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TRITERPENOIDS FROM *ANAMIRTA COCCULUS*

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Key Word Index—*Anamirta cocculus*; Menispermaceae; epoxyoleanolide; oleanene; oleanene glycosides; arjunolic acid.

Abstract—One new triterpenoid, 2α,3β,23-trihydroxy-11α,12α-epoxyolean-28,13β-olide and two new triterpenoid glycosides, β-D-glucopyranosyl-2β,3β,23-trihydroxyolean-12-en-28-oate and 2α,3β-dihydroxy-23-β-D-glucopyranosyloxyolean-12-en-28-oic acid, are reported from the stem of *Anamirta cocculus*. The major compounds isolated are the already known 2α,3β,23-trihydroxyolean-12-en-28-oic acid (arjunolic acid) and its 28-*O*-β-D-glucopyranoside. None of the isolated compounds showed any molluscicidal or antifungal activity.

INTRODUCTION

Anamirta cocculus (L.) Wight et Arn. is a liana occurring in several regions of South-East Asia. The berries of the plant are the source of picrotoxin, a commercial sesquiterpene mixture. They are used as fish poisons and have figured in a number of pharmacopoeias. Four quaternary alkaloids, berberine, palmatine, magnoflorine and columbamine, and one tertiary alkaloid, (−)-8-oxotetrahydropalmatine, have been reported from the stem and root of the plant [1]. Another tertiary alkaloid, oxypalmatine, and a secondary alkaloid, stepharine, have later been isolated from the stem of the plant [2].

RESULTS AND DISCUSSION

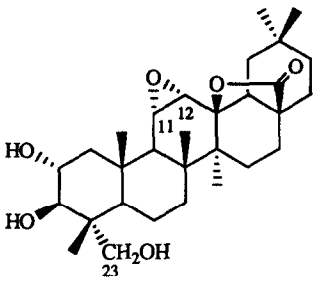
The *n*-butanol extract of the methanol extract of the defatted stem of *A. cocculus* showed strongly positive froth and haemolysis tests for saponins. Although the extract did not show any antifungal activity, it showed marginal molluscicidal activity (100% lethal to *Biomphalaria glabrata* snails at a minimum concentration of 200 ppm). A chromatographic separation over silica gel of the *n*-butanol extract gave two triterpenoids, **1** and **2**, and three isomeric triterpenoid glycosides, **3**–**5**, in yields of 0.026, 0.51, 0.022, 0.23 and 0.035%, respectively. The purity of **3**–**5** was checked by HPLC.

The major triterpenoid **2** was identified as arjunolic acid (2α,3β,23-trihydroxyolean-12-en-28-oic acid), first isolated from *Terminalia arjuna* (Combretaceae) [3]. Arjunolic acid has been reported from several other plant sources, including *Cochlospermum tinctorium* A. Rich, (Cochlospermaceae) [4], but this constitutes the first report of its isolation from the Menispermaceae. The identification of **2** as arjunolic acid was based on comparison of its physical and spectral data with an authentic specimen, as well as physical and spectral data of its triacetate **2a**, methyl ester **2b** and the methyl ester of the triacetate **2c** with recorded data [4].

The ^{1}H NMR spectrum of the triacetate **2a** recorded at 500 MHz gave evidence for the 2α,3β-configuration of the hydroxyl groups in **2**. In the triacetate **2a**, H-3 appeared as a clear doublet ($J_{2a,3a}$ = 10.6 Hz) centred at δ 5.12, while H-2 appeared as a doubled doubled doublet ($J_{2a,3a}$ = 10.6 Hz, $J_{2a,1a}$ = 11.8 Hz and $J_{2a,1e}$ = 4.3 Hz), centred at δ 5.17. The triacetate **2a** and its methyl ester **2c** have shown inhibitory effects on skin tumour promotors [4].

The less polar triterpenoid **1**, molecular formula $C_{30}H_{46}O_6$ (HRMS), showed IR absorptions at 3450, 1775, 1220, 930 and 870 cm^{-1} indicating the presence of hydroxyl, γ-lactone and epoxide groups. It gave a triacetate, **1a** ($C_{36}H_{52}O_9$), and failed to react with diazomethane. Signals in the ^{13}C NMR spectrum of **1** at δ 179.3, 52.4 and 56.9 confirmed the presence of a γ-lactone and an epoxide group. A broad singlet for two protons at δ 3.06 in the ^{1}H NMR spectrum indicated attachment of the epoxide ring to methine carbon atoms. Both the ^{13}C and ^{1}H NMR spectra indicated an oleanane structure for **1** with the same hydroxyl substitution (2α,3β,23) as in **2**. The ^{1}H NMR spectrum indicated the absence of an olefinic proton. These observations together with 2D ^{13}C–^{1}H COSY data enabled identification of **1** as 2α,3β,23-trihydroxy-11α,12α-epoxyolean-28,13β-olide.

