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A SULPHATED TRITERPENOID SAPONIN FROM *SCHEFFLERA OCTOPHYLLA*

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Key Word Index—*Schefflera octophylla*; Araliaceae; lupane glycoside 3-*O*-sulphate.

Abstract—Dried leaves of *Schefflera octophylla* afforded a new sulphated triterpene glycoside. From spectroscopic data and chemical transformations the structure of the new constituent was determined as 3-*epi*-betulinic acid 3-*O*-sulphate 28-*O*-[α-L-rhamnopyranosyl(1→4)-*O*-β-D-glucopyranosyl(1→6)]-β-D-glucopyranoside.

INTRODUCTION

In recent papers [1, 2] the isolation and structures of some triterpene glycosides of the lupane series were described from *Schefflera octophylla* which is used in the folk medicine of southeast Asia as an anti-inflammatory and a tonic drug [3, 4]. In continuation of this studies we now report a new sulphated triterpenoid saponin isolated from the same source. Based on spectroscopic data and chemical transformations, the structure of this constituent was shown to be 3-*epi*-betulinic acid 3-*O*-sulphate 28-*O*-[α-L-rhamnopyranosyl(1→4)-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (**1**).

RESULTS AND DISCUSSION

Repeated flash chromatography of the methanol extract of dried leaves followed by gel filtration on Sephadex LH 20 provided saponin **1** in 0.10% yield. Compound **1** showed IR absorption for hydroxyl (3520), ester carbonyl (1730) and exo-methylene group (1640, 890 cm^{-1}). Its ^1H NMR spectrum exhibited signals for six tertiary methyls, one of them at δ 1.69 and one secondary methyl (δ 1.26, *d*, $J=6.3$ Hz). The signal of H-3β appeared downfield shifted at δ 4.04 in comparison to that of 3-*epi*-betulinic acid (**3**, δ 3.38) [1] indicating that the sulphate group is located at C-3 [5, 6]. This was supported by the ^{13}C NMR spectrum with a signal at δ 85.4 (C-3), while for 3-*epi*-betulinic acid this signal appeared at δ 76.4. Except for the signals due to C-2, C-3 and C-5 the ^{13}C NMR spectrum of **1** was shown to be identical with that of the glycoside **4**, isolated recently from this plant [1] by direct comparison (see Table 1). Obviously, on heating with pyridine–dioxane [5] compound **1** desulphated to give **4**, identified by the IR spectrum and co-TLC with an authentic sample.

Furthermore, the FAB mass spectrum of **1** showed a base peak at *m/z* 1005 $[M-H]^-$ as well as peaks indicating the stepwise cleavage of the sugar residue at *m/z* 859 $[M-H-Rha]^-$, 697 $[M-H-Rha-Glc]^-$, and 535 $[M-H-Rha-Glc-Glc]^-$, while the FAB mass spectrum of peracetate **1a** contained peaks at *m/z* 1383 $[M-H]^-$, 851 $[(Rha-Glc-Glc)Ac_9]^-$, 535 $[M-H-$sugar residue$]^-$, and 97 $[HSO_3]^-$.

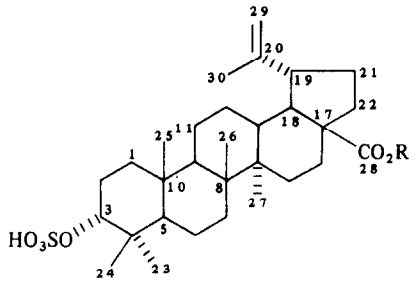
The $LiAlH_4$ reduction of peracetate **1a** yielded 3-*epi*-betulin (**2b**) and alcohol **2**, which was acetylated to **2a**. Alkaline hydrolysis of **1** provided aglycone **1c** and via desulphation 3-*epi*-betulinic acid (**3**) in a 1:1 ratio. Compound **1c** gave a positive reaction with the potassium rhodizonate reagent [6, 7] and remained unchanged under usual acetylation conditions (Ac_2O–pyridine). With diazomethane, however, a methyl ester **1d** was obtained. It exhibited a distinctive IR absorption at ν_{max} 1240 cm^{-1} due to the S–O bond stretching vibration [8].

Upon permethylation [9], **1** afforded the nonamethyl derivative **1b** as main product. On acid hydrolysis, **1b** gave methyl pyranosides of 2,3,4-tri-*O*-methylrhamnose,2,3,4- and 2,3,6-tri-*O*-methylglucose, identified by GC. The EI mass spectra of all compounds **1c**, **1d**, **2** and **2a** showed no molecular ion peaks, they always contained the $[M-H_2SO_4]^+$ peaks, determined by high resolution.

