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THREE CYCLOASTRAGENOL GLUCOSIDES FROM *ASTRAGALUS VERRUCOSUS*

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Key Word Index—*Astragalus verrucosus*; Fabaceae; aerial parts; triterpene cycloartane-type glucosides; astraverrucin I, II and III.

Abstract—Three new cycloartane-triterpene glucosides, astraverrucin I, II and III, were isolated from the aerial parts of *Astragalus verrucosus*. The structures of these compounds were elucidated by spectroscopic methods.

INTRODUCTION

Astragali Radix, prepared from the dried roots of *Astragalus membranaceus* Bunge, *A. mongholicus* Bunge and other *Astragalus* spp., is one of the most famous oriental crude drugs used in traditional medicine as an antiperspirant, a diuretic or a tonic [1].

A number of chemical studies on the constituents of *Astragalus* plants have been reported, and many cycloartane-type triterpenoid glycosides, named astragalosides, have been isolated and characterized [2].

This paper describes the isolation and structural elucidation of three new glucosides, astraverrucin I (**1**), II (**2**) and III (**3**), isolated from the aerial parts of *Astragalus verrucosus* Moris, a perennial herb, located only in a restricted area of the Sardinia region, in Italy [3].

RESULTS AND DISCUSSION

Astraverrucin I (**1**), the most polar of the three new glucosides, showed a peak at *m/z* 675 $[M+Na]^+$ in its FAB-mass spectrometry spectrum, corresponding to the molecular formula $C_{36}H_{60}O_{10}$, and exhibited strong hydroxyl absorption bands characteristic of a glycoside in its IR spectrum.

The ^1H NMR spectrum showed signals due to a cyclopropane-methylene at δ 0.21 and 0.53 (each *d*, $J = 3.8$ Hz, H_2-19) and seven tertiary methyls at δ 0.98, 1.28, 1.33, 1.40, 1.56 and 2.01. The base peak at *m/z* 143 in the EI-mass spectrometry spectrum of **1** resulted from the cleavage between C-17 and C-20 and suggested the presence of a 25-hydroxy-20,24-epoxy residue. Furthermore, the ^1H NMR spectrum of **1** clearly showed only one anomeric doublet at δ 5.00 ($J = 7.5$ Hz) in the downfield region, indicative of the presence of one β-linked sugar [4]. This was supported by the ^{13}C NMR spectrum, which revealed one anomeric carbon at δ 107.0. Thus astraverrucin I (**1**) was identified as a glucoside of cycloastragenol [20(*R*),24(*S*)-epoxy-9β,19cyclolanostan-3β,6α,16β,25-tetrol], an aglycone commonly found in many astragalosides isolated from *Astragalus* species [1].

The ^{13}C NMR spectrum of **1** displayed a total of 36 carbon signals; on the basis of DEPT experiment, ^1H-^1H COSY and HETCOR spectra and by comparison with ^{13}C data of related astragalosides [2, 5], all signals were assigned (Table 1).

The 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. Astraverrucin I (**1**) is, therefore, 3-*O*-β-D-glucopyranosil-cycloastragenol, and has been isolated here for the first time as a genuine saponin. It had previously been obtained, after mild acid hydrolysis, from astrailienin A [6].

Astraverrucin II (**2**) and III (**3**) showed the same molecular ion at *m/z* 717 $[M+Na]^+$ on FAB-MS, corresponding to the molecular formula $C_{38}H_{62}O_{11}$. The ^1H and ^{13}C NMR spectra of **2** and **3** were very similar to those of compound **1**, except for the presence of an acetyl group in both compounds (Table 1). The FAB-MS spectra of **2** and **3** exhibited a peak at *m/z* 472 $[M-Glc-OAc]^+$, due to the loss of an acetylated glucose moiety, indicating that the acetyl group was linked to the sugar. The positions of the acetoxy functions were determined on the basis of the acetylation induced shifts in the ^{13}C NMR spectra,

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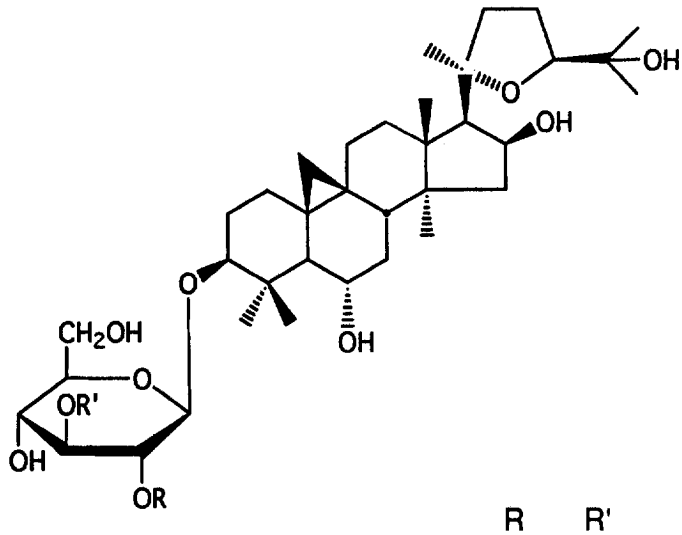

