema*
Molecular Formula : $C_{40}H_{64}O_{14}$
Molecular Weight : 791
Optical Rotation : $[\alpha]_D^{25} +20$ (c 0.1, MeOH)
 ^{13}C -NMR Solvent : CD_3OD
Name : 3-O-[α -L-arabinopranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl]-3 β , 6 α , 23 α , 25-tetrahydroxy-20(R), 24(R)-16 β , 24; 20, 24-diepoxy-cycloartane

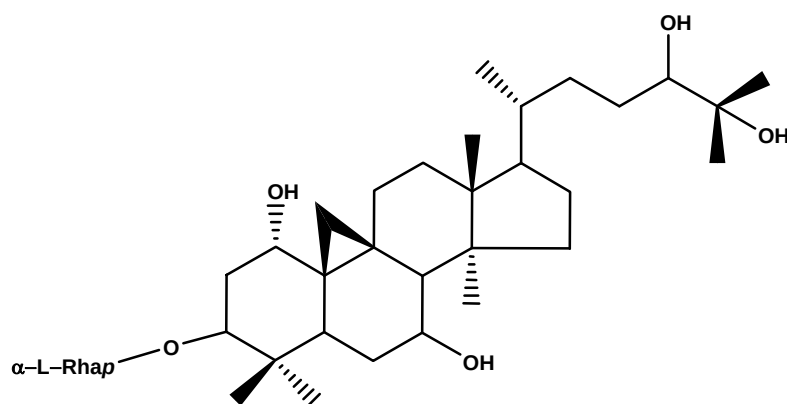
C-1	33.1	C-13	45.0	25	75.0		
2	30.4	14	45.8	26	24.3		
3	89.3	15	43.7	27	25.7		
4	43.0	16	75.5	28	28.5		
5	54.6	17	61.7	29	16.0		
6	69.3	18	23.5	30	20.2		
7	39.1	19	33.1	β -D-Xylp-1	105.8	α -L-Arap-1	106.4
8	48.0	20	84.6	2	83.2	2	73.4
9	21.3	21	30.1	3	76.7	3	73.9
10	30.1	22	43.6	4	70.8	4	69.3
11	27.2	23	77.6	5	65.8	5	67.0
12	34.2	24	108.6				

No Name (Çalış et al., 2008)



Plant Material : *Astragalus campylosema* Boiss. ssp. *campylosema*
Molecular Formula : C₄₆H₇₆O₁₈
Molecular Weight : 939
Optical Rotation : [α]_D²⁵ +24 (c 0.1, MeOH)
¹³C-NMR Solvent : CD₃OD
Name : 3-*O*-[α-L-arabinopranosyl-(1→2)-β-D-xylopyranosyl]-25-*O*-β-D-glucopyranosyl-3β,6α,16β,25-tetrahydroxy-20(*R*),24(*S*)-epoxycycloartane

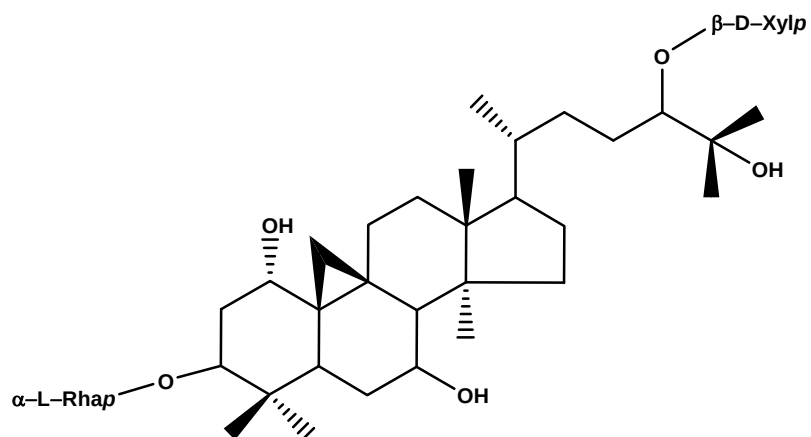
C-1	32.9	C-13	45.8	25	79.8	α-L-Arap-1	106.2
2	30.2	14	46.9	26	23.0	2	73.3
3	89.4	15	46.3	27	25.2	3	73.8
4	43.2	16	74.3	28	28.4	4	69.3
5	54.6	17	58.9	29	16.1	5	66.9
6	69.1	18	22.0	30	20.2	β-D-Glup-1	98.2
7	38.8	19	31.8	β-D-Xylp-1	105.6	2	74.8
8	48.5	20	88.4	2	83.1	3	77.9
9	21.5	21	28.2	3	76.7	4	71.1
10	30.2	22	35.3	4	70.7	5	77.3
11	26.6	23	26.1	5	65.7	6	62.4
12	33.6	24	82.9				

Cyclomacroside C (Iskenderov et al., 2009)

Plant Material : *Astragalus macropus* Bunge
 Molecular Formula : $C_{36}H_{62}O_9$
 Molecular Weight : 638
 ^{13}C -NMR Solvent : C_5D_5N
 Name : 3-*O*- α -L-rhamnopyranoside-24*R*-cycloartan-
 1 α ,3 β ,7 β ,24,25-pentaol
 M.P : 281-283 $^{\circ}C$

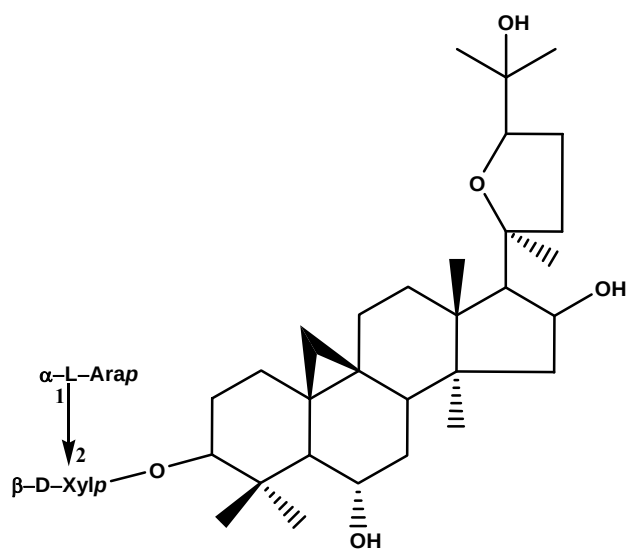
C-1	72.4	C-13	45.9	25	72.7
2	37.0	14	49.0	26	25.9
3	84.2	15	37.9	27	26.0
4	40.8	16	28.9	28	18.9
5	39.1	17	52.3	29	25.6
6	32.0	18	17.9	30	14.4
7	70.0	19	28.9	α -L-Rhap-1	104.4
8	55.3	20	36.4	2	72.3
9	21.0	21	18.6	3	72.8
10	30.9	22	34.2	4	74.0
11	26.3	23	28.2	5	69.7
12	33.2	24	79.0	6	18.3

Cyclomacroside D (Iskenderov et al., 2009)



Plant Material	: <i>Astragalus macropus</i> Bunge
Molecular Formula	: C ₄₁ H ₇₀ O ₁₃
Molecular Weight	: 770
¹³ C-NMR Solvent	: C ₅ D ₅ N
Name	: 24 <i>R</i> -cycloartan-1α,3β,7β,24,25-pentaol-3- <i>O</i> -α-L-rhamnopyranoside-24- <i>O</i> -β-D-xylopyranoside
M.P	: 151-153 °C

C-1	72.3	C-13	46.0	25	71.9	β -D-Xylp-1	106.0
2	37.0	14	49.0	26	25.6	2	75.1
3	84.1	15	37.9	27	26.7	3	78.3
4	40.7	16	29.5	28	18.5	4	70.8
5	39.1	17	52.1	29	25.6	5	67.2
6	32.5	18	17.9	30	14.4		
7	69.9	19	28.9	α -L-Rhap-1	104.8		
8	55.5	20	36.7	2	72.3		
9	21.0	21	19.0	3	72.8		
10	30.9	22	33.5	4	74.0		
11	26.4	23	28.2	5	69.7		
12	33.1	24	89.5	6	18.3		

Elongatoside (Çalış et al., 2008)

Plant Material	: <i>Astragalus elongatus</i>
Molecular Formula	: $C_{40}H_{64}O_{13}$
Molecular Weight	: 752
Name	: 3-O-[α -arabinopyranosyl-(1 $\rightarrow$ 2)- β -xylopyranosyl]-cycloastragenol

2. MATERIAL AND METHODS

2.1. General

Optical rotations were measured on a JASCO DIP 1000 polarimeter. IR measurements were obtained on a Bruker IFS-48 spectrometer. NMR experiments were performed on a Bruker DRX-600 spectrometer (Bruker BioSpinGmbH, Rheinstetten, Germany) equipped with a Bruker 5 mm TCI CryoProbeat 300 K. All 2D-NMR spectra were acquired in CD₃OD (99.95%, SigmaAldrich) and standard pulse sequences and phase cycling were used for DQF-COSY, HSQC, and HMBC spectra. The NMR data were processed using UxNMR software. Exact masses were measured by a Voyager DE mass spectrometer. Samples were analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDITOF) mass spectrometry. A mixture of analyte solution and α -cyano-4-hydroxycinnamic acid (Sigma) was applied to the metallic sample plate and dried. Mass calibration was performed with the ions from ACTH (fragment 18-39) at 2465.1989 Da and angiotensin III at 931.5154 Da as internal standard. ESIMS analyses were performed using a ThermoFinnigan LCQ Deca XP Max iontrap mass spectrometer equipped with Xcalibur software. GC analysis was performed on a Thermo Finnigan Trace GC apparatus using a I-Chirasil-Val column (0.32 mm x 25 m). Column chromatography was carried out on Silica gel (JT Baker, 40 μ m), Sephadex LH-20 (Amersham Biosciences, 17-0090-02) and RP (C-18, 40 μ m) (Merck). TLC analyses were carried out on Silica

gel 60 F₂₅₄ (Merck) and RP-18 F_{254s} (Merck) plates. Compounds were detected by UV and 20% H₂SO₄/water spraying reagent followed by heating at 110 °C for 1-2 min.

During chromatographic studies the following solvent systems were used;

I	CHCl ₃ :MeOH:H ₂ O	80:20:2
II	CHCl ₃ :MeOH:H ₂ O	80:20:1
III	CHCl ₃ :MeOH	80:20
IV	CHCl ₃ :MeOH:H ₂ O	70:30:3
V	CHCl ₃ :MeOH:H ₂ O	61:32:7
VI	CHCl ₃ :MeOH:H ₂ O	64:50:10
VII	MeOH:H ₂ O	8:2
VIII	MeOH:H ₂ O	7:3
IX	MeOH:H ₂ O	4:6
X	MeOH:H ₂ O	3:7
XI	MeOH:H ₂ O	2:8
XII	EtOAc:MeOH:H ₂ O	100:17:13

2.2. Plant Material

2.2.1. General Knowledge on *Astragalus amblolepis* Fischer

Astragalus amblolepis Fischer (Sect. Rhacophorus) was collected from Ersele Village, Pütürge-Malatya, East Anatolia, Turkey in October 2006 and identified by Serdar G. Senol (Department of Biology, Faculty of Sciences, Ege University, Izmir, Turkey). A voucher specimen was deposited in the Herbarium of Ege University, Izmir, Turkey (EGE 32574).

Cushion-forming shrub, 20-30 cm. Leaf rachis spiny, 5-15 cm, curved. Leaflets 15-25 mm, narrowly elliptic, spine-tipped, densely simple-adpressed-tomentose, grey, sometimes becoming glabrescent with age, 5-7-paired. Stipules 10-15 mm, ovate-triangular, white-tomentose. Flowers sessile, 5-10 per leaf axil. Inflorescence ovoid, 2-4 cm diam., 20-50-flowered. Bracts 7-8 mm, ovate-oblong to broadly ovate, white-tomentose. Bracteoles absent, or 1-3 mm, linear, white-tomentose. Calyx 7-9 mm, densely and shortly white-villous. Corolla dark pink; standart 12-15 mm, abruptly contracted below the auricles. *Fl.* 6-8. *Hilly steppe, pasture, open woods, 810-1850 m.*

E. and adjacent parts of S. Anatolia. **B6** Maraş: 19 km E from Göksun to Elbistan, 1280 m, *Hub.-Mor.* 11926! **B8** Diyarbakır: Diyarbakır, *D.* 22146! Erzurum: 10 km N of Hınıs, 1630 m, *D.* 46239! Siirt: Halkis Da., above Sason, 1850 m, *Cheese&Watson* 1274! **B9** Muş: between Bulanık and Muş, 3 km W of Kasangüran, 1820 m, *Hub.-Mor.* 11452! Bitlis: Reşadiye to Tatvan, Gözler 14410. **C6** Maraş: 30 km from Maraş to Gaziantep, *M. Zohary* 2019! **C7** Diyarbakır: Karacadağ, 1050 m, *E. S. Brown* 619!

N. Iraq. Ir.-Tur. Element.



Figure 2.1. *Astragalus amblolepis*

2.2.2. General Knowledge on *Astragalus wiedemannianus* Fischer

Astragalus wiedemannianus Fischer (Sect. Pterophorus) was collected from Yaka Village, 6 km from Burdur to Antalya, South Anatolia, Turkey in July 2003 and identified by Serdar G. Senol (Department of Biology, Faculty of Sciences, Ege University, Izmir, Turkey). A voucher specimen was deposited in the Herbarium of Ege University, Izmir, Turkey (EGE 18847).

Dwarf cushion-forming shrub, 6-20 cm. Leaf rachis spiny, 1-6 cm, straight or curved. Leaflets 3-9 mm, elliptic, $\pm$ spreading densely white-simple-pilose, 4-6-paired. Stipules (4-)8-14 mm, triangular-lanceolate, pilose. Flowers sessile, 4-5 per leaf axil. Inflorescence globose, 2.5-4 cm diam., 6-30-flowered. Bracts 9-13 mm, oblong to lanceolate, navicular. Bracteoles 12-15 mm, linear, densely pilose, resembling the calyx lobes. Calyx 13-15(-17) mm, densely white-pilose; lobes divided to the base. Flowers pink; standard (20-)22-25 mm. Style hairy. *Fl.* 5-7. *Steppe, fields, hills, 900-2200 m.*

Syntypes: [Turkey **A4** Kastamonu] prope Kastamuni (Kastamonu); [**A6** Tokat] in ditone Tokatensi; [**B4** Ankara] in M. trachytico Hussein-Kasi; [**B4** Ankara] in M. calcareo Dykmen pr. Angoram, *Wiedemann* (G!).

Mainly N., C. & S. Anatolia. **A4** Kastamonu: 5 km N of Kastamonu, 900 m, *D.* 21638! **A5** Kastamonu: Tosya, Giaurdagh, *Sint.* 1892: 3889 (type of *A. verae*)! **A7** Sivas: 39 km Suşehri to Refahiye, 1520 m, *Hub.-Mor.* 11922! **B2** Uşak: Mourad-Da (Murat Da.), 2200 m, *Bal.* 320 (type of *A. muradicus*!) **B3** Afyon: 32 km S of Sandıklı, *Sorger* 62-36-3! **B4** Ankara: Ankara, *Bornm.* 1892:3215 (type of *A. ankarensis*)! **B5** Kayseri: Yuralı, Erciyes Da., 1300 m, *Kotschy* 186 (type of *A. yuralicus*)! Nr. Tchomakli (Çomaklı), 1680 m, *Kotschy* 203 (type of *A. porphyraemus*)! **B6** Sivas: d. Kangal, 36 km from Tecer to Gürün, 1520 m, *Hub.-Mor.* 13024! **C2** Burdur: 6 km from Yeşilova to Kızılhisar, 1100 m, *Hub.-Mor.* 16856! **C3** Burdur: 4 km from Burdur, 950 m. *Hub.-Mor.* 5308! **C4** Konya: Barikurd, c. 30 km N of Konya, 7 vi 1937, Reese (type of *A. macroptilus* var. *longilaciniatus*)! **C5** Niğde: nr. Niğde, *Zederbauer*!

Endemic; Ir.-Tur. Element.



Figure 2.2. *Astragalus wiedemannianus*

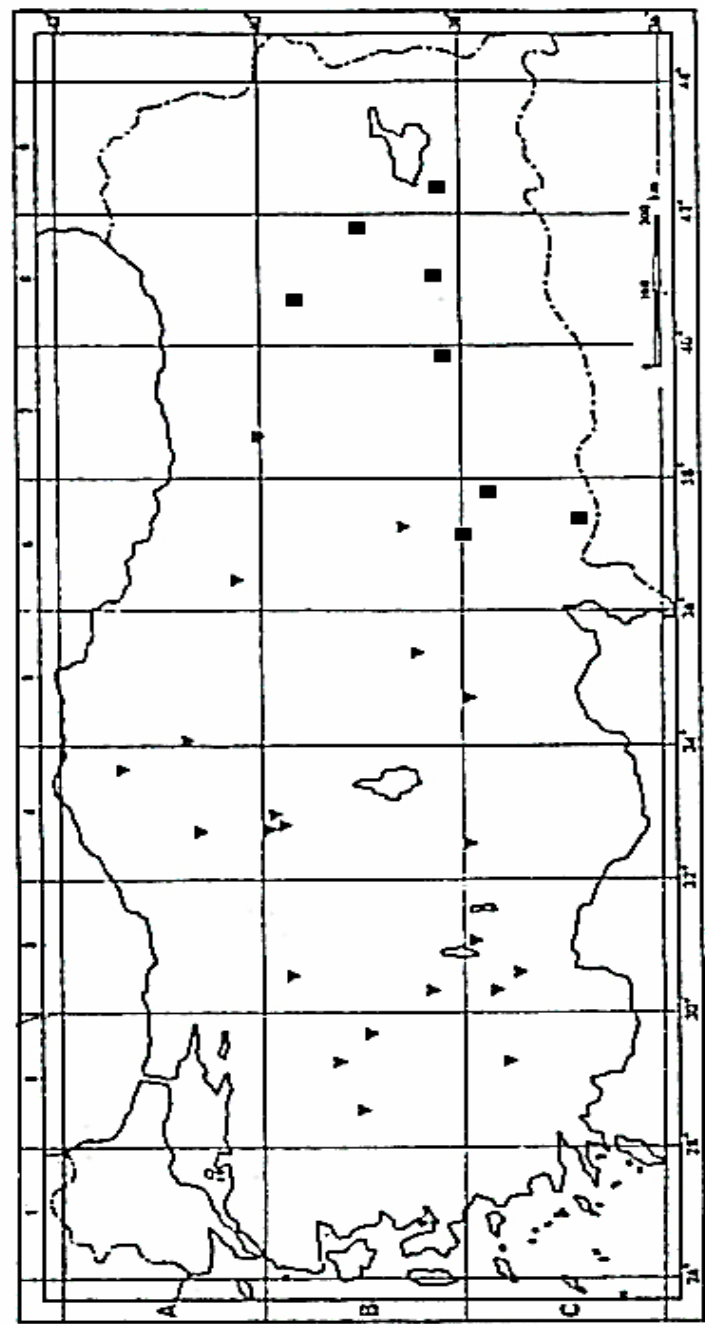


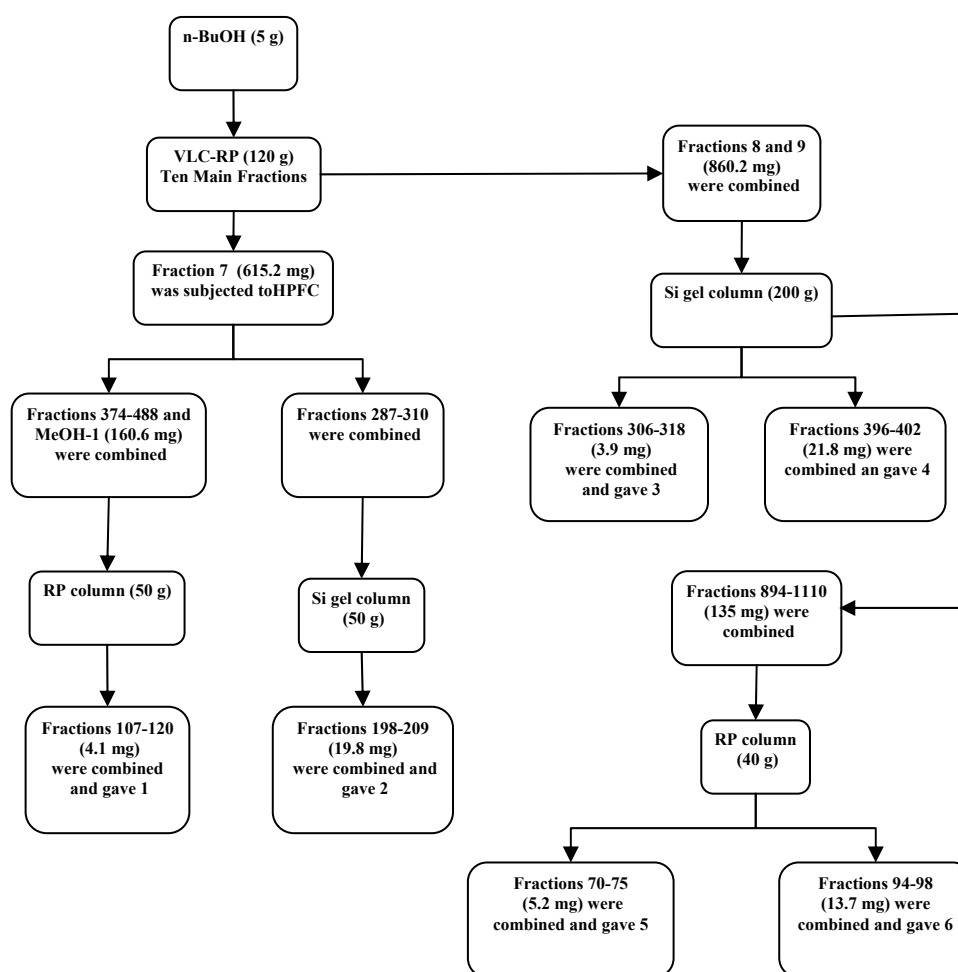
Figure 2.3. Location of *Astragalus amblolepis* ■ and *Astragalus wiedemannianus* ▼

2.3. Isolation and Purification

2.3.1. *Astragalus amblolepis*

Air-dried and powdered roots of *A. amblolepis* (600 g) were extracted with MeOH (3 x 3.5 l) at 60 °C. After filtration, the solvent was removed by rotary evaporation yielding 36 g of extract. The MeOH extract was dissolved in H₂O (400 ml), and successively partitioned with n-hexane (3 x 100 ml), CH₂Cl₂ (4 x 100 ml), and n-BuOH saturated with H₂O (6 x 100 ml). The n-BuOH extract (5 g) was subjected to vacuum liquid chromatography (VLC) using reversed-phase material (Lichroprep RP-18, 25-40 µm, 120 g) employing H₂O (850 ml), H₂O-MeOH (8:2, 200 ml; 6:4, 400 ml; 4:6, 1600 ml) and MeOH (800 ml) to give ten main fractions (1-10). Fractions 7, 8 and 9 eluted with H₂O:MeOH (4:6) were rich in saponins. Fraction 7 (615.2 mg) was subjected to High Performance Flash Chromatography (HPFC, Biotage, Inc., A Dyax Corp. Company; Biotage SI 40 M column), and eluted with CHCl₃-MeOH mixtures [80% CHCl₃, 5 column volume (CV); 80-70% CHCl₃, 5 CV; 70% CHCl₃, 4 CV; 70-60% CHCl₃, 4 CV; 50% CHCl₃, 3 CV] and MeOH (4 CV) to yield 510 fractions. Fractions 374-488 and the first MeOH fraction (160.6 mg) were combined and applied to a reversed-phase column (Lichroprep RP-18, 25-40 µm, 50 g) using MeOH-H₂O (5:5, 400 ml; 6:4, 300 ml) to give **1** (4.1 mg). Fractions 287-310 (81.4 mg) were combined and fractionated over an open column chromatography using Si gel (50 g) as stationary phase. Elution was

performed with CHCl_3 -MeOH- H_2O mixtures (80:20:2, 300 ml; 70:30:3, 1700 ml; 61:32:7, 400 ml) to afford **2** (19.8 mg). Fractions 8 and 9 (860.2 mg) from VLC were combined and subjected to Si gel (200 g) column chromatography. Elution was carried out with CHCl_3 -MeOH- H_2O (85:15:0.5, 400 ml), CHCl_3 -MeOH (80:20, 400 ml) and CHCl_3 -MeOH- H_2O (80:20:1, 500 ml) yielding **3** (3.9 mg) and **4** (21.8 mg). Fractions 894-1110 (135 mg) were applied to column chromatography using reversed-phase material (Lichroprep RP-18, 25-40 μm , 40 g) employing MeOH: H_2O (6:4, 350 ml) to give **5** (5.2 mg) and **6** (13.7 mg).



Scheme 2.1. Isolation of the new cycloartanes (**1**, **3**, **4**, **5**, and **6**) from *Astragalus amblelepis*

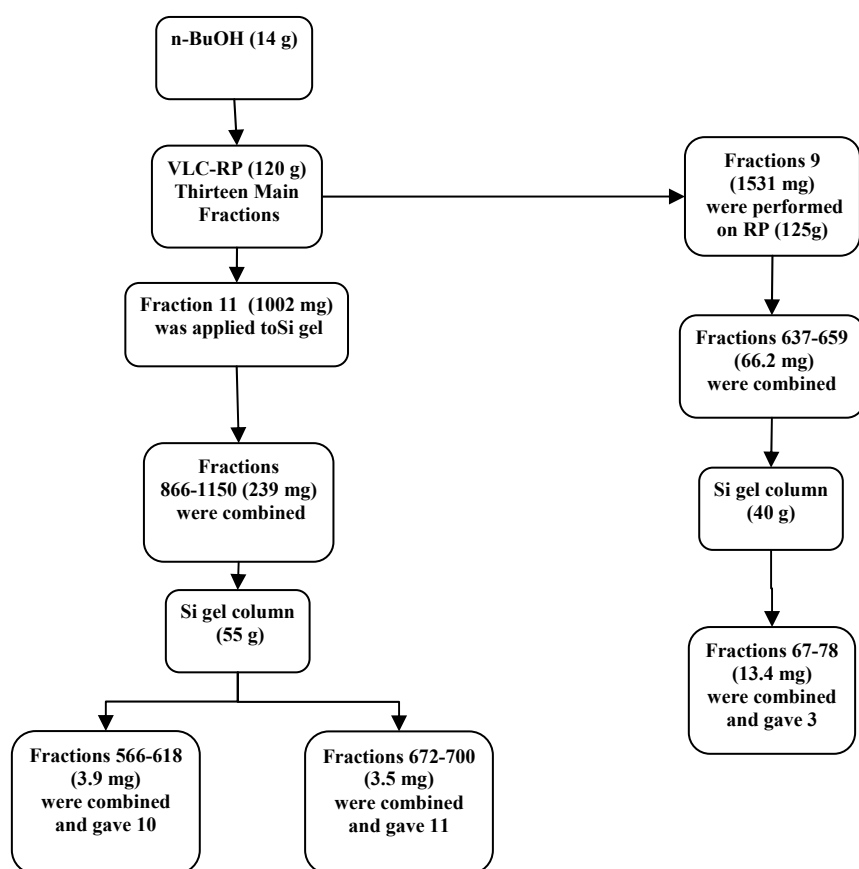
2.3.2. *Astragalus wiedemannianus*

The air-dried and powdered plant material (*Astragalus wiedemannianus*, 1410 g) was extracted with MeOH (3x2.5 L) for 5 days, under reflux. After filtration, the solvent was removed by rotary evaporation to afford 45 g of crude extract. The MeOH extract was dissolved in H₂O (600 mL), and successively partitioned with n-hexane (4x350 mL), CH₂Cl₂ (4x350 mL), and n-BuOH saturated with H₂O (4x350 mL). The crude BuOH extract (14 g) was subjected to vacuum liquid chromatography (VLC) on reversed-phase material (Lichroprep RP-18, 25-40 µm, 800 g) employing H₂O (1150 mL), H₂O-MeOH (80:20, 1000 mL; 70:30, 100 mL; 6:4, 2000 mL; 50:50, 1650 mL), and MeOH (900 mL) to give thirteen main fractions (1-13). After TLC controls, fractions 9, 10, 11, and 12 eluted with H₂O:MeOH (20:80) were determined to be rich in saponins. Fraction 11 (1002 mg) was applied to an open column chromatography using silica gel (230 g) as stationary phase. Elution was carried out with CHCl₃-MeOH (90:10, 1200 mL), CHCl₃-MeOH (85:15, 1200 mL), CH₃Cl-MeOH-H₂O (80:20:1, 1800 mL), CHCl₃-MeOH-H₂O (80:20:2, 1100 mL), CHCl₃-MeOH-H₂O (70:30:3, 1000 mL), and CHCl₃-MeOH-H₂O (61:32:7, 1100 mL) yielding **1** (5.6 mg). Fraction 9 was chromatographed over a reversed-phase column (Lichroprep RP-18, 25-40 µm, 125 g) using H₂O:MeOH (70:30, 500 mL; 60:40, 800 mL; 50:50, 1450 mL; 45:55, 600 mL). Total of 724 fractions were collected. Fractions 637-659 (66.2 mg) were combined and applied to a silica gel column (40 g) eluting with CHCl₃-

MeOH-H₂O mixture (70:30:3, 500 mL) to give compounds **2** (18.2 mg) and **3** (13.4 mg).

Fractions 471-550 (288.2 mg) obtained from the first silica gel column were combined and further purified on an open column chromatography using reversed-phase material (Lichroprep RP-18, 25-40 μ m, 40g) and eluted with H₂O-MeOH (40:60, 1100 mL) to give **4** (25.7 mg). Finally, fractions 1480-1589 (46.9 mg) was chromatographed on a reversed-phase column (Lichroprep RP-18, 25-40 μ m, 40g) using H₂O-MeOH (60:40, 400 mL; 50:50, 400 mL; 47.5:52.5, 200 mL; 45:55, 200 mL) as mobile phase to afford **5** (24.5 mg). Fractions 372-415 (27.6 mg) was subjected to silica gel column (40 g). Elution was started with CHCl₃-MeOH (90:10, 600 mL), and followed by CHCl₃-MeOH (85:15, 700 mL), and CHCl₃-MeOH-H₂O (80:20:2, 200 mL) to afford **6** (5.1 mg).

Finally, fractions 866-1150 (239 mg) were combined and fractionated over an open column chromatography using silica gel (55 g) as stationary phase employing with CHCl₃-MeOH-H₂O (80:20:2, 1000 mL) to give 702 fractions. The fractions 130-136, 144-152, 156-164, 566-618, and 672-700 afforded **7** (2.0 mg), **8** (2.7 mg), **9** (3.2 mg), **10** (3.9 mg), and **11** (3.5 mg), respectively.



Scheme 2.2. Isolation of the new cycloartanes (**3**, **10**, and **11**) from *Astragalus wiedemannianus*

3. RESULTS AND DISCUSSIONS

3.1. *Astragalus amblolepis*

3.1.1. Structural Identification of Compound **1** (AA₁)

The HRMALDITOF mass spectrum of compound **1** (m/z 809.4669 $[M+Na]^+$, calc. for $C_{41}H_{70}O_{14}Na$, 809.4663) supported a molecular formula of $C_{41}H_{70}O_{14}$. The ESI mass spectrum showed the major ion peak at m/z 809.4 which was assigned to $[M+Na]^+$. The MS/MS of this ion showed peaks at m/z 629.3 $[M+Na-180]^+$, corresponding to the loss of an hexose unit and at m/z 479.0 $[M+Na-180-150]^+$, due to the loss of a pentose unit. Taking into account the results of our comprehensive ^1H and ^{13}C -NMR studies, the main features of a cyclopropane-type triterpene possessing an acyclic side chain were evident for compound **1**: characteristic signals due to cyclopropane-methylene protons as an AX system (δ 0.41, 0.55, $J_{AX}=4.2$ Hz, H₂-19), six tertiary methyl groups (δ 1.05, 1.21, 0.98, 1.32, 1.24, 1.29; respectively, H₃-29, H₃-18, H₃-30, H₃-28, H₃-27, H₃-26) and a secondary methyl group (δ 0.97, d , $J=6.5$, H₃-21) (Bedir et al., 2001b) (**Table 3.1.**). Additionally, the resonances for two anomeric protons were observed at δ 4.30 (d , $J=7.5$ Hz) and 4.55 (d , $J=7.5$ Hz), indicative of the presence of two β -linked sugar units (Table 2). Thus, **1** was considered to be a cycloartane-type triterpene diglycoside.

The ^{13}C NMR spectrum contained 41 resonances; 30 of them, attributed to the sapogenol moiety (**Table 3.1.**), were in good agreement with cyclocanthogenin (Isaev et al., 1992). Full assignment of the ^1H and ^{13}C signals of the aglycon part of **1**, accomplished by DQF-COSY and HSQC spectra, showed glycosylation shifts for C-3 (δ 89.6) and C-25 (δ 81.4). All connectivities within **1** were also confirmed by HMBC spectrum, which showed a long range correlation between the anomeric proton signal at δ 4.30 (*d*, $J=7.5$ Hz) and the carbon resonance at δ 89.6 (C-3), while the anomeric proton at δ 4.55 (*d*, $J=7.5$ Hz) exhibited a long range correlation with the carbon resonance at δ 81.4 (C-25), confirming the bidesmosidic character of **1**. Furthermore the following stereochemical data confirmed the presence of cyclocanthogenol as aglycon of compound **1**. The relative configurations of the oxygenated carbon atoms were determined from the magnitude of the vicinal proton-proton coupling constants: C-3-(β -OH) (δ 3.24, *dd*, $J=11.3$, 4.0 Hz, $H_{\text{ax}}-3$), C-6-(α -OH) (δ 3.48, *ddd*, $J=9.5$, 9.5, 4.5 Hz, $H_{\text{ax}}-6$), C-16-(β -OH) (δ 4.47, *ddd*, $J=8.0$, 8.0, 5.2 Hz, $H_{\text{ax}}-16$). Moreover, ^{13}C -NMR data for C-24 was comparable to those reported for analogous compounds having a 24(*S*) configuration (Bedir et al., 2000; Fadeev et al., 1987; Hirotani et al., 1994).

As concerning the sugar moiety, examination of the remaining signals allowed us to identify one β -xylopyranosyl unit and one β -glucopyranosyl unit (Agrawal et al., 1985; Bedir et al., 2001b). The configuration of glucose and xylose units was established as D after

hydrolysis of **1** with 1 N HCl, trimethylsilation and determination of retention time by GC.

Consequently, the structure of **1** was established as 3-*O*- β -D-xylopyranosyl-25-*O*- β -D-glucopyranosyl-3 β ,6 α ,16 β ,24(*S*),25-pentahydroxy-cycloartane, a new natural product (**Figure 3.1**).

Amorphous powder; $[\alpha]_D^{25} +34.0$ (*c* 0.1, MeOH); IR (KBr): $\nu_{\max}$ = 3480 (>OH), 3025 (cyclopropane ring), 2870 (>CH), 1290-1030 (C-O-C) cm^{-1} ; ESI-MS m/z 809.4 $[\text{M}+\text{Na}]^+$; MS/MS m/z 629.3 $[\text{M}+\text{Na}-180]^+$, m/z 479.0 $[\text{M}+\text{Na}-180-150]^+$; HRMALDITOFMS $[\text{M}+\text{Na}]^+$ m/z 809.4669 (calc. for $\text{C}_{41}\text{H}_{70}\text{O}_{14}\text{Na}$, 809.4663); ^1H NMR (CD_3OD , 600 MHz) and ^{13}C NMR (CD_3OD , 150 MHz) data: see **Tables 3.1.-3.2.**

Table 3.1. ^1H and ^{13}C assignments of sapogenol moiety of **AA₁** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
1	33.1	H₂-1	1.56, 1.26, m
2	30.3	H₂-2	1.97, 1.69, m
3	89.6	H-3	3.24, dd (11.3, 4.0)
4	42.7	-----	-----
5	54.5	H-5	1.40, d (9.5)
6	69.4	H-6	3.48, ddd (9.5, 9.5, 4.5)
7	38.7	H₂-7	1.50, 1.37, m
8	48.7	H-8	1.81, dd (11.9, 4.2)
9	21.6	-----	-----
10	29.8	-----	-----
11	26.7	H₂-11	2.00, 1.22, m
12	33.5	H₂-12	1.73, 1.68, m
13	46.1	-----	-----
14	47.5	-----	-----
15	48.6	H₂-15	2.05, dd (12.7, 8.0) 1.42, dd (12.7, 5.2)
16	73.2	H-16	4.47, ddd (8.0, 8.0, 5.2)
17	57.9	H-17	1.73, dd (9.9, 8.0)
18	18.9	H₃-18	1.21, s
19	31.5	H₂-19	0.55, d (4.2) 0.41, d (4.2)
20	29.8	H-20	1.90, m
21	17.9	H₃-21	0.97, d (6.5)
22	33.6	H₂-22	1.81, 1.24, m
23	28.4	H₂-23	1.59, 1.51, m
24	76.7	H-24	3.61, dd (2.4, 10.5)
25	81.4	-----	-----
26	22.9	H₃-26	1.29, s
27	22.6	H₃-27	1.24, s
28	28.3	H₃-28	1.32, s
29	16.3	H₃-29	1.05, s
30	20.2	H₃-30	0.98, s

Table 3.2. ^1H and ^{13}C assignments of sugar residues of **AA₁** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
$\beta\text{-D-xylose-1'}$	107.4	H-1'	4.30, d (7.5)
2'	75.1	H-2'	3.22, dd (9.2, 7.5)
3'	77.7	H-3'	3.31, dd (9.2)
4'	70.8	H-4'	3.49, m
5'	66.3	H₂-5'	3.84, dd (11.7, 5.2) 3.20, t (11.7)
$\beta\text{-D-glucose-1''}$	97.8	H-1''	4.55, d (7.5)
2''	75.0	H-2''	3.20, dd (7.5, 9.0)
3''	78.0	H-3''	3.39, dd (9.0, 9.0)
4''	71.2	H-4''	3.30, dd (9.0, 9.0)
5''	77.8	H-5''	3.30, m
6''	62.2	H₂-6''	3.67, dd (4.5, 12.0) 3.86, dd (3.5, 12.0)

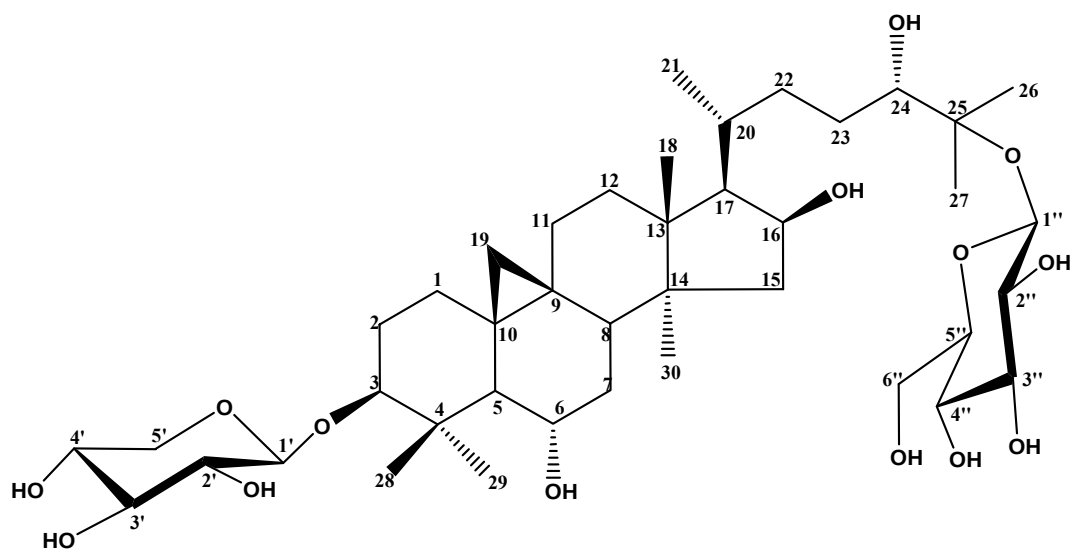
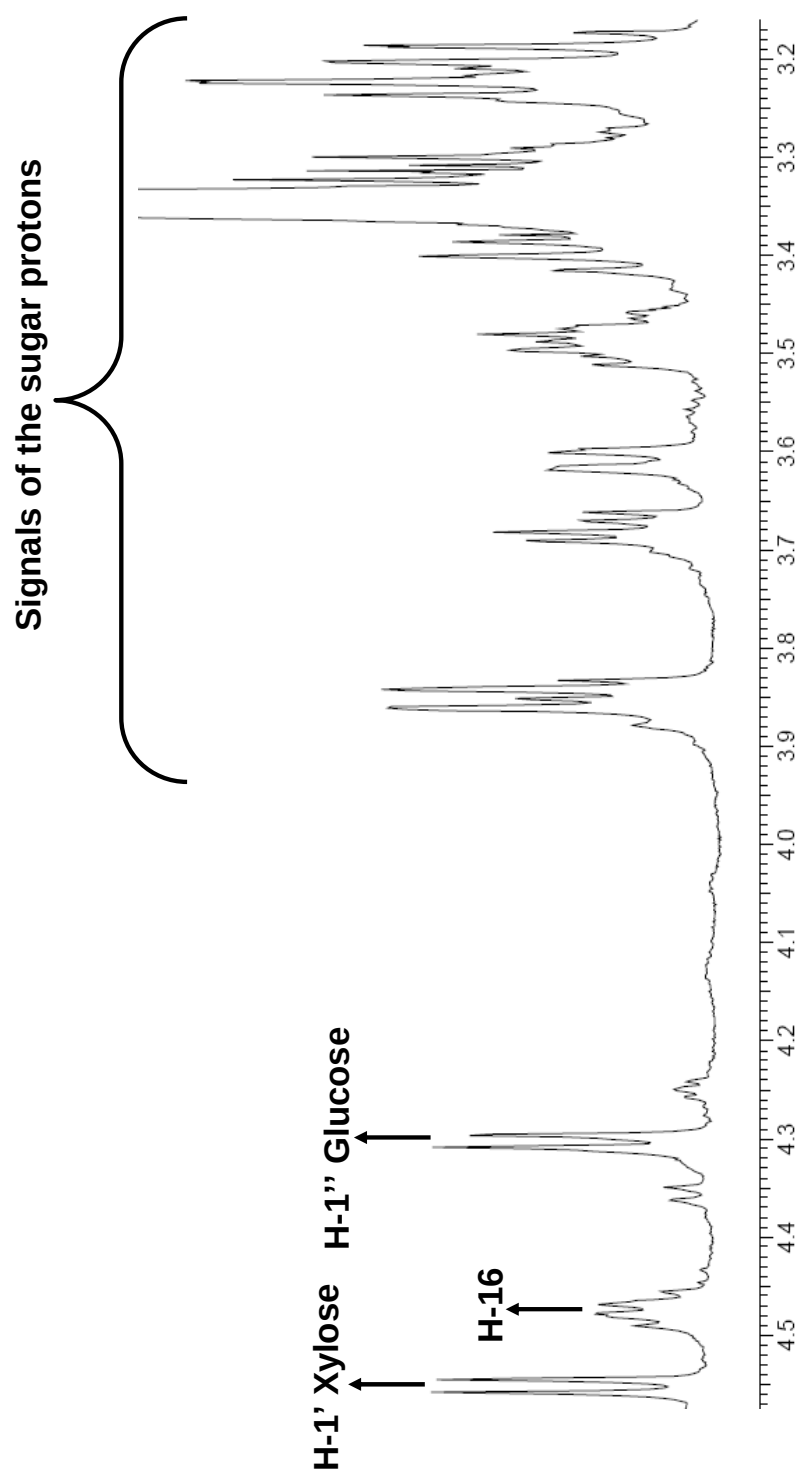
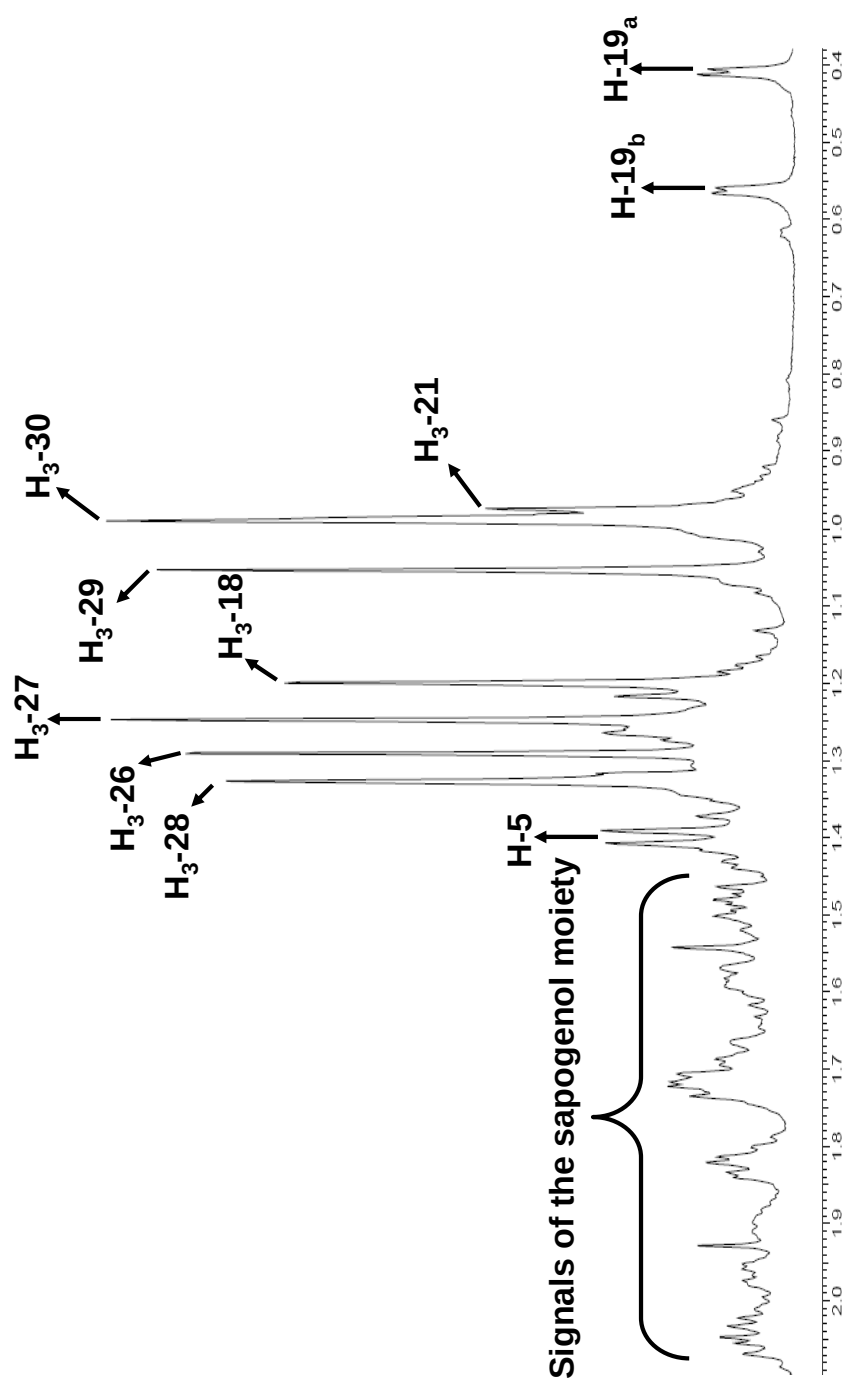


