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3,4-Seco-lupane Type Triterpene Glycosyl Esters from a Korean Medicinal Plant, *Acanthopanax chiisanensis* (Araliaceae)

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The experimental details of the isolation and structural determination of chiisanoside (**1**), a new 3,4-seco-lupane type triterpene glycosyl ester, from leaves and stem bark of *Acanthopanax chiisanensis* are described. From leaves of this plant, two homologous glycosyl esters, named isochiisanoside and its methyl ester (**2** and **3**), were also isolated. Their structures were elucidated on the basis of chemical and spectral data and confirmed by the derivation of these compound from **1**.

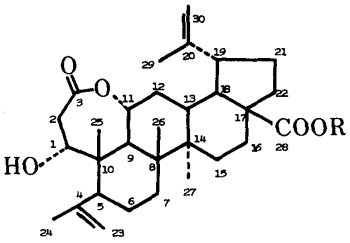
Keywords——*Acanthopanax chiisanensis*; Araliaceae; chiisanoside; isochiisanoside; chiisanogenin; anhydro-chiisanogenoic acid; 3,4-secotriterpenoid; lupane derivative; oligo-glycosyl ester

A folk medicine, leaves and stem-bark of *Acanthopanax chiisanensis* NAKAI (智異山五加, Araliaceae), is used as an anti-rheumatic, an anti-inflammatory and a tonic in Korea. From this folk medicine, a new glycosyl ester (**1**) named chiisanoside was isolated and identified as the α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside of (1*R*)-1,11α-dihydroxy-3,4-seco-lupa-4(23),20(29)-diene-3,28-dioic acid 3,11-lactone (Chart 1). This is the first example of the occurrence of a 3,4-seco-lupane type triterpene glycoside in nature, as was reported in a communication to the editors of *Chem. Pharm. Bull.*[1] The present paper presents the experimental details of this study, including the preparation of 3,4-seco-betulinic acid derivatives (Chart 2) for use as model compounds in the structure determination of **1**. The isolation and structure determination of additional new glycosides (**2** and **3**) from the leaves of this plant are also described.

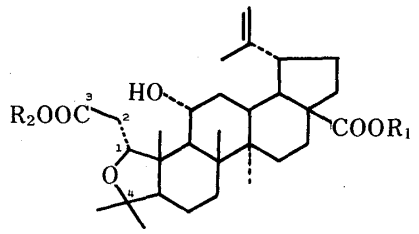
A suspension of a methanolic extract of the leaves collected in Korea was washed with ether and then extracted with 1-butanol saturated with water to give a glycoside mixture, which was subjected to repeated chromatography, affording **1** and two new glycosides (**2** and **3**) in yields of 0.04, 0.05 and 0.01%, respectively. The structure determination of **1** by means of physical and chemical procedures has already been reported.[1]

On hydrolysis with crude hesperidinase,[2] **2** afforded an aglycone (**4**). The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra indicated the presence of one terminal methylene, two carbonyl and six methyl groups. On treatment with diazomethane in ether, **4** afforded a dimethyl ester (**5**).

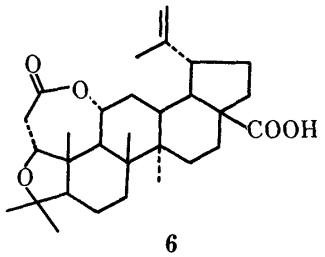
In the previous communication,[1] it was reported that on acid hydrolysis with 1.5% H_2SO_4 in an aprotic solvent, a mixture of dichloromethane (CH_2Cl_2) and dimethyl sulfoxide (DMSO), **1** gave an aglycone, named anhydro-chiisanogenin (**6**); an artifact formed from the genuine aglycone, chiisanogenin (**7**), together with several other minor products. The structure having a 2,2-dimethyltetrahydrofuran ring was proposed for **6** on the basis of ^{1}H- and ^{13}C-NMR spectrometry as shown in Chart 1. Further examination of the acid hydrolysis



