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ASTRAILIENIN A FROM *ASTRAGALUS ILIENSIS*

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Key Word Index—*Astragalus iliensis*, Leguminosae; cycloartane triterpene, astrailienin A, triterpenoid saponins.

Abstract—A new triterpenoid glycoside, astrailienin A, was isolated from the roots of *Astragalus iliensis*. By heterogeneous acid hydrolysis an aglycone was obtained which was identified as cycloastragenol. On the basis of spectral analysis and chemical reactions, the structure of the new compound was assigned as the 3-*O*-[*β*-D-apio-D-furanosyl(1→2)]-*β*-D-glucopyranoside of cycloastragenol.

INTRODUCTION

We have isolated two glycosides from the Chinese traditional medical plant, *Astragalus membranus* [1], and are now studying other species of *Astragalus* grown in Xin Jian: there are more than 100 species of *Astragalus* in this region. We have isolated 16 cycloartane triterpenoid glycosides from *A sievesianus* Pall [2, 3]. In this paper we report the structure of a new glycoside isolated from *A. iliensis* L.

RESULTS AND DISCUSSION

Astrailienin A (**1**) was isolated as the main glycoside component from a methanol extract of the roots of *A iliensis*. Strong absorption bands related to hydroxy groups were present in the IR spectrum. Compound **1** was indicated to have the molecular formula $C_{41}H_{68}O_{14}$ by FABMS (*m/z* 877 $[M+92]^+$), CIMS (*m/z* 785 $[M]^+$) and ^{13}C NMR [INEPT and ATP] The presence of ^1H NMR signals at δ0.282, 0.443 (each 1H, $J=4.4$ Hz) and 0.885–1.214 (21H, *s*, 7 × Me) established the presence of a cyclopropane methylene and seven angular methyl groups. The two doublets at δ5.313 ($J=2.1$ Hz) and 4.342 ($J=7.7$ Hz) in the ^1H NMR spectrum and at δ111.03 (*d*) and 105.54 (*d*) in the ^{13}C NMR spectrum indicated the presence of two anomeric protons and carbons of the glycoside. These results suggested that **1** might be a triterpenoid disaccharide with cycloastragenol [4] as aglycone. Heterogenous acid hydrolysis of **1** afforded an aglycone (**2**) identical in all respects with cycloastragenol. Glucose and a less polar sugar were detected by PC of the aqueous solution of hydrolysis of **1**.

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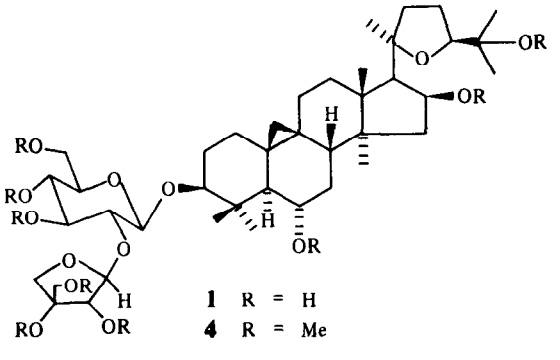

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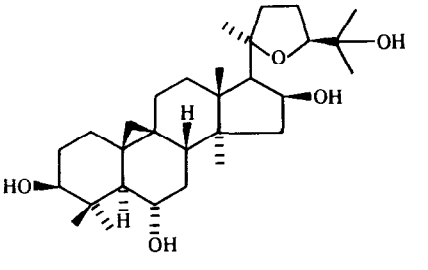
4 R = Me



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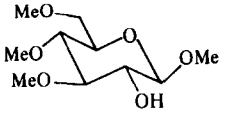

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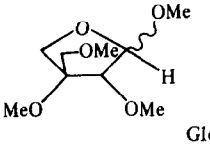

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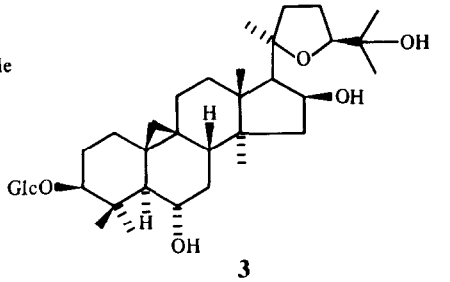