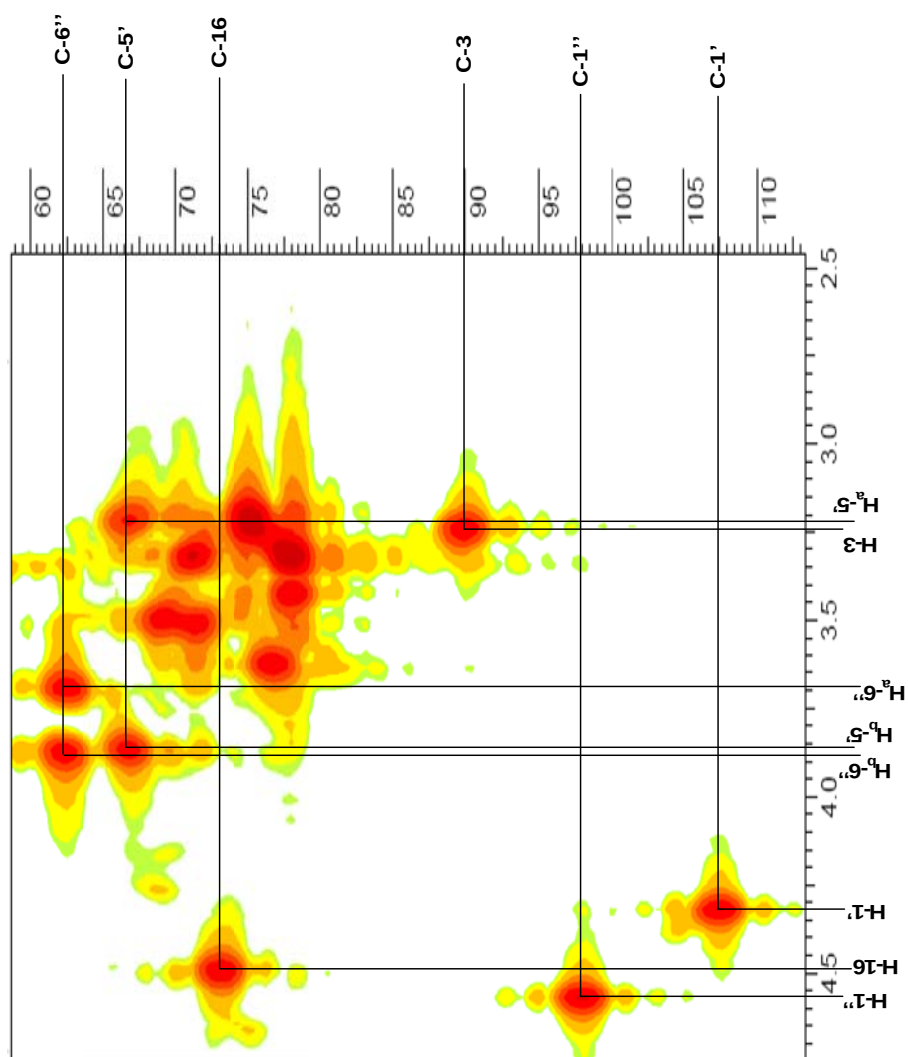
Figure 3.1. Structure of AA₁

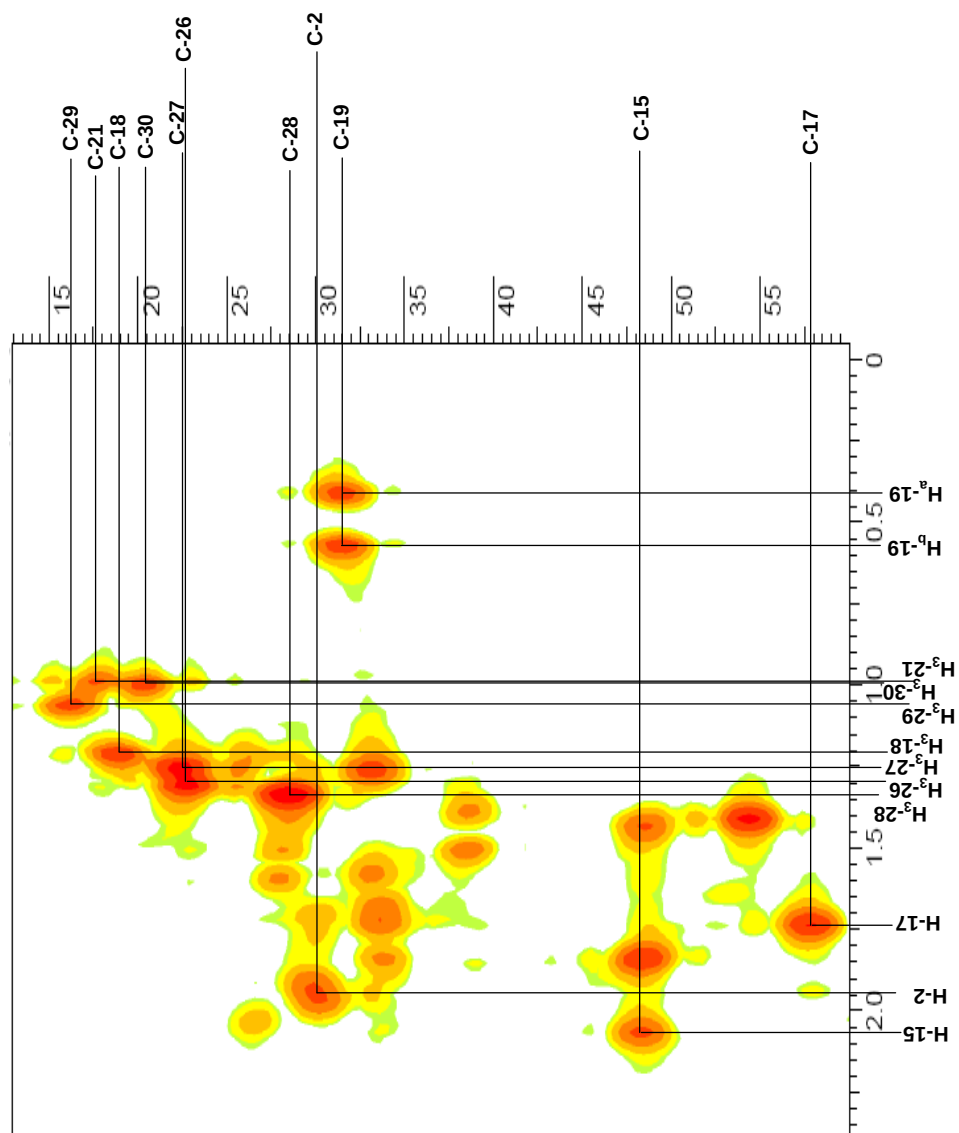


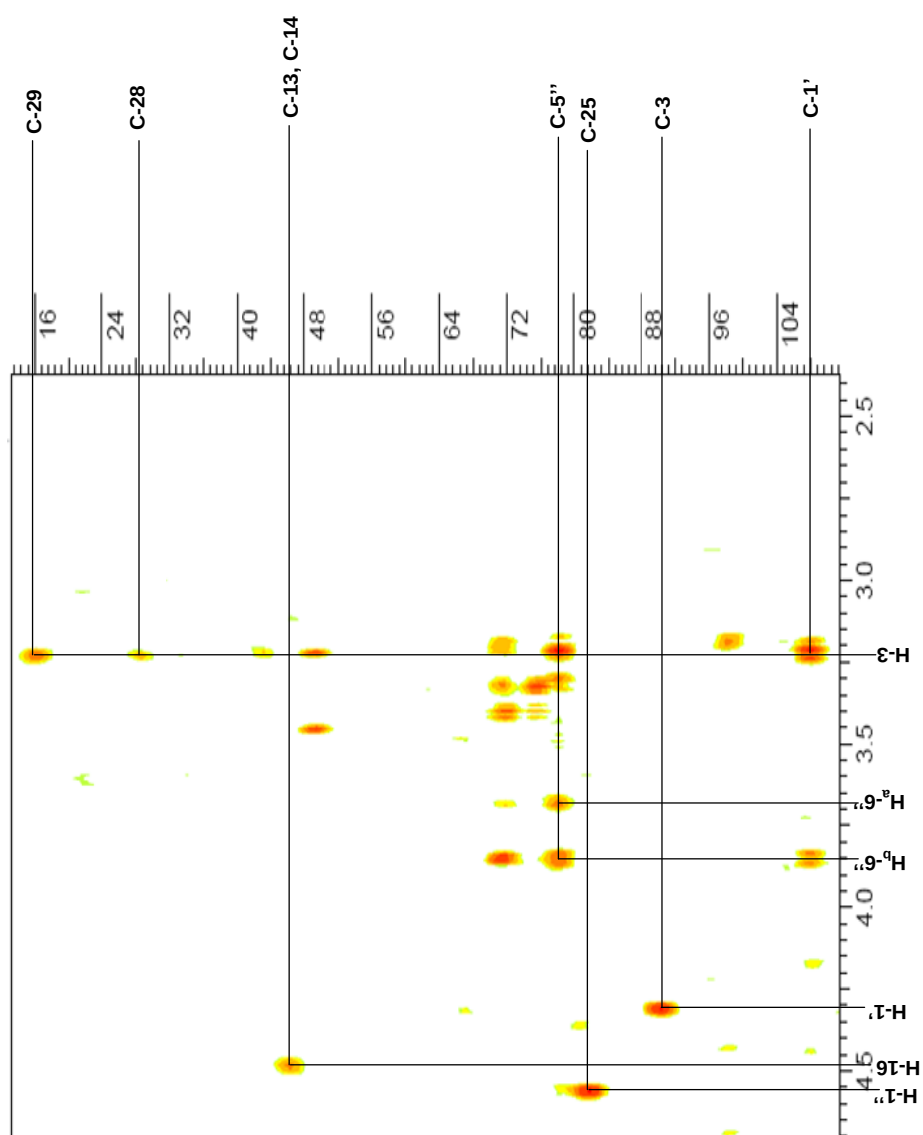
Spectrum 3.1. ^1H spectrum of **AA₁** in the downfield region

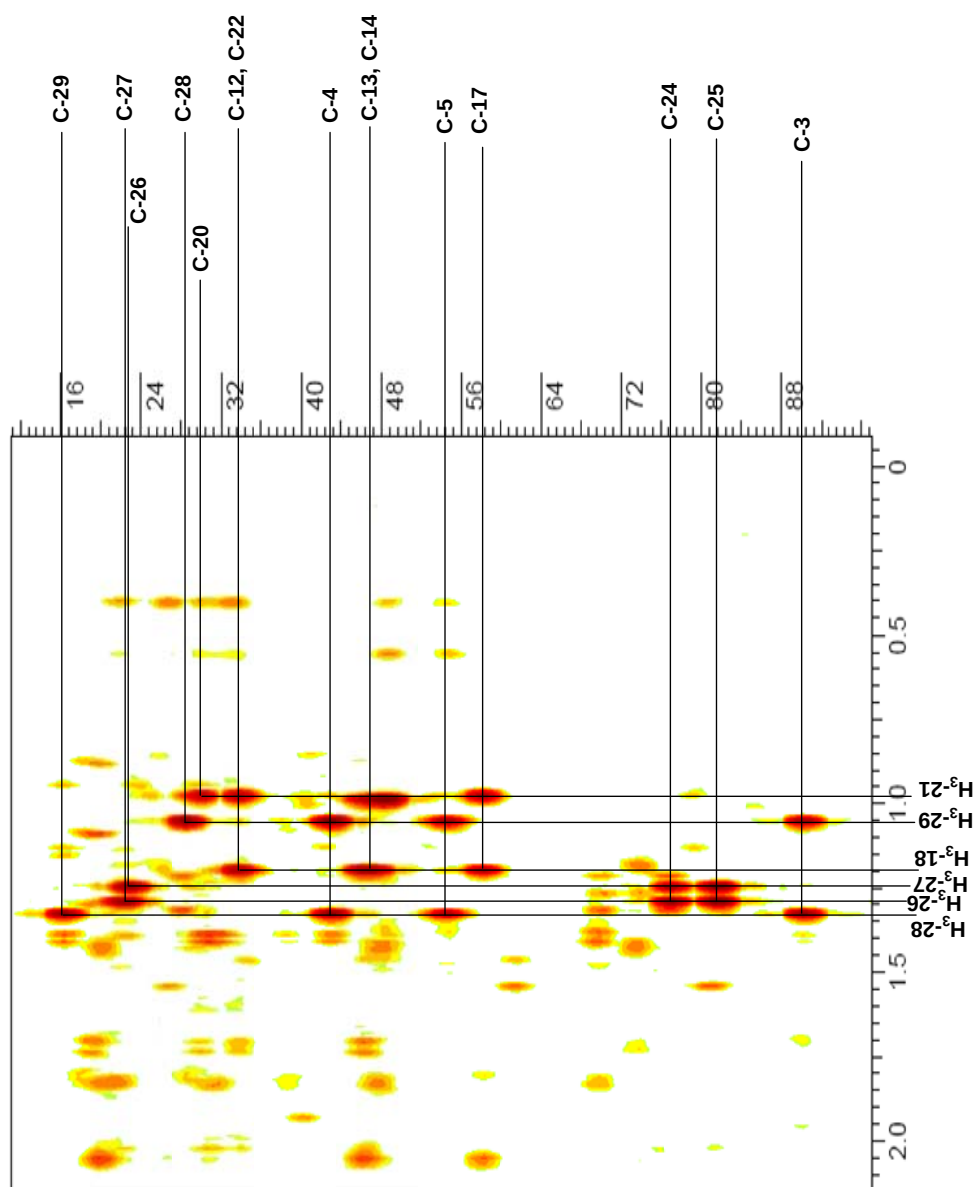


Spectrum 3.2. ^1H spectrum of AA_1 in the upfield region

Spectrum 3.3. HSQC spectrum of AA₁

Spectrum 3.4. HSQC spectrum of AA₁

Spectrum 3.5. HMBC spectrum of AA₁

Spectrum 3.6. HMBC spectrum of AA₁

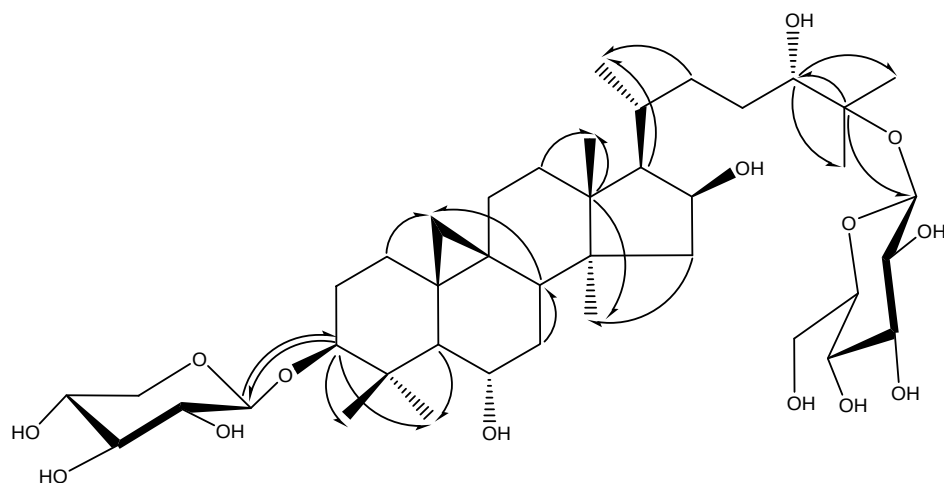


Figure 3.2. Key HMBC correlations of AA₁

3.1.2. Structural Identification of Compound 2 (AA₂)

The HRMALDITOF mass spectrum of compound **2** showed a major ion peak at m/z 809.4665 $[M+Na]^+$ ascribable to molecular formula $C_{41}H_{70}O_{14}$ (calc. for $C_{41}H_{70}O_{14}Na$, 809.4663). The NMR spectral data of **2** were consistent with **1** being a cycloartane-type triterpene diglycoside. Full assignments of ^1H and ^{13}C NMR data of the aglycon of **2** were deduced from its HSQC and HMBC spectra. All 1D- and 2D-NMR data of **2** were superimposable with those of the known compound 3-*O*- β -D-xylopyranosyl,16-*O*- β -D-glucopyranosyl-3 β ,6 α ,16 β ,24(*S*),25-pentahydroxy-cycloartane, isolated previously from *Tragacantha stipulosa* Boviss (Karimov et al., 1998) (**Figure 3.3.**).

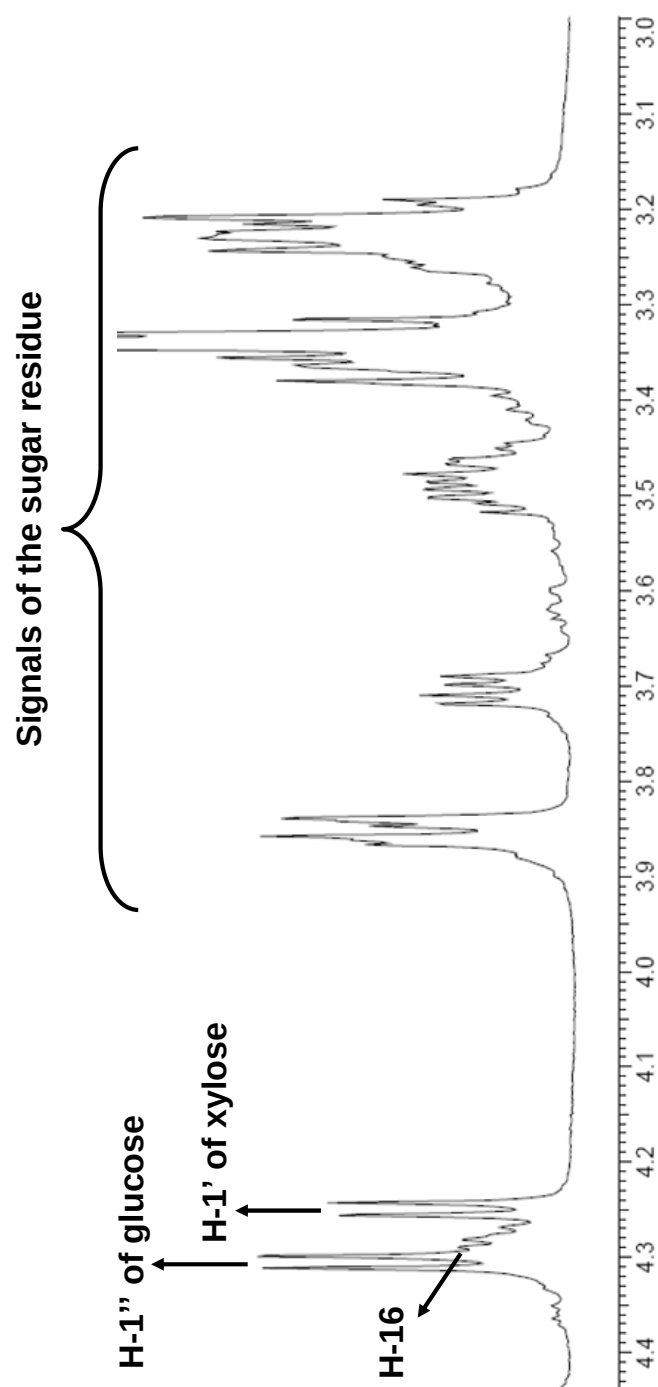
Amorphous powder; $[\alpha]_D^{25}$ 29.3 (c 0.1, MeOH); IR (KBr): ν_{max} = 3490 ($>OH$), 3033 (cyclopropane ring), 2889 ($>CH$), 1271-1025 (C-O-C) cm^{-1} ; ESI-MS m/z 809.3 $[M+Na]^+$; MS/MS m/z 629.3 $[M+Na-180]^+$; HRMALDITOFMS $[M+Na]^+$ m/z 809.4665 (calc. for $C_{41}H_{70}O_{14}Na$, 809.4663); ^1H NMR (CD_3OD , 600 MHz) and ^{13}C NMR (CD_3OD , 150 MHz) data: see **Tables 3.3.-3.4.**

Table 3.3. ^1H and ^{13}C assignments of sapogenol moiety of **AA₂** (in CD_3OD)

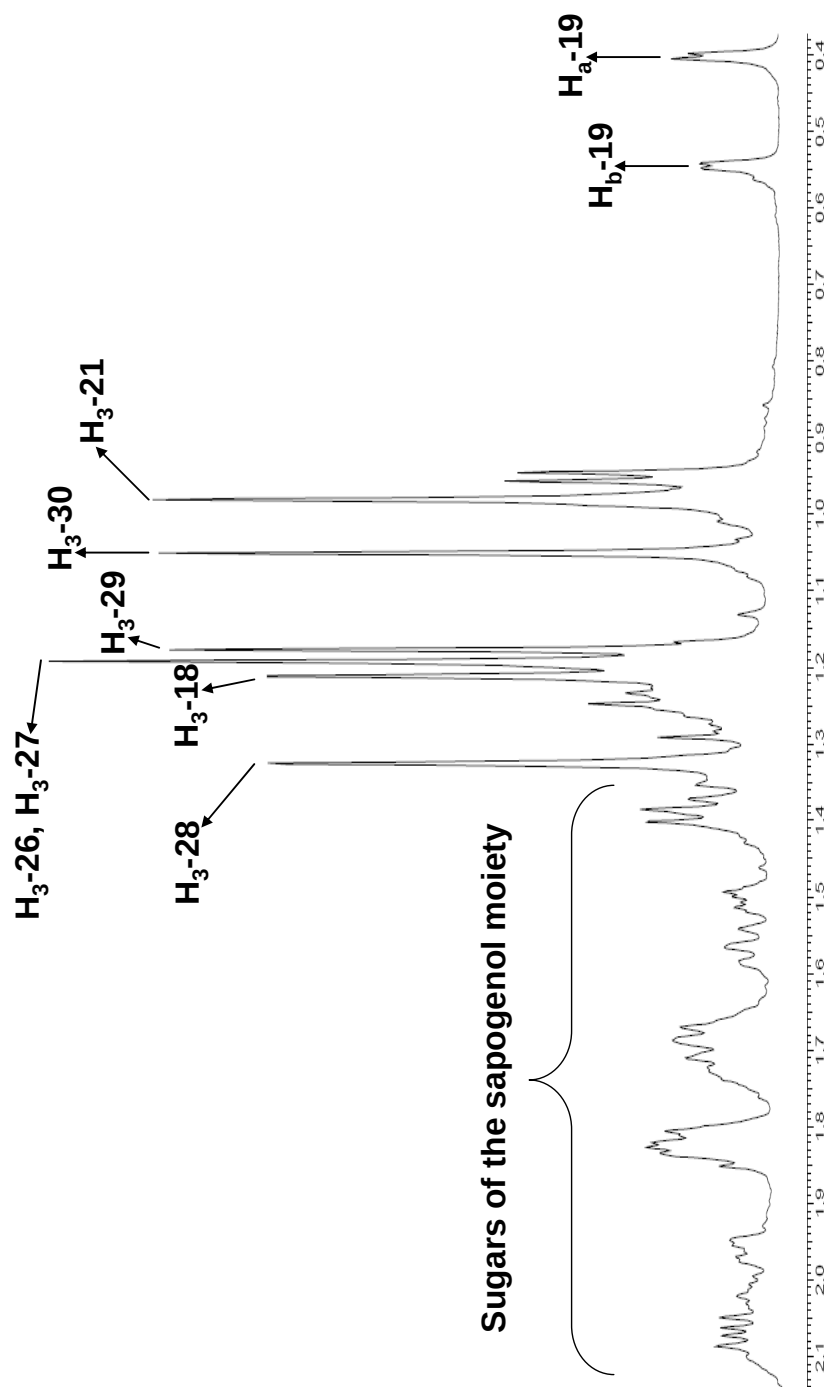
Position	δC (ppm)	Position	δH ppm
1	33.1	H₂-1	1.56, 1.26, m
2	30.3	H₂-2	1.97, 1.69, m
3	89.6	H-3	3.24, dd (11.3, 4.0)
4	42.7	-----	-----
5	54.7	H-5	1.40, d (9.5)
6	69.4	H-6	3.48, ddd (9.5, 9.5, 4.5)
7	38.7	H₂-7	1.50, 1.37, m
8	48.7	H-8	1.81, dd (11.9, 4.2)
9	21.6	-----	-----
10	29.8	-----	-----
11	26.7	H₂-11	2.02, 1.22, m
12	33.5	H₂-12	1.73, 1.68, m
13	46.1	-----	-----
14	47.5	-----	-----
15	48.4	H₂-15	2.08, dd (12.7, 8.0) 2.06, dd (12.7, 5.2)
16	83.4	H-16	4.27, ddd (8.0, 8.0, 5.2)
17	58.1	H-17	1.84, dd (9.9, 8.0)
18	18.9	H₃-18	1.21, s
19	31.5	H₂-19	0.55, d (4.2) 0.41, d (4.2)
20	29.8	H-20	2.10, m
21	17.9	H₃-21	0.96, d (6.5)
22	33.7	H₂-22	1.81, 1.24, m
23	28.6	H₂-23	1.67, 1.40, m
24	78.6	H-24	3.41, dd (2.4, 10.5)
25	73.6	-----	-----
26	24.9	H₃-26	1.18, s
27	24.9	H₃-27	1.18, s
28	28.3	H₃-28	1.32, s
29	16.3	H₃-29	1.05, s
30	20.1	H₃-30	0.98, s

Table 3.4. ^1H and ^{13}C assignments of sugar residues of **AA₂** (in CD_3OD)

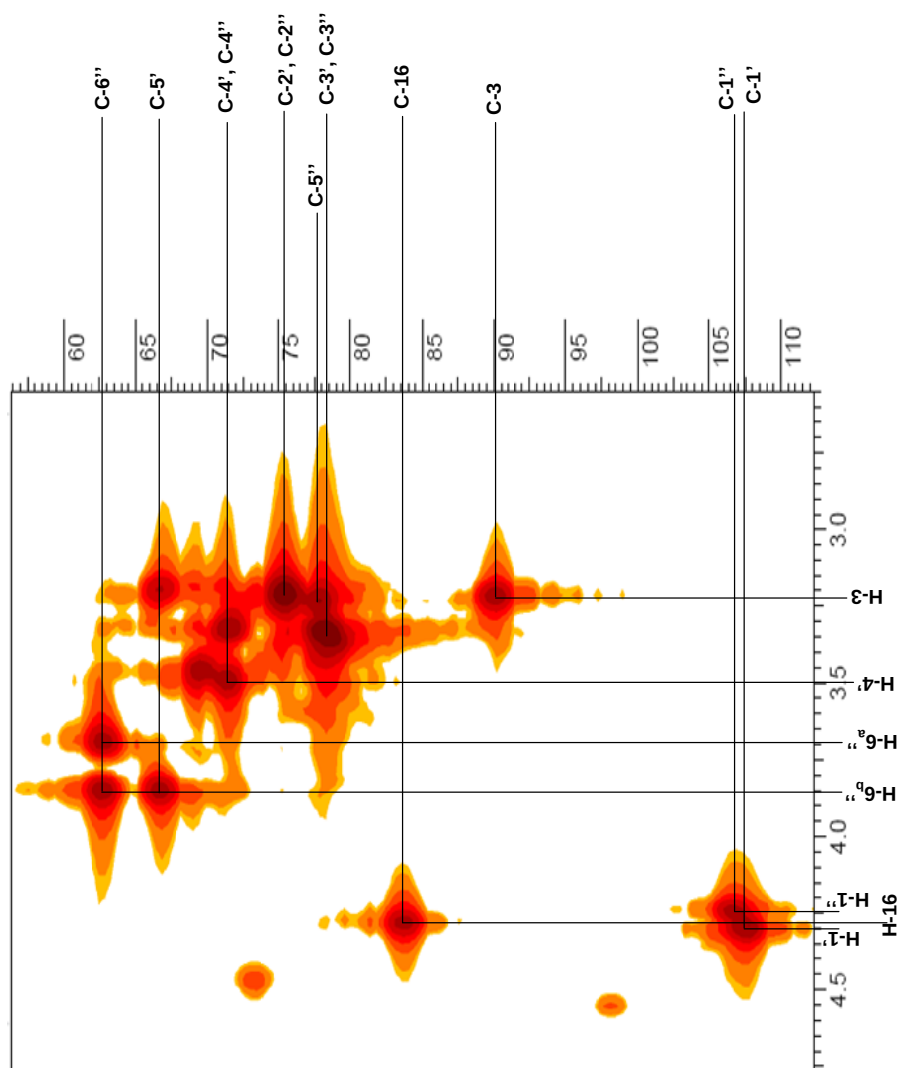
Position	δC (ppm)	Position	δH ppm
<i>β-D-xylose-1'</i>	107.2	H-1'	4.31, d (7.5)
2'	75.0	H-2'	3.22, dd (9.2, 7.5)
3'	77.7	H-3'	3.34, dd (9.2)
4'	70.8	H-4'	3.49, m
5'	66.2	H₂-5'	3.85, dd (11.7, 5.2) 3.21, t (11.7)
<i>β-D-glucose-1''</i>	106.2	H-1''	4.24, d (7.5)
2''	75.0	H-2''	3.22, dd (7.5, 9.0)
3''	78.2	H-3''	3.38, dd (9.0, 9.0)
4''	71.2	H-4''	3.32, dd (9.0, 9.0)
5''	77.5	H-5''	3.25, m
6''	62.4	H₂-6''	3.71, dd (4.5, 12.0) 3.85, dd (3.5, 12.0)

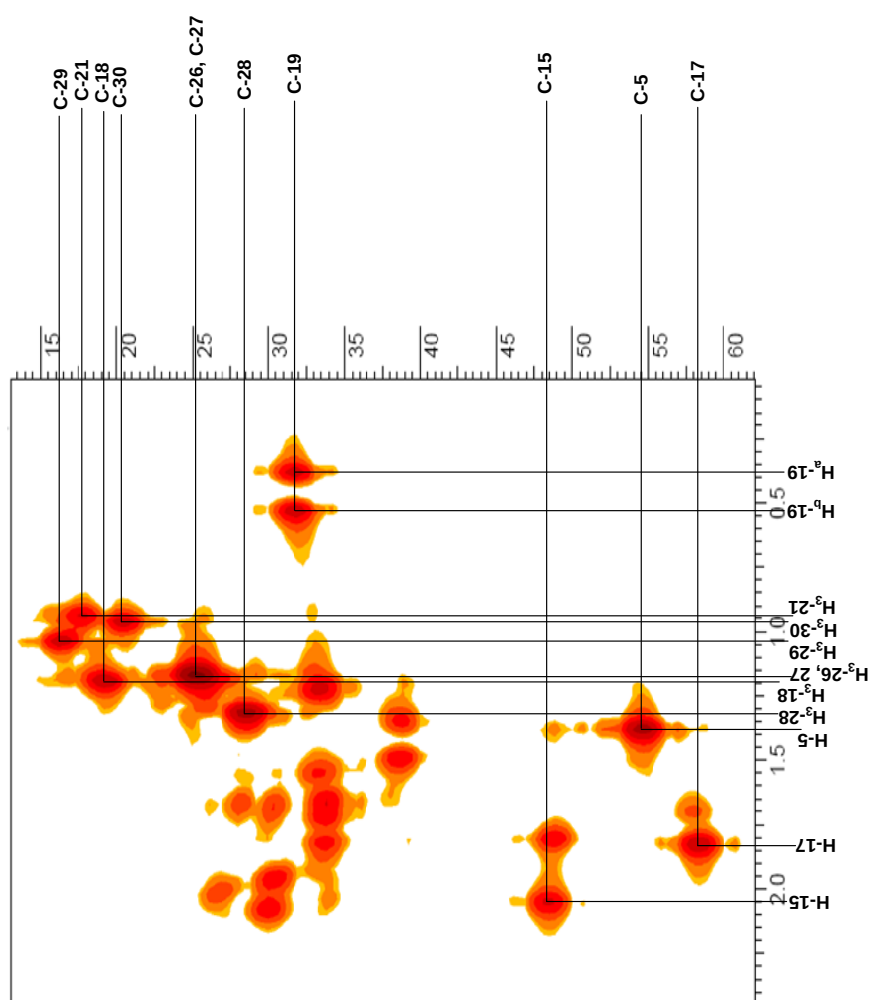


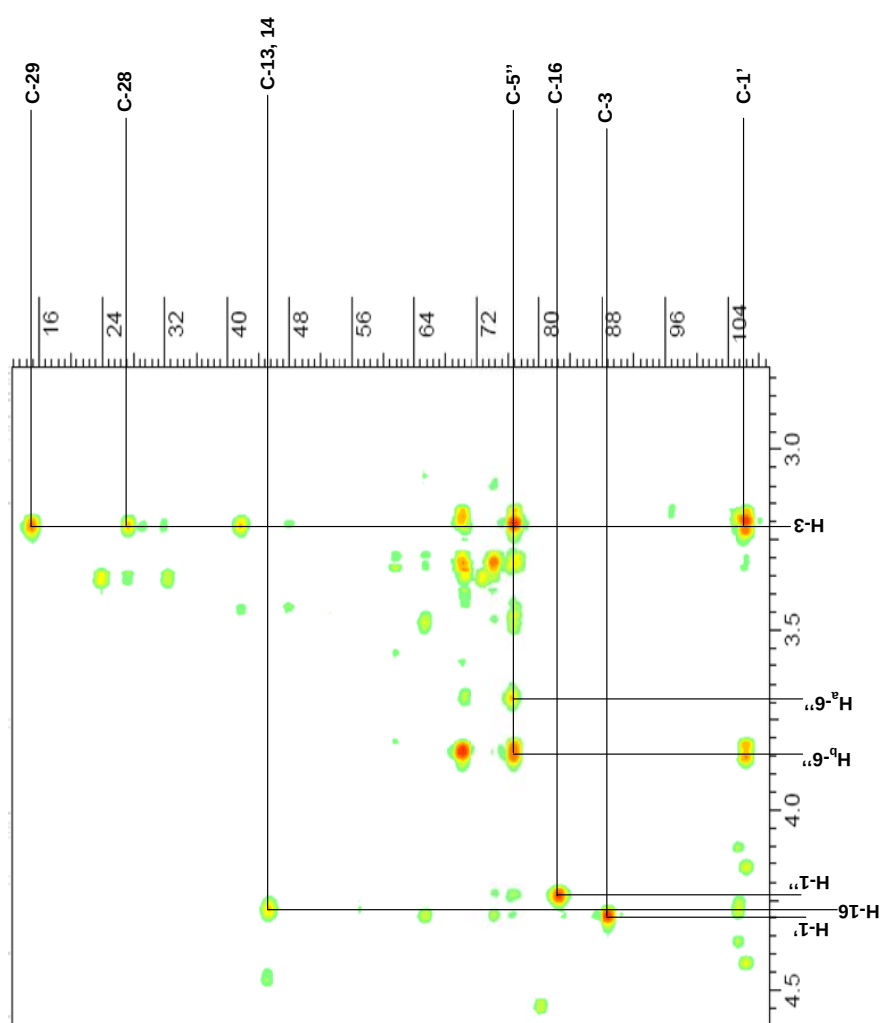
Spectrum 3.7. ^1H spectrum of AA_2 in the downfield region

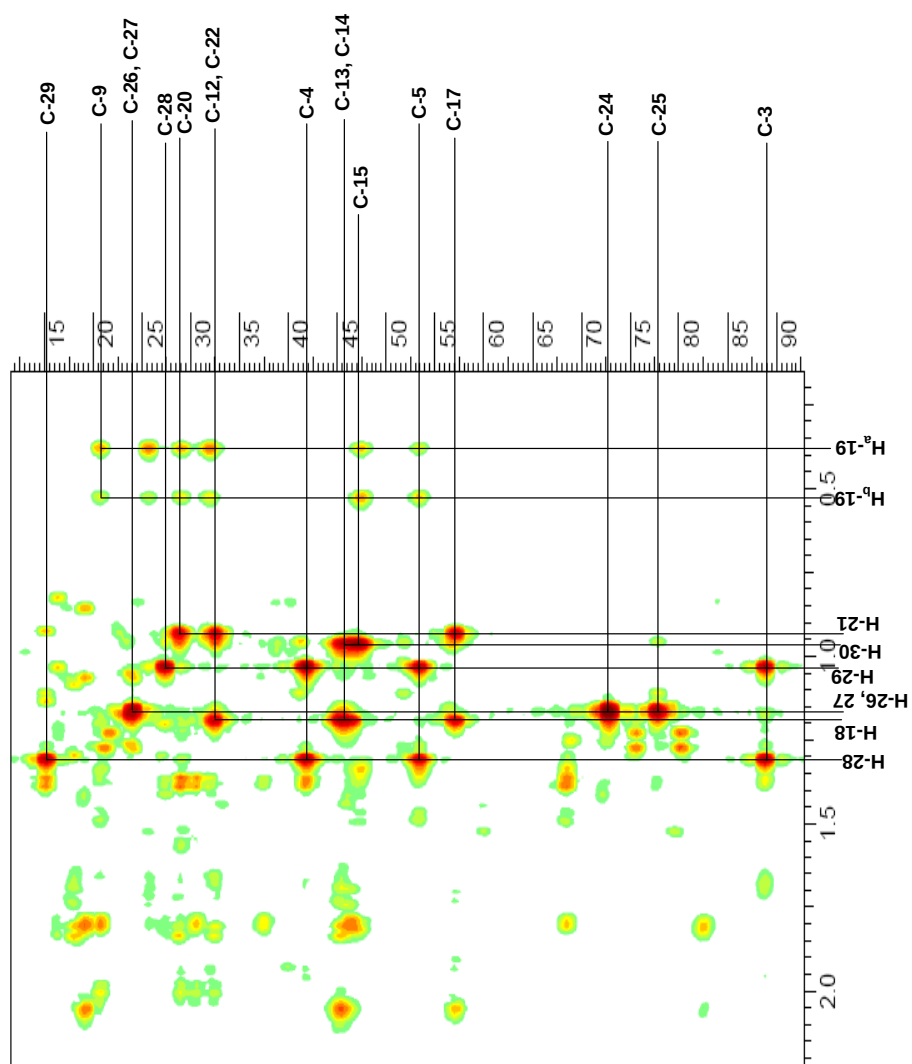


Spectrum 3.8. ^1H spectrum of AA_2 in the upfield region

Spectrum 3.9. HSQC spectrum of AA₂

Spectrum 3.10. HSQC spectrum of AA₂

Spectrum 3.11. HMBC spectrum of AA₂

Spectrum 3.12. HMBC spectrum of AA₂

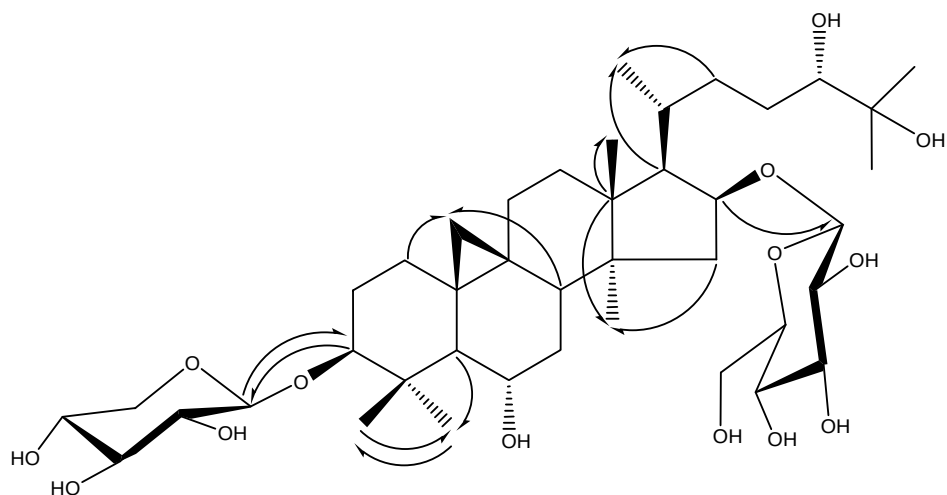


Figure 3.4. Key HMBC correlations of AA_2

3.1.3. Structural Identification of Compound **3** (AA₃)

The molecular formula of compound **3** was unequivocally established to be C₄₇H₇₈O₂₀ by HRMALDITOFMS analysis (m/z 985.4979 [M+Na]⁺, calc. for C₄₇H₇₈O₂₀Na, 985.4984).

The NMR spectra of compounds **3** and **4** were also characteristic of cyclocanthogenol type glycosides (**Tables 3.5.-3.7.**). Furthermore, the ¹H-NMR spectrum of **3** clearly showed three anomeric proton doublets at δ 4.66 ($J= 7.5$ Hz), 4.56 ($J= 7.5$ Hz), and 4.55 ($J= 7.5$ Hz) in the downfield region, indicative of β -linked sugar units. The ¹³C-NMR spectrum contained 47 resonances; 30 of them attributed to cyclocanthogenol moiety. Full assignment of the ¹H and ¹³C signals of the aglycon part of **3** showed glycosylation shifts for C-3 (δ 89.8) and C-25 (δ 81.3). These results suggested that **3** was also a bidesmosidic saponin with sugar residues linked to C-3 and C-25 of cyclocanthogenol. A combination of 1D-TOCSY and HSQC experiments allowed us to unambiguously determine all sugar signals and to identify the sugar moieties as consisting of β -xylopyranosyl, β -glucopyranosyl and β -glucuronic acid units, respectively.

All connectivities, including the sites of attachment of sugar moieties on the aglycon of **3**, were determined by HMBC experiment (**Figure 3.6.**). In the HMBC spectrum, the anomeric proton signal at δ 4.56 (H-1'), assigned to the β -xylopyranose, showed long-range

correlation with the carbon resonance at δ 89.8 (C-3). The second anomeric proton signal at δ 4.66 (H-1''), assigned to the β -glucuronic acid, showed long-range correlation with the carbon resonance at δ 81.4 (C-2'). The third anomeric proton signal at δ 4.55 (H-1'''), assigned to the β -glucopyranose, showed long-range correlation with the carbon resonance at δ 81.3 (C-25). The D configuration of glucose, xylose and glucuronic acid units was determined by acid hydrolysis followed by GC analysis.

Thus the structure of **3** was elucidated as 3-*O*-[β -D-glucuronopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl]-25-*O*- β -D-glucopyranosyl-3 β ,6 α ,16 β ,24(*S*),25-pentahydroxy-cycloartane, a new secondary metabolite. This compound (**3**) represents the first report of the series of cycloartane-type compound possessing a glucuronic acid residue (**Figure 3.5.**).

Amorphous powder; $[\alpha]_D^{25}$ 15.8 (*c* 0.1, MeOH); IR (KBr): $\nu_{\max}$ = 3477 (>OH), 3048 (cyclopropane ring), 2895 (>CH), 1285-1043 (C-O-C) cm^{-1} ; ESI-MS m/z 985.3 $[\text{M}+\text{Na}]^+$; MS/MS m/z 805.3 $[\text{M}+\text{Na}-180]^+$, m/z 641 $[\text{M}+\text{Na}-180-164]^+$; HRMALDITOFMS $[\text{M}+\text{Na}]^+$ m/z 985.4979 (calc. for $\text{C}_{47}\text{H}_{78}\text{O}_{20}\text{Na}$, 985.4984); ^1H NMR (CD_3OD , 600 MHz) and ^{13}C NMR (CD_3OD , 150 MHz) data: see **Tables 3.5.-3.6.**

Table 3.5. ^1H and ^{13}C assignments of sapogenol moiety of **AA₃** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
1	33.1	H₂-1	1.56, 1.26, m
2	30.3	H₂-2	1.97, 1.69, m
3	89.8	H-3	3.25, dd (11.3, 4.0)
4	43.1	-----	-----
5	54.6	H-5	1.39, d (9.5)
6	69.4	H-6	3.46, ddd (9.5, 9.5, 4.5)
7	38.7	H₂-7	1.48, 1.38, m
8	48.7	H-8	1.82, dd (11.9, 4.2)
9	22.3	-----	-----
10	30.0	-----	-----
11	26.6	H₂-11	2.00, 1.23, m
12	34.0	H₂-12	1.71, 1.68, m
13	46.8	-----	-----
14	47.6	-----	-----
15	48.4	H₂-15	2.05, dd (12.7, 8.0) 1.42, dd (12.7, 5.2)
16	73.0	H-16	4.48, ddd (8.0, 8.0, 5.2)
17	58.2	H-17	1.72, dd (9.9, 8.0)
18	19.0	H₃-18	1.20, s
19	31.5	H₂-19	0.54, d (4.2) 0.40, d (4.2)
20	29.8	H-20	1.90, m
21	18.7	H₃-21	0.97, d (6.5)
22	33.6	H₂-22	1.81, 1.24, m
23	28.4	H₂-23	1.59, 1.51, m
24	76.8	H-24	3.61, dd (2.4, 10.5)
25	81.3	-----	-----
26	23.0	H₃-26	1.29, s
27	22.6	H₃-27	1.25, s
28	28.7	H₃-28	1.31, s
29	16.5	H₃-29	1.02, s
30	20.2	H₃-30	0.99, s

Table 3.6. ^1H and ^{13}C assignments of sugar residues of AA_3 (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
$\beta\text{-D-xylose-1'}$	105.0	H-1'	4.56, d (7.5)
2'	81.4	H-2'	3.65, dd (9.2, 7.5)
3'	76.4	H-3'	3.58, dd (9.2)
4'	71.0	H-4'	3.54, m
5'	66.3	H₂-5'	3.91, dd (11.7, 5.2) 3.24, t (11.7)
$\beta\text{-D-glucuronicacid-1''}$	104.3	H-1''	4.66, d (7.5)
2''	75.5	H-2''	3.35, dd (7.5, 9.0)
3''	77.2	H-3''	3.46, dd (9.0, 9.0)
4''	73.6	H-4''	3.49, dd (9.0, 9.0)
5''	77.3	H-5''	3.64, d (9.0)
6''	177.0	H-6''	-----
$\beta\text{-D-glucose-1'''}$	97.7	H-1'''	4.55, d (7.5)
2'''	75.0	H-2'''	3.20, dd (7.5, 9.0)
3'''	78.0	H-3'''	3.39, dd (9.0, 9.0)
4'''	71.2	H-4'''	3.30, dd (9.0, 9.0)
5'''	77.8	H-5'''	3.30, m
6'''	62.2	H₂-6'''	3.67, dd (4.5, 12.0) 3.86, dd (3.5, 12.0)

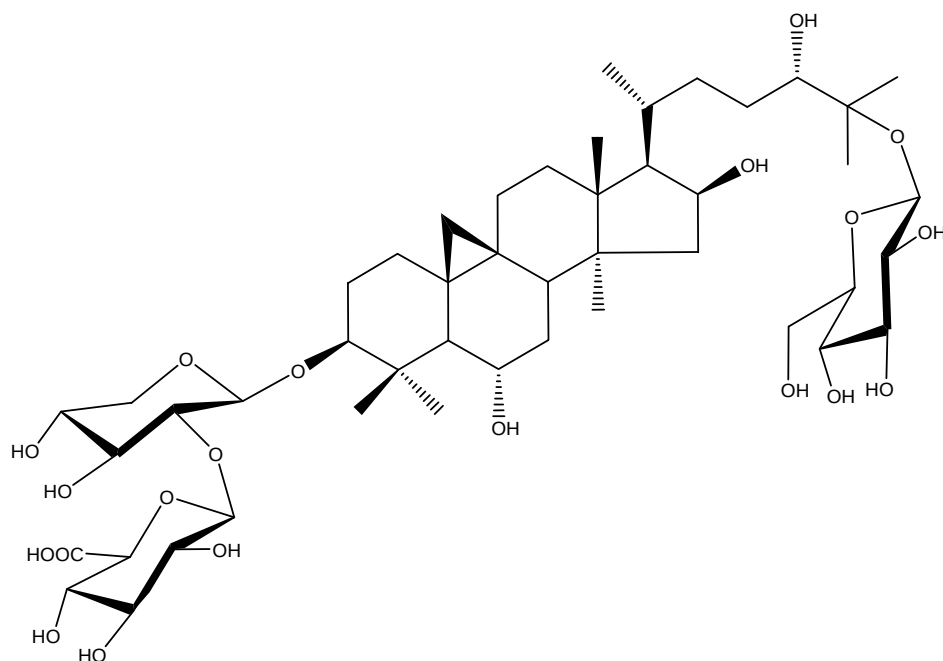
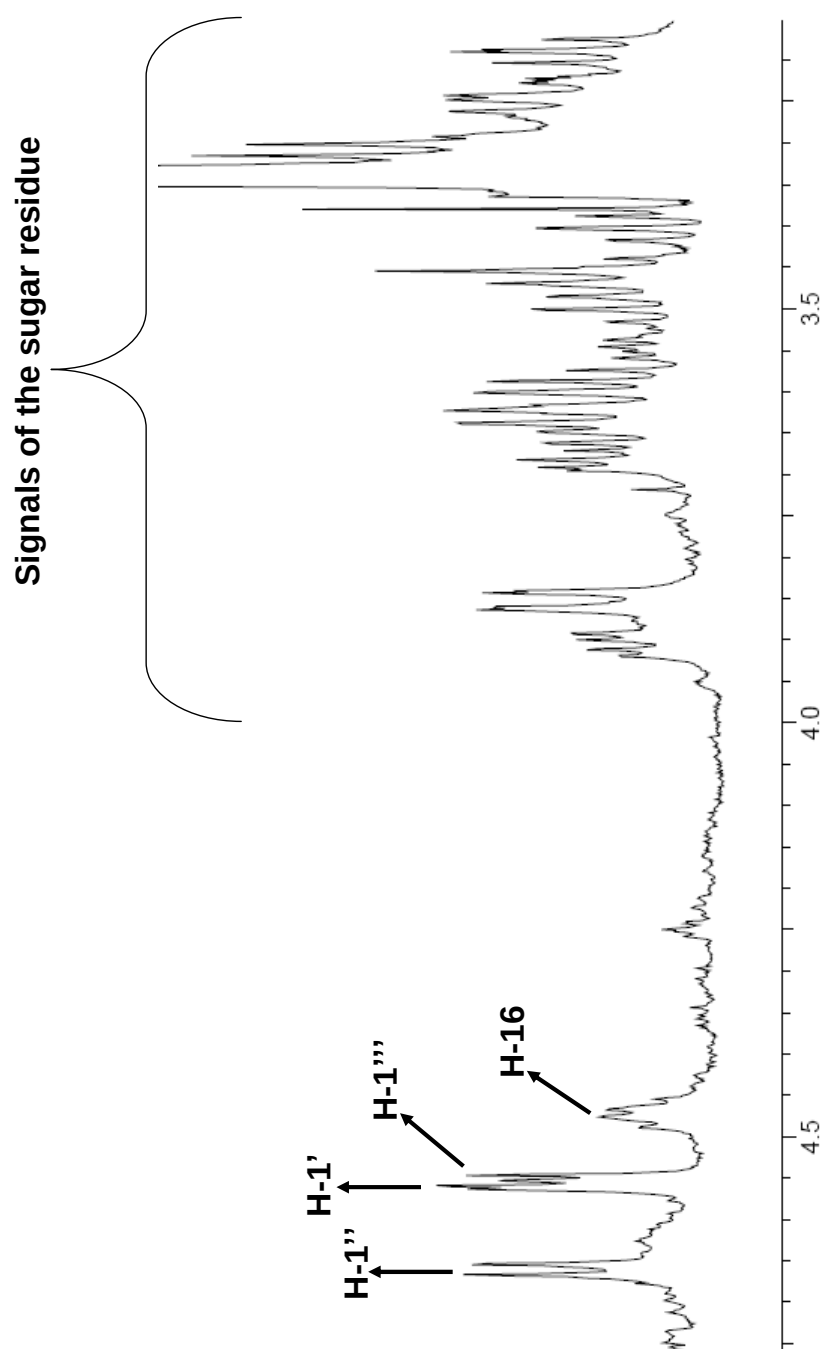
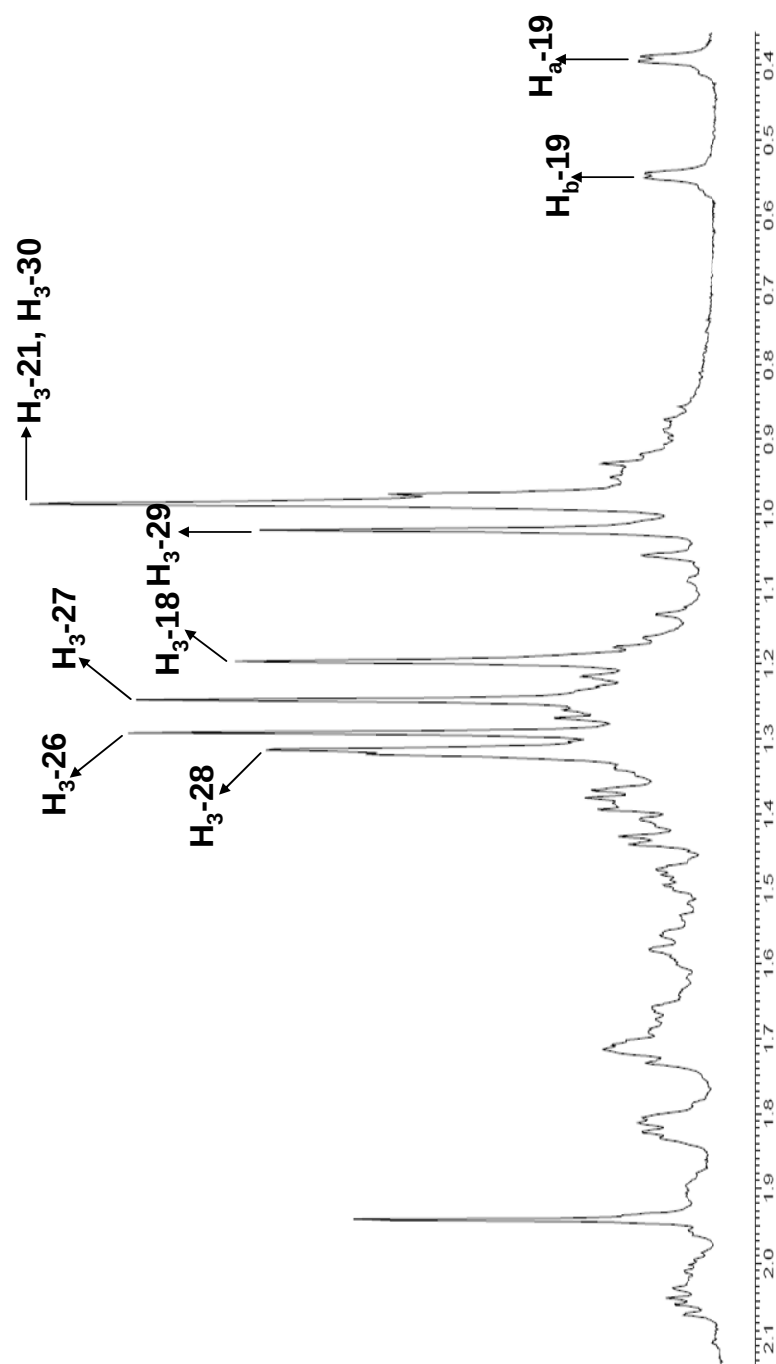