	R	R'
1	H	H
2	Ac	H
3	H	Ac

determined by comparison with the corresponding signals in the spectrum of **1**. As reported in Table 1, significant acetylation shifts occurred for the signals of C-1′, C-2′ and C-3′ for **2** and of C-2′, C-3′ and C-4′ for **3**. Therefore, the structures of astraverrucin II (**2**) and III (**3**) were determined to be 3-*O*-β-D-(2′-*O*-acetyl)glucopyranosyl-cycloastragenol and 3-*O*-β-D-(3′-*O*-acetyl)glucopyranosyl-cycloastragenol, respectively.

EXPERIMENTAL

IR: in nujol mulls; ^1H and ^{13}C NMR: 200 and 50 MHz, respectively, in pyridine-d_5, using TMS as int. standard; FAB-MS: dithiodiethanol matrix in the positive ion mode (Xe atoms of energy of 2.6 KV); EI-MS: direct inlet (20 eV); TLC: silica gel; CC: Sephadex LH-20 and silica gel 60 (70–230 and 230–400 mesh).

Plant material. Astragalus verrucosus. Moris, harvested in Italy, (Is Pisittus, Sardinia island) was collected in June 1994 and identified by one of us (A. M.). A voucher specimen is lodged in the Herbarium of the Istituto di Botanica ed Orto botanico (Università di Urbino).

Extraction and isolation. Air-dried powdered aerial parts of the plant (1110 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 six crude frs, A–G. Fr. B on flash chromatography (eluted with MeOH–$CHCl_3$ 9:1) furnished 10 frs, B_1-B_{10}. Compounds **2** (44.1 mg) and **3** (50.0 mg) were obtained from fraction B_3 (0.29 g) by silica gel CC (eluted with a gradient of solvent from MeOH–$CHCl_3$ 17:3 to MeOH).

The *n*-BuOH soln was evapd to dryness (crude extract 102. 6 g); a part of the residue (32.2 g) was subjected to Sephadex LH-20 CC (eluted with MeOH–H_2O 8:2) to yield compound **1** (28.4 mg).

Astraverrucin I (**1**). $[\alpha]_D^{20} = +10.52$ (pyridine, *c* 0.75); TLC ($CHCl_3$–MeOH–H_2O (6:3:0.1): R_f 0.34; IR ν_{max}^{nujol} cm^{-1}: 3420, 1740, 1050; FAB-MS *m/z*: 675 $[M+Na]^+$, 653 $[M+H]^+$; EI-MS *m/z* (rel. int.): 454 (1.60), 395 (1.52), 199 (2.20) 143 (100), 125 (23.56), 107 (6.37); ^1H NMR (pyridine-d_5): δ 0.21 (1H, *d*, *J* = 3.8 Hz, H-19a), 0.53 (1H, *d*, *J* = 3.8 Hz, H-19b), 0.98 (3H, *s*, Me-30), 1.28 (6H, *s*, Me-21 and Me-27), 1.33 (3H, *s*, Me-29), 1.40 (3H, *s*, Me-18), 1.56 (3H, *s*, Me-26), 2.01 (3H, *s*, Me-28), 2.52 (1H, *d*, *J* = 7.5 Hz, H-17), 3.09 (1H, *m*, H-6), 3.61 (1H, *m*, H-3), 3.84 (1H, *dd*, *J* = 5.5 and 8.8 Hz, H-24), 3.96 (1H, *m*, H-5′), 4.08 (1H, *m*, H-2′), 4.25 (1H, *m*, H-3′), 4.27 (1H, *m*, H-4′), 5.00 (1H, *d*, *J* = 7.5 Hz, H-1′); ^{13}C NMR (pyridine-d_5): Table 1.

