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ISOLATION OF NORBERGENIN FROM *SAXIFRAGA STOLONIFERA**

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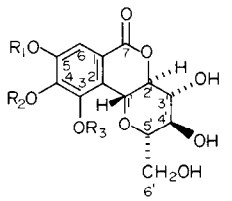
Key Word Index—*Saxifraga stolonifera*; Saxifragaceae; desmethylbergenin; norbergenin; bergenin; tri-*O*-methylnorbergenin.

Abstract—Norbergenin, a *C*-glucoside, was isolated from *Saxifraga stolonifera* and its structure assigned as 2β-D-glucopyranosylgallic acid δ-lactone.

Bergenin (**2**), one of the simplest *C*-glucosides, occurs in a number of plants [1–4]. In previous publications [5, 6], its distribution and biosynthesis have been reported. The results of incorporation of [^{14}C]glucose into **2** by leaves of *Saxifraga stolonifera* showed that gallic acid accepted a glucosyl moiety and was a precursor of **2**. During this study an unidentified phenolic, having high radioactivity, was isolated which was thought to be a demethylation product of **2**; it was named desmethylbergenin (**1**) [6]. While preparing a manuscript on its identification, Kalidhar *et al.* [7] reported the same compound in *Woodfordia fruticosa* (Lythraceae) and called it norbergenin. We now report the confirmation of its structure, as isolated from *S. stolonifera*.



1 $R_1 = R_2 = R_3 = H$
Norbergenin

2 $R_1 = R_3 = H$, $R_2 = Me$
Bergenin

3 $R_1 = R_2 = R_3 = Me$
Tri-*O*-methylnorbergenin

Our compound and its derivatives were identical in every way with norbergenin and its derivatives as described by Kalidhar *et al.* (see Experimental). In addition, the absolute configuration of **1** was determined by the aromatic chirality method [8, 9]. The three Cotton effects at $\Delta\varepsilon_{210} + 2.0$, $\Delta\varepsilon_{228} - 12.5$ and $\Delta\varepsilon_{290} + 0.8$ showed the chiral lactone ring to be a β-glucosyl moiety of **1**.

Table 1. ^{13}C NMR chemical shift of norbergenin (**1**), bergenin (**2**) and tri-*O*-methylnorbergenin (**3**)

	1		**2**	**3**
Carbon No.	CD_3OD–D_2O (2:1)	DMSO-d_6	DMSO-d_6	DMSO-d_6
1	117.49 *s*	116.08 *s*	118.03 *s*	126.45 *s*
2	114.08 *s*	112.76 *s*	115.93 *s*	119.05 *s*
3	143.31 *s*	142.43 *s*	148.04 *s*	150.80 *s*
4	140.93 *s*	139.61 *s*	140.59 *s*	147.70 *s*
5	146.58 *s*	145.94 *s*	150.92 *s*	152.82 *s*
6	111.25 *d*	109.30 *d*	109.50 *d*	109.00 *d*
7	166.65 *s*	163.73 *s*	163.34 *s*	163.54 *s*
1′	73.88 *d*	72.27 *d*	72.17 *d*	71.00 *d*
2′	75.14 *d*	73.78 *d*	73.73 *d*	73.97 *d*
3′	80.99 *d*	79.82 *d*	79.82 *d*	80.16 *d*
4′	71.44 *d*	70.90 *d*	70.71 *d*	70.41 *d*
5′	82.40 *d*	81.57 *d*	81.77 *d*	81.60 *d*
6′	62.33 *t*	61.25 *t*	61.16 *t*	61.11 *t*
OMe	—	—	—	61.10 *q*
	—	—	59.84 *q*	60.60 *q*
	—	—	—	56.00 *q*

21.15 MHz, TMS as int. standard.

*Part 3 in the series "Studies on *C*-Glycosides in Higher Plants". For Part 2 see Taneyama, M. and Yoshida, S. (1979) *Bot. Mag.* **92**, 69.

EXPERIMENTAL

General methods. Mps were uncorr. TLC was carried out on Si gel and on an Avicel plate (0.25 mm). The following solvents were used for PC and TLC: (A) *n*-BuOH–HOAc–H_2O (4:1:2); (B) 6% HOAc; (C) 70% PhOH; (D) EtOAc–MeOH–H_2O (77:13:10).

Isolation. Saxifraga stolonifera was collected at Kanagawa Prefecture, Japan, in Dec. 1978. The whole plants (2.1 kg fr. wt) of *S. stolonifera* were extracted twice with 16 l. hot 80% EtOH. The combined extract was concd under red. pres. to 500 ml and the soln was filtered. The filtrate was extracted with Et_2O and EtOAc successively. The EtOAc-soluble fraction was concd to dryness and then dissolved in 50 ml H_2O. The H_2O-soluble portion was fractionated through the polyamide column (30 × 2 cm, Wako C-200) with 6% HOAc. The fractions containing **1** were concd under red. pres. to a small vol. An aliquot was streaked on paper, which was developed with solvent A followed by solvent B. The band corresponding to **1** was cut out and extracted with 80% EtOH. After concn, the extract was applied to TLC (Avicel plate, solvent A). The band corresponding to **1** was extracted with 80% EtOH. The extract was concd under red. pres. and crystals appeared. Recrystallization from hot H_2O with a small amount of MeOH, gave colourless prisms. Mp 277–278° (mmp 276–277°). Yield 110 mg.