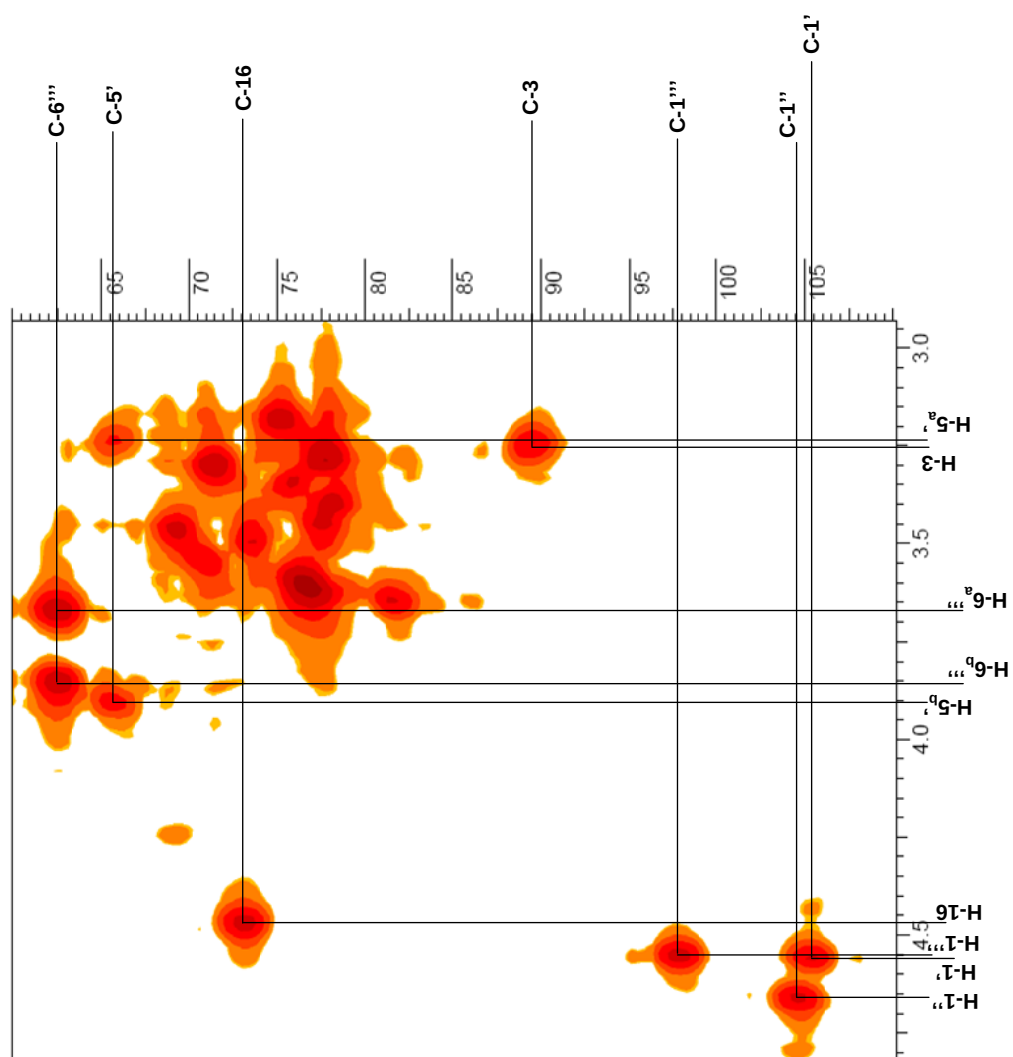
Figure 3.5. Structure of AA₃

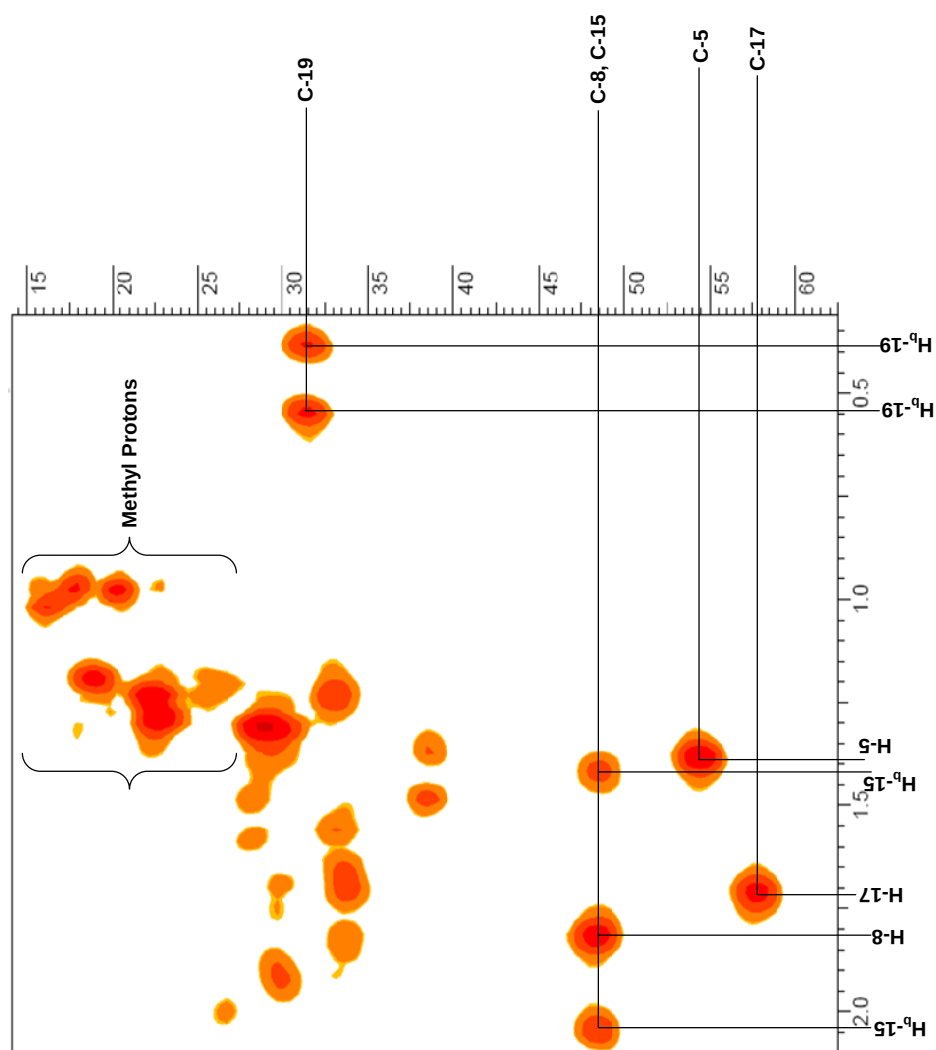


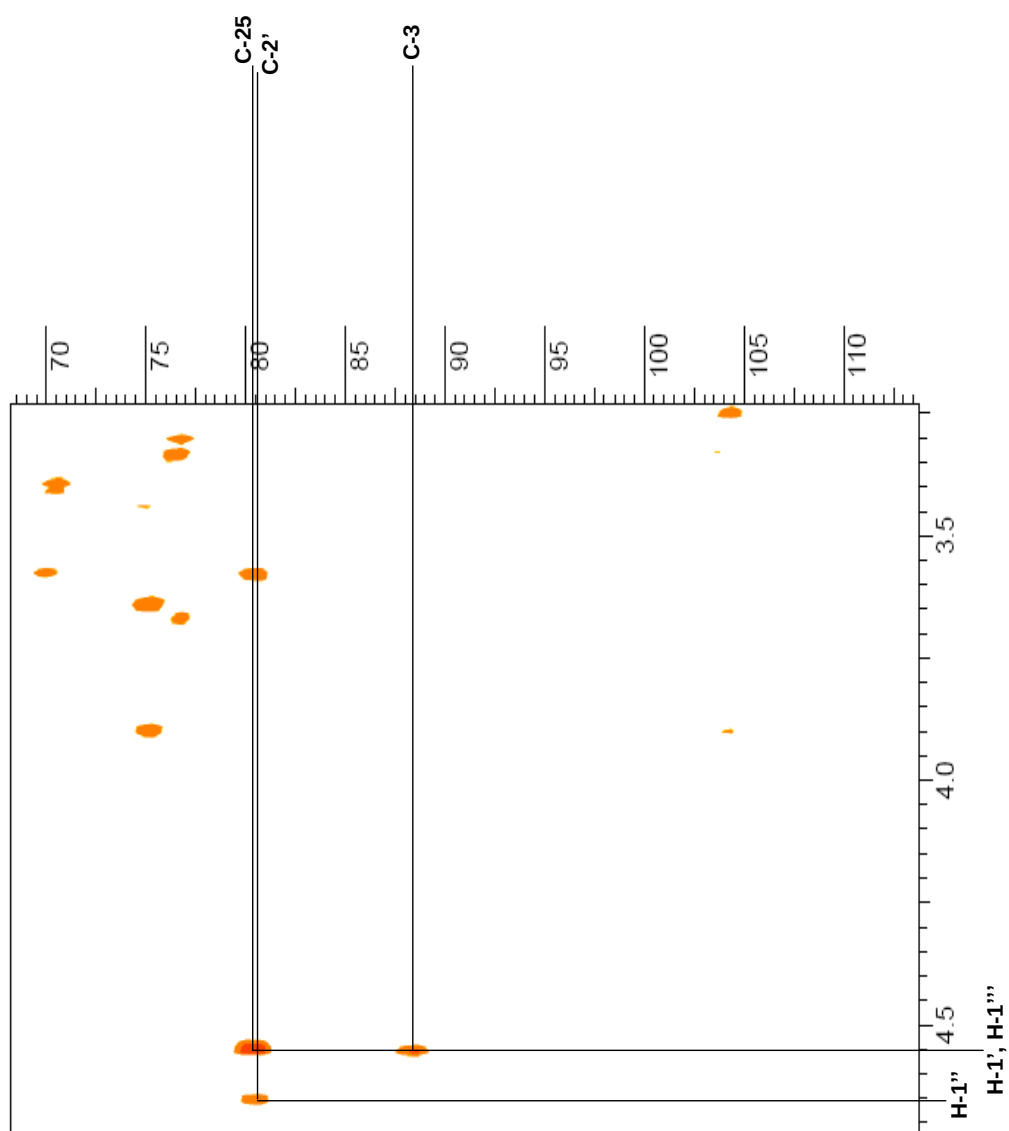
Spectrum 3.13. ^1H spectrum of AA_3 in the downfield region

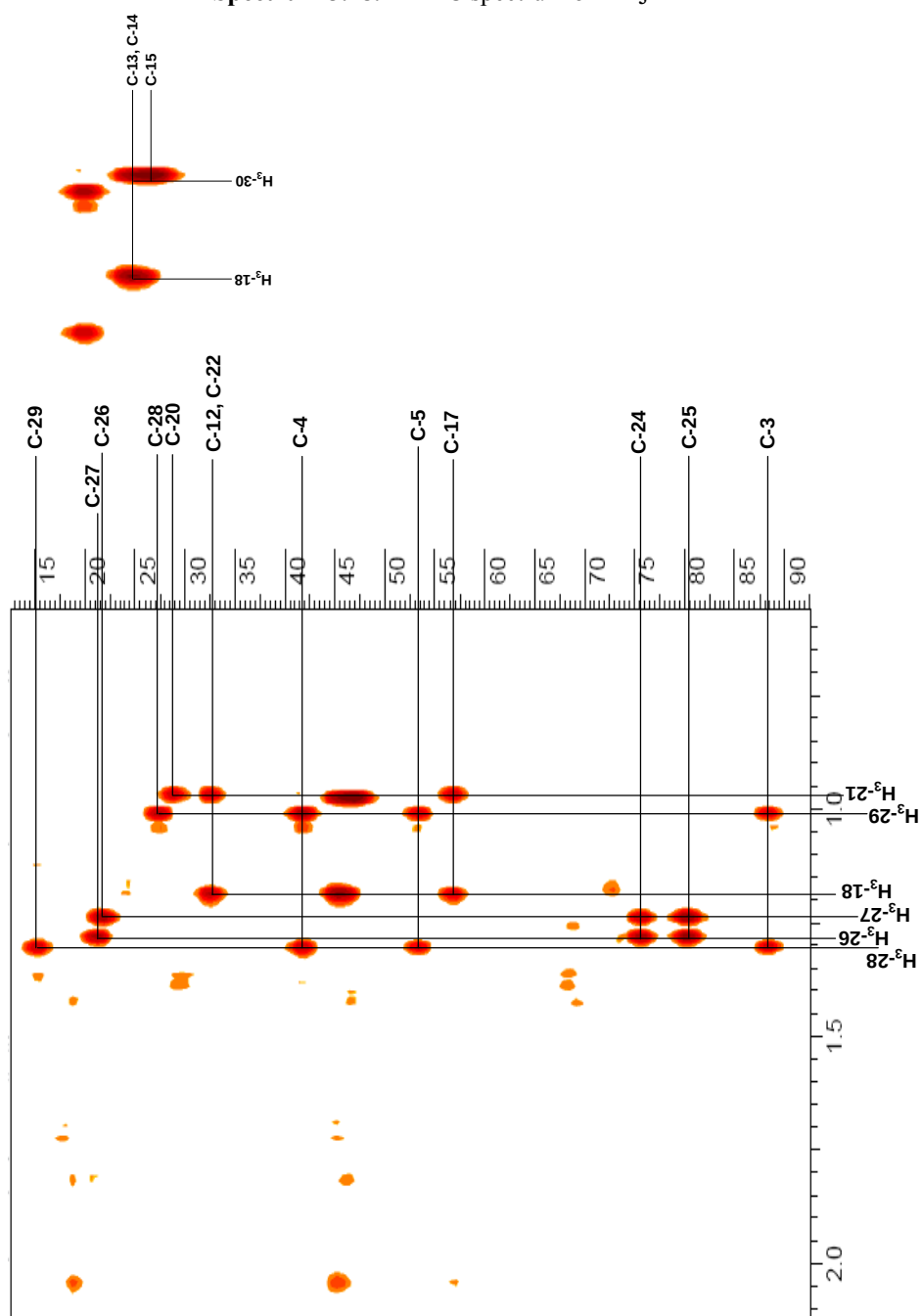


Spectrum 3.14. ^1H spectrum of AA₃ in the upfield region

Spectrum 3.15. HSQC spectrum of AA₃

Spectrum 3.16. HSQC spectrum of AA₃

Spectrum 3.17. HMBC spectrum of AA₃

Spectrum 3.18. HMBC spectrum of AA₃

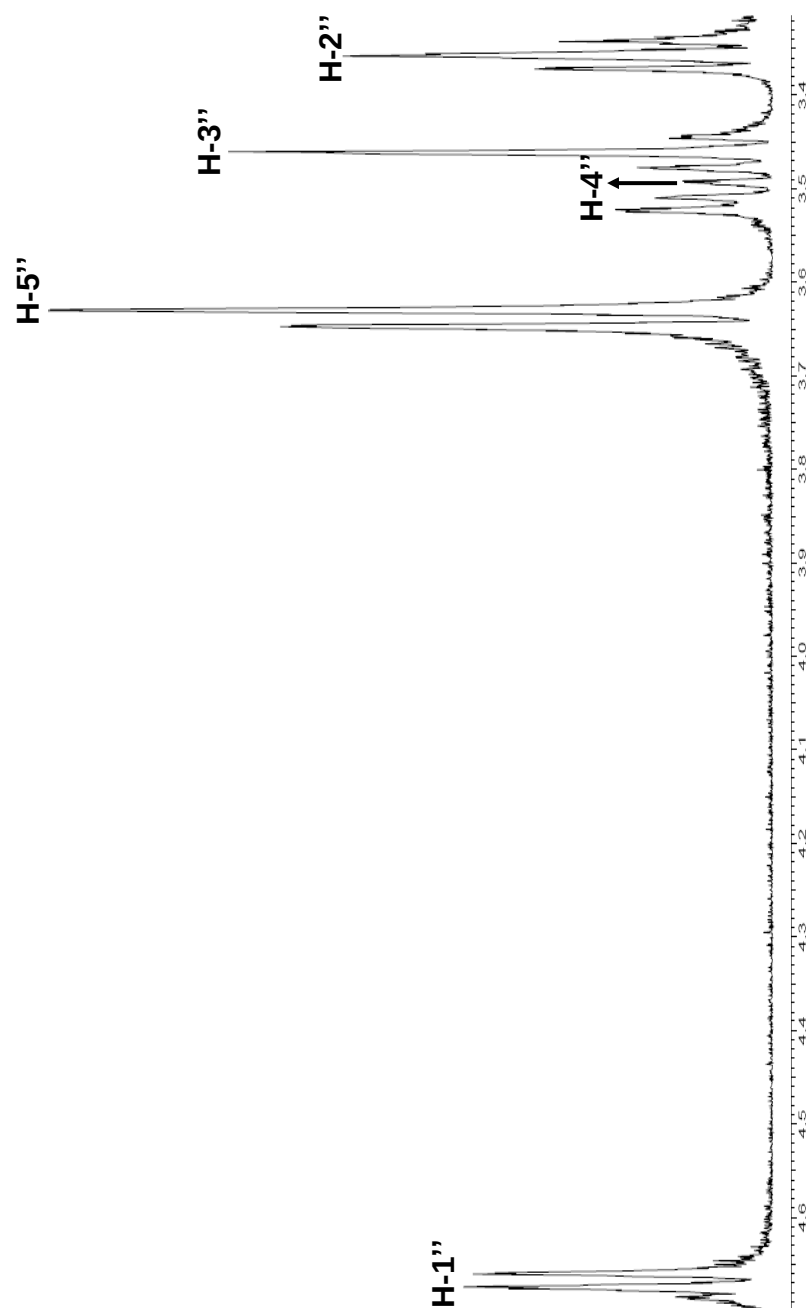
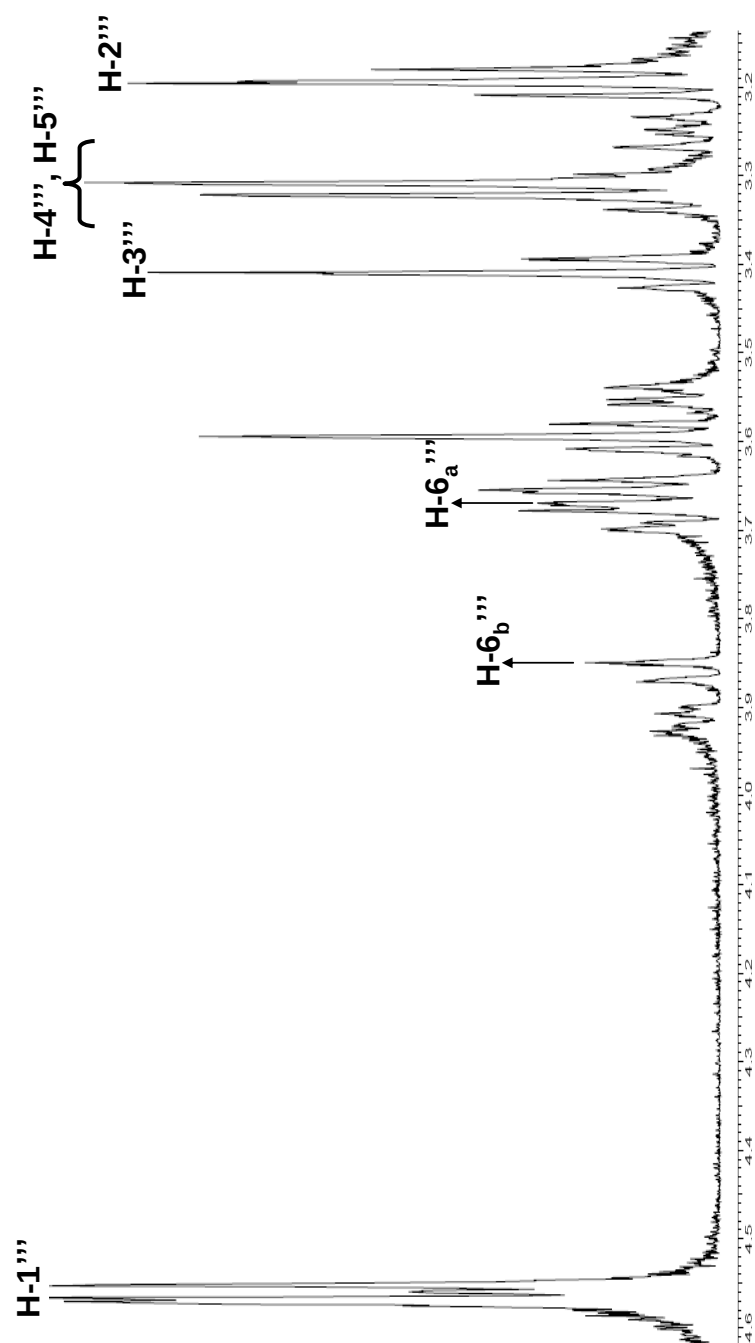


Figure 3.19. 1D-TOCSY spectrum of glucuronic acid residue of AA₃



Spectrum 3.20. 1D-TOCSY spectrum of glucose residue of AA₃

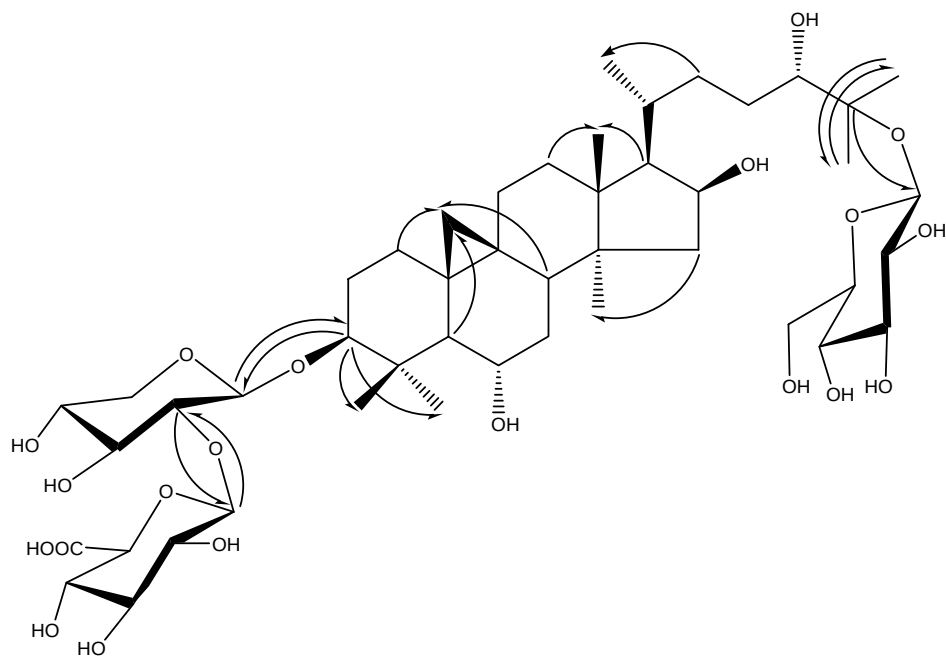


Figure 3.6. Key HMBC correlations of **AA₃**

3.1.4. Structural Identification of Compound 4 (AA₄)

The molecular formula of compound **4** was unequivocally established to be C₄₇H₈₀O₁₉ by HRMALDITOFMS analysis (m/z 971.5199 [M+Na]⁺, calc. for C₄₇H₈₀O₁₉Na, 971.5192).

The ¹H-NMR spectrum of **4** showed three anomeric proton resonances at δ 4.30 (*d*, *J*= 7.5 Hz), 4.45 (*d*, *J*= 7.5 Hz), and 4.60 (*d*, *J*= 7.5 Hz) correlated by HSQC to the resonances at δ 107.1, 104.8 and 98.4 (Tables 2 and 3). The three terminal sugar units were identified as a β -D-xylopyranose and two β -D-glucopyranoses, respectively, using a combination of 1D-TOCSY and HSQC spectra.

The ¹³C-NMR resonances arising from the sapogenol moiety were similar to those of **3**, except for the signal assigned to C-24 (δ 85.2) exhibiting a significant glycosidation shift. These results suggested another tridesmosidic structure in which the three sugar units were attached to the hydroxyl groups at C-3, C-24 and C-25. The glycosidation sites were established by the HMBC experiment which showed correlations between H-3 (δ 3.24) and C-1' (δ 107.1) of the β -D-xylopyranosyl unit, H-24 (δ 3.77) and C-1'' (δ 104.8) of the first β -D-glucopyranosyl and between H-1''' (δ 4.60) and C-25 (δ 81.0).

On the basis of these evidence, the structure of compound **4** was established as 3-*O*- β -D-xylopyranosyl-24,25-di-*O*- β -D-glucopyranosyl-3 β ,6 α ,16 β ,24(*S*),25-pentahydroxy-cycloartane (**Figure 3.7.**). Based on the database search (SciFinderScholar) compound **4** was identified as a new chemical entity.

Amorphous powder; $[\alpha]_D^{25}$ 22.7 (*c* 0.1 MeOH); IR (KBr): $\nu_{\max}$ = 3483 (>OH), 3027 (cyclopropane ring), 2877 (>CH), 1279-1038 (C-O-C) cm^{-1} ; ESI-MS m/z 971.4 $[\text{M}+\text{Na}]^+$; MS/MS m/z 791.4 $[\text{M}+\text{Na}-180]^+$, m/z 613.3 $[\text{M}+\text{Na}-180-180]^+$; HRMALDITOFMS $[\text{M}+\text{Na}]^+$ m/z 971.5199 (calc. for $\text{C}_{47}\text{H}_{80}\text{O}_{19}\text{Na}$, 971.5192); ^1H NMR (CD_3OD , 600 MHz) and ^{13}C NMR (CD_3OD , 150 MHz) data: see **Tables 3.7.-3.8.**

Table 3.7. ^1H and ^{13}C assignments of sapogenol moiety of **AA₄** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
1	33.1	H₂-1	1.56, 1.26, m
2	30.3	H₂-2	1.97, 1.69, m
3	89.6	H-3	3.24, dd (11.3, 4.0)
4	42.7	-----	-----
5	54.5	H-5	1.40, d (9.5)
6	69.4	H-6	3.48, ddd (9.5, 9.5, 4.5)
7	38.7	H₂-7	1.50, 1.37, m
8	48.7	H-8	1.81, dd (11.9, 4.2)
9	21.6	-----	-----
10	29.8	-----	-----
11	26.7	H₂-11	2.02, 1.22, m
12	33.5	H₂-12	1.73, 1.68, m
13	46.1	-----	-----
14	47.5	-----	-----
15	49.0	H₂-15	2.04, dd (12.7, 8.0) 1.43, dd (12.7, 5.2)
16	72.7	H-16	4.46, ddd (8.0, 8.0, 5.2)
17	57.6	H-17	1.75, dd (9.9, 8.0)
18	18.9	H₃-18	1.21, s
19	31.5	H₂-19	0.55, d (4.2) 0.41, d (4.2)
20	31.6	H-20	1.84, m
21	18.0	H₃-21	0.99, d (6.5)
22	34.1	H₂-22	1.81, 1.24, m
23	28.4	H₂-23	1.85, 1.79, m
24	85.2	H-24	3.77, dd (2.4, 10.5)
25	81.0	-----	-----
26	25.3	H₃-26	1.37, s
27	22.7	H₃-27	1.32, s
28	28.3	H₃-28	1.32, s
29	16.3	H₃-29	1.05, s
30	20.1	H₃-30	0.98, s

Table 3.8. ^1H and ^{13}C assignments of sugar residues of **AA₄** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
<i>β-D-xylose-1'</i>	107.1	H-1'	4.30, d (7.5)
2'	75.1	H-2'	3.22, dd (9.2, 7.5)
3'	77.7	H-3'	3.31, dd (9.2)
4'	70.8	H-4'	3.49, m
5'	66.3	H₂-5'	3.84, dd (11.7, 5.2) 3.20, t (11.7)
<i>β-D-glucose-1''</i>	104.8	H-1''	4.45, d (7.5)
2''	75.4	H-2''	3.25, dd (7.5, 9.0)
3''	77.8	H-3''	3.42, dd (9.0, 9.0)
4''	71.3	H-4''	3.33, dd (9.0, 9.0)
5''	77.8	H-5''	3.33, m
6''	62.4	H₂-6''	3.69, dd (4.5, 12.0) 3.89, dd (3.5, 12.0)
<i>β-D-glucose-1'''</i>	98.4	H-1'''	4.60, d (7.5)
2'''	75.0	H-2'''	3.20, dd (7.5, 9.0)
3'''	78.0	H-3'''	3.39, dd (9.0, 9.0)
4'''	71.2	H-4'''	3.30, dd (9.0, 9.0)
5'''	77.8	H-5'''	3.30, m
6'''	62.2	H₂-6'''	3.67, dd (4.5, 12.0) 3.86, dd (3.5, 12.0)

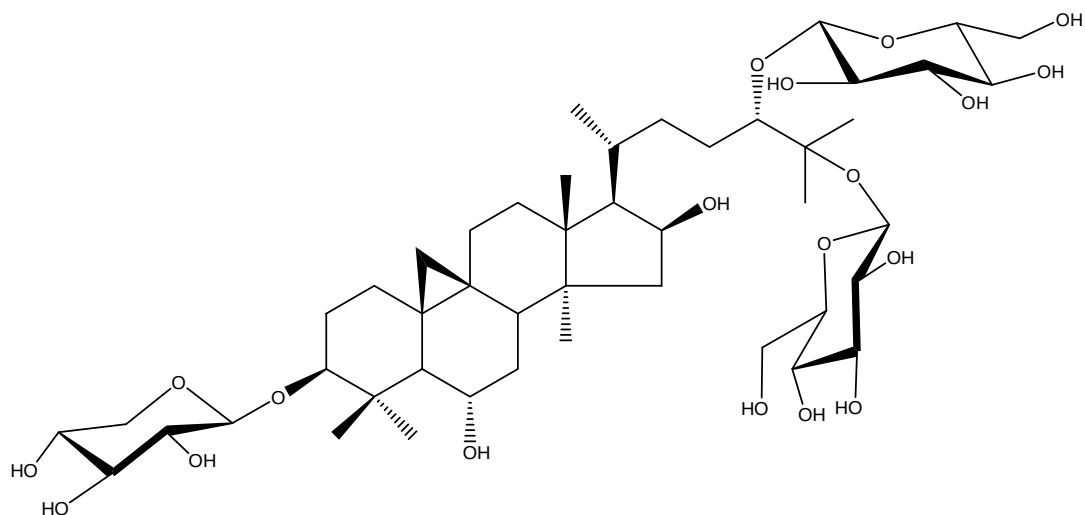
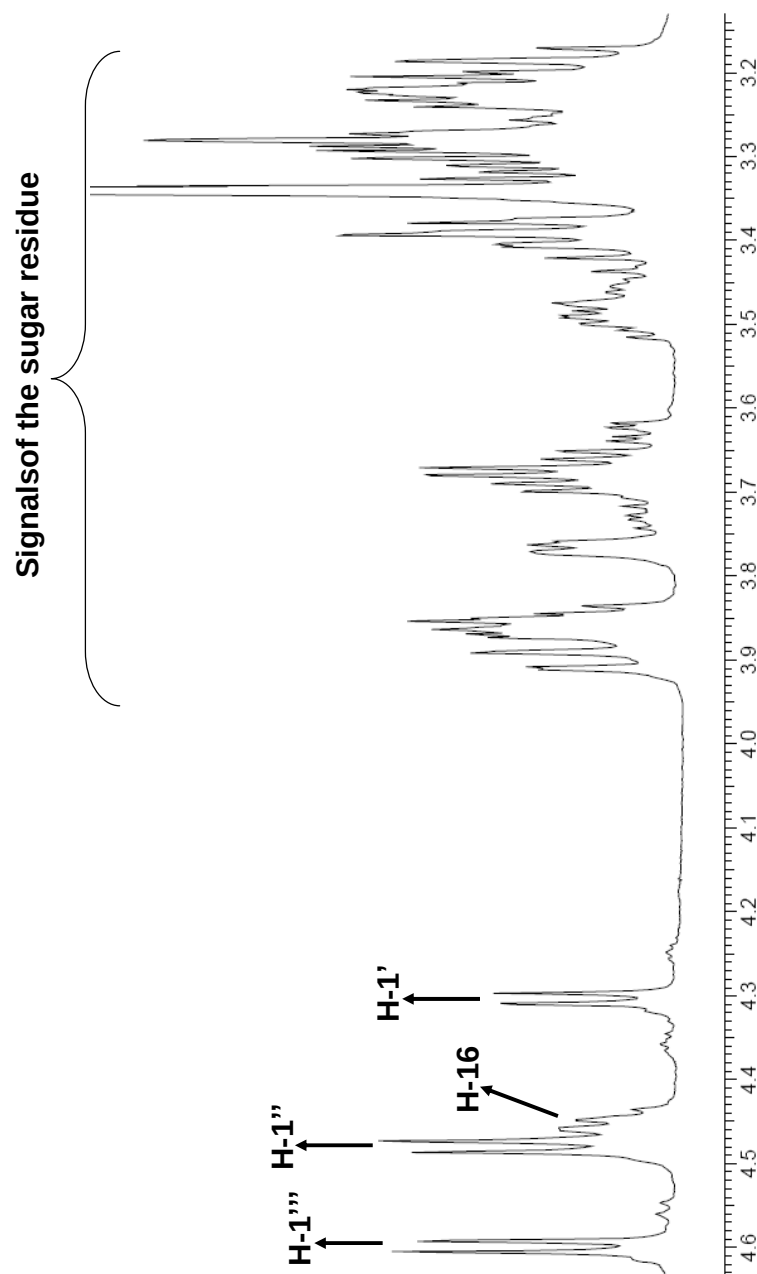
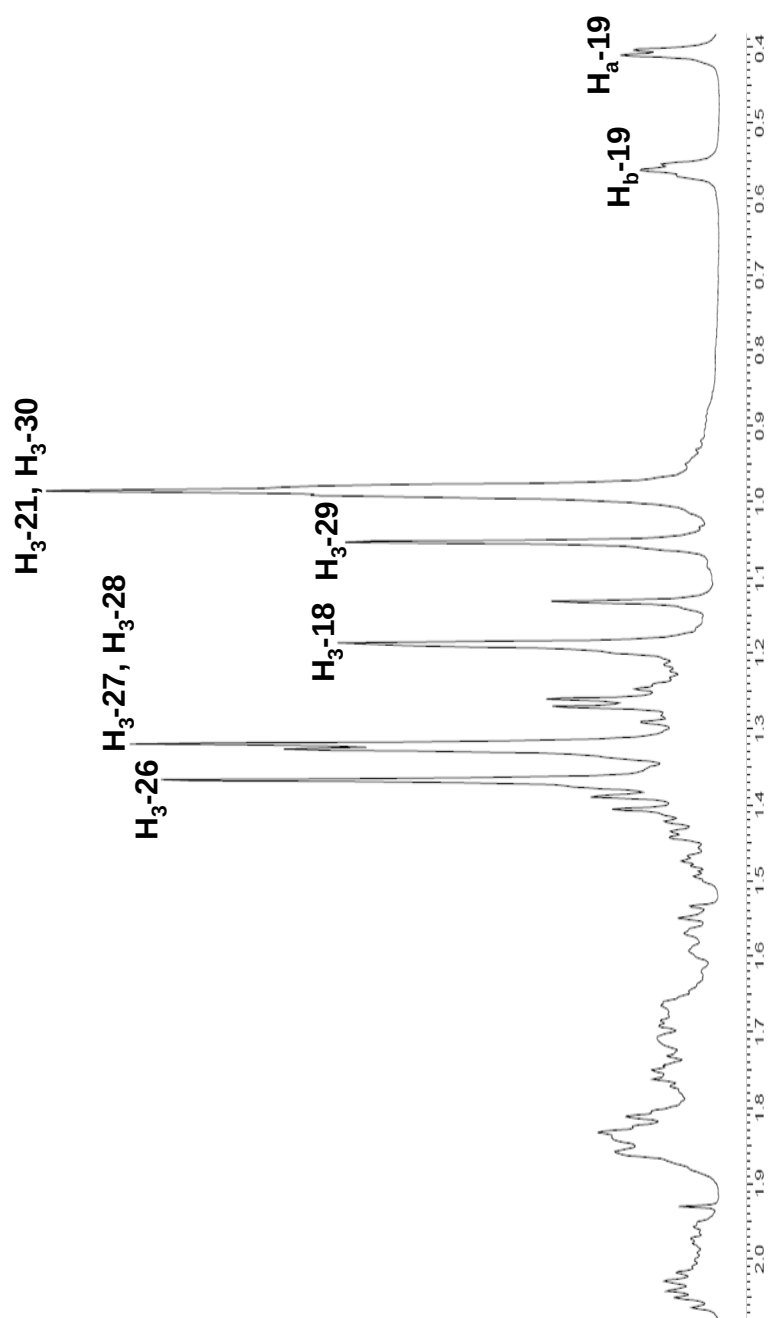


Figure 3.7. Structure of AA₄

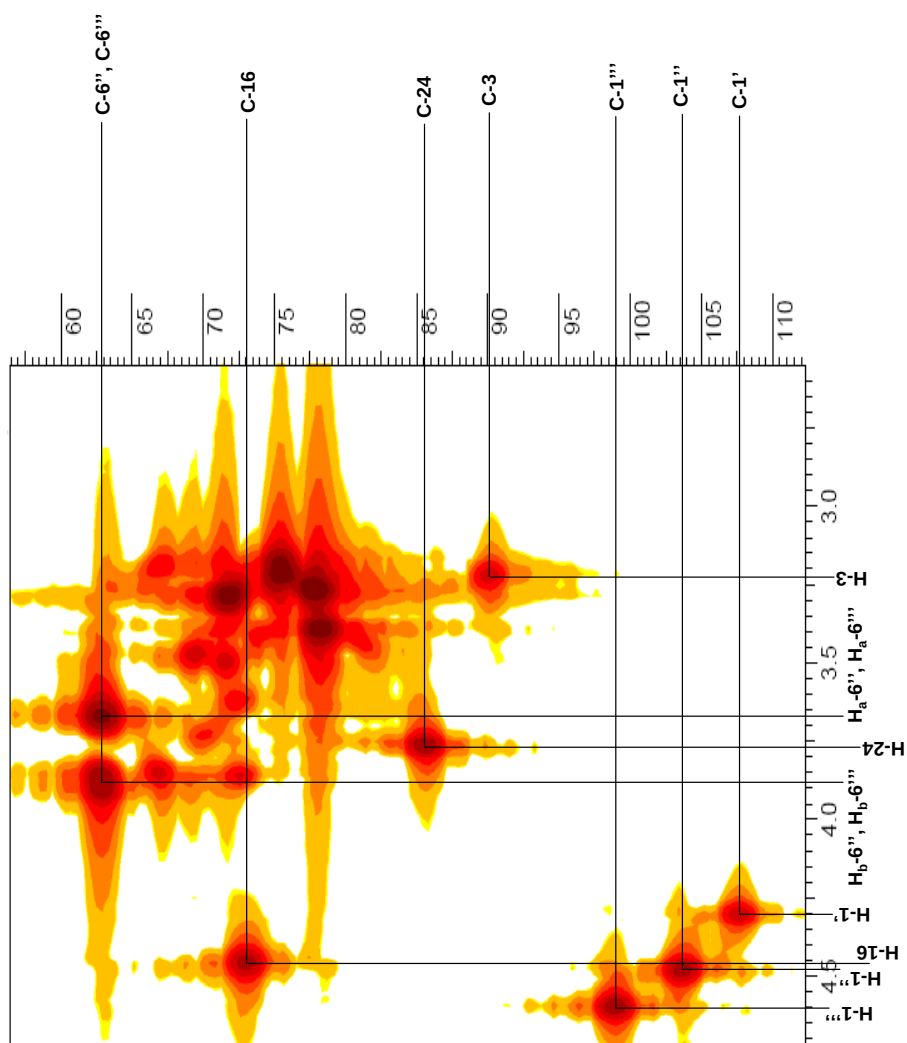


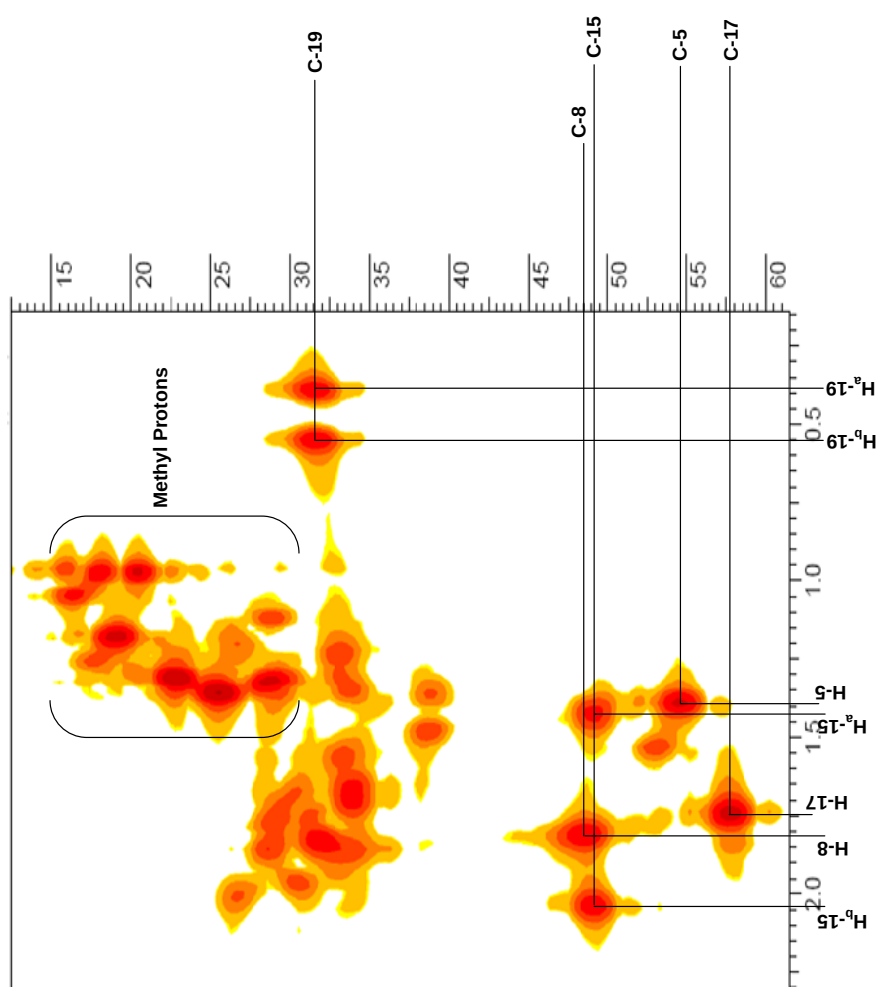
Spectrum 3.21. ^1H spectrum of AA_4 in the downfield region

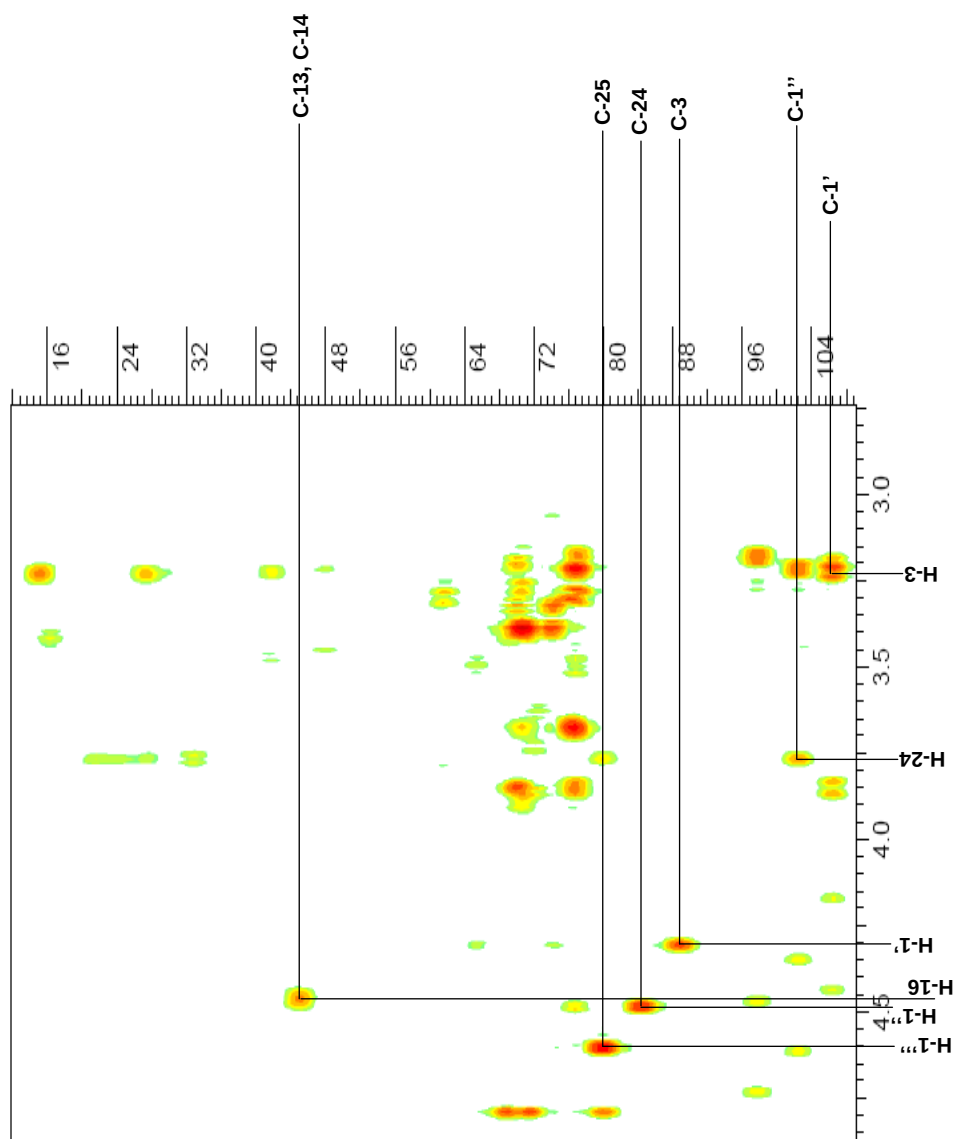


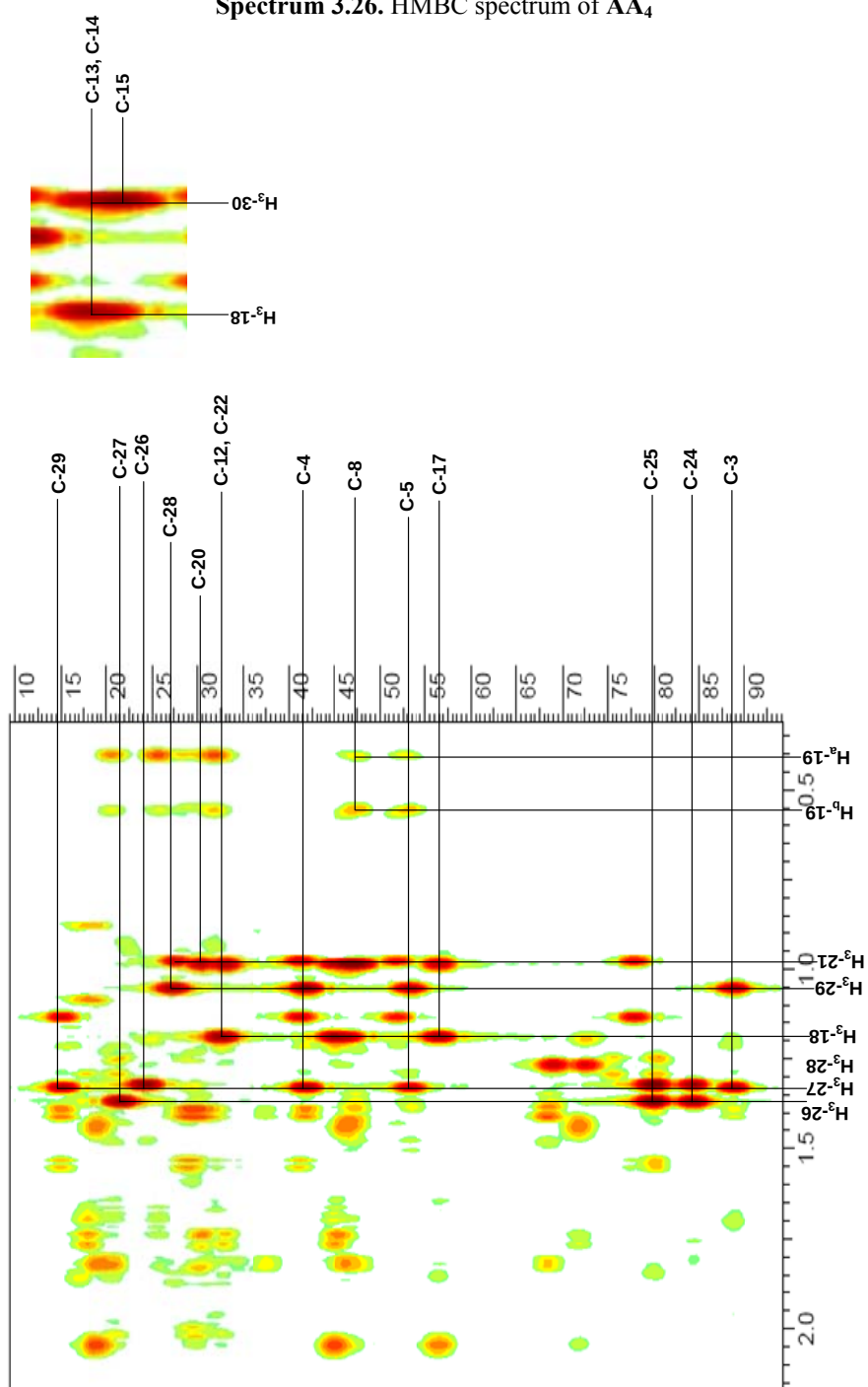
Spectrum 3.22. ^1H spectrum of AA₄ in the upfield region