The α-epoxide configuration is preferred for **1**, since the lactone ring is necessarily β. Nevertheless, confirmation of the α-epoxide configuration was obtained by reducing **1** with lithium aluminium hydride to a hexol (**1b**) which on acetylation gave a penta-acetate (**1c**) [5]. A β-epoxide would have been reduced to a 11β-hydroxy compound, known to be very resistant to acetylation. Epoxy lactones e.g. **1** have previously been isolated from other plant sources [5–7], but none from the Menispermaceae.



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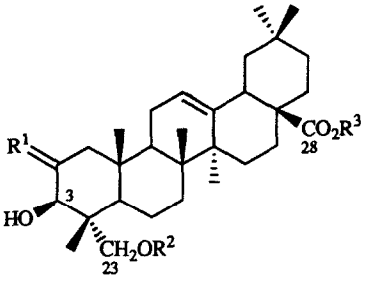
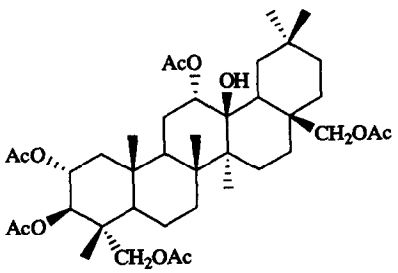
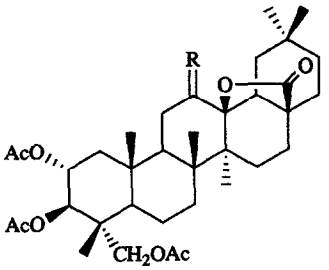


	R^1	R^2	R^3
2	α-OH, β-H	H	H
3	β-OH, α-H	H	β-D-glucopyranosyl
4	α-OH, β-H	H	β-D-glucopyranosyl
5	α-OH, β-H	β-D-glucopyranosyl	H



1c



	R
2d	α-OH, β-H
2e	O

In order to interrelate **1** with **2**, the triacetate **2a** was oxidized with *m*-chloroperbenzoic acid [8] to the 12α-hydroxylactone **2d**, which was in turn oxidized with pyridinium chlorochromate to the 12-ketolactone **2e**, identical with the product of isomerization of the triacetate **1a** with sulphuric acid in ethanol [7].

FAB-MS showed that **3–5** are isomeric compounds with molecular formula $C_{36}H_{58}O_{10}$. Compounds **3** and **5** are new natural products, and **4**, identified as the 28-*O*-β-D-glucopyranoside of arjunolic acid (**2**), has been previously isolated from *T. arjuna* [9].

Treatment with 4 M HCl hydrolysed **3** to bayogenin (2β,3β,23-trihydroxyolean-12-en-28-oic acid (**3a**) and D-glucose, while both **4** and **5** underwent similar hydrolysis to **2** and D-glucose. The ^{1}H NMR spectrum of the triacetate (**3b**) of bayogenin gave confirmatory evidence for the 2β,3β-configuration of the hydroxyl groups in ring A of **3**. The H-3 signal appeared as a doublet ($J_{2e,3a}$ = 3.9 Hz) at δ 4.92 coupled (^{1}H–^{1}H COSY) to H-2 at δ 5.40. The latter showed multiple splitting due to coupling with the axial and equatorial protons on C-1 as well as with H-3. The ^{1}H NMR spectrum of **3a** has been described [10].

Identification of **3–5** as pyranosides was carried out by permethylation of each compound by a modified Hakomori procedure [11], followed by acid hydrolysis, reduction with sodium borohydride and acetylation to give 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol which was identified by GC and GC-MS [12]. The three compounds were evidently mono-D-glucopyranosides as their ^{1}H NMR spectra showed only one anomeric doublet in each case. Further, the magnitude of the coupling constants of the doublets showed that **3–5** were β-D-glucopyranosides [13].

