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Phytochemical Studies of Seeds of Medicinal Plants. I. Two Sulfated Triterpenoid Glycosides, Sulfapatrinosides I and II, from Seeds of *Patrinia scabiosaefolia* FISCHER

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Two new sulfated glycosides were isolated as major constituents from seeds of *Patrinia scabiosaefolia* FISCHER (Valerianaceae). These compounds, termed sulfapatrinosides I (**1**) and II (**2**), have been established to be 23-sulfates (in the forms of the sodium salts) of 3β-hydroxyurs-12-en-28-oic acid 28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester and 3β-hydroxyolean-12-en-28-oic acid 28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester, respectively, based on chemical and spectral evidence.

Keywords——*Patrinia scabiosaefolia*; Valerianaceae; seed; sulfated triterpenoid glycoside; sulfapatrinoside I; sulfapatrinoside II

Whole plants of *Patrinia scabiosaefolia* FISCHER (ominaeshi in Japanese) (Valerianaceae), a Chinese crude drug Bai Jiang in Chinese; Haisho in Japanese), have been used in China as a diuretic and for "Qing Re Jie Du" (treatment of fever and inflammation along with detoxication), "Huo Xue Hua Yu" (mobilization of blood circulation and treatment of stasis), *etc.*[1)]

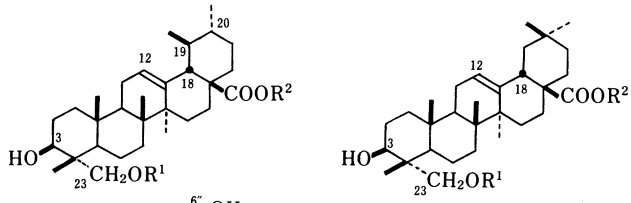
A number of triterpenoid glycosides,[2)] and coumarins,[2b)] together with an iridoid glycoside,[3)] have been identified from roots and rhizomes of *P. scabiosaefolia*, but no phytochemical study on seeds of this plant has appeared to date. This paper describes the structure elucidation of two new sulfated triterpenoid glycosides isolated from the seeds.

The total glycoside mixture from the methanol extracts was purified by chromatographic and high-pressure liquid chromatographic (HPLC) separations to give two new sulfated triterpenoid glycosides named sulfapatrinosides I (**1**) and II (**2**).

Both glycosides, **1**[4)] [$C_{42}H_{67}NaO_{17}S$, mp 239—242 °C, $[\alpha]_D$ +19.2° (pyridine)] and **2**[4)] [$C_{42}H_{67}NaO_{17}S$, mp 242—244 °C $[\alpha]_D$ +25.3° (pyridine)], showed bands due to an ester carbonyl (**1** and **2**, 1720 cm^{-1}) and a typical sulfate S=O stretching vibration (**1**, 1220 cm^{-1} and **2**, 1230 cm^{-1}) in the infrared (IR) spectra.

The presence of a sulfate group in **1** and **2** was further confirmed by the elemental analysis and the positive color test to the pinacryptol yellow reagent.[5)] Negative ion fast atom bombardment mass (FAB-MS) spectral data suggested that both glycosides (**1** and **2**) possess the same molecular weight [$(M-H)^-$, m/z 897]. In addition, both compounds also afforded two common significant fragment peaks at m/z 713 and 551, which arise from the $(M-Na)^-$ ion by the loss of one and two hexose units, respectively.

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** showed signals due to four tertiary methyls, two secondary methyls (δ 0.85, d, J=6.4 Hz and 0.93, d, J=6.4 Hz), an olefinic proton, and two anomeric protons (δ 5.04, d, J=7.9 Hz and 6.18, d, J=7.9 Hz) (Table



1 : $R^1=SO_3^-Na^+$, $R^2=$ (β-D-gentiobiosyl)

2 : $R^1=SO_3^-Na^+$, $R^2=\beta$-D-gentiobiosyl

3 : $R^1=R^2=H$

3a : $R^1=H$, $R^2=CH_3$

4 : $R^1=R^2=H$

5 : $R^1=SO_3^-Na^+$, $R^2=H$

6 : $R^1=SO_3^-Na^+$, $R^2=H$

Chart 1

TABLE I. ^{1}H-NMR Spectral Data for **1**—**6** (400 MHz, in Pyridine-d_5, δ)[a]