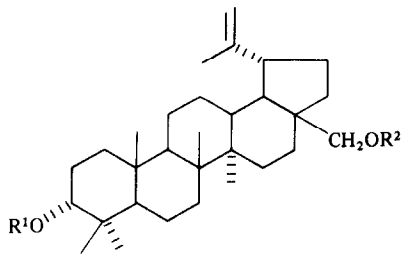
From these data the structure of the new saponin should be 3-*epi*-betulinic acid 3-*O*-sulphate 28-*O*-[α-L-rhamnopyranosyl (1→4)-*O*-β-D-glucopyranosyl (1→6)]-β-D-glucopyranoside (**1**). During the preparation of this manuscript we became aware of a recent paper of Kitajima *et al.* [10], who described the isolation and structures of two free triterpenoid sulphates from *Schefflera octophylla*. The 3-*O*-sulphated saponins of oleanolic acid, echinocystic acid [6] and withanolides [5] were reported.

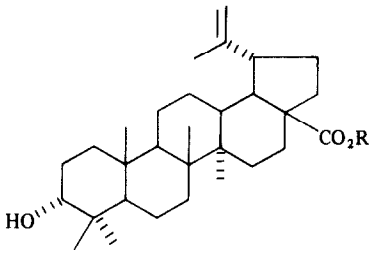
EXPERIMENTAL

Isolation of compound **1**. Dried leaves (250 g) were extracted with hot MeOH for 10 hr at 70° and then filtered and evapd. The residue was dissolved in H_2O and extracted with $CHCl_3$ to remove non-polar material. The aq. layer was evapd to dryness and repeatedly flash chromatographed ($CHCl_3$–MeOH–H_2O, 60:35:8) to give a product which was passed through Sephadex LH 20 (MeOH, 800 ml). The later frs from the Sephadex LH 20 column containing **1** were collected and the procedure was repeated once more to give pure **1** (250 mg, 0.10%). R_f 0.29 (FS 1), powder mp 175–185° (dec.), $[\alpha]_D^{20}$ −33.8°



1 R = (α-L-Rham^{4}β-D-Glc^{6}β-D-Glc-)
1a R = (α-L-Rham^{4}β-D-Glc^{6}β-D-Glc-)Ac_9
1b R = (α-L-Rham^{4}β-D-Glc^{6}β-D-Glc-)Me_9
1c R = H
1d R = Me





2 R^1 = HSO_3, R^2 = H
2a R^1 = HSO_3, R^2 = Ac
2b R^1 = R^2 = H

3 R = H
4 R = (α-L-Rham^{4}β-D-Glc^{6}β-D-Glc-)

(MeOH; *c* 0.455). IR_{max}^{KBr} cm^{-1}: 3420 (OH), 1730 (ester), 1640, 880 (>C=CH_2), 1220 (S-O). FAB-MS (negative ion) *m/z* (rel. int.): 1005 $[M-H]^-$ (100). 859 $[M-H-Rham]^-$ (8), 697 $[M-H-Rham-Glc]^-$ (8), 535 $[M-H-Rham-Glc-Glc]^-$ (33), 489 (22), 1027 (34), 955 (12), 881 (6), 725 (10). ^1H NMR (200 MHz, CD_3OD): δ 0.88 (6H, *s*). 0.93 (3H, *s*), 0.98 (6H, *s*), 1.69 (3H, *s*), 1.26 (3H, *d*, *J* = 6.3 Hz, Rham-Me), 2.99 (1H, *t*-like, H-19), 4.04 (1H, *br s*, H-3β), 4.40 (1H, *d*, *J* = 7.6 Hz, anomeric proton), 4.60, 4.73 (each 1H, *br s*, >C=CH_2), 4.57 (1H, *d*, *J* = 7.6 Hz anomeric proton). ^{13}C NMR: see Table 1.