Compounds **3** and **4** gave the hepta-acetates **3c** and **4a**, respectively, on acetylation, failed to react with diazomethane, and showed IR absorption at 1730 cm^{-1}. These findings indicate attachment of the β-D-glucopyranosyl group as an ester through C-28. This was confirmed by the ^{13}C NMR spectrum which showed signals at δ 95.7 and 91.6 for **3** and **3c**, respectively, and at δ 95.7 and 91.6 for **4** and **4a**, respectively. The C-3 signals were at δ 73.4 and 72.0 for **3** and **3c**, and at δ 78.7 and 74.7 for **4** and **4a**. Hence, **3** and **4** are the β-D-glucopyranosyl esters of 2β,3β,23-trihydroxyolean-12-en-28-oic acid (bayogenin) and 2α,3β,23-trihydroxyolean-12-en-28-oic acid (arjunolic acid), respectively. In accordance with their ester nature, **3** and **4** were hydrolysed with 0.5 M potassium hydroxide to bayogenin and arjunolic acid, respectively. Glycosides of bayogenin have been found in several plants [14], but this constitutes the first report of the isolation of a bayogenin glycoside with the sugar attached through a carbon atom other than C-3.

Glycoside **5** showed an IR absorption at 1690 cm^{-1} indicating the presence of a free carboxyl group, which was also evident from the ^{13}C NMR signal at δ 181.0, stability to alkali and formation of a methyl ester **5a** on treatment with diazomethane. The ^{13}C NMR spectrum provided evidence for the position of attachment of the

sugar residue. Attachment through C-2, C-3 or C-23 was evident from the anomeric carbon signal which appeared at δ 104.7 for **5**, but at δ 95.7 for **4**. Attachment to C-23 was indicated by an appreciable downfield shift of the C-23 signal from δ 66.3 in **4** to δ 75.2 in **5**, whereas the signals for C-2 and C-3 for **5** were approximately at the same positions as for **4**. Hence, **5** was identified as 2α,3β-dihydroxy-23-β-D-glucopyranosyloxyolean-12-en-28-oic acid.

Glycosylation through C-23 is rare among natural products. Examples are the 23-*O*-arabinoside and 23-*O*-glucoside of hederagenin isolated from *Clematis chinensis* (Ranunculaceae) [15].

Compounds **1**–**5** are inactive as molluscicides and fungicides.

EXPERIMENTAL

Mps: uncorr; $^1H/^{13}C$ NMR: at 300/75 MHz (unless otherwise stated). TMS as int. standard; EIMS and FABMS: 70 eV and 30 kV, respectively; GC: DB 225 capillary column, oven temp. programmed from 150 to 250° at 3° min^{-1}, H_2 as carrier gas (6 ml min^{-1}). Injector and detector temp. were both maintained at 250°; GC-MS: 12 m HP1 bonded methyl silicone column, oven temp. programmed from 100 to 250° at 10° min^{-1}; HPLC: 300 × 7.8 mm μ Bondapak Tm/C_{18} column with MeOH–H_2O (3:7), flow rate 1.5 ml min^{-1}.

Plant material. Anamirta cocculus was identified and collected in July from the Uva Province of Sri Lanka by the late Prof. S. Balasubramaniam. A voucher specimen has been deposited at the Department of Botany, University of Peradeniya, Sri Lanka.

Extraction and isolation. The dry, ground, mature stem of *A. cocculus* (650 g) was sequentially extracted with petrol (40–60°) and hot MeOH. Evapn of the MeOH gave a brown solid (40 g), which was partitioned between *n*-BuOH and H_2O. Evapn of the *n*-BuOH gave a brown solid (21 g). A portion (15 g) was sepd by MPLC on a column of silica gel (200 g) eluted with petrol, EtOAc and MeOH. Compounds **1** (120 mg), **2** (2.3 g), **3** (100 mg), **4** (1.04 g) and **5** (160 mg) were obtained. All 5 compounds were further purified by CC over silica gel.

2α,3β,23-Trihydroxy-11α,12α-epoxyolean-28,13β-olide (**1**). Light yellow microcrystalline needles, mp 284–286°, $[\alpha]_D^{22}$ +37.1° ($CHCl_3$; *c* 0.35); IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 1775, 1510, 1460, 1390, 1360, 1320, 1220, 1140, 1040, 930, 870; ^1H NMR ($CDCl_3$): δ 0.80, 0.92, 1.00, 1.05, 1.10, 1.12 (each 3H, *s*, Me), 2.12 (2H, *m*, H-1), 2.3 (1H, *m*, H-18), 3.06 (2H, *br s*, H-11, H-12), 3.35, 3.59 (each 1H, *d*, *J* = 10.8 Hz, H-23), 3.45 (1H, *d*, *J* = 9.5 Hz, H-3), 3.85 (1H, *m*, H-2); ^{13}C NMR: Table 1; HRMS *m/z* 502.3287 ([M]$^+$ calcd for $C_{30}H_{46}O_6$: 502.3294).