1 : R = -β-Glc^{6}-β-Glc^{4}-α-Rha
7 : R = -H
9 : R = -CH_3



2 : R_1 = -β-Glc^{6}-β-Glc^{4}-α-Rha R_2 = -H
3 : R_1 = -β-Glc^{6}-β-Glc^{4}-α-Rha R_2 = -CH_3
4 : R_1 = -H R_2 = -H
5 : R_1 = -CH_3 R_2 = -CH_3
8 : R_1 = -H R_2 = -CH_2CH_3



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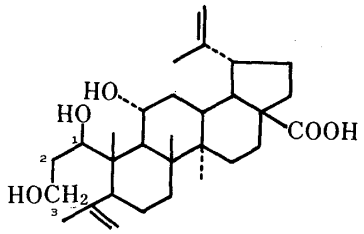

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Chart 1

of **1** disclosed that on acid treatment with 1.5% H_2SO_4 using 50% ethanol as a solvent, instead of CH_2Cl_2–DMSO, **1** afforded not **6**, but **8**. On the basis of a comparison of the NMR spectral data with those of **6**, **8** was formulated as an ethyl ester of a hydroxy-acid formed from the lactone (**6**) (Chart 1). A comparison of the ^{13}C-NMR spectra showed that, on going from **8** to **4**, the signal due to C-3 was shifted by +2.4 ppm, while other carbon resonances of both compounds appeared at the same positions except for the additional signals due to the carboethoxyl group of **8**. Based on these results, **4** was concluded to have the structure illustrated in Chart 1; it is a hydroxy-acid of **6**, and was named anhydro-chiisanogenoic acid.

The ^{1}H- and ^{13}C-NMR spectra of **2** showed the presence of three monosaccharides units. Acid hydrolysis of **2** gave D-glucose and L-rhamnose. The absolute configurations of both monosaccharides were confirmed by the procedure reported by Oshima *et al.*[3] The electron impact mass spectrum (EI-MS) of a peracetate of **2** exhibited fragment ions at *m/z* 849 ((Glc–Glc–Rha)Ac_9), 561 ((Glc–Rha)Ac_6) and 273 ((Rha)Ac_3). The ^{13}C-NMR signals due to the sugar moiety of **2** were observed at almost the same positions as those of **1**. It follows that **2** can be formulated as the α-L-rhamnopyranosyl (1→4)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl ester of **4**; this compound was named isochiisanoside. Finally, the structure of **2** was confirmed by conversion of **1** into **2** under mile acidic conditions.

Glycoside **3** was obtained as a white powder. The ^{1}H- and ^{13}C-NMR spectra of **3** are essentially similar to those of **2** except for the presence of signals due to a carbomethoxyl group (proton signal at δ 3.60 (3H, s,) and carbon signals at δ 51.2 and 173.4) of **3**. This indicates that **3** is a methyl ester of **2**. On treatment with diazomethane, **2** afforded a methyl ester which was proved to be identical with **3**.

The possibility that **2** and **3** might be artifacts formed from **1** during the process of extraction can not be completely excluded.

Experimental

General Procedure——Melting points were determined on a Yanaco micro hot stage and are uncorrected. Optical rotations were measured with a Union PM-101 automatic digital polarimeter. Infrared (IR) spectra were

TABLE I. ^{13}C-Chemical Shifts (δ) in C_5D_5N (Parentheses in $CDCl_3$)

