Two New Triterpenoid Sulfates from the Leaves of *Schefflera octophylla*[1)]

Junichi KITAJIMA,* Masami SHINDO and Yasuko TANAKA

Showa College of Pharmaceutical Sciences, Tsurumaki 5-1-8, Setagaya-ku, Tokyo 154, Japan. *Received September 22, 1989*

Two new triterpenoid sulfates were isolated from the fresh leaf blades of *Schefflera octophylla* (Araliaceae). On the basis of results of spectral and chemical investigations, their structures were characterized as free forms of 3-*epi*-betulinic acid 3-*O*-sulfate and betulinic acid 3-*O*-sulfate.

Keywords *Schefflera octophylla*; Araliaceae; lupane-type triterpenoid 3-*O*-sulfate; FAB-MS; NMR

In the previous paper,[1)] we reported the isolation and characterization of two new triterpenoid glycosides, 3α-hydroxy-lup-20(29)-ene-23,28-dioic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside and 3-*epi*-betulinic acid 3-*O*-β-D-glucopyranoside together with lup-20(29)-ene-23,28-dioic acid[2)] from the fresh leaf blades and petioles of *Schefflera octophylla* (Araliaceae, fukanoki in Japanese), which is used in Chinese and Vietnamese folk medicines, for example, as an anti-inflammatory agent. In this paper, we describe the isolation and structure elucidation of triterpenoid sulfates from the fresh leaf blades of this plant.

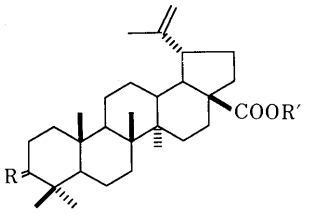
A methanolic extract of the fresh leaf blades was heated under reflux successively with hexane, ether, acetone and methanol, and the methanol-soluble fraction was subjected to column chromatography on Amberlite XAD-II and then silica gel to afford a sulfate fraction. This fraction was separated by repeated column chromatography on silica gel and Sephadex LH-20, and two new triterpenoid sulfates (compounds I and II) were isolated.

Compound I (**1**) was positive for the Liebermann–Burchard reaction and showed the $(M-H)^-$ ion peak at *m/z* 535 ($C_{30}H_{47}O_6S$) in the negative fast-atom bombardment mass spectrum (FAB-MS). The presence of sulfate function in **1** has been suggested by a positive result in the potassium rhodizonate reagent test,[3)] and the distinctive absorption (1230 cm^{-1}) in its infrared (IR) spectrum due to the S–O bond stretching vibration, together with carboxylic (1700 cm^{-1}) and *exo*-methylene (1640, 880 cm^{-1}) groups.[3a,4)] The proton nuclear magnetic resonance (^{1}H-NMR) spectrum (in C_5D_5N) exhibited signals due to six tertiary methyl, and two olefinic protons (δ 4.79 and 4.94, multiplet) and a broad singlet signal of one-proton intensity at δ 4.61, which is ascribable to a methine proton attached to carbon bearing a sulfate group.[3a,5)] From these properties, **1** was considered to be a triterpenoid sulfate with one carboxylic acid group and one *exo*-methylene group. Compound I (**1**) was not acetylated by the usual method (Ac_2O–pyridine at room temperature for 20 h), but **1**-methylate (**2**) was produced by methylation with ethereal diazomethane in methanol. By both hydrolysis using 1 N HCl and solvolysis with dioxane containing potassium carbonate, **1** provided the same product, which was identical with 3-*epi*-betulinic acid (**3**).[1,6)] A comparative investigation of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral data of **1** with those of **2** and **3** resulted in the assignment of carbon signals as given in Table I.[1,7)] The spectral data of **1** showed a good coincidence with those of **3** except for signals due to C-3, C-4 and C-5, an effect of shifts due to the presence of sulfate at 3α-OH.[8)] From these results, **1** was characterized as 3-*epi*-betulinic acid 3-*O*-sulfate.

