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SAPONINS FROM *OXYTROPIS BICOLOR*

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Key Word Index—*Oxytropis bicolor*; Leguminosae; triterpenoid saponins.

Abstract—Two new trace saponins were isolated from *Oxytropis bicolor* by column chromatography and preparative TLC on silica gel. The structures were elucidated as 16-*O*-[β-D-glucopyranosyl(1 → 3)-β-D-glucopyranosyl]-(20*S*, 24*S*)-3β,16β,20,24,25-pentahydroxy-9,19-cyclolanostane and 16-*O*-[β-D-glucopyranosyl(1 → 3)-β-D-glucopyranosyl-20-*O*-α-L-arabinopyranosyl-(20*S*,24*S*)-3β,16β,20,24,25-pentahydroxy-9,19-cyclolanostane on the basis of chemical evidence and spectral analysis.

INTRODUCTION

Most of the *Oxytropis* species growing in China are highly poisonous to livestock. For sheep and cattle, lengthy exposure will result in chronic intoxication with symptoms such as emaciation, acyesis, abortus, still birth or abnormalities, and it may even cause death. There are more than 20 species of *Oxytropis* distributed in different regions of China. The poisonous species of this genus mainly grow in southwestern and northwestern China. For example, *O. glabra*, *O. ochrocephala*, *O. kansuensis* and *O. deflexa* are all known to be very detrimental weeds for domestic animals in Gansu Province. In our early study of these plants, we found that the saponins from different *Oxtropis* varied from each other in both the number of saponins and the type of aglycones [1–3]. As a continuing study, we now report two new minor saponins of the cyclopropane type from *O. bicolor*.

RESULTS AND DISCUSSION

An ethanolic extract of the aerial parts of *O. bicolor* was first treated with ethyl acetate and after that the aqueous solution was extracted several times with *n*-butanol saturated with water. The butanol extract was pretreated on a non-polar macroreticular resin column, eluting with aqueous ethanol, and the fraction containing saponins was separated on a dry silica gel column to give two fractions, A and B. Saponin **1** [1] was isolated by column chromatography from fraction A.

Two new trace saponins (**2** and **3**) were isolated on preparative silica gel TLC from fraction B. The molecular formula of **2** was $C_{42}H_{72}O_{15}$ (FAB mass spectrometry). Acidic hydrolysis of **2** gave D-glucose as the sugar moiety and an artefact (**4**) as the aglycone moiety, the same as in the case of saponin **1**. Enzymic hydrolysis of both saponins **1** and **2** liberated aglycone **5** [2]. The evidence above revealed that saponins **1** and **2** were composed of D-glucose and sapogenol (**5**). However, the chemical behaviour and ^{13}C NMR spectrum of **2** differed from that of **1** as shown below. The sugar chain of **2** could not be completely acetylated because of the steric effect of the side chain of the aglycone part. For the same reason, methylation of **2** afforded two main derivatives. One was the corresponding undeca-*O*-methylate of **2** afforded two main derivatives. One was the corresponding undeca-*O*-methylate, the other deca-*O*-methylate. Under the same conditions, permethylation of **1** furnished an undeca-*O*-methyl derivative [2]. In the ^{13}C NMR spectrum of **2** (Table 1), the significant glycosylation shifts [4–6] were observed at C-16 of the aglycone part and C-3 of the glucosyl moiety by comparison of its ^{13}C NMR data with those of **1** and **4** and the methyl sugars [7], indicating the attachment sites of the sugar chain to the aglycone and between the two glucoses. The ^{1}H NMR spectrum of the methylate of **2** showed the β-glucosidic nature of both sugar moieties (4.20, *d*, $J = 8.0$ Hz; 4.55, *d*, $J = 8.0$ Hz). Based on the above mentioned details, the structure of saponin **2** was elucidated as 16-*O*-[β-D-glucopyranosyl(1 → 3)-β-D-glucopyranosyl]-(20*S*,24*S*)-3β,16β,20,24,25-pentahydroxy-9,19-cyclolanostane, a new naturally occurring saponin.

