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SCROKOELZISIDE A, A TRITERPENE GLYCOSIDE FROM *SCROPHULARIA KOELZII**

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Key Word Index—*Scrophularia koelzii*; Scrophulariaceae; triterpenoid saponin; scrokoelziside A.

Abstract—Scrokoelziside A, isolated from the aerial parts of *Scrophularia koelzii* was shown to be 3-*O*-{[α-L-rhamnopyranosyl-(1 → 3), β-D-glucopyranosyl-(1 → 2)]-β-D-fucopyranosyl-(1 → 4)-β-D-glucopyranosyl}-13β,28-epoxyolean-11-en-23-ol, on the basis one- and two-dimensional NMR homo- and hetero-nuclear spectroscopic evidence.

INTRODUCTION

In continuation of our chemical studies on *Scrophularia koelzii* [1], we now report the isolation and structural elucidation of a triterpene glycoside, scrokoelziside A.

RESULTS AND DISCUSSION

The chloroform extract of the dried aerial parts on chromatographic purification on a silica gel column and preparative TLC yielded one glycoside, scrokoelziside A (**1**). The glycosidic nature of compound **1** was indicated by the broad absorption bands at 3400 and 1070 cm^{-1} for hydroxyl groups in its infrared spectrum and many resonances in the region 61–78 ppm in its ^{13}C NMR spectrum. The mass spectrum of this compound was successfully determined by negative-ion FAB mass spectrometry in which the $[M]^+$ peak appeared at *m/z* 1071, which is compatible with the molecular formula $C_{54}H_{88}O_{21}$.

The ^1H NMR spectrum (Table 1) displayed a pattern characteristic of a pentacyclic triterpene (six tertiary methyl singlets at δ0.80, 0.90, 0.93, 0.94, 1.06 and 1.31), two AB quartets (J = 11.2 and 11.6 Hz) at δ3.32 and 3.73, and at δ3.72 and 4.38, respectively, and an oxymethine at δ4.15. There were two olefinic protons (δ5.53, 1H, *d*, J = 10.4 Hz; 5.53 *dd*, J = 10.4, 1.5 Hz) of an endocyclic disubstituted olefinic bond which indicated a Δ^{11}-oleanene skeleton [2, 3]. The sugar moiety showed in the ^1H NMR spectrum two doublets (J = 6.2 Hz) for secondary methyl groups at δ1.38 and 1.73, three doublets for the anomeric protons (δ5.56, J = 7.2 Hz, Glc-1; 4.89, *d*, J = 7.5 Hz, Fuc-1; 5.24, *d*, J = 7.5 Hz, Glc-1) indicative of β-anomeric configuration [4] of three monosaccharides and a broad singlet ($W_{1/2}$ = 1.8 Hz) at δ5.82 attributed to H-1 of a monosaccharide (Rha) with α-anomeric configuration [4]. The assignment of the methyl resonances at δ1.38 and 1.73 was based upon the fact that these exhibited their correlation with the methine resonance at δ3.59 and 4.94 in the COSY spectrum. The cross-peak connectivity was further elucidated, which led to the assignment of the upfield methyl resonance (δ1.38) to a fucopyranosyl and the resonance at lower field (δ1.73) to a rhamnopyranosyl residue. Many overlapping multiplets were observed between δ3.5–5.0, the assignments of which were performed by means of two dimensional ^1H–^1H HOMOCOSY and ^1H–^{13}C HETCOR spectra. The analysis of these spectral data confirmed the presence of a tetrasaccharide residue composed of two hexopyranose (2 × glucopyranose) and two 6-deoxyhexopyranose (fuc + rha) moieties in **1**.

Starting with H-3 at δ4.15, the resonances at δ2.02 and 1.95 could be assigned to CH_2-2 which, in turn, exhibited their connectivity to CH_2-1 at δ0.97 and 1.78. Among the olefinic methine resonances, the high-field methine resonance at δ5.53 corresponded to H-11 as it displayed a cross-peak with H-9 at δ2.03. The H-23 and H-28 methylene protons were assigned to the resonances at δ3.72 and 4.38, and δ3.32 and 3.73, respectively, related by the geminal couplings.

