Phytochemistry, Vol. 30, No. 7, pp. 2349–2356, 1991
Printed in Great Britain.

0031-9422/91 $3.00+0.00

TRITERPENE GLYCOSIDES FROM *SCHEFFLERA OCTOPHYLLA*

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(*Received* 15 *November* 1990)

Key Word Index—*Schefflera octophylla*; Araliaceae, triterpene glycosides; 3-*epi*-betulinic acid.

Abstract—In addition to 3-*epi*-betulinic acid, three triterpene glycosides were isolated from leaves of *Schefflera octophylla*. The structures of the glycosides have been determined as 28-*O*-[α-L-rhamnopyranosyl(1→4)-*O*-β-D-glucopyranosyl(1→6)-]-β-D-glucopyranosides of 3α-hydroxy-lup-20(29)-ene-23,28-dioic acid, 3α,11α-dihydroxy-lup-20(29)-ene-23,28-dioic acid and 3-*epi*-betulinic acid by spectroscopic data and chemical transformations. The last two compounds were found for the first time in the plant kingdom.

INTRODUCTION

Schefflera octophylla (Lour.) Harms (Araliaceae) is used in Vietnamese folk medicine as a tonic drug, an antirheumatic agent and for liver diseases [1]. In earlier communications the isolation and structures of two free triterpenes from the leaves of this plant have been described [2, 3]. In continuation of our investigations on Vietnamese medicinal plants we now report the isolation of three triterpene glycosides and 3-*epi*-betulinic acid from the same source. Two of these glycosides are new compounds [4].

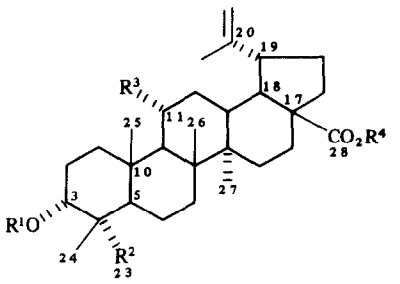
RESULTS AND DISCUSSION

Repeated flash chromatography of the methanol extract of dried leaves afforded, besides the known triterpenes **1–3**, their glycosides **4–6** in 5, 1 and 0.5% yield, respectively. The main glycoside, **4**, showed IR absorptions at 3500 (OH), 1730, 1700 (>C=O) as well as 1640 and 880 cm^{-1} (>C=CH_2). Its ^1H and ^{13}C NMR spectra indicated the presence of one secondary and five tertiary methyls, two carboxylic groups and three anomeric carbons (δ_C 184.7, 176.6, 103.9, 102.1, 94.7). Acid hydrolysis of **4** gave an aglycone identical with 3α-hydroxy-lup-20(29)-ene-23,28-dioic acid (**1**) together with D-glucose and L-rhamnose as sugar components. The same aglycone was obtained on alkaline hydrolysis of **4**, indicating the linkage of the sugar residue to the aglycone through an ester bond. To decide the location of the glycosyl ester, **4** was methylated with diazomethane to yield **4e**, which afforded **4f** on alkaline hydrolysis [5, 6]. The EI mass spectrum of **4f** exhibited peaks at *m/z* 264 [**c**], 251 [**a**], 248 [**b**], 234 [**d**], 233 [**a**−H_2O] and 219 [**e**] [7–9], which showed that the methyl group is located at C-23. The sugar residue must therefore be linked to C-28. This fact was also supported by the EI mass spectrum of **4d** (see Scheme 1).

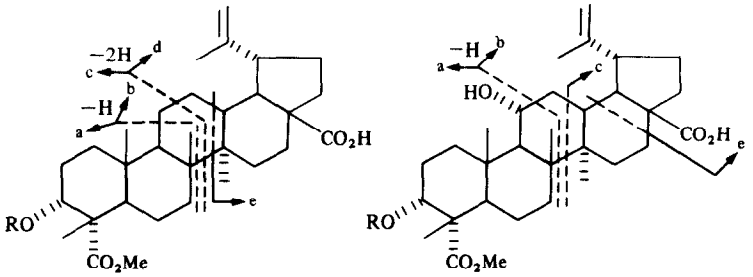
Peracetylation of **4** in the usual manner provided peracetate **4a**. Both the negative ion FAB mass spectra of **4** and **4a** showed intense $[M-H]^-$ peaks at *m/z* 955 and 1375, as well as $[M-H-$sugar residue$]^-$ at *m/z* 485 and 527, respectively. The positive ion FAB spectrum of **4a** exhibited peaks due to the successive loss of sugars at *m/z* 273 $[RhaAc_3]^+$, 561 $[(Rha\text{-}Glc)Ac_6]^+$ and 849 $[(Rha\text{-}Glc\text{-}Glc)Ac_9]^+$.

Permethylation of **4** with *t*-BuONa, NaOH in DMSO [10, 11] afforded permethyl derivative **4b** besides the minor product **4e**. On acid hydrolysis **4b** gave **4d** and three methylated sugars: methyl pyranosides of 2,3,4-tri-*O*-methylrhamnose, 2,3,4- and 2,3,6-tri-*O*-methylglucose (identified by ^1H NMR and GC). For elucidation of the sugar chain, **4b** was reduced with $LiAlH_4$ to give **4g** and **7**, which on methanolysis yielded methyl pyranosides of 2,3,4-tri-*O*-methylrhamnose and 2,3,6-tri-*O*-methylglucose (identified by GC). The EI mass spectrum of **7** exhibited peaks at *m/z* 617 $[M+1]^+$ and 189 [terminal permethylated rhamnose]. In the ^1H NMR spectrum of **7** the anomeric proton signals appeared at 4.26 (*d*, *J* = 7.2 Hz) and 4.91 (*d*, *J* = 1.8 Hz) and were assigned to those of β-D-glucopyranose and α-L-rhamnose, respectively. Therefore, compound **7** is 2,3,4-tri-*O*-methyl-α-L-rhamnopyranosyl(1→4)-2,3,6-tri-*O*-methyl-β-D-glucopyranosyl (1→6)-2,3,4-tri-*O*-methyl-D-sorbitol [12, 13]. Consequently, the glucoside **4** is 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, isolated recently by a Japanese group from the same source [14].