	1	3	5	2	4	6
3α-H	4.20—4.30[b]	4.21 (dd, 11.0, 5.5)	4.25 (dd)[c]	4.20—4.30[b]	4.18 (dd, 4.0, —[c])	4.20 (dd)[c]
12-H	5.41 (m)	5.51 (m)	5.46 (m)	5.39 (m)	5.50 (m)	5.45 (m)
18β-H	2.49 (d, 11.6)	2.64 (d, 11.6)	2.62 (d, 11.3)	3.13 (dd, 10.1, 3.5)	3.30 (dd, 10.1, 3.4)	3.27 (dd, 10.4, 3.4)
23-H_2	4.25 (d, 10.7)	3.73 (d, 10.2)	4.23 (d, 10.4)	4.20 (d, 10.3)	3.72 (d, 10.2)	4.21 (d, 10.3)
	4.73 (d, 10.7)	4.19 (d, 10.2)	4.76 (d, 10.4)	4.67 (d, 10.3)	4.18 (d, 10.2)	4.73 (d, 10.3)
CH_3	0.87, 0.92, 0.99, 1.13 (all s)	0.98, 1.07, 1.09, 1.19 (all s)	0.86, 0.88, 1.01, 1.05 (all s)	0.85 (6H), 0.87, 0.90, 1.04, 1.10 (all s)	0.94, 0.98, 1.01, 1.06, 1.07, 1.25 (all s)	0.87, 0.88, 0.92, 0.98, 0.99, 1.15 (all s)
	0.85 (d, 6.4)	0.94 (d, 6.1)	0.94 (d, 6.0)			
	0.93 (d, 6.4)	1.00 (d, 6.4)	1.00 (d, 6.0)			
Anomeric H	5.04 (d, 7.9)			4.95 (d, 7.8)		
	6.18 (d, 7.9)			6.14 (d, 8.2)		

a) Signal multiplicities and coupling constants (Hz) in parentheses. *b*) The multiplicity and coupling constant of these signals are obscure, due to partial overlap. *c*) This coupling constant could not be determined due to partial signal overlap.

I). Similarly, that of **2** exhibited the presence of six tertiary methyls, an olefinic proton, and two anomeric protons (δ 4.95, d, $J=7.8$ Hz and 6.14, d, $J=8.2$ Hz) (Table I). These lines of evidence suggest that both **1** and **2** are sulfated triterpenoid diglycosides.

Acidic hydrolysis of **1** with 10% H_2SO_4–EtOH (1 : 2) gave 23-hydroxyursolic acid (**3**) [mp 264—265 °C, $[\alpha]_D$ +64.0° (MeOH)][6-8] and 2 mol of glucose. Analogous acidic hydrolysis of **2** afforded hederagenin (**4**) and 2 mol of glucose. However, on enzymic hydrolysis with protease [type XIII from *Aspergillus saitoi* (Molsin)],[9] **1** and **2** furnished the corresponding genuine aglycones, **5** [a white powder, $[\alpha]_D$ +49.0° (MeOH)] and **6** [a white powder, $[\alpha]_D$ +54.5° (MeOH)], respectively. Compounds **5** and **6** both showed absorptions due to a carboxyl [**5** and **6**, 1690 cm^{-1}] and sulfate S=O stretching [**5** and **6**, 1240 cm^{-1}] in the respective IR spectra. Negative ion FAB-MS experiments showed that **5** and **6** possess the same molecular formula $C_{30}H_{47}NaO_7S$ [$(M-H)^-$, m/z 573]. These spectral data together with ^{1}H- and carbon-13 nuclear magnetic resonance (^{13}C-NMR) data (Table II) (*vide infra*), indicated that **5** and **6** are monosulfates of **3** and **4**, respectively.