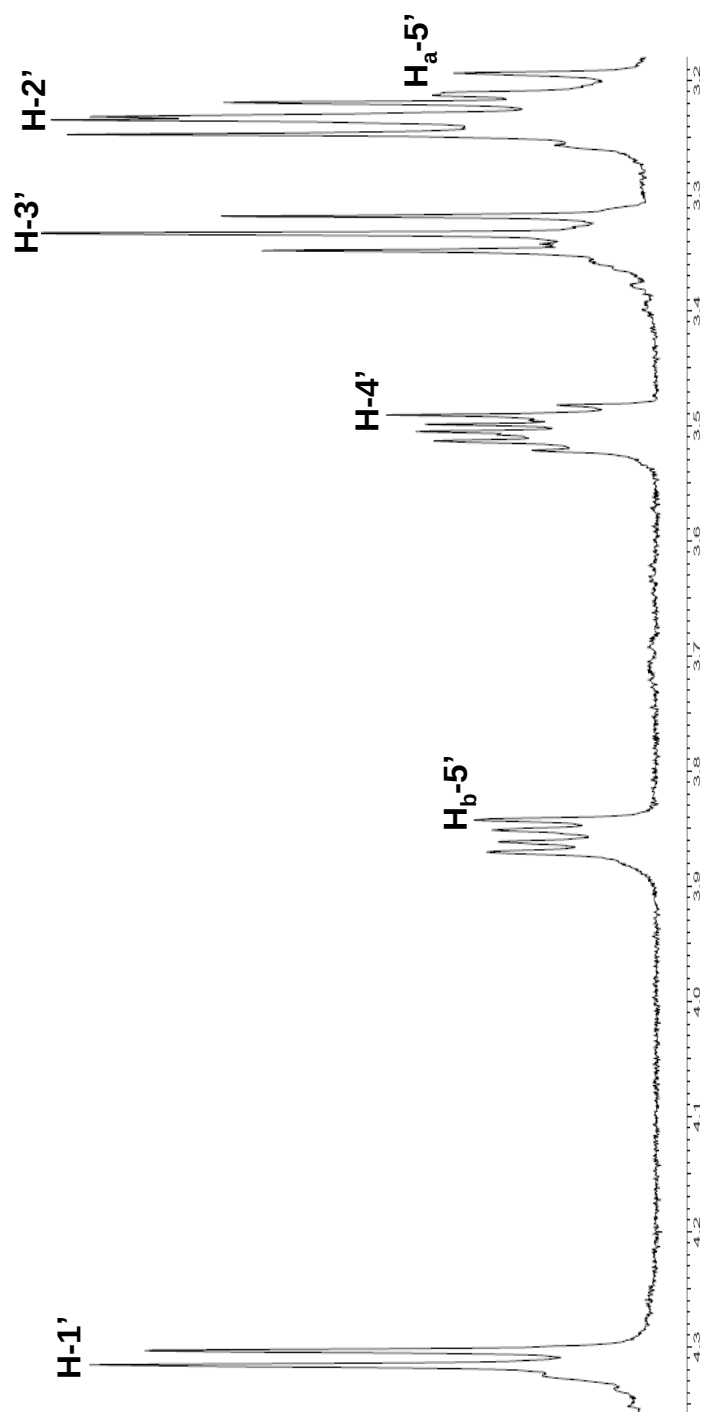
Spectrum 3.23. HSQC spectrum of **AA**₄



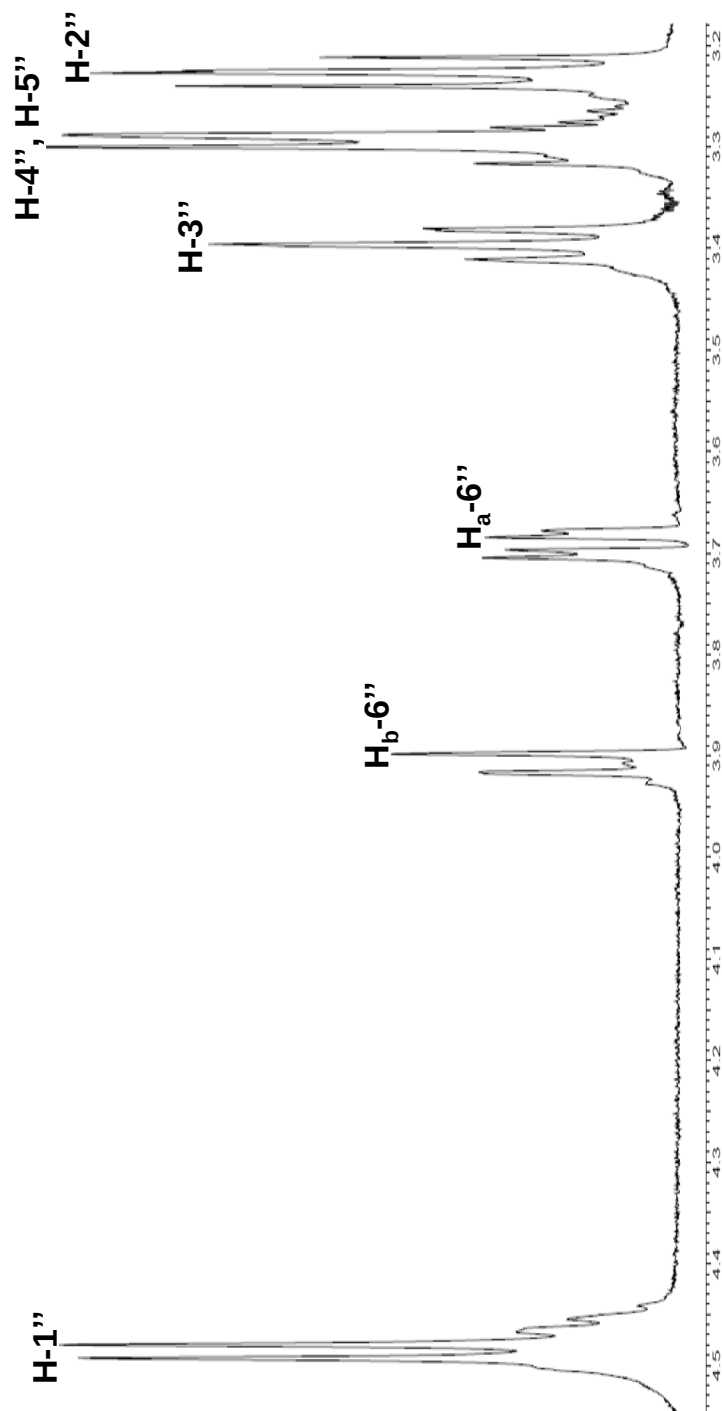
Spectrum 3.24. HSQC spectrum of AA₄

Spectrum 3.25. HMBC spectrum of AA₄

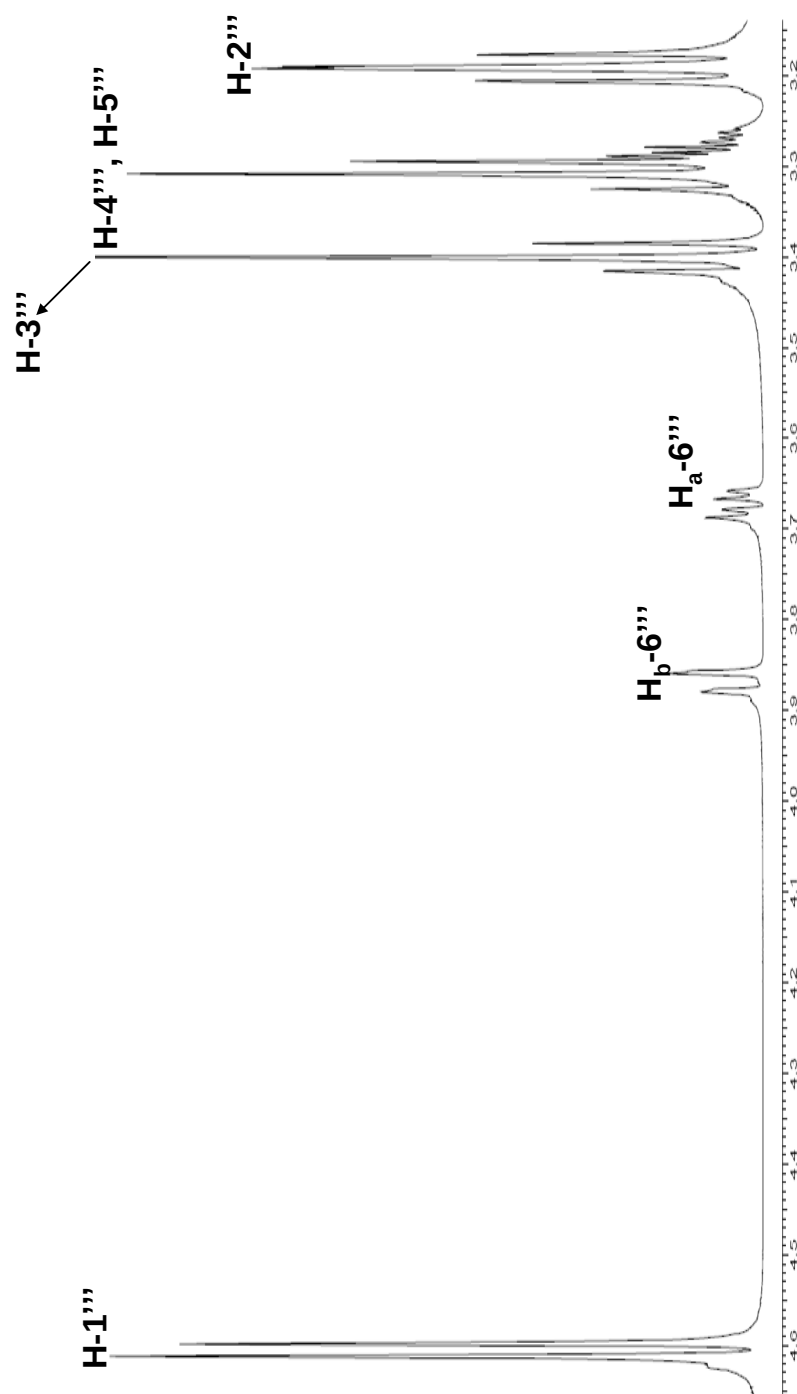
Spectrum 3.26. HMBC spectrum of AA₄



Spectrum 3.27. 1D-TOCSY spectrum of xylose residue of AA₄



Spectrum 3.28. 1D-TOCSY spectrum of glucose residue of AA₄ (at C-24)



Spectrum 3.29. 1D-TOCSY spectrum of glucose residue of AA₄ (at C-25)

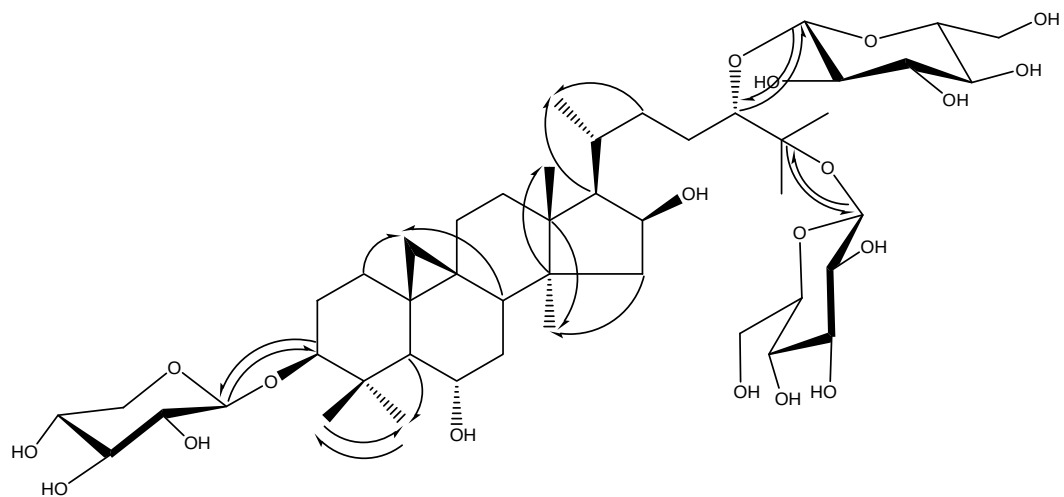


Figure 3.8. Key HMBC correlations of AA₄

3.1.5. Structural Identification of Compound **5** (AA₅)

The HRMALDITOF mass spectrum of **5** (m/z 985.5350 $[M+Na]^+$, calc. for $C_{48}H_{82}O_{19}Na$, 985.5348) supported a molecular formula of $C_{48}H_{82}O_{19}$. The ESIMS mass spectrum showed the major ion peak at m/z 985.3 which was assigned to $[M+Na]^+$. The MS/MS of this ion showed peaks at m/z 805.3 $[M+Na-180]^+$, corresponding to the loss of an hexose unit and at m/z 641.4 $[M+Na-180-164]^+$, ascribable to the loss of a 6-deoxyhexose. The ^1H -NMR spectrum of aglycon moiety of **5** displayed the typical signals of cycloartanes having acyclic side chain: six tertiary methyl groups, from signals at δ 0.98, 1.20, 0.98, 1.13, 1.21, 1.19, and a secondary methyl group from signal at δ 0.96 (d , $J=6.5$ Hz, H_3-21), as well as the cyclopropane-methylene protons at δ 0.40, 0.54 ($J_{AX}=4.2$ Hz, H_2-19) (**Tables 3.9.-3.10.**). In addition, the resonances of three anomeric protons, indicative of the presence of one α -linked and two β -linked sugar moieties, were observed in the downfield region (δ 4.78, d , $J=1.2$ Hz, $H-1'$; δ 4.32, d , $J=7.5$ Hz, $H-1''$; δ 4.45, d , $J=7.5$ Hz, $H-1'''$).

Combination of 1D-TOCSY and HSQC experiments allowed the sequential assignments of all proton resonances of each sugar residue, starting from anomeric protons. Furthermore, on the basis of chemical shifts, the multiplicity of the signal and the coupling constants, the three sugar residues were identified as α -rhamnopyranose and two β -glucopyranoses. The D configuration of glucose units and the L

configuration of rhamnose unit were determined by acid hydrolysis followed by GC analysis.

The remaining ^1H and ^{13}C resonances of **5** arising from the sapogenol moiety were evident for the presence of cyclocanthogenol except for the signals ascribable to linkage sites exhibiting typical glycosidation shifts. Key correlation peaks observed in the HMBC spectrum of **5** between H-1' of the rhamnosyl at δ 4.78 (*d*, $J=1.2$, Hz) and C-6 (δ 81.4) of the aglycon, between H-1'' of the glucosyl at δ 4.32 (*d*, $J=7.5$ Hz) and C-16 (δ 83.7), and between H-1''' of the second glucosyl unit at δ 4.45 (*d*, $J=7.5$ Hz) and C-24 (δ 90.4), revealed the locations of glycosidic linkages to be C-6, C-16 and C-24 indicating the tridesmosidic nature of compound **5**.

Consequently, the structure of **5** was established as 6-*O*- α -L-rhamnopyranosyl-16,24-di-*O*- β -D-glucopyranosyl-3 β ,6 α ,16 β ,24(*S*),25-pentahydroxy-cycloartane, a new cycloartane-type triterpene saponin (**Figure 3.9**).

Amorphous powder; $[\alpha]_D^{25}$ 17.9 (*c* 0.1, MeOH); IR (KBr): ν_{max} = 3472 (>OH), 3040 (cyclopropane ring), 2883 (>CH), 1283-1029 (C-O-C) cm^{-1} ; ESI-MS m/z 985.3 $[\text{M}+\text{Na}]^+$; MS/MS m/z 805.3 $[\text{M}+\text{Na}-180]^+$, m/z 641.4 $[\text{M}+\text{Na}-180-164]^+$; HRMALDITOFMS $[\text{M}+\text{Na}]^+$ m/z 985.5350 (calc. for $\text{C}_{48}\text{H}_{82}\text{O}_{19}\text{Na}$, 985.5348); ^1H NMR (CD_3OD , 600 MHz) and ^{13}C NMR (CD_3OD , 150 MHz) data: see **Tables 3.9.-3.10**.

Table 3.9. ^1H and ^{13}C assignments of sapogenol moiety of **AA₅** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
1	33.2	H₂-1	1.59, 1.29, m
2	30.9	H₂-2	1.72, 1.65, m
3	79.0	H-3	3.23, dd (11.3, 4.0)
4	42.5	-----	-----
5	53.1	H-5	1.53, d (9.5)
6	81.4	H-6	3.42, ddd (9.5, 9.5, 4.5)
7	35.6	H₂-7	1.85, 1.39, m
8	48.0	H-8	1.79, dd (11.9, 4.2)
9	21.7	-----	-----
10	29.8	-----	-----
11	26.5	H₂-11	2.00, 1.24, m
12	33.3	H₂-12	1.71, 1.65, m
13	46.2	-----	-----
14	48.0	-----	-----
15	49.0	H₂-15	2.07, dd (12.7, 8.0) 1.80, dd (12.7, 5.2)
16	83.7	H-16	4.27, ddd (8.0, 8.0, 5.2)
17	57.1	H-17	1.89, dd (9.9, 8.0)
18	18.9	H₃-18	1.20, s
19	31.3	H₂-19	0.54, d (4.2) 0.40, d (4.2)
20	31.4	H-20	2.09, m
21	17.9	H₃-21	0.96, d (6.5)
22	34.3	H₂-22	1.69, 1.24, m
23	29.4	H₂-23	1.60 (2H), m
24	90.4	H-24	3.58, dd (2.4, 10.5)
25	73.7	-----	-----
26	24.7	H₃-26	1.21, s
27	26.5	H₃-27	1.19, s
28	28.9	H₃-28	1.13, s
29	16.2	H₃-29	0.98, s
30	20.1	H₃-30	0.98, s

Table 3.10. ^1H and ^{13}C assignments of sugar residues of **AA₅** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
$\alpha\text{-L-rhamnose-1'}$	103.9	H-1'	4.78, d (1.2)
2'	72.5	H-2'	3.86, dd (1.2, 3.2)
3'	72.3	H-3'	3.62, dd (3.2, 9.3)
4'	73.6	H-4'	3.42, t (9.3)
5'	69.9	H-5'	3.72, m
6'	17.7		1.29, s
$\beta\text{-D-glucose-1''}$	106.8	H-1''	4.32, d (7.5)
2''	75.5	H-2''	3.18, dd (7.5, 9.0)
3''	78.0	H-3''	3.38, dd (9.0, 9.0)
4''	71.2	H-4''	3.39, dd (9.0, 9.0)
5''	77.6	H-5''	3.27, m
6''	62.4	H₂-6''	3.69, dd (4.5, 12.0) 3.89, dd (3.5, 12.0)
$\beta\text{-D-glucose-1'''}$	104.8	H-1'''	4.45, d (7.5)
2'''	75.4	H-2'''	3.25, dd (7.5, 9.0)
3'''	77.8	H-3'''	3.42, dd (9.0, 9.0)
4'''	71.3	H-4'''	3.33, dd (9.0, 9.0)
5'''	77.8	H-5'''	3.33, m
6'''	62.4	H₂-6'''	3.69, dd (4.5, 12.0) 3.89, dd (3.5, 12.0)

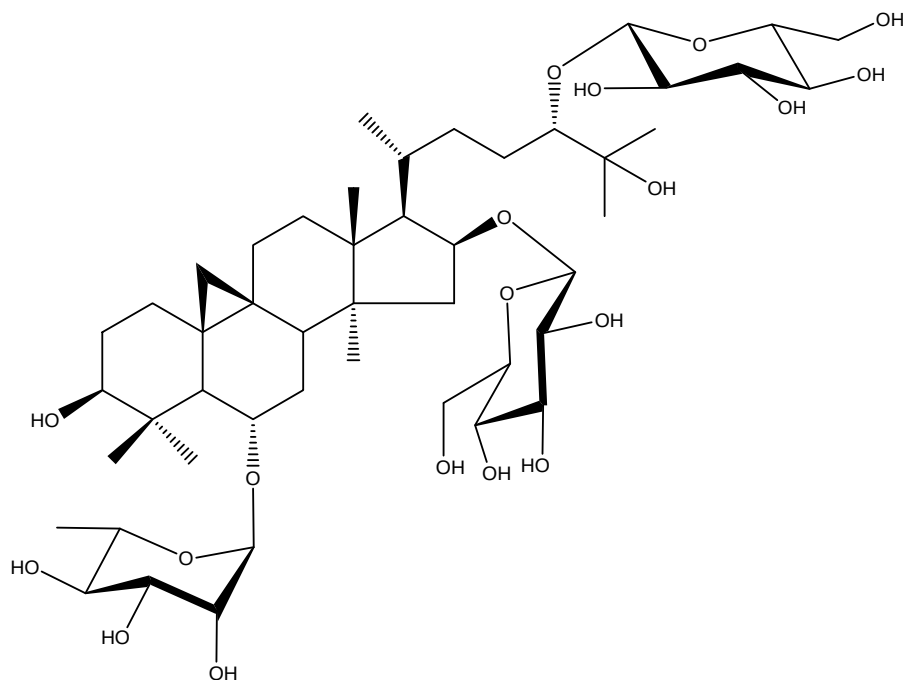
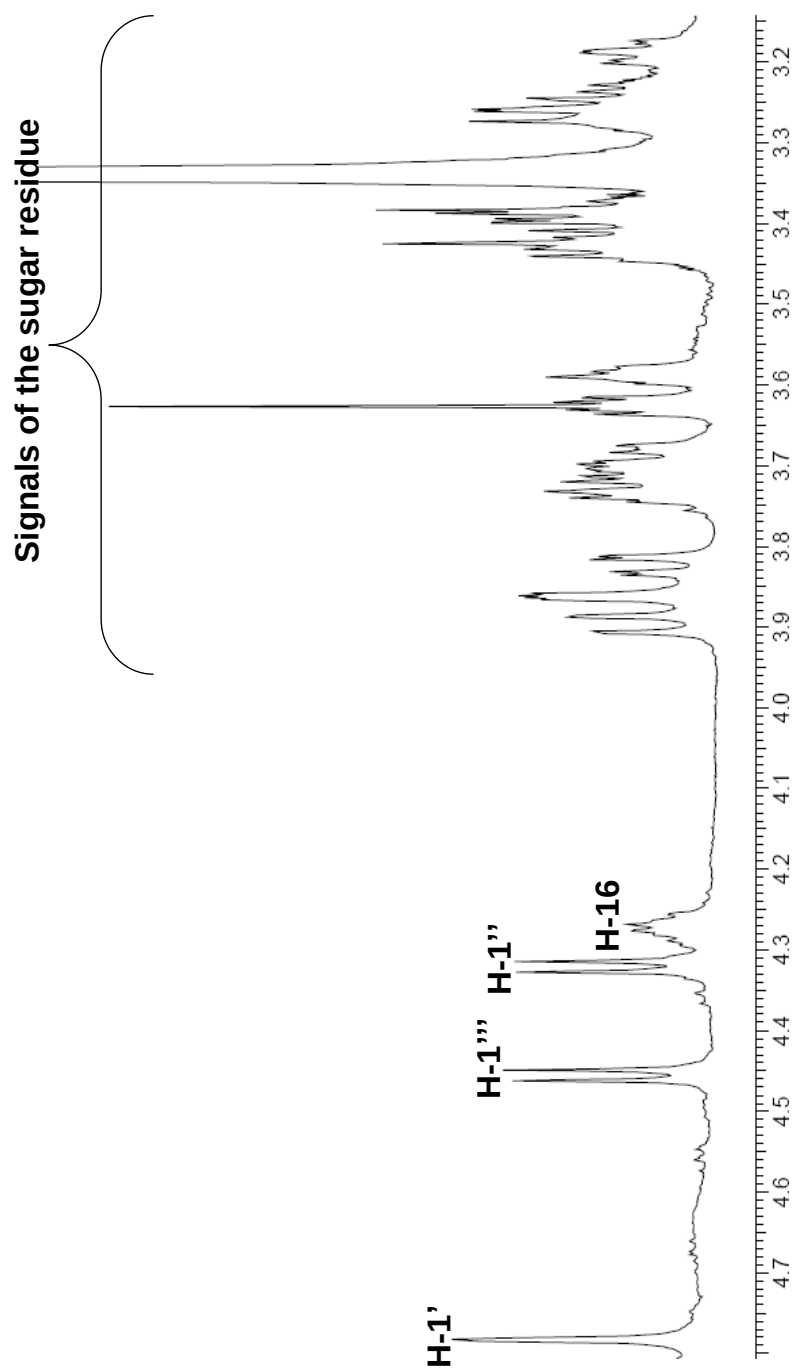
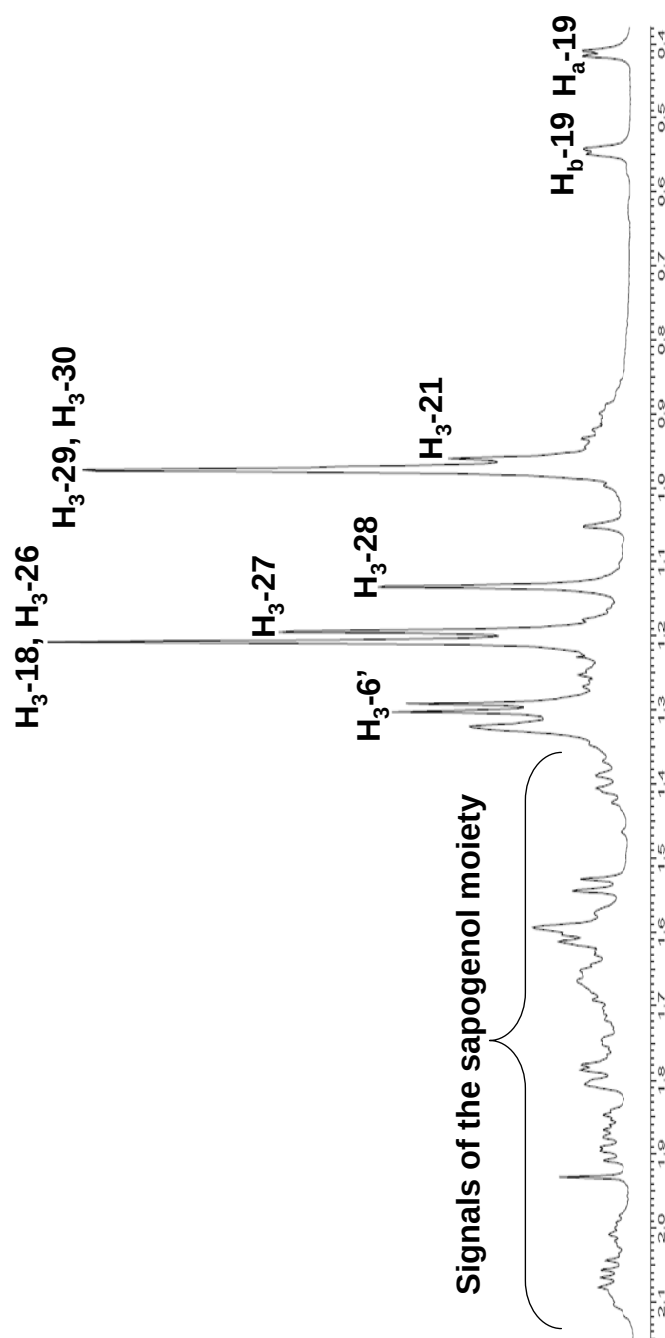


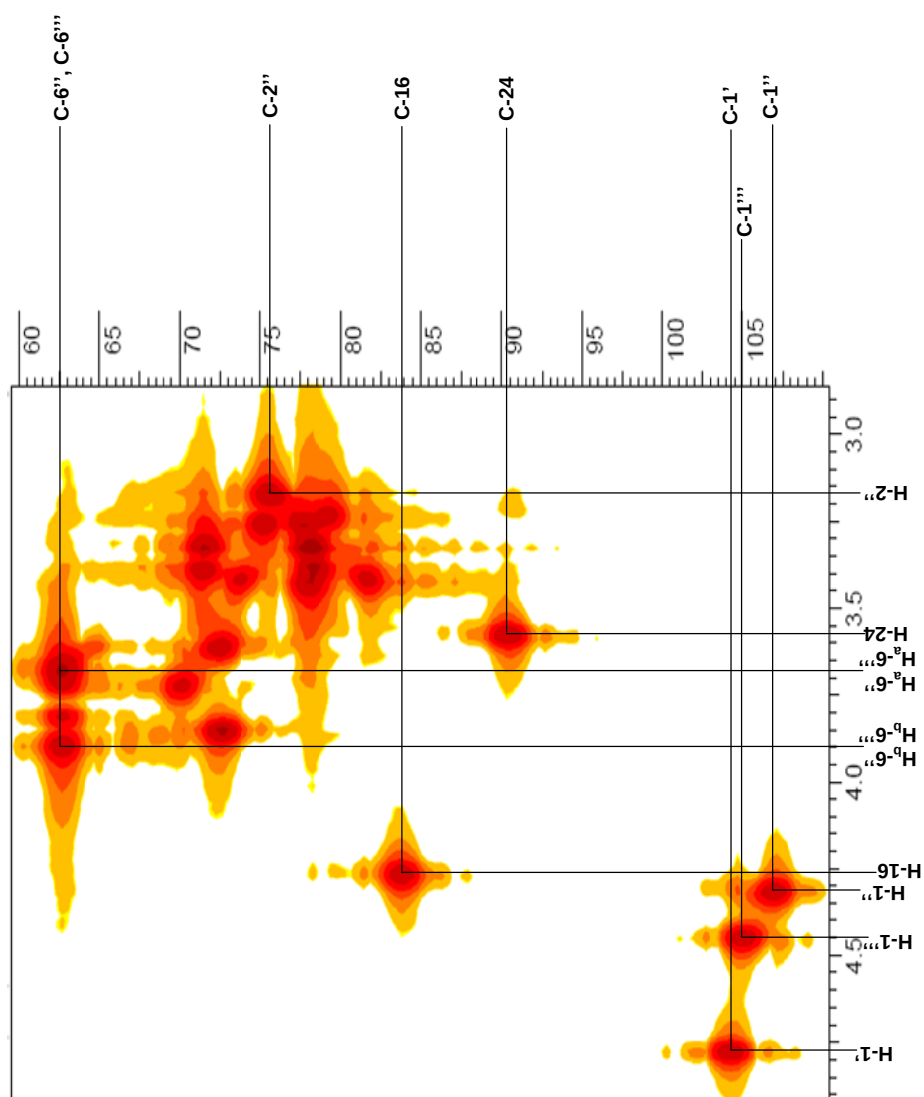
Figure 3.9. Structure of AA₅

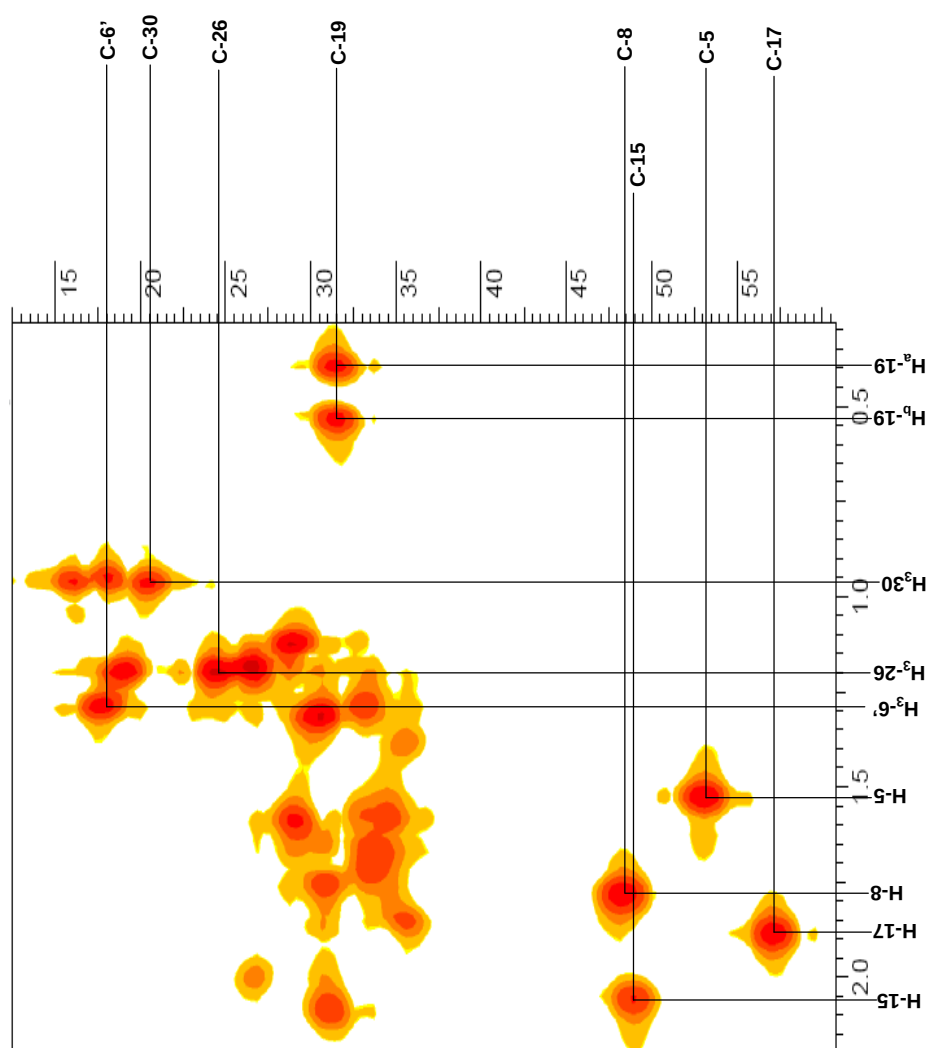


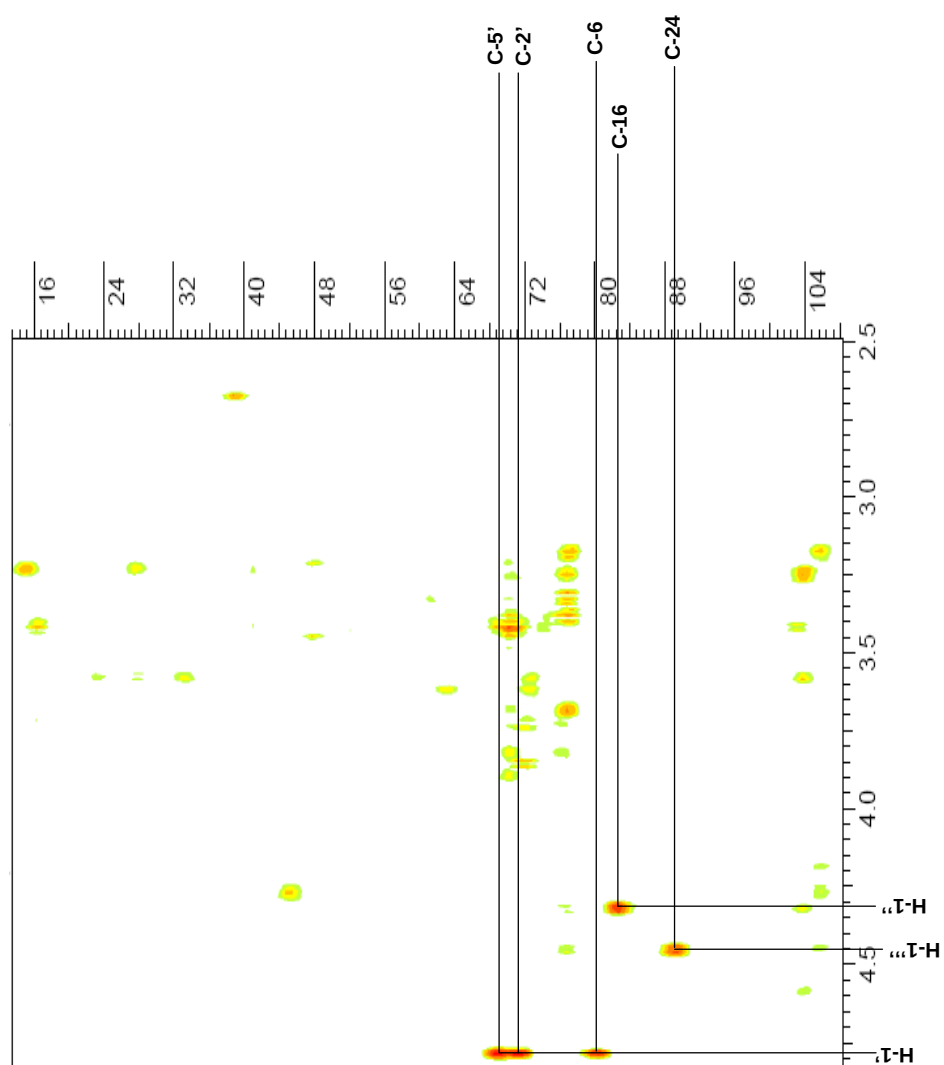
Spectrum 3.30. ^1H spectrum of AA_5 in the downfield region

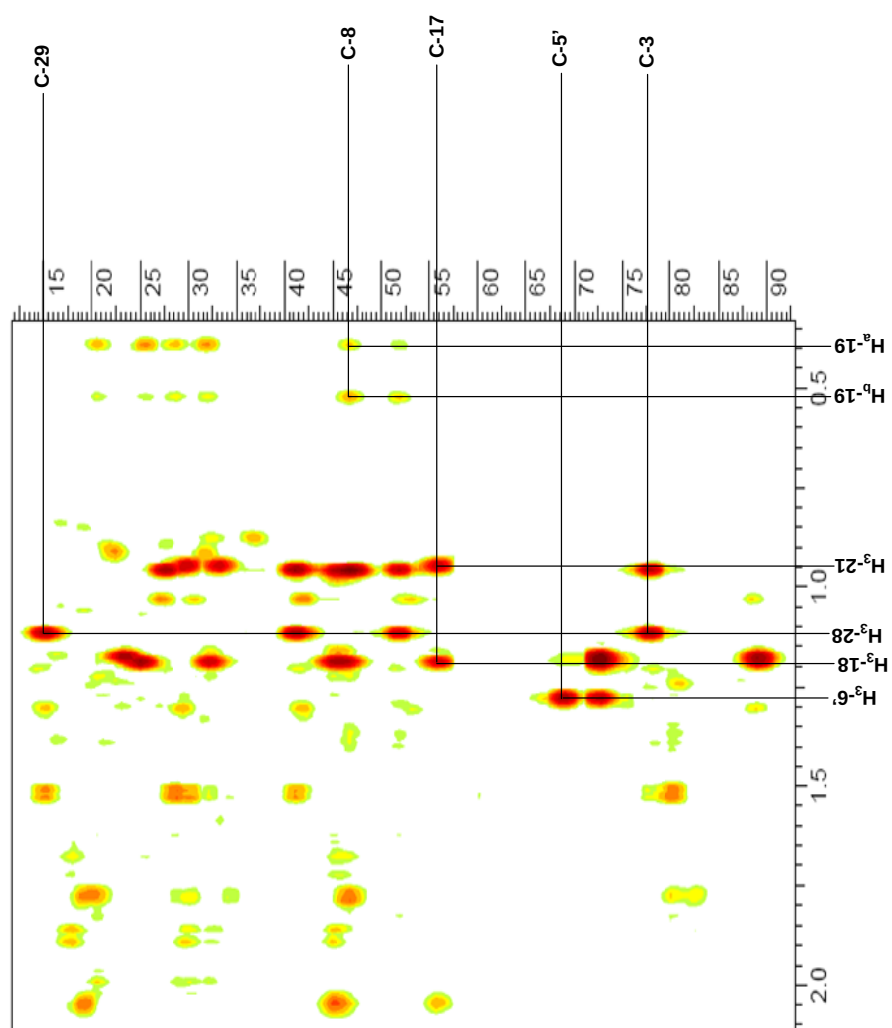


Spectrum 3.31. ^1H spectrum of AA_5 in the upfield region

Spectrum 3.32. HSQC spectrum of AA₅

Spectrum 3.33. HSQC spectrum of AA₅

Spectrum 3.34. HMBC spectrum of AA₅

Spectrum 3.35. HMBC spectrum of AA₅

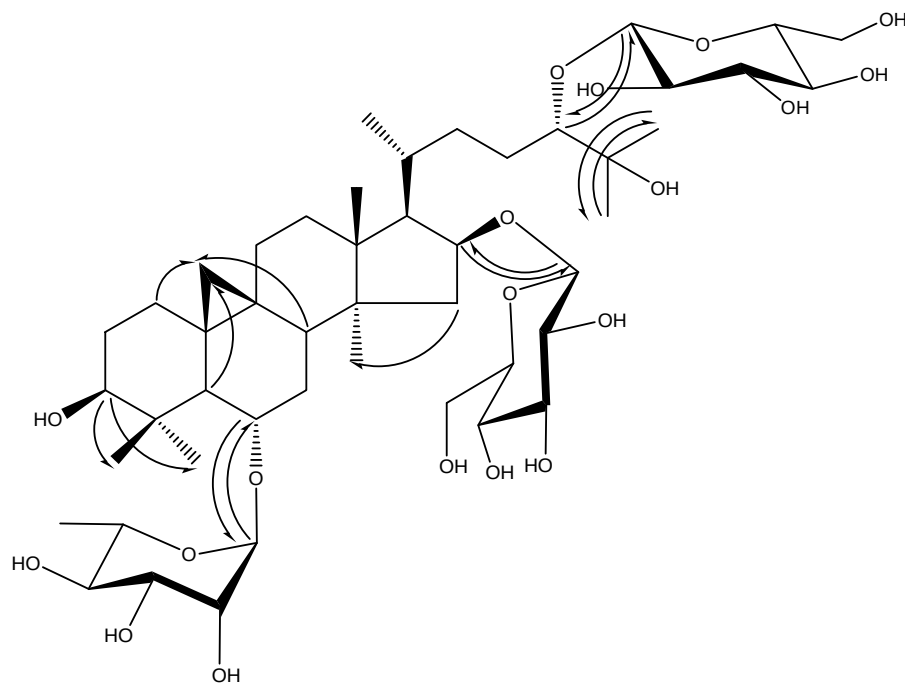


Figure 3.10. Key HMBC correlations of AA₅

3.1.6. Structural Identification of Compound 6 (AA₆)

The molecular formula of **6** was determined to be C₄₈H₈₂O₁₉ by HRMALDITOFMS analysis (m/z 985.5341 [M+Na]⁺, calc. for C₄₈H₈₂O₁₉Na, 985.5348). In the positive ESIMS spectrum, compound **6** showed a major ion peak at m/z 985.4 [M+Na]⁺ and a significant fragment in MS/MS analysis at m/z 805.3 [M+Na-180]⁺, ascribable to the loss of an hexose unit. The MS³ fragmentation showed a peak at m/z 641.3 [M+Na-180-164]⁺ corresponding to the loss of a 6-deoxyhexose unit. Full assignments of the proton and carbon signals of the aglycon and sugar parts of **6** in comparison with those of **5** showed their considerable structural similarity (**Tables 3.9.-3.11.**). The difference consisted only in the position of one of the glucose moieties. The sapogenol moiety showed an unambiguous glycosidation shift at C-25 (δ 81.4), while C-24 displayed no downfield shift due to the sugar attachment (δ 79.1). Furthermore, the HMBC experiment showed key correlation peaks between the anomeric proton of rhamnosyl unit (δ 4.78, d , $J=1.2$ Hz, H-1') and C-6 (δ 81.4), between the anomeric proton of the first glucose moiety (δ 4.27, d , $J=7.5$ Hz, H-1'') and C-16 (δ 83.7), and H-1''' (δ 4.55, d , $J=7.5$ Hz) of the second glucose residue and C-25 (δ 81.6). Thus, compound **6** displayed the glycosidation at C-25 instead of the glycosidation at C-24 as for compound **5**.

Consequently, the structure of **6** was established as 6-*O*- α -L-rhamnopyranosyl-16,25-di-*O*- β -D-glucopyranosyl-3 β ,6 α ,16 β ,24(*S*),25-

pentahydroxy-cycloartane, a new cycloartane-type glycoside (**Figure 3.11.**).

Amorphous powder; $[\alpha]_D^{25}$ 24.4 (*c* 0.1, MeOH); IR (KBr): $\nu_{\max}$ = 3486 (>OH), 3035 (cyclopropane ring), 2874 (>CH), 1292-1040 (C-O-C) cm^{-1} ; ESI-MS m/z 985.4 $[\text{M}+\text{Na}]^+$; MS/MS m/z 805.3 $[\text{M}+\text{Na}-180]^+$, m/z 641.3 $[\text{M}+\text{Na}-180-164]^+$; HRMALDITOFMS $[\text{M}+\text{Na}]^+$ m/z 985.5341 (calc. for $\text{C}_{48}\text{H}_{82}\text{O}_{19}\text{Na}$, 985.5348); ^1H NMR (CD_3OD , 600 MHz) and ^{13}C NMR (CD_3OD , 150 MHz) data: see **Tables 3.11.-3.12.**

Table 3.11. ^1H and ^{13}C assignments of sapogenol moiety of **AA₆** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
1	33.2	H₂-1	1.59, 1.29, m
2	30.9	H₂-2	1.72, 1.65, m
3	79.0	H-3	3.23, dd (11.3, 4.0)
4	42.5	-----	-----
5	53.1	H-5	1.53, d (9.5)
6	81.4	H-6	3.42, ddd (9.5, 9.5, 4.5)
7	35.6	H₂-7	1.85, 1.39, m
8	48.0	H-8	1.79, dd (11.9, 4.2)
9	21.7	-----	-----
10	29.8	-----	-----
11	26.5	H₂-11	2.00, 1.24, m
12	33.3	H₂-12	1.71, 1.65, m
13	46.2	-----	-----
14	48.0	-----	-----
15	49.0	H₂-15	2.07, dd (12.7, 8.0) 1.80, dd (12.7, 5.2)
16	83.7	H-16	4.27, ddd (8.0, 8.0, 5.2)
17	57.1	H-17	1.89, dd (9.9, 8.0)
18	18.9	H₃-18	1.20, s
19	31.3	H₂-19	0.54, d (4.2) 0.40, d (4.2)
20	31.4	H-20	1.92, m
21	17.9	H₃-21	0.96, d (6.5)
22	34.3	H₂-22	2.00, 0.89, m
23	29.4	H₂-23	1.83, 1.19, m
24	79.1	H-24	3.41, dd (2.4, 10.5)
25	81.6	-----	-----
26	20.9	H₃-26	1.25, s
27	23.2	H₃-27	1.27, s
28	28.9	H₃-28	1.13, s
29	16.2	H₃-29	0.98, s
30	20.1	H₃-30	0.98, s

Table 3.12. ^1H and ^{13}C assignments of sugar residues of **AA₆** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
α-L-rhamnose-1'	103.9	H-1'	4.78, d (1.2)
2'	72.5	H-2'	3.86, dd (1.2, 3.2)
3'	72.3	H-3'	3.62, dd (3.2, 9.3)
4'	73.6	H-4'	3.42, t (9.3)
5'	69.9	H-5'	3.72, m
6'	17.7		1.29, s
β-D-glucose-1''	106.8	H-1''	4.30, d (7.5)
2''	75.5	H-2''	3.15, dd (7.5, 9.0)
3''	77.8	H-3''	3.39, dd (9.0, 9.0)
4''	71.2	H-4''	3.37, dd (9.0, 9.0)
5''	77.2	H-5''	3.24, m
6''	62.4	H₂-6''	3.69, dd (4.5, 12.0) 3.89, dd (3.5, 12.0)
β-D-glucose-1'''	98.4	H-1'''	4.55, d (7.5)
2'''	75.0	H-2'''	3.20, dd (7.5, 9.0)
3'''	78.0	H-3'''	3.39, dd (9.0, 9.0)
4'''	71.2	H-4'''	3.30, dd (9.0, 9.0)
5'''	77.8	H-5'''	3.30, m
6'''	62.2	H₂-6'''	3.67, dd (4.5, 12.0) 3.86, dd (3.5, 12.0)

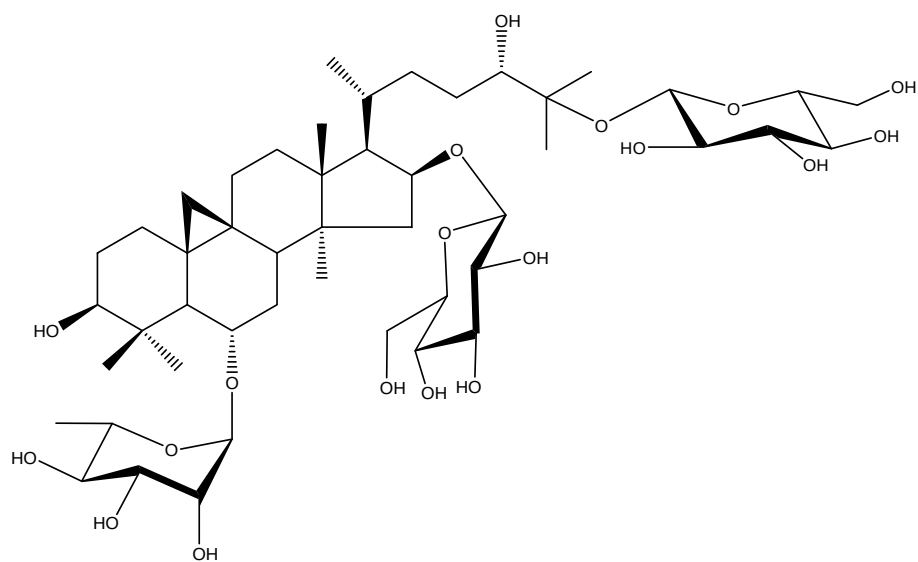
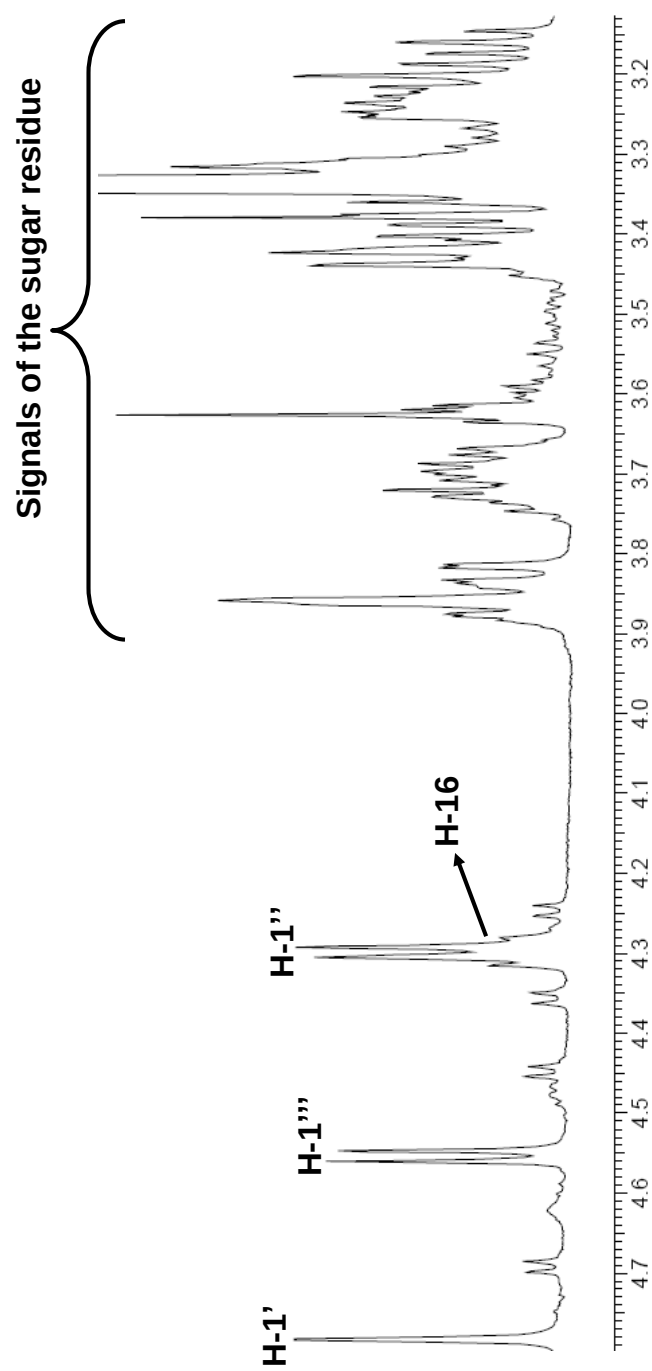
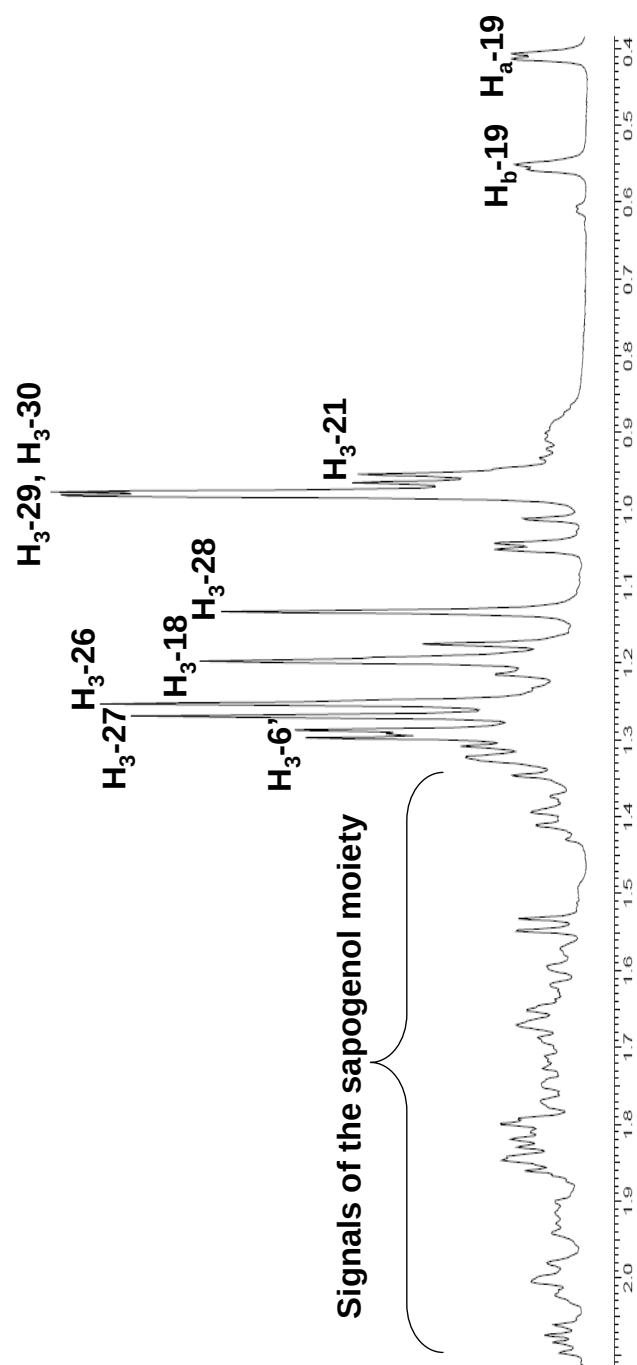