Compound II (**4**) was positive for the Liebermann–Burchard reaction and positive for the potassium rhodizonate reagent test. The negative FAB-MS of **4** exhibited an $(M-H)^-$ ion peak at *m/z* 535 ($C_{30}H_{47}O_6S$), and the IR spectrum showed distinctive absorption due to carboxylic (1695 cm^{-1}), *exo*-methylene (1630, 875 cm^{-1}) and sulfate (1230 cm^{-1}) groups. The ^{1}H-NMR of **4** exhibited signals due to six tertiary methyl, and two olefinic protons (δ 4.78 and 4.93, multiplet) and a multiplet of totally one-proton intensity between δ 4.3 and 4.6, which was ascribable to a methine-proton attached to a carbon bearing a sulfate group.[3a,5)] All of these spectral properties were similar to those of **1**, and **4** was suggested to be a triterpenoid sulfate with one carboxylic acid group and one *exo*-methylene group. Compound II (**4**) was methylated with ethereal diazomethane [**4**-methylate (**5**)] and **4** was solvolysed with dioxane containing potassium carbonate. From the solvolyzate, betulinic acid (**6**) was obtained as the desulfate product. As described in Table I, the ^{13}C-NMR sectral data of **4** were shown to be almost identical with those of **6**[7)] except for the signals due to C-2 and C-3 (an effect of shifts due to the presence of sulfate at 3-β-OH).[8)] Thus, **4** was characterized as betulinic acid 3-*O*-sulfate.

From the negative FAB-MS data [no detection of a $(C_{30}H_{47}O_6SNa)^+$ ion peak] and the results of atomic absorption spectrometry (no significant amount of Na^+, Mg^+ or K^+ ion detectable), **1** and **4** were considered to be free sulfate forms of the natural products.

This is the first example of naturally occurring lupane-



1 : R = 3β-OSO$_3$H, H R′ = H
2 : R = 3β-OSO$_3$H, H R′ = CH_3
3 : R = 3β-OH, H R′ = H
4 : R = 3α-OSO$_3$H, H R′ = H
5 : R = 3α-OSO$_3$H, H R′ = CH_3
6 : R = 3α-OH, H R′ = H

Chart 1

TABLE I. ^{13}C-NMR Assignments of **1**—**6** (δ) (**1**—**5** in C_5D_5N, **6** in C_5D_5N:$CDCl_3$ (1:1))

	1	2	3	4	5	6[7]
C-1	34.2	34.0	34.0	38.5	38.9	39.0
C-2	23.2	23.0	23.2	24.9	25.0	27.6
C-3	82.9	83.9	75.5	86.0	85.7	78.2
C-4	37.8	37.6	39.0	38.8	39.0	39.0
C-5	50.4	50.2	49.3	56.1	56.2	55.5
C-6	18.4	18.2	18.6	18.6	18.6	18.4
C-7	34.6	34.3	34.8	34.6	34.6	34.5
C-8	41.2	41.0	41.3	41.0	41.0	40.8
C-9	50.6	50.3	50.7	50.7	50.7	50.7
C-10	37.6	37.2	37.7	37.2	37.3	37.3
C-11	21.0	20.8	21.0	21.1	21.1	21.0
C-12	26.0	25.8	26.1	26.0	26.0	25.6
C-13	38.5	38.3	38.5	38.8	38.5	38.2
C-14	42.9	42.6	42.9	42.8	42.7	42.5
C-15	31.2	30.8	31.2	31.2	31.0	30.8
C-16	32.9	32.3	32.8	32.8	32.4	32.6
C-17	56.6	56.7	56.6	56.6	56.8	56.3
C-18	47.7	47.3	47.7	47.7	47.6	47.1
C-19	49.7	49.7	49.7	49.7	49.8	49.4
C-20	151.3	150.7	151.2	151.2	150.2	150.9
C-21	30.3	29.9	29.9	30.2	30.0	29.9
C-22	37.6	37.0	37.5	37.6	37.1	37.3
C-23	28.9	28.6	29.2	28.4	28.5	28.2
C-24	22.3	22.1	22.5	16.7	16.9	15.6
C-25	16.3	16.2[a]	16.4	16.3[a]	16.3[a]	16.1
C-26	16.3	16.1[a]	16.4	16.2[a]	16.2[a]	16.1
C-27	15.1	15.0	14.9	14.9	14.9	14.7
C-28	178.9	176.3	178.7	179.0	176.5	178.9
C-29	19.5	19.5	19.4	19.4	19.4	19.4
C-30	109.8	109.9	109.8	109.8	110.2	109.4
COOMe		51.2			51.4	