Triacetate (**1a**) *of* **1**. Pale yellow microcrystalline needles, mp 77–79°, $[\alpha]_D^{22}$ +27.1° ($CHCl_3$; *c* 0.7); IR ν_{max}^{KBr} cm^{-1}: 2950, 1770, 1740, 1380, 1250, 1020, 790; ^1H NMR (60 MHz, $CDCl_3$): δ 0.95 (6H, *s*, 2 × Me), 1.03, 1.13, 1.23, 1.28 (each 3H, *s*, Me), 2.03, 2.08. 2.13 (each 3H, *s*, OAc), 3.1 (2H, *br s*, H-11, H-12), 3.67, 3.93 (each 1H, *d*, *J* = 12 Hz, H-23), 5.27 (2H, *m*, H-2, H-3); EIMS *m/z* (rel. int.): 628 [M]$^+$ (20), 613 (25), 217 (30), 204 (30), 189 (30), 133 (30), 119 (60), 107 (50), 95 (70), 81 (70), 69 (100), 55 (95); HRMS *m/z* 628.3586 ([M]$^+$, calcd for $C_{36}H_{52}O_9$: 628.3611).

$LiAlH_4$ *reduction of* **1** *to* **1b**. $LiAlH_4$ (80 mg) was added in portions to a stirred soln of **1** (80 mg) in THF (10 ml). The reaction mixt. was filtered, the solvent evapd and the residue purified by CC over silica gel. Compound **1b** was obtained as a sticky solid, $[\alpha]_D^{22}$ +90° (MeOH; *c* 0.11); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2950, 1370, 1210, 1000.

Penta-acetate (**1c**) *of* **1b**. Sticky solid, $[\alpha]_D^{22}$ +11.4° ($CHCl_3$; *c* 0.7); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2900, 1735, 1550, 1410, 1370, 1230, 1030; ^1H NMR ($CDCl_3$): δ 0.82, 0.85 (each 3H, *s*, Me), 1.9 (12H, *br s*, 4Me), 1.98, 2.01, 2.05, 2.07, 2.10 (each 3H, *s*, OAc), 3.8–4.1 (4H, *m*, H-23, H-28), 5.0–5.4 (3H, *m*, H-2, H-3, H-12).

2α,3β,23-Trihydroxyolean-12-en-28-oic acid (**2**). Microcrystalline needles, mp 245–247°, $[\alpha]_D^{22}$ +20.8° (MeOH; *c* 0.24); IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 1690, 1460, 1380, 1300, 1265, 1185, 1040; ^1H NMR (C_5D_5N): δ 0.89, 1.03, 1.05 (each 3H, *s*, Me), 1.08 (6H, *s*, 2Me), 1.22 (3H, *s*, Me), 3.3 (1H, *m*, H-18), 3.73, 4.25 (each 1H, *d*, *J* = 12 Hz, H-23), 5.43 (1H, *m*, H-12); ^{13}C NMR: Table 1; HRMS *m/z* 488.3491 ([M]$^+$, calcd for $C_{30}H_{48}O_5$: 488.3502).

Triacetate (**2a**) *of* **2**. Microcrystalline needles, mp 134–136°, $[\alpha]_D^{22}$ +32.9° ($CHCl_3$; *c* 0.7); IR ν_{max}^{KBr} cm^{-1}: 2950, 1745, 1700, 1460, 1370, 1230, 1040; ^1H NMR (500 MHz, $CDCl_3$): δ 0.75, 0.87, 0.91, 0.93, 1.09, 1.12 (each 3H, *s*, Me), 1.99, 2.03, 2.09 (each 3H, *s*, OAc), 2.83 (1H, *dd*, *J* = 13.8 Hz, 3.8 Hz, H-18), 3.59, 3.85 (each 1H, *d*, *J* = 12 Hz, H-23), 5.12 (1H, *d*, $J_{2a,3a}$ = 10.6 Hz, H-3), 5.17 (1H, *ddd*, $J_{2a,3a}$ = 10.6 Hz, $J_{2a,1a}$ = 11.8 Hz, $J_{2a,1e}$ = 4.3 Hz, H-2), 5.28 (1H, *m*, H-12); EIMS *m/z* (rel. int.): 614 (2), 568 (18), 248 (80), 203 (100), 187 (26), 173 (19), 133 (38), 119 (49), 105 (44), 91 (44), 81 (53), 69 (75), 55 (85).