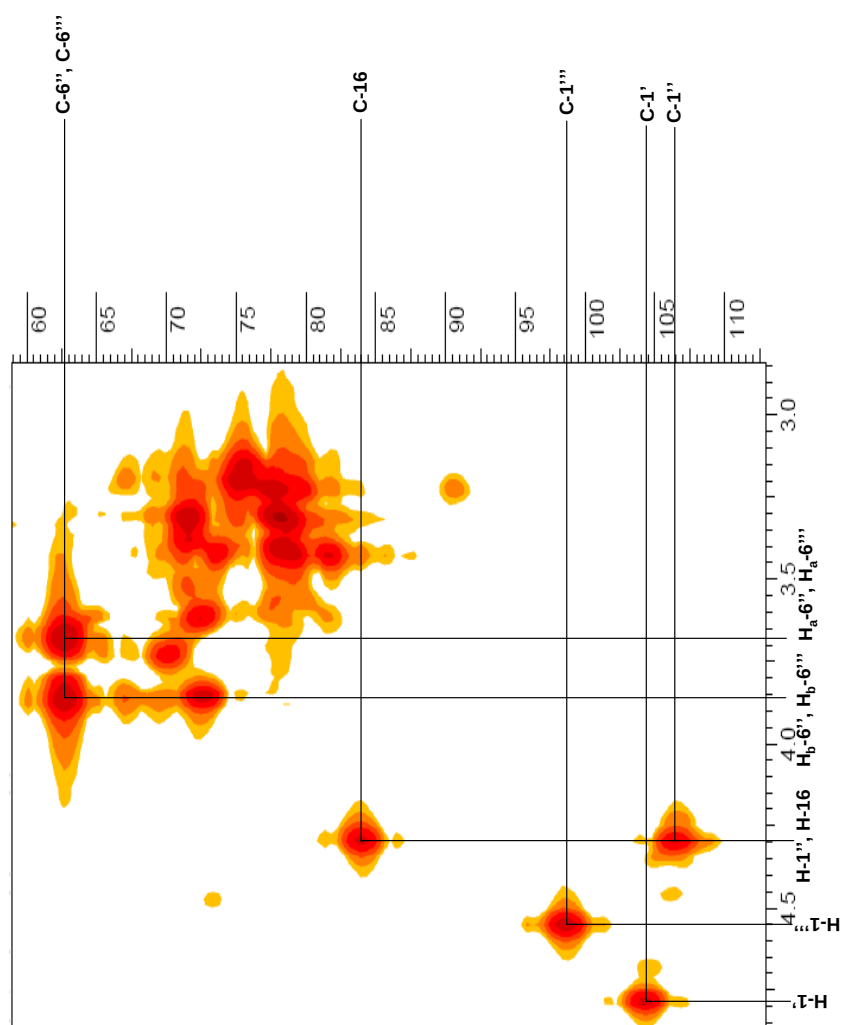
Figure 3.11. Structure of $\mathbf{AA}_6$

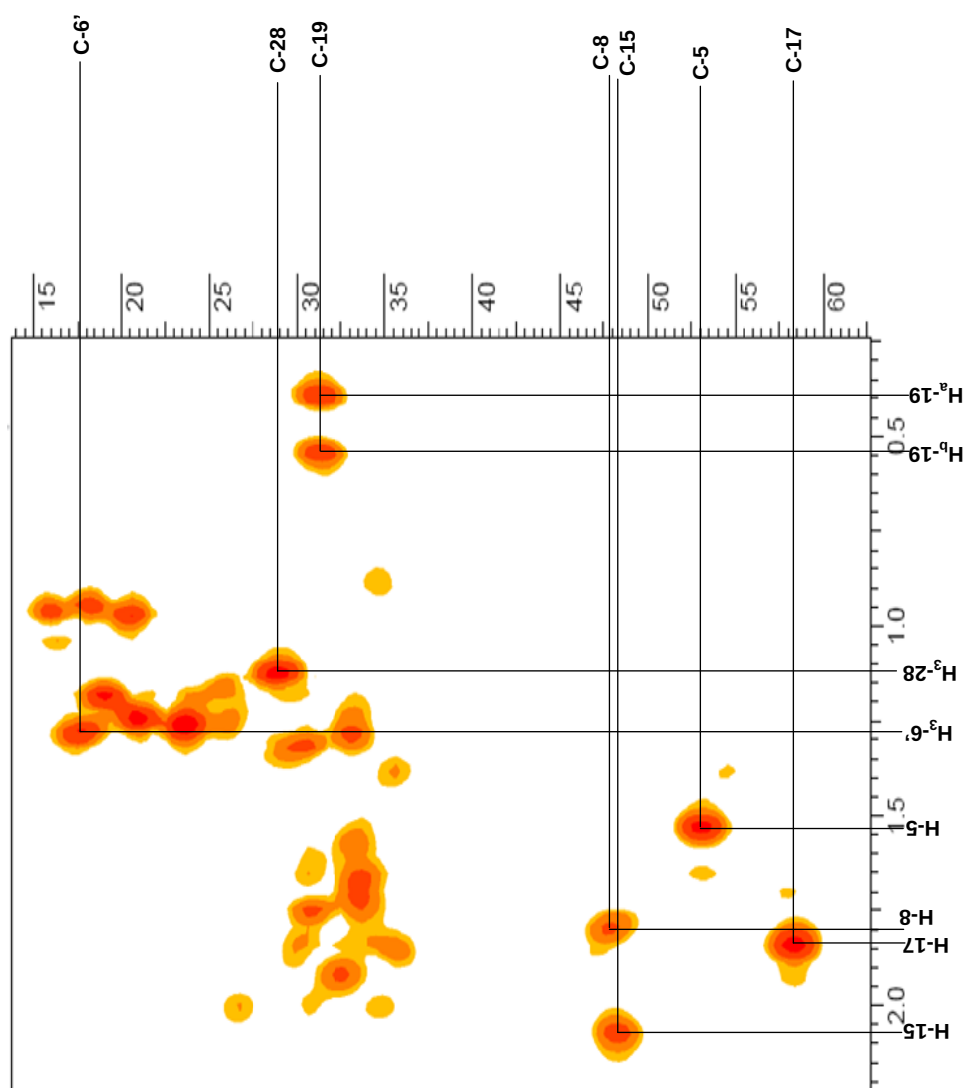


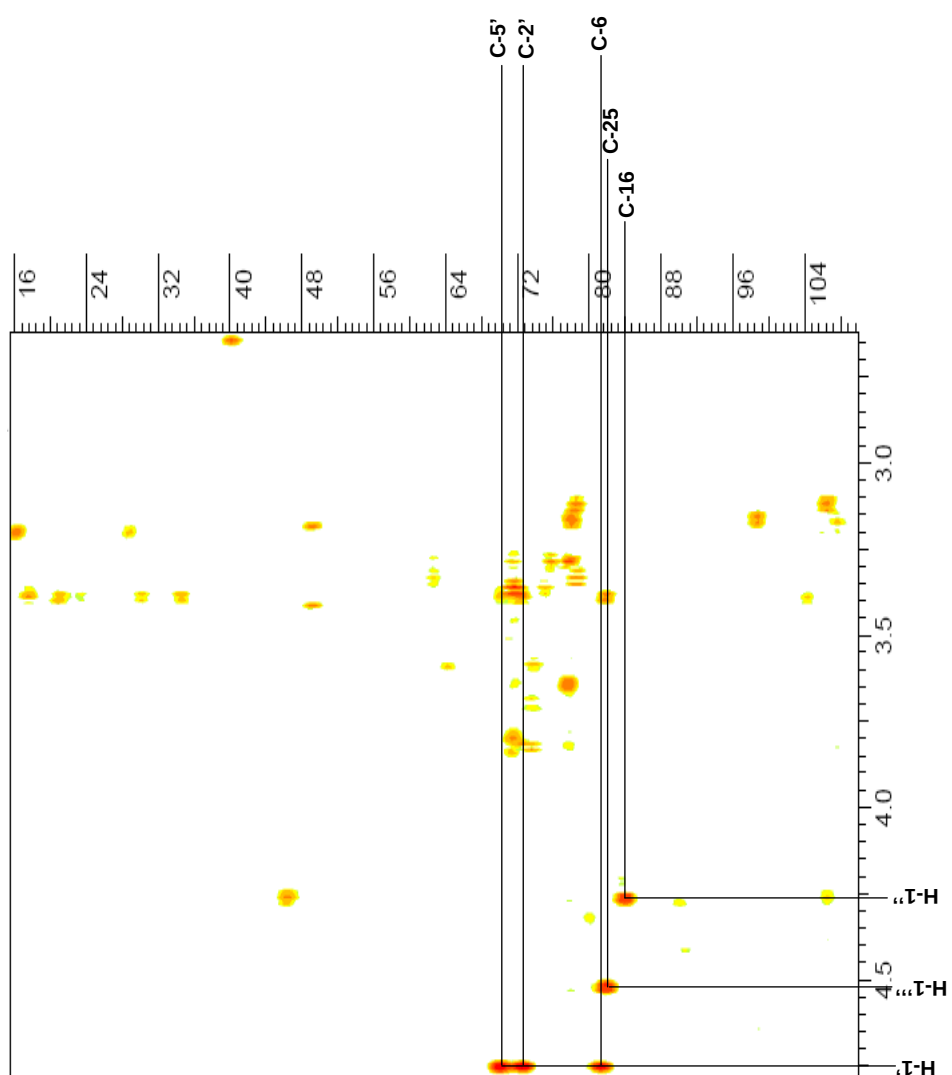
Spectrum 3.36. ^1H spectrum of AA₆ in the downfield region

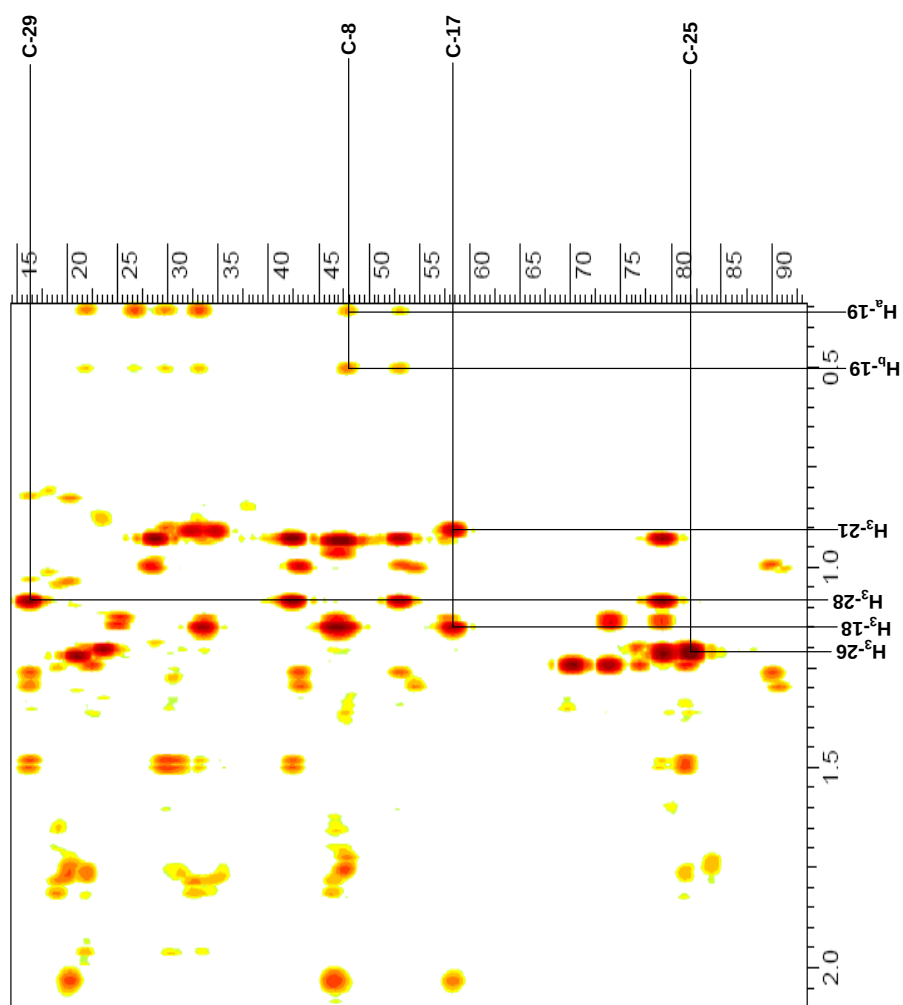


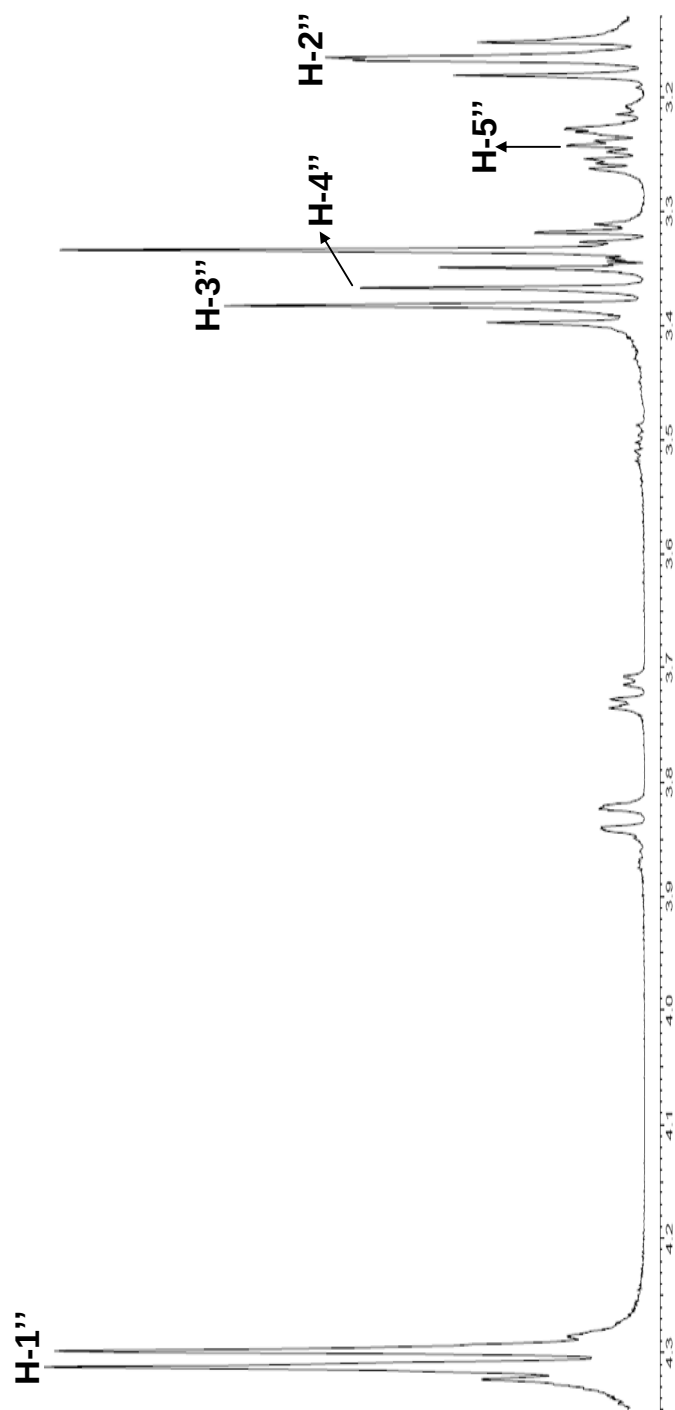
Spectrum 3.37. ^1H spectrum of **AA₆** in the upfield region

Spectrum 3.38. HSQC spectrum of AA₆

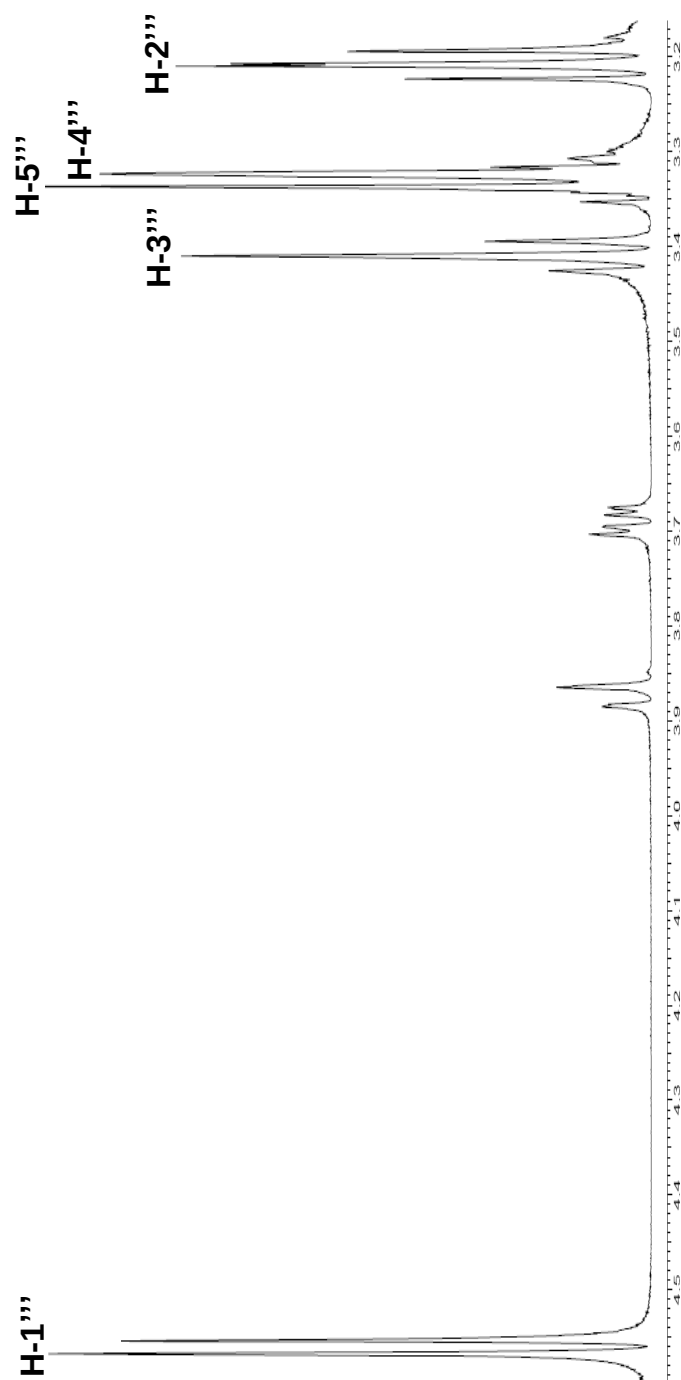
Spectrum 3.39. HSQC spectrum of AA₆

Spectrum 3.40. HMBC spectrum of AA₆

Spectrum 3.41. HMBC spectrum of AA₆



Spectrum 3.42. 1D-TOCSY spectrum of glucose residue of AA₆ (at C-16)



Spectrum 3.43. 1D-TOCSY spectrum of glucose residue of AA₆ (at C-25)

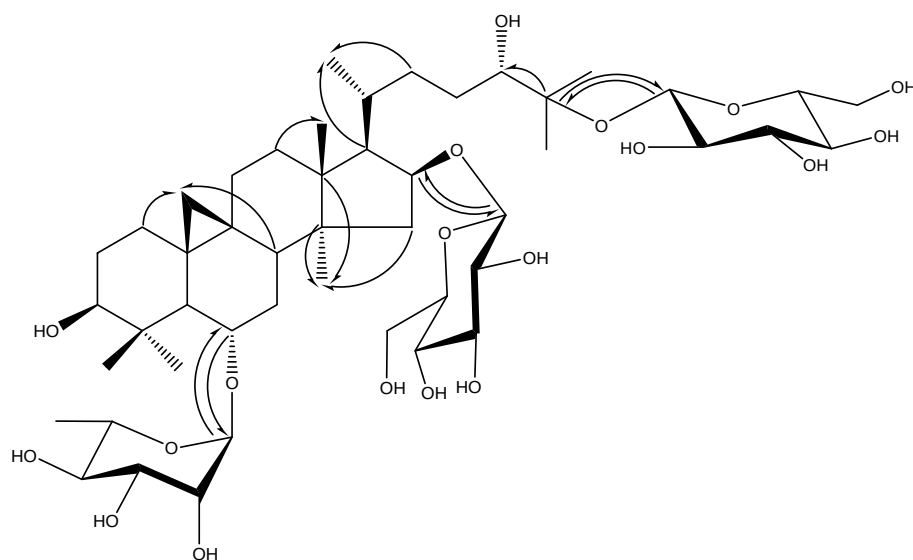


Figure 3.12. Key HMBC correlations of **AA₆**

3.1.7. Acid Hydrolysis of Compounds of *Astragalus amblolapis*

A solution (1 mg each) of compounds **1**, **3** and **5** in 1 N HCl (0.5 ml) was stirred at 80 °C for 4 h. After cooling, the solution was concentrated by blowing with N₂. The residue was dissolved in 1-(trimethylsilyl)-imidazole and pyridine (0.1 ml), and the solution was stirred at 60 °C for 5 min.

After drying the solution with a stream of N₂, the residue was partitioned between H₂O and CH₂Cl₂ (1 ml, 1:1 v/v). The CH₂Cl₂ layer was analysed by GC using an L-Chirasil-Val column (0.32 mm x 25 m). Temperatures of the injector and detector were 200 °C for both. A temperature gradient system was used for the oven, starting at 100 °C for 1 min and increasing up to 180 °C at a rate of 5°C/min. The peaks of the hydrolysate of **1** were detected at 10.96 and 12.01 (D-xylose) and 14.72 min (D-glucose). The peaks of D-glucuronic acid (15.81 min), D-glucose (14.70 min) and D-xylose (10.94 and 12.00 min) were detected in the hydrolysate of **3**. The peaks of the hydrolysate of **5** were detected at 14.71 min (D-glucose), 9.67 and 10.70 (L-rhamnose). Retention times for authentic samples after being treated in the same manner with 1-(trimethylsilyl)-imidazole in pyridine were detected at 15.81 (D-glucuronic acid), 14.71 min (D-glucose), 9.68 and 10.71 (L-rhamnose), 10.96 and 12.00 min (D-xylose).

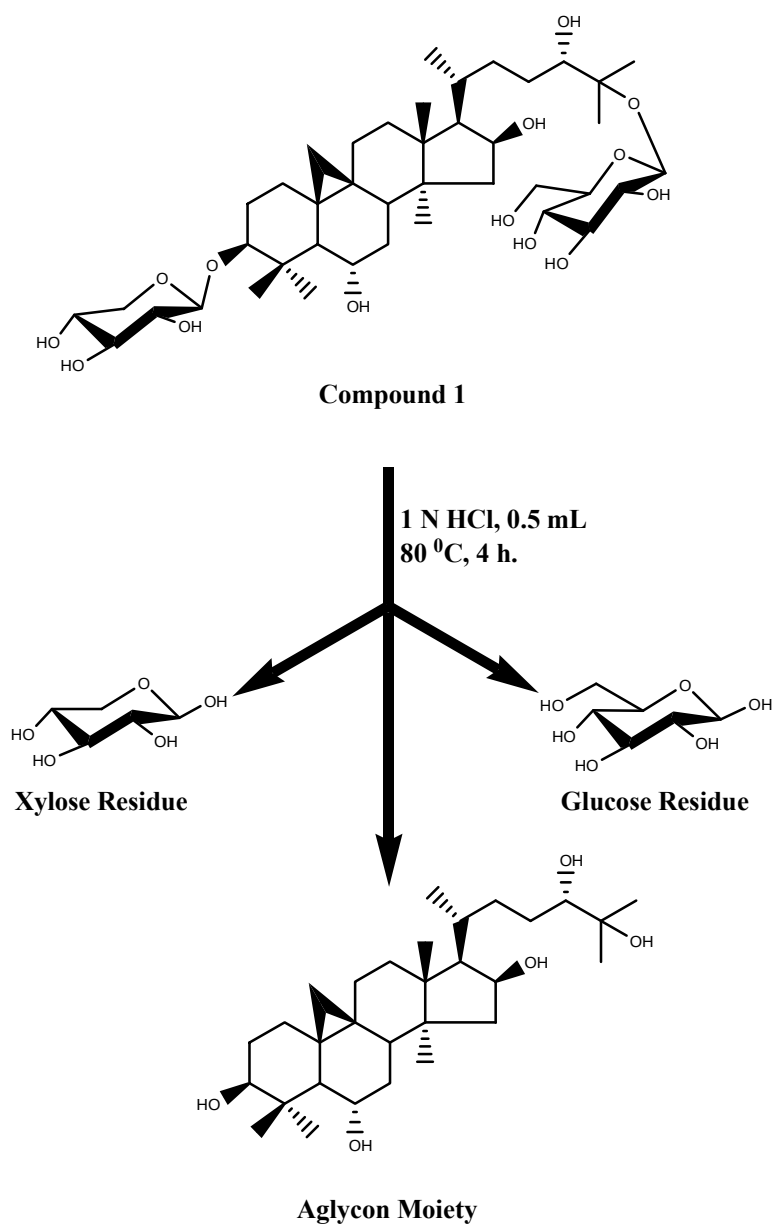


Figure 3.13. Acid Hydrolysis of AA₁

3.2. *Astragalus wiedemannianus*

3.2.1. Structural Identification of Compound **1** (AW₁)

The ¹H NMR spectrum of **1** showed characteristic signals due to cyclopropane-methylene protons as an AX system (δ 0.42 and 0.58, AX system, J_{AX} = 4.2 Hz; H₂-19) and seven tertiary methyl groups (δ 0.98, 1.03, 1.16, 1.24, 1.26, 1.29, and 1.31). No anomeric proton was observed. This observation was supported by the HMQC and HMBC spectra. Thus, compound **1** was considered to be a cycloartane-type triterpen saponin in genin form. The remaining carbon and proton resonances consistent with C₃₀H₅₀O₅ for **1**. This implied six saturated ring systems because there were no olefinic protons. Additional functionalities on the genin included three geminal methine proton on oxygen-bearing carbon atoms (H-3, H-6, and H-16). The resonances for the oxygenated carbons also indicated the presence of three oxymethine carbons (δ 78.3, 68.5, and 73.5; C-3, C-6, and C-16, respectively) and three oxygenated quaternary carbons (δ 88.6, 81.4, and 72.5; C-20, C-24, and C-25, respectively). Consequently, the structure of **1** was established as cycloastragenol (**Figure 3.14.**).

Table 3.13. ^1H and ^{13}C assignments of sapogenol moiety of **AW₁** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
1	32.2	H₂-1	1.24, 1.61, m
2	29.5	H₂-2	1.70, 2.10, m
3	78.0	H-3	3.24, dd (11.3, 4.0)
4	42.6	-----	-----
5	53.2	H-5	1.36, d (9.5)
6	68.7	H-6	3.47, ddd (9.5, 9.0, 4.5)
7	38.7	H₂-7	1.37, 1.48, m
8	47.4	H-8	1.82, dd (11.9, 4.2)
9	21.6	-----	-----
10	30.8	-----	-----
11	26.9	H₂-11	1.24, 2.06, m
12	34.0	H₂-12	1.71, (2H) m
13	45.9	-----	-----
14	46.8	-----	-----
15	45.4	H₂-15	1.41, dd (12.7, 5.2) 1.99, dd (12.7, 8.0)
16	73.5	H-16	4.69, ddd (8.0, 8.0, 5.2)
17	57.9	H-17	2.39, d (8.0)
18	29.0	H₃-18	1.31, s
19	30.8	H₂-19	0.42, d (4.2) 0.58, d (4.2)
20	88.4	H-20	-----
21	27.1	H₃-21	1.24, s
22	34.1	H₂-22	1.66, m 2.64, m
23	26.2	H₂-23	1.98, m 2.19, m
24	81.5	H-24	3.79, dd (8.2, 6.0)
25	72.5	-----	-----
26	20.4	H₃-26	1.29, s
27	24.9	H₃-27	1.16, s
28	26.2	H₃-28	1.26, s
29	14.1	H₃-29	0.98, s
30	18.8	H₃-30	1.03, s

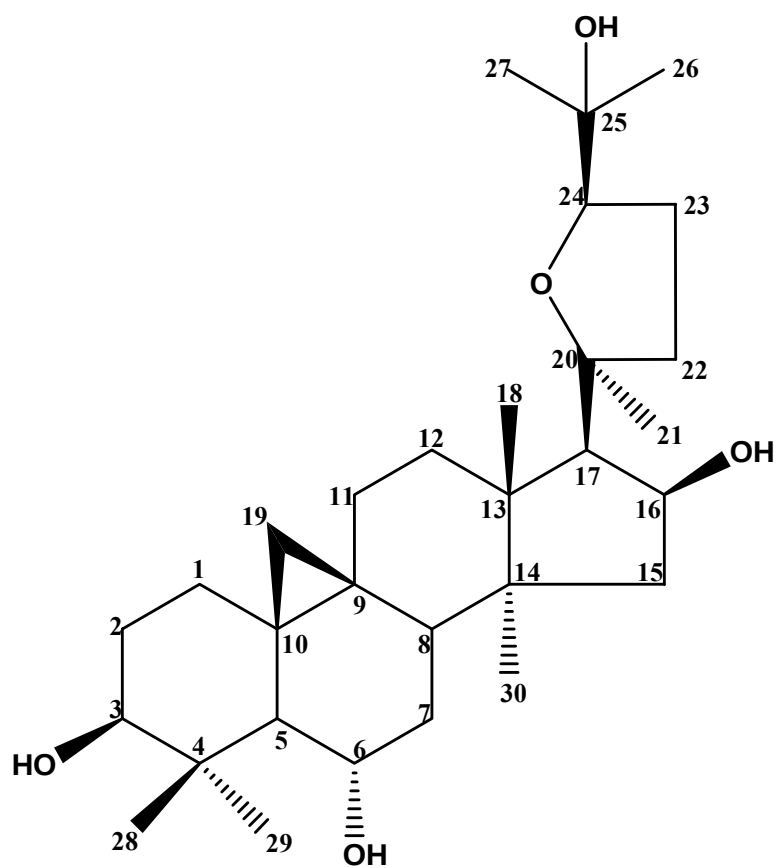
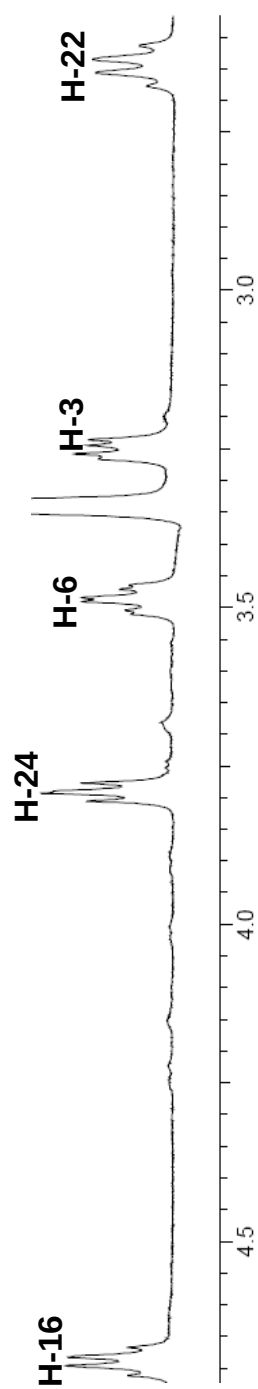
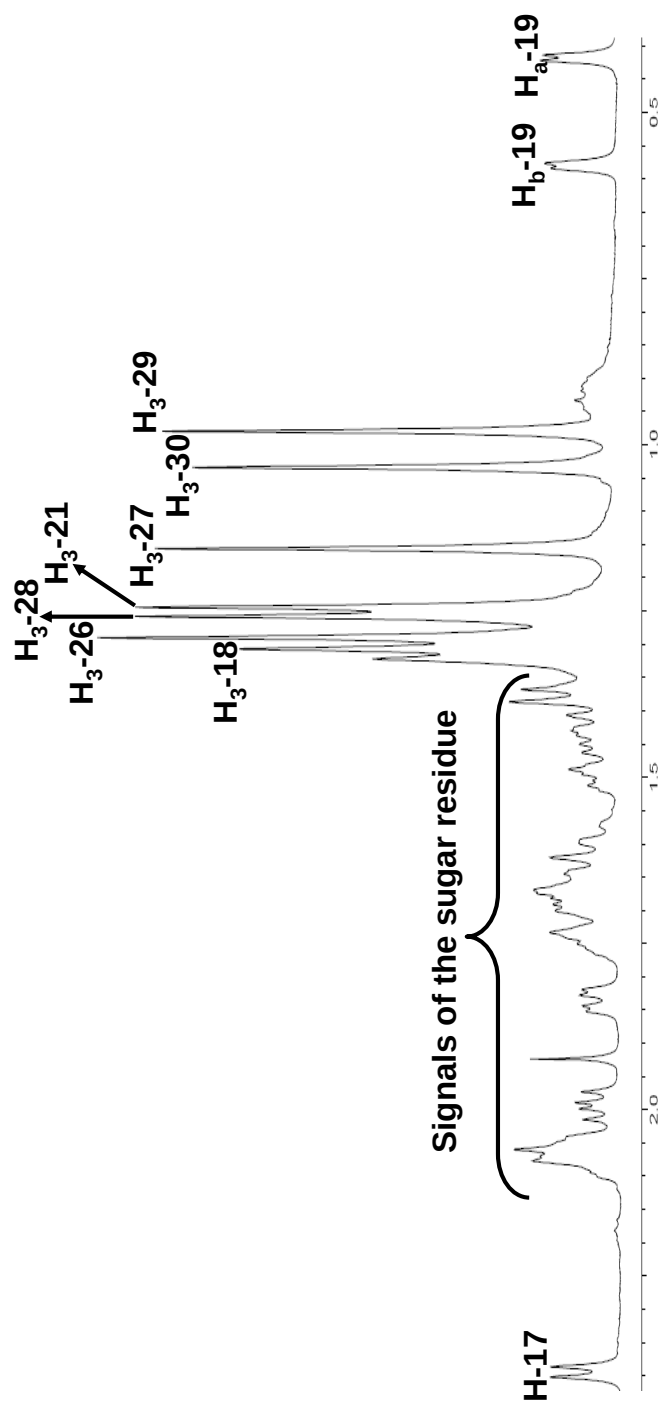


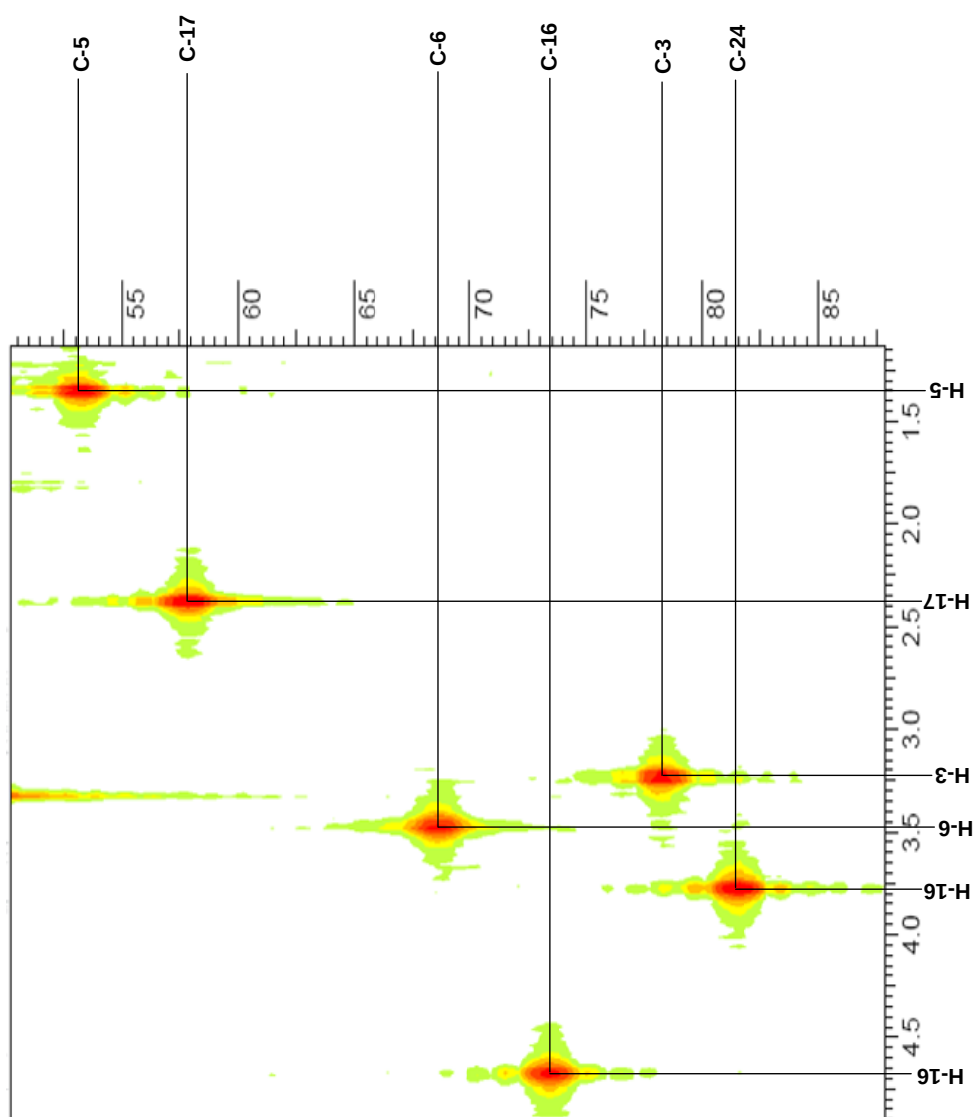
Figure 3.14. Structure of **AW₁**

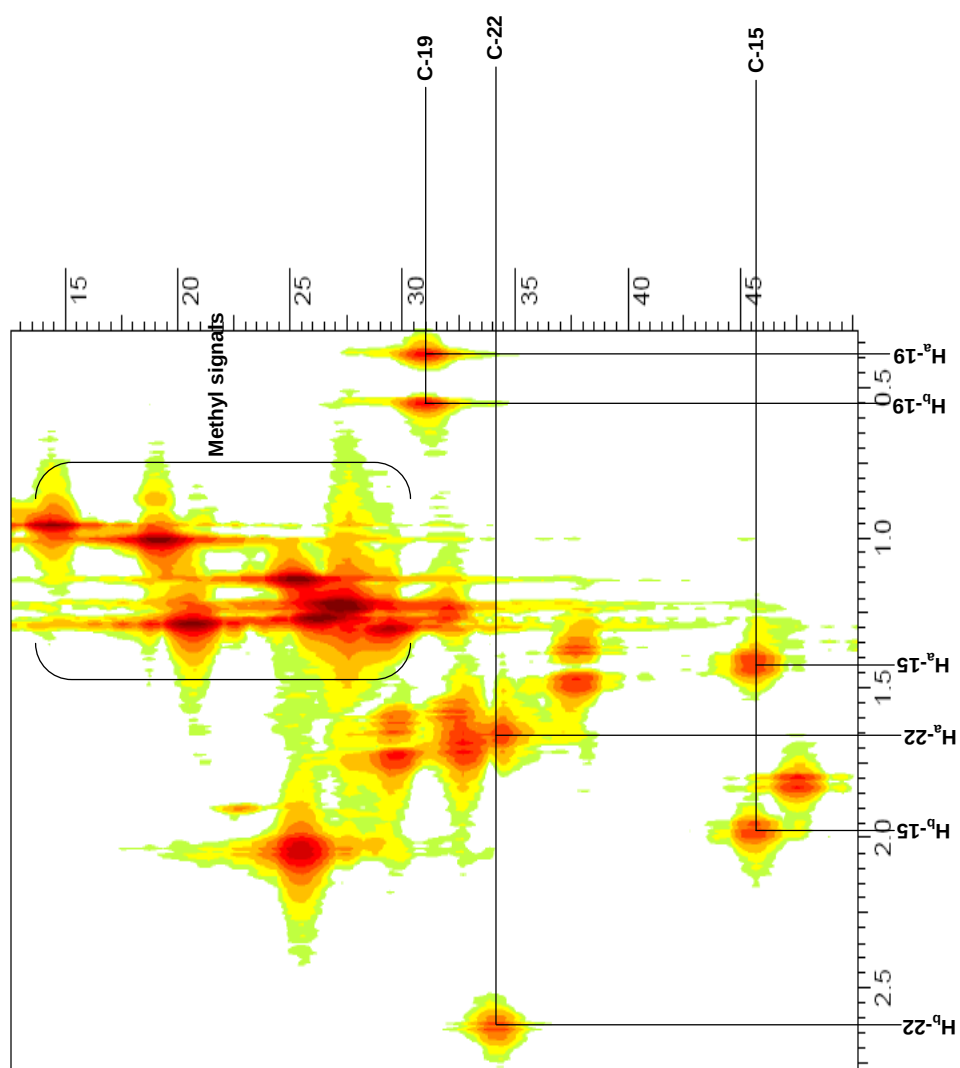


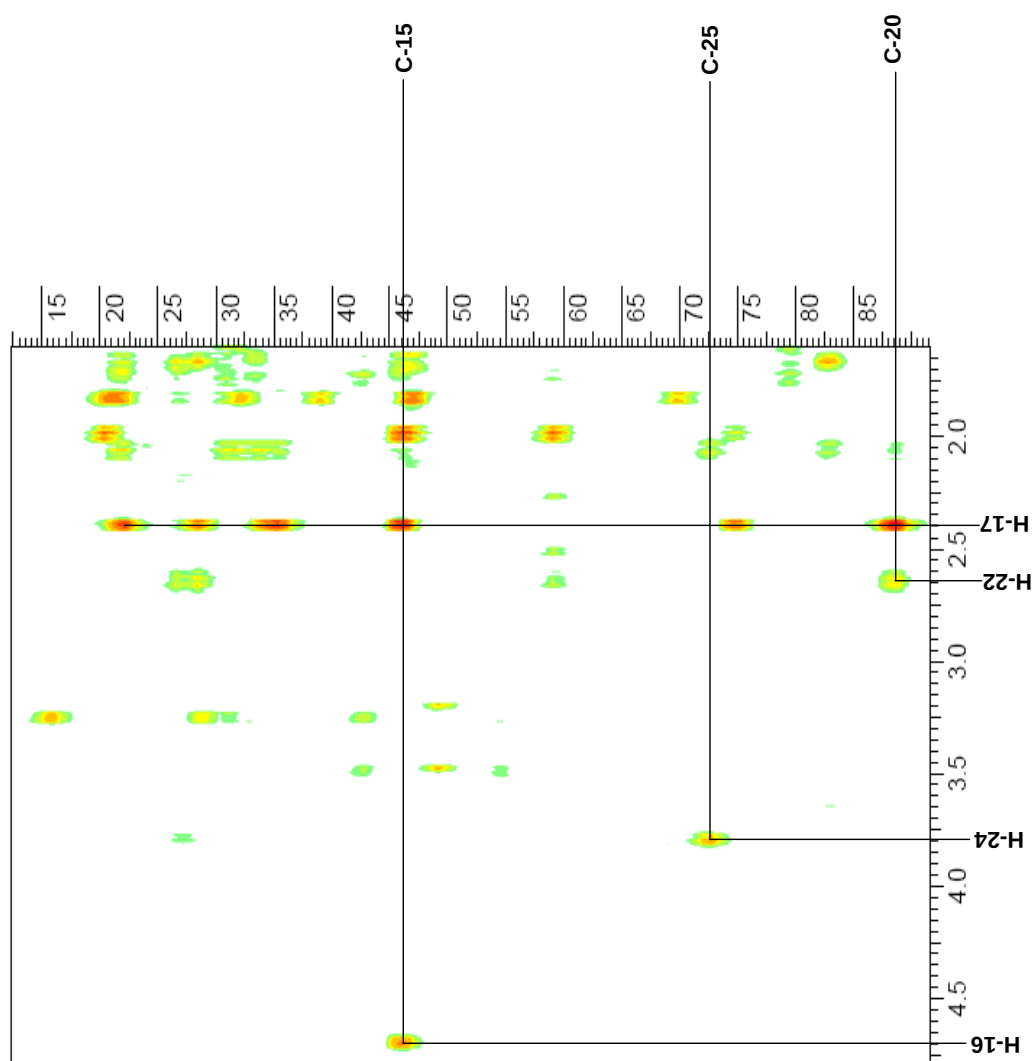
Spectrum 3.44. ^1H spectrum of **AW**₁ in the downfield region

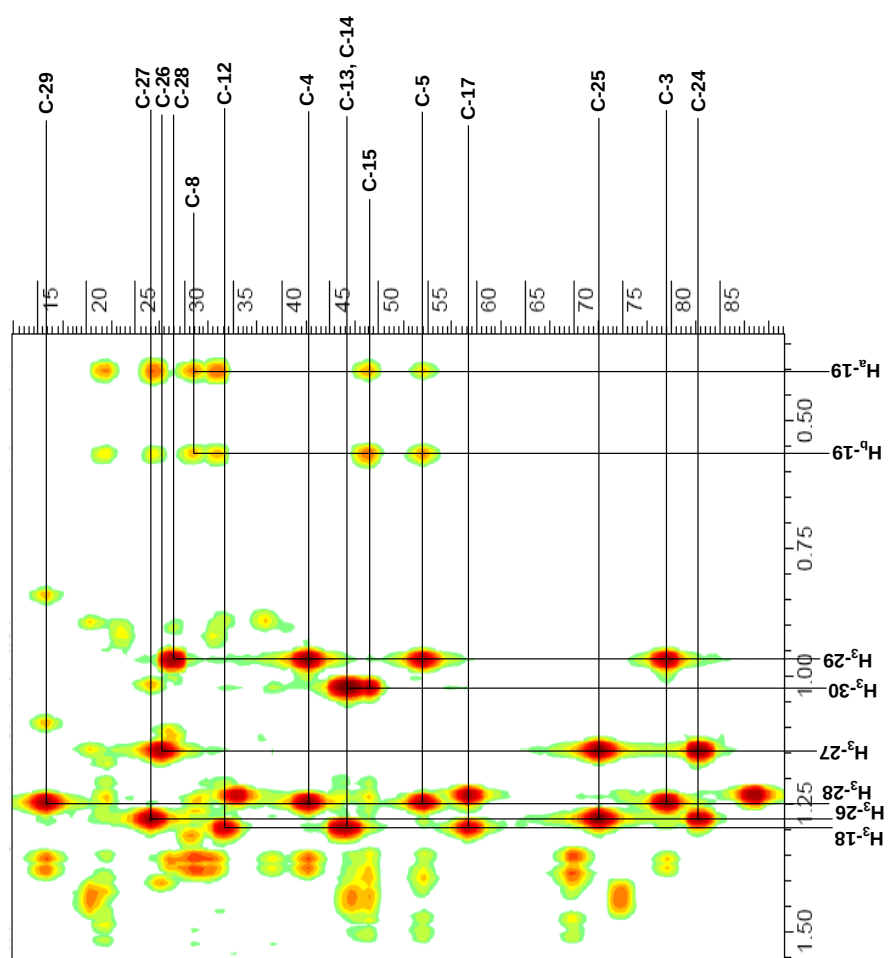


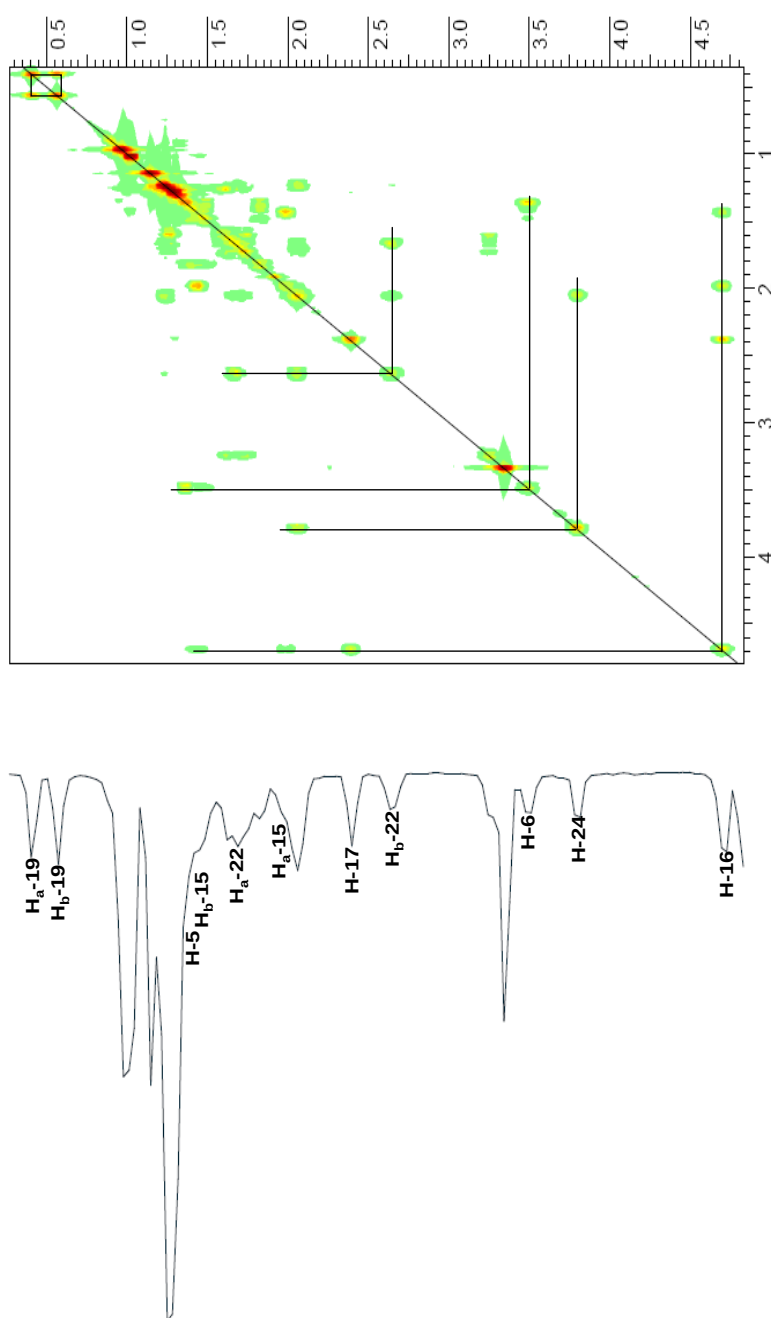
Spectrum 3.45. ^1H spectrum of AW_1 in the upfield region

Spectrum 3.46. HSQC spectrum of **AW₁**

Spectrum 3.47. HSQC spectrum of AW₁

Spectrum 3.48. HMBC spectrum of AW₁

Spectrum 3.49. HMBC spectrum of AW₁

Spectrum 3.50. COSY spectrum of AW₁

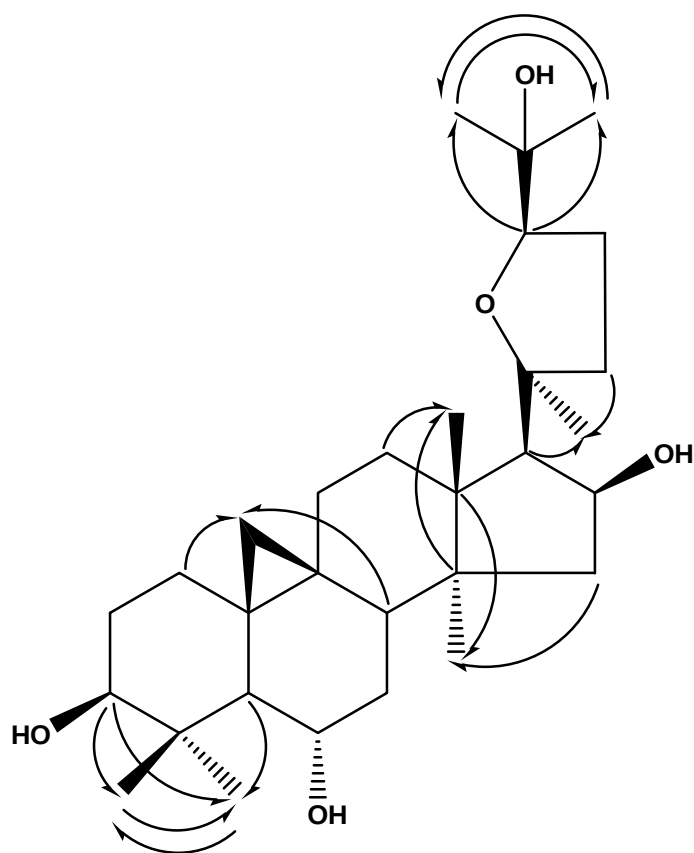


Figure 3.15. Key HMBC correlations of **AW₁**

3.2.2. Structural Identification of Compound 2 (AW₂)

The ¹H NMR spectrum of **2** showed signals characteristic of cyclopropane-ring methylene protons as an AX system (δ 0.43, 0.57, $J_{AX}=4.2$ Hz, H₂-19), seven tertiary methyl groups at δ 0.97, 1.06, 1.22, 1.31, 1.33, 1.48, and 1.54. Additionally, two anomeric protons at δ 4.44 (d, $J=7.5$ Hz, H-1') and 5.41 (d, $J=1.2$ Hz, H-1'') indicated the presence of one β - and α -linked sugar units. The chemical shifts and coupling constants of the signals assigned to the sugar units revealed the presence of one glucose and one rhamnose residue. The NMR spectroscopic data attributed to the sapogenol moiety were characteristic for a cycloartane-type triterpenoid skeleton and in good agreement with cycloascauloside B (Alaniya et al., 2008). Thus, compound **2** (cycloascauloside B) had the structure 20*R*,25-epoxy-24*S*-cycloartan-3 β ,6 α ,16 β ,24-tetraol-3-*O*-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)]- β -D-glucopyranoside (**Figure 3.16.**).

