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CICLOASTRAGENOL GLYCOSIDES FROM *ASTRAGALUS VERRUCOSUS*

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Key Word Index—*Astragalus verrucosus*; Leguminosae aerial parts; triterpene cycloartane-type glycosides; astraverrucin IV, V and VI; D-pinitol.

Abstract—Three new cycloartane-triterpene glycosides, astraverrucin IV, V and VI, were isolated from the aerial parts of *Astragalus verrucosus*. Based on spectroscopic analysis (IR, 1D and 2D-NMR, FABMS), the structures of the saponins were established to be cycloastragenol 3-*O*-[α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranoside], cycloastragenol 3-*O*-[α-L-rhamnopyranosyl(1 → 4)]-β-D-(3-*O*-acetyl)glucopyranoside and cycloastragenol 3-*O*-[α-L-rhamnopyranosyl (1 → 4)]-β-D-(6-*O*-acetyl)-glucopyranoside. D-pinitol and astraverrucin I were also obtained. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Astragalus L., the largest genus in the family Leguminosae, is represented by 34 species in the Italian flora [1]. The roots of various *Astragalus* species represent a very old and well-known drug used in traditional Chinese medicine as an antiperspirant, diuretic and tonic and as an anticancer agent [2].

Previous studies performed on this genus have resulted in the isolation of a series of cycloartane-type triterpenoid glycosides [3]. An earlier chemical investigation of the aerial parts of *Astragalus verrucosus* Moris led to the isolation of three saponins, astraverrucin I, II and III [4]. This paper deals with the isolation and structural elucidation of three novel cycloartane-type triterpenoid glycosides, named astraverrucin IV (**1**), V (**2**) and VI (**3**), from the same source. In addition, D-pinitol and astraverrucin I were isolated and identified.

RESULTS AND DISCUSSION

After repeated column chromatography of the EtOAc- and *n*-BuOH- soluble parts of a MeOH extract, three new cycloartane-type triterpenoids (**1**–**3**) were obtained together with astraverrucin I and D-pinitol.

Astraverrucin IV (**1**), the most polar astraverrucin, exhibited a strong IR absorption band at 3370 cm^{-1} suggesting the presence of hydroxyl groups. The base peak at *m/z* 143 in the EI-mass spectrum of **1** resulted from cleavage of the cycloartane skeleton between C-17 and C-20 and suggested the presence of a 25-hydroxy-20,24-epoxy residue, as in astraverrucins I-III [4]. The FAB-mass spectrum contained a $[M + Na]^+$ peak at *m/z* 821, corresponding to the molecular formula $C_{47}H_{70}O_{14}$.

The ^1H NMR spectrum (300 MHz, Py-d_5) showed signals characteristic of cyclopropane-methylene protons (δ 0.25 and 0.6, each *d*, *J* = 3.8 Hz, H_2-19), seven tertiary methyls (singlets at δ 1.0, 1.30, 1.30, 1.35, 1.35, 1.60 and 2.05) and one secondary methyl group (δ 1.70, *d*, *J* = 6.1) assigned to an α-L-rhamnose unit. Additionally, the resonances for two anomeric protons (δ 4.90, *d*, *J* = 7.6 Hz, β-D-glucose; δ 5.80, *br s*, α-L-rhamnose) were observed. This was supported by the ^{13}C NMR spectrum, which revealed two anomeric carbons at δ 106.8 (C-1′) and 102.7 (C-1″).

Thus astraverrucin IV (**1**) appeared to be a glycoside of cycloastragenol [20(*R*),24(*S*)-epoxy-9,19-cyclolanostan-3β,6α,16β,25-tetrol], an aglycone commonly found in many astragalosides isolated from *Astragalus* species and already found in astraverrucins I-III [4]. The ^1H and ^{13}C NMR data for H-24 and C-24 were comparable to those reported

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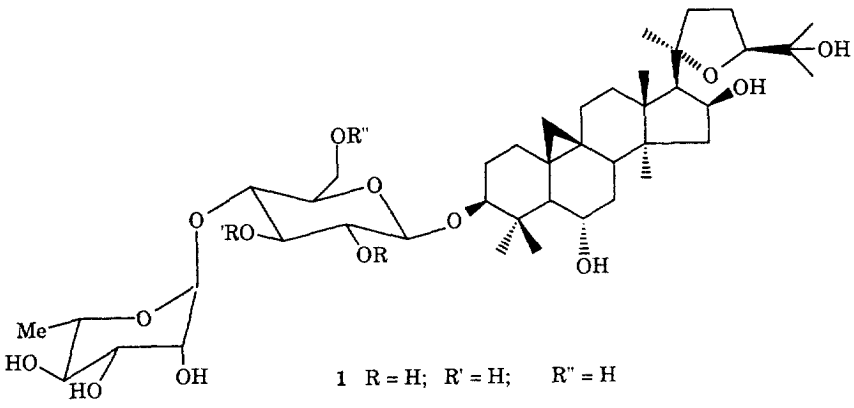

1 R = H; R' = H; R'' = H

2 R = H; R' = OAc; R'' = H

3 R = H; R' = H; R'' = OAc

for analogous compounds having a 24*S* configuration.