Astraverrucin II (**2**). $[\alpha]_D^{20} = +2.27$ (pyridine, *c* 1.94); TLC ($CHCl_3$–MeOH 8:2) R_f 0.43; IR ν_{max}^{nujol} cm^{-1}: 3425, 1750, 1250, 1040; FAB-MS *m/z*: 717 $[M+Na]^+$, 695 $[M+H]^+$, 472 $[M-Glc-OAc]^+$, 143; EI-MS *m/z* (rel. int.): 454 (1.37), 395 (1.33), 201 (2.04), 187 (4.81), 143 (100), 125 (21.43), 107 (5.94), 85 (7.17); ^1H NMR (pyridine-d_5): δ 0.17 (1H, *d*, *J* = 3.9 Hz, H-19a), 0.48 (1H, *d*, *J* = 3.9 Hz, H-19b), 0.98 (3H, *s*, Me-30), 1.25 (3H, *s*, Me-29), 1.28 (3H, *s*, Me-21), 1.29

Table 1. ^{13}C NMR data for compounds **1**–**3** (in pyridine-d_5)

	C	1	2	3
Aglycone	1	32.4	32.2	32.3
	2	30.2	30.0	30.1
	3	89.0	89.3	89.3
	4	42.6	42.2	42.6
	5	54.1	53.8	54.0
	6	68.0	68.0	67.9
	7	38.7	38.7	38.6
	8	47.0	47.1	47.0
	9	21.0	21.0	20.9
	10	29.5	29.4	29.5
	11	26.3	26.2	26.2
	12	33.4	33.4	33.3
	13	45.1	45.0	45.0
	14	46.2	46.1	46.1
	15	46.7	46.7	46.6
	16	73.5	73.5	73.7
	17	58.1	58.4	58.3
	18	21.6	21.5	21.5
	19	30.6	30.6	30.5
	20	87.3	87.3	87.2
	21	27.2	27.1	27.1
	22	35.0	34.9	34.9
	23	26.5	26.5	26.4
	24	81.7	81.7	81.6
	25	71.3	71.3	71.3
	26	28.2	28.2	28.2
	27	28.6	28.6	28.6
	28	29.0	28.8	28.9
	29	16.7	16.5	16.6
	30	20.2	20.2	20.1
D-Glucose moiety	1′	107.0	104.0	106.7
	2′	76.0	75.8	73.4
	3′	78.8	76.3	79.7
	4′	71.9	71.8	69.4
	5′	78.2	78.4	78.1
	6′	63.1	62.6	62.3
Acetoxyl group	*Me*CO	—	21.5	21.3
	Me*C*O	—	170.1	170.9

(3H, *s*, Me-27), 1.39 (3H, *s*, Me-18), 1.56 (3H, *s*, Me-26), 1.77 (3H, *s*, Me-28), 1.98 (3H, *s*, MeCO), 2.51 (1H, *d*, $J = 7.7$ Hz, H-17), 3.49 (1H, *dd*, $J = 4.1$ and 11.3 Hz, H-3), 3.71 (1H, *m*, H-6), 3.86 (1H, *dd*, $J = 5.5$ and 8.8 Hz, H-24), 4.52 (1H, *m*, H-6′), 5.0 (1H, *d*, $J = 7.7$ Hz, H-1′); ^{13}C NMR (pyridine-d_5): Table 1.

Astraverrucin III (**3**). $[\alpha]_D^{20} = +1.10$ (pyridine, *c* 0.92); TLC ($CHCl_3$–MeOH 8:2) R_f 0.5; IR ν_{max}^{nujol} cm^{-1}: 3400, 1735, 1250, 1040; FAB-MS *m/z*: 717 $[M+Na]^+$, 695 $[M+H]^+$, 472 $[M-Glc-OAc]^+$, 143; EI-MS *m/z* (rel. int.): 454 (1.21), 187 (3.52), 159 (3.07), 143 (100), 125 (26.83), 109 (8.69), 107 (7.30), 85 (7.77); ^1H NMR (pyridine-d_5): δ 0.20 (1H, *d*, $J = 3.5$ Hz, H-19a), 0.52 (1H, *d*, $J = 3.5$ Hz, H-19b), 0.97 (3H, *s*, Me-30), 1.28 (6H, *s*, Me-21 and Me-27), 1.33 (3H, *s*, Me-29), 1.40 (3H, *s*, Me-18), 1.56 (3H, *s*, Me-26), 1.98 (6H, *s*, Me-28 and MeCO), 2.51 (1H, *d*, $J = 7.8$ Hz, H-17), 3.72 (1H, *m*, H-6), 3.60 (1H, *m*, H-3), 3.72 (1H, *m*, H-6), 3.86 (1H, *dd*, $J = 5.5$ and 8.8 Hz, H-24), 5.00 (1H, *d*, $J = 7.7$ Hz, H-1′); ^{13}C NMR (pyridine-d_5): Table 1.

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