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The CI mass spectrum of **1** exhibited a molecular ion peak at *m/z* 785 $[M]^+$ and a fragment ion peak at *m/z* 653 $[M-132]^+$ related to [M − pentose]$^+$ The lack of an ion peak at *m/z* 623 $[M-162]^+$ related to [M − glucose]$^+$, indicated that the glucose is linked to the aglycone directly and a pentose is attached to the glucose The attachment of the sugar chain at the C-3 position of the aglycone (**2**) was evident by the downfield shift [5] (Δ + 10.9) observed in the ^{13}C NMR signal of C-3 in **1** Mild acid hydrolysis gave a second glycoside (**3**) as the sole product and in good yield This confirmed that the two sugars were linked together The FAB mass spectrum of **3** exhibited a quasi molecular ion peak and a molecular ion peak at *m/z* 745 $[M+92]^+$ and 653 $[M]^+$ respectively The signals at δ 4 342 (*d*, *J* = 7 7 Hz) in the ^{1}H NMR spectrum, and at δ 106 89 (*d*) in ^{13}C NMR spectrum, were attributed to the anomeric proton and carbon of glucoside **3**. On the basis of the chemical shift of the anomeric carbon (δ 106 89) and the coupling constant ($J_{1,2}$ = 7 7 Hz) of the anomeric proton of **3**, the glucosyl moiety had to be β-oriented [6]

By comparison of the ^{13}C NMR data (Table 1) of glycoside **1** with those of glycoside **3**, the upfield shift at C′-1 (Δ − 1 35) and at C′-3 (Δ − 1.03), and the downfield shift at C′-2 (Δ + 3 49) indicated that the pentose might be attached to C′-2 of the glucosyl moiety. It also revealed that the remaining five signals at δ 111 03 (*d*), 80.49 (*s*), 78.03 (*d*), 75 61 (*t*), 66 13 (*t*) in **1** must be attributed to the pentose Due to the split pattern of the five carbons, the pentose was proved to be a branched chain sugar According to the coupling constant of the anomeric proton (H-1″, δ 5.313, *d*, *J* = 2 1 Hz), a secondary hydroxy group must be located on C″-2 [δ 78 03 (*d*)], and a hydroxy and hydroxy methylene on C″-3 [δ 80.49 (*s*)]. The pentose was thus indicated to be apiose [7] For further determination of the configuration of the pentose, methanolysis of the permethylate **4** afforded two methylated sugars, **5** and **6** Compound 5 was identified as methyl 3,4,6-tri-*O*-methyl-2-hydroxy-D-glucoside by TLC and GC There are four isomers of permethylated apioside [8] According to the chemical shift and coupling constant of the anomeric proton (H-1″, δ 4 95, *d*, *J* = 2 6 Hz) and to the specific rotation $[\alpha]_D^{1.5}$ −65° ($CHCl_3$, *c* 0 58), **6** was shown to be methyl-2,3,5-tri-*O*-methyl-β-D-apio-D-furanoside. Thus the structure of **1** was shown to be the 3-*O*-[β-D-apio-D-furanosyl(1→2)]-β-D-glucopyranoside of cycloastragenol

EXPERIMENTAL

Mps uncorr ^{1}H NMR $CDCl_3$ with TMS as int standard ^{13}C NMR (50 3 MHz) pyridine-d_5 (secondary ref values converted to TMS scale)

The spots in TLC were detected by spraying with the following reagents: a soln of vanillin (3 g) and conc H_2SO_4 (3 ml) in 95% EtOH (100 ml), 1% $Ce(SO_4)_2$ soln in 10% H_2SO_4, 1% aniline phthalate soln in 70% EtOH Prep HPLC was run on a Waters 421, column YWG C18, 4 6 × 200 mm eluted with MeOH–H_2O (7 3) GC was run on 10% PEG column 170, N_2, 40 ml/min

Isolation of astrathenin A (**1**) Dried powdered (10 kg) roots of *Astragalus illiensis* were extracted (× 5) with MeOH The MeOH soln was evapd to dryness, dissolved in H_2O and extracted with *n*-BuOH The BuOH extract was evapd under red pres to give a dark-brown tarry mass (235 g) The residue (22 g) was chromatographed on silica gel eluted with $CHCl_3$–MeOH–H_2O (13 7 2) Fractions 4–11 gave a residue (7 51 g) which on CC on silica gel eluted with $CHCl_3$–MeOH (4 1) afforded **1** as needles (2 36 g, 0 252%), mp 226–227° (MeOH-H_2O) **1** was further purified by semi-prep HPLC for ^{13}C NMR and elemental analysis (Found C, 60 98, H, 8 96. $C_{41}H_{68}O_{14}·H_2O$ requires C, 61 33, H, 8 78%). IR ν_{max}^{Nujol} cm^{-1} 3320 (OH) CIMS *m/z* (rel int) 785 $[M]^+$ (0 5), 749 $[M-2\times H_2O]^+$ (0 5), 653 $[M-132]^+$ (0 5), 617 [M − 132