The ^{13}C NMR spectrum of **1** displayed a total of 42 carbon signals, including eight methyl groups and seven quaternary carbons. All signals were assigned on the basis of a combination of 1D and 2DNMR techniques: ^{1}H-^{1}H COSY, DEPT, HETCOR, COLOC and ROESY experiments and by comparison with ^{13}C data of related compounds [5]. The chemical shifts of astraverrucin IV (**1**) are reported in Table 1. The 2D ^{13}C-^{1}H correlation spectrum obtained through $^{3}J_{C,H}$ showed significant cross-peaks between the quaternary C-20 (δ 87.2) and of H-21, and the quaternary C-25 (δ 71.3) and H-26 and H-27. The quaternary carbon C-4 (δ 42.6) also correlated with H-28 and H-29. Table 1 summarizes also the most significant cross-peaks observed in the 2D ROESY experiment. This experiment made it possible to confirm the location and the stereochemistry of the ^{1}H resonances of the molecule.

All the above data suggest that astraverrucin IV (**1**) contained one more sugar moiety (α-L-rhamnose) than astraverrucins I-III. The nature of each sugar residue and the interglycosidic linkage were established by the combined use of the COSY and HOHAHA spectra and by analysis of ^{1}H-^{1}H coupling constants. The site of attachment of the glucose moiety of **1** at C-3 of the aglycone was determined by means of the diagnostic glycosidation shifts of this carbon atom. This assignment was confirmed by an intense cross-peak correlation in the ROESY spectrum between H-3 and H-1′ (Table 1). Since no other aglycone carbons showed glycosylation shifts, the second sugar moiety (α-L-rhamnose) had to be attached to one hydroxyl group of glucose. Thus, astraverrucin IV (**1**) had a monodesmosidic structure, as indicated by the fragmentation peak at *m/z* 356 $[M - glc - rha]^+$.

C-4′ of glucose showed a glycosidation shift (78.4 ppm), while none of the carbons of rhamnopyranose showed any shift. Therefore, it followed that the glucopyranose moiety must be linked to the 3-hydroxyl group of the aglycone and rhamnopyranose to the 4′-hydroxyl group of glucopyranose. As shown in Table 1, strong cross-peaks were detectable between H-1″ and H-3″, H-4″, H-5″ in the ROESY spectrum of **1**. Based on the foregoing evidence, the structure of astraverrucin IV (**1**) was determined as cycloastragenol 3-*O*-[α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranoside].

Astraverrucins V (**2**) and VI (**3**) were found to have the same molecular formular, $C_{44}H_{72}O_{15}$ (FAB-MS, *m/z* 863 $[M + Na]^+$ and 841 $[M + H]^+$). The $[M + Na]^+$ peak was 42 amu higher than in **1**, indicating the presence of an acetoxy group which was confirmed in the ^{1}H NMR spectra (Table 2). The ^{1}H and ^{13}C NMR spectra of **2** and **3** were very similar to those of compound **1**, except for the presence of an acetoxy group in both compounds. Additional fragmentation peaks in the FAB-mass spectrum at *m/z* 473 $[M + H - glc - rha - OAc]^+$, 495 $[M + Na - glc - rha - OAc]^+$ and 675 $[M - rha]^+$ indicated that the acetyl group was linked to the glucose in the sugar portion. The positions of the acetoxy function were determined on the basis of the acetylation shifts in the ^{13}C NMR spectra, in comparison with those observed for **1**. As reported in Table 2, significant acetylation shifts occurred for the signals of C-2′ (−2.0), C-3′ (+0.7) and C-4′ (−2.4) for **2** and of C-5″ (−2.0) and C-6′ (+1.5 in comparison with compound **1**, +1.3 if compared with astraverrucin I) for **3**, while none of the carbons of rhamnopyranose showed a significant shift.

