Pergamon

0031-9422(94)00833-7

Phytochemistry, Vol. 38, No. 6, pp. 1407–1410, 1995

0031-9422/95 $9.50 + 0.00

TWO TRIGLYCOSIDIC TRITERPENE ASTRAGALOSIDES FROM HAIRY ROOT CULTURES OF *ASTRAGALUS MEMBRANACEUS**

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(*Received* 5 *September* 1994)

Key Word Index—*Astragalus membranaceus*; Leguminosae; hairy root cultures; *Agrobacterium rhizogenes*; triterpenoids; agroastragalosides III and IV.

Abstract—Two new triglycosidic triterpene astragalosides, named agroastragaloside III and agroastragaloside IV, were isolated from the hairy root cultures of *Astragalus membranaceus*. From their spectroscopic data, the structures of the two compounds were elucidated as 3-*O*-*β*-(2′,3′-di-*O*-acetyl)-D-xylopyranosyl-6-*O*-*β*-D-glucopyranosyl-25-*O*-*β*-D-glucopyranosyl-cycloastragenol and 3-*O*-*β*-(2′-*O*-acetyl)-D-xylopyranosyl-6-*O*-*β*-D-glucopyranosyl-25-*O*-*β*-D-glucopyranosyl-cycloastragenol, respectively. Both are triterpenetridesmosides.

INTRODUCTION

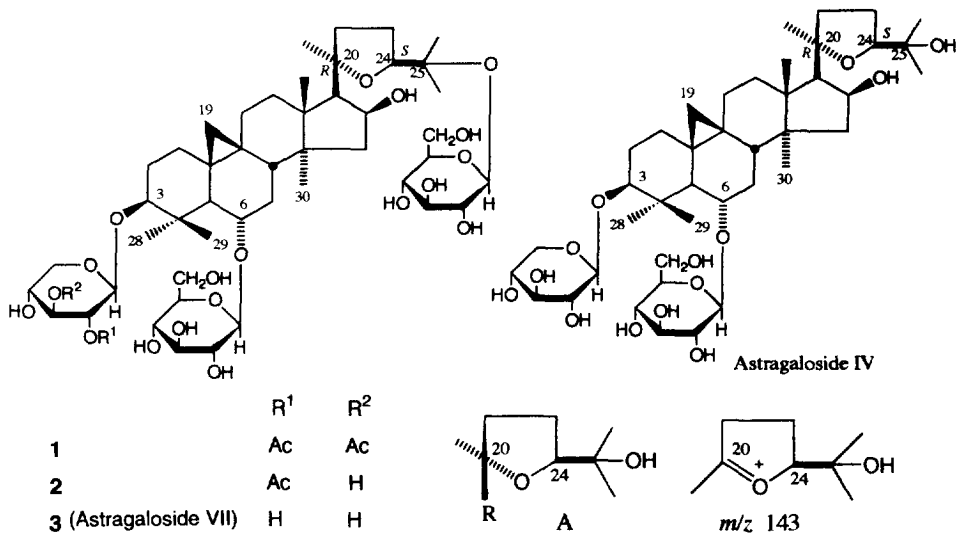
In preceding papers [1, 2], we reported the isolation of nine triterpene oligoglycosides: acetylastragaloside I, astragaloside I–IV, agroastragaloside I, II, isoastragaloside I and 3-*O*-*β*-D-xylopyranosyl-cycloastragenol from hairy root cultures of *Astragalus membranaceus*. We now describe the structural elucidation of an additional two triglycosidic triterpene oligoglycosides, agroastragaloside III (**1**) and agroastragaloside IV (**2**) from the same culture source.