Peracetylation of compound **1**. Compound **1** (50 mg) was peracetylated with Ac_2O–pyridine (each 0.6 ml) for 18 hr at room temp. The mixture was evapd to dryness and the residue was flash chromatographed ($CHCl_3$–MeOH, 9:1) to give peracetate **1a** (50 mg). R_f 0.48 (FS 6). powder mp 153–155°, $[\alpha]_D^{20}$ −18° ($CHCl_3$; *c* 0.775). FAB-MS (negative ion) *m/z* (rel. int.): 1383 $[M-H]^-$ (80), 1341 (8.8), 1035 (2.2), 851 [(Rham−Glc−Glc)Ac_9]$^-$ (3.2), 821 (2.4), 535 [M−H−(Rham−Glc−Glc)Ac_9]$^-$ (26.4), 489 (20), 299 (8.8), 219 (16), 97 $[HSO_3]^-$ (57.6), 59 (100). FAB-MS (positive ion) *m/z* (rel. int.): 561 [(Rham−Glc)Ac_6]$^+$ (0.8), 273 [(Rham)Ac_3]$^+$ (17.2), 153 (25.6), 111 (65.6), 43 (100). ^1H NMR (200 MHz, $CDCl_3$): δ 0.79–0.90 (5 × *tert.* Me), 1.11 (3H, *d*, *J* = 6.4 Hz, Rham-Me), 1.62 (3H, *s*), 2.91 (1H, *br*, H-19), 4.47 (1H, *d*, *J* = 8 Hz, anomeric proton), 4.55, 4.69 (each 1H, *br s*, >C=CH_2), 5.64 (1H, *d*, *J* = 8 Hz, anomeric proton).

Permethylation of compound **1**. Compound **1** (58 mg) was stirred in DMSO (4.7 ml) with *t*-BuONa (491 mg), finely powdered NaOH (144 mg) and MeI (3.9 ml) for 45 min at room temp. The mixture was poured into ice-water and extracted with BuOH. The BuOH layer was washed with satd NaCl soln and evapd. The residue was passed through Sephadex LH 20 (MeOH) to give permethyl **1b** (38 mg). R_f 0.44 (FS 6) powder mp 153–157°, $[\alpha]_D^{20}$ −2.2° (MeOH; *c* 0.37). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1735 (ester), 1640, 890 (>C=CH_2). ^1H NMR (400 MHz, CD_3OD): δ 0.86, 0.87, 0.95, 0.99, 1.01, 1.69 (each 3H, *s*, *tert.* CH_3), 1.21 (3H, *d*, *J* = 6 Hz, Rham-Me), 3.00 (1H, *br s*, H-19), 4.04 (1H, *br s*, H-3β), 4.36 (1H, *d*, *J* = 8 Hz, anomeric proton), 4.60, 4.71 (each 1H, *br s*, >C=CH_2), 4.91 (1H, *d*, *J* = 8 Hz, anomeric proton).

Acid hydrolysis of compound **1b**. Compound **1b** (5 mg) was hydrolysed in MeOH with 6% H_2SO_4 (1.5 ml) for 3 hr at 80°. The mixture was evapd, diluted with H_2O and extracted with $CHCl_3$. On GC analysis the $CHCl_3$ extract showed the presence of methyl pyranosides of: 2,3,4-tri-*O*-methylrhamnose; 2,3,4- and 2,3,6-tri-*O*-methylglucose.

Alkaline hydrolysis of compound **1**. Compound **1** (100 mg) was heated in MeOH with 5% KOH (15 ml) for 3.5 hr at 80°. The soln was passed through Dowex 50 w × 4 (H^+ form, MeOH) and evapd. The residue was dissolved in H_2O and extracted with BuOH. BuOH was evapd and the solid passed through Sephadex LH 20 (MeOH, 600 ml) to give aglycone **1c** (20 mg) and 3-*epi*-betulinic acid (**3**) (20 mg).

Compound **1c**. R_f 0.30 (FS 6), powder mp 200–205°, $[\alpha]_D^{20}$ −6.7° (MeOH; *c* 0.49 [lit. [10]: mp 260–265°; $[\alpha]_D^{27}$ −12.2° (EtOH; *c* 0.50)]. IR ν_{max}^{KBr} cm^{-1}: 3550 (OH), 3080, 1640, 895 (>C=CH_2), 1697 (CO_2H), 1240 (S-O, vs). MS *m/z* (rel. int.): 438.3490 $C_{30}H_{46}O_2$ calc. 438.3497 $[M-H_2SO_4]^+$ (47.4), 423

