Pergamon

PII: S0031-9422(97)00256-2

Phytochemistry, Vol. 45, No. 8, pp. 1717–1719, 1997
© 1997 Elsevier Science Ltd
All rights reserved. Printed in Great Britain
0031-9422/97 $17.00+0.00

A TRITERPENE GLYCOSIDE FROM *SCROPHULARIA KOELZII**

S. P. S. BHANDARI,† U. V. BABU and H. S. GARG✠

Medicinal Chemistry Division, Central Drug Research Institute, Lucknow-226001, India

(*Received in revised form* 12 *February* 1997)

Key Word Index—*Scrophularia koelzii*; Scrophulariaceae; scrokoelziside 'B'.

Abstract—A new triterpene glycoside, scrokoelziside B, was isolated from the aerial parts of *Scrophularia koelzii* and characterized as 3β-*O*-([β-D-glucopyranosyl (1 → 2), α-L-rhamnopyranosyl (1 → 3)]-β-D-fucopyranosyl (1 → 4)-β-D-glucopyranosyl)-olean-11,13 (18)-diene 23α, 28 diol on the basis of its ^{13}C NMR and 2D NMR data. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In continuation of our earlier studies on *Scrophularia koelzii* [1, 2], we report here on the isolation and characterization of a new triterpene glycoside, scrokoelziside B (**1**).

RESULTS AND DISCUSSION

The chloroform extract of the dried aerial parts yielded the glycosidic compounds scrokoelziside B (**1**) and scrokoelziside A [2]. The glycosidic nature of compound **1** was indicated by the broad absorption bands at 3400 and 1075 cm^{-1} for hydroxyl groups in its IR spectrum. In the negative-ion FAB mass spectrometry the molecular ion peak appeared at *m/z* 1071 compatible with the molecular formula $C_{54}H_{88}O_{21}$.

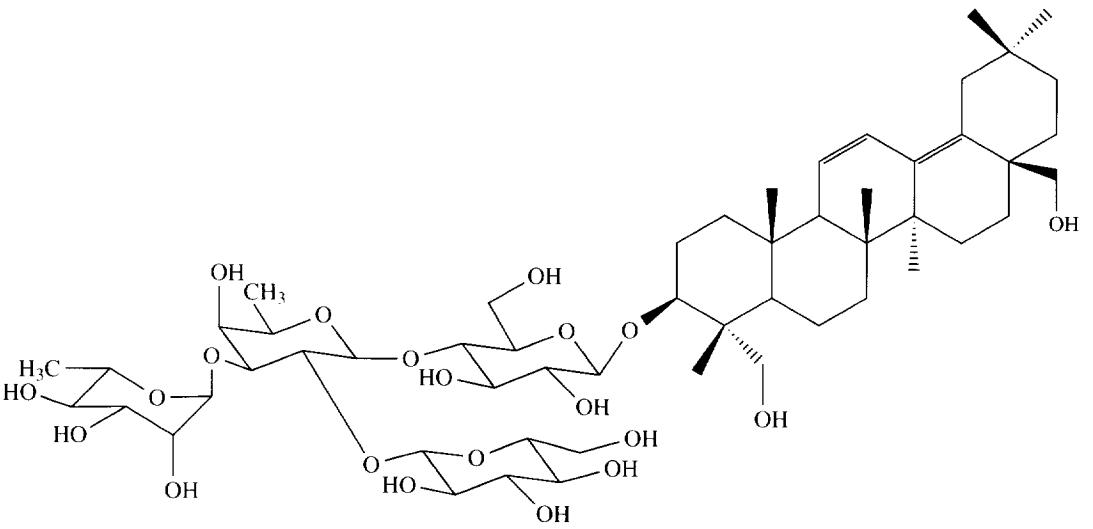
The ^1H NMR spectrum (Table 1) displayed a pattern characteristic of a pentacyclictriterpene (six tertiary methyl singlets at δ 0.84, 0.92, 0.94, 0.94, 1.02 and 1.07), an oxymethine at δ 4.15 as a multiplet and two oxymethylenes, one as a singlet at δ 3.73 and the other as an AB quartet (J = 11.2 Hz) at δ 3.59 and 4.20, respectively. Two olefinic protons resonating at δ 5.67 (1H, *d*, J = 10.6 Hz) and 6.57 (1H, *dd*, J = 10.6, 1.5 Hz) were indicative of an endocyclic disubstituted olefinic bond in a Δ^{11} oleanene skeleton [3, 4]. The downfield shift of the olefinic proton at δ 6.57 was due to conjugation with a tetrasubstituted olefinic bond [9]. The sugar part of ^1H NMR spectrum showed two doublets (J = 6.2 Hz) for secondary methyl groups at δ 1.40 and 1.75, three doublets for anomeric protons (δ 5.28, J = 7.5 Hz, Glc-1; 4.85, J = 7.5 Hz, Fuc-1; 5.61, J = 7.2 Hz, Glc-2), indicative of a β-anomeric configuration [5] of three monosaccharides, and a broad singlet ($W_{1/2}$ = 1.8 Hz) at δ 5.88 attributed to a monosaccharide (Rha) with an α-anomeric configuration [5]. Many overlapping multiplets were observed between δ 3.59–4.88, the assignment of which was performed by ^1H-^1H COSY. The analysis of these spectral data confirmed the presence of a tetrasaccharide residue composed of two hexopyranoses (2 × glucopyranose) and two 6-deoxyhexopyranoses (Fuc + Rha) in **1**.