Therefore, the structures of astraverrucin V (**2**) and VI (**3**) were established to be cycloastragenol

Table 1. ^{1}H and ^{13}C NMR data for of astraverrucin IV (**1**) in pyridine-d_5

	H	^{1}H(δ ppm)	Multiplicity (J)	C	^{13}C (δ ppm)	Correlation in the ROESY spectrum
Aglycone	1ax;eq	1.58; 1.15		1	32.4	
	2ax;eq	1.92; 2.4		2	30.1	
	3ax;α	3.63	*dd (4.6; 11.8)*	3	89.2	1ax, 5, 28, 1′
	4			4	42.6	
	5ax;α	1.75		5	54.0	
	6ax;β	3.75		6	68.0	19β, 28
	7ax;eq	1.6; 1.8		7	38.7	
	8	1.9		8	47.2	
	9			9	21.6	
	10			10	29.5	
	11ax;eq	2.05; 2.32		11	26.5	
	12ax;eq	1.7; 3.1		12	34.9	
	13			13	45.0	
	14			14	46.9	
	15ax;eq	1.78; 2.17		15	46.1	
	16α	5.02		16	73.5	15eq, 30, 17
	17α	2.55	*d (7.5)*	17	58.4	12ax, 21, 16, 30
	18	1.35		18	20.9	
	19α	0.25	*d (3.8)*	19	30.6	1eq
	19β	0.6	*d (3.8)*			2ax, 6, 29
	20			20	87.2	
	21	1.30		21	27.1	
	22ax;eq	1.3; 1.7		22	33.4	
	23ax;eq	2.05; 2.38		23	26.2	
	24 (*S*)	3.93		24	81.7	26 (27)
	25			25	71.3	
	26 (27)	1.6		26	28.2	24
	27 (26)	1.3		27	28.6	24
	28	2.05		28	29.0	29
	29	1.35		29	16.7	6ax, 19 *b*, 28
	30	1.0		30	20.2	15eq
b-D-glc	1′	4.9	*d (7.6)*	1′	106.8	3
	2′	4.05	*dd (7.6; 8.2)*	2′	76.1	
	3′	4.2	*dd (8.2; 9.3)*	3′	76.8	
	4′	4.42	*dd (9.3; 8.9)*	4′	78.4	
	5′	3.7	*m*	5′	77.0	
	6′a,b	4.12; 4.35		6″	63.0	
a-L-rha	1″	5.8	*br s*	1″	102.7	3′, 4′, 5′
	2″	4.7	*d (3.0)*	2″	72.6	
	3″	4.58	*dd (3.0; 9.3)*	3″	72.8	
	4″	4.35	*t (9.3; 9.6)*	4″	74.0	
	5″	5.00	*dq (9.6; 6.1)*	5″	70.3	
	6″	1.7	*(6.1)*	6″	19.0	

*The structure of astraverrucin IV (**1**) was established by a combination of 1D and 2D NMR techniques. The ^{1}H NMR signals were assigned by 2D-^{1}H-^{1}H correlation (COSY and TOCSY experiments)

3-*O*-[α-L-rhamnopyranosyl(1 → 4)]-β-D-(3-*O*-acetyl)-glucopyranoside (**2**) and cycloastragenol 3-*O*-[α-L-rhamnopyranosyl(1 → 4)]-β-D-(6-*O*-acetyl)glucopyranoside (**3**).

Together with these three new saponins, astraverrucin I [4] and D-pinitol [6] were also isolated and identified by comparison with authentic samples.

Table 2. ^{1}H and ^{13}C NMR chemical shifts in pyridine-d_5 solution of sugar moiety

H	**1**	**2**	**3**	C	**1**	**2**	**3**
1′	4.9	4.9	4.9	1′	106.8	106.5	105.5
2′	4.05	4.05	4.25	2′	76.1	74.1	74.2
3′	4.2	5.75	4.15	3′	76.8	77.5	79.7
4′	4.42	4.45	4.20	4′	78.4	76.0	77.6
5′	3.7	3.65	3.95	5′	77.0	77.0	74.8
6′	4.12–4.35	4.12–4.35	4.78–4.84	6′	63.0	62.0	64.5
1″	5.8	5.75	6.5	1″	102.7	103.0	101.9
2″	4.7	4.63	4.8	2″	72.5	73.0	72.0
3″	4.58	4.55	4.68	3″	73.0	72.5	72.5
4″	4.35	4.3	4.32	4″	74.0	74.0	74.2
5″	5.00	4.85	4.88	5″	70.0	72.5	69.7
6″	1.7	1.65	1.7	6″	19.0	19.0	18.8
*CO*Me						170.9	170.8
CO*Me*		2.19	*2.1*			21.7	21.5