Methyl ester (**2b**) *of* **2**. Microcrystalline needles, mp 145–147°, $[\alpha]_D^{22}$ +43.4° ($CHCl_3$; *c* 0.53); IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 1730, 1460, 1380, 1260, 1160, 1040; ^1H NMR ($CDCl_3$): δ 0.71, 0.84, 0.90, 0.94, 1.02, 1.13 (each 3H, *s*, Me), 2.86 (1H, *dd*, *J* = 13.5 Hz, 4 Hz, H-18), 3.34, 3.72 (each 1H, *d*, *J* = 12 Hz, H-23), 3.63 (3H, *s*, C-28 OMe), 3.75 (1H, *m*, H-2), 5.29 (1H, *m*, H-12); EIMS *m/z* (rel. int.): 502 (1), 442 (1), 203 (100), 189 (20), 133 (25), 119 (25), 105 (25), 95 (25), 81 (30), 69 (40), 55 (55).

Methyl ester (**2c**) *of triacetate* **2a**. Light yellow crystals, mp 83–85°, $[\alpha]_D^{22}$ +14.1° ($CHCl_3$; *c* 3.2); IR ν_{max}^{KBr} cm^{-1}: 2950, 1740, 1460, 1360, 1230, 1030, 960, 900; ^1H NMR (60 MHz, $CDCl_3$): δ 0.73 (3H, *s*, Me), 0.90 (6H, *s*, 2Me), 1.12, 1.27, 1.38 (each 3H, *s*, Me), 2.0, 2.03, 2.1 (each 3H, *s*, OAc), 2.83 (1H, *m*, H-18), 3.67 (3H, *s*, C-28 OMe), 3.60, 3.93 (each 1H, *d*, *J* = 11 Hz, H-23), 5.17 (2H, *m*, H-2, H-3), 5.37 (1H, *m*, H-12); EIMS *m/z* (rel. int.): 628 (13), 613 (2), 568 (13), 262 (43), 203 (100), 189 (24), 119 (31), 95 (27), 81 (35), 69 (48), 55 (55).

Synthesis of hydroxylactone (**2d**). Compound **2a** (80 mg) was treated with *m*-chloroperbenzoic acid (100 mg) in $CHCl_3$ (20 ml) at 0° for 24 hr. After removal of excess *m*-chloroperbenzoic acid with 10% Na_2CO_3, the $CHCl_3$ layer was washed (× 3) with H_2O. Evapn of the $CHCl_3$ gave **2d** (80 mg) as needles, mp 94°, $[\alpha]_D^{22}$ +20° ($CHCl_3$; *c*