Carboxy compounds **5** and **6** were also derived from **1** and **2**, respectively, by alkaline

TABLE II. ^{13}C-NMR Spectral Data for **1**—**6** (100.5 MHz, in Pyridine-d_5, δ_C)

Carbon No.	**1**	**3**	**5**	**2**	**4**	**6**
Aglycone						
C-1	38.84	38.83	38.79	38.86	38.53	38.63
C-2	26.98	27.58	26.99	26.98	27.40	26.96
C-3	71.62	73.52	71.69	71.58	73.32	71.78
C-4	42.50	42.45	42.52	42.89	42.59	42.84
C-5	47.70[a)]	47.95	47.71[a)]	48.00[a)]	48.48[a)]	48.00[a)]
C-6	18.38	18.49	18.36	18.42	18.34	18.35
C-7	33.16	33.19	33.19	32.59	32.74	32.86
C-8	40.12	39.90	39.96	39.90	39.54	39.74
C-9	47.92[a)]	47.95	47.90[a)]	47.90[a)]	47.92[a)]	47.89[a)]
C-10	36.85	37.03	37.14	37.27	37.00	37.27
C-11	23.63	23.56	23.59	23.41	23.50	23.71
C-12	125.81	125.57	125.46	122.73	122.23	122.36
C-13	138.61	139.16	139.41	144.40	144.57	145.08
C-14	42.87	42.72	42.83	42.12	41.95	42.15
C-15	28.68	28.60	28.64	28.18	28.10	28.22
C-16	24.63	24.82	24.95	23.72	23.45	23.81
C-17	48.48	48.59	48.10	47.06	46.21	46.48
C-18	53.29	53.47	53.62	41.66	41.75	41.97
C-19	39.32[b)]	39.37[b)]	39.50[b)]	46.21	46.40	46.66
C-20	39.10[b)]	39.30[b)]	39.47[b)]	30.74	30.67	30.94
C-21	30.82	30.96	31.13	33.98	33.96	34.24
C-22	37.16	37.33	37.49	32.75	32.96	33.23
C-23	70.12	68.08	70.19	70.26	67.92	70.33
C-24	12.95	12.95	12.95	12.97	12.78	12.99
C-25	16.44	15.97	16.24	16.26	15.69	16.08
C-26	17.32	17.41	17.48	17.58	17.23	17.44
C-27	23.68	23.77	23.87	26.12	25.88	26.20
C-28	176.46	179.70	179.92	176.68	179.86	180.25
C-29	17.76	17.36	17.48	33.07	32.96	33.23
C-30	21.21	21.23	21.38	23.72	23.59	23.81
Inner glucose						
C-1′	95.72			95.76		
C-2′	73.79			73.85		
C-3′	78.36[c)]			78.35[c)]		
C-4′	71.20			71.07		
C-5′	77.89			77.94		
C-6′	69.70			69.53		
Terminal glucose						
C-1″	105.25			105.21		
C-2″	75.20			75.16		
C-3″	78.71[c)]			78.73[c)]		
C-4″	71.62			71.71		
C-5″	78.46[c)]			78.44[c)]		
C-6″	62.69			62.64		

a—*c*) Assignments may be interchanged in each column.

hydrolysis. Furthermore, in the ^{13}C-NMR spectra, the C-28 signals (δ 176.46 and 176.68 ppm, respectively) of **1** and **2** appeared at higher field[10] than those (δ 179.92 and 180.25 ppm, respectively) of **5** and **6**. These chemical and spectral results proved that both **1** and **2** contain a diglucosyl moiety linked (*via* an ester bond) with the 28-COOH group on the triterpene aglycone (**5** and **6**). The position of the sulfate group in **1** and **2** (also on **5** and **6**) was determined as follows. In the ^{1}H-NMR studies (Table I), the 23-H_2 signals of **1** [δ 4.25 and

4.73 (1H each, both d, $J=10.7$ Hz] and **5** [δ 4.23 and 4.76 (1H each, both d, $J=10.4$ Hz)] appeared downfield (by *ca.* 0.5 ppm[11]) from the corresponding signals of **3** [δ 3.73 and 4.19 (1H each, both d, $J=10.2$ Hz)].