The ^1H, ^{13}C NMR and IR spectra of compound **5** were very similar to those of **4**. The negative ion FAB mass spectra of **5** and peracetate **5a** showed peaks at *m/z* 971 and 1433 $[M-H]^-$ as well as 501 and 585 $[M-H-$sugar residue$]^-$, respectively, indicating that **5** contained an additional hydroxy group. The positive ion FAB mass spectrum of **5a** confirmed the presence of the same sugar residue as in **4a**. This fact was supported by comparative GC studies of the methylated sugars formed by acid hydrolysis of permethyl **5b**, as well as reduction of **5b** with $LiAlH_4$, which in addition afforded **7** and **5g**. Acid and alkaline hydrolysis of **5** yielded 3α,11α-dihydroxy-lup-20(29)-ene-23,28-dioic acid as aglycone, identified by comparison with an authentic sample. In the same manner as shown for compound **4**, the structure of the new



	R^1	R^2	R^3	R^4
1	H	CO_2H	H	H
2	H	CO_2H	OH	H
3	H	Me	H	H
4a	Ac	CO_2H	H	(α-L-Rham^{4}β-D-Glc^{6}β-D-Glc-)Ac$_9$
4d	Me	CO_2Me	H	H
4e	H	CO_2Me	H	(α-L-Rham^{4}β-D-Glc^{6}β-D-Glc-
4f	H	CO_2Me	H	H
4h	Ac	CO_2Me	H	(α-L-Rham^{4}β-D-Glc^{6}β-D-Glc-)Ac$_9$
5a	Ac	CO_2H	OAc	(α-L-Rham^{4}β-D-Glc^{6}β-D-Glc-)Ac$_9$
5d	Me	CO_2Me	OH	H
5e	H	CO_2Me	OH	(α-L-Rham^{4}β-D-Glc^{6}β-D-Glc-
5f	H	CO_2Me	OH	H
5h	Ac	CO_2Me	OAc	(α-L-Rham^{4}β-D-Glc^{6}β-D-Glc-)Ac$_9$
6a	Ac	Me	H	(α-L-Rham^{4}β-D-Glc^{6}β-D-Glc-)Ac$_9$
6d	Me	Me	H	H



4d R = Me
4f R = H

5d R = Me
5f R = H

Scheme 1. Mass spectral fragmentation of compounds **4d**, **4f**, **5d** and **5f**.

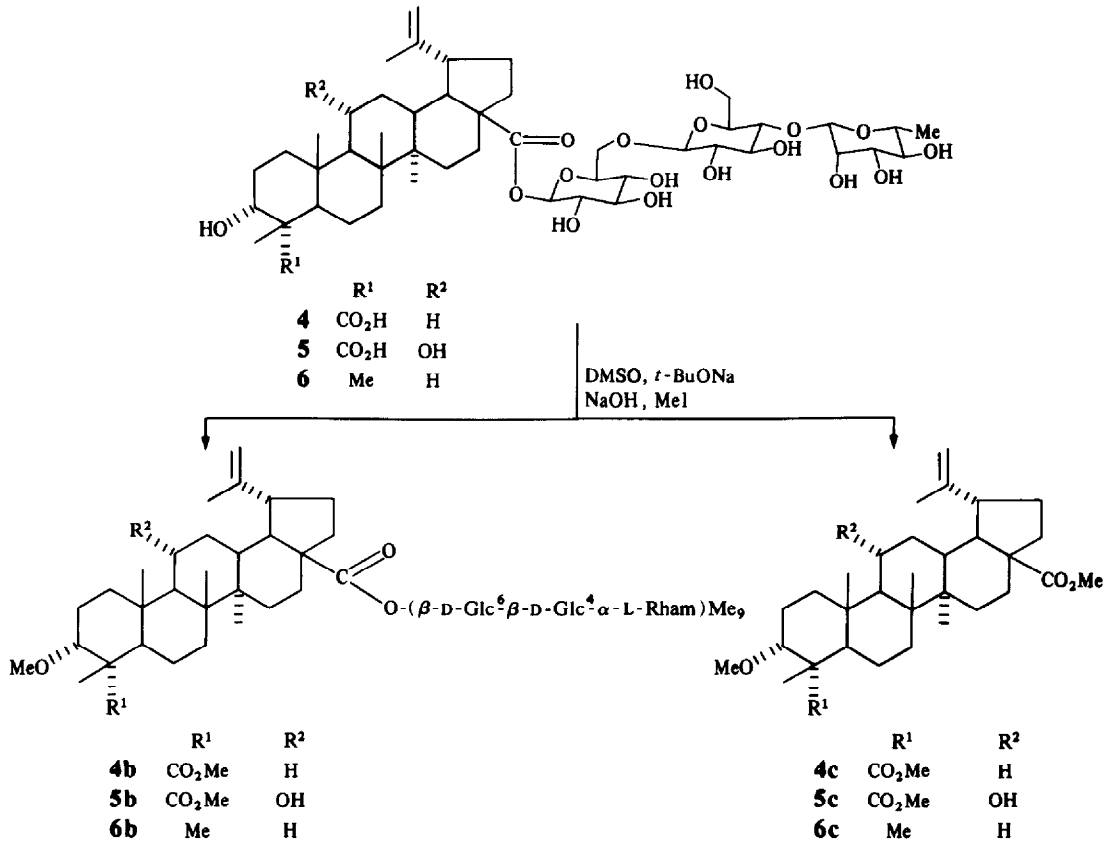
glucoside was determined as 3α,11α-dihydroxylup-20(29)-ene-23,28-dioic acid 28-*O*-[α-L-rhamnopyranosyl(1→4)-*O*-β-D-glucopyranosyl(1→6)]-β-D-glucopyranoside (**5**). It should be noted that upon permethylation of **5** under the applied conditions [10] the 11α-hydroxy group was not methylated.

The ^1H and ^{13}C NMR spectrum of **6** showed the presence of an additional tertiary methyl group and one carboxyl group less than **5**. This was confirmed by the FAB mass spectra of **6** and its peracetate **6a** with peaks at *m/z* 925 $[M-H]^-$ for **6** and 1453 $[M+thioglycerol]^+$ for **6a**. The analysis of the ^{13}C NMR and the FAB mass spectra of **6** and **6a** indicated that **6** has the same sugar residue as found in **4** and **5**.

