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Research Article

URSANE AND OLENANE TRITERPENES FROM *ASTRAGALUS PROPINQUUS*

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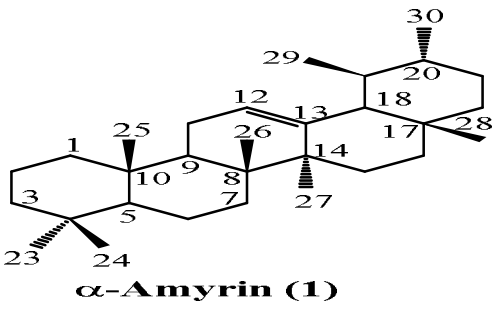
ABSTRACT

Continuation on the phytochemical studies of the dichloromethane (CH_2Cl_2) fraction of the aqueous extract of *Astragalus propinquus* furnished two triterpenes namely α-amyrin and β-amyrin. The structures of the two isolated compounds were characterized on the basis of extensive spectral studies and literature search. The complete ^1H and ^{13}C NMR spectral assignments of the two isolated compounds are reported on the basis of 1D (^1H and ^{13}C) and 2D (COSY, HSQC, and HMBC) NMR spectral data.

Keywords: *Astragalus propinquus*, Fabaceae, Triterpenes, NMR, MS, Structure elucidation.

INTRODUCTION

Astragalus root (also known as *Huang Qi*) is a staple of Traditional Chinese Medicine (TCM), it is considered as a sweet, warming herb with many medicinal properties including the treatment of fatigue, decreased appetite, general debility (particularly in the elderly), susceptibility to viral infections, non-healing wounds, fever, sweating, uterine prolapse, uterine bleeding, edema (nephritis), numbness, muscle pain, diabetes mellitus, and uterine, ovarian or colon cancer [1]. The gummy sap of astragalus (tragacanth) has been used since ancient times as a thickener and emulsifier and used today as a thickening agent for ice cream [2]. In our continuing research on the isolation of natural sweeteners from the commercial extracts of various plants obtained from across the world, we have isolated several diterpene



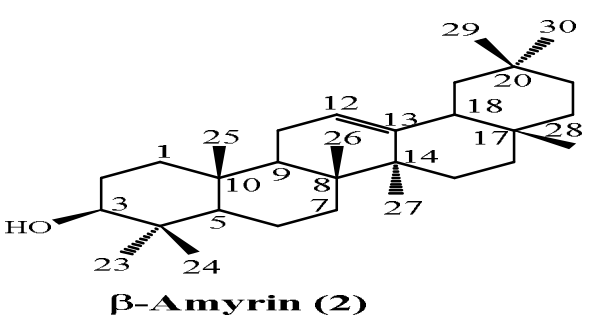


Figure 1: Structures of α-Amyrin (**1**) and β-Amyrin (**2**)

glycosides from *Stevia rebaudiana* and *Rubus suavissimus* [3-9], triterpene and phenolic glycosides from *Siraitia grosvenorii* [10-11] whose structures were characterized based on the extensive NMR and Mass spectroscopic studies as well as chemical studies. Recently we have reported the presence of three flavonoids namely salvigenin, apigenin, and luteolin from the phytochemical studies of *Astragalus propinquus* [12]. In this paper we are describing the isolation and purification of two triterpenes namely α-amyrin (**1**) and β-amyrin (**2**), from the commercial aqueous extract of *Astragalus propinquus*; their structures were characterized on the basis of COSY, HSQC, and HMBC spectral data.

EXPERIMENTAL

General Methods

NMR spectra were acquired on a Varian Unity Plus 600 MHz instrument using standard pulse sequences at ambient temperature. Chemical shifts are given in δ (ppm), and coupling constants are reported in Hz. Mass spectral (MS) data was generated with a Thermo LTQ Orbitrap Discovery mass spectrometer in the positive ion mode electrospray. Instrument was mass calibrated with a mixture of Ultramark 1621, MRFA [a peptide], and caffeine immediately prior to accurate mass measurements of the samples. Samples were diluted with water:acetonitrile:methanol (1:2:2) and prepared a stock solution of 50 ul concentration for each sample. Each sample (25 ul) was introduced via infusion using the on-board syringe pump at a flow injection rate of 120 ul/min. Low pressure chromatography was performed on a Biotage Flash system using a C-18 cartridge (40+ M, 35-70 m). TLC was performed on Baker Si-C_{18}F plates with mobile phase H_2O-MeOH (35:65). Identification of the spots on the TLC plate was carried out by spraying 10% H_2SO_4 in EtOH and heating the plate at about 80° C.