Analogously, the 23-H_2 signals of **2** and **6** were also shifted downfield compared with those of **4**. In the ^{13}C-NMR studies (Table II), the C-23 atom of **1**, **2**, **5**, and **6** (δ 70.12, 70.26, 70.19, and 70.33 ppm, respectively) resonated at lower field than that (δ 68.08 and 67.92 ppm, respectively) of **3** and **4**.[11,12] Based on these spectral data, the sulfate group in **1** and **2** is indicated to be located at C-23 on the genuine aglycone (**5** and **6**, respectively) for **1** and **2**. The structure of the diglucosyl moiety in **1** and **2** was established as follows. In the ^{13}C-NMR spectra, both C-6′ atoms on the inner glucoses in **1** and **2** resonated at δ 69.70 and 69.53 ppm, respectively, downfield (by 7.01 and 6.89 ppm), compared with the C-6′′ signals (δ 62.69 and 62.64 ppm, respectively) on the terminal glucoses in **1** and **2**, suggesting that the terminal glucoses in **1** and **2** are linked to the 6′-OH group of the inner glucose by glycosidic linkages, *i.e.*, indicative of the presence of a glucopyranosyl-(1→6)-glucopyranosyl group in both **1** and **2**.

The large J-values of a pair of anomeric proton doublets (Table I) and the chemical shifts of a pair of anomeric carbons (Table II) observed in the ^{1}H- and ^{13}C-NMR spectra for **1** and **2** were indicative of the presence of a β-D-gentiobiosyl moiety (=β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl) as the common glycone part in **1** and **2**. Furthermore, all the carbon signals due to the β-D-gentiobiosyl ester in **1** and **2** were consistent, in chemical shifts, with those reported for other analogous β-D-gentiobiosides, *i.e.*, β-D-gentiobiosyl esters of triterpene 28-oic acids.[11b,13] Based on the combined evidence, the structures of sulfapatrinosides I (**1**) and II (**2**) are now defined as the 23-sulfates (in the forms of the sodium salts) of 3β-hydroxyurs-12-en-28-oic acid 28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester and 3β-hydroxyolean-12-en-28-oic acid 28-*O*-[β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl] ester, respectively. To our knowledge, this is the first report of the isolation of triterpenoids with a sulfate group at C-23 and their glycosides from plant sources.

Furthermore, sulfapatrinoside I (**1**) is the first instance of the occurrence of an ursane-type glycoside in the genus *Patrinia*.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were run with a JASCO A-302 instrument. Unless otherwise stated, ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100.5 MHz) spectra were measured with a JEOL JNM-GX400 spectrometer with pyridine-d_5 as a solvent and tetramethylsilane as an internal standard. Electron impact (EI)-MS spectra were obtained with a JEOL JNM-DX300 spectrometer at 30 eV, and negative ion FAB-MS with the same spectrometer under the following conditions: accelerating voltage, 2—3 kV; matrix, triethanolamine; collision gas, Xe. Optical rotations were determined on a JASCO DIP-140 digital polarimeter. Gas liquid chromatography (GLC) was carried out on a Shimadzu GC-7AG gas chromatograph under the following conditions: column, 1.5% SE-52 on Chromosorb WAW DMCS (2 m × 3 mm i.d.); detector, FID; column temperature, 180 °C; carrier N_2 gas, 30 ml/min. For column chromatography and thin layer chromatography (TLC), Kieselgel 60 (Merck; 230—400 mesh) and precoated silica gel plates (Merck HF-254) were used, respectively. Preparative HPLC was carried out on a Waters instrument with an M 6000A pump, a U6K septum-less injector, and a series R-401 differential refractometer. A reversed-phase Waters ODS column (μ-Bondapack-C_{18}; 7.8 mm × 30 cm) was used, with an eluant flow of 1—1.5 ml/min of H_2O–MeOH (1 : 1).

Plant Material——Seeds of *P. scabiosaefolia*, cultivated at the Medicinal Plant Garden of Setsunan University (Faculty of Pharmaceutical Sciences), were harvested in 1985. The plant used in this study was identified by one of us (H. M.).