Table 1. ^{13}C NMR spectral data of compounds **1**, **1c**, **1d** and **4**

C	1*	4*	1c†	1d*
1	35.2	35.4	34.6	35.4
2	23.6	26.4	23.2	23.7
3	85.4	74.5	84.1	85.7
4	38.3	38.4	37.8	38.5
5	51.5	49.3	50.6	51.6
6	19.0	19.2	18.4	19.1
7	34.7	34.4	34.2	34.8
8	42.0	42.0	41.2	42.0
9	51.0	51.4	50.6	51.1
10	38.1	38.2	37.4	38.2
11	21.8	21.8	21.0	21.9
12	26.7	26.7	26.1	26.8
13	39.2	39.2	38.6	39.6
14	43.5	43.5	42.9	43.6
15	30.7	30.7	30.3	30.7
16	32.7	32.7	32.9	33.1
17	57.8	57.8	56.7	57.9
18	50.4	50.4	49.8	50.6
19	48.2	48.3	47.8	48.5
20	151.6	151.5	151.5	151.7
21	31.4	31.4	31.3	31.6
22	37.5	37.5	37.7	37.7
23	28.9	29.0	28.9	29.0
24	22.4	22.7	22.2	22.4
25	16.7[a]	16.7[a]	16.4[a]	16.7[a]
26	16.6[a]	16.6[a]	16.3[a]	16.5[a]
27	15.0	15.2	15.1	15.1
28	176.2	176.0	179.3	178.1
29	110.5	110.5	109.9	110.3
30	19.5	19.6	19.6	19.5
				51.8 (CO_2Me)
Sugar moiety				
Glc 1				
1′	95.1	95.0		
2′	73.5	73.8		
3′	79.3	79.2		
4′	70.8	70.6		
5′	77.9	78.0		
6′	69.4	69.4		
Glc 2				
1″	104.3	104.3		
2″	75.1	75.1		
3″	76.6	76.4		
4″	77.8	77.7		
5″	76.5	76.6		
6″	61.6	61.7		
Rham				
1‴	102.7	102.7		
2‴	72.2	72.2		
3‴	72.0	72.0		
4‴	73.8	73.6		
5‴	70.5	70.4		
6‴	17.8	17.8		

*50.3 MHz in methanol-d_4.
†50.3 MHz in pyridine-d_5.
[a]Assignments may be interchanged in each column.

$[M-H_2SO_4-Me]^+$ (12), 395 (7), 259 (46), 248 (16), 234 (12), 219 (10), 202 (30), 189 (100), 150 (10), 121 (68). ^1H NMR (200 MHz, $CDCl_3$–CD_3OD 1:1): δ 0.83, 0.85, 0.93, 0.97, 0.99, 1.66 (each 3H, *s*, *tert.* Me), 2.98 (1H, *m*, H-19), 3.65 (1H, *s*, OH), 4.05 (1H, *br s*, H-3β), 4.56, 4.69 (each 1H, *br s*, $>C=CH_2$). ^{13}C NMR: see Table 1.

Compound **3** showed identical ^1H NMR, IR and R_f with those of 3-*epi*-betulinic acid [1, 11].

Detection of sulphate group in compound **1c**. Compound **1c** (2 mg) was refluxed with 2 M HCl (1.5 ml) for 3 hr. The soln was stirred with Dowex 501 × 8 (OH^- form), filtered and evapd to dryness. The residue was subjected to PC (Whatman No. 1, solvent system: MeOH–H_2O, 9:1). After drying in the air, the paper was sprayed with a soln of $BaCl_2$ (100 mg in 50 ml 70% MeOH) and dried again. The paper was then sprayed with a soln of K rhodizonate (10 mg in 50 ml 50% MeOH) to develop the light yellow colour.

Methylation of compound **1c**. A soln of **1c** (29 mg) in MeOH (10 ml) was treated with ethereal diazomethane for 30 min at room temp. After evapn the residue was passed through Sephadex LH 20 (250 ml, MeOH) to give methyl ester **1d** (23 mg), R_f 0.34 (FS 6), mp 173–175° (from $CHCl_3$–MeOH), $[\alpha]_D^{20}$ −15.7° (MeOH; *c* 0.30). MS *m/z* (rel. int.): 452.3657 $C_{31}H_{48}O_2$ calc. 452.3654 $[M-H_2SO_4]^+$ (54.3), 437 $[M-H_2SO_4-Me]^+$ (12), 393 $[M-H_2SO_4-CO_2Me]^+$ (18), 377 (8), 273 (44), 262 (22), 249 (15), 248 (15), 233 (8), 203 (32), 202 (29), 189 (100), 121 (47), 107 (38). ^1H NMR (200 MHz, $CDCl_3$–CD_3OD, 1:1): δ 0.76, 0.80, 0.83, 0.89, 0.92, 1.61 (each 3H, *s*, *tert.* Me), 2.91 (1H, *dt* J = 11.2, 4.2 Hz, H-19), 3.60 (3H, *s*, CO_2Me), 4.02 (1H, *br s*, H-3β), 4.53, 4.67 (each 1H, *br s*, $>C=CH_2$). ^{13}C NMR: see Table 1.