Acid hydrolysis of permethyl **6b** provided the same three methylated sugars as **4b** and **5b** (identified by GC). Alkaline hydrolysis of **6** gave an aglycone identified as 3-*epi*-betulinic acid (**3**), which was also isolated from the chloroform extract of the plant in 0.08% yield (see Experimental). 3-Epibetulinic acid was first isolated from *Picramnia pentandra* (Simaroubaceae) by Herz *et al.* [15]. From this data the structure of this new glucoside is 3-*epi*-betulinic acid 28-*O*-[α-L-rhamnopyranosyl(1→4)-*O*-β-D-glucopyranosyl(1→6)]-β-D-glucopyranoside (**6**).

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded at 200 and 50.3 MHz and 400 and 100.6 MHz; chemical shifts are given in ppm, TMS was used as int. standard. FAB-MS were performed by the Institut für physiologische Chemie, University of Bonn, F.R.G. in negative and positive ion mode. HRMS: 70 eV. Analytical GC: column Chrompack CP Sil 19 CB. Operating conditions were: temp. programmed from 60 to 180° at 10° min^{-1}, P_{N_2}: 0.7 bar;



Scheme 2. Permethylation of glycosides **4**–**6**.

inj. temp. was held at 200° and detector at 250°. TLC was carried out with silica gel 60 F254 Merck; for flash CC silica gel 60, 63–100 μm (Merck) was used. Spray reagent: vanilline–H_2SO_4 for glycosides and aglycones, orcine or aniline hydrogen phthalate for sugars. Solvent systems for TLC: FS1: $CHCl_3$–MeOH–H_2O (60:35:8), FS2; $CHCl_3$–MeOH–H_2O (70:30:3), FS3: $CHCl_3$–MeOH (9:1), FS4: $CHCl_3$–MeOH (49:1), FS5: $CHCl_3$–MeOH (19:1), FS6: $CHCl_3$–MeOH (4:1).

Isolation. Dried leaves (250 g) collected in province Nghe Tinh, Vietnam, were extracted with boiling MeOH. The extract was concd. diluted with H_2O and extracted with $CHCl_3$. Chromatography of the $CHCl_3$ extract (petrol–EtOAc, 4:1) gave 3-*epi*-betulinic acid (**3**, 200 mg 0.08%) besides the known triterpenes **1** and **2**.

Compound **3**. Mp 277–281° (hexane–EtOAc); $[\alpha]_D^{20}$ −12° ($CHCl_3$, *c* 1.285). IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 3070, 1644, 890 (>C=CH_2), 1700 (CO_2H). MS *m/z* (rel. int.): 456.3600 $C_{30}H_{48}O_3$ calc. 456.3603 $[M]^+$ (9.6), 438 $[M-H_2O]^+$ (38), 421 $[M-H_2O-Me]^+$ (16), 410 $[M-HCO_2H]^+$ (8), 248 (20), 234 (10), 233 (16), 207 (32), 203 (30), 189 (100). ^1H NMR: Table 1.

The aq. layer was evapd to dryness and the residue repeatedly flash chromatographed. Elution with $CHCl_3$–MeOH–H_2O (70:30:3) gave glucoside **6** (1.25 g, 0.5%). R_f 0.67 (FS2), powder mp 182–184° $[\alpha]_D^{20}$ −39.5° (MeOH, *c* 1.365). FAB-MS (negative ion) *m/z* (rel. int.): 925 $[M-H]^-$ (33), 455 [M−H−(Rha-Glc-Glc)] (100), 659 (10.8). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1730 (ester), 1640, 880 (>C=CH_2), 1060, 1030. ^1H and ^{13}C NMR: Tables 2 and 3.

Peracetate **6a**. Compound **6** (50 mg) was acetylated with Ac_2O–pyridine (each 0.6 ml) for 20 hr at room temp. to give **6a** (35 mg) after CC. R_f 0.83 (FS5), powder mp 138–140°, $[\alpha]_D^{20}$ −23° ($CHCl_3$; *c* 0.985). (Found: C, 58.00; H, 7.27. $C_{66}H_{96}O_{26}\cdot 4H_2O$ requires C, 57.56; H, 7.56). FAB-MS (positive ion) *m/z* (rel. int.): 1453 $[M+thioglycerol]^+$ (0.04), 849 [(Rha-Glc-Glc)Ac_9]$^+$ (0.18), 561 [(Rha-Glc)Ac_6]$^+$ (4.1), 273 [(Rha)Ac_3]$^+$ (32.4), 43 (100). ^1H NMR: Table 2.

Permethylation of compound **6**. Compound **6** (58 mg) was permethylated with dry DMSO (4.7 ml), dry *t*-BuONa (491 mg), finely powdered NaOH (144 mg) and MeI (3.9 ml). The mixt. was stirred at room temp. for 1 hr, poured into ice-water and extracted with Et_2O. The Et_2O layer was washed with a satd NaCl soln, dried (Na_2SO_4) and evapd. Complete methylation was checked by IR. Flash chromatography of the residue ($CHCl_3$–MeOH, 49:1) gave permethyl **6b** (40 mg) and **6c** (5 mg).

Compound **6b**. Amorphous, R_f 0.58 (FS4), $[\alpha]_D^{20}$ −23.9° ($CHCl_3$; *c* 1.05). ^1H NMR: Table 2.

Compound **6c**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3070, 1640, 890 (>C=CH_2), 1725 (ester). MS *m/z* (rel. int.) 484 $[M]^+$ (2), 248 (20), 234 (8). 189 (100), 101 (66), 88 (98), 75 (74). ^1H NMR: Table 1.

Acid hydrolysis of permethyl **6b**. Compound **6b** (40 mg) was hydrolysed in MeOH with 6% H_2SO_4 (6 ml) for 4 hr at 80°. MeOH was evapd and H_2O added. The soln was extracted with $CHCl_3$. Chromatography of the residue of the $CHCl_3$ layer ($CHCl_3$–MeOH, 49:1) gave **6d** (15 mg), methyl pyranosides of 2,3,4-tri-*O*-methylrhamnose (4 mg); 2,3,4- and 2,3,6-tri-*O*-methylglucose (each 5 mg). The methylated sugars were identified by GC and ^1H NMR.