Isolation of 1 and 2——The crushed seeds (178 g) were extracted three times with MeOH (1.8 l) at room temperature for two weeks, and the solvent was evaporated off under reduced pressure. The combined extracts (21.7 g) were washed with Et_2O (200 ml) and then, with AcOEt (300 ml). The resultant insoluble precipitate (total glycoside mixture) (11.3 g) was collected by filtration, and a part (8.0 g) of it was chromatographed on silica gel (300 g), eluting with the lower phase of $CHCl_3$–MeOH–H_2O (65 : 35 : 10). Of the 12 separated fractions (Nos. 1 to 12),

fraction 10 afforded a glycoside mixture (1.57 g) comprised of **1** and **2**. A portion (0.5 g) of it was subjected to preparative HPLC separation to give **1** (144 mg), **2** (155 mg), and a mixture (101 mg) of them. The physical and spectral data are as follows. Sulfapatrinoside I (**1**), colorless fine crystals of mp 239—242 °C (EtOH), $[\alpha]_D^{20}$ +19.2° (pyridine, $c=0.33$). IR ν_{max}^{KBr} cm^{-1}: 1720, 1220, 1060, 825. Negative ion FAB-MS *m/z* (%): 897 ($[M(C_{42}H_{67}NaO_{17}S)-H]^-$, 3), 875 [$(M-Na)^-$, 22], 713 [$(M-Na-162)^-$, 2], 551 [$(M-Na-324)^-$, 9], 507 (50), 297 (100).[4] ^{1}H- and ^{13}C-NMR: given in Tables I and II, respectively. *Anal.* Calcd for $C_{42}H_{67}NaO_{17}S\cdot 2H_2O$: C, 53.96; H, 7.60; S, 3.43. Found: C, 54.17; H, 7.35; S, 3.21.[4] Sulfapatrinoside II (**2**), colorless fine crystals of mp 242—244 °C (EtOH), $[\alpha]_D^{20}$ +25.3° (pyridine, $c=0.39$). IR ν_{max}^{KBr} cm^{-1}: 1720, 1230, 1050, 825. Negative ion FAB-MS *m/z* (%): 897 ($[M(C_{42}H_{67}NaO_{17}S)-H]^-$, 9), 875 [$(M-Na)^-$, 47], 713 [$(M-Na-162)^-$, 1], 551 [$(M-Na-324)^-$, 10], 507 (9), 297 (100).[4] ^{1}H- and ^{13}C-NMR: given in Tables I and II, respectively. *Anal.* Calcd for $C_{42}H_{67}NaO_{17}S\cdot H_2O$: C, 55.02; H, 7.53; S, 3.49. Found: C, 54.81; H, 7.18; S, 3.38.[4]

Acidic Hydrolysis of 1——A solution of **1** (30 mg) in 10% H_2SO_4–MeOH (1 : 2, 15 ml) was refluxed for 5 h, then poured into ice-water, and extracted with AcOEt. The AcOEt layer was washed with H_2O, dried over $MgSO_4$, and concentrated to give a crude product (20 mg). Recrystallization from MeOH furnished pure **3** (8 mg), colorless needles, mp 264—265 °C, $[\alpha]_D^{25}$ +64.0° (MeOH, $c=0.27$). IR ν_{max}^{KBr} cm^{-1}: 1680. Negative ion FAB-MS *m/z* (%): 471 [$(M-H)^-$, 64], 297 (100). ^{1}H- and ^{13}C-NMR: given in Tables I and II, respectively.[6—8] The mother liquor of the recrystallization was treated with diazomethane–ether as usual. A product was recrystallized from MeOH to give the methyl ester (**3a**)[7] of **3**, colorless prisms, mp 249—251 °C (ref. 6, mp 254—257 °C), $[\alpha]_D^{25}$ +69.7° ($CHCl_3$, $c=0.22$) [ref. 6, +65.7° ($CHCl_3$)]. IR ν_{max}^{KBr} cm^{-1}: 1717. EI-MS *m/z* (%): 486 [$M^+(C_{31}H_{50}O_4)$, 2], 262 (100), 203 (83). ^{1}H-NMR ($CDCl_3$) δ: 0.74, 0.90, 0.97, 1.07 (3H each, all s, 4 × *tert*-Me), 0.85, 0.94 (3H each, both d, $J=6.4$ Hz, 19β- and 20α-Me), 2.23 (1H, d, $J=11.7$ Hz, 18β-H), 3.60 (3H, s, COOMe), 3.62 (1H, dd, $J=8.2$ Hz, —,[14] 3α-H), 3.43, 3.73 (1H each, both d, $J=10.4$ Hz, 23-H_2), 5.24 (1H, m, 12-H). The melting point, optical rotation, and IR, ^{1}H-NMR, and EI-mass spectra for **3a** were consistent with the published data for authentic 23-hydroxyursolic acid methyl ester.[7] The aqueous layer of the hydrolysate was neutralized with Amberlite IRA-45 (OH^- form) and evaporated under reduced pressure. The residue was subjected to paper partition chromatography (PPC) [iso-PrOH–*n*-BuOH–H_2O (7 : 1 : 2) as the developing solvent system; aniline hydrogen phthalate for detection] and GLC [after trimethylsilylation with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide-pyridine] to demonstrate the presence of 2 mol of glucose.