a) Assignment may be reversed.

type triterpenoid sulfates and the first report of isolation of triterpenoid sulfates from Araliaceae plants. It is interesting that triterpenoid sulfates were obtained as free forms from nature.

Experimental

Melting points were measured with a Büchi 510 melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-140 automatic polarimeter. IR spectra were obtained with a JASCO A-103 infrared spectrophotometer. Atomic absorption spectra were taken using a HITACHI polarized Zeeman atomic absorption spectrophotometer. Electron impact (EI) and chemical ionization mass spectra (CI-MS) were recorded on a JEOL JMS D-300 spectrometer. ^{1}H- and ^{13}C-NMR spectra were run on a JEOL JNM FX-100 spectrometer (100 MHz for ^{1}H-NMR, 25 MHz for ^{13}C-NMR) and a JEOL JNM GX-270 spectrometer (270 MHz for ^{1}H-NMR, 67.5 MHz for ^{13}C-NMR). Column chromatography was carried out using Kieselgel 60 (0.063—0.2 mm, Merck), Silica Woelm TSC (silica gel for dry column, Woelm), Sephadex LH-20 (25—100 μm, Pharmacia) and Amberlite XAD-II (Organo). Thin layer chromatography (TLC) was conducted on DC-Fertigplatten Kiselgel 60 (Merck) and spots were located using anisaldehyde reagent.[9]

Extraction and Isolation As we reported in the previous paper,[1] fresh leaf blades (320 g) were extracted with MeOH at room temperature.[10] After evaporation of the solvent under reduced pressure, the syrupy residue (19.4 g) was extracted successively with hot hexane, hot ether, hot acetone and hot MeOH. The hot MeOH-soluble fraction (11.0 g) was subjected to column chromatography on Amberlite XAD-II ($H_2O\rightarrow$MeOH), and the MeOH eluate fraction was separated by silica gel column chromatography [$CHCl_3$–MeOH–H_2O (8:2:0.1)] to obtain the sulfate fraction (0.28 g). This fraction was repeatedly purified by silica gel [$CHCl_3$–MeOH (9:1→8:2)] and Sephadex LH-20 (MeOH), and finally compounds I (0.11 g) and II (0.07 g) were isolated.

Compound I (1) White needles, mp 260—265 °C (contracted at 127—130 °C with black coloring), $[\alpha]_D^{27}$ −12.2° (c=0.5, EtOH). Negative FAB-MS m/z: 535 $(M-H)^-$. EI-MS m/z: 438 $(M-H_2SO_4)^+$, 423, 395 (base), 259, 189. CI-MS m/z: 439 $(M-H_2SO_4+H)^+$ (base), 395, 189. IR ν_{max}^{Nujol} cm^{-1}: 1700, 1640, 1230, 880. ^{1}H-NMR (100 MHz, C_5D_5N) δ: 0.76, 0.81, 0.93, 1.03, 1.33, 1.83 (each 3H, s, *tert*-CH_3), 4.61 (1H, br s, 3β-H), 4.79, 4.94 (each 1H, m, $>C=CH_2$). ^{13}C-NMR (25 MHz, C_5D_5N) δ: Table I. The amounts of Na^+, K^+ and Mg^+ ions detected by atomic absorption spectrometry were less than 1% of the calculated values.