Acetylation of compound **1c**. Compound **1c** (7 mg) was acetylated with Ac_2O–pyridine (each 0.1 ml) at room temp. for 21 hr. The starting material was obtained after evapn of the soln.

$LiAlH_4$ *reduction of peracetate* **1a**. A soln of **1a** (63 mg) in dry THF (10 ml) and $LiAlH_4$ (60 mg) was stirred for 3 hr at 60°. The excess of reagent was decomposed with H_2O and the mixt. acidified with H_2SO_4 (6%) and extracted with BuOH. The organic layer was washed with H_2O and evapd. The residue was chromatographed ($CHCl_3$–MeOH 19:1 and 8:2) to give 3-*epi*-betulin (**2b**, 10 mg) and alcohol **2** (14 mg).

3-epi-*Betulin* (**2b**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 3060, 1640, 885 ($>C=CH_2$). MS *m/z* (rel. int.): 442.3821 $C_{30}H_{50}O_2$ calc. 442.3810 $[M]^+$ (3), 424 $[M-H_2O]^+$ (30), 409 $[M-H_2O-Me]^+$ (15), 393 $[M-H_2O-CH_2OH]^+$ (38), 245 (14), 234 (17), 221 (12), 220 (10), 207 (16), 203 (56), 189 (100). ^1H NMR (200 MHz, $CDCl_3$): δ 0.84, 0.86, 0.93, 0.99, 1.04, 1.67 (each 3H, *s*, *tert.* Me) 3.33, 3.80 (each 1H, *d*, J = 10.4 Hz, CH_2OH), 3.47 (1H, *br s*, H-3β), 4.58, 4.68 (each 1H, *m*, $>C=CH_2$) (see ref. [11]).

Alcohol **2**. R_f 0.30 (FS 6), $[\alpha]_D^{20}$ +2.6° (MeOH; *c* 0.42). MS *m/z* (rel. int.): 424.3739 $C_{30}H_{48}O$ calc. 424.3705 $[M-H_2SO_4]^+$ (23.3), 409 $[M-H_2SO_4-Me]^+$ (8), 393 $[M-H_2SO_4-CH_2OH]^+$ (46), 245 (50), 234 (17), 220 (6), 203 (88), 189 (100). ^1H NMR (200 MHz, CD_3OD–$CDCl_3$ 1:1): δ 0.83 (3H, *s*), 0.85 (3H, *s*), 0.98 (3H, *s*), 1.00 (3H, *s*), 1.03 (3H, *s*), 1.67 (3H, *s*), 3.25 (1H, *d*, J = 12 Hz, H-28 A), 3.72 (1H, *d*, J = 12 Hz, H-28 B), 4.05 (1H, *br s*, H-3β), 4.54, 4.66 (each 1H, *m*, $>C=CH_2$).

Acetylation of alcohol **2**. Compound **2** (4 mg) was acetylated with Ac_2O–pyridine (each 0.2 ml) for 6.5 hr at room temp. The mixt. was evapd to dryness to give **2a**. R_f 0.35 (FS 6), mp 167–168° (from MeOH), $[\alpha]_D^{20}$ +24.2° (MeOH; *c* 0.335). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420 (OH), 3060, 1640, 890 ($>C=CH_2$), 1725 (ester), 1220 (vs S-O and OAc). MS *m/z* (rel. int.): 466.3823 $C_{32}H_{50}O_2$ calc. 466.3811 $[M-H_2SO_4]^+$ (28), 451 $[M-H_2SO_4-Me]^+$ (4), 406 $[M-H_2SO_4-MeCO_2H]^+$ (8), 393 $[M-H_2SO_4-CH_2OAc]^+$ (19), 287 (14), 262 (16), 248 (14), 236 (14), 235 (13), 203 (76), 189 (100). ^1H NMR (200 MHz, $CDCl_3$–CD_3OD): δ 0.87 (6H, *s*), 1.00 (6H, *s*), 1.05 (3H, *s*), 1.68

(3H, *s*), 2.05 (1H, *s*, OAc), 3.83, 4.32 (each 1H, *d*, $J = 10.8$ Hz, CH_2OAc), 4.05 (1H, *br s*, H-3β), 4.53 (*s*, OH), 4.57, 4.68 (each 1H, *m*, $>C=CH_2$).

Solvolysis of compound **1**: A soln of **1** (15 mg) in pyridine (1.5 ml) and dioxane (0.4 ml) was heated for 5 hr at 80°. After evapn to dryness the residue was dissolved in H_2O and extracted with BuOH. The BuOH layer indicated the presence of **4** (co. TLC (FS 2) and IR comparison with authentic sample).

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