The molecular formula of **3** was $C_{47}H_{80}O_{19}$ (FAB mass spectrometry). Acid hydrolysis of **3** liberated D-glucose, L-arabinose and **4**. In the EI mass spectra of **2** and **3**, the fragment ion peaks [i], [ii] and [iii] were observed. However, in the EI mass spectrum of **2**, [i] was one of the main fragments and [ii] was the base peak, whereas in the EI mass spectrum of **3**, both [i]

Table 1. ^{13}C NMR spectral data for compounds **1**–**4** in pyridine-d_5

C	1	2	3	4	C	1	2	3	4
1	33.7	33.8	33.5	34.9	16	73.5	83.0	83.6	73.4
2	32.0	32.1	32.4	32.4	17	55.5	55.9	56.5	58.5
3	88.8	78.0	78.2	77.9	18	21.2	20.8	21.2	21.9
4	41.2	41.2	41.5	41.1	19	30.2	29.7	30.0	30.6
5	47.3	47.7	48.0	48.2	20	76.7	72.5	80.8	87.2
6	21.0	20.8	20.4	21.3	21	26.1	26.0	24.2	27.2
7	26.3	26.0	26.5	26.4	22	42.7	42.4	41.5	31.2
8	47.6	46.7	47.0	47.5	23	27.3	30.1	30.0	26.6
9	19.7	19.9	20.0	19.7	24	79.9	79.8	78.5	81.7
10	26.2	26.0	26.5	26.8	25	72.8	76.5	76.7	71.2
11	26.5	26.6	26.8	26.4	26	25.7	25.4	26.5	28.2
12	29.9	27.2	27.0	33.4	27	25.8	25.6	26.9	28.6
13	47.2	47.2	47.3	45.0	28	20.4	20.3	20.4	20.3
14	46.6	47.4	46.0	46.3	29	15.3	15.1	15.5	14.9
15	49.1	47.7	48.0	47.0	30	26.2	26.0	25.9	26.2
C	1	2	3	C	1	2	3	C	3
1′	104.8	104.3	104.7	1″	105.9	105.3	105.8	1‴	98.7
2′	83.3	73.7	73.8	2″	76.9	76.7	77.6	2‴	71.5
3′	77.8	88.8	89.0	3″	77.8	77.5	78.2	3‴	73.5
4′	71.4	71.7	72.2	4″	71.5	71.9	72.3	4‴	68.0
5′	78.2	77.7	77.9	5″	78.1	78.0	78.5	5‴	65.5
6′	62.2	62.9	63.3	6″	62.6	62.9	63.3		

and [ii] were weaker peaks than found for **2**. The reason for this difference is the attachment of one sugar unit to the side chain in **3**, which increased the difficulty for the formation of [ii] from [i]. This conclusion was reinforced by the facts that in the ^{13}C NMR spectrum of **3**, the glycosylation shift at C-16 and the signals due to two β-D-glucopyranosyls remained nearly identical to those of compound **2**. In addition, carbon signals due to an α-L-arabinopyranosyl and the significant glycosylation shift on C-20 in **3** were observed. On the basis of all the above results, the structure of **3** was established as 16-*O*-[β-D-glucopyranosyl(1 → 3)-β-D-glucopyranosyl]-20-*O*-α-L-arabinopyranosyl(20*S*, 24*S*)-3β,16β,20,24,25 - pentahydroxy - 9,19 - cyclolanostane, also a new naturally occurring saponin.

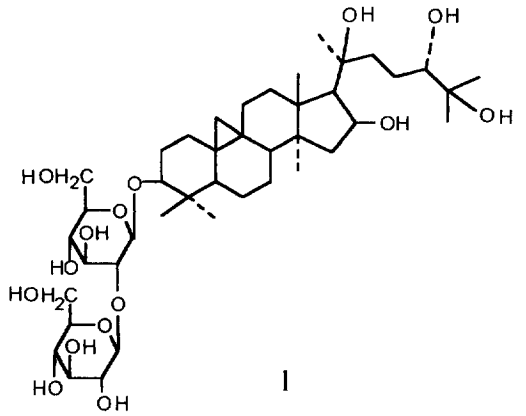
EXPERIMENTAL

General. Oxytropis bicolor was collected from Gansu Province, China. Mps: uncorr. ^1H and ^{13}C NMR with TMS as int. standard, EIMS: 70 eV. Silica gel (10–40 μm, Qingdao Marine Chemical Factory, China) was used for CC and prep. TLC with the solvent systems: (*a*) EtOAc–EtOH–H_2O (8:2:1); (*b*) $CHCl_3$–MeOH–H_2O (6:3:1, lower layer).

