BETULINIC ACID AND STEROLS FROM *Astragalus altaicus*

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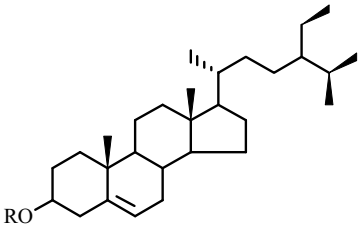
UDC 547.918:547.926

Astragalus altaicus Bunge (Leguminosae) (root of *Astragalus*, *Huangqi*) is used in Uyghur traditional medicine as an immunostimulant, hepatoprotector, diuretic, antidiabetic, expectorant, and sedative [1]. However, there are no data on the chemical composition of this plant. Therefore, we studied roots of *A. altaicus*.

The plant was collected during flowering in July 2008 in the Altay, in the Uyghur Autonomous Region of Xinjiang, P. R. China. The plant was identified by Prof. Doc. Yan Fu Zhang (Institute of Medicine Inspection Department of Altay City Xinjiang of China).

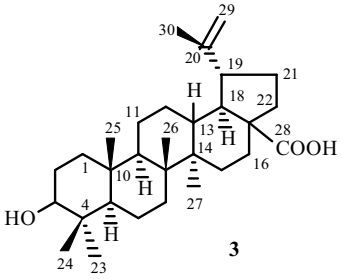
Air-dried roots (400 g) were extracted exhaustively with MeOH (1.5 L × 4). The MeOH extract was evaporated to dryness to afford total extracted compounds (10% of air-dried raw material). The whole extract was placed on a column of silica gel and eluted successively with $CHCl_3$, $CHCl_3$:CH_3OH (20:1 and 10:1), and $CHCl_3$:CH_3OH:H_2O (70:12:1)

Fractions eluted by $CHCl_3$ and containing slightly polar compounds were combined and rechromatographed over the column using C_6H_6:$CHCl_3$:EtOAc (5:1:1) to afford β-sitosterol (**1**, 25 mg, 0.00625%), $C_{29}H_{50}O$, mp 131–133°C (MeOH) [2]. Mass spectrum (ES PI, *m*/*z*): $[M + Na]^+$ 437; (ES NI, *m*/*z*): $[M - H]^-$ 413.



1, 2

1: R = H; **2:** R = β-D-Glc*p*



3

Fractions eluted by $CHCl_3$:CH_3OH (20:1) and containing triterpenoid **3** were combined and recrystallized from MeOH. Several recrystallizations afforded **3** (700 mg, 0.175%), $C_{30}H_{48}O_3$, mp 316-318°C. IR spectrum (KBr, ν, cm^{-1}): 3449 (OH), 1692 (COOH).

Mass spectrum (ES PI, *m*/*z*): $[M + Na]^+$ 479; (ES NI, *m*/*z*) $[M - H]^-$ 455.

PMR, ^{13}C NMR, DEPT, COSY, HSQC, and HMBC spectra were interpreted (Table 1) and determined that triterpenoid **3** was betulinic acid [3-5].

The ^1H resonances at δ 1.75 (t, $^3J_1 = {}^3J_2 = 11.4$ Hz) and δ 3.53 (td, $^3J_1 = {}^3J_2 = 11.4$ Hz, $^3J_3 = 5.4$ Hz) in the PMR spectrum of betulinic acid (C_5D_5N) for H-18 and H-19 were interesting. Judging from the multiplicity, the first resonance belongs to H-18; the second, H-19. The resonance for H-18 in the HSQC spectrum of **3** had a correlation peak with the resonance at δ 49.73; the resonance for H-19, at δ 47.76. This indicated that they belonged to C-18 and C-19, respectively. This conclusion was confirmed by the HMBC spectrum, where resonances for CH_3-30 and the olefinic proton (δ 4.94) correlated with that for C-19. Thus, we re-examined the assignment of the resonances for C-18 and C-19 given in the literature [3-5].

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 0009-3130/09/4504-0592

TABLE 1. Chemical Shifts of C and H Atoms of Betulinic Acid (**3**), DEPT Data, and Parameters of 2D ^1H–^1H COSY, HMQC, and HMBC Spectra (δ, ppm, J/Hz, C_5D_5N and $CDCl_3$, 0 = TMS)