EXPERIMENTAL

General

^1H and ^{13}C NMR: Bruker AC-300 spectrometer with chemical shifts referenced to the residual solvent signal (Py: δH = 8.71/7.55/7.19; δC = 149.9/135.5/123.5). Carbon multiplicities were determined by DEPT 90° and 135° pulse sequences. Homonuclear 1H connectivities were determined by COSY and TOCSY experiments. HETCOR experiments were performed using standard Bruker software. The ROESY spectrum [7] was run with analogous resolution features. A mixing time of 0.4 sec was adopted for the time-shared spin-lock duration. FAB-MS: a dithiodiethanol matrix in the positive ion mode on a VG ZAB instrument (Xe atoms of energy of 2,6 KV); EI-MS: direct inlet (20 eV); TLC: silica gel (Merck); CC: Sephadex LH-20 (Pharmacia) and Silica gel 60 (70–230 and 230–400 mesh, Merck).

Plant material

Astragalus verrucosus Moris, harvested in Italy (Is Pisittus, Sardinia island), was collected in June 1994 and a voucher specimen is deposited in the Herbarium of the Istituto di Botanica ed Orto botanico, Università di Urbino, Italy.

Extraction and isolation

Air-dried powdered aerial parts of the plant (1100 g) were defatted with *n*-hexane and extracted in a Soxhlet apparatus with $CHCl_3$ followed by MeOH. The MeOH residue (293.1 g) was suspended in H_2O and then successively extracted with EtOAc and *n*-BuOH.

The EtOAc-soluble part (60.6 g) was chromatographed on a Sephadex LH-20 column (MeOH–$CHCl_3$ 9:1 as solvent) to give seven crude fractions A-G. Fr. B, on repeated flash chromatography (eluted with MeOH–$CHCl_3$ 9:1), furnished ten frs. B_1–B_{10}. Part of the fraction B_5 (175.5 mg) was rechromatographed by silica gel CC eluting with $CHCl_3$–MeOH–H_2O (7:13:8, lower layer) to obtain six subfractions (I–VI). Fr. III (35.8 mg) contained compound **2**. Fr. B_6 was purified by prep. TLC on silica gel G developed in $CHCl_3$–MeOH (17:3) to give 21.3 mg of **3**.

The *n*-BuOH extract (10.9 g) was fractionated on a Sephadex LH-20 column (eluted with MeOH–H_2O 4:1) to give nine frs (Frs. A–I). Pure D-pinitol (62.8 mg) was sepd. from Fr. C by crystallization; the remaining part of was separated into six subfractions (Frs. C_1–C_6), by repeated CC on silica gel eluting with $CHCl_3$–MeOH–H_2O (70:23:4, lower layer). Compound **1** (94.7 mg), along with compound **3** (28.4 mg) and astraverrucin I (27.7 mg) [4], was obtained from Fr. C_3 by CC on silica gel eluting with $CHCl_3$–MeOH–H_2O (6:2:0.1).

Astraverrucin IV (**1**)

$[\alpha]_D^{20}$ = +10.5 (MeOH, *c* 0.18); TLC ($CHCl_3$–MeOH–H_2O 6:2:0.1): R_f 0.37; IR ν_{max}^{nujol} cm^{-1} 3370, 1732, 1050; FAB-MS *m/z*: 821 $[M + Na]^+$, 799 $[M + H]^+$; EI-MS *m/z* (rel. int.): 185 (8.39), 143 (82.32), 125 (93.50), 107 (70.73), 95 (55.39), 85 (71.71, 71 87.12), 59 (100.00); ^1H and ^{13}C NMR (pyridine-d_5): Tables 1 and 2.

Astraverrucin V (**2**)