Isolation of saponins. Air-dried powdered aerial parts of *O. bicolor* (2 kg) were extracted 3× with 75% EtOH under reflux. After removal of solvent, the aq. phase was first treated with EtOAc and then extracted several times with *n*-BuOH satd with H_2O. The *n*-BuOH extract (2 g) was pretreated with a non-polar macroreticular resin column, eluting with H_2O–EtOH (from 100:0 to 1:4). Frs eluted with H_2O–EtOH (from 1:1 to 3:7) afforded a crude saponin mixt. (1 g), which was sepd on a dry silica gel column with solvent system (*a*) to give two frs A and B. From fr. A, **1** (300 mg) [2] was isolated by repeat CC, and fr. B was first subjected to CC on silica gel with solvent system (*b*) and then to prep. silica gel TLC to give **2** (30 mg) and **3** (16 mg).

Saponin **2**. Crystals from MeOH; mp 270–272°; $[\alpha]_D^{20}$ + 5.0 (MeOH; *c* 0.5). FABMS (positive ion mode) *m/z* 839 $[M + Na]^+$. EIMS (rel. int.): *m/z* 313 [iii] (2), 161 [i] (20), 143 [ii] (100), 125 [ii − H_2O] (29), 107 [ii − H_2O × 2] (76). ^{13}C NMR: Table 1. According to ref. [8], **2** was methylated to provide two methylates, the undeca-*O*-methylate and deca-*O*-methylate. ^1H NMR of the undeca-*O*-methylate of **2** [200 MHz, $(CD_3)_2CO$]: 0.80, 0.94, 1.05 (6H each, all *s*, Me × 6), 1.20 (3H, *s*, Me), 3.05, 3.15, 3.22, 3.23, 3.32, 3.34, 3.36, 3.43, 3.44, 3.48, 3.53 (3H each, all *s*, OMe × 11), 4.20, 4.57 (1H each, all *d*, *J* = 8 Hz, anomeric protons of two methylated β-D-glucopyranosyls).

Saponin **3**. Powder from MeOH; mp 208–211°; $[\alpha]_D^{20}$ − 4.0 (MeOH; *c* 0.3). FABMS (positive ion mode) *m/z* 971 $[M + Na]^+$. EIMS *m/z* (rel. int.): 313 [iii] (2), 161 [i] (6), 143 [ii] (18), 125 [ii-H_2O] (8), 107 [ii − H_2O × 2] (20). ^{13}C NMR: Table 1. Acid hydrolysis of **3** with dilute HCl liberated an aglycone with mp 240–242°, identified as (20*R*, 24*S*)-3β,16β,25-trihydroxy-20,24-epoxy-9,19-cyclolanostane by mp and TLC comparison with an authentic sample.



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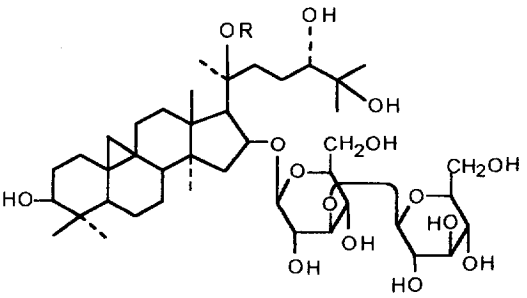
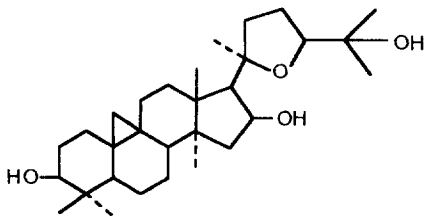


2 R = H

3 R = arabinosyl



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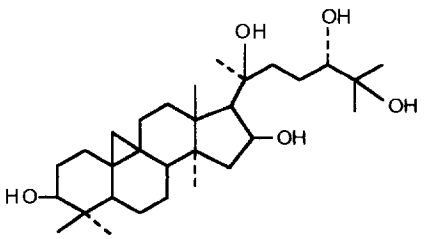

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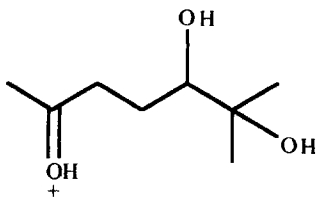

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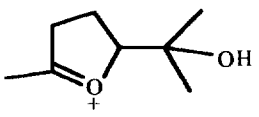

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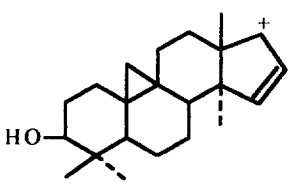

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