	1	2	3	7	4	6	8
Aglycone moiety							
1	75.0	87.4	87.1	75.3	87.5 (85.9)	(86.4)	87.2
2	38.6	36.8	36.7	38.5	37.0 (36.8)	(37.9)	37.0
3	173.1	175.5	173.4	172.8	175.4 (177.3)	(177.3)	173.0
4	147.6	79.1	79.3	147.7	79.1 (80.7)	(79.7)	79.2
5	49.5	48.8	48.8	49.5	48.9 (47.9)	(48.1)	48.9
6	25.1	18.7	18.7	25.1	18.8 (19.0)	e)	18.8
7	33.4	35.5	35.4	33.5	35.6 (35.1)	(36.1)	35.6
8	41.6	42.7	42.7	41.6	42.7 (42.4)	e)	42.7
9	44.0	56.9	56.9	44.1	56.2 (55.6)	(55.6)	56.0
10	44.0	46.8	46.8	43.9	46.9 (46.4)	(46.5)	46.9
11	70.2	67.6	67.5	70.5	67.7 (68.1)	(68.3)	67.6
12	32.2	38.9	38.5	32.5	38.9 (37.3)	(35.2)	38.7
13	35.2	37.5	37.4	35.3	37.7 (37.1)	(37.0)	37.7
14	42.1	42.7	42.7	42.3	42.9 (42.4)	(42.5)	42.9
15	30.7	30.5	30.3	30.9	30.5 (30.6)	(30.5)	30.5
16	32.5	32.2	32.3	32.6	32.8 (32.3)	(32.2)	32.6
17	56.7	56.1	56.0	56.3	56.6 (56.3)	(56.3)	56.6
18	47.5	47.2	47.2	47.8	47.6 (46.8)	(46.8)	47.6
19	49.5	49.8	49.4	49.5	49.5 (48.7)	(48.8)	49.5
20	150.7	150.6	150.7	150.5	150.9 (149.8)	(149.9)	150.9
21	29.5	30.3	30.8	29.6	31.2 (29.7)	(29.9)	31.2
22	36.8	36.8	36.9	37.3	37.4 (37.3)	(37.4)	37.4
23	113.9	24.9[b]	24.9[b]	113.9	25.0 (24.4)[b]	(24.4)[b]	24.9[b]
24	23.5	32.8[b]	32.6[b]	23.5	32.8 (32.4)[b]	(32.3)[b]	32.8[b]
25	18.9[a]	19.2[a]	19.1[a]	18.9[a]	19.3 (18.7)[a]	(18.5)[a]	19.2[a]
26	17.9[a]	17.8[a]	17.8[a]	17.8[a]	17.9 (17.5)[a]	(17.6)[a]	17.9[a]
27	13.8	15.1	15.1	13.7	15.2 (14.9)	(15.0)	15.2
28	175.0	174.8	174.8	178.7	178.8 (180.6)	(179.6)	178.8
29	18.9	19.4	19.5	18.9	19.5 (19.4)	(18.9)	19.6
30	110.6	110.2	110.2	110.6	110.1 (110.4)	(110.2)	110.1
$-OCH_3$			51.2				
$-OCH_2\underline{C}H_3$							14.5
$-O\underline{C}H_2CH_3$							59.9
Sugar moiety							
Glc-1	95.2	95.2	95.2				
-2	73.8	73.9	73.8				
-3	78.4	78.5[c]	78.5[c]				
-4	70.5	70.6	70.7				
-5	76.3	76.3	76.3				
-6	69.2	69.3	69.3				
Glc-1′	104.7	104.8	104.9				
-2′	75.2	75.2	75.2				
-3′	76.9	76.9	77.0				
-4′	78.4	78.2[c]	78.2[c]				
-5′	77.8	77.9	77.9				
-6′	61.3	61.2	61.2				
Rha-1	102.5	102.5	102.5				
-2	72.5[b]	72.5[d]	72.6[d]				
-3	72.3[b]	72.4[d]	72.4[d]				
-4	73.8	73.9	73.8				
-5	70.2	70.2	70.2				
-6	18.3	18.4	18.4				

a—*d*) These assignments may be interchanged in each column; Glc, β-D-glucopyranosyl; Rha, α-L-rhamnopyranosyl.
e) Obscure.

taken on a Shimadzu IR-408 spectrometer. NMR spectra were recorded on a JEOL FX-100, GX-270, or FX-400 spectrometer using tetramethylsilane (TMS) as an internal standard. For gas liquid chromatography (GLC), a Shimadzu GC-8A or GC-6A was used. MS were taken on a JEOL JMS-01-SG-2 or JMS-DX-300 spectrometer by the direct inlet method; ionization voltage 75 or 70 eV. For column chromatography, Kieselgel 60 (70—230 mesh, Merck), LiChroprep RP-8 (40—63 μm, Merck) and Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) were used. All solvent systems for chromatography were homogeneous.