Compound **6d**. MS *m/z* (rel. int.): 470 $[M]^+$ (4), 438 $[M-MeOH]^+$ (36), 423 $[M-HCO_2H]^+$ (17), 248 (20), 189 (100). ^1H NMR: Table 1.

Table 1. ^{1}H NMR spectral data of triterpenes **1**–**3** and their derivatives*

	H_2-29	CO_2Me	OMe	H-11β	H-3β	H-19 [17]	tert. Me	CH_2OH-23	CH_2OH-28
1†	4.59, 4.71				3.72	3.02	0.89, 0.97,		
	m				*t*, (2.8)	*dt*, (11.2, 4.8)	1.06, 1.14, 1.69		
2†	4.60, 4.75			3.86	3.62	3.04	0.96, 1.07 (2×), 1.15,		
	m			six line (10.4, 5.3)	*t*, (2.6)	*dt*, (12; 4.5)	1.71		
3‡	4.59, 4.72				3.39	3.00	0.80, 0.81, 0.92		
	m				*t*, (2.7)	*dt*, (11.0; 4.5)	(2×), 0.98, 1.68		
4c‡	4.59, 4.73	3.64, 3.65	3.20		3.24	2.98	0.85, 0.91, 1.00		
	m	*s*	*s*		*t*, (2.6)	*dt*, (12.2, 4.9)	1.13, 1.68		
4d‡	4.59, 4.72	3.66	3.20		3.24	2.98	0.85, 0.92, 1.00		
	m	*s*	*s*		*t*, (2.4)	*dt*, (11.2; 4.9)	1.13, 1.68		
4f†	4.58, 4,71	3.63			3.69	3.02	0.90, 0.97, 1.07		
	m	*s*			*t*, (2.8)	*dt*, (11.2; 4.8)	1.14, 1.69		
4g‡	4.54, 4.66		3.30		3.05		0.63, 0.84, 0.98,	3.53, 3.78	3.27, 3.30
	m		*s*		*t*, (2.7)		1.00, 1.66	AB_q, (10.8)	AB_q, (10.8)
5c‡	4.60, 4.75	3.65, 3.66	3.21	3.95	3.24	§	0.93, 1.02, 1.08		
	m	*s*	*s*	six line (10.8, 5.4)	*t*, (2.8)		1.18, 1.68		
5d‡	4.61, 4.75	3.65	3.20	3.96	3.21	2.98	0.93, 1.02, 1.07		
	m	*s*	*s*	six line (10.5; 5.3)	*t*, (2.7)	*dt*, (11.2; 4.5)	1.17, 1.68		
5f†	4.61, 4.76	3.64		3.87	§	304	0.96, 1.08, 1.09		
	m	*s*		six line (10.5, 5.3)		*dt*, (11.2; 4.8)	1.17; 1.71		
5g‡	4.58, 4.69		3.30	3.71	3.04		0.66, 1.01 (2×)	3.72, 3.55	3.35, 3.26
	m		*s*	six line (10.8, 5.4)	*t*, (2.7)		1.06, 1.67	AB_q, (10)	AB_q, (10)
6c‡	4.59, 4.71		3.39		3.37	3.00	0.80, 0.81, 0.91		
	m		*s*		*t*, (2.7)	*br*	(2×), 0.98, 1.68		
6d‡	4.59, 4.71	3.61	3.39		3.37	3.00	0.80 (2×), 0.91 (2×)		
	m	*s*	*s*		*t*, (2.7)	*br*	0.97, 1.68		

*Values in parentheses are coupling constants in Hz.
†200 MHz, CD_3OD.
‡200 MHz, $CDCl_3$.
§Overlapped.

Table 2. ^{1}H NMR spectral data of glucosides **4–6** and their derivatives*

	Glc 1H-1	Glc 2H-1	Rha H-1	H_2-29	CO_2Me	H-19 [17]	Rha Me	tert. Me
4†	5.45	4.39	4.84	4.73, 4.60		2.98	1.26	1.70, 1.10, 1.03
	d (8)	*d* (8)	*d* (1.8)	*br s*		*t*-like, *br*	*d* (6)	0.94, 0.88
4a‡	5.60	4.46	4.87	4.66, 4.52		2,88	1.07	1.61, 1.14, 0.98
	d (8)	*d* (8)	*s*	*br s*		*t*-like, *br*	*d* (6.2)	0.86, 0.83
4b§	5.42	4.22	4.93	4.68, 4.55		**	1.24	1.64, 1.12, 0.97
	d (8.1)	*d* (8.1)	*d* (1.8)	*m*			*d* (6.3)	0.90, 0.83
4e‖	5.44	4.37	4.83	4.72, 4.59	3.63 *s*	2.99	1.23	1.68, 1.13, 1.04
	d (7.6)	*d* (7.6)	*d* (1.7)	*br s*		*br s*	*d* (6)	0.94, 0.88
5¶	5.46	4.44	4.86	4.64,**		2.96	1.26	1.69, 1.10, 1.06
	d (7.8)	*d* (7.8)	*s*	*br s*		*br s*	*d* (5.7)	1.04, 0.85
5a‡	5.60	4.43	4.93	4.64, 4.50		2.85	1.04	1.58, 1.10, 1.00
	d (7.8)	*d* (7.8)	*s*	*br s*		*br s*	*d* (6.2)	0.92, 0.87
5b§	5.40	4.23	4.94	4.72, 4.58		**	1.26	1.66, 1.16, 1.06
	d (7.7)	*d* (7.7)	*s*	*br s*			*d* (6.2)	1.01, 0.92
5e‖	5.45	4.38	4.84	4.78, 4.62	3.62 *s*	3.01	1.24	1.72, 1.16,
	d (7.8)	*d* (7.8)	*s*	*br s*		*br s*	*d* (6)	1.07, (2×), 0.94
6‡	5.47	4.37	5.00	4.73, 4.60		3.00	1.25	1.67, 1.00, 0.93
	d (8)	*d* (8)	*d* (1.7)	*br s*		*t*-like, *br*	*d* (6.5)	0.88, 0.84, 0.80
6a§	5.64	4.52	5.10	4.69, 4.56		2.90	1.10	1.64, 0.98, 0.88
	d (8)	*d* (8)	*s*	*br s*		*br s*	*d* (6.4)	0.83 (2×), 0.78
6b§	5.43	**	4.93	4.68, 4.55		**	1.24	1.64, 0.79–0.94
	d (8)		*s*	*br s*			*d* (6)	(×5)

*Values in parentheses are coupling constants in Hz.
†200 MHz, CD_3OD–D_2O (4:1).
‡400 MHz, $CDCl_3$.
§200 MHz, $CDCl_3$.
‖200 MHz, CD_3OD.
¶400 MHz, CD_3OD–D_2O (4:1).
**Overlapped.