Acidic Hydrolysis of 2——A solution of **2** (30 mg) in 10% H_2SO_4–EtOH (1 : 2, 15 ml) was refluxed for 5 h. The reaction mixture was worked up in the same manner as in the case of acidic hydrolysis of **1**. The resultant product (**4**) (8 mg) was shown to be identical with authentic hederagenin by mixed melting point determination, and by TLC and IR, ^{1}H-, and ^{13}C-NMR spectral comparisons. From the aqueous layer of the hydrolysate, 2 mol of glucose was identified by PPC and GLC.

Enzymic Hydrolysis of 1——A solution of **1** (40 mg) and protease [type XIII from *Aspergillus saitoi* (Molsin)][9] (500 mg) in 0.2 M citric acid–0.2 M Na_2HPO_4 buffer (pH 4.0, 10 ml) was stirred at 37 °C for 48 h, then poured into H_2O, and extracted with AcOEt. The AcOEt layer was washed with H_2O, dried over $MgSO_4$, and evaporated to dryness. The residue (40 mg) was chromatographed on silica gel with the lower phase of $CHCl_3$–MeOH–H_2O (7 : 3 : 1) as the eluant to give pure **5** (14 mg), $C_{30}H_{47}NaO_7S$, as a white powder of $[\alpha]_D^{25}$ +49.0° (MeOH, $c=0.14$). IR ν_{max}^{Nujol} cm^{-1}: 1690, 1240, 830. Negative ion FAB-MS *m/z* (%): 573 [$(M-H)^-$, 29], 551 [$(M-Na)^-$, 38], 505 (24), 297 (100). ^{1}H- and ^{13}C-NMR: given in Tables I and II, respectively.

Enzymic Hydrolysis of 2——A solution of **2** (40 mg) and protease (500 mg) in 0.2 M citric acid–0.2 M Na_2HPO_4 buffer (pH 4.0, 10 ml) was reacted and worked up in the same manner as in the case of enzymic hydrolysis of **1** to afford pure **6** (13 mg), $C_{30}H_{47}NaO_7S$, as a white powder of $[\alpha]_D^{25}$ +54.5° (MeOH, $c=0.26$). IR ν_{max}^{Nujol} cm^{-1}: 1690, 1240, 825. Negative ion FAB-MS *m/z* (%): 573 [$(M-H)^-$, 22], 551 [$(M-Na)^-$, 67], 505 (17), 297 (100). ^{1}H- and ^{13}C-NMR: given in Tables I and II, respectively.

Alkaline Hydrolysis of 1——A solution of **1** (6 mg) in 10% aqueous NaOH–EtOH (1 : 1, 2 ml) was refluxed for 3 h, poured into ice-water, acidified with 5% H_2SO_4, and extracted with AcOEt. The AcOEt layer was washed with H_2O, dried over $MgSO_4$, and evaporated to dryness. The residue was purified by silica gel column chromatography to give the corresponding aglycone (2.0 mg), which was identical with the genuine aglycone (**5**), obtained by enzymic hydrolysis of **1** (*vide supra*), as judged from IR and ^{1}H-NMR spectral and TLC comparisons.

Alkaline Hydrolysis of 2——On alkaline hydrolysis of **2** in the same manner as in the case of alkaline hydrolysis of **1**, **2** (6 mg) afforded the corresponding aglycone (2.0 mg), which was identical with the genuine aglycone (**6**) obtained by enzymic hydrolysis of **2** (*vide supra*) as judged from IR and ^{1}H-NMR spectral and TLC comparisons.

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