Materials

The commercial extract of *Astragalus propinquus* was supplied by Jia Herb, Parsippany, NJ 07054. A voucher specimen is deposited at The Coca Cola Company, No. VSPC-3166-169.

Isolation and Purification

The aqueous extract of the roots of *A. propinquus* (20 g) was suspended in 200 ml water and extracted successively with *n*-hexane (3 x 100 ml), CH_2Cl_2 (3 x 100 ml) and *n*-BuOH (2 x 100 ml). The CH_2Cl_2 layer was concentrated under vacuum furnished a residue (2.5 g) which was purified on a Biotage flash chromatography system using C-18 (100 g) column (solvent system: gradient from 80-20 MeOH-water to 100% MeOH at 60 ml/min. detection at UV 210 nm) for 40 min. Fractions 61-70 were combined to get a residue 0.32 g, which on repeated purification using the gradient 90-100% MeOH in water at 10 ml/min for 60 min resulted α-amyrin (**1**, 130 mg), and β-amyrin (**2**, 85 mg), respectively.

Identification of α-Amyrin (1) and β-Amyrin (2)

α-Amyrin (1): White powder (130 mg); mp: 270-272 °C; ^{1}H NMR ($CDCl_3$, 600 MHz): see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz): see Table 1; MS (*m/z*): 427 $[M+H]^+$, 419, 365, 325, 271, 163, 97.

β-Amyrin (2): White powder (85 mg); mp: 283-285 °C; ^{1}H NMR ($CDCl_3$, 600 MHz): see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz): see Table 1; MS (*m/z*): 427 $[M+H]^+$, 409, 365, 325, 271, 105, 97.

RESULTS AND DISCUSSION

Compound **1** was isolated as a white powder. The mass spectral data of compound **1** gave a molecular ion peak at m/z 427 corresponding to its $(M+H)^+$ ion suggesting the molecular formula as $C_{30}H_{50}O$, which was supported by the ^{13}C NMR spectral data. The ^{1}H NMR spectra of compound **1** showed the presence of six methyl sinlgets at δ 0.71, 0.74, 0.87, 0.91, 0.96, and 1.03, as well as two methyl doublets that appeared at δ 0.72 and 0.86. Liebermann-Burchard reaction indicated compound **1** is having a terpenoid skeleton [13-14]. The proton corresponding to a secondary hydroxyl group of a terpene moiety was appeared as a doublet of doublets at δ 3.24. Compound **1** also showed a proton at δ 5.13 as a triplet suggesting the presence of a trisubstituted olefinic bond. The ^{1}H and ^{13}C NMR values for all the protons and carbons were assigned on the basis of COSY, HMQC and HMBC correlations and were given in Table 1.

Table 1. ^{1}H and ^{13}C NMR chemical shift values for α-Amyrin (**1**) and β-Amyrin (**2**) recorded in $CDCl_3$ [a-c].

Position	1		2	
	^{1}H	^{13}C	^{1}H	^{13}C
1		39.2		39.3
2		28.1		28.3
3	3.24 (dd, 1H, *J* = 10.8, 5.4 Hz)	79.4	3.26 (dd, 1H, *J* = 10.8, 5.7 Hz)	79.6
4		39.0		39.1
5	0.71 (d, 1H, *J* = 11.4 Hz)	55.2	0.69 (d, 1H, *J* = 11.1 Hz	55.1
6		18.2		18.8
7		32.8		33.2
8		40.5		39.8
9		48.1		48.0
10		36.8		37.4
11		23.2		23.9
12	5.13 (t, 1H, *J* = 3.1 Hz)	123.8	5.18 (t, 1H, *J* = 3.4 Hz)	122.2
13		139.1		145.7
14		42.1		42.1
15		27.4		26.0
16		26.0		26.3
17		34.1		32.6
18		59.3		48.1
19		39.8		47.3
20		39.2		31.3
21		31.5		34.3
22		41.5		37.4
23	0.91 (s, 3H)	28.3	0.77 (s, 3H)	28.2
24	0.74 (s, 3H)	15.8	0.91 (s, 3H)	16.1
25	0.71 (s, 3H)	15.8	0.76 (s, 3H)	16.1
26	0.87 (s, 3H)	16.7	0.94 (s, 3H)	17.2
27	1.03 (s, 3H)	23.5	1.21 (s, 3H)	24.4
28	0.96 (s, 3H)	28.2	1.09 (s, 3H)	28.2
29	0.86 (d, 3H, *J* = 6.4 Hz)	17.8	0.85 (s, 3H)	33.9
30	0.72 (d, 3H, *J* = 7.2 Hz)	22.1	0.78 (s, 3H)	23.8