C atom	DEPT	Data in C_5D_5N			Data in $CDCl_3$		
		δ_C	δ_H (J/Hz)	HMBC (C atoms)	δ_C	δ_H (J/Hz)	HMBC (C atoms)
1	CH_2	39.26	1.65 dt (13.2, 3), 1.88		38.68	1.19 dt (13.8, 3)	
2	CH_2	28.30	1.84, 1.84		27.36	1.56, 1.56	
3	CH	78.09	3.45 t (7.8)	23, 24	78.99	3.19 dd (11.4, 4.8)	
4	C	39.51	–		38.84	–	
5	CH	55.89	0.82 dd (9.6, 1.8)		55.31	0.68 br.d	1, 24
6	CH_2	18.76	0.97, 1.36		18.26	1.40	
7	CH_2	34.80	1.38, 1.42		34.29		
8	C	41.09	–		40.66	–	
9	CH	50.93	1.40	18, 21, 30	50.49	1.26	
10	C	37.50	–		37.18	–	
11	CH_2	21.18	1.42, 1.20		20.83	1.24	
12	CH_2	26.09	1.19, 1.93		25.47	1.30, 1.68	
13	CH	38.59	2.73 td (12.6, 3.6)		38.35	2.20 td (12.2, 3.6)	
14	C	42.83	–		42.41	–	
15	CH_2	31.18	2.26, 1.53		30.52		
16	CH_2	32.85	2.62 dt (12.6, 3.6), 1.55		32.13		
17	C	56.61	–		56.26	–	
18	CH	49.73	1.75 t (11.4)		49.23	1.61 t (11.4)	13, 17, 19
19	CH	47.76	3.53 td (11.4, 5.4)		46.87	3.00 td (10.2, 5.4)	
20	C	151.32	–		150.41	–	
21	CH_2	30.26	1.25, 1.53		29.68	1.42, 1.97	
22	CH_2	37.57	1.58, 2.26		37.01	1.97	18
23	CH_3	28.65	1.22 s	3, 4, 5, 24	27.95	0.96 s	4, 5, 24
24	CH_3	16.40	1.00 s	3, 4, 5, 23	15.31	0.75 s	4, 5, 23
25	CH_3	16.40	0.81 s	1, 5, 9, 10	16.12	0.82 s	1, 5, 9, 10
26	CH_3	16.34	1.05 s	7, 9, 14	16.01	0.94 s	7, 8, 9, 14
27	CH_3	14.88	1.06 s	13, 15	14.66	0.98 s	8, 14, 15
28	C	178.86	–		*	–	
29	CH_2	109.95	4.76,	30	109.67	4.61 br.s, 4.47 br.s	
			4.94 d (2.4)	19	19.36	1.69 s	19
30	CH_3	19.45	1.78 s	19			

Chemical shifts of protons without multiplicities and SSCC were found from 2D spectra; *this spectral region was not recorded.

Fractions eluted by $CHCl_3$:CH_3OH (10:1) and $CHCl_3$:CH_3OH:H_2O (70:12:1) that contained a pure compound were combined. Recrystallization of the combined fraction from MeOH produced a glycoside **2** (75 mg, 0.01875%), $C_{35}H_{60}O_6$, mp 277–279C, that was identified as *β*-sitosterol *β*-D-glucopyranoside also by direct comparison with an authentic sample on TLC and PMR and ^{13}C NMR spectra [2].

PMR spectrum (C_5D_5N, 600 MHz, δ, ppm, J/Hz, 0 = TMS): 0.65 (CH_3-18, s), 0.85 and 0.87 (CH_3-26, CH_3-27, d, ^3J = 6.6), 0.88 (CH_3-29, t, $^3J_1 = {}^3J_2$ = 7.2), 0.92 (CH_3-19, s), 0.98 (CH_3-21, d, ^3J = 6.6), 2.47 and 2.73 (2H-4, m), 3.95 (H-3, m), 3.99 (H-5 of D-Glc*p*, m), 4.07 (H-2 of D-Glc*p*, t, $^3J_1 = {}^3J_2$ = 7.8), 4.29-4.31 (H-3 and H-4 of D-Glc*p*, m), 4.42 (H-6a of D-Glc*p*, dd, ^2J = 12, ^3J = 4.8), 4.57 (H-6b of D-Glc*p*, dd, ^2J = 12, ^3J = 1.2), 5.06 (H-1 of D-Glc*p*, d, ^3J = 7.8), 5.34 (H-6, m).

^{13}C NMR spectrum (150 MHz, C_5D_5N, δ, ppm, 0 = TMS): 37.48 (C-1), 30.26 (C-2), 78.63 (C-3), 39.34 (C-4), 140.90 (C-5), 121.94 (C-6), 32.18 (C-7), 23.05 (C-8), 50.34 (C-9), 36.93 (C-10), 21.29 (C-11), 39.95 (C-12), 42.48 (C-13), 56.82 (C-14), 24.51 (C-15), 28.55 (C-16), 56.24 (C-17), 12.16 (C-18), 19.99 (C-19), 36.40 (C-20), 19.42 (C-21), 34.21 (C-22), 26.37 (C-23), 46.03 (C-24), 29.45 (C-25), 19.01 (C-26), 19.21 (C-27), 23.39 (C-28), 11.99 (C-29), 102.59 (C-1′), 75.36 (C-2′), 78.53 (C-3′), 71.69 (C-4′), 78.08 (C-5′), 62.84 (C-6′).

Thus, roots of *A. altaicus* contained two steroids (*β*-sitosterol and *β*-sitosterol *β*-D-glucopyranoside) and one lupane triterpenoid (betulinic acid). A triterpenoid of the lupane class was found for the first time in plants of the genus *Astragalus*.

ACKNOWLEDGMENT

The study was supported by CAS/SAFEA International Partnership Program for Creative Research Teams; Key Project of Knowledge Innovation Program of Chinese Academy of Sciences (Contract Grant No. KGCX2-SW-507) and a grant from "Western Light" (XIBUZHIGUANG) Program of Talent Cultivation of Chinese Academy of Sciences at 2007.

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