Table 1. ^{13}C NMR of **1–3**, **3c**, **4**, **4a**, **5**

C	**1*** ($CDCl_3$)	**2*** (C_5D_5N)	**3** (CD_3OD)	**3c** ($CDCl_3$)	**4** (CD_3OD)	**4a** ($CDCl_3$)	**5** (CD_3OD)
1	45.6	46.3	47.2	45.6	43.1	43.4	43.0
2	68.1	68.8	73.9[a]	69.5	69.7	69.7	69.5
3	79.0	78.2	73.4[a]	72.0	78.7	74.7	78.4
4	42.6	43.5	43.1	41.6	44.1	41.8	44.1
5	47.6	47.8[a]	—	47.6	—	47.5	—
6	17.3	18.4	18.7	17.1	19.1	17.7	18.9
7	34.1	32.8	33.4	31.6	33.1	31.5	33.5
8	40.4	39.7	40.7	39.4	39.0	39.2	39.0
9	50.4	48.1[a]	—	48.1	—	47.4	—
10	—	38.3	37.7	36.5	36.9	37.7	37.0
11	52.4[a]	23.8[b]	23.9	22.8	24.6	23.3[a]	24.6
12	56.9[a]	122.5	123.9	122.6	123.6	122.4	123.5
13	87.4	144.8	145.0	143.1	145.1	143.0	145.5
14	41.2	42.1	42.6	40.6	40.7	41.5	40.6
15	26.5[b]	28.2	28.7	27.4	28.8	27.4	28.8
16	21.1	23.6[b]	23.9	21.2	24.0	22.6[a]	24.1
17	43.7	47.6	48.0	46.7	47.2	46.6	—
18	49.4	41.9	45.3	41.0	42.6	40.8	42.7
19	37.6[c]	42.1	42.7	41.7	44.1	45.5	44.1
20	30.6	29.9	31.5	30.5	31.5	30.4	31.6
21	26.8[b]	30.8	33.5	33.3	33.3	33.6	33.6
22	37.3[c]	34.1	34.8	32.4	34.9	32.2	34.9
23	68.1	66.4	67.4	65.5	66.3	65.1	75.2
24	12.3	14.2	14.0	13.8	13.9	13.7	13.9
25	18.6	17.2	17.5	16.5	17.6	16.7	17.6
26	18.7	17.4	17.7	17.0	17.8	16.7	17.7
27	19.9	26.0	26.3	25.4	26.4	25.4	26.5
28	179.3	180.3	178.1	175.6	178.1	175.5	181.0
29	33.0	33.1	33.1	32.9	34.5	32.8	33.9
30	23.4	23.7	24.6	23.4	23.9	23.3	24.0
1′			95.7	91.6	95.9	91.6	104.7
2′			72.2	67.8	73.9	67.8	73.4
3′			78.3	69.9	78.2	69.8	77.6
4′			71.1	72.8	71.1	72.7	71.8
5′			78.7	72.5	78.3	72.7	78.0
6′			62.4	61.5	62.4	61.4	63.0

[a–c] Values with the same superscript in the same column are interchangeable, signals were edited by APT.

*C–H connectivities were established by 2D ^{13}C–^{1}H COSY, blank denotes masking by solvent.

1.1); IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 1770, 1740, 1460, 1360, 1230, 1030; ^{1}H NMR (60 MHz, $CDCl_3$): δ0.9, 1.0, 1.07, 1.1, 1.27, 1.30 (each 3H, *s*, Me), 2.0, 2.03, 2.1 (each 3H, *s*, OAc), 3.67, 3.80, 3.93 (each 1H, *m*, H-12, H-23), 5.10 (2H, *m*, H-2, H-3).

Oxidation of **2d** *to ketolactone* **2e**. Compound **2d** (50 mg) in CH_2Cl_2 (10 ml) was stirred with pyridinium chlorochromate (50 mg) for 2 hr. The reaction mixt. was sepd by passing through a Celite column and **2e** obtained as microcrystalline needles, mp 78–80°, $[\alpha]_D^{22}$ −1.4° ($CHCl_3$; *c* 0.7); IR ν_{max}^{KBr} cm^{-1}: 2925, 1780, 1740, 1710, 1460, 1360, 1230, 1030; ^{1}H NMR (60 MHz, $CDCl_3$): δ0.93 (3H, *s*, Me), 1.02 (6H, *s*, 2Me), 1.13, 1.27, 1.30 (each 3H, *s*, Me), 2.0, 2.03, 2.1 (each 3H, *s*, OAc), 3.67, 3.83 (each 1H, *s*, H-23), 5.13 (2H, *m*, H-2, H-3). The product was identical with the product of isomerization of **1a**.

Isomerization of **1a**. Compound **1a** (40 mg) was treated with conc. H_2SO_4 (0.3 ml) in EtOH (10 ml) at room temp. for 48 hr. The reaction mixt. was neutralized ($BaCO_3$), filtered and evapd. The residue was re-acetylated and purified by prep. TLC (silica gel) to give **2e**.

Glycoside **3**. Needles, mp 220–222°, $[\alpha]_D^{22}$ +31.3° (MeOH; *c* 0.8); IR ν_{max}^{KBr} cm^{-1}: 3550, 3400, 2925, 1730, 1460, 1380, 1070; ^{1}H NMR (CD_3OD): δ0.72, 0.80, 0.82, 0.84, 1.05, 1.17 (each 3H, *s*, Me), 2.75 (1H, *m*, H-18), 3.38, 3.13 (each 1H, *d*, J = 10.9 Hz, H-23), 3.15–3.5 (5H, *m*, H-2, H-3, sugar CHOH), 3.57, 3.71 (each 1H, *d*, J = 12 Hz, H-6′), 5.16 (1H, *m*, H-12), 5.27 (1H, *d*, J = 7.9 Hz, H-1′); ^{13}C NMR: Table 1; positive-ion FABMS [*m*-nitrobenzyl alcohol] *m/z* 673.2 $[M+Na]^+$, negative-ion FABMS [*m*-nitrobenzyl alcohol] *m/z* 649.3 $[M-H]^-$ (M for $C_{36}H_{58}O_{10}$: 650).