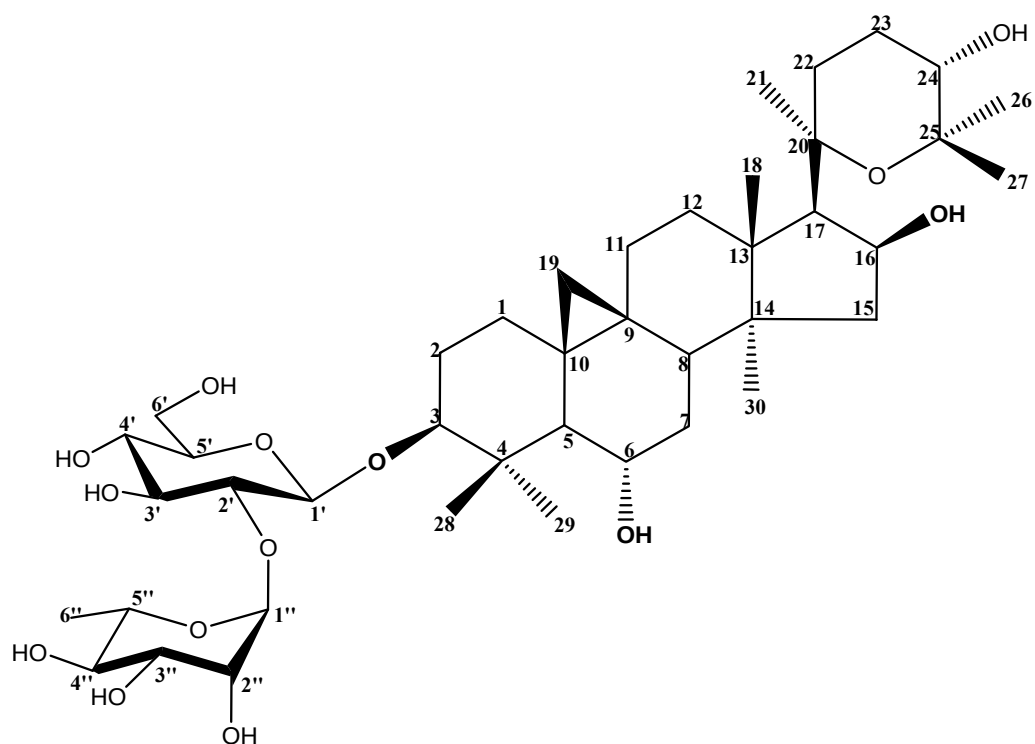
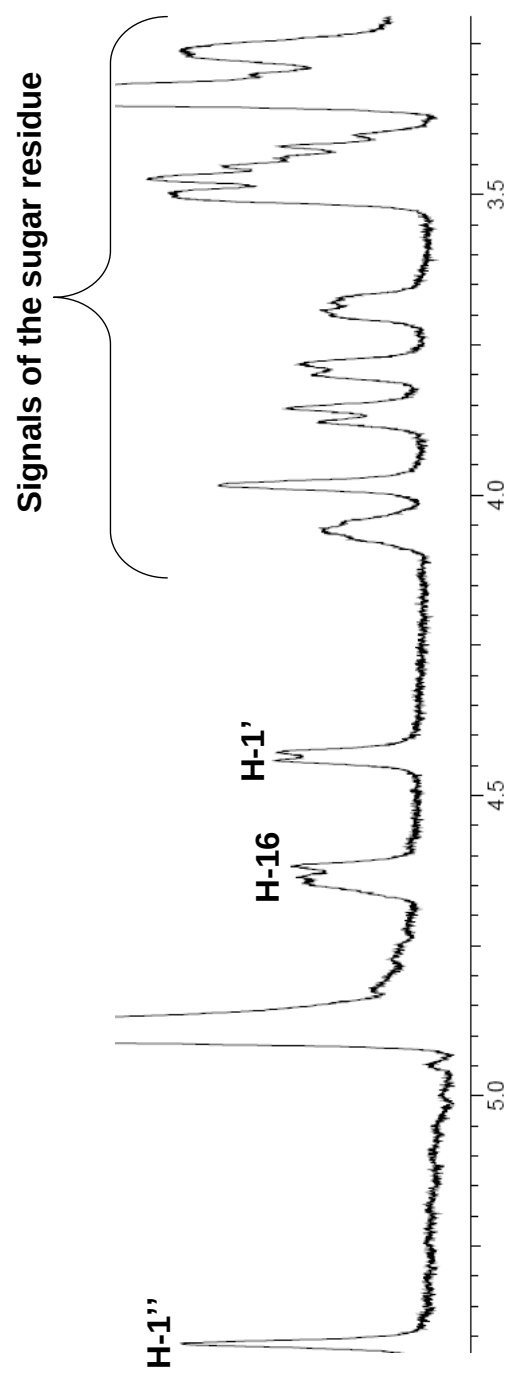
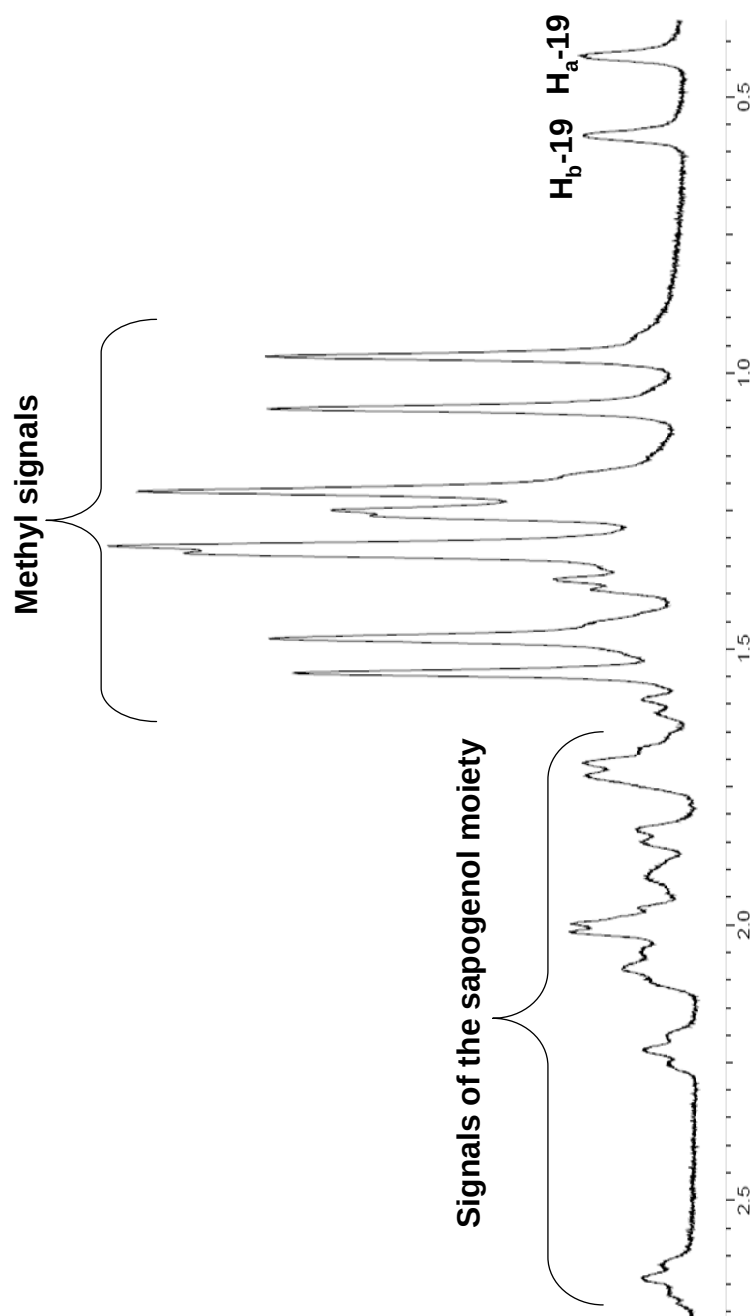


Figure 3.16. Structure of AW_2



Spectrum 3.51. ^1H spectrum of **AW₂** in the downfield region



Spectrum 3.52. ^1H spectrum of **AW₂** in the upfield region

3.2.3. Structural Identification of Compound **3** (AW₃)

The ¹H NMR spectrum of **3** showed for the aglycon moiety signals a cyclopropane methylene at δ 0.59 and 0.41 (each 1H, d, J = 4.2 Hz), seven tertiary methyl groups at δ 1.41, 1.33, 1.31, 1.26, 1.26, 1.07 and 1.03, and three methine proton signals at δ 4.69 (ddd, J = 8.0, 8.0, 5.2 Hz), 3.49 (ddd, J = 9.5, 9.0, 4.5 Hz) and 3.27 (dd, J = 11.3, 4.0 Hz) indicative of secondary alcoholic functions.

Furthermore, the ¹H NMR spectrum of **3** clearly showed three anomeric proton doublets at δ 4.44 (J = 7.5 Hz), 4.54 (J = 7.5 Hz), and 5.41 (J = 1.2 Hz) in the downfield region suggesting two β -linked and one α -linked sugar units. These correlated to carbons at δ 105.3, 98.2, and 101.5, respectively, in the HSQC spectrum. The ¹³C spectrum contained 48 resonances 30 of which attributed to the sapogenol moiety were in good agreement with cycloastragenol. Full assignment of the ¹H and ¹³C signals of the aglycon part of **3**, secured by ¹H-¹H DQF-COSY and HSQC spectra, showed marked glycosilation shifts for C-3 (δ 89.3), and C-25 (δ 79.8).

All connectivities within **3** were also confirmed by a HMBC experiment. These results suggested that compound **3** was a bidesmosidic saponin in which the sugar residues were linked to C-3 and C-25 of cycloastragenol. A combination of DQF-COSY and 1D-TOCSY experiments allowed unambiguous assignment of all sugar signals: one

α -L-rhamnopyranosyl and two β -D-glucopyranosyl units. The HSQC experiments correlated each proton sugar signal to the corresponding carbon resonances and showed the absence of glycosylation shifts for the carbon resonances of rhamnopyranosyl and one of the glucopyranosyl units; a glycosylation shift (ca. 4.9 ppm) was observed for C-2' (δ 78.9) of glucopyranosyl unit. All connectivities, including the sites of attachment of sugar moieties on the aglycon of **3** as well as the position of the interglycosidic linkage, were determined by a HMBC experiment. In the HMBC spectrum, the anomeric proton signal at δ 4.54 (H-1'''), assigned to the β -D-glucopyranosyl, showed long-range correlation with the carbon at δ 79.8 (C-25). The second anomeric proton signal at δ 4.44 (H-1'), assigned to the 2-substituted β -D-glucopyranosyl, showed long-range correlation with the carbon resonance at δ 89.3 (C-3). Thus, one of glucose units must be linked to C-25 and the other one (bridging glucose residue) should be attached to C-3. The third anomeric proton signal at δ 5.41 (H-1''), assigned to the terminal α -L-rhamnopyranosyl unit, showed long-range correlation with the carbon resonance at δ 78.9 (C-2' of the bridging glucose unit), revealing the presence of a disaccharide unit at C-3. Thus, the structure of compound **3** was elucidated as 3-*O*-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranosyl]-25-*O*- β -D-glucopyranosyl-20(*R*),24(*S*)-epoxy-3 β ,6 α ,16 β ,25-tetrahydroxycycloartane (**Figure 3.17.**), a new cycloartane triterpenoid.

Table 3.14. ^1H and ^{13}C assignments of sapogenol moiety of **AW₃** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
1	33.3	H₂-1	1.61, 1.24, m
2	30.4	H₂-2	2.10, 1.70, m
3	89.3	H-3	3.27, dd (11.3, 4.0)
4	42.8	-----	-----
5	54.7	H-5	1.40, d (9.5)
6	69.3	H-6	3.49, ddd (9.5, 9.0, 4.5)
7	38.7	H₂-7	1.51, 1.41, m
8	48.3	H-8	1.84, dd (11.9, 4.2)
9	21.4	-----	-----
10	30.4	-----	-----
11	26.9	H₂-11	2.06, 1.24, m
12	34.0	H₂-12	1.71, (2H) m
13	45.9	-----	-----
14	46.8	-----	-----
15	46.2	H₂-15	1.98, dd (12.7, 8.0) 1.45, dd (12.7, 5.2)
16	74.5	H-16	4.69, ddd (8.0, 8.0, 5.2)
17	58.6	H-17	2.39, d (8.0)
18	22.0	H₃-18	1.31, s
19	31.9	H₂-19	0.59, d (4.2) 0.41, d (4.2)
20	88.3	H-20	-----
21	27.8	H₃-21	1.26, s
22	35.4	H₂-22	2.58, m 1.68, m
23	26.2	H₂-23	2.18, m 2.02, m
24	82.9	H-24	3.86, dd (8.2, 6.0)
25	79.8	-----	-----
26	23.1	H₃-26	1.26, s
27	25.3	H₃-27	1.41, s
28	28.6	H₃-28	1.33, s
29	16.9	H₃-29	1.07, s
30	20.1	H₃-30	1.03, s

Table 3.15. ^1H and ^{13}C assignments of sugar residues of **AW₃** (in CD_3OD)

Position	δC (ppm)	Position	δH (ppm)
β-D-glucose-1'	105.3	H-1'	4.44 d (7.5)
2'	78.9	H-2'	3.45, dd (9.0, 7.5)
3'	79.5	H-3'	3.49, t (9.0)
4'	71.8	H-4'	3.32, t (9.0)
5'	77.5	H-5'	3.27, ddd (9.0, 4.5, 2.5)
6'	62.8	H₂-6'	3.86, dd (11.0, 2.5) 3.68, dd (11.0, 4.5)
α-L-rhamnose-1''	101.5	H-1''	5.41, d (1.2)
2''	72.0	H-2''	3.98, dd (1.2, 3.2)
3''	72.2	H-3''	3.79, dd (3.2, 9.3)
4''	73.9	H-4''	3.42, t (9.3)
5''	70.0	H-5''	4.06, m
6''	18.0	H₃-6''	1.26, d (6.5)
β-D-glucose-1'''	98.2	H-1'''	4.54 d (7.5)
2'''	75.0	H-2'''	3.16 dd (9.0, 7.5)
3'''	78.1	H-3'''	3.37 t (9.0)
4'''	71.2	H-4'''	3.35 t (9.0)
5'''	77.3	H-5'''	3.27 ddd (9.0, 4.5, 2.5)
6'''	62.8	H₂-6'''	3.86 dd (11.0, 2.5) 3.68 dd (11.0, 4.5)

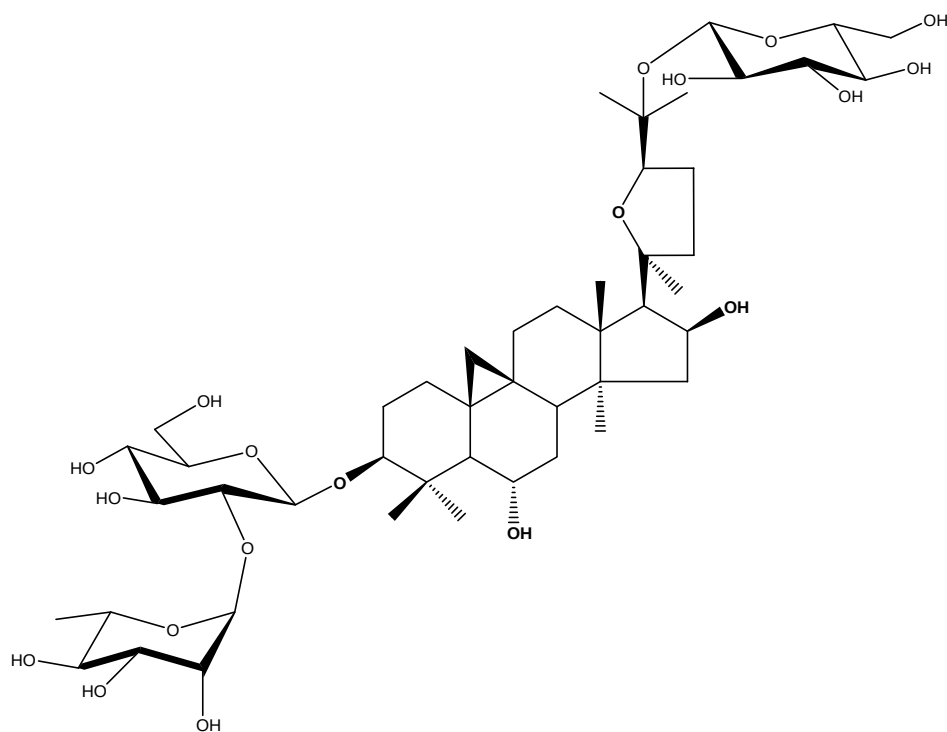
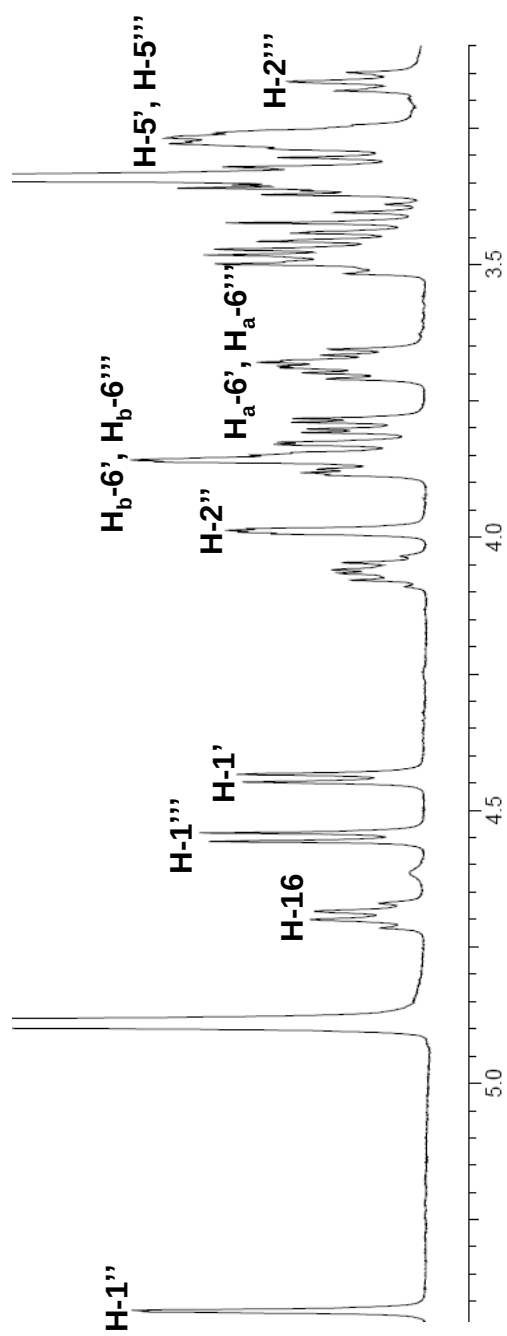
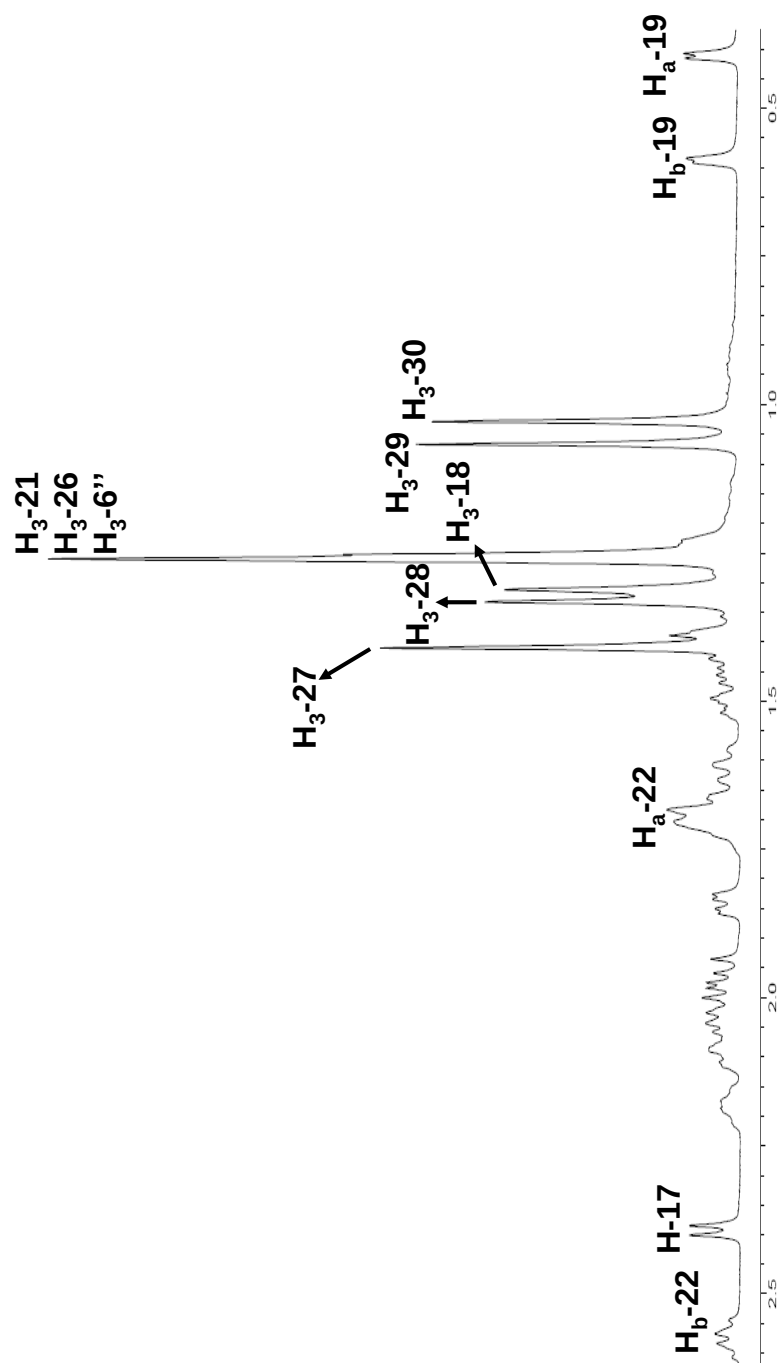


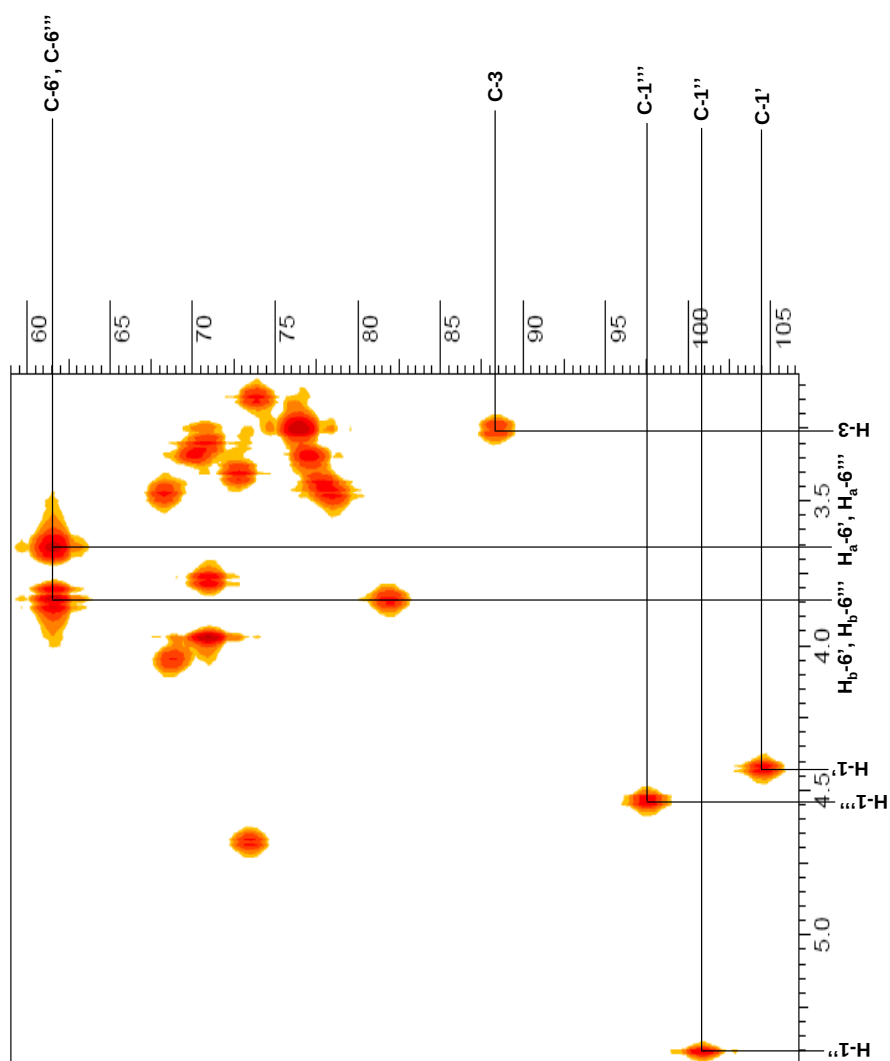
Figure 3.17. Structure of AW₃

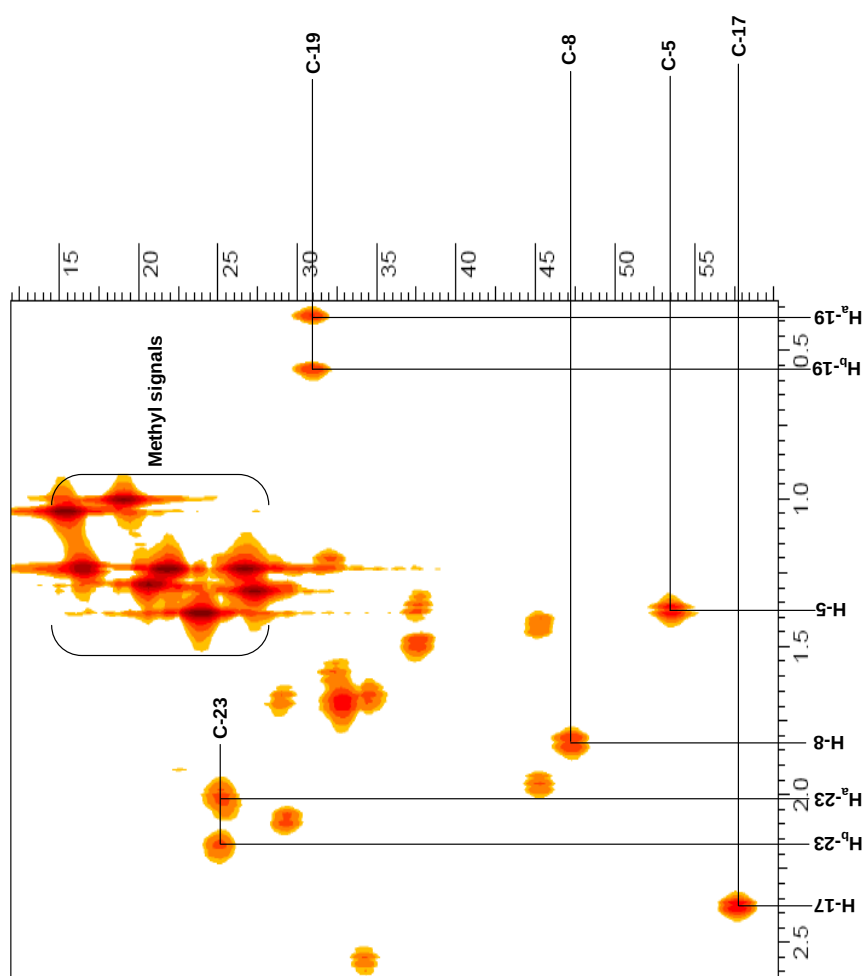


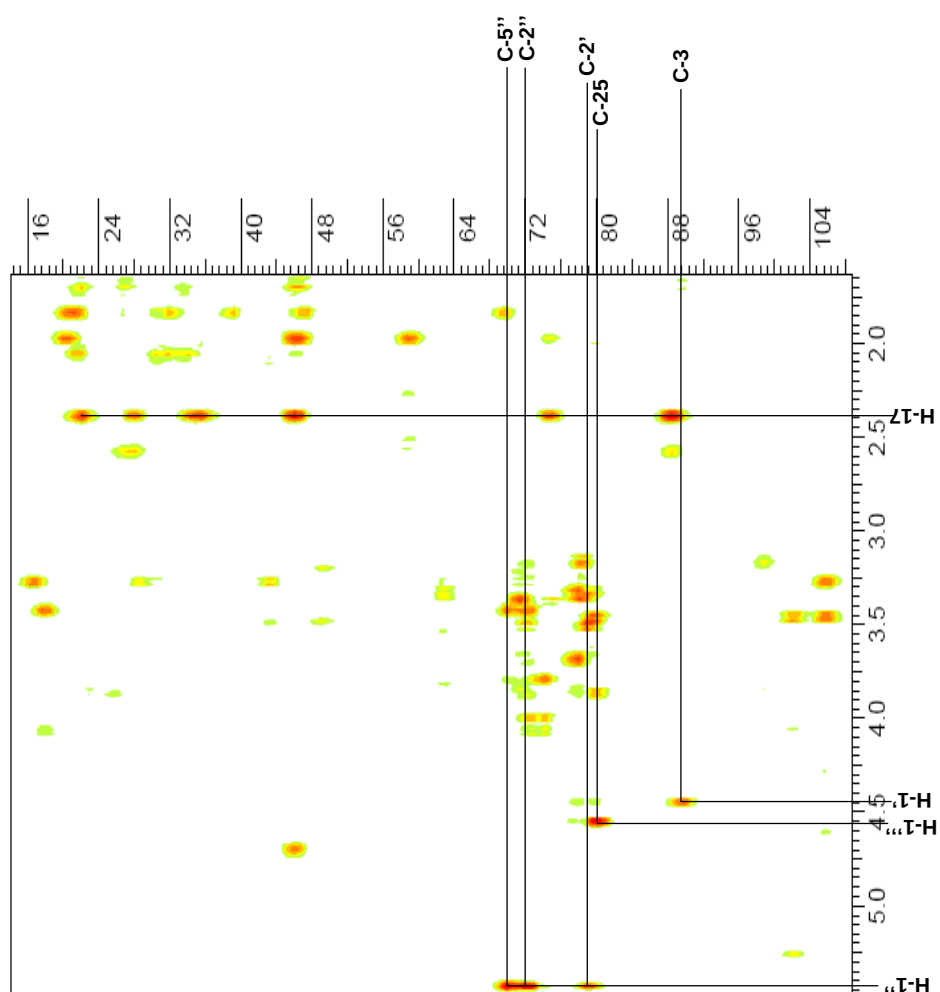
Spectrum 3.53. ^1H spectrum of **AW**₃ in the downfield region

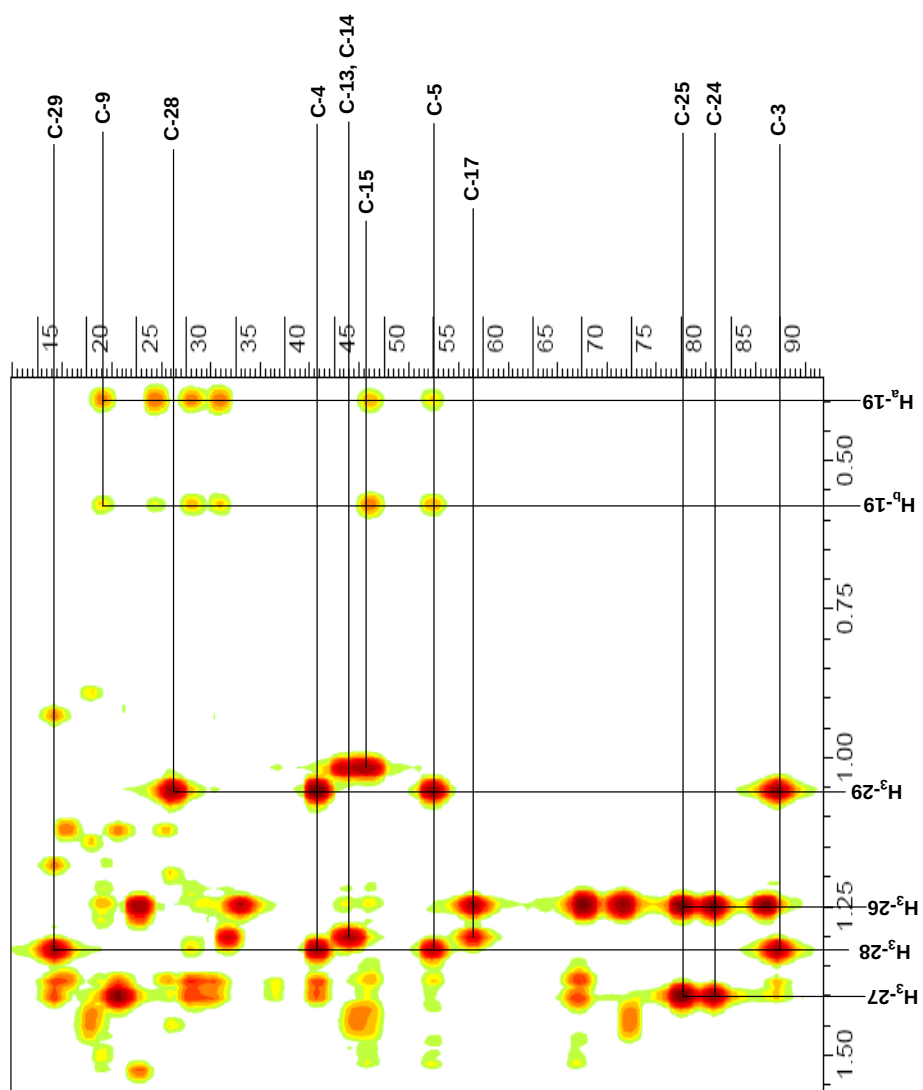


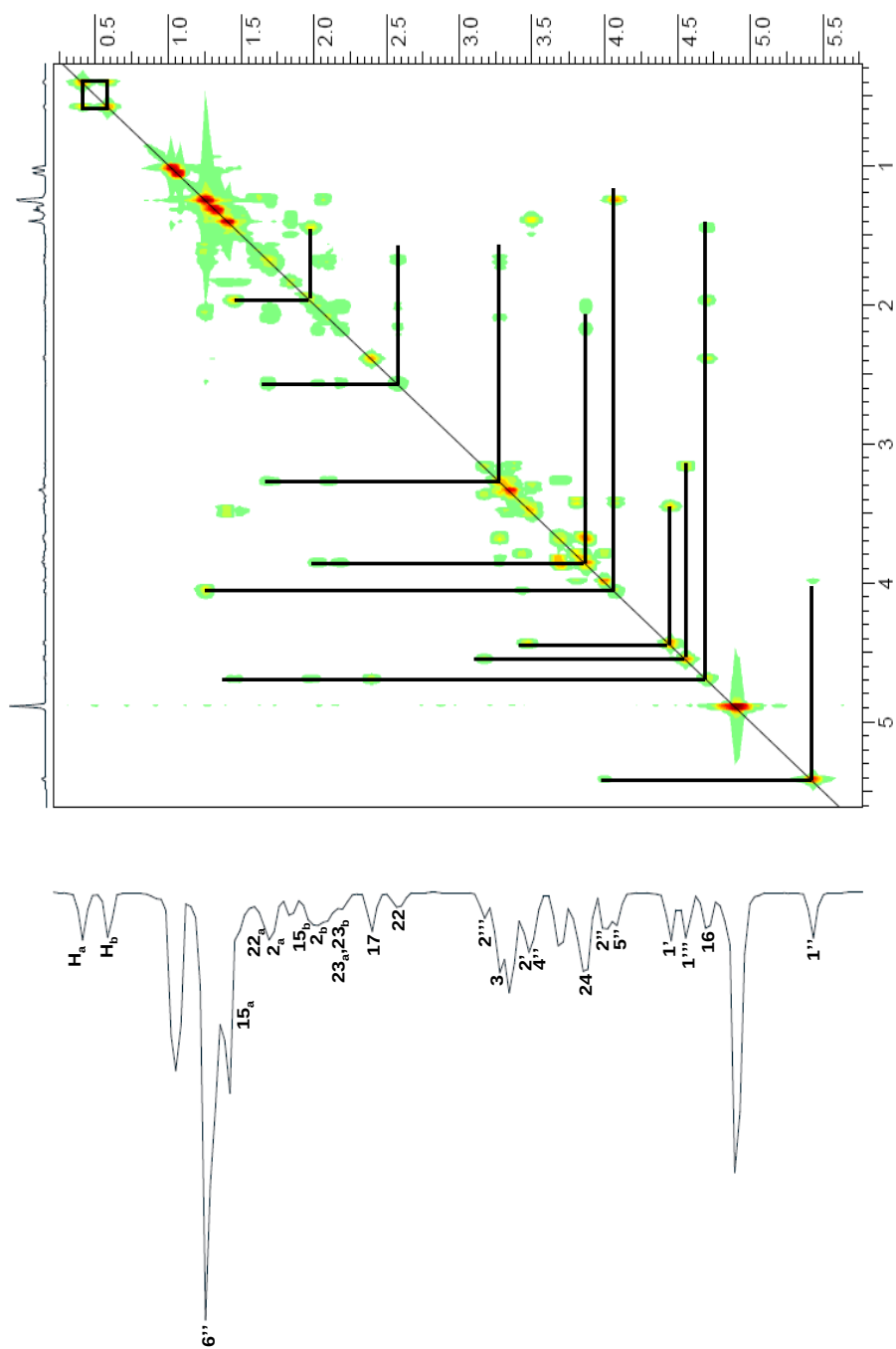
Spectrum 3.54. ^1H spectrum of AW_3 in the upfield region

Spectrum 3.55. HSQC spectrum of AW₃

Spectrum 3.56. HSQC spectrum of AW₃

Spectrum 3.57. HMBC spectrum of AW₃

Spectrum 3.58. HMBC spectrum of AW₃

Spectrum 3.59. COSY spectrum of AW_3 

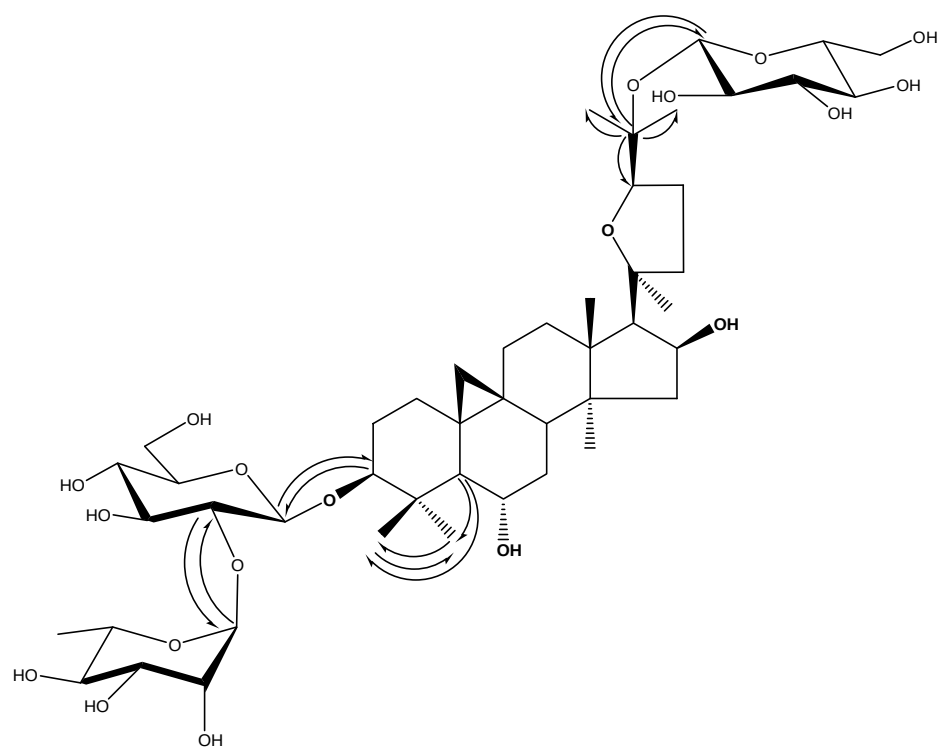


Figure 3.18. Key HMBC correlations of AW₃

3.2.4. Structural Identification of Compound 4 (AW₄)

The ¹H NMR spectrum of **4** showed for the aglycon moiety signals due to a cyclopropane methylene at δ 0.63 and 0.29 (each 1H, d, J = 4.2 Hz), seven tertiary methyl groups at δ 1.32, 1.29, 1.28, 1.24, 1.15, 1.05 and 1.04, and three methine proton signals at δ 4.69 (ddd, J = 8.0, 8.0, 5.2 Hz), 3.57 (ddd, J = 9.5, 9.0, 4.5 Hz) and 3.23 (dd, J = 11.3, 4.0 Hz) indicative of secondary alcoholic functions.

Furthermore, the ¹H NMR spectrum of **4** clearly showed two anomeric proton doublets at δ 4.32 (J = 7.5 Hz), and 4.35 (J = 7.5 Hz) in the downfield region suggesting two β -linked units. These correlated to carbons at δ 105.1, and 107.8, respectively, in the HSQC spectrum. The ¹³C spectrum contained 41 resonances 30 of which attributed to the sapogenol moiety were in good agreement with cycloastragenol. Full assignment of the ¹H and ¹³C signals of the aglycon part of **4**, secured by ¹H-¹H DQF-COSY and HSQC spectra, showed marked glycosilation shifts for C-3 (δ 90.7), and C-6 (δ 79.9).

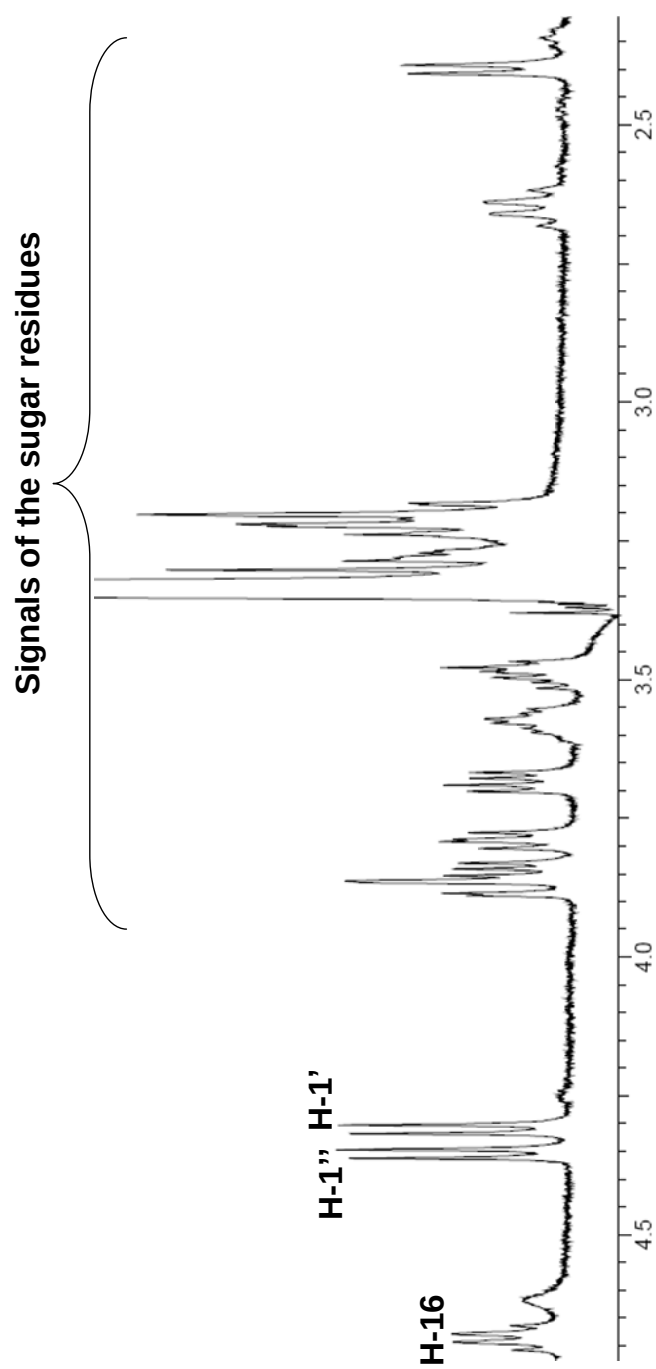
All connectivities within **4** were also confirmed by a HMBC experiment. These results suggested that compound **4** was a bidesmosidic saponin in which the sugar residues were linked to C-3 and C-6 of cycloastragenol. A combination of DQF-COSY and 1D-TOCSY experiments allowed unambiguous assignment of all sugar signals: one β -D-xylopyranosyl and one β -D-glucopyranosyl units.

Thus, the structure of compound **4** was elucidated as 3-*O*- β -D-xylopyranosyl-25-*O*- β -D-glucopyranosyl-20(*R*),24(*S*)-epoxy-3 β ,6 α ,16 β ,25-tetrahydroxycycloartane by comparing its physical properties and spectral data with those reported previously for astragaloside IV (**Figure 3.19.**) (Kitagawa et al., 1983).