The ^{13}C NMR spectrum of compound **1** showed 54 resonances of which 30 were accounted for by the sapogenin moiety and the rest (24) by the oligosaccharide moiety (4 × hexoses). The presence of six methyl groups in the aglycone moiety was supported by carbon resonances at δ 12.86, 17.27, 18.77, 20.81, 24.70 and 33.07. The two oxymethylene carbons resonating at δ 63.21 and 64.69, the oxymethine carbon at δ 82.71, a disubstituted double bond at δ 125.90 and 126.49, and a tetrasubstituted double bond at δ 133.30 and 136.36 revealed the presence of the olean 11,13(18) diene-3β, 23α,28 triol skeleton in compound **1**, which was in good agreement with the reported data of triterpenoid glycosides having the same skeleton [6]. The presence of glycosidation at C-23 and C-28 was ruled out whereas glycosidation at C-3 was supported by comparison of the chemical shifts with those of scrokoelziside A [2]. The appearance of four anomeric carbon resonances at δ 105.02, 104.01, 104.17 and 102.82, and in the ^1H NMR spectrum at δ 5.61, 5.28, 3.85 and 5.88 further confirmed the existence of a tetrasaccharide moiety in compound **1**. Additionally, there were 16 methine resonances between δ 70.44 and 84.76, two oxymethylenes at δ 61.39 and 63.21, and two methyl resonances at δ 17.02 and 18.59, supporting the existence of two hexopyranose and two 6-deoxyhexopyranose residues. Both the hexopyranose

* CDRI Communication No. 5608

† Author to whom correspondence should be addressed.

✠ Deceased.



residues were identified as glucopyranose and the 6-deoxyhexopyranose residues as fucopyranose and rhamnopyranose by analysis of the COSY spectrum. Moreover, on acid hydrolysis **1** yielded rhamnose, fucose and glucose in the ratio of 1:1:2. The anomeric configuration of the glucopyranose and fucopyranose

Table 1. ^{13}C and ^{1}H NMR data of compound **1** (C_5D_5N, TMS as int. standard)

Atom. no.	Aglycone moiety ^{13}C	DEPT	^{1}H*
1	38.65	(CH_2)	1.08, 1.78
2	26.11	(CH_2)	2.05, 2.30
3	82.71	(CH)	4.15 (1H, *m*)
4	43.84	(C)	
5	47.84	(CH)	1.70 (*m*)
6	18.37	(CH_2)	1.80, 1.62
7	32.53	(CH_2)	1.23, 1.30
8	40.5	(C)	
9	54.84	(CH)	2.50
10	36.60	(C)	
11	126.49	(CH)	6.57 (1H, *dd*, J = 10.6, 1.5 Hz)
12	125.90	(CH)	5.67 (1H, *d*, J = 10.6 Hz)
13	136.36	(C)	
14	42.59	(C)	
15	32.53	(CH_2)	1.66, 1.80
16	26.11	(CH_2)	1.64, 1.78
17	40.64	(C)	
18	133.30	(C)	
19	38.39	(CH_2)	2.07 (2H, *d*, J = 10 Hz)
20	33.07	(C)	
21	35.44	(CH_2)	1.27 (2H, *m*)
22	29.33	(CH_2)	1.20, 1.36
23	63.21	(CH_2)	3.59, 4.20 (*q*, J = 11.2 Hz)
24	12.86	(CH_3)	1.02 (3H, *s*)
25	17.27	(CH_3)	0.84 (3H, *s*)
26	18.77	(CH_3)	0.92 (3H, *s*)
27	20.81	(CH_3)	1.07 (3H, *s*)
28	64.69	(CH_2)	3.73 (2H, *s*)
29	33.07	(CH_3)	0.94
30	24.70	(CH_3)	0.94 (6H, *s*)

* Individual protons assigned by 2D ^{1}H-^{1}H HOMOCOSY and ^{1}H-^{13}C HETCOR.

units was determined as β, due to the appearance of anomeric resonances as doublets ($J_{1,2}$ = 7.2 7.5 Hz) in the ^{1}H NMR spectrum.