Acetylation for MS: A solution of a few milligram of glycoside in 5 drops of C_5H_5N and Ac_2O was allowed to stand for 24 h at room temperature. The reaction mixture was concentrated to dryness by blowing N_2 gas over it, and the residue was subjected to MS.

Acid Hydrolysis of Glycosides and Identification[3] of Resulting Monosaccharides: A sample of glycoside (10 mg) was heated with 3.5% HCl in H_2O–dioxane (1 : 1) (1 ml) in a sealed microtube at 80 °C for 3 h. The reaction mixture was diluted with H_2O and then washed with $CHCl_3$. The aqueous layer was neutralized with Amberlite MB-3 ion-exchange resin and then concentrated to give a sugar fraction. A solution of the sugar fraction (1 mg) in 50 μl of H_2O was treated with a solution of α-methylbenzylamine (9 mg) and $NaBH_3CN$ (0.6 mg) in 50 μl of EtOH, and the mixture was kept at 40 °C for 4 h. Then several drops of acetic acid were added, and the whole was concentrated to dryness. The residue was heated with several drops of *N*-trimethylsilylimidazole in a sealed microtube at 80 °C for 30 min. The reaction mixture was diluted with H_2O and then extracted with *n*-C_6H_{14}. The hexane layer was washed with H_2O and concentrated to dryness. A solution of the residue in *n*-C_6H_{14} was subjected to GLC analysis (dual flame ionization detector (FID); carrier gas, He 50 ml/min; WCOT glass capillary column (0.25 mm × 25 m) coated with Carbowax 20M; isothermal 150 °C; injection temperature, 190 °C).

Extraction and Separation of Glycosides——i) Isolation of **1** from the Stem-bark: The stem-bark (2 kg) was defatted with Et_2O and then extracted with MeOH. The MeOH extract was concentrated to dryness and the residue was partitioned between H_2O and 1-BuOH. The 1-BuOH layer was concentrated to give a crude glycoside fraction, which was chromatographed on a silica-gel column ($CHCl_3$–MeOH (10 : 1), (5 : 1) and (3 : 1)). Elution with $CHCl_3$–MeOH (3 : 1) afforded **1** (yield, 0.2%).

ii) Isolation of **1**,**2** and **3** from the Leaves: Dried leaves (3 kg) collected in Korea were extracted with hot MeOH, and the MeOH extract was concentrated to dryness. The resulting extract was suspended in H_2O and then washed with Et_2O. The aqueous layer was extracted with water-saturated 1-BuOH to give a crude glycoside fraction (49.5 g). This fraction was chromatographed on a column of highly porous polymer (Diaion HP-20) and eluted with H_2O, 60% MeOH, 80% MeOH, MeOH and Me_2CO, successively. The fraction eluted with 80% MeOH was subjected to chromatography on silica gel. Elution with $CHCl_3$–MeOH–H_2O (10 : 5 : 1) provided three fractions (frs. 1—3 in order of elution). Fraction 1 was subjected to chromatography on a silica gel column ($CHCl_3$–MeOH–H_2O (10 : 5 : 1) and then a reversed-phase column (LiChroprep RP-8, 70% MeOH) to give **1** (yield: 0.04%) and **3** (yield: 0.01%). Fraction 3 was chromatographed on a reversed-phase column (LiChroprep RP-8, 65% MeOH) and further purified by chromatography on a silica gel column ($CHCl_3$–MeOH–H_2O (10 : 5 : 1)) to give **2** (yield: 0.05%).