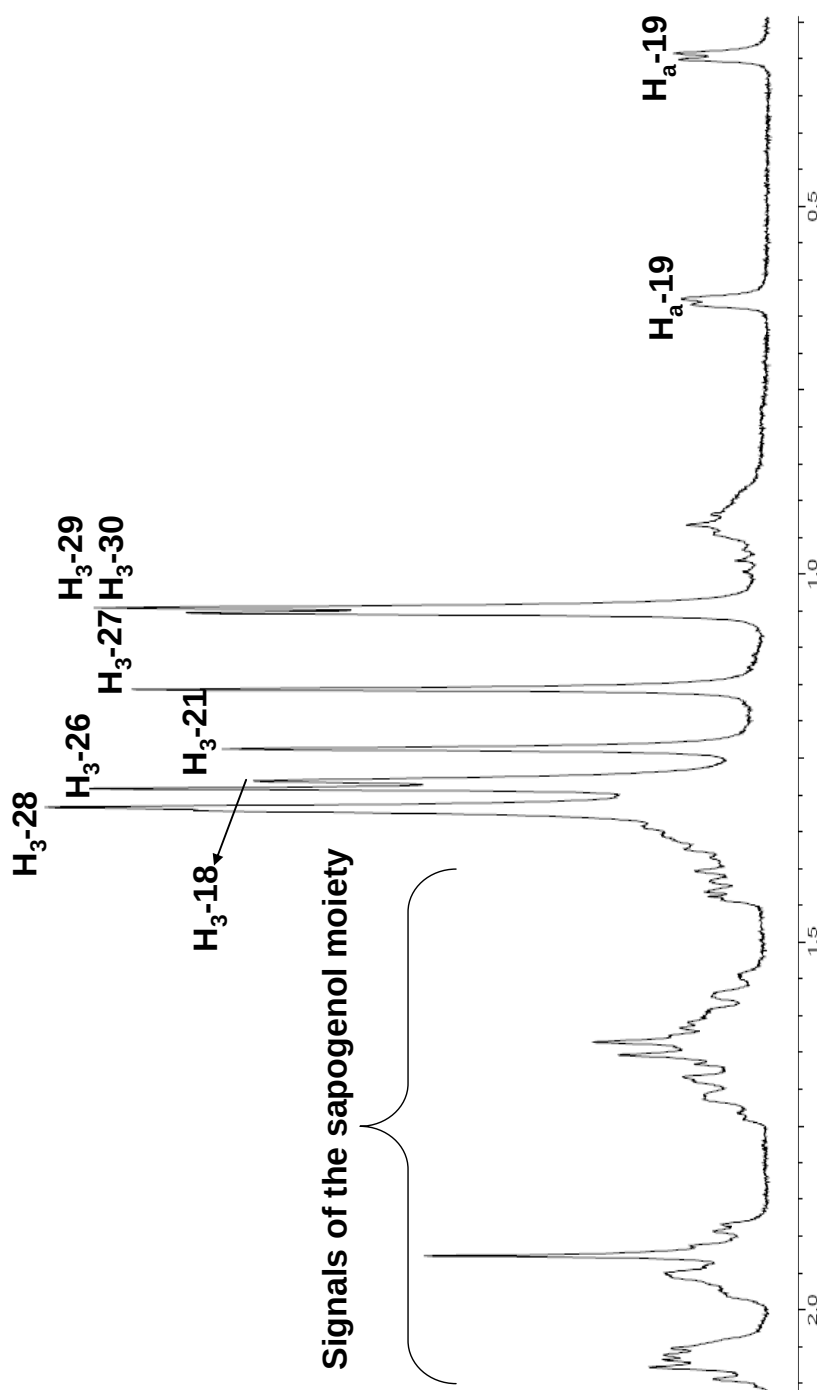
Table 3.16. ^1H and ^{13}C assignments of sapogenol moiety and sugar residues of **AW₄** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
3	90.7	H-3	3.23, dd (11.3, 4.0)
5	53.7	-----	-----
6	79.9	H-6	3.57, ddd (9.5, 9.0, 4.5)
16	-----	H-16	4.69, ddd (8.0, 8.0, 5.2)
17	59.2	-----	-----
18	26.4	H₃-18	1.28, s
20	88.9	-----	-----
21	27.0	H₃-21	1.24, s
24	82.5	-----	-----
25	72.7	-----	-----
26	27.0	H₃-26	1.29, s
27	25.1	H₃-27	1.15, s
28	29.5	H₃-28	1.32, s
29	15.1	H₃-29	1.04, s
30	18.8	H₃-30	1.05, s
β-D-xylose-1'	105.1	H-1'	4.32, d (7.5)
β-D-glucose-1''	107.8	H-1''	4.35, d (7.5)

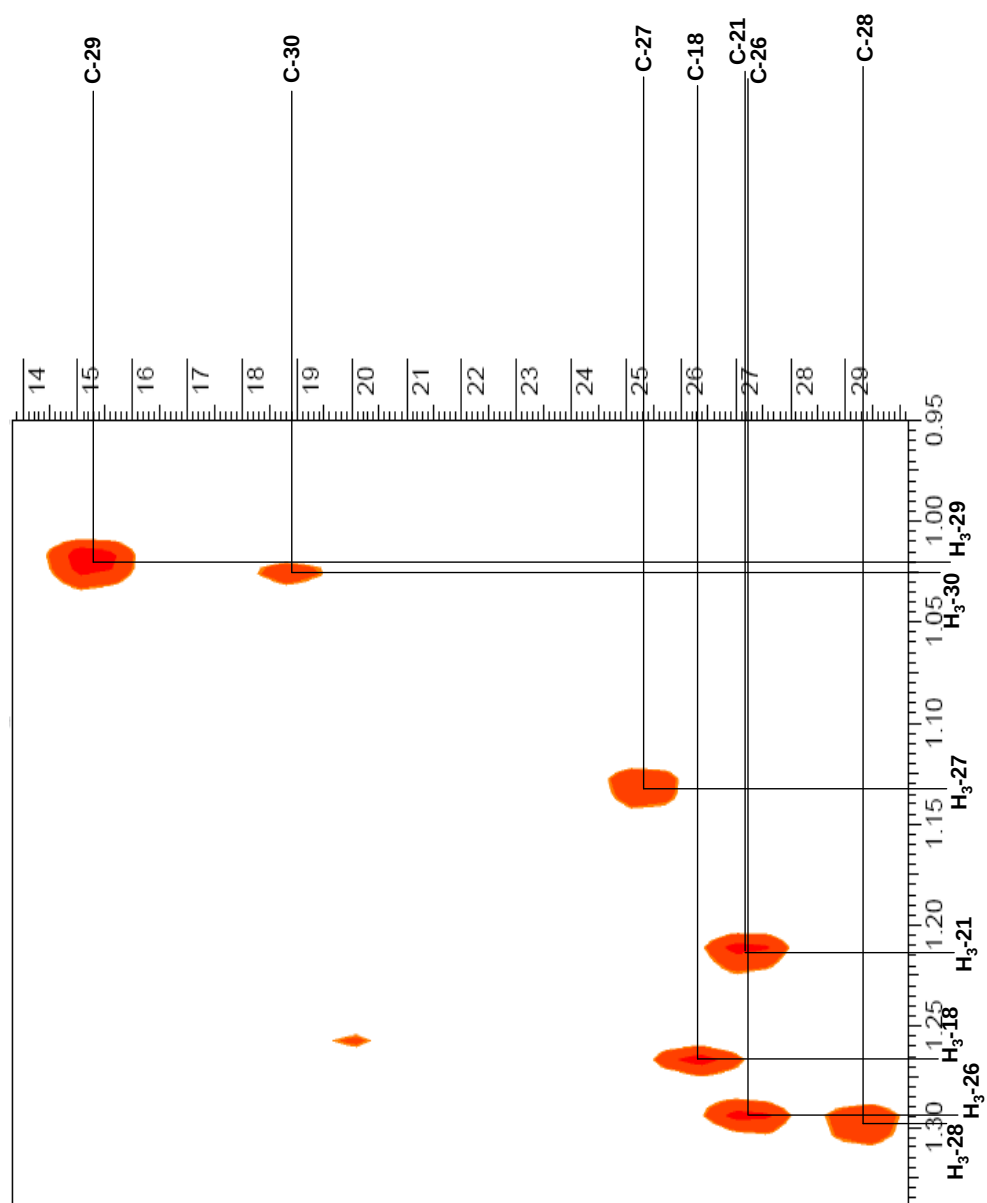
Figure 3.19. Structure of AW_4

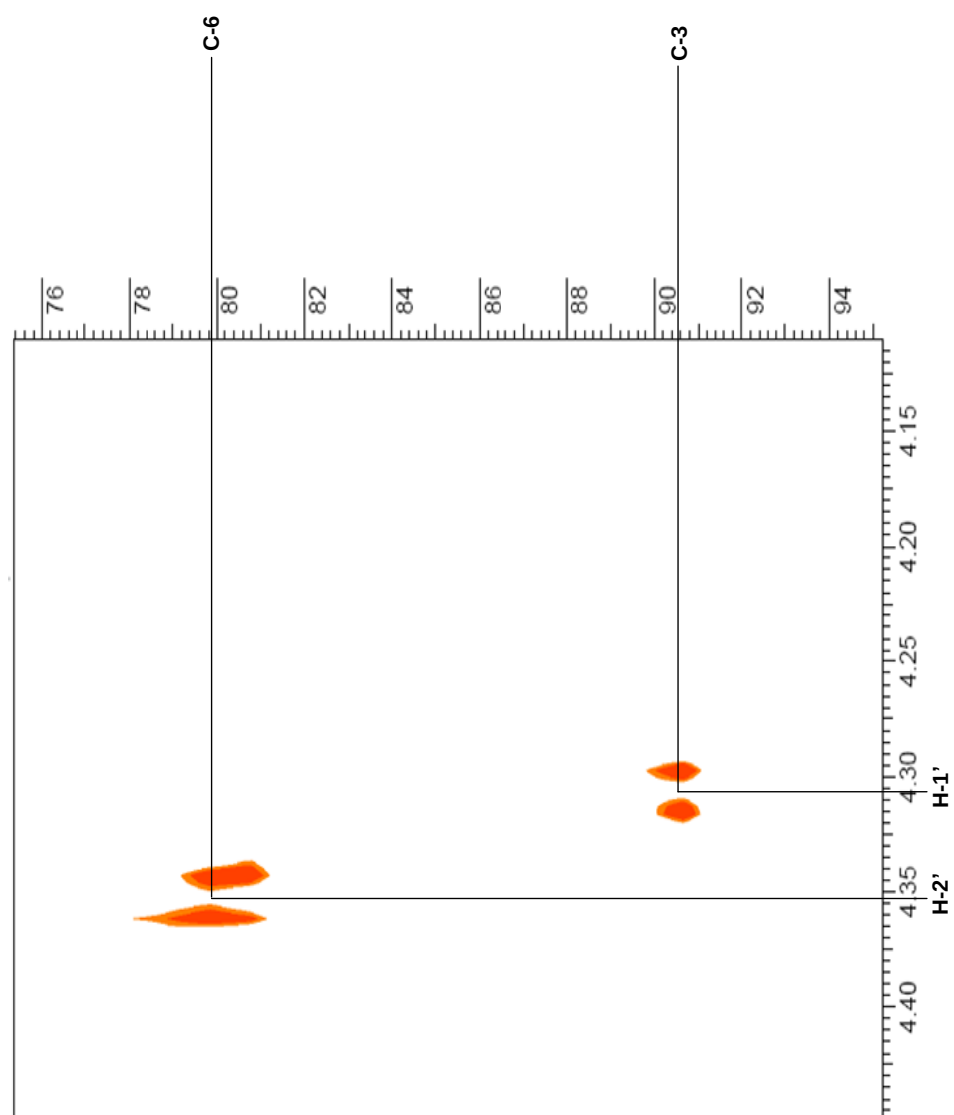


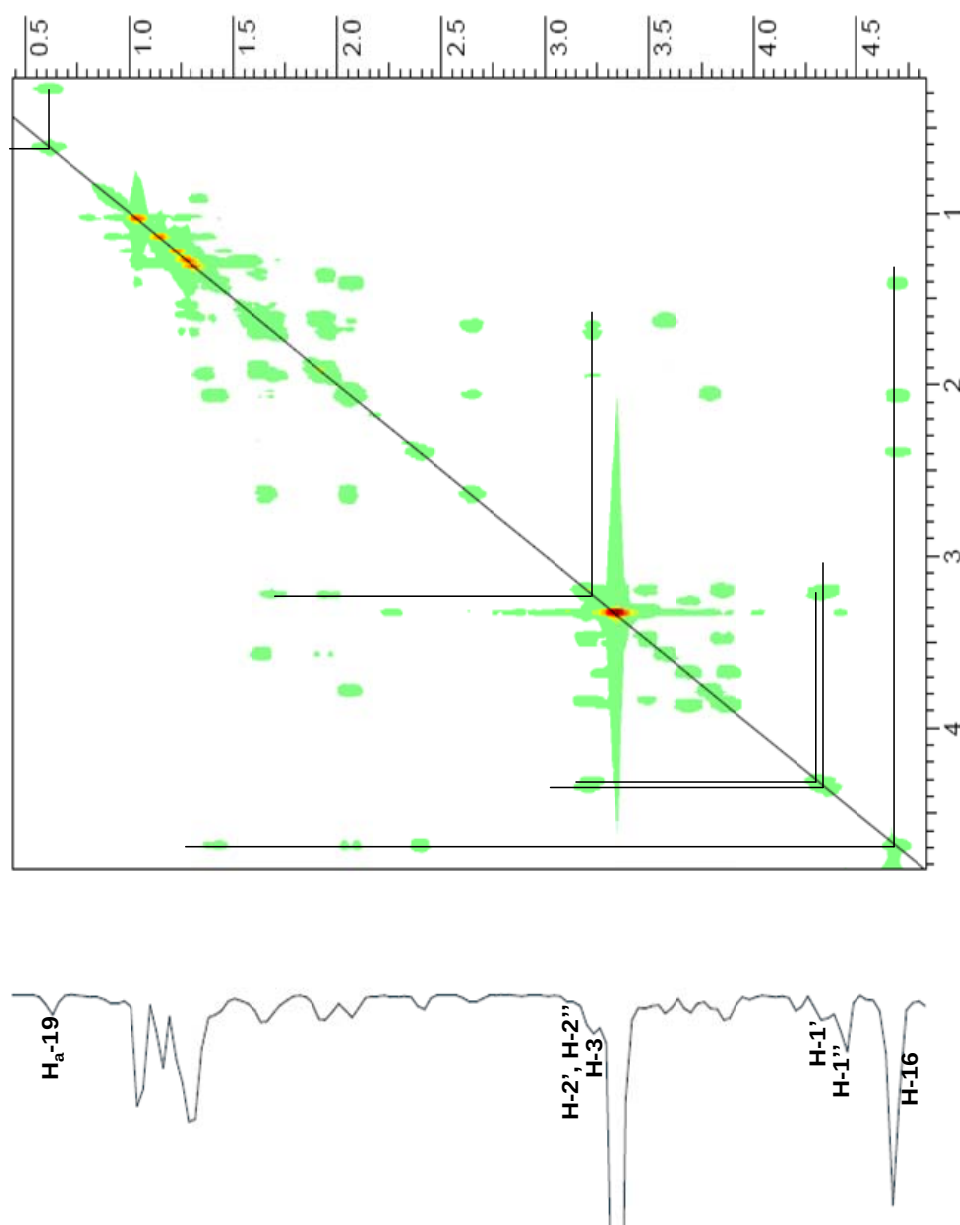
Spectrum 3.60. ^1H spectrum of **AW₄** in the downfield region



Spectrum 3.61. ^1H spectrum of AW_4 in the upfield region

Spectrum 3.62. HSQC spectrum of AW₄

Spectrum 3.63. HMBC spectrum of **AW₄**

Spectrum 3.64. COSY spectrum of AW_4 

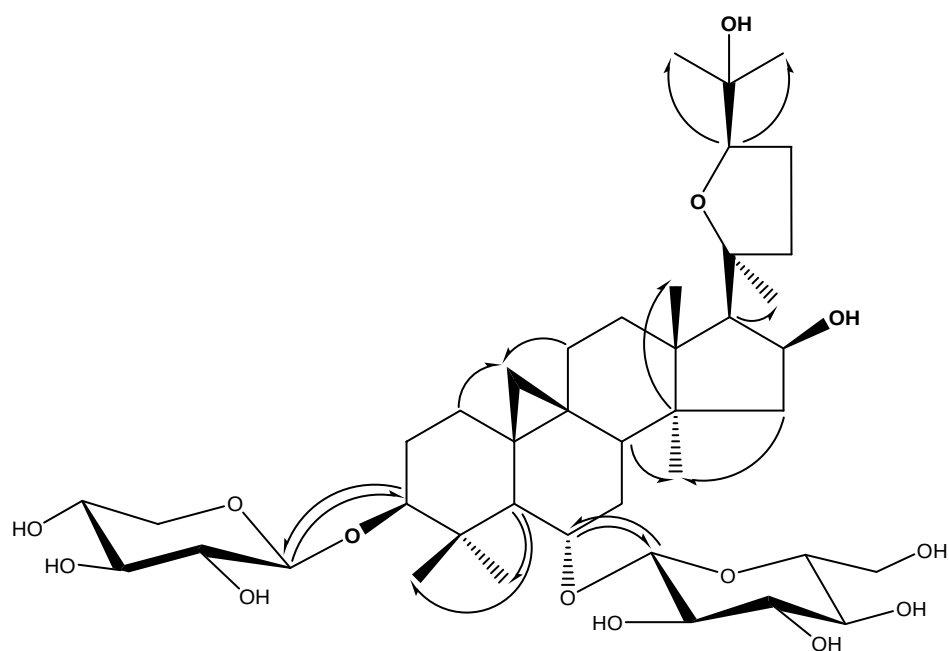


Figure 3.20. Key HMBC correlations of AW₄

3.3.5. Structural Identification of Compound 5 (AW₅)

The NMR spectral data of **5** indicated that this compound was a oleanene type triterpene bearing three sugar residues. A combination of 1D and 2D NMR techniques led to the assignment of the ¹H and ¹³C NMR signals due to the A, B, C, and D rings of a 3 β -24-dihydroxyolean-12-ene sapogenol moiety, glycosylated at C-3. Ring E was shown to contain an oxymethine at C-22 (δ_C 76.9, δ_H 3.64 br, m). 1D and 2D NMR data (**Table 3.17.**) also indicated the presence of a terminal α -L-rhamnopyranosyl as well as an inner 2-substituted β -D-glucuronopyranosyl (δ 78.2, C-2') and a β -D-xylopyranosyl (δ 78.2, C-2''). The sequence of the trisaccharide chain at C-3 was derived by the observed HMBC correlations between H-1' (δ 4.43) of the glucuronic acid residue and C-3 (δ 92.0) of the aglycon, as well as between H-1''' (δ 5.23) of the terminal rhamnose residue and C-2'' (δ 78.2) of the inner xylopyranosyl. All the sugar signals assigned for the trisaccharide chain at C-3 were also in good agreement with those reported for astragaloside VIII (Kitagawa et al., 1983). Based on the above-mentioned results, the structure of astragaloside VIII was determined to be 3-O-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl-(1 $\rightarrow$ 2)- β -D-glucuronopyranosyl] soyasapogenol B (**Figure 3.21.**).

Table 3.17. ^1H and ^{13}C assignments of sapogenol moiety of **AW₅** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
1	39.1	H₂-1	1.03, 1.66
2	25.6	H₂-2	1.81, 2.17
3	92	H-3	3.34
4	43.9	-----	-----
5	56.7	H-5	0.94
6	17.7	H-6	1.40, 1.65
7	33.9	H₂-7	1.43, 1.59
8	39.9	H-8	-----
9	48.7	-----	1.58
10	36.9	-----	-----
11	23.5	H₂-11	1.23, 1.89
12	122.6	H₂-12	5.28
13	145.1	-----	-----
14	43.1	-----	-----
15	26.3	H₂-15	1.24, 1.83
16	29.3	H-16	1.34, 1.76
17	38.6	H-17	-----
18	46.4	H₃-18	2.07
19	47.1	H₂-19	0.97, 1.78
20	31.1	H-20	-----
21	42	H₃-21	1.34, 1.46
22	76.9	H₂-22	3.64
23	21.3	H₂-23	1.25
24	62.4	H-24	-CH ₂ (3.23) -OH (4.10)
25	14.8	-----	0.91
26	16.0	H₃-26	1.01
27	23.9	H₃-27	1.16
28	18.6	H₃-28	0.86
29	31.0	H₃-29	0.95
30	27.5	H₃-30	1.04

Table 3.18. ^1H and ^{13}C assignments of sugar residues of **AW₅** (in CD_3OD)

Position	δC (ppm)	Position	δH (ppm)
<i>β-D-glucuronicacid-1'</i>	104.1	H-1'	4.43
2'	78.2	H-2'	3.40
3'	77.4	H-3'	3.56
4'	72.9	H-4'	3.41
5'	77.5	H-5'	3.63
6'	177.3	-----	-----
<i>β-D-xylose-1''</i>	101.8	H-1''	4.81
2''	78.2	H-2''	3.39
3''	77.6	H-3''	3.60
4''	70.0	H-4''	3.53
5''	62.6	H₂-5''	3.22, 4.10
6''	-----	H-6''	-----
<i>α-L-rhamnose-1'''</i>	100.9	H-1'''	5.23
2'''	71.1	H-2'''	3.96
3'''	75.7	H-3'''	3.40
4'''	73.8	H-4'''	3.42
5'''	69.4	H-5'''	3.43
6'''	16.3	H₃-6'''	1.29

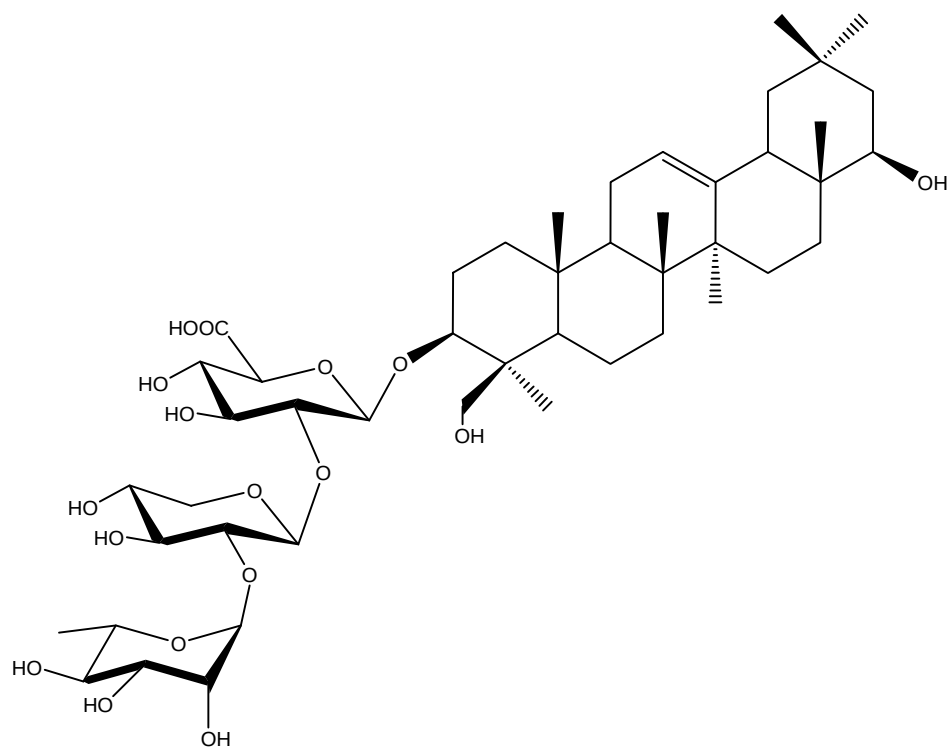
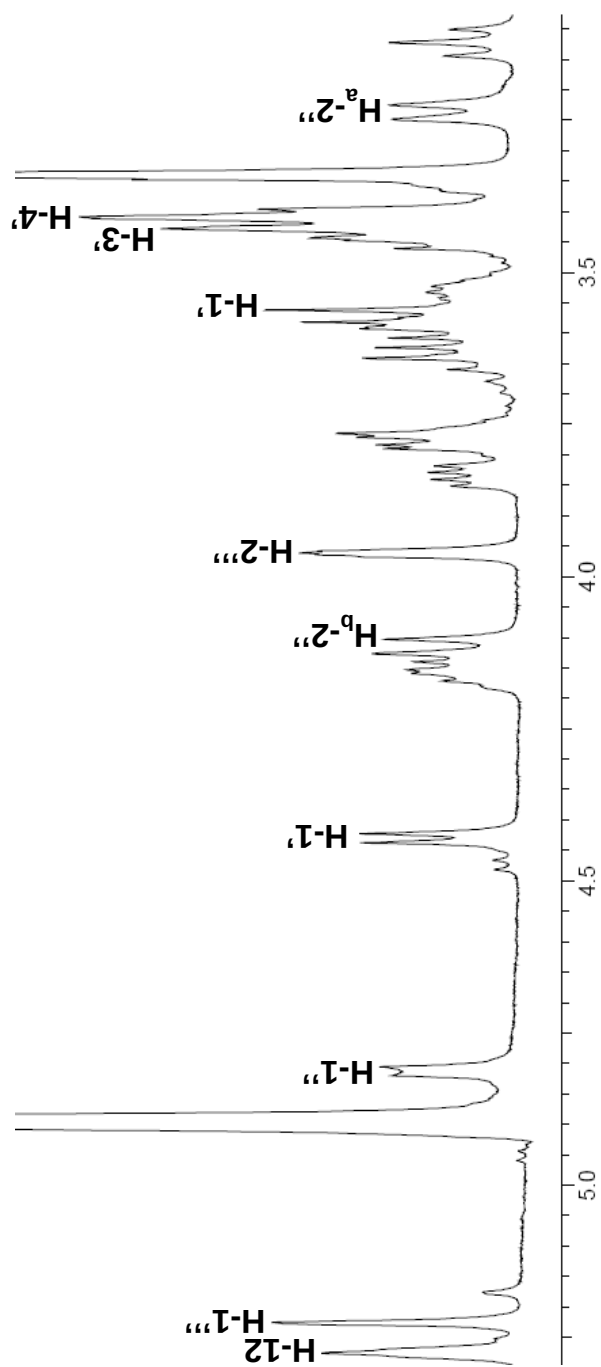
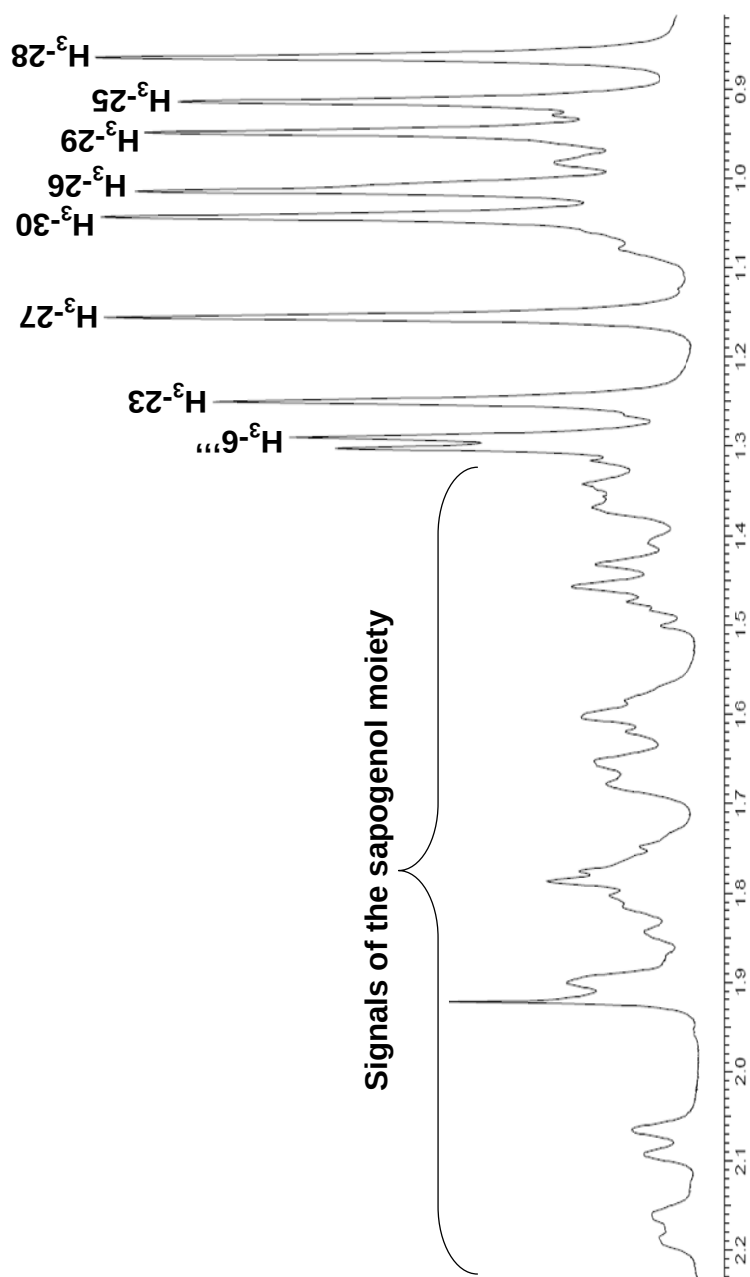


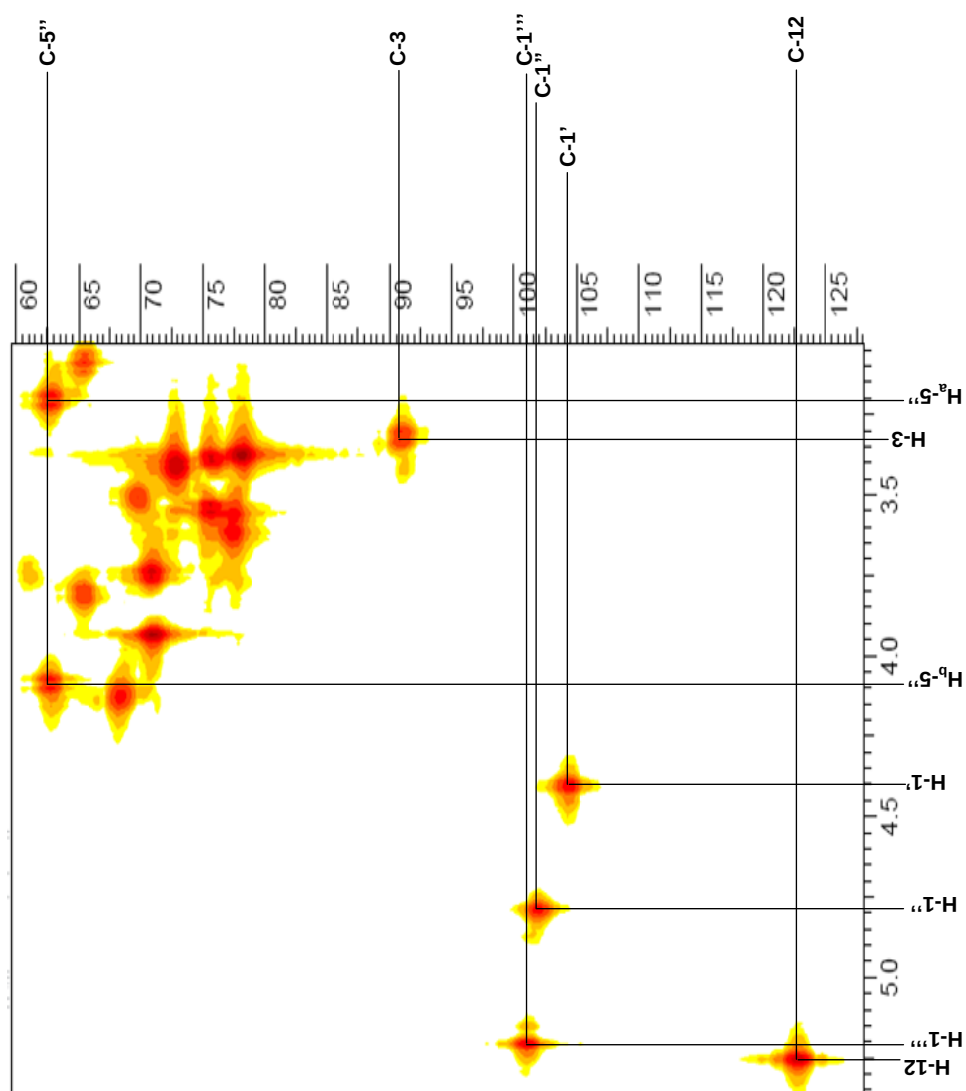
Figure 3.21. Structure of AW₅

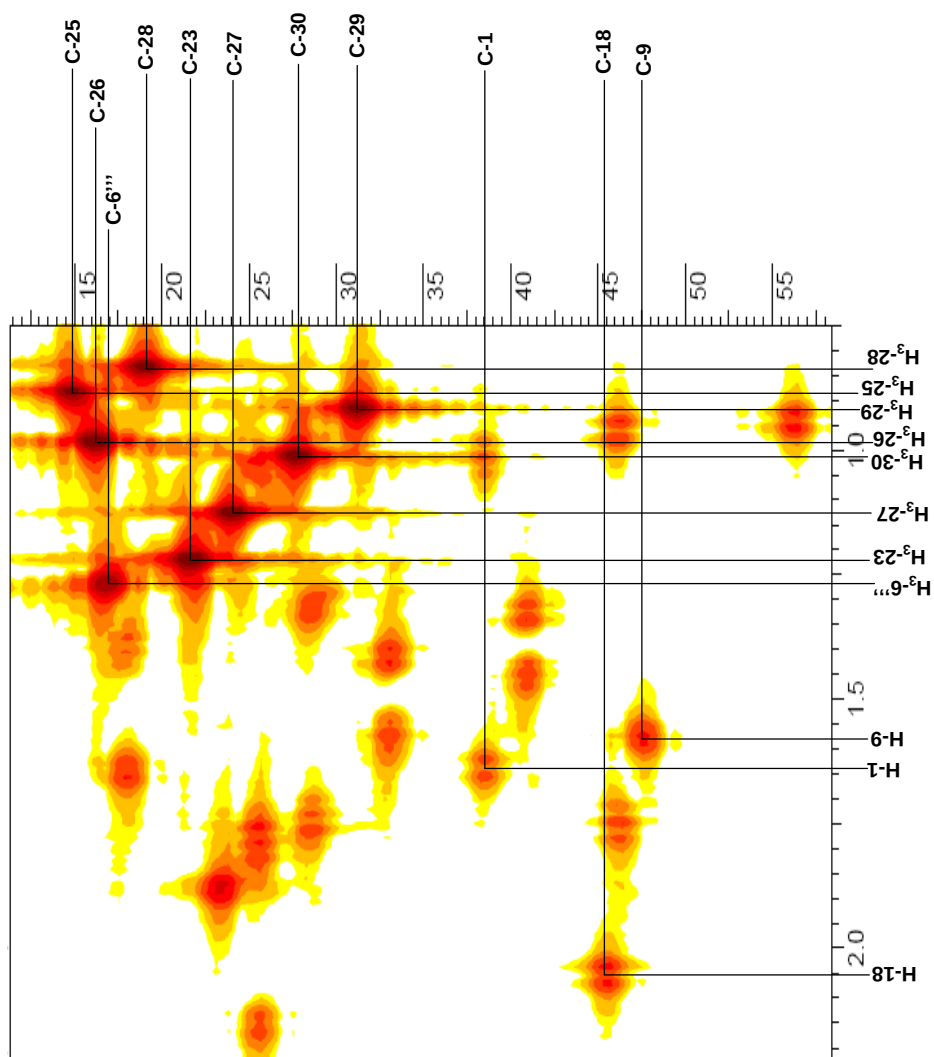


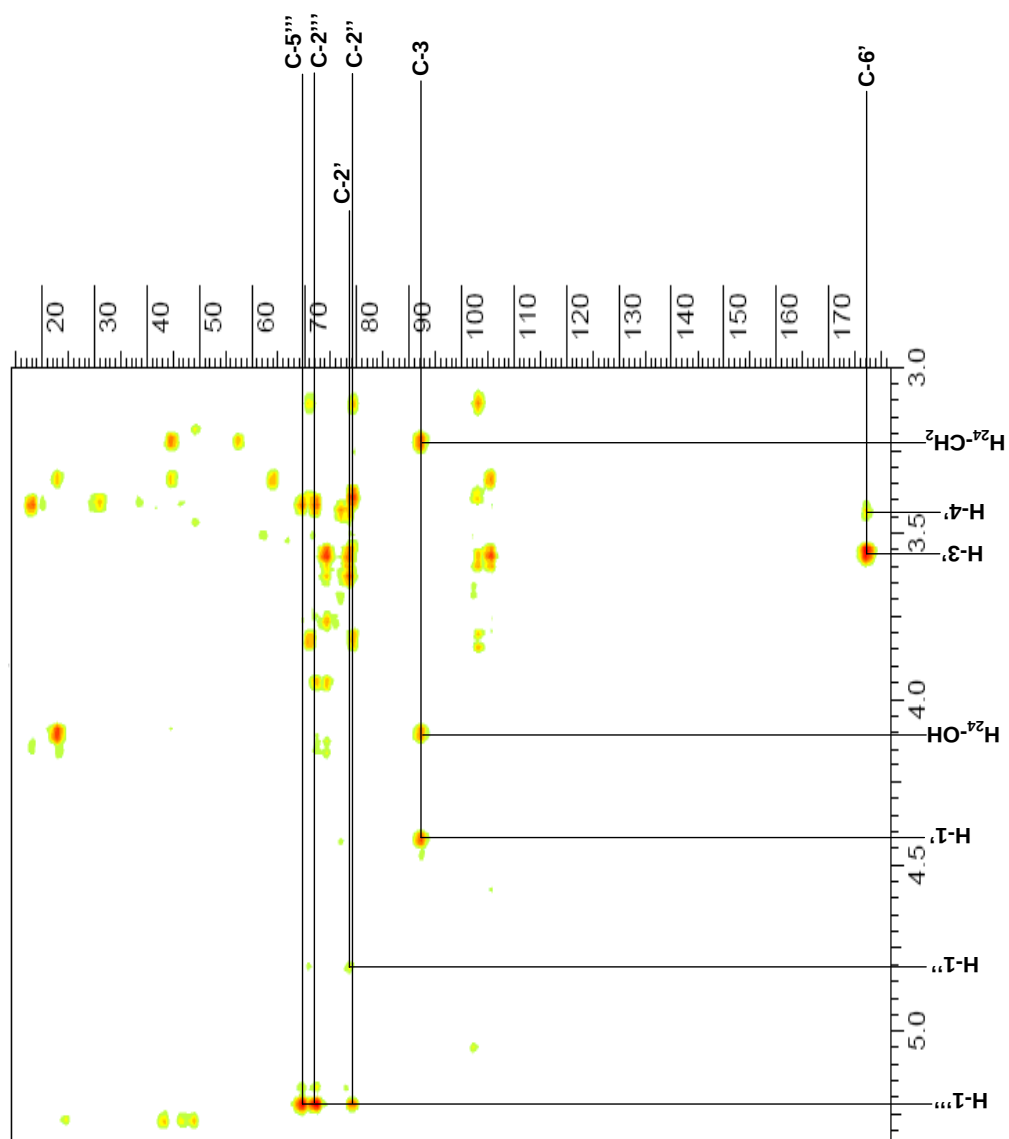
Spectrum 3.65. ^1H spectrum of **AW**₅ in the downfield region

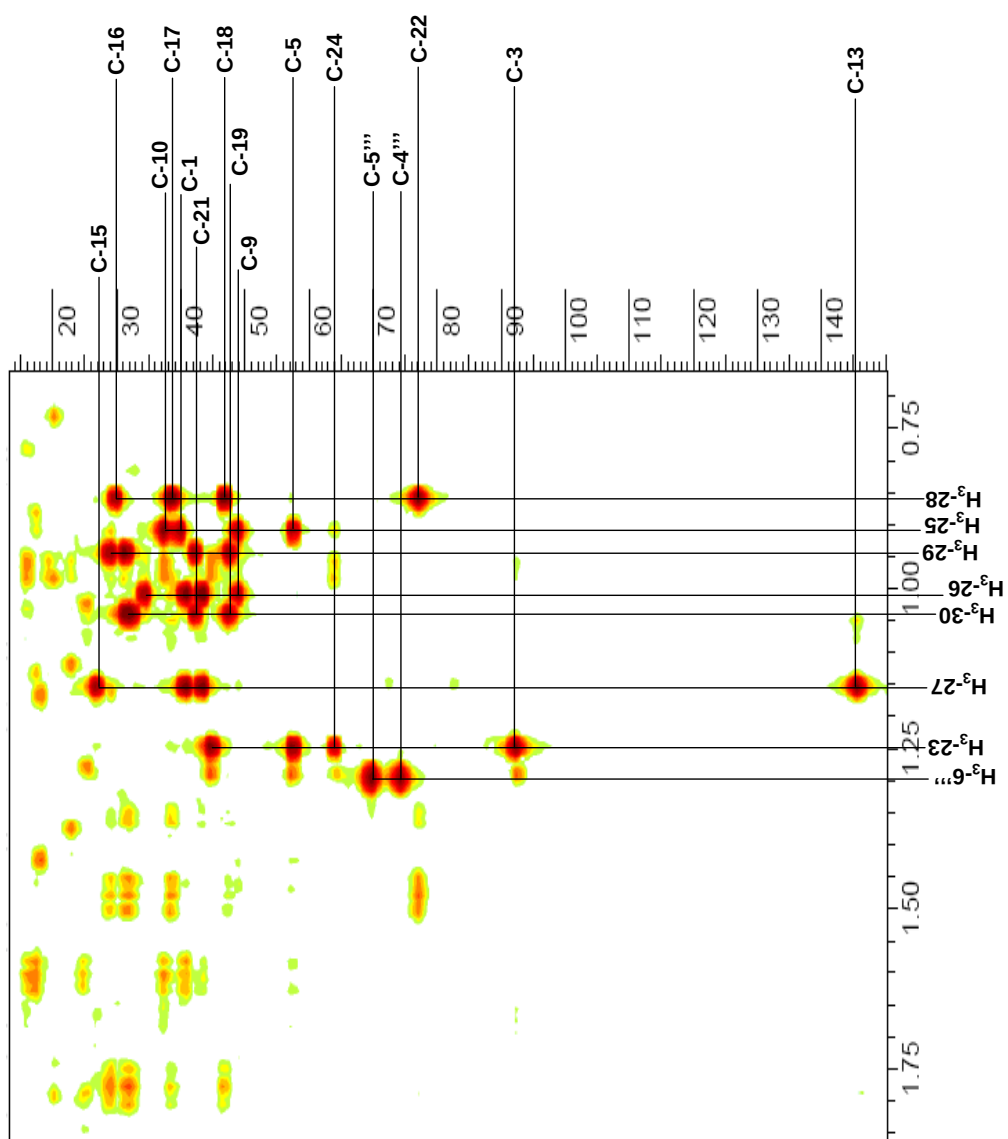


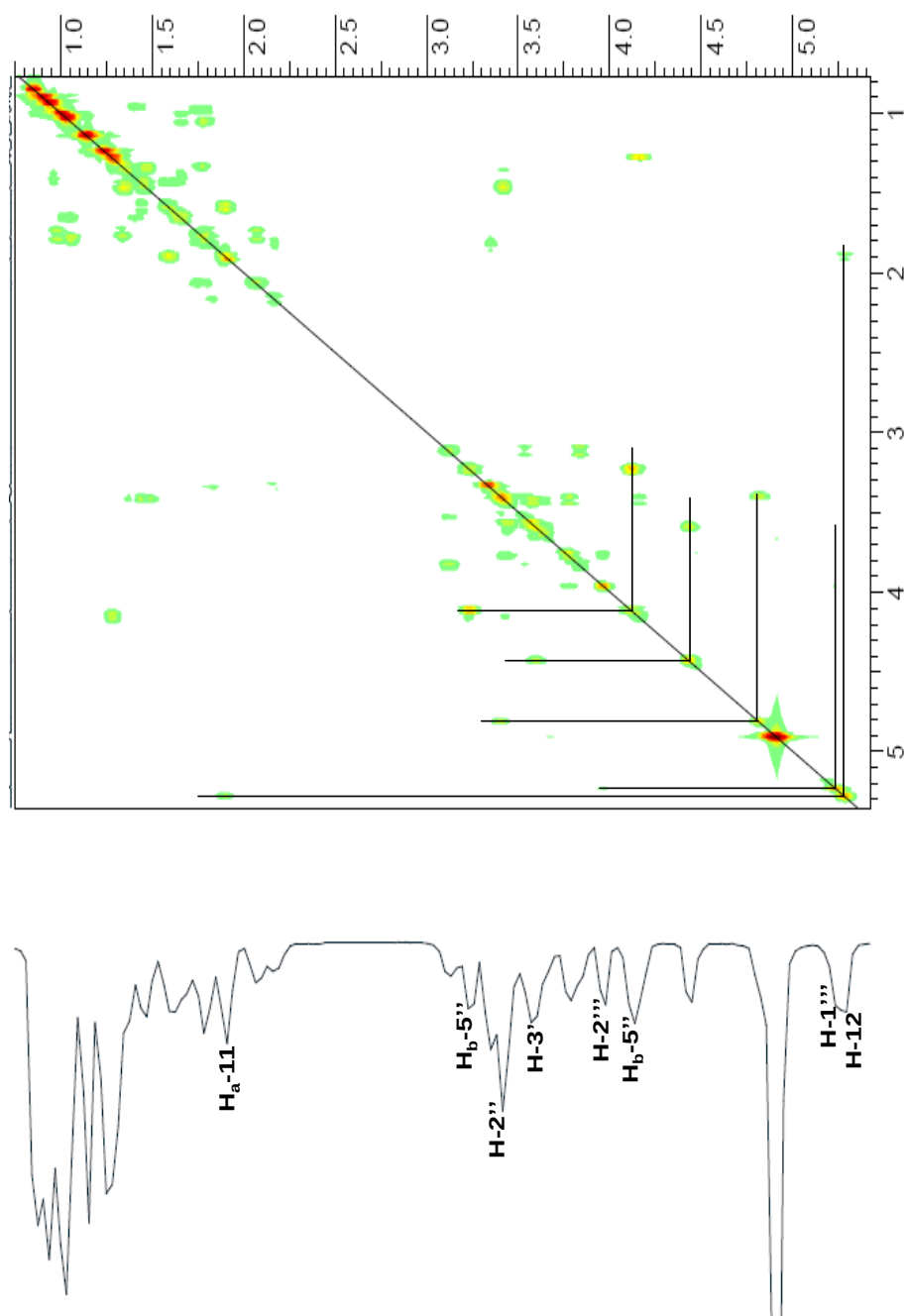
Spectrum 3.66. ^1H spectrum of **AW₅** in the upfield region

Spectrum 3.67. HSQC spectrum of AW_5 

Spectrum 3.68. HSQC spectrum of AW_5 

Spectrum 3.69. HMBC spectrum of AW_5 

Spectrum 3.70. HMBC spectrum of AW₅

Spectrum 3.71. COSY spectrum of **AW₅**

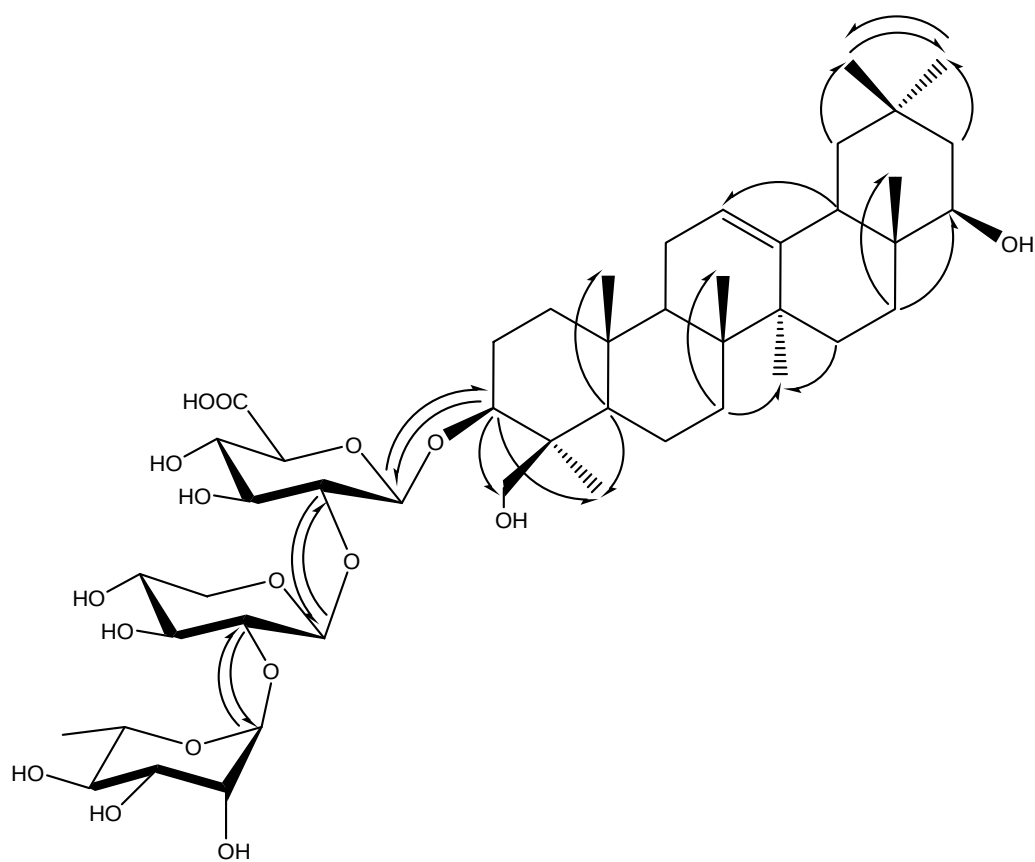


Figure 3.22. Key HMBC correlations of **AW₅**

3.2.6. Structural Identification of Compound 6 (AW₆)

The structure of compound **6** was elucidated as 6-*O*- β -D-glucopyranosyl-20(*R*),24(*S*)-epoxy-3 β ,6 α ,16 β ,25-tetrahydroxy cycloartane by comparing its physical properties and spectral data with those reported previously for brachyoside B (**Figure 3.23.**) (Bedir et al., 1998).

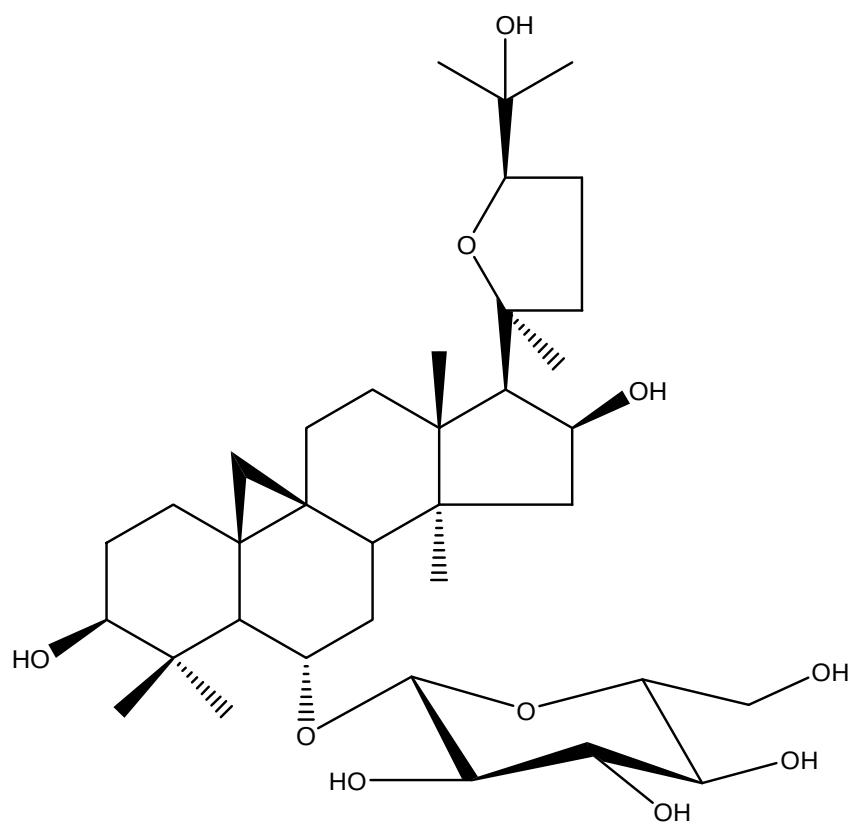


Figure 3.23. Structure of AW₆

3.2.7. Structural Identification of Compound 7 (AW₉)

The structure of compound **7** was elucidated as 3-*O*-(2'-*O*-acetyl)- β -D-xylopyranosyl-6-*O*- β -D-glucopyranosyl-20(*R*),24(*S*)-epoxy-3 β ,6 α ,16 β ,25-tetrahydroxy cycloartane by comparing its physical properties and spectral data with those reported previously for astragaloside II (**Figure 3.24.**) (Kitagawa et al., 1983).

3.2.8. Structural Identification of Compound **8** (AW₁₀)

The ¹H NMR spectrum of **8** showed signals characteristic of cyclopropane-ring methylene protons as an AX system (δ 0.41, 0.58, $J_{AX}=4.2$ Hz, H₂-19), seven tertiary methyl groups at δ 1.03, 1.06, 1.15, 1.29, 1.30, 1.32, and 1.32. Additionally, two anomeric protons at δ 4.41 (d, $J=7.5$ Hz, H-1') and 5.37 (d, $J=1.2$ Hz, H-1'') indicated the presence of one β - and α -linked sugar units. The chemical shifts and coupling constants of the signals assigned to the sugar units revealed the presence of one xylose and one rhamnose residue. The NMR spectroscopic data attributed to the sapogenol moiety were characteristic for a cycloartane-type triterpenoid skeleton and in good agreement with astrachrysoside A (Wang et al., 1990). Thus, compound **8** was determined to be 3-*O*-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl]-20(*R*),24(*S*)-epoxy-3 β ,6 α ,16 β ,25-tetrahydroxy cycloartane known as astrachrysoside A (Figure 3.25.).