RESULTS AND DISCUSSION

Compound **1**, a new astragaloside derivative, named agroastragaloside III, analysed for $C_{51}H_{82}O_{21}$ by HR-FAB-mass spectrometry. The IR spectrum of **1** showed the presence of a hydroxyl group (3425 cm^{-1}) and an ester carbonyl group (1750 cm^{-1}). The base peak at *m/z* 143 in the mass spectrum of **1** resulted from cleavage between C-17 and C-20 suggesting the presence of the partial structure A (a 25-hydroxy-20, 24-epoxy residue) as found in astragaloside IV [1]. In the ^1H NMR spectrum, **1** showed signals from a cyclopropane-methylene at δ0.18 and 0.55 (each *d*, $J = 4.2$ Hz, H_2-19), seven tertiary methyl groups at δ0.90, 1.25, 1.27, 1.33, 1.42, 1.66 and 1.76, and two acetyl methyl groups at δ1.97 and 2.03, respectively (Table 1). Furthermore, the ^1H NMR spectrum of **1** clearly showed three anomeric doublets at δ4.81 ($J = 7.6$ Hz), δ4.91 ($J = 7.2$ Hz) and δ5.06 ($J = 7.3$ Hz) in the downfield region, indicative of the presence of three *β*-linked sugars [3, 4]. This was supported by the ^{13}C NMR spectrum, which showed three anomeric carbon signals at δ99.0, 104.2 and 105.1. The ^{13}C NMR spectrum of **1** displayed a total of 51 carbon signals. Based on a DEPT experiment, ^1H–^1H and ^{13}C–^1H COSY spectra and comparison with ^{13}C NMR data of related astragalosides [1, 2], all signals could be assigned (Table 2). These data suggest that **1** contained one additional glucose moiety relative to that of astragaloside IV. In the ^1H NMR spectrum, signals at δ1.97 (3H, *s*) and 2.03 (3H, *s*), and ^{13}C NMR signals at δ170.0 and 170.7, showed the presence of two acetoxyl groups in **1**. The sites of attachment of the xylose and glucose moieties of **1** were determined by means of a HMBC spectrum to be at C-3, C-6 and C-25, respectively [5]. In the HMBC spectrum, the first anomeric proton signal at δ4.81 (H-1′) showed long-range correlation with the carbon at δ89.4 (C-3). Also the second and third anomeric proton signals at δ4.91 (H-1″) and 5.06 (H-1‴) showed long-range correlation with the carbons at δ79.4 (C-6) and 78.8 (C-25), respectively. Consequently, xylose and glucose residues should be attached to the hydroxyl groups at C-3, C-6 and C-25 of **1**, respectively. Furthermore, the positions of the acetoxyl groups of **1** were elucidated from its HOHAHA spectrum. In this spectrum the effects of the anomeric proton (δ4.81) of the xylopyranose were observed on all other protons after a long mixing time and clearly two acetoxyl groups were located at C-2′ and C-3′ of the xylose moiety. These data led us to the conclusion that **1** was 3-*O*-*β*-(2′,3′-di-*O*-acetyl)-D-xylopyranosyl-6-*O*-*β*-D-glucopyranosyl-25-*O*-*β*-D-glucopyranosyl-cycloastragenol.

Compound **2**, also a new astragaloside derivative, named agroastragaloside IV, analysed for $C_{49}H_{80}O_{20}$ by HR-FAB-mass spectrometry. The IR spectrum of **2** showed the presence of a hydroxyl group (3430 cm^{-1}) and an ester carbonyl group (1755 cm^{-1}). The base peak at *m/z*

*Part 102 in the series 'Studies on Plant Tissue Cultures'. For Part 101 see ref. [2].



143 in the mass spectrum resulted from cleavage between C-17 and C-20, again suggesting the presence of the partial structure A (a 25-hydroxy-20,24-epoxy residue) as found in **1**. The ^{1}H NMR spectrum was very similar to that of **1** except for the absence of one acetyl methyl signal at δ1.97 (Table 1). The ^{13}C NMR spectrum was also closely similar to that of **1** except for the absence of the ester carbonyl carbon signal (δ170.7) and for the carbon signals owing to the xylose moiety. Furthermore, the ^{1}H NMR spectrum of **2** clearly showed three anomeric doublets at δ4.80 (J = 7.7 Hz), δ4.92 (J = 7.5 Hz) and 5.07 (J = 7.7 Hz) in the downfield region, indicative of the presence of three β-linked sugars, as in **1** [3, 4]. The presence of three β-linked sugars was supported by the ^{13}C NMR spectrum, which showed three anomeric carbon signals at δ99.0, 104.9 and 105.1. The ^{13}C NMR spectrum of **2** displayed a total of 49 carbon signals. Based on a DEPT experiment, ^{1}H–^{1}H and ^{13}C–^{1}H COSY spectra and comparison with ^{13}C NMR data of related astragalosides[1, 2], all signals could be assigned (Table 2). These data suggest that **1** was identical to **2**, except for the presence of one less acetyl group. Furthermore, the sites of attachment of the xylose and glucose moieties of **2** were confirmed by means of a HMBC spectrum to be at C-3, C-6 and C-25, respectively [5]. The position of the acetoxy group in **2** was elucidated from its HOHAHA spectrum. In this spectrum, the effects of the anomeric proton (δ4.80) of the xylopyranose were observed on all other protons after a long mixing time and clearly one acetyl group was located at C-2′ of the xylose moiety. Therefore, the structure of **2** was determined to be 3-*O*-β-(2′-*O*-acetyl)-D-xylopyranosyl-6-*O*-β-D-glucopyranosyl-25-*O*-β-D-glucopyranosyl-cycloastragenol.