Compound **1**. R_fs (A, B, C, D) of **1** PC 0.22, 0.40, 0.42, 0.43 (detecting with $FeCl_3$–$K_3Fe(CN)_6$ (1:1)). R_fs (A, D) on TLC (Si gel) 0.53, 0.93. Purple colour in UV light, blue-purple under UV light + NH_3 vapour. Analysis: calcd for $C_{13}H_{14}O_9$: C, 49.68; H, 4.49%; found: C, 49.39; H, 4.62%. UV λ_{max}^{EtOH} nm (log ε): 222 (4.27), 289 (3.93); $\lambda_{max}^{EtOH+NaOAc}$ nm: 212, 245 (sh), 338; $\lambda_{max}^{EtOH+AlCl_3}$ nm: 223, 240 (sh), 325; $\lambda_{max}^{EtOH+AlCl_3+HCl}$ nm: 222, 289, IR ν_{max}^{Nujol} cm^{-1}: 3470, 3310, 3250 (OH), 2850 (CH_2), 1690 (C=O), 1618, 1595, 1530 (aromatic), 1250 (aromatic C–O) and 860 (C–H). ^1H NMR (100 MHz, DMSO-d_6, TMS): δ 3.20–4.04 [6H, C-2′–C-5′, C-6′ (CH_2)], 4.96 (1H, *d*, *J* = 10.3 Hz, C-1′), 7.13 (1H, *s*, C-6). For ^{13}C NMR data see Table 1. CD (H_2O; *c* 0.64). ORD (H_2O; *c* 0.61): $[\phi]_{215} + 15.5 \times 10^5$, $[\phi]_{222}$ 0, $[\phi]_{233} - 14.4 \times 10^5$. $[\alpha]_D^{17} - 22.9°$ (H_2O; *c* 0.393).

Demethylation of **2**. This was achieved by HI and **1** was obtained. It gave a single spot on PC, R_fs (A, B, C, D) 0.22, 0.40, 0.42, 0.43 and TLC (Si gel), R_fs (A, D) 0.53, 0.93. Analysis: calcd for $C_{13}H_{14}O_9$: C, 49.68; H, 4.49%; found: C, 49.20; H, 4.45%. Mp 278°. UV λ_{max}^{EtOH} nm (log ε): 222 (4.35), 289 (3.96). $\lambda_{max}^{EtOH+NaOAc}$ nm: 212, 245 (sh), 338. $\lambda_{max}^{EtOH+AlCl_3}$ nm: 223, 240 (sh), 325. $\lambda_{max}^{EtOH+AlCl_3+HCl}$ nm: 222, 289. The results of the IR, ^1H NMR, CD and ORD spectra agreed with those of **1**. ^{13}C NMR data are shown in Table 1.

Hydrolysis of **1**. The hydrolysis was carried out by the method of ref. [10]. Compound **1** (2 mg) in satd $Ba(OH)_2$ soln (2 ml) was heated at 100° for 2 hr in a boiling water-bath in a sealed tube. The gallic acid was isolated from the Et_2O-soluble fraction of the hydrolysate. Gallic acid was confirmed by PC, TLC, UV and IR.

Methylation of **1** *and* **2**. Compounds **1** and **2** were methylated with ethereal CH_2N_2, and tri-*O*-methylnorbergenin was obtained, mp 194–196°. It gave a single spot on PC, R_fs (A, B) 0.67, 0.76 and TLC (Si gel), R_fs (A, B) 0.80, 0.75. Analysis: Calcd $C_{16}H_{20}O_9$: C, 53.93, H, 5.66%; found: C, 53.80, H, 5.55%. UV λ_{max}^{MeOH} nm: 227, 267. $\lambda_{max}^{MeOH+NaOAc}$ nm: 227, 267. $\lambda_{max}^{MeOH+AlCl_3}$ nm: 226, 267. ^1H NMR (100 MHz, DMSO-d_6, TMS as int. standard): δ 3.26–4.02 [6H, C-2′–C-5′, C-6′ (CH_2)], 3.79 (3H, *s*, C-3, OMe), 3.84 (3H, *s*, C-5, OMe), 4.78 (1H, *d*, *J* = 10.7 Hz, C-1′), 7.32 (1H, *s*, C-6). For ^{13}C NMR data see Table 1. GC/MS (probe), 70 eV, *m/z* (rel. int.): 572 $[M]^+$ (0.07), 557 $[M-Me]^+$ (0.3), 500 (50), 499 $[M-73]^+$ (21), 356 (15), 399 (29), 311 (37), 309 (57), 237 (47), 224 (100), 208 (26), 117 (83), 103 (48), 75 (68).

From **2** a dimethyl ether was prepared by the same method which had the same characteristics as **1** trimethyl ether.

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