The methyl signals at δ0.80, 0.90, 0.93, 0.94, 1.06 and 1.31 were correlated with the ^{13}C NMR signals at δ23.62, 33.66, 18.70, 19.63, 12.75 and 19.90, respectively. The chemical shift values of the rest of the methylene and methine resonances could readily be established from the COSY and HETCOR spectra.

The ^{13}C NMR spectrum exhibited 54 carbon signals and of these, 30 carbons accounted for the aglycone moiety while the remaining 24 carbon resonances were

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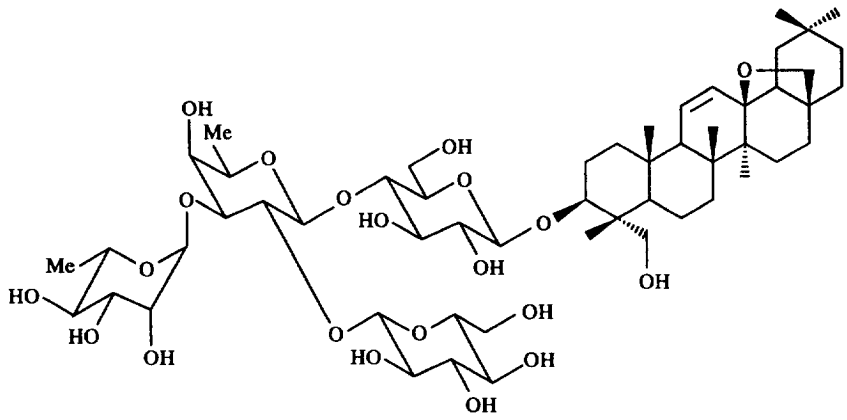

Table 1. ^{13}C NMR and ^{1}H NMR spectral data for compound **1***

	Aglycone residue†			Sugar residue†	
Atom No.	^{13}C	^{1}H	Atom No.	^{13}C	^{1}H
1	38.66 (CH_2)	0.97, 1.78	Glucose (internal) (Glc)		
2	25.88 (CH_2)	1.95, 2.02	1	103.97 (CH)	5.24 (1H, *d*, J = 7.5 Hz)
3	82.63 (CH)	4.15 (1H, *m*)			
4	43.88 (C)	—	2	76.30 (CH)	3.91
5	47.84 (CH)	1.52	3	77.64 (CH)	4.16
6	17.71 (CH_2)	1.50, 1.76	4	78.48 (CH)	4.37
7	31.55 (CH_2)	1.31, 1.40	5	76.48 (CH)	3.70
8	42.05 (C)	—	6	63.19 (CH_2)	4.05 (2H, *m*)
9	53.75 (CH)	2.03	Fucose (Fuc)		
10	36.33 (C)	—	1	104.14 (CH)	4.89 (1H, *d*, J = 7.5 Hz)
11	132.07 (CH)	5.53 (1H, *dd*, J = 10.4 Hz and 1.5 Hz)	2	77.24 (CH)	4.62
12	131.75 (CH)	5.93 (1H, *d*, J = 10.4 Hz)	3	84.82 (CH)	4.03
			4	77.26 (CH)	4.15
13	84.75 (C)	—	5	70.53 (CH)	3.59
14	43.88 (C)	—	6	17.25 (CH_3)	1.38 (3H, *d*, J = 6.2 Hz)
15	31.09 (CH_2)	0.89, 1.84			
16	25.68 (CH_2)	1.01, 1.94	Glucose (terminal) (Glc)		
17	41.73 (C)	—	1	105.02 (CH)	5.56 (1H, *d*, J = 7.2 Hz)
18	51.49 (CH)	1.69			
19	37.37 (CH_2)	1.26, 1.68	2	75.98 (CH)	4.08
20	31.77 (C)	—	3	78.87 (CH)	4.18
21	31.55 (CH_2)	1.27 × 2	4	72.06 (CH)	4.31
22	26.08 (CH_2)	1.20, 1.49	5	77.24 (CH)	3.62
23	64.19 (CH_2)	3.72, 4.38 (AB, *q*, J = 11.6 Hz)	6	61.37 (CH_2)	4.25, 4.30 (each 1H, *m*)
24	12.75 (CH_3)	1.06 (3H, *s*)	Rhamnose (Rha)		
25	18.70 (CH_3)	0.93 (3H, *s*)	1	102.82 (CH)	5.82 (1H, broad *s*, $W_{1/2}$ = 1.8 Hz)
26	19.63 (CH_3)	0.94 (3H, *s*)			
27	19.90 (CH_3)	1.31 (3H, *s*)	2	72.82 (CH)	4.85
28	77.13 (CH_2)	3.32, 3.73 (AB, *q*, J = 11.2 Hz)	3	72.62 (CH)	4.51
			4	73.98 (CH)	4.34
29	33.66 (CH_3)	0.90 (3H, *s*)	5	70.44 (CH)	4.94
30	23.62 (CH_3)	0.80 (3H, *s*)	6	18.58 (CH_3)	1.73 (3H, *d*, J = 6.2 Hz)