Kitagawa *et al.* [6] reported the structural elucidation of astragaloside VII (**3**) as 3-*O*-β-D-xylopyranosyl-6-*O*-β-D-glucopyranosyl-25-*O*-β-D-glucopyranosyl-cycloastragenol isolated from an extract of Astragali Radix. It has quite a similar structure to the two new compounds agroastragaloside III (**1**) and agroastragaloside IV (**2**).

Table 1. ^{1}H NMR spectral data of compounds **1** and **2** (400 MHz, δ, pyridine-d_5)

H	1	2
3α	3.65 *dd* (11.6, 4.4)	3.41 *dd* (11.5, 4.2)
5α	1.88 *m*	1.89 *d* (8.1)
6β	3.78 *ddd* (8.5, 8.5, 4.0)	3.80 *ddd* (7.8, 7.8, 3.5)
16α	4.88 *m*	4.87 *m*
17α	2.42 *d* (7.2)	2.42 *d* (7.7)
H_3-18	1.33 *s*	1.33 *s*
19a	0.18 *d* (4.2)	0.16 *d* (4.1)
19b	0.55 *d* (4.2)	0.54 *d* (4.1)
H_3-21	1.25 *s*	1.26 *s*
22a	1.61 *m*	1.60 *m*
22b	2.79 *dd* (19.8, 11.5)	2.80 *dd* (19.6, 11.5)
24	3.91 *m*	3.89 *m*
H_3-26	1.42 *s*	1.42 *s*
H_3-27	1.66 *s*	1.66 *s*
H_3-28	1.76 *s*	1.82 *s*
H_3-29	1.27 *s*	1.29 *s*
H_3-30	0.90 *s*	0.91 *s*
1′(xylose)	4.81 *d* (7.6)	4.80 *d* (7.7)
1″(6-C-glucose)	4.91 *d* (7.2)	4.92 *d* (7.5)
1‴(25-C-glucose)	5.06 *d* (7.3)	5.07 *d* (7.7)
Ac	1.97 *s*	2.04 *s*
Ac	2.03 *s*	

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR: 400 and 100 MHz. FAB- and EI-MS: 20 eV. Specific rotations: MeOH. CC: silica gel (C-200).

Table 2. ^{13}C NMR data of compounds **1–3** (100 MHz, δ, pyridine-d_5)

	C	1	2	3*
Aglycone	1	32.1	32.2	
	2	29.9	30.1	
	3	89.4	89.0	88.6
	4	42.4	42.4	
	5	52.5	52.6	52.4
	6	79.4	79.4	79.1
	7	34.4	34.4	
	8	45.7	45.6	
	9	21.0	21.3	
	10	28.8	29.0	
	11	26.3	26.3	
	12	33.6	33.6	
	13	45.4	45.4	
	14	46.3	46.3	
	15	45.7	45.7	
	16	73.7	73.7	73.5
	17	58.2	58.1	58.1
	18	21.4	21.4	
	19	29.0	28.8	
	20	87.4	87.3	87.2
	21	28.0	27.9	
	22	35.2	35.2	
	23	26.2	26.2	
	24	82.2	82.2	82.2
	25	78.8	78.7	78.6
	26	23.1	23.1	
	27	25.9	25.8	
	28	28.4	28.4	
	29	16.7	16.7	
	30	20.0	19.9	
3-*O*-β-D-Xylopyranosyl moiety	1′	104.2	104.9	107.3
	2′	73.3	75.8	75.3
	3′	77.0	76.4	77.8
	4′	71.5	72.0	71.4
	5′	66.9	67.3	66.8
6-*O*-β-D-Glucopyranosyl moiety	1″	105.1	105.1	104.8
	2″	75.7	75.8	75.5
	3″	79.2	79.2	78.7
	4″	72.0	71.5	72.0
	5″	78.7	78.7	77.8
	6″	63.2	63.2	62.8
25-*O*-β-D-Glucopyranosyl moiety	1‴	99.0	99.0	98.8
	2‴	75.3	75.3	75.1
	3‴	78.3	78.3	78.4
	4‴	71.5	71.5	71.5
	5‴	78.1	78.2	77.8
	6‴	62.9	62.9	62.8
Acetoxyl group		170.0	170.2	
		170.7		
		21.0	21.2	
		21.3		

*Data for compound **3** (astagaloside VII) from ref. [6].