Compound **1**: Colorless needles (from 1-BuOH), mp 228 °C, $[\alpha]_D^{14}$ +7.7 °(c=1.69, MeOH). *Anal.* Calcd for $C_{48}H_{74}O_{19}\cdot 3H_2O$: C, 57.13; H, 7.99. Found: C, 57.12. H, 7.98. IR (Nujol): 3450 (OH), 1750, 1710 (COOR), 1640, 890 (C=CH_2) cm^{-1}. ^{1}H-NMR (C_5D_5N, 100 MHz) δ: 5.21 (1H, d, J=8 Hz, anomeric H of Glc′), 5.82 (1H, s, anomeric H of Rha), 6.40 (1H, d, J=8 Hz, anomeric H of Glc). EI-MS (peracetate) m/z: 849 ((Glc–Glc–Rha)Ac_9), 561 ((Glc–Rha)Ac_6), 273 (terminal–Rha–Ac_3). On mineral acid hydrolysis, **1** yielded D-glucose and L-rhamnose. ^{13}C-NMR data are given in Table I.

Compound **2**: A white powder, $[\alpha]_D^{17}$ −12.9 ° (c=1.01, MeOH). *Anal.* Calcd for $C_{48}H_{76}O_{20}\cdot 5/2H_2O$: C, 56.62; H, 8.02. Found: C, 56.43; H, 7.94. IR (Nujol): 3300 (OH), 1740 (COOR), 1700 (COOH), 1640, 890 (C=CH_2) cm^{-1}. ^{1}H-NMR (C_5D_5N, 100 MHz) δ: 4.93 (1H, d, J=6.5 Hz, anomeric H of Glc′), 5.78 (1H, s, anomeric H of Rha), 6.28 (1H, d, J=7 Hz, anomeric H of Glc). EI-MS (peracetate) m/z: 849 (Glc–Glc–Rha)Ac_9), 561 ((Glc–Rha)Ac_6), 273 (terminal–Rha–Ac_3). On mineral acid hydrolysis, **2** yielded D-glucose and L-rhamnose. ^{13}C-NMR data are given in Table I.

Compound **3**: A white powder, $[\alpha]_D^{17}$ −10.5 ° (c=0.95, MeOH). *Anal.* Calcd for $C_{49}H_{78}O_{20}\cdot H_2O$: C, 58.55; H, 8.02. Found: C, 58.49; H, 8.18. IR (Nujol): 3300 (OH), 1740, 1720 (COOR), 1640, 880 (C=CH_2) cm^{-1}. ^{1}H-NMR (C_5D_5N, 100 MHz) δ: 4.83 (1H, d, J=6.5 Hz, anomeric H of Glc′), 5.77 (1H, s, anomeric H of Rha), 6.26 (1H, d, J=7 Hz, anomeric H of Glc). EI-MS (peracetate) m/z: 849 ((Glc-Glc-Rha)Ac_{10}), 561 ((Glc–Rha)Ac_6), 273 (terminal–Rha–Ac_3). ^{13}C-NMR data are listed in Table I.

Enzymic Hydrolysis of 1 and 2——A solution of **1** (150 mg) and crude hesperidinase (150 mg, Tanabe Pharm., Co., Ltd., Osaka, Japan)[2] in H_2O (15 ml) was incubated at 37 °C for 40 h. The reaction mixture was subjected to column chromatography on Diaion HP-20 (H_2O and MeOH). The MeOH eluate was concentrated to dryness, and the residue was purified by column chromatography on silica gel ($CHCl_3$–MeOH (10 : 1)) to give **7** (62 mg). Compound **2** (120 mg) was also hydrolyzed in same way, yielding **4** (yield, 43 mg).