[a] assignments made on the basis of COSY, HMQC and HMBC correlations;

[b] Chemical shift values are in δ (ppm);

[c] Coupling constants are in Hz.

A search in literature found that the spectral data of **1** was supportive to the structure of a ursane triterpene skeleton having a hydroxyl group at C-3 position with a double bond at C-12/C-13.

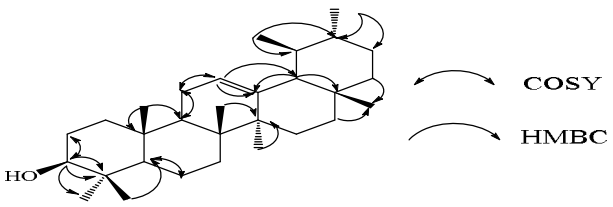


Fig. 2: Key COSY and HMBC correlations of α-Amyrin (**1**)

Thus, the structure of **1** was assigned as the known compound α-amyrin. The physical and spectral data are consistent to the reported literature values [15-16] of α-amyrin which was further confirmed by the key COSY and HMBC correlations as shown in Figure 2.

Compound **2** was also isolated as a white powder and its mass spectral data suggested the molecular formula as $C_{30}H_{50}O$, identical to **1**, which was supported by the ^{13}C NMR spectral data. Compound **2** also showed positive Liebermann-Burchard reaction for terpenes as in **1**. The ^{1}H NMR spectra of compound **2** showed the presence of eight methyl sinlgets at δ 0.76, 0.77, 0.78, 0.85, 0.91, 0.94, and 1.21. The ^{1}H NMR spectra of compound **2** also showed a proton corresponding to the H-3 of a terpene moiety which was appeared as a doublet of doublets at δ 3.26 and a proton at δ 5.18 as a triplet suggesting the presence of a trisubstituted olefinic bond. The ^{1}H and ^{13}C NMR values for all the protons and carbons were assigned on the basis of COSY, HMQC and HMBC correlations and were given in Table 1.

The above spectral data supported the presence of oleanane triterpene skeleton having a hydroxyl group at C-3 position with a double bond at C-12/C-13 with eight methyl groups. A search in literature found that the spectral data of **2** was supportive to the structure of β-amyrin, an oleanane triterpene skeleton having a hydroxyl group at C-3 position with a double bond at C-12/C-13 which was further supported by the key COSY and HMBC correlations as shown in Figure 3. Thus, the structure of **2** was assigned as β-amyrin that was consistent to the reported literature values [15-17].

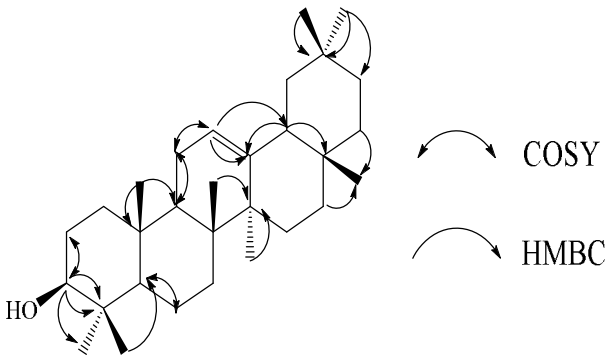


Fig. 3: Key COSY and HMBC correlations of β-Amyrin (**2**)

CONCLUSION

Two known triterpenes were isolated from the commercial aqueous extract of *Astragalus propinquus*. The structures of the isolated compounds were identified as α-amyrin (**1**), and β-amyrin (**2**), on the basis of spectroscopic and chemical studies as well as by comparing their physical and spectral properties reported in the literature.

ACKNOWLEDGEMENT

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