Table 1 ^{13}C NMR data for compounds **1**–**3** and reference substances (glycosidation shift)

C	2	1	3	Apinin [7]	Muscaroside C [9]
3	78 2	89 10 (+10 9)	88 95		
6	68 3	68 11	67 94		
16	73 4	73 53	73 40		
20	87 2	87 34	87 20		
24	81 6	81 79	81 64		
25	71 2	71 38	71 26		
($CDCl_3$)					
Glc-1		105 54 (−1 35)	106 89	99 7	103 7
2		79 33 (+3 49)	75 84	76 8	79 8
3		77 64 (−1 03)	78 67	76 6	77 6
4		71 88	71 79	70 2	71 2
5		78 46	78 13	77 2	78 2
6		62 91	62 97	60 9	63 2
Api-1		111 03		109 0	111 2
2		78 03		76 5	78 2
3		80 49		79 1	80 3
4		75 61		74 0	75 4
5		66 13		64 4	66 0

All in pyridine-d_5 except apinin in DMSO-d_6

$-2\times H_2O]^+$ (1 2), 599 $[M-132-3\times H_2O]^+$ (1 0), 491 [M $-132-162]^+$ (1 0), 473 $[M-132-162-H_2O]^+$ (20), 455 [M $-132-162-2\times H_2O]^+$ (55), 437 $[M-132-162-3\times H_2O]^+$ (50), 419 $[M-132-162-4\times H_2O]^+$ (15), 143 $[C_8H_{14}O_2+1]^+$ (100) ^{1}H NMR (500 MHz) δ0 282, 0 443 (1H each, *d*, $J=4$ 4, H-19), 0 885, 0 934, 1 016, 1 139, 1 160, 1 207, 1 214 (3H each, 7 × Me), 2 274 (1H, *d*, $J=8$ 5, H-17a), 3 114 (1H, *dd*, $J=4$ 0, 11 5, H-3), 3 166 (1H, *td*, $J=3.6$, 10 0, 10 0, H-6), 3 332, 3 369 (1H, each, AB, $J=9$ 0, H-5″), 3 548, 3 604 (1H, each, AB, $J=9.0$, H-4″), 3 675 (1H, *dd*, $J=6$ 0, 9 0, H-24B), 4 342 (1H, *d*, $J=7$ 7, H-1′), 4 600 (1H, *dd*, $J=7$ 7, 14 8, H-16a), 5 313 (1H, *d*, $J=2$ 1, H-1″) ^{13}C NMR· δ58 34 (*d*, C-17), 54 08 (*d*, C-5), 47 14 (*d*, C-8), 46 65 (*t*, C-15), 46.23 (*s*, C-14), 45.10 (*s*, C-13), 42.64 (*s*, C-4), 38 63 (*t*, C-7), 34.95 (*t*, C-22), 33 50 (*t*, C-12), 32 58 (*t*, C-1), 30 81 (*t*, C-19), 30 29 (*t*, C-2), 29 57 (*s*, C-10), 28 80 (*q*, C-29), 28 71 (*q*, C-21), 28 28 (*q*, C-26), 27 23 (*q*, C-27), 26 55 (*t*, C-23), 26 35 (*t*, C-11), 21 65 (*q*, C-28), 20 97 (*s*, C-9), 20 35 (*q*, C-18), 16 66 (*q*, C-30) For the other signals see Table 1

Heterogeneous acidic hydrolysis of **1**. A soln of **1** (50 mg) in EtOH (2 6 ml) was mixed with 10% HCl (2 ml) and C_6H_6 (5 7 ml) and refluxed for 5 hr The reaction mixture was poured into ice-H_2O and extracted with EtOAc The EtOAc extract was washed with 4% $NaHCO_3$, H_2O and dried over Na_2SO_4, and worked-up as usual The crude product (38 7 mg) was subjected to CC on silica gel ($CHCl_3$–EtOAc–MeOH, 10 10.1) to give **2** (15.7 mg) as needles, mp 238–240°, $[\alpha]_D^{15}$ + 46° (MeOH, *c* 1 0) By comparison (TLC, HPLC and mmp) with an authentic sample, **2** was proved to be identical with cycloastragenol From the aq layer, after neutralization with Ag_2CO_3, one of the sugars was identified as glucose by PC (*n*-BuOH–HOAc–H_2O, 4 1 5) The less polar component which was not sensitive to aniline phthalate was not identified