Hepta-acetate (**3c**) *of* **3**. Needles, mp 80–82°, $[\alpha]_D^{22}$ +60° ($CHCl_3$; *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 2925, 1740, 1460, 1360, 1220, 1030; ^1H NMR ($CDCl_3$): δ0.78, 0.89, 0.91, 1.02, 1.1, 1.21 (each 3H, *s*, Me), 1.9–2.1 (21H, overlapping *s*, 7 × OAc), 2.83 (1H, *m*, H-18), 3.67, 3.88 (each 1H, *d*, *J* = 11.8 Hz, H-23), 3.8 (1H, *m*, H-5′), 4.05, 4.28 (each 1H, *d*, *J* = 12 Hz, H-6′), 4.95 (1H, *br s*, H-3), 5.42 (1H, *br s*, H-2), 5.1–5.3 (3H, *m*, sugar CHOAc), 5.34 (1H, *m*, H-12), 5.58 (1H, *d*, *J* = 7.9 Hz, H-1′); ^{13}C NMR: Table 1; positive-ion FABMS [*m*-nitrobenzyl alcohol] *m/z* 967 $[M+Na]^+$ (M^+ for $C_{50}H_{72}O_{17}$:944).

Acid hydrolysis of **3**. Compound **3** (50 mg) was refluxed with 4 M HCl (50 ml) for 2 hr. The mixt. was extracted with EtOAc. The organic layer was evapd to dryness to give bayogenin (**3a**, 30 mg) as microcrystalline needles, mp 294–296°, $[\alpha]_D^{22}$ + 12.5° (MeOH; *c* 0.6); IR ν_{max}^{KBr} cm^{-1}: 3575, 3475, 2925, 1675, 1460, 1380, 1260, 1040; ^1H NMR DMSO-d_6): δ0.7, 0.75, 0.85, 0.87, 1.06, 1.16 (each 3H, *s*, Me), 2.8 (1H, *m*, H-18), 3.0–4.0 (4H, *m*, H-2, H-3, H-23), 5.17 (1H, *m*, H-12); ^{13}C NMR (DMSO-d_6): δ 70.6, 70.2 (C-1/C-3), 121.8 (C-12), 144.0 (C-13), 64.8 (C-23), 178.9 (C-28); EIMS *m/z* (rel. int.): 488 (7), 470 (5), 446 (20), 411 (15), 248 (45), 203 (60).

The aq. phase was worked up as described in an earlier investigation [16] and the presence of D-glucose confirmed. The ring size of the D-glucose moiety was also established as described. The permethylation procedure used was however a modification [11] of the original Hakomori procedure.

Permethylation of **3**. Compound **3** (5 mg) in DMSO (2 ml) was treated with dry NaOH (25 mg), stirred at room temp. for 10 min and MeI (2 ml) added. After standing for 20 min, the excess of MeI was blown off. The mixt. was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was evapd to dryness to give the permethylated glycoside.

Triacetate (**3b**) *of* **3a**. Microcrystalline needles, mp 108–110°, $[\alpha]_D^{22}$ + 40° ($CHCl_3$; *c* 0.6); IR ν_{max}^{KBr} cm^{-1}: 2900, 1740, 1710, 1680, 1460, 1370, 1230, 1030; ^1H NMR ($CDCl_3$): δ0.80, 0.88, 0.90, 0.94, 1.08, 1.15 (each 3H, *s*, Me), 2.0, 2.05, 2.1 (each 3H, *s*, OAc), 2.83 (1H, *m*, H-18), 3.36, 3.69 (each 1H, *d*, *J* = 11.9 Hz, H-23), 5.28 (1H, *m*, H-12), 4.92 (1H, *d*, $J_{2e,3a}$ = 3.9 Hz, H-3), 5.40 (1H, *m*, $W_{1/2}$ = 12 Hz, H-2); EIMS *m/z* (rel. int.): 614 (0.5), 568 (2.5), 265 (20), 248 (30), 203 (50), 55 (100).