Compound **7**: Colorless needles (from Et_2O), mp 232—234 °C, $[\alpha]_D^{22}$ +86.4 °(c=0.66, MeOH). IR ($CHCl_3$): 3340 (OH), 1700 (C=O), 1640, 890 (C=CH_2) cm^{-1}. *Anal.* Calcd for $C_{30}H_{44}O_5$: C, 74.34; H, 9.15. Found: C, 74.14; H, 9.22. ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 0.91 (3H), 1.02 (3H), 1.08 (3H) (each s, *tert*-CH_3), 1.73 (3H, s, C_{24}-CH_3), 1.69

(3H, s, C_{29}-CH_3), 2.72 (1H, dd, J=15, 8 Hz C_2-Ha), 2.93 (1H, d, J=15 Hz, C_2-Hb), 2.97 (1H, ddd, J=11, 11, 4 Hz, C_{19}), 3.57 (1H, d, J=8 Hz, C_1-H), 4.53 (1H, ddd, J=8, 8, 8 Hz, C_{11}-H), 4.83 (1H, brs, C_{23}-Ha), 4.86 (1H, brs, C_{23}-Hb), 4.64 (1H, brs, C_{30}-Ha), 4.76 (1H, brs, C_{30}-Hb). EI-MS m/z: 484 (M^+), 469 ($M^+ - CH_3$), 466 ($M^+ - H_2O$), 41 (base peak, isopropenyl). ^{13}C-NMR data are listed in Table I.

Compound **4**: A white powder, $[\alpha]_D^{20}$ +49.3° (c=0.89, MeOH). *Anal.* Calcd for $C_{30}H_{46}O_6 \cdot 1/2H_2O$: C, 70.42; H, 9.26. Found: C, 70.49; H, 9.31. IR ($CHCl_3$): 3400 (OH), 1695 (COOH), 1640, 890 ($C=CH_2$) cm^{-1}. ^{1}H-NMR ($(CD_3)_2CO$, 270 MHz) δ: 0.99 (3H), 1.08 (3H), 1.09 (3H), 1.19 (3H), 1.24 (3H) (each s, *tert*-CH_3), 1.72 (3H, s, C_{29}-CH_3), 3.10 (1H, dd, J=14, 3 Hz, C_2-Ha), 2.29 (1H, dd, J=11, 14 Hz, C_2-Hb), 3.05 (1H, ddd, J=11,11, 3 Hz, C_{19}-H), 4.25 (1H, dd, J=11, 3 Hz, C_1-H), 3.89 (1H, ddd, J=11, 11, 5 Hz, C_{11}-H), 4.60 (1H, brs, C_{30}-Ha), 4.73 (1H, brs, C_{30}-Hb). EI-MS m/z: 502 (M^+), 487 (M^+-CH_3), 41 (base peak, isopropenyl). ^{13}C-NMR data are given in Table I.

Methylation of 4 and 7——Compound **7** (76 mg) was methylated with CH_2N_2 in Et_2O, and the usual work up gave **9** (yield, 53 mg). Compound **4** (17 mg) was also methylated by a similar method to give **5** (yield, 18 mg).

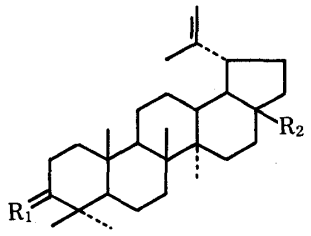
Compound **9**: Colorless needles (from benzene), mp 245—247.5 °C, $[\alpha]_D^{20}$ +84.0° (c=0.5, MeOH). IR ($CHCl_3$): 3400 (OH), 1720 (COOR), 1640, 890 ($C=CH_2$) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 0.90 (3H), 1.01 (3H), 1.06 (3H) (each s, *tert*-CH_3), 1.74 (3H, s, C_{24}-CH_3), 1.69 (3H, s, C_{29}-CH_3), 3.58 (1H, d, J=8 Hz, C_1-H), 3.67 (3H, s, -$COOCH_3$), 4.85 (2H, brs, C_{23}-H_2), 4.63 (1H, brs, C_{30}-Ha), 4.76 (1H, brs, C_{30}-Hb).