Mild acid hydrolysis of **1** A soln of **1** (262 mg) in EtOH–10% HCl (8 1 ml) was refluxed for 15 min The EtOH was evapd below 30° after H_2O (25 ml) was added to the reaction mixture The mixture was extracted with *n*-BuOH and worked-up to give a residue which was purified by CC on silica gel ($CHCl_3$–MeOH–H_2O, 15 2.1) to give **3** as needles (112 mg, MeOH), mp 215–216°, $[\alpha]_D^{12}$ +38° (MeOH, *c* 0 50) Found: C, 64.84, H, 9 68 $C_{36}H_{60}O_{10}$ H_2O requires C, 64.44, H, 9.32 FABMS *m/z* (rel int): 745 $[M+92]^+$ (7 0), 653 $[M]^+$ (22 0), 491 $[M-162]^+$ (2 5), 473 $[M-162-H_2O]^+$ (18 0), 455 $[M-162-2\times H_2O]^+$ (50 0), 437 $[M-162-3\times H_2O]^+$ (58 0), 419 $[M-162-4\ H_2O]^+$ (20 0), 143 $[C_8H_{14}O_2+1]$ (100) ^{1}H NMR (500 MHz) δ0 381, 0 533 (1H each, *d*, $J=3$ 75, H-19), 0 980, 1.025, 1 146, 1 226, 1 251, 1 292, 1 305 (3H each, *s*, 7 × Me), 2 364 (1H, *d*, $J=7$ 5, H-17a), 3 251 (1H, *dd*, $J=4$ 0, 11 5, H-3), 3 293 (1H, *td*, $J=3$ 6, 10 0, 10.0, H-6), 3 745 (1H, *dd*, $J=5$ 0, 11 5, H-6′), 3 772 (1H, *dd*, $J=4.0$, 11 5, H-6′), 3 748 (1H, *dd*, $J=5$ 5, 9 5, H-24B), 4.342 (1H, *d*, $J=7$ 7, H-1′), 4 683 (1H, *dd*, $J=7$ 0, 14 5, H-16α)

Permethylation of **1** *and methanolysis of* **4** Compound **1** (349 mg) was permethylated by the Hakomori method [10] The crude product (543 mg) on CC on silica gel (petrol–EtOAc, 2 1) gave a TLC pure powder (**4**) (132 mg) No hydroxy absorption band was detectable by IR ^{1}H NMR (200 MHz) δ0.22, 0 48 (1H each, *d*, $J=4$ 2, H-19), 0 93, 0 96, 1 09, 1 14, 1 17, 1.21, 1 26 (3H each, *s*, 7 × Me), 3 13, 3 23, 3 26, 3 38, 3 39, 3 43, 3 50, 3 51, 3.62 (3H each, *s*, 9 × OMe), 4 30 (1H, *d*, $J=7.5$, H-1′), 5.50 (1H, *d*, $J=2$ 6, H-1″) A soln of 4 (100 mg) was refluxed in 2 M dry HCl–MeOH (5 ml) for 30 min. The reaction mixture was neutralized with Ag_2CO_3 The filtrate was evapd to dryness to afford a residue which was separated by prep TLC on silica gel (petrol–EtOAc, 2 1) Sugar **5** (R_f 0 2, green colour with vanillin reagent) was identified as methyl 3,4,6-tri-*O*-methyl-D-glucoside (*RR*, 9′30″ and 11′42″) by comparison with the authenic sample on GC A less polar sugar **6** (R_f 0 50, orange colour with vanillin reagent) was obtained as a syrup, $[\alpha]_D^{15}$ −65° ($CHCl_3$, *c* 0.58) ^{1}H NMR (200 MHz) δ3 38, 3 40, 3 44, 3 48 (3H each, *s*, 4 × OMe), 3 59, 3 49 (1H each, *d*, $J=10.2$, H-5″), 3 58 (1H, *d*, $J=2$ 6, H-2″), 3 93, 4 08 (1H each, *d*, $J=11$ 0, H-4″), 4 95 (1H, *d*, $J=2$ 6, H-1″)

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