$[\alpha]_D^{20}$ = +12.2 (MeOH; *c* 0.27); TLC ($CHCl_3$–MeOH–H_2O 6:2:0.1): R_f 0.83; IR ν_{max}^{nujol} cm^{-1}: 3430, 1732, 1250, 1070; FAB-MS *m/z*: 863 $[M + Na]^+$, 841 $[M + H]^+$, 676 $[M - rha]^+$, 495 $[M + Na - glc - rha - OAc]^+$, 473 $[M + H - glc - rha - OAc]^+$, 495, 473, 437, 143, 85; EIMS *m/z* (rel. int.): 185 (4.27), 157 (6.49), 143 (40.39), 125 (14.38), 105 (17.54), 91 (20.44), 71 (48.34), 59 (100.00); ^1H NMR aglycone (pyridine-d_5): *d* 0.21 (1H, *d*, *J* = 3.4 Hz, H-19a), 0.54 (1H, *d*, *J* = 3.5 Hz, H-19b), 0.97 (3H, *s*, Me-30), 1.28 (6H, *s*, Me-21 and Me-29), 1.37 (3H, *s*, Me-18), 1.40 (3H, *s*, Me-29), 1.56 (3H, *s*, Me-26), 1.66 (3H, *d*, *J* = 5.7 Hz, Me-6″), 1.96 (3H, *s*, Me-28), 2.19 (3H, *s*, MeCO), 2.52 (1H, *d*, *J* = 7.6 Hz, H-17), 3.65 (1H, *m*, H-6), 3.50 (1H, *m*, H-3), 3.72 (1H, *m*, H-6), 4.80 (1H, *m*, H-24); ^{13}C NMR aglycone (pyridine-d_5): *d* 16.7 (C-29), 20.2 (C-30), 21.0 (C-18), 21.4 (C-9), 21.7 (*Me*CO), 26.3 (C-11), 26.5 (C-23), 27.3 (C-21), 28.3 (C-26), 28.7 (C-27), 29.0 (C-28), 29.5 (C-10), 30.1 (C-2), 30.8 (C-19), 32.4 (C-1), 33.4 (C-12), 35.0 (C-22), 38.8 (C-7), 42.6 (C-4), 45.1 (C-13), 46.2 (C-14), 46.7 (C-15), 47.3 (C-8), 54.0 (C-5), 58.4 (C-17), 68.2 (C-6), 71.4 (C-25), 73.6 (C-16), 81.8 (C-24), 87.3 (C-20), 89.5 (C-3), 170.9 (Me*CO*). For sugar ^1H and ^{13}C NMR (pyridine-d_5), see Table 2.

Astraverrucin VI (**3**)

$[\alpha]_D^{20}$ = +2.86 (MeOH, *c* 0.7); TLC ($CHCl_3$–MeOH–H_2O 6:2:0.1): R_f 0.34; IR ν_{max}^{nujol} cm^{-1}: 3410, 1730, 1250, 1030; FAB-MS *m/z*: 863 $[M + Na]^+$, 841 $[M + H]^+$, 550, 495 $[M + Na - glc - rha - OAc]^+$, 473 $[M + H - glc - rha - OAc]^+$, 437, 143, 85; EIMS *m/z* (rel. int.): 149 (25.62), 109 (27.48), 97 (44.32), 85 (51.27), 69 (78.19), 57 (100.00); ^1H NMR aglycone (pyridine-d_5): δ 0.21 (1H, *m*, H-19a), 0.54 (1H, *m*, H-19b), 0.99 (3H, *s*, Me-30), 1.30 (3H, *s*, Me-27), 1.30 (3H, *s*, Me-21), 1.38 (3H, *s*, Me-18), 1.56 (3H, *s*, Me-29), 1.6 (3H, *s*, Me-26), 1.68 (3H, *d*, *J* = 6.1 Hz, Me-6″), 1.70 (1H, *m*, H-5), 1.97 (3H, *s*, Me-28), 2.1 (3H, *s*, MeCO), 2.51 (1H, *d*, *J* = 7.7 Hz, H-17), 3.05 (1H, *m*, H-12eq), 3.55 (1H, *dd*, *J* = 4.2–12 Hz, H-3ax), 3.86 (1H, *m*, H-24), 5.00 (1H, *m*, H-16); ^{13}C NMR aglycone (pyridine-d_5): δ 16.7 (C-29), 20.2 (C-30), 21.0 (C-18), 21.4- (C-9), 21.5 (*Me*CO), 26.2 (C-23), 26.5 (C-11), 27.1 (C-21), 28.2 (C-26), 28.6 (C-27), 29.0 (C-28), 29.5 (C-10), 30.0 (C-2), 30.4 (C-19), 32.7 (C-1), 33.4 (C-22), 34.9 (C-12), 38.6 (C-7), 42.6

(C-4), 45.0 (C-13), 46.1 (C-14), 46.6 (C-15), 46.9 (C-8), 54.3 (C-5), 58.3 (C-17), 67.8 (C-6), 71.3 (C-25), 73.4 (C-16), 81.7 (C-24), 87.2 (C-20), 89.2 (C-3), 170.8 (Me*CO*). For sugar ^{1}H and ^{13}C NMR (pyridine-d_5), see Table 2.

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