The interglycosidic linkage and sequence in the sugar chain in **1** was established by negative-ion FAB Mass spectrometry [2] together with 2D-NOESY spectrometry and by the glycosylation effects [3, 4, 8, 9] observed in the ^{13}C NMR spectrum, as {-β-D-Glc-(1-2),α-L-Rha-(1-3)}-β-D-Fuc-(1-4)-Glc. The positions of the interglycosidic linkages between the monosaccharide units in **1** were found to be similar by comparison with the ^{13}C chemical shifts of scrokoelziside A [2] and in view of the glycosylation effects observed in the ^{13}C NMR spectrum [5, 7].

Thus the structure of scrokoelziside B could be assigned as 3β-*O*-([β-D-glucopyranosyl (1 → 2)-α-L-rhamnopyranosyl (1 → 3)]-β-D-fucopyranosyl (1 → 4)-β-D-glucopyranosyl)-11,13(18)diene-olean-23α, 28 diol. It is noteworthy that scrokoelziside A [2] and scrokoelziside B isolated from *Scrophularia koelzii* have the same oligosaccharide chain but differ in the aglycone moiety, whereas sangaro saponin A from *Verbascum sangaricum* [4] and ilwensisaponin B from *Scrophularia ilwensis* [10] have the same aglycone moiety but differ in the sugar sequence.

EXPERIMENTAL

General. Mps: uncorr; TLC: silica gel G (SISCO). Spots were visualized by spraying with 1% $Ce(SO_4)_2$ in 1 M 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-25 on gas chrom. Q FID. temp [80–120]: N_2 at 50 ml min^{-1}; ^{1}H, ^{13}C and 2D NMR: C_5D_5N, using TMS as int. standard; MS: 70 eV. The matrix for the FAB-MS was glycerol. The isolation and experimental conditions were the same as those reported earlier [1].

Extraction and Isolation. The $CHCl_3$-soluble fr. (57.5 g) from the aerial parts of the plant *Scrophularia koelzii* was chromatographed over silica gel G with a discontinuous gradient of $CHCl_3$ and MeOH. The residue (18.1 g) from the (25–100%) $CHCl_3$–MeOH eluted frs was subjected to CC over silica gel with $CHCl_3$–MeOH (10–30%) gradient. The frs eluted with $CHCl_3$–MeOH (25–30%) yielded the compound **1**-containing fr. which on flash chromatography over silica gel $CHCl_3$–MeOH 18–19% followed by prep. TLC $CHCl_3$–MeOH–H_2O 35:12:2 afforded compound **1** (0.18 g) as an amorphous powder, $[\alpha]_D^{27}$ + 14° (*c*, 0.09, pyridine).

Scrokoelziside B (**1**). IR (KBr) cm^{-1}: 3400, 1075 (OH), 2920; FAB-MS (negative) 3 kV, *m/z* (rel. int.): 1071 [M − H]$^-$ (100), 1069 (15), 927 (18), 925 (24), 909 (16), 763 (35), 619 (10), 457 (20); ^{1}H and ^{13}C NMR (400 MHz: C_5D_5N): Table 1.

Hydrolysis of compound **1**. Compound **1** (40 mg) refluxed with MeOH–1 M HCl for 5 hr and work up as usual. The aq hydrolysate was neutralized with $BaCO_3$ and filtered. The filtrate was evapd under red. pres. to dryness and acetylated with Py/Ac_2O. GC, with comparison with reference compounds, showed the presence of peracetyl rhamnose, peracetyl fucose and peracetyl glucose (1:1:2).

Acknowledgements—Thanks are due to the staff of RSIC for spectral data and the Director, CDRI for his interest in the work.

REFERENCES

1. Bhandari, S. P. S., Mishra, A., Roy, R. and Garg, H. S., *Phytochemistry*, 1992, **31**, 689.
2. Bhandari, S. P. S., Roy, Raja, Agrawal, P. K. and Garg, H. S., *Phytochemistry*, 1996, **41** (3), 879.
3. Ding, N., Yahara, N. and Nohara, T., *Chemical and Pharmaceutical Bulletin*, 1992, **40**, 780.
4. Seifert, K., Preiss, A., Johne, S., Schmidt, J., Lien, N., Lavaud, C. and Massiot, G., *Phytochemistry*, 1991, **30**, 3395.
5. Agrawal, P. K., *Phytochemistry*, 1992, **31**, 3307.
6. Liu, Z., Li, Du, Owen, N. L., Grant, D. M., Cates, R. G. and Jia, Q., *Journal of Natural Products*, 1995, **58** (10), 1600.
7. Agrawal, P. K., Jain, D. C., Gupta, R. K. and Thakur, R. S., *Phytochemistry*, 1985, **24**, 2479.
8. Agrawal, P. K. and Bansal, M. C., in *Carbon-13 NMR of Flavonoids*, ed. P. K. Agrawal, Elsevier, Amsterdam, 1989, p. 283.
9. Bhandari, S. P. S., Agrawal, P. K. and Garg, H. S., *Phytochemistry*, 1990, **29**, 3889.
10. Calis, I., Zor, M., Basaran, A. A., Wright, A. D. and Sticher, O., *Helvetia Chimica Acta*, 1993, **76** (3), 1352.