Alkaline hydrolysis of compound **6**. Compound **6** (75 mg) was hydrolysed in MeOH with 5% KOH (8 ml) for 4 hr at 80°. The soln was passed through Dowex 50 W × 4 (H^+ form, MeOH), evapd to dryness, diluted with H_2O and extracted with $CHCl_3$. The organic layer was dried (Na_2SO_4) and evapd to give aglycone **3** (45 mg), which was identical with 3-*epi*-betulinic acid isolated from the $CHCl_3$ extract. Further elution with $CHCl_3$–MeOH–H_2O (60:35:8) gave the main glycoside **4** (12.5 g, 5%); R_f 0.38 (FS1), powder mp 279–284° (dec.), $[\alpha]_D^{20}$ −35° (MeOH; *c* 0.41) (lit. [14] mp 274–277°; $[\alpha]_D^{18}$ −40°). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1730 (ester), 1700 (CO_2H), 1640, 880 (>C=CH_2). (Found: C, 54.52; H, 7.68, $C_{48}H_{76}O_{19}\cdot 5H_2O$ requires: C, 55.05; H, 8.28). FAB-MS (negative ion) *m/z* (rel. int.): 955 [M−H]$^-$ (100), 485 [M−H−sugar residue]$^-$ (43). ^{1}H and ^{13}C NMR: Tables 2 and 3.

Acid hydrolysis of compound **4**. A soln of **4** (160 mg) in 2 M HCl (10 ml) was heated on a water bath at 80° for 3.5 hr. The ppt. was filtered and chromatographed ($CHCl_3$–MeOH, 19:1) to give aglycone **1** (79 mg). The filtrate was passed through Dowex 50 1 × 8 (OH^- form, MeOH) and evapd. PC (paper Whatman No. 1, descending mode, solvent: EtOAc–pyridine–H_2O 10:4:4, lit. [16] and TLC (solvent: *n*-BuOH–*iso*-PrOH–H_2O, 5:3:1) of the residue indicated the presence of glucose and rhamnose.

Alkaline hydrolysis of compound **4**. Compound **4** (62 mg) was hydrolysed in MeOH with 5% KOH (15 ml) for 3 hr at 80° to give aglycone **1** (33 mg), mp 260–263° ($CHCl_3$) (dec.), $[\alpha]_D^{20}$ −11° (MeOH; *c* 0.58). IR ν_{max}^{KBr} cm^{-1}: 3320 (OH), 3070, 1640, 880 (>C=CH_2), 1705 (CO_2H), MS *m/z* (rel. int.): 486.3334 $C_{30}H_{46}O_5$ calc. 486.3345 $[M]^+$ (36.5). ^{1}H NMR: Table 1.

Permethylation of compound **4**. Compound **4** (105 mg) was permethylated with DMSO (8.2 ml), *t*-BuONa (850 mg), finely powdered NaOH (250 mg) and MeI (6.5 ml) as above. Flash chromatography of the product ($CHCl_3$–MeOH 49:1) gave permethyl **4b** (89 mg) and a minor compound **4c** (9 mg).

Compound **4b**. R_f 0.50 (FS 4), mp 114–115° (from H_2O), $[\alpha]_D^{20}$ −43.6° ($CHCl_3$; *c* 0.58). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730 (ester), 1640, 890 (>C=CH_2). ^{1}H NMR: Table 2.

Compound **4c**. Mp 156–161° (from $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (ester), 1650, 890 (>C=CH_2). MS *m/z* (rel. int.): 528.3816 $C_{33}H_{52}O_5$ calc. 528.3814 $[M]^+$ (7.1), 496 $[M-MeOH]^+$ (2), 468 $[M-HCO_2Me]^+$ (2), 436 $[M-HCO_2Me-MeOH]^+$ (6), 279 (20), 262 (6), 233 (15), 279 (54), 167 (36), 149 (100). ^{1}H NMR: Table 1.

Acid hydrolysis of permethyl **4b**. Compound **4b** (50 mg) was hydrolysed as above to give **4d** (20 mg), methyl pyranosides of 2,3,4-tri-*O*-methylrhamnose (6 mg); 2,3,4- and 2,3,6-tri-*O*-methylglucose as a mixt. of α- and β-form (12 mg).

Compound **4d**. $[\alpha]_D^{20}$ −11° ($CHCl_3$; *c* 0.50), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1725 (>C=O), 1640, 890 (>C=CH_2). MS *m/z* (rel. int.): 514.3661 $C_{32}H_{50}O_5$ calc. 514.3663 $[M]^+$ (34.2); MS 16 eV: 514 $[M]^+$ (42), 482 $[M-MeOH]^+$ (27), 468 $[M-HCO_2H]^+$ (15), 450 (17), 443 (75), 422 $[M-HCO_2Me-MeOH]^+$ (17), 411 (12), 397 (13), 365 (11.5), 355 (11.5), 281 (13), 278 [**c**] (29), 265 [**a**] (13), 248 [**b**] (33), 234 [**d**] (37.5), 233 [**a**−MeOH] (75), 219 [**e**], (14), 189 (55), 173 (52.6), 121 (64), 95 (58), 80 (100). ^{1}H and ^{13}C NMR: Tables 1 and 3.