*C_5D_5N, TMS as internal standard, ppm (multiplicity).
†From the one-bond HETCOR spectrum and COSY spectrum.

due to four hexose residues. The ^{13}C NMR and DEPT spectra of **1** showed that 30 carbons of the aglycone consisted of six methyls (δ12.75, 18.70, 19.63, 19.90, 23.62, 33.66), nine methylenes, 7 aliphatic methines, including an oxymethine (δ82.63), and seven quaternary carbons. There were also two olefinic methines (δ131.75 and 132.07) for an endocyclic olefinic bond, an oxy-substituted quaternary carbon atom (δ84.93, C-13) and an

oxy-methylene (δ77.13, C-28) indicative of a 13β,28-epoxyoleanane skeleton [5]. The ^{13}C NMR resonances due to the aglycone moiety were in good agreement with those of 13β,28-epoxyolea-11-en-3β-23-diol (16-dehydrosaikogenin G) [2, 3]. The appearance of four anomeric signals at δ105.02, 104.14, 103.97 and 102.82, evident in the ^{13}C NMR spectra and correlating with the ^{1}H NMR resonances at δ5.56, 4.89, 5.24 and 5.82, respectively, in the HETCOR spectrum, further confirmed the existence of a tetrasaccharide moiety in **1** [4–6]. In addition there were 16 methine resonances between δ69–82, two hydroxymethylene resonances (δ63.19, 61.37) and two methyl resonances (δ17.25 and 18.58) thus supporting the existence of two hexopyranose and two 6-deoxyhexopyranose residues. Both the hexopyranose residues were identified as glucopyranose whereas the 6-deoxyhexopyranose moieties were identified as fucopyranose and rhamnopyranose by analysis of the COSY spectrum [4, 7]. Moreover, on acid hydrolysis compound **1** yielded rhamnose, fucose and glucose in the ratio of 1:1:2. The anomeric configuration of both of the glucopyranose and fucopyranose units was determined as β due to: (1) the appearance of anomeric resonance as a doublet ($^{3}J_{1,2}$ = 7.2–7.5 Hz) in the ^{1}H NMR spectrum; (2) the ^{13}C NMR chemical shift of the anomeric carbon resonances (δ105.02, 104.14 and 103.97); and (3) the NOE cross-peaks between H-1, H-3, H-5 in the two-dimensional NOESY spectrum [4, 8].

The interglycosidic linkage and sequence in the sugar chain was established by the negative ion, FAB mass spectrum together with two-dimensional NOE spectroscopy and also in view of the glycosylation effects observed in the ^{13}C NMR spectrum [4, 9]. The fragment ions at *m/z* 925 and *m/z* 909 were in accord with the terminal position of the rhamnopyranose and glucopyranose in the glycosidic part of **1**. The fragment ion at *m/z* 763 further confirmed the loss of the rhamnose and glucopyranose. Therefore, these were terminal sugar residues directly attached to the fucopyranosyl residue and in agreement with the fragment ion at *m/z* 619. In the two-dimensional NOESY spectrum, the cross-peaks were observed between H-1 of a terminal glucose (δ5.56) and H-2 (δ4.62) of the fucose moiety, and H-1 of rhamnose (δ5.82) and H-3 (δ4.03) of the fucose residue. The H-1 of fucose (δ4.89) exhibited a NOESY cross peak with the H-4 (δ4.37) of the internal glucose moiety revealing a (1 → 4) interglycosidic linkage. Observation of NOESY cross-peaks between H-3 of the aglycone (δ4.15) and H-1 of glucose (δ5.24) led to the identification of glucose as the internal sugar involved in linking the aglycone and the 2,3-disubstituted fucopyranosyl residue. Thus, the sequence of the monosaccharide residues and their interglycosidic linkage could be deduced as {β-D-Glc(1 → 2), α-L-Rha(1 → 3)}-β-D-Fuc-(1 → 4)-Glc, which was in full agreement with the ^{13}C NMR chemical shifts. The C-2 and C-3 of the fucose were observed at δ77.24 and δ84.82, respectively, revealing significant deshielding (4.54 and 10.92 ppm) in comparison with the reported values for methyl-β-D-fucopyranoside [4] due to the α-effect of glycosylation [6, 9]. Moreover, analogous chemical shift values have been reported for the C-2 and C-3 of fucopyranose in 2,3-diglycosylated fucopyranosyl-containing naturally occurring glycosides [2, 3]. The C-4 of the internal glucose (δ78.48) was also in complete accordance with the glycosidation at this position [2, 3, 6, 10].