Culture of hairy roots. Methods of isolation and culture of hairy roots of *A. membranaceus* were performed according to ref. [1]. Roots were subcultured every 4 weeks on modified Ganborg B5 medium [7] containing 30 g sucrose, 45 g $CaCl_2 \cdot 2H_2O$, 38 g $MgSO_4 \cdot 7H_2O$ in 1 l H_2O at 25° in the dark at 60 rpm on a rotary shaker. Large-scale culture was carried out using 500 ml conical flask containing 250 ml of the medium described above.

Extraction and separation procedures. After 4-weeks culture (143 flasks), roots (7.14 kg fr. wt) were harvested with nylon mesh and dried at 60° for 7 days. Dried roots (549 g) were refluxed (× 3) with 70% EtOH. After filtration, the extracts was combined and the solvent evapd under red. pres. The residue was partitioned between *n*-BuOH and H_2O, and the *n*-BuOH fr. evapd to dryness (crude saponin fr. 30.8 g). The crude saponin fr. was subjected to Diaion HP 20 CC and eluted successively with 20, 50, 80 and 100% MeOH. After removal of solvent, the 100% MeOH eluate (12.3 g) was subjected to CC over silica gel (*ca* 1 kg Wako gel C-200) and eluted with a $CHCl_3$–MeOH solvent system to yield 7 frs (frs A–G).

Agroastragaloside III (**1**). Fr. E (0.8 g) was purified by HPLC-1 and the fr. containing the peak at R_t 16.5 mins was collected. Further purification of the collected fr. was achieved by repeated HPLC-1 and **1** was isolated from the fr. containing the peak at R_t 16.5 min. Compound **1** (41.1 mg), needles. Mp 191–193° (from MeOH). $[\alpha]_D^{25}$ + 5.9 (MeOH; *c* 2.74). FAB-MS *m/z*:1053 $[M + Na]^+$. HR-FAB-MS: $C_{51}H_{82}O_{21}Na$ (required 1053.5248, $[M + Na]^+$ at *m/z* 1053.5277). EIMS (direct inlet) 20 eV, *m/z* (rel. int.): 472 (9), 454 (21), 395 (10), 271 (10), 187 (16), 143 (100), 125 (78). IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 1750 (CO_2), 1250, 1035. ^1H and ^{13}C NMR: Tables 1 and 2.

Agroastragaloside IV (**2**). Fr. E (0.8 g) was purified by HPLC-1 and the fraction containing the peak at R_t 12.5 mins was collected. Further purification of the collected fr. was achieved by repeated HPLC-2 and **2** was isolated from the fr. containing the peak at R_t 12.1 min. Compound **2** (16.4 mg), needles. Mp 187–189° (from MeOH). $[\alpha]_D^{25}$ + 13.9 (MeOH; *c* 0.75). FAB-MS *m/z*: 1011 $[M + Na]^+$. HR-FAB-MS: $C_{49}H_{80}O_{20}Na$ (required 1011.5140, $[M + Na]^+$ at *m/z* 1011.5167). EIMS (direct inlet) 20 eV, *m/z* (rel. int.): 472 (2), 454 (11), 395 (10), 311 (5), 270 (9), 143 (100), 125 (30). IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 1755 (COO). ^1H and ^{13}C NMR: Tables 1 and 2.

HPLC analysis. Analysis and isolation of astragaloside derivatives in system HPLC-1: column (19 mm × 150 mm) packed with μ-Bondasphere 5μ C_{18}-100 Å solvent 82.5% MeOH in H_2O, flow rate 6 ml min^{-1}; HPLC-2: column (19 mm × 150 mm) packed with μ-Bondasphere 5μ C_{18}-100 Å solvent 80% MeOH in H_2O, flow rate 6 ml min^{-1}. Absorbance and differential refraction detection.

Acknowledgement—We are indebeted to the members of the Analytical Centre of this University for NMR and MS.

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