Reduction of compound **4b** *with* $LiAlH_4$. Compound **4b** (100 mg) in dry THF (10 ml) was reduced with $LiAlH_4$ (50 mg)

Table 3. ^{13}C NMR chemical shifts of compounds **4–6**, **4g**, **4d** and **5g**

C		4*	5*	6†	4g‡	4d§	5g‖
1		33.7	35.5	35.3	33.1	32.2	35.2
2		25.7	25.6	26.4	20.8	19.4	19.9
3		73.1	73.3	74.5	87.5	83.3	87.1
4		51.9	52.4	38.4	47.8	51.6	47.8
5		46.4	45.8	49.3	43.1	45.0	43.1
6		21.9	22.0	19.2	18.0	21.4	17.8
7		34.9	35.5	34.4	33.7	34.0	33.8
8		42.1	43.1	42.0	41.0	41.4	42.6
9		51.4	56.2	51.4	50.2	50.4	55.5
10		37.8	39.5	38.2	36.9	37.1	38.7
11		20.7	70.6	21.8	19.8	20.8	70.5
12		26.5	37.8	26.5	25.2	25.5	37.6
13		39.1	37.8	39.2	37.3	38.4	36.3
14		43.4	43.7	43.5	41.1	42.8	42.8
15		30.5	30.2	30.7	27.0	29.8	27.0
16		32.6	32.6	32.7	29.8	30.6	29.7
17		57.8	57.6	57.6	42.9	56.4	41.2
18		50.3	49.8	50.4	48.8	49.3	48.2
19		48.0	47.8	48.3	47.9	47.0	47.6
20		151.5	151.0	151.5	150.7	150.6	150.0
21		31.1	31.2	31.4	29.2	29.8	29.1
22		37.5	37.3	37.5	34.0	37.1	34.7
23		184.7	184.7	29.0	71.6	177.1	71.9
24		18.2	18.2	22.7	17.9	17.3	18.1
25		17.7	17.7	16.7	16.4	16.4	17.3
26		17.3	17.4	16.6	16.0	16.3	16.7
27		15.4	14.8	15.2	15.0	14.9	14.9
28		176.6	176.8	176.0	60.5	180.5	60.4
29		110.9	110.9	110.5	109.7	109.8	110.2
30		19.9	19.5	19.6	19.1	19.4	19.1
OMe		—	—	—	56.0	56.8	55.8
CO_2Me						51.6	
Glc-1:	1	94.7	94.8	95.0			
	2	73.1	73.1	73.8			
	3	78.4	78.6	79.2			
	4	70.2	70.3	70.6			
	5	77.2	77.4	78.0			
	6	69.1	69.0	69.4			
Glc-2:	1	103.9	103.7	104 3			
	2	74.6	74.7	75.1			
	3	76.3	76.3	76.4			
	4	77.2	77.3	77.7			
	5	75.8	76.0	76.7			
	6	61.3	61.3	61.7			
Rha	1	102.1	102.1	102.7			
	2	71.7	71.8	72.2			
	3	71.5	71.5	72.0			
	4	73.1	72.8	73.6			
	5	70.2	70.2	70.4			
	6	17.7	17.7	17.8			

*100.6 MHz, in CD_3OD/D_2O (4:1).
†50.3 MHz, in CD_3OD.
‡100.6 MHz, $CDCl_3$.
‖50.3 MHz, $CDCl_3$.
§22.63 MHz, $CDCl_3$.

for 3 hr at 60°. The excess of reagent was decomposed with H_2O. The soln was acidified with 6% H_2SO_4 and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over Na_2SO_4, evapd and chromatographed ($CHCl_3$–MeOH, 19:1) to give diol **4g** (39 mg) and **7** (34 mg). The diol **4g**: mp 100–102° (MeOH), $[\alpha]_D^{20}$ −13° ($CHCl_3$; *c* 1.505), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3480 (OH), 3060, 1640, 890 (>C=CH_2). MS *m/z* (rel. int.): 472.3934 $C_{31}H_{52}O_3$ calc. 472.3952 $[M]^+$ (32), 454 $[M-H_2O]^+$ (15), 441 $[M-CH_2OH]^+$ (19), 422 $[M-MeOH-H_2O]^+$ (18), 410 $[M-2CH_2OH]^+$ (46), 391 $[M-MeOH-H_2O-CH_2OH]^+$ (12), 379 (13), 245 (21), 234 (22), 220 (22), 189 (68), 187 (72), 175 (67), 88 (100). ^1H and ^{13}C NMR: Tables 1 and 3.

Compound **7**. Oil, $[\alpha]_D^{20}$ −31° ($CHCl_3$; *c* 1.35), lit. [12]: $[\alpha]_D$ −33° ($CHCl_3$; *c* 0.88). MS *m/z* (rel. int.): 617.3390 $C_{17}H_{53}O_{15}$ calc. 617. 3384 $[M+H]^+$ (0, 02), 235 (48), 207 (14), 189 [terminal permethylated rhamnose] (72), 157 (22), 101 (64), 88 (100). ^1H NMR (200 MHz, $CDCl_3$): δ4.91 (1H, *d*, *J* = 1.8 Hz, H-1 of Rha), 4.26 (1H, *d*, *J* = 7.2 Hz, H-1 of Glc), 3.55, 3.54, 3.52, 3.51, 3.48, 3.46, 3.44, 3.43, 3.34 (each 3H, 9 × OMe), 2.37, 1.91 (*br s*, OH), 1.26 (3H, *d*, *J* = 6.3 Hz, Rha-Me). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ104.1 (C-1 of Glc), 97.5 (C-1 of Rha), 84.2, 84.1, 82.1, 81.2, 80.8, 80.5, 79.4, 77.8, 75.2, 74.5 (all *d*), 72.5 (*t*), 70.2 (*t*), 69.9 (*d*), 68.2 (*d*), 61.7 (*t*), 60.9, 60.5, 60.47, 60.43, 59.6, 59.4, 58.9, 58.8, 57.8, 17.6 (all *q*).

Methanolysis of compound **7**. Compound **7** (10 mg) was heated in MeOH with 5% HCl (5 ml) for 4 hr at 80°. The mixt. was passed through Dowex 50 1 × 8 (OH^- form, MeOH), evapd and examined by GC. Methyl pyranosides of 2,3,4-tri-*O*-methylrhamnose and 2,3,6-tri-*O*-methylglucose were detected.