The structure of scrokoelziside A was thus deduced to be 3-*O*-{[α-L-rhamnopyranosyl-(1 → 3), β-D-glucopyranosyl-(1 → 2)]-β-D-fucopyranosyl-(1 → 4)-β-D-glucopyranosyl}-13β,28-epoxyolean-11-en-23-ol. It is notable that scrokoelziside A is similar to thapsuine A from *Verbascum thapsus* and *V. lychnitis* [11–13], ilwensisaponin 1 from *Scrophularia ilwensis* [14], mimengoside A from *Buddleia officinalis* [2] and songarosapin C from *V. songaricum* [3] and *V. nigrum* [15], as it has same aglycone and the same sugar composition but a different arrangement of sugar sequence. This is in accord with a taxonomically interesting relationship between the plants of the genera *Verbascum* and *Scrophularia* [15].

EXPERIMENTAL

Mps: uncorr. TLC: silica gel G (SISCO). Spots were visualized by spraying with 1% $Ce(SO_4)_2$ in 1M H_2SO_4. CC: silica gel (60–120 mesh) (SISCO). Flash CC: EF-10 (EYELA) A.S.C. silica gel (230–400 mesh). PC: Whatman paper No. 1. GC (steel column 2 m × 4 mm packed with 3% OV-225 on GC Q. FID. temp. 80–120°C; N_2 at 50 ml min^{-1}). ^{1}H, ^{13}C and other NMR experiments were carried out in $CDCl_3$ and/or C_5D_5N, using TMS as int. standard. Chemical shifts were expressed in δ values. Mass spectra were recorded at 70 eV. The matrix for the FAB-MS was glycerin. The isolation and experimental conditions were same as reported earlier [1].

Extraction and Isolation. The $CHCl_3$ fr. (57.5 g) of the aerial parts of the plant [1] was chromatographed over silica gel G with a stepwise increase of MeOH content in $CHCl_3$. The residue (18.1 g) the 25–100% MeOH–$CHCl_3$ frs was subjected to CC over silica gel with MeOH–$CHCl_3$ (10–30%) gradient. The fractions eluted with MeOH–$CHCl_3$ (18–25%) yielded **1**, which, on flash CC over silica gel (MeOH–$CHCl_3$, 18–19%), provided compound **1** (1.32 g) as an amorphous powder $[\alpha]_D^{27}$ + 27° (*c* 0.8; pyridine).

Scrokoelziside A (**1**). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2920, 1070, FAB-MS negative-ion 3 kV, *m/z* (rel. int.): 1071 [M − 1]$^{-}$ (100), 1069 (15), 927 (19), 925 (26), 909 (18), 763 (40), 619 (12); ^{1}H and ^{13}C NMR (400 MHz: C_5D_5N): see Table 1.

Hydrolysis of compound **1**. Compound **1** (50 mg) was treated with 50% methanolic HCl (10 ml for 5 hr) under reflux and worked up as usual. The aq. hydrolysate was neutralized with $BaCO_3$ and filtered. The filtrate was evapd under red. press. to dryness and acetylated with pyridine-Ac_2O. GC analysis showed, in comparison with reference compounds, the presence of peracetylrhamnose, peracetylfucose and peracetylglucose (1:1:2).

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