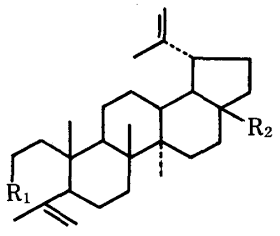
Compound **5**: A white powder, $[\alpha]_D^{19}$ +44.2° (c=0.65, MeOH). IR ($CHCl_3$): 3400 (OH), 1720 (COOR), 1640, 890 ($C=CH_2$) cm^{-1}. ^{1}H-NMR ($(CD_3)_2CO$, 270 MHz) δ: 0.96 (3H), 1.04 (3H), 1.09 (3H), 1.17 (3H), 1.28 (3H) (each s, *tert*-CH_3), 1.71 (3H, s, C_{29}-CH_3), 3.57 (3H, s, -$COOCH_3$), 3.65 (3H, s, -$COOCH_3$), 4.23 (1H, dd, J=11, 3 Hz, C_1-H), 4.26 (1H, brs, C_{30}-Ha), 4.75 (1H, brs, C_{30}-Hb).

Acid Hydrolysis of 1——i) Formation of **6**: Concentrated H_2SO_4 (0.3 ml) and CH_2Cl_2 (18 ml) were added to a solution of **1** (100 mg) in DMSO (2 ml), and the mixture was refluxed for 2 h at 80 °C. After cooling, the reaction mixture was washed with H_2O and then the organic layer was dried and evaporated. The residue was chromatographed on a silica gel column ($CHCl_3$–MeOH (10 : 1)) to give **6** (yield, 6 mg). A white powder, $[\alpha]_D^{15}$ +32.6° (c=0.46, MeOH). IR ($CHCl_3$): 1730 (COOR), 1690 (COOH), 1640, 870 ($C=CH_2$) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.96 (3H), 1.02 (3H), 1.11 (3H), 1.18 (3H), 1.25 (3H) (each s, *tert*-CH_3), 1.69 (3H, s, C_{29}-CH_3), 2.34 (1H, dd, J=11, 14 Hz, C_2-Ha), 3.09 (1H, dd, J=14, 3 Hz, C_2-Hb), 3.00 (1H, ddd, J=11, 11, 4 Hz, C_{11}-H), 4.31 (1H, dd, J=11, 3 Hz, C_1-H), 3.94 (1H, ddd, J=11, 11, 5 Hz, C_{11}-H), 4.64 (1H, brs, C_{30}-Ha), 4.76 (1H, brs, C_{30}-Hb). High-resolution MS m/z: 484.3166 (M^+, Calcd for $C_{30}H_{44}O_5$: 484.3189). ^{13}C-NMR data are given in Table I.

ii) Formation of **8**: A solution of **1** (90 mg) in 1.5 % H_2SO_4/H_2O–EtOH (1 : 1) (20 ml) was refluxed for 4 h and the reaction mixture was neutralized with Amberlite MB-3 resin and then concentrated to dryness. The crude product was subjected to chromatography on silica gel ($CHCl_3$–MeOH–H_2O (10 : 5 : 1)) to give **8** (yield, 13 mg). A white powder, $[\alpha]_D^{19}$ +40.3° (c=0.90, MeOH). IR ($CHCl_3$): 1720 (COOR), 1690 (COOH), 1640, 870 ($C=CH_2$) cm^{-1}. ^{1}H-NMR (C_5D_5N, 100 MHz) δ: 1.11 (3H, t, J=6 Hz, $C\underline{H}_3CH_2$-), 1.12 (3H), 1.16 (3H), 1.32 (3H), 1.40 (3H) (each s, *tert*-CH_3), 1.76 (3H, s, C_{29}-CH_3), 3.84 (2H, q, J=6 Hz, $CH_3C\underline{H}_2O$-), 4.16 (1H, dd, J=12, 6 Hz, C_1-H). 4.68 (1H, brs, C_{30}-Ha), 4.84 (1H, brs, C_{30}-Hb). ^{13}C-NMR data are given in Table I.