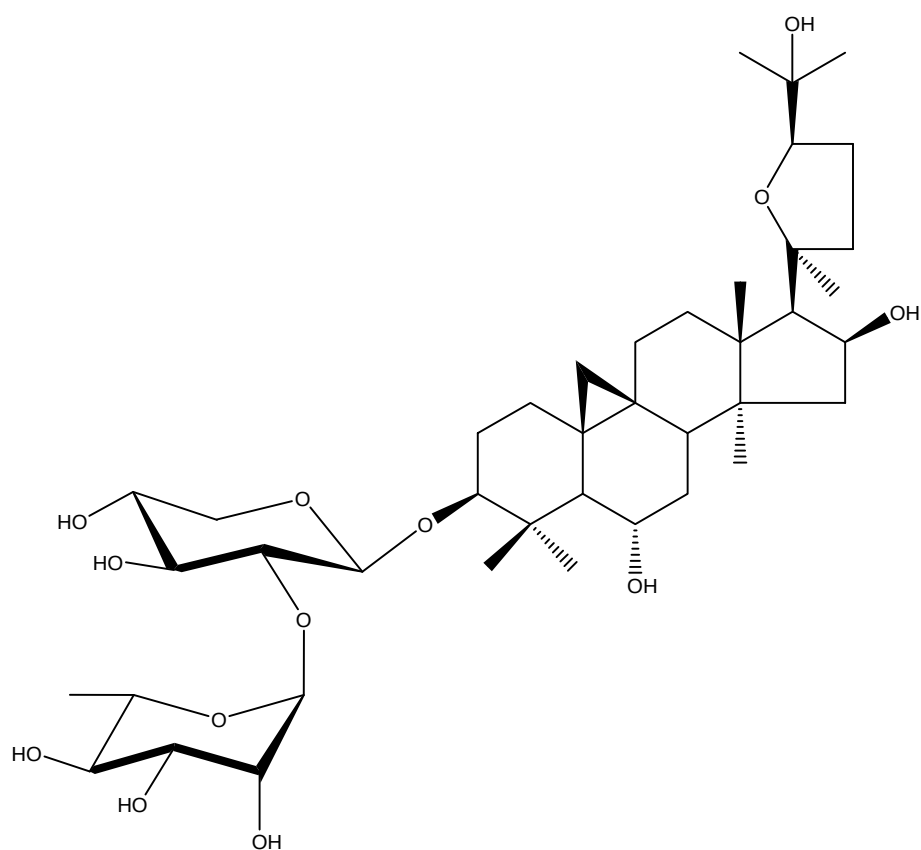
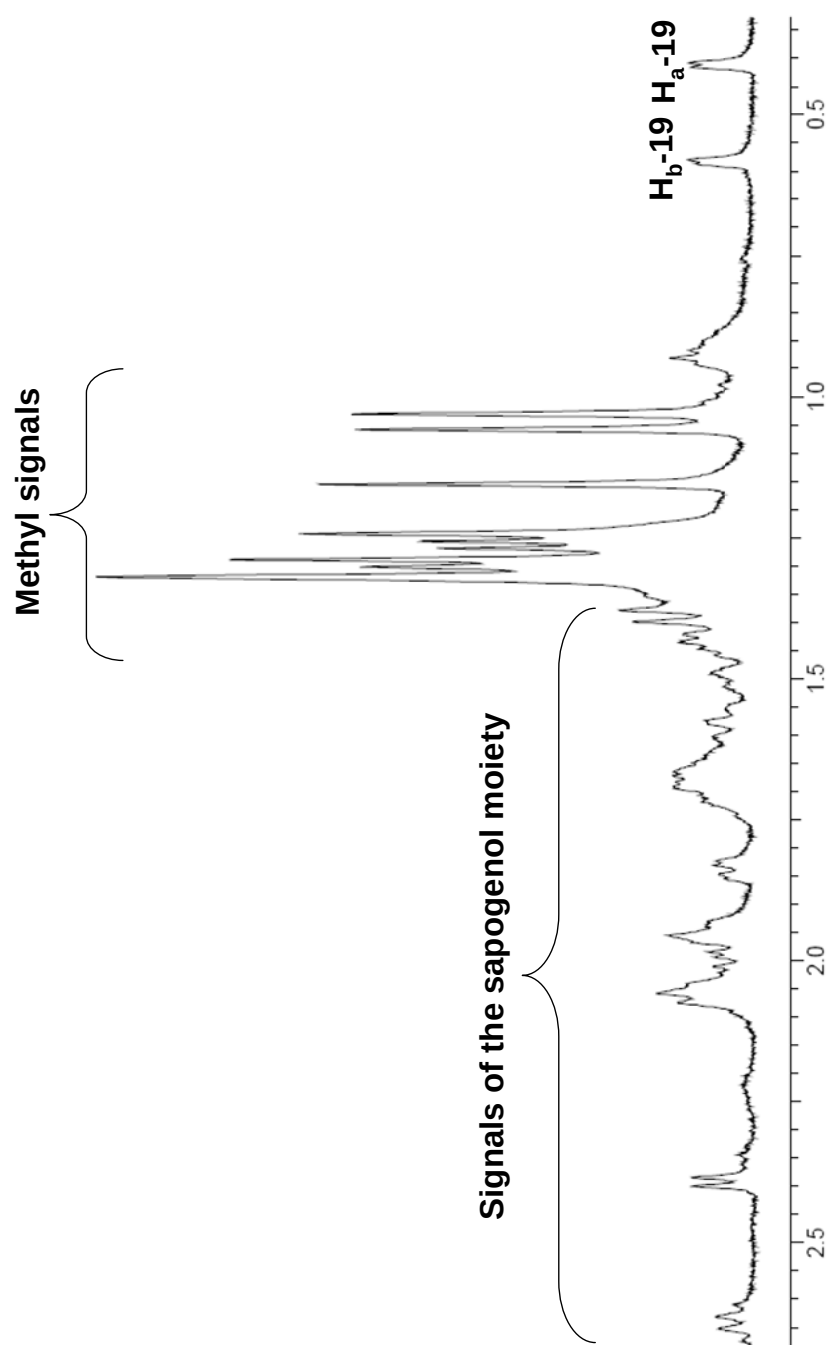
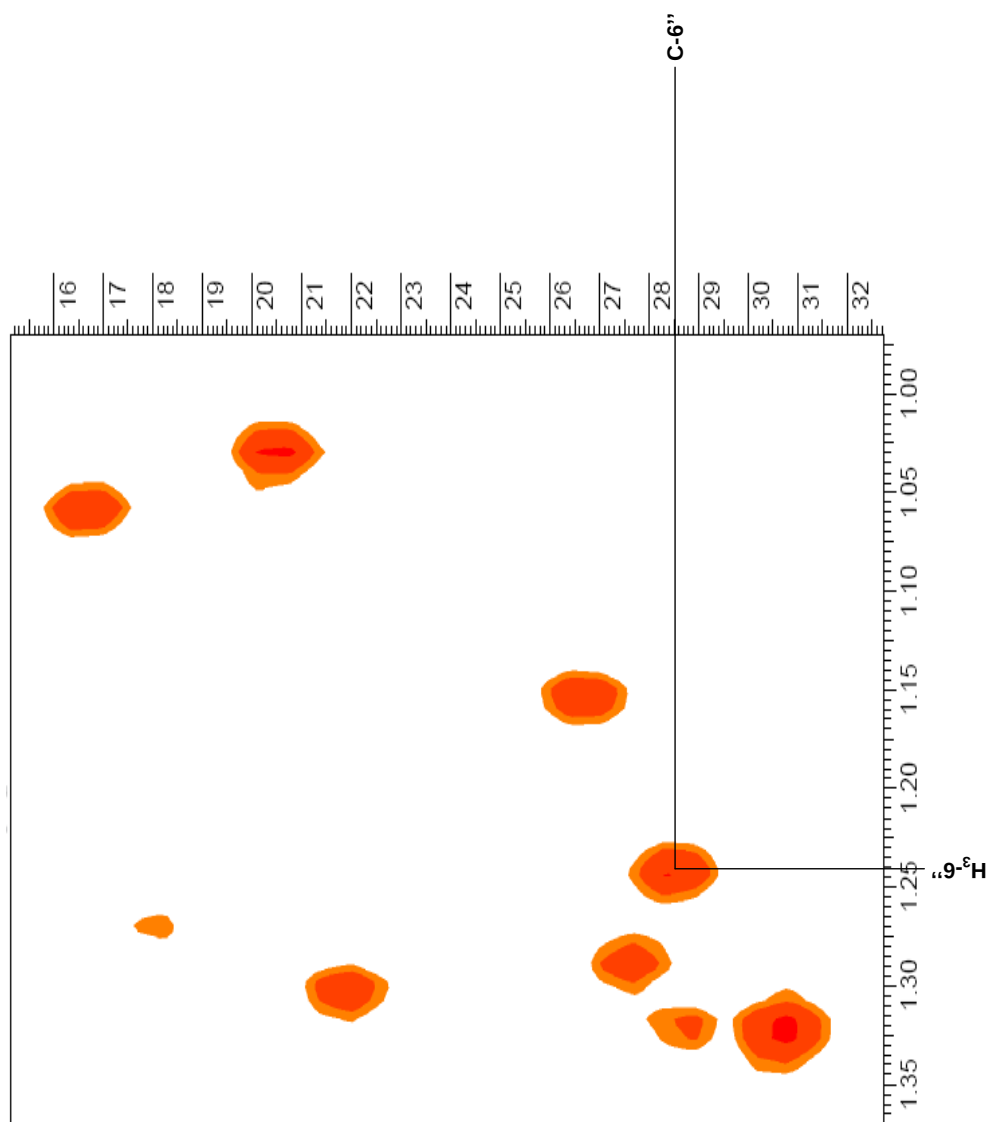


Figure 3.25. Structure of AW₁₀



Spectrum 3.72. ^1H spectrum of **AW₁₀** in the upfield region

Spectrum 3.73. HSQC spectrum of **AW₁₀**

3.2.9. Structural Identification of Compound 9 (AW₁₁)

The structure of compound **9** was elucidated as 3-*O*- β -D-xylopyranosyl-6-*O*- β -D-xylopyranosyl-20(*R*),24(*S*)-epoxy-3 β ,6 α ,16 β ,25-tetrahydroxy cycloartane by comparing its physical properties and spectral data with those reported previously for astrasieversianin X (**Figure 3.26.**) (Lixian et al., 1986).

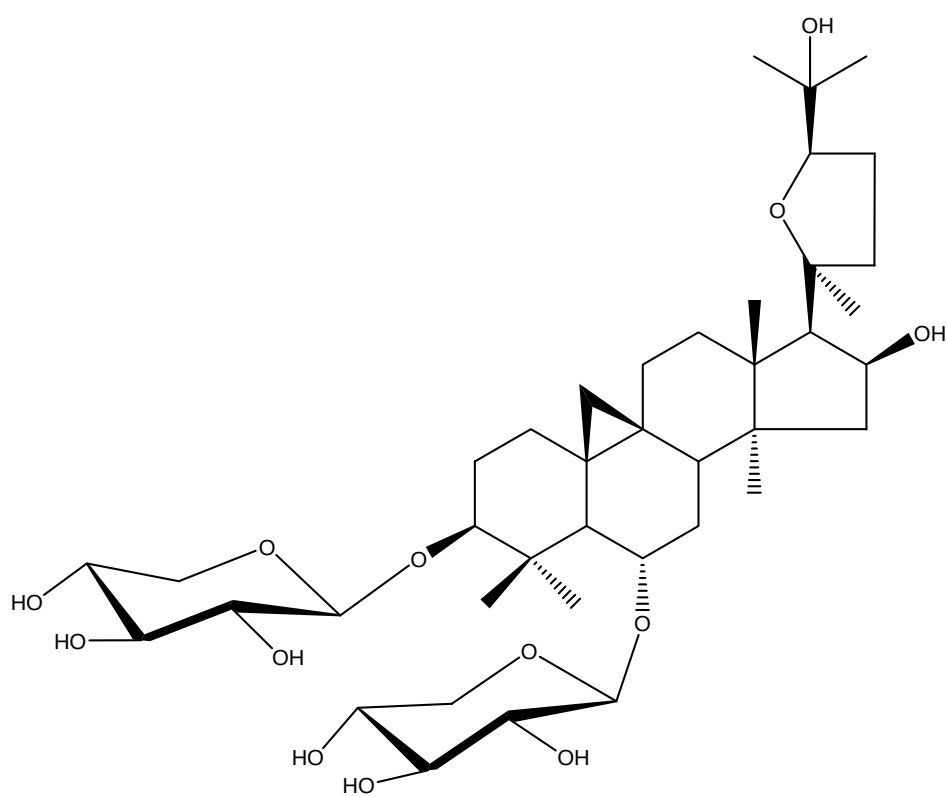


Figure 3.26. Structure of AW_{11}

3.2.10. Structural Identification of Compound **10** (AW₁₂)

Taking into account the results of our comprehensive ¹H- and ¹³C NMR studies, and previous knowledge derived from metabolites isolated from the genus *Astragalus*, the main features of a cycloartane-type triterpene were evident for **10**: characteristic signals due to cyclopropane-methylene protons as an AX system (δ 0.22, 0.62, each d, J_{AX} = 4.2, H2-19), seven tertiary methyl groups (δ 1.28, 1.21, 1.18, 1.15, 1.06, 1.03, and 0.97; respectively, H₃-28, H₃-27, H₃-26, H₃-18, H₃-30, H₃-29, and H₃-21). Additionally, the resonance for two acetyl methyls at δ 2.07 and 2.22, and four anomeric protons at δ 4.41 (d, J = 7.5 Hz, H-1'), 4.33 (d, J = 7.5 Hz, H-1''), 4.34 (d, J = 6.3 Hz, H-1'''), and 5.42 (d, J = 1.2 Hz, H-1'''), indicative of the presence of two α -linked and two β -linked sugar units, were observed. Thus, **10** was considered to a cycloartane-type triterpene tetraglycoside.

On the basis of chemical shifts, the multiplicity of the signals, and the coupling constants, the four sugar residues were identified as β -xylopyranose, β -glucopyranose, α -arabinopyranose, and α -rhamnopyranose. The HMQC experiments correlated each ¹H NMR sugar signal to the corresponding carbon resonance and showed the absence of any glycosilation shift for the ¹³C NMR resonances of the rhamnopyranosyl residue, suggesting this sugar to be terminal. Additionally, in the ¹H NMR spectrum, the H-4'''' proton of the arabinose residue was observed at δ 4.97 (m) showing the downfield shift due to acetylation. Key correlation peaks observed in the HBMC

spectrum of **10** between H-1' of the xylosyl at δ 4.41 (d, J = 7.5 Hz) and C-3 (δ 89.2) of the aglycon, between H-1'' of the rhamnosyl at δ 5.42 (d, J = 1.2 Hz) and C-2' (δ 78.7) of the xylosyl allowed the disaccharide chain at C-3 to be determined as α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranoside. The cross peak of the ^3J long-range coupling between H-1''' (δ 4.33, d, J = 7.5, Hz) of the glycosyl and C-6 (δ 79.5), between H-1'''' (4.34, d, J = 6.3, Hz) of the arabinosyl and C-24 (δ 92.4) provided evidence for the other glycosidation sites on the sapogenol moiety.

Based on these results, compound **10** (**Figure 3.27.**) was assigned as 3-*O*-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl]-6-*O*- β -D-glucopyranosyl-24-*O*- α -(4'-*O*-acetyl)-L-arabinopyranosyl-16-*O*-acetoxy-3 β ,6 α ,16 β ,24(*S*),25-pentahydroxy cycloartane. Based on the database search (SciFinderScholar) compound **10** was identified as a new cycloartane-type glycoside.

Table 3.19. ^1H and ^{13}C assignments of sapogenol moiety of **AW₁₂** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
1	32.9	H₂-1	1.58, 1.31, m
2	30.4	H₂-2	1.95, 1.70, m
3	89.2	H-3	3.21, dd (11.3, 4.0)
4	42.6	-----	-----
5	52.9	H-5	1.64, d (9.5)
6	79.5	H-6	3.58, ddd (9.5, 9.5, 4.5)
7	33.5	H₂-7	1.84, 1.74, m
8	45.2	H-8	1.93, dd (11.9, 4.2)
9	21.4	-----	-----
10	29.4	-----	-----
11	26.1	H₂-11	1.89, 1.38, m
12	33.5	H₂-12	1.69, (2H) m
13	46.4	-----	-----
14	47.8	-----	-----
15	46.2	H₂-15	2.36, dd (12.7, 8.0) 1.31, dd (12.7, 5.2)
16	77.6	H-16	5.04, ddd (8.0, 8.0, 5.2)
17	56.2	H-17	1.92, dd (9.9, 8.0)
18	17.6	H₃-18	1.15, s
19	27.0	H₂-19	0.62, d (4.2), 0.22, d (4.2)
20	32.9	H-20	1.79, m
21	18.7	H₃-21	0.97, d (6.5)
22	34.1	H₂-22	2.06, 0.80, m
23	30.7	H₂-23	1.74, 1.15, m
24	92.4	H-24	3.23, dd (2.4, 10.5)
25	74.9	-----	-----
26	26.2	H₃-26	1.18, s
27	23.7	H₃-27	1.21, s
28	28.1	H₃-28	1.28, s
29	16.3	H₃-29	1.03, s
30	20.0	H₃-30	1.06, s
COCH₃	172.5	-----	-----
COCH₃	21.3	H₃-Ac	2.07, s

Table 3.20. ^1H and ^{13}C assignments of sugar residues of **AW₁₂** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
<i>β-D-xylose-1'</i>	106.2	H-1'	4.41, d (7.5)
2'	78.7	H-2'	3.47, dd (9.2, 7.5)
3'	78.8	H-3'	3.46, t (9.2)
4'	71.5	H-4'	3.49, m
5'	66.3	H₂-5'	3.88, dd (11.7, 5.2) 3.21, t (11.7)
<i>α-L-rhamnose-1''</i>	101.8	H-1''	5.42, d (1.2)
2''	72.1	H-2''	3.97, dd (1.2, 3.2)
3''	72.1	H-3''	3.78, dd (3.2, 9.3)
4''	74.2	H-4''	3.43, t (9.3)
5''	69.8	H-5''	4.06, m
6''	17.9	H₃-6''	1.26, d (6.5)
<i>β-D-glucose-1'''</i>	104.8	H-1'''	4.33, d (7.5)
2'''	75.4	H-2'''	3.21, dd (7.5, 9.0)
3'''	78.5	H-3'''	3.35, t (9.0)
4'''	71.5	H-4'''	3.32, t (9.0)
5'''	77.6	H-5'''	3.26, ddd (3.5, 4.5, 9.0)
6'''	63.1	H₂-6'''	3.86, dd (3.5, 12.0) 3.69, dd (4.5, 12.0)
<i>α-L-arabinose-1''''</i>	107.1	H-1''''	4.34, d (6.3)
2''''	73.7	H-2''''	3.56, dd (8.5, 6.3)
3''''	73.2	H-3''''	3.66, dd (8.5, 3.0)
4''''	73.1	H-4''''	4.97, m
5''''	64.9	H₂-5''''	3.94, dd (12.7, 3.2) 3.52, dd (12.7, 2.3)
4-COCH₃	172.9	-----	-----
4-COCH₃	21.3	H₃-Ac	2.22, s

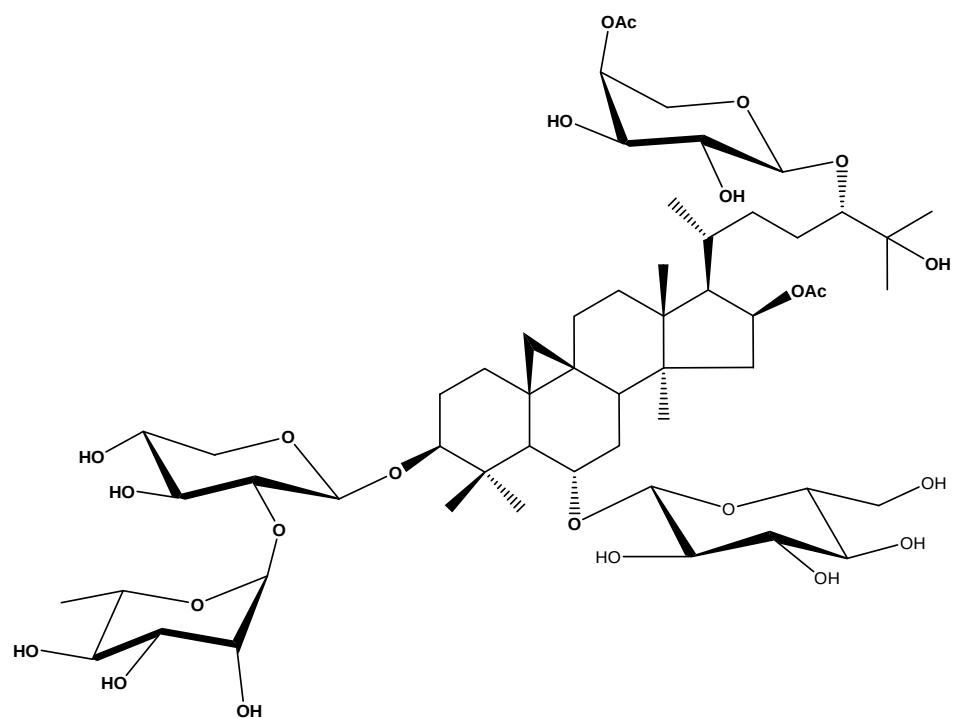
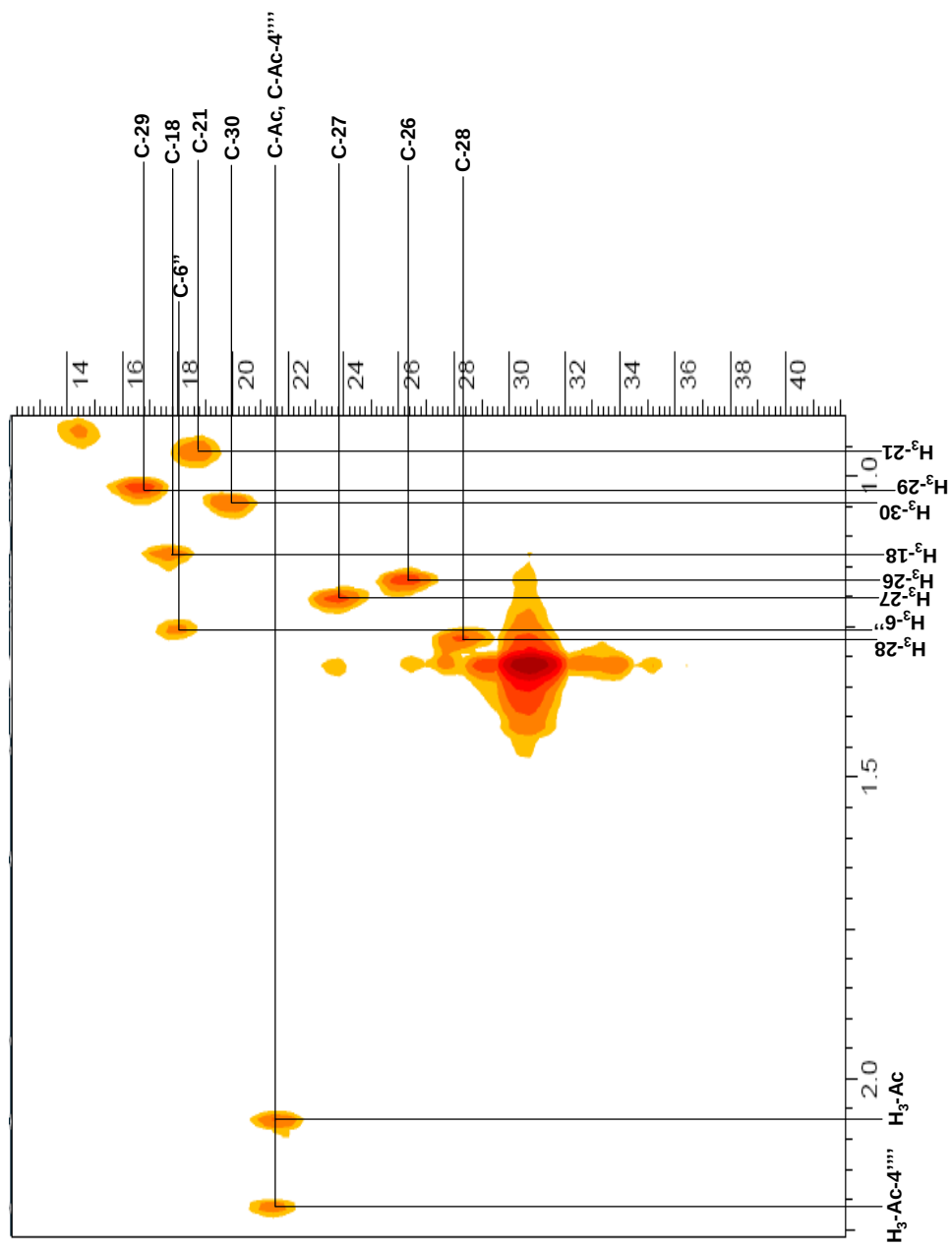
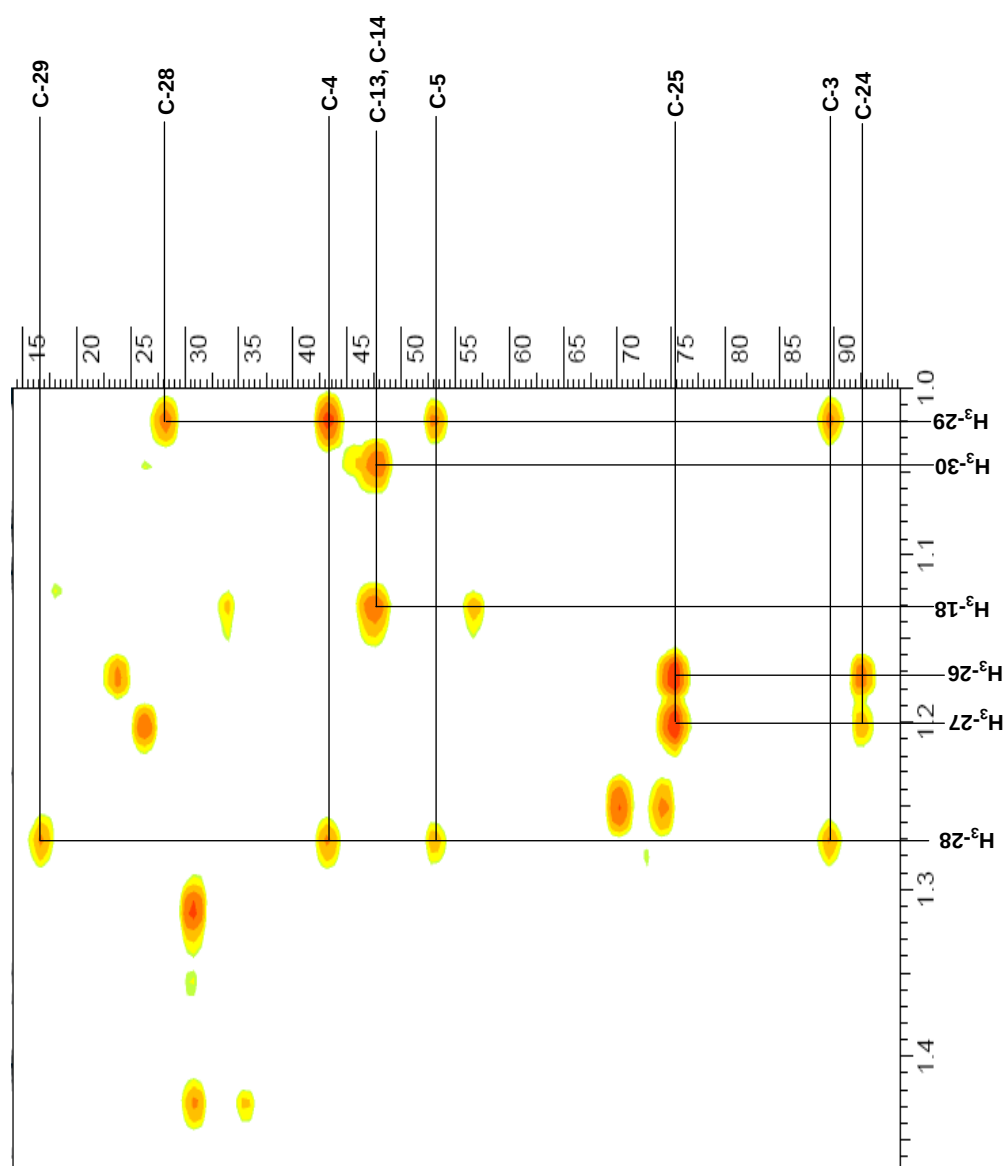


Figure 3.27. Structure of AW₁₂

Spectrum 3.74. HSQC spectrum of AW₁₂

Spectrum 3.75. HMBC spectrum of AW₁₂

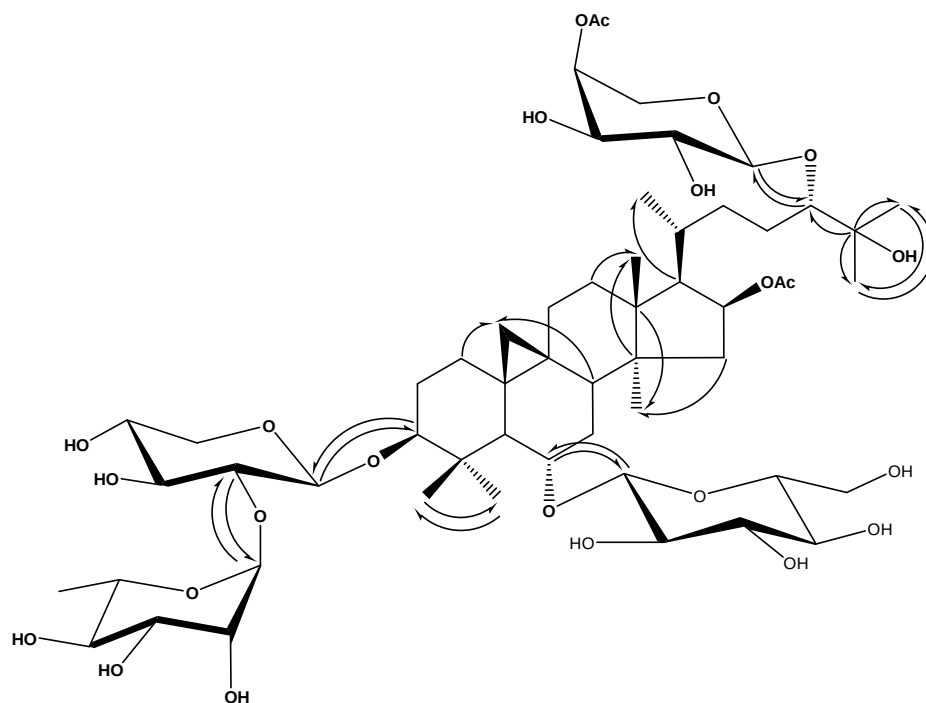


Figure 3.28. Key HMBC correlations of **AW₁₂**

3.2.11. Structural Identification of Compound **11** (AW₁₃)

Detailed examination of 1D- and 2D-NMR spectra of **11** and comparison with those of **10** showed their considerable structural similarity. As a result of this, the chemical shift values of the aglycon moiety of **11** were superimposable with those reported for **10**. The differences consisted only in the signals of the sugar residue which was attached at C-24 of the sapogenol moiety. The difference appeared to be due to the absence of the acetyl group at C-4'''' of the arabinopyranosyl unit, as determined by ¹H- and ¹³C-NMR. Additionally, the resonance of the proton, indicative of the absence of the acetyl group, was observed in the highfield region at δ 3.81 (m, H-4'''). Detailed examination of DQF-COSY, HMQC and HMBC spectra of **11** allowed unambiguous assignment of sugar residue at C-24, identified as α -L-arabinopyranosyl. The position of each sugar residue was unambiguously determined by the HMBC experiment which showed long-range correlations between H-1' (δ 4.40, d, J = 7.5, Hz) and C-3 (δ 89.2), H-1''' (δ 4.34, d, J = 7.5, Hz) and C-6 (δ 79.5), H-1'''' (δ 4.33, d, J = 6.3, Hz) and C-24 (δ 92.4), and H-1'' (5.43, d, J = 1.2, Hz) and C-2' (δ 78.5), indicating the tridesmosidic nature of **11**.

Consequently, the structure of compound **11** (**Figure 3.29.**) was established as 3-*O*-[α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- β -D-xylopyranosyl]-6-*O*- β -D-glucopyranosyl-24-*O*- α -L-arabinopyranosyl-16-*O*-acetoxy-3 β ,6 α ,16 β ,24(*S*),25-pentahydroxycycloartane, a new chemical entity.

Table 3.21. ^1H and ^{13}C assignments of sapogenol moiety of **AW₁₃** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
1	32.9	H₂-1	1.58, 1.31, m
2	30.4	H₂-2	1.95, 1.70, m
3	89.2	H-3	3.21, dd (11.3, 4.0)
4	42.6	-----	-----
5	52.9	H-5	1.64, d (9.5)
6	79.5	H-6	3.58, ddd (9.5, 9.5, 4.5)
7	33.5	H₂-7	1.84, 1.74, m
8	45.2	H-8	1.93, dd (11.9, 4.2)
9	21.4	-----	-----
10	29.4	-----	-----
11	26.1	H₂-11	1.89, 1.38, m
12	33.5	H₂-12	1.69, (2H) m
13	46.4	-----	-----
14	47.8	-----	-----
15	46.2	H₂-15	2.36, dd (12.7, 8.0) 1.31, dd (12.7, 5.2)
16	77.6	H-16	5.04, ddd (8.0, 8.0, 5.2)
17	56.2	H-17	1.92, dd (9.9, 8.0)
18	17.6	H₃-18	1.15, s
19	27.0	H₂-19	0.62, d (4.2), 0.22, d (4.2)
20	32.9	H-20	1.79, m
21	18.7	H₃-21	0.97, d (6.5)
22	34.1	H₂-22	2.06, 0.80, m
23	30.7	H₂-23	1.74, 1.15, m
24	92.4	H-24	3.23, dd (2.4, 10.5)
25	74.9	-----	-----
26	26.2	H₃-26	1.18, s
27	23.7	H₃-27	1.21, s
28	28.1	H₃-28	1.28, s
29	16.3	H₃-29	1.03, s
30	20.0	H₃-30	1.06, s
COCH₃	172.5	-----	-----
COCH₃	21.3	H₃-Ac	2.07, s

Table 3.22. ^1H and ^{13}C assignments of sugar residues of **AW₁₃** (in CD_3OD)

Position	δC (ppm)	Position	δH ppm
β-D-xylose-1'	106.3	H-1'	4.40, d (7.5)
2'	78.5	H-2'	3.47, dd (9.2, 7.5)
3'	78.6	H-3'	3.49 t (9.2)
4'	71.3	H-4'	3.52, m
5'	66.3	H₂-5'	3.89, dd (11.7, 5.2) 3.20, t (11.7)
α-L-rhamnose-1''	101.6	H-1''	5.43, d (1.2)
2''	72.1	H-2''	3.97, dd (1.2, 3.2)
3''	71.8	H-3''	3.78, dd (3.2, 9.3)
4''	73.6	H-4''	3.44, t (9.3)
5''	69.7	H-5''	4.06, m
6''	17.9	H₃-6''	1.26, d (6.5)
β-D-glucose-1'''	104.5	H-1'''	4.34, d (7.5)
2'''	75.2	H-2'''	3.22, dd (7.5, 9.0)
3'''	78.4	H-3'''	3.34, t (9.0)
4'''	71.7	H-4'''	3.33, t (9.0)
5'''	77.5	H-5'''	3.26, ddd (3.5, 4.5, 9.0)
6'''	63.0	H₂-6'''	3.90, dd (3.5, 12.0) 3.69, dd (4.5, 12.0)
α-L-arabinose-1''''	106.7	H-1''''	4.33, d (6.3)
2''''	73.5	H-2''''	3.57, dd (8.5, 6.3)
3''''	73.5	H-3''''	3.55, dd (8.5, 3.0)
4''''	69.3	H-4''''	3.81, m
5''''	64.9	H₂-5''''	3.91, dd (12.7, 3.2) 3.56, dd (12.7, 2.3)

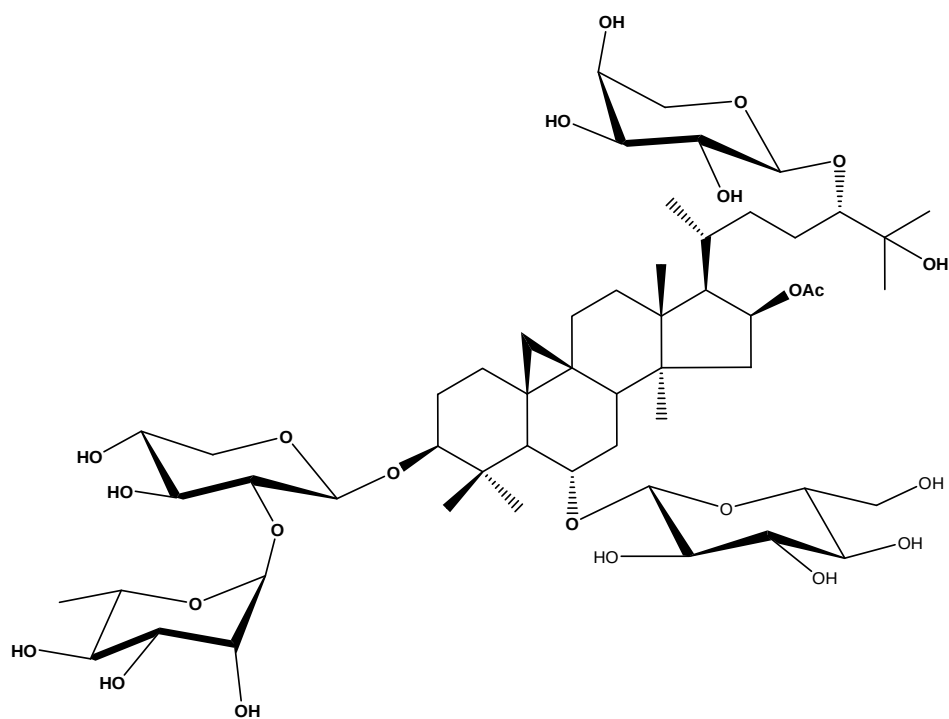
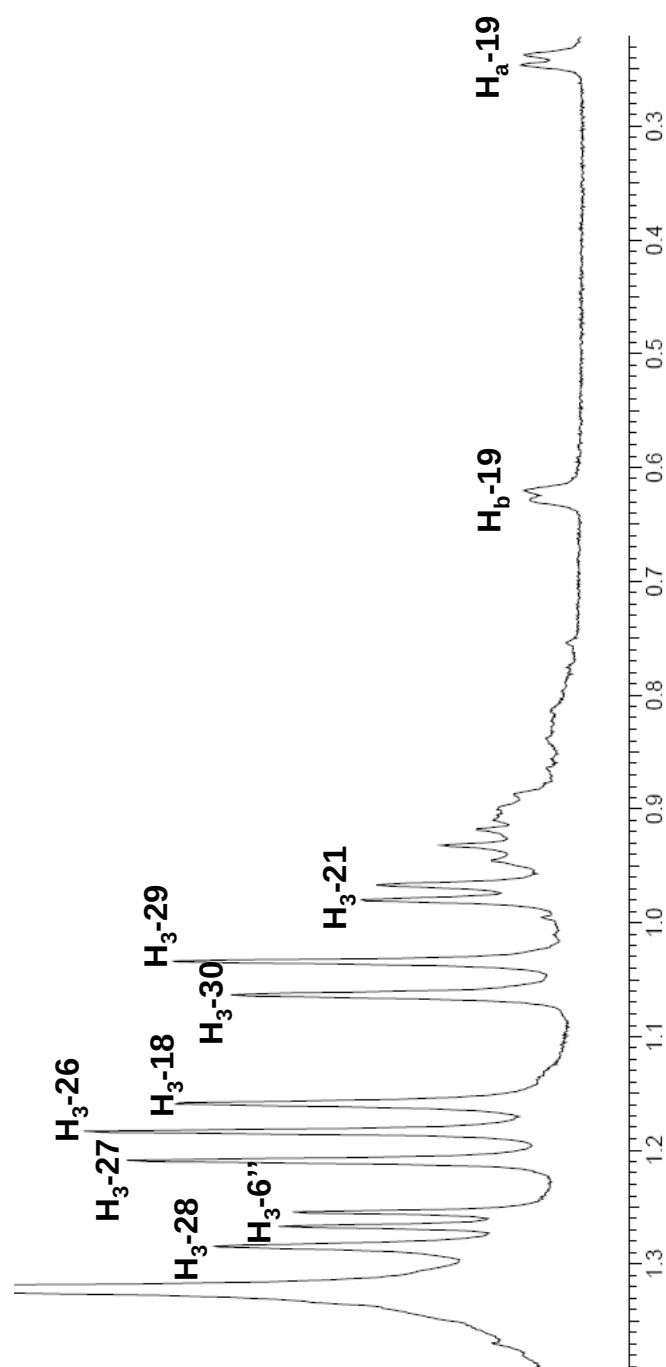
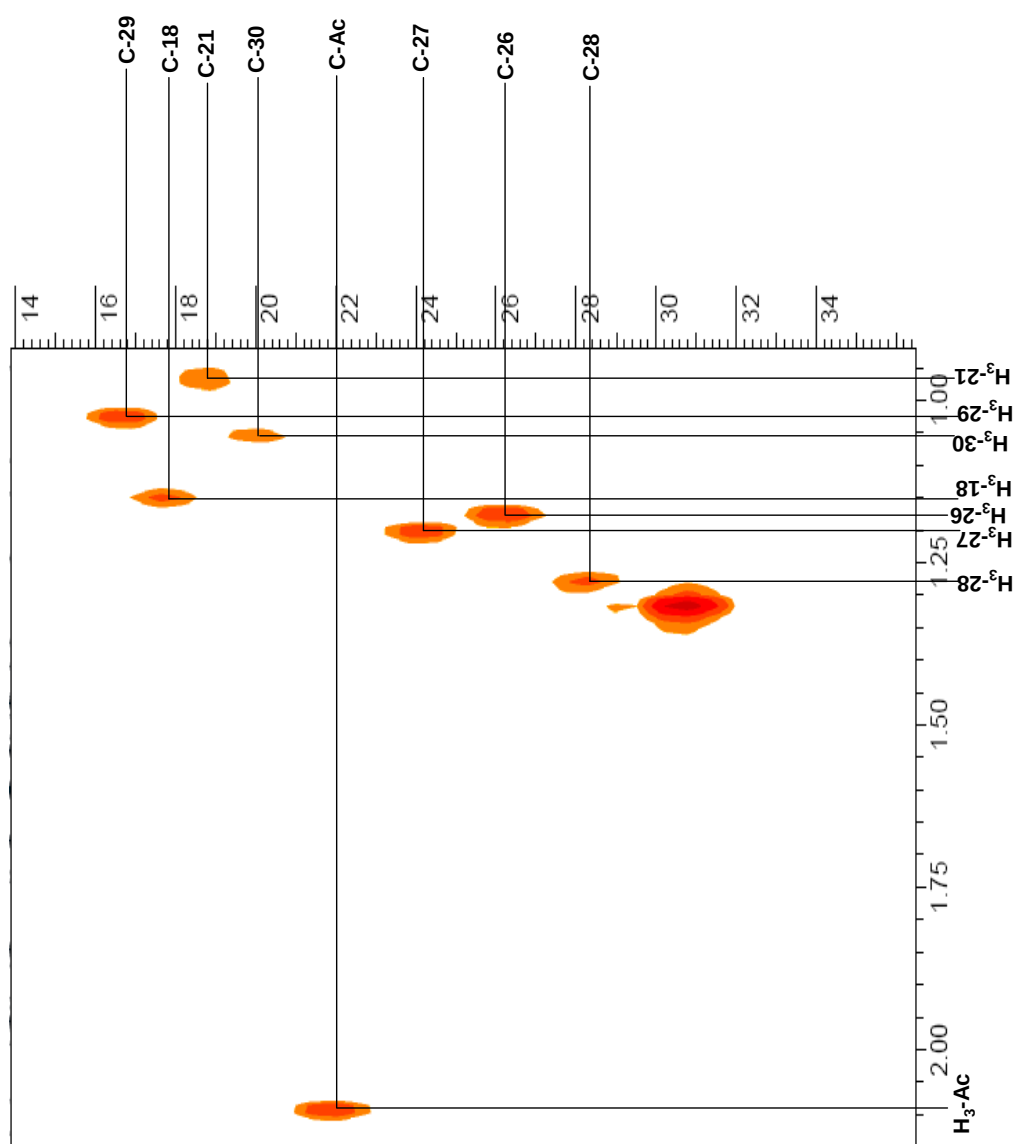
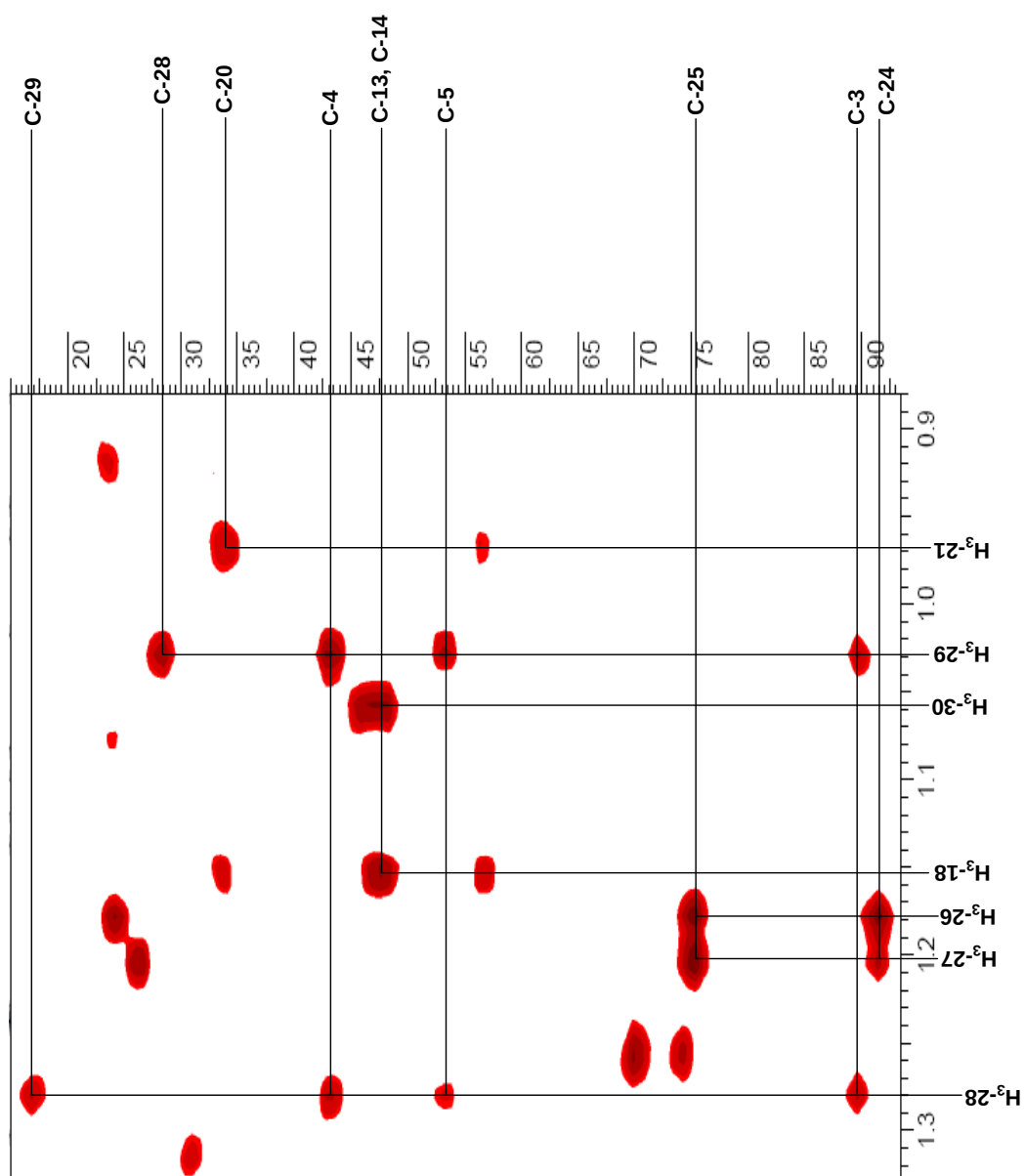


Figure 3.29. Structure of AW₁₃



Spectrum 3.76. ^1H spectrum of **AW**₁₃ in the upfield region

Spectrum 3.77. HSQC spectrum of AW₁₃

Spectrum 3.78. HMBC spectrum of AW₁₃

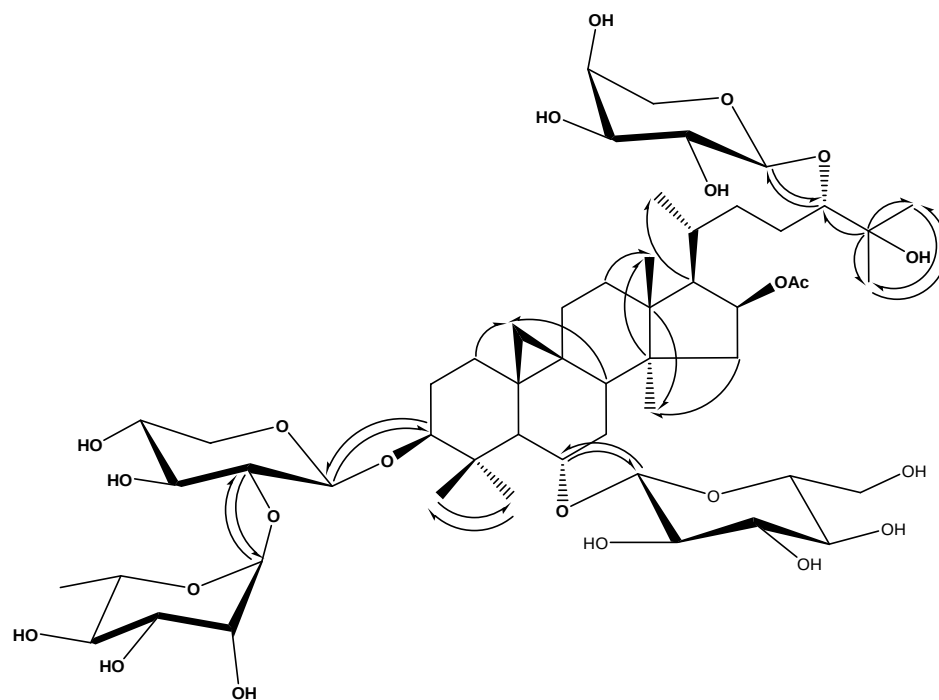


Figure 3.30. Key HMBC correlations of **AW₁₃**

Saponins are a vast class of natural products whose structural diversity includes a wide array of functional groups. Many compounds of this group are reported to have hypocholesterelomic, anti-coagulant, hepatoprotective, hypoglycemic, immunomodulatory, neuroprotective, anti-inflammatory, anti-oxidant, and anti-carcinogenic activities (Hostetmann and Marston, 1995; Rao and Gurfinkel, 2000).

Among these cycloartanes occupy a special position among low molecular bioregulators because they are produced by photosynthesizing organisms only, and one from the initial representatives of this range cycloartenol serves as key link in the biosynthesis of different phytosterols. In this connection, cycloartenol and its weakly polar derivatives widespread in the plant kingdom. In the main, the plants of *Astragalus* genera proved to be the richest source of this class of compounds.

Up to now, about four hundred cycloartane-type saponins were determined and one hundred and sixty of them were isolated from *Astragalus* genus. A total of 30 aglycones have been found in the plants under discussion. Some of these have not been characterized as pure compounds but as the corresponding glycosides (Mamedova and Isaev, 2004).

Astragalus L., the largest genus in the family Leguminosae, is represented by 380 species in the flora of Turkey (Davis, 1970).

The roots of *Astragalus* species represent a very old and well-known drug in traditional medicine for its usage as an antiperspirant,

tonic and diuretic. It has also been used in the treatment of diabetes mellitus, nephritis, leukemia and uterine cancer (Tang and Eisenbrand, 1992). In the district of Anatolia, located in South Eastern Turkey, an aqueous extract of the roots of *Astragalus* is traditionally used against leukemia and for its wound-healing properties. Known biologically active constituents of *Astragalus* roots represent two major classes of chemical compounds, polysaccharides and saponins (Tang and Eisenbrand, 1992).

Cycloartanes from *Astragalus* genus are found to possess cardiogenic, hypocholesteremic, anti-depressive and antitumor actions as well as immunomodulatory activity (Mamedova and Isaev 2004; Calis et al., 1997; Bedir et al., 2000). Especially semi-synthetic glycosides demonstrated strong cardiogenic activity and had several advantages over cardenolides owing to lack of toxicity and cumulative effects. (Mamedova and Isaev, 2004).

Earlier investigations of Turkish *Astragalus species* resulted in the isolation of about 60 cycloartane glycosides including five different aglycones (Bedir et al., 1998a, 1998b, 1999a, 1999b, 2000a, 2000b, 2001a, 2001b, and 2005; Calis et al., 1996, 1997, and 1999).

As part of our continuing studies on Turkish *Astragalus species*, we decided to carry out a full phytochemical study on two *Astragalus* species, *A. amblelepis* and *A. wiedemannianus*

Our studies resulted in the isolation of 6 metabolites from *A. amblelepis* and 11 from *A. wiedemannianus*. By using mainly 1D and 2D

NMR methods, structures of the compounds were determined to be cycloartane-type saponins except an oleanane-type glycoside from *A. wiedemannianus* (**AW₅**).

The purified cycloartanes were primarily from two different classes, 20,24-epoxy (7 number) or acyclic side chain (8 number). Additionally (1 number) a 20,25-epoxy side chain cycloartane was isolated (**AW₂**).

Apart from this, *A. wiedemannianus* and *A. amblolapis* belonging to sections Pterophorus and Rhacophorus, respectively, are evaluated from chemotaxonomy point of view. Previous studies on the members of Turkish Rhacophorus section revealed 20,24-epoxy, 20,25-epoxy and acyclic side chain cycloartanes (Bedir et al., 2001; Çalış et al., 2001; Çalış et al., 1999). Our study has afforded only acyclic side chain cycloartanes from *A. amblolapis*; however, based on preliminary works, it is not possible to draw a chemotaxonomic conclusion and state that acyclic side chain cycloartanes are characteristic feature of Rhacophorus section. Pterophorus section is correspondingly rich in terms of cycloartanes having 20,24-epoxy side-chain and also includes cycloartanes having acyclic side-chain.

When we evaluate our results from chemistry point of view, the following should be stressed. Secondary metabolite **AA₃** from *Astragalus amblolapis* has a unique feature as being the first cycloartane-type triterpene glycoside including a glucuronic acid residue. Although the cycloartanes including rhamnose unit were obtained in preliminary

studies, compounds **AA₅** and **AA₆** isolated from *Astragalus amblolepis* are the first two cycloartane-type triterpene glycosides containing rhamnose residue at C-6 position.

Tridesmosidic saponins are rarely encountered in the nature and their numbers are less than 10. Therefore, compounds **AW₁₂** and **AW₁₃** isolated from *Astragalus wiedemannianus* are exceptional as being tridesmosidic molecules. Besides, there has been only a few tetraglycosidic cycloartanes isolated so far, and both compounds **AW₁₂** and **AW₁₃** are also significant molecules due to their glycosidic nature. Moreover, the presence of arabinose unit located on acyclic side chain (**AW₁₂** and **AW₁₃**) observed for the first time in cycloartane class.

Consequently, isolation of 16 cycloartane type saponins proves once more that *Astragalus* genus is the richest source of cycloartanes. Our results are noteworthy not only for chemistry end due to the elucidation of 8 new compounds, but also for providing information on the subject of *Astragalus* chemotaxonomy.

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