Peracetate **4a**. Compound **4** (116 mg) was acetylated with Ac_2O–pyridine (each 2.5 ml) at room temp. for 20 hr to give peracetate **4a** (150 mg). R_f 0.39 (FS5), powder mp 156–158°, $[\alpha]_D^{20}$ −30° ($CHCl_3$; *c* 0.54). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1645, 890 (>C=CH_2), 1735 (ester), 1700 (>C=O), 1250 (OAc). FAB-MS (positive ion) *m/z* (rel. int.): 849 [(Rha-Glc-Glc)Ac_9]$^+$ (0.29), 561 [(Rha-Glc)Ac_6]$^+$ (6.9), 501 (1.6), 273 [(Rha)Ac_3]$^+$ (100). FAB-MS (negative ion) *m/z* (rel. int.): 1375 $[M-H]^-$ (40), 1333 (13.6), 527 $[M-H-$sugar residue$]^-$ (10), 423 (3.6), 59 (100). ^1H NMR: Table 2.

Compound **4h**. Compound **4a** (10 mg) in Et_2O was treated with ethereal CH_2N_2 to give **4h**, mp 153–154° (MeOH–$CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3070, 1640, 890 (>C=CH_2), 1745 (ester), 1245 (OAc).

Mythylation of **4** *and hydrolysis of methylester* **4e**. Compound **4** (16 mg) in MeOH (5 ml) was treated with ethereal CH_2N_2 to yield methyl ester **4e**, R_f 0.73 (FS 2), powder mp 188–190°, $[\alpha]_D^{20}$ −51.5° (MeOH; *c* 0.53). ^1H NMR: Table 2.

Hydrolysis of **4e**. Compound **4e** (16 mg) was heated in MeOH with 5% KOH (1.5 ml) for 25 min at 80°. The mixt. was passed through Dowex 50 W × 4 (H^+ form, MeOH) and evapd. The residue was dissolved in H_2O and extracted with BuOH. The extract was evapd to dryness to give **4f** (8 mg); R_f 0.70 (FS 5), amorphous, $[\alpha]_D^{20}$ −30.6° (MeOH; *c* 0.36). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430 (OH), 1640, 890 (>C=CH_2), 1715 (ester), 1700 (CO_2H). MS 16 eV *m/z* (rel. int.): 500 $[M]^+$ (39), 482 $[M-H_2O]^+$ (13), 468 $[M-MeOH]^+$ (11), 454 $[M-HCO_2H]^+$ (18), 450 (12), 422 $[M-H_2O-HCO_2Me]^+$ (14), 264 [**c**] (30), 251 [**a**] (21), 248 [**b**] (41), 234 [**d**] (52), 233 $[a-H_2O]^+$ (64), 219 [**e**] (20), 203 (39), 189 (65), 175 (53), 173 (56), 161 (25), 147 (32), 133 (37), 121 (52), 107 (43), 95 (47), 80 (100). ^1H NMR: Table 1.

Further elution with $CHCl_3$–MeOH–H_2O (60:35:8) gave glucoside **5** (2.5 g, 1%). R_f 0.27 (FS 1), powder mp > 316°, $[\alpha]_D^{20}$ −25.8° (MeOH, *c* 0.345). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730 (ester), 1700 (CO_2H), 1640, 880 (>C=CH_2). FAB-MS (negative ion) *m/z* (rel. int.): 971 $[M-H]^-$ (100), 501 $[M-H-$sugar residue$]^-$ (39.2). ^1H and ^{13}C: Tables 2 and 3.

Acetylation of compound **5**. Compound **5** (250 mg) was acetylated with Ac_2O–pyridine (each 2.5 ml) for 18 hr at room temp. to give peracetate **5a** (237 mg) after flash chromatography ($CHCl_3$–MeOH, 19:1). R_f 0.34 (FS 5), powder mp 163–165°, $[\alpha]_D^{20}$ −31° ($CHCl_3$; *c* 1.08), (Found: C, 54.72; H, 6.67 $C_{66}H_{94}O_{29}\cdot 5H_2O$ requires: C, 54.99; H, 7.27). FAB-MS (positive ion) *m/z* (rel. int.): 1457 $[M+Na]^+$ (0.18), 1145 $[M-(Rha)Ac_3]^+$ (0,89), 849 [(Rha−Glc−Glc)Ac_9]$^+$ (1.6), 561 [(Rha−Glc)Ac_6]$^+$ (22), 273 [(Rha)Ac_3]$^+$ (76), 153 (70.4) 111 (68.8), 43 (100). FAB-MS (negative ion) *m/z* (rel. int.) 1433 $[M-H]^-$ (26.4), 1391 (10.7), 585 $[M-H-$sugar residue$]^-$ (12.8), 59 (100). ^1H NMR: Table 2.

Methylation of peracetate **5a**. Compound **5a** (15 mg) in Et_2O (25 ml) was treated with ethereal CH_2N_2 to give **5h**, mp 163–164° ($CHCl_3$–MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1640, 890 (>C=CH_2), 1745 (ester), 1245 (OAc).

Acid hydrolysis of compound **5**. Compound **5** (180 mg) was hydrolysed with 2 M HCl (20 ml) for 3.5 hr at 80°. The ppt. was filtered to give an aglycone identified as 3α,11α-dihydroxy-lup-20(29)-ene-23,28-dioic acid (**2**, 72 mg). The aq. layer was passed through Dowex 50 1 × 8 (OH^- form), evapd to dryness and acetylated with Ac_2O–pyridine. Glucose- and rhamnose-peracetate were identified by TLC and GC.

Alkaline hydrolysis of compound **5**. Compound **5** (69 mg) was heated in MeOH with 5% KOH (15 ml) for 4 hr at 80°. The mixt. was evapd, diluted with H_2O and extracted with BuOH. The BuOH layer was washed with H_2O, evapd to dryness to give aglycone **2** (28 mg), mp 224–225° (from MeOH). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1640, 890 (>C=CH_2), 1700 (CO_2H). MS *m/z* (rel. int.): 440 $[M-H_2O-CO_2]^+$ (14), 438 $[M-H_2O-HCO_2H]^+$ (6), 425 (20), 422 $[M-2H_2O-CO_2]^+$ (29), 411 (12), 407 (22), 385 (20), 367 (14), 303 (8), 285 (12), 234 (18), 220 (12), 219 (16), 201 (26), 189 (56), 175 (68), 161 (40), 147 (42), 133 (46), 121 (64), 107 (96), 95 (100). ^1H NMR: Table 1.