Detection of Sulfate Function in 1 A solution of **1** (1 mg) in aq. 2 N HCl (1 ml) was heated for 2 h, neutralized with dil. NaOH and evaporated to dryness under reduced pressure. The residue was subjected to paper partition chromatography (Toyo Filter Paper No. 50), being developed with MeOH–H_2O (9:1) mixture. After drying in the air, the paper was sprayed with a solution of $BaCl_2$ (100 mg) in 70% MeOH (50 ml) and dried again in air. The paper was then sprayed with a solution of potassium rhodizonate (10 mg) in 50% MeOH (50 ml) to develop the positive coloration (yellow).

Methylation of 1 A solution of **1** (30 mg) in MeOH was treated with ethereal diazomethane and the reaction mixture was left to stand at room temperature overnight. The reaction mixture was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography [$CHCl_3$–MeOH (9:1)] to give **1**-methylate (**2**). **2**: White powder. ^{1}H-NMR (270 MHz, C_5D_5N) δ: 0.76, 0.80, 0.82, 0.94, 1.31, 1.75 (each 3H, s, *tert*-CH_3), 3.66 (3H, s, $COOCH_3$), 4.46 (1H, br s, 3β-H), 4.73, 4.85 (each 1H, m, $>C=CH_2$). ^{13}C-NMR (67.5 MHz, C_5D_5N) δ: Table I.

Acid Hydrolysis of 1 A solution of **1** (20 mg) in aq. 1 N HCl (3 ml) was heated for 2 h, then added with water and extracted with ether. The ether solution was washed with water, dried (Na_2SO_4) and concentrated. The residue was chromatographed on silica gel [$CHCl_3$–MeOH (9:1)] to give the desulfate (**3**), which was identical with 3-*epi*-betulinic acid by ^{1}H-NMR and TLC comparison with an authentic sample.

Solvolysis of 1 A solution of **1** (30 mg) in pyridine (3 ml) was left to stand at 40 °C for 2 h. After removal of pyridine under reduced pressure, the residue was dissolved in 50% MeOH (1 ml), followed by addition of dioxane (30 ml) and K_2CO_3 (3.5 mg), and heated under reflux for 20 min. The reaction mixture was diluted with EtOAc, then filtered, and evaporated under reduced pressure to give the desulfate, which was purified in the same way as described for the acid hydrolysis to give **3**.

Compound II (4) White powder [mp 253—257 °C (contracted at 140—142 °C with black coloring)], $[\alpha]_D^{27}$ +4.3° (c=0.5, EtOH). Negative FAB-MS m/z: 535 $(M-H)^-$. IR ν_{max}^{Nujol} cm^{-1}: 1695, 1640, 1230, 875. ^{1}H-NMR (100 MHz, C_5D_5N) δ: 0.73, 0.88, 1.00, 1.07, 1.26, 1.80 (each 3H, s, *tert*-CH_3), 4.46 (1H, m, 3α-H), 4.78, 4.93 (each 1H, m, $>C=CH_2$). ^{13}C-NMR (25 MHz, C_5D_5N) δ: Table I.

Detection of Sulfate Function in 4 A slution of **4** (1 mg) in aq. 2 N HCl (1 ml) was treated in the same way as described for **1** and a positive coloration result obtained.

Methylation of 4 A solution of **4** (20 mg) in MeOH was treated in the same way as described for **1** to give **4**-methylate (**5**). **5**: White powder. ^{1}H-NMR (270 MHz, C_5D_5N) δ: 0.77, 0.93, 0.95, 1.02, 1.30, 1.76 (each 3H, s, *tert*-CH_3), 3.72 (3H, s, $COOCH_3$), 4.49 (1H, m, 3α-H), 4.76, 4.91 (each 1H, m, $>C=CH_2$). ^{13}C-NMR (67.5 MHz, C_5D_5N) δ: Table I.

Solvolysis of 4 A solution of **4** (20 mg) in pyridine (3 ml) was treated in the same way as described for the solvolysis of **1** to give a desulfate (**6**), which was identified as betulinic acid by ^{1}H-NMR and TLC comparison with an authentic sample.

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