Glycoside **4**. Needles, mp 222–224°, $[\alpha]_D^{22}$ + 22° (MeOH; *c* 0.5); IR ν_{max}^{KBr} cm^{-1}: 3425, 2950, 1730, 1630, 1455, 1380, 1255, 1070; ^1H NMR (CD_3OD): δ0.65, 0.78, 0.88, 0.92, 1.01, 1.15 (each 3H, *s*, Me), 2.82 (1H, *m*, H-18), 3.23, 3.46 (each 1H, *d*, *J* = 11 Hz, H-23), 3.3–3.4 (5H, *m*, H-2, H-3, sugar CHOH), 3.5–3.7 (2H, *m*, H-6′), 5.23 (1H, *m*, H-12), 5.34 (1H, *m*, *J* = 7.9 Hz, H-1′); ^{13}C NMR: Table 1; positive-ion FABMS [glycerol] *m/z* 673.4 $[M+Na]^+$, negative-ion FABMS [glycerol] *m/z* 649.2 $[M-H]^-$ (M for $C_{36}H_{58}O_{10}$: 650).

Acid hydrolysis of **4**. Compound **4** (120 mg) was refluxed with 4 M HCl (100 ml) for 2 hr. The mixt. was extracted with EtOAc. The organic layer was evapd to dryness to give arjunolic acid (**2**, 80 mg). The aq. phase was worked up as described above and the presence of D-glucose established.

Permethylation of **4**. As described above.

Hepta-acetate (**4a**) *of* **4**. Needles, mp 135°, $[\alpha]_D^{22}$ + 30° ($CHCl_3$; *c* 0.5); IR ν_{max}^{KBr} cm^{-1}: 2950, 1740, 1460, 1360, 1220, 1030, 960, 890; ^1H NMR ($CDCl_3$): δ0.75 (3H, *s*, Me), 0.87 (3H, *s*, Me), 0.92 (6H, *s*, 2 × Me), 1.08 (3H, *s*, Me), 1.12 (3H, *s*, Me), 1.98 (3H, *s*, OAc), 2.00, 2.01 (each 3H, *s*, OAc), 2.02 (6H, *s*, 2 × OAc), 2.06, 2.08 (each 3H, *s*, OAc), 2.82 (1H, *m*, H-18), 3.56, 3.86 (each 1H, *d*, *J* = 11.7 Hz, H-23), 3.78 (1H, *m*, H-5′), 4.0–4.3 (2H, *m*, H-6′), 5.00–5.3 (5H, *m*, H-2, H-3, sugar CHOAc), 5.33 (1H, *m*, H-12), 5.57 (1H, *d*, *J* = 7.6 Hz, H-1′); ^{13}C NMR: Table 1; positive-ion FABMS [*m*-nitrobenzyl alcohol] *m/z* 967.2 $[M+Na]^+$ (M^+ for $C_{50}H_{72}O_{17}$:944).

Glycoside **5**. Microcrystalline needles, mp 174°, $[\alpha]_D^{22}$ + 3.3° (MeOH; *c* 0.3); IR ν_{max}^{KBr} cm^{-1}: 3400, 2900, 1690, 1460, 1370, 1260, 1070; ^1H NMR (CD_3OD): δ0.68, 0.75, 0.85, 0.90, 1.00, 1.12 (each 3H, *s*, Me), 2.8 (1H, *m*, H-18), 3.43, 3.82 (each 1H, *d*, *J* = 12 Hz, H-23), 3.2–3.7 (5H, *m*, H-2, H-3, sugar CHOH), 4.18 (1H, *d*, *J* = 7.9 Hz, H-1′), 5.19 (1H, *m*, H-12); ^{13}C NMR: Table 1; negative-ion FABMS [glycerol] *m/z* 649.2 $[M-H]^-$ (M^- for $C_{36}H_{58}O_{10}$:650).

Acid hydrolysis of **5**. Compound **5** (10 mg) was refluxed with 4 M HCl (10 ml) for 2 hr. After extraction of the mixt. with EtOAc, the organic phase was evapd to dryness to give arjunolic acid (**2**, 5 mg). The aq. phase was shown to contain D-glucose.

Permethylation of **5**. As described above.

Methyl ester (**5a**) *of* **5**. Light brown microcrystalline needles, mp 120–122°, $[\alpha]_D^{22}$ − 20° (MeOH; *c* 0.2); IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 1730, 1510, 1460, 1380, 1270, 1120, 1070.

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