Methylation of **5** *and hydrolysis of methyl ester* **5e**. Compound **5** (16 mg) was methylated with ethereal CH_2N_2 to give **5e**, R_f 0.57 (FS 2), powder mp 168–170°, $[\alpha]_D^{20}$ −61° (MeOH; *c* 0.77). ^1H NMR: Table 2.

The methyl ester **5e** was hydrolysed in MeOH with 5% KOH (1.5 ml) for 25 min at 80° as above to give the monoester **5f** (8 mg), R_f 0.40 (FS 5), $[\alpha]_D^{20}$ −19 (MeOH; *c* 0.475), amorphous. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1720 (ester), 1710 (CO_2H), 1640, 890 (C=CH_2). MS *m/z* (rel. int.): 516 $[M]^+$ (5.6), 498 $[M-H_2O]^+$ (73.4), 480 $[M-2H_2O]^+$ (25.4), 466 (14.1), 454 $[M-H_2O-CO_2]$ (15), 438 $[M-H_2O-HCO_2Me]^+$ (19.7), 372 (25.4), 281 [**a**] (21.4), 263 [**a**−H_2O] (28.2), 248 [**a**−H_2O-CH_3] (51.4), 234 [**b**] (83), 220 [**c**] (40.6), 219 [**c**−H] (43.5), 203 [**a**−H_2O-CO_2Me] (63.8), 201 (64), 189 [**b**−CO_2H] (86.4), 175 [**c**−CO_2H] (77.4), 152 [**e**] (21.4), 121 (89.9), 107 [**e**−CO_2H] (79), 80 (100). ^1H NMR: Table 1.

Permethylation of compound **5**. Compound **5** (182 mg) was methylated with DMSO (14.2 ml), *t*-BuONa (1.473 g), finely powdered NaOH (433 mg) and MeI (11.5 ml) for 1 hr at room temp. as above. Repeated flash chromatography of the residue ($CHCl_3$–MeOH, 49:1) gave permethyl **5b** (95 mg) besides a minor product **5c** (16 mg).

Compound **5b**. R_f 0.42 (FS4), powder mp 117–118°, $[\alpha]_D^{20}$ −41° ($CHCl_3$; *c* 0.975). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460 (OH), 1730 (ester), 1640, 890 (>C=CH_2). ^1H NMR: Table 2.

Compound **5c**. Amorphous, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1720 (ester), 1670, 890 (>C=CH_2). MS *m/z* (rel. int.): 544.3739 $C_{33}H_{52}O_6$ calc. 544.3763 $[M]^+$ (6.9), 526 $[M-H_2O]^+$ (50), 502 $[M-MeOH]^+$ (10), 494 $[M-H_2O-MeOH]^+$ (18), 487 (10), 473 (50), 455 (100), 435 (10), 413 (16), 395 (30), 309 (10), 295 (10), 287 (10), 263 (12), 247 (36), 233 (18). ^1H NMR: Table 1.

Acid hydrolysis of permethyl **5b**. Compound **5b** (45 mg) was

hydrolysed with H_2SO_4 (6 ml) for 4 hr at 80° as above to give **5d** (20 mg), methyl pyranosides of 2,3,4-tri-*O*-methylrhamnose (7 mg) and a mixt. of 2,3,4- and 2,3,6-tri-*O*-methylglucose (15 mg), identified by GC.

Compound **5d**. Amorphous, $[\alpha]_D^{20}$ −9° ($CHCl_3$; *c* 0.30). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430 (OH), 1725 (ester), 1700 (CO_2H), 1640, 890 (C=CH_2), MS *m/z* (rel. int.): 530.3611 $C_{32}H_{50}O_6$ calc. 530.3607 $[M]^+$ (2.6), 512 $[M-H_2O]^+$ (52), 498 $[M-MeOH]^+$ (18), 480 $[M-H_2O-MeOH]^+$ (16), 459 (76), 441 (100), 421 (12), 409 (28), 399 (24), 395 (36), 381 (10), 353 (12), 295 [**a**] (8), 273 (12), 263 [**a** −MeOH] (12), 245 [**a**−MeOH−H_2O] (12), 235 [**a** −HCO_2Me] (16), 234 (**b**] (26), 235 [**b**−H] (30), 189 [**b**−CO_2H] (24), 175 [**c**−CO_2H] (38), 107 [e−CO_2H] (46). ^1H NMR: Table 1.

Reduction of compound **5b** *with* $LiAlH_4$. Compound **5b** (80 mg) in dry THF (10 ml) was reduced with $LiAlH_4$ (50 mg) as above. Flash chromatography of the residue ($CHCl_3$–MeOH, 19:1) gave triol **5g** (42 mg) and **7** (22 mg). The triol **5g**: mp 110–112° ($CHCl_3$–MeOH), $[\alpha]_D^{20}$ −19° (MeOH; *c* 1.61). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3460 (OH), 3060, 1640, 890 (C=CH_2). MS *m/z* (rel. int.): 488.3845 $C_{31}H_{52}O_4$ calc. 488.3866 $[M]^+$ (1.1), 470 $[M-H_2O]^+$ (4), 457 $[M-CH_2OH]^+$ (6), 452 $[M-2H_2O]^+$ (3), 440 (6), 426 $[M-2CH_2OH]^+$ (38), 408 $[M-2CH_2OH-H_2O]^+$ (18), 205 (8), 201 (16), 190 (12), 88 (100). ^1H and ^{13}C NMR: Tables 1 and 3. Compound **7** was identical with that obtained from $LiAlH_4$ reduction of **4b** by direct comparison.

Acknowledgements—We thank Dr J. Peter-Katalinic (Bonn), for the FAB-MS, Dr G. Eckhardt (Bonn) and Dr J. Schmidt, (Halle/Saale) for the HREI and LREI mass spectra 70 eV and 16 eV. Dr T. V. Sung (Institute of Natural Products Chemistry Hanoi, Vietnam) is indebted to the Alexander v. Humboldt Foundation for a research fellowship in Bonn during this work.

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