Reductive Lactone Ring Cleavage[4] **of 7**——A solution of **7** (40 mg), $NaBH_4$ (5.8 mg) and $AlCl_3$ (20 mg) in diglyme (4 ml) was heated for 2 h at 70 °C. After cooling, the reaction mixture was poured into 2 N HCl (50 ml) and the whole was extracted with $CHCl_3$. The organic layer was washed with H_2O, dried and evaporated to dryness. The residue was purified by silica gel column chromatography ($CHCl_3$–MeOH (10 : 1)) to give **17** (yield, 18 mg). A white powder, $[\alpha]_D^{16}$ +40.0° (c=0.30, MeOH). ^{1}H-NMR ($CDCl_3$, 270 MHz) δ: 1.01 (3H), 1.03 (3H), 1.31 (3H)(each s, *tert*-CH_3), 1.84 (3H, s, C_{24}-CH_3), 1.70 (3H, s, C_{29}-CH_3), 3.00 (1H, ddd, J=11, 11, 4 Hz, C_{19}-H), 3.97 (1H, ddd, J=11, 11, 6 Hz, C_{11}-H), 4.76(1H × 2, brs, C_{23}-Ha and C_{23}-Hb), 4.88 (1H, brs, C_{30}-Hb), 4.63 (1H, brs, C_{30}-Ha). High-resolution MS m/z: 488.3512 (M^+, Calcd for $C_{30}H_{48}O_5$: 488. 3502).





10 : R_1 = R_2 = -CH_2OH
11 : R_1 = =O R_2 = -$COOH$
12 : R_1 = =NOH R_2 = -$COOH$

13 : R_1 = -CN R_2 = -$COOH$
14 : R_1 = -$COOH$ R_2 = -$COOH$
15 : R_1 = -$COOCH_3$ R_2 = -$COOCH_3$
16 : R_1 = -CH_2OH R_2 = -CH_2OH

Chart 2

Synthesis of 16——i) Cleavage of Ring A of **11** (Abnormal Beckmann Rearrangement): According to the reported procedure,[5] a solution of **11** (455 mg), derived from betulin (**10**),[6] CH_3COONa (160 mg) and $NH_2OH \cdot HCl$ (100 mg) in EtOH (50 ml) was refluxed for 20 h. The reaction mixture was diluted with H_2O and then extracted with Et_2O. The organic layer was subjected to silica gel column chromatography ($CHCl_3$) to give **12** (438 mg). A mixture of **12** (438 mg) and *p*-toluenesulfonyl chloride (1 g) in dry pyridine (20 ml) was refluxed for 17 h under an N_2 stream. The reaction mixture was poured into 3 N HCl (50 ml) and then extracted with Et_2O. The organic layer was concentrated to dryness and the residue was chromatographed on silica gel ($CHCl_3$) to afford **13** (375 mg).

ii) Hydrolysis of **13**: A solution of **13** (375 mg) in 20% KOH–EtOH (10 ml) was refluxed for 3 h. The reaction mixture was neutralized with Amberlite MB-3 resin and concentrated to dryness. The residue was purified by silica gel chromatography ($CHCl_3$–MeOH (10 : 1)) to give **14** (202 mg).

iii) Methylation of **14**: Compound **14** was methylated with CH_2N_2 in Et_2O and after work up as usual, the product was subjected to silica gel chromatography ($CHCl_3$) to give **15** (198 mg). Colorless syrup, $[\alpha]_D^{24}$ +15.2° (c=3.0, MeOH). IR ($CHCl_3$): 1720 ($COOCH_3$), 1635, 890 ($C=CH_2$) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 0.82 (3H), 0.96 (3H), 0.98 (3H), 1.70 (3H), 1.72 (3H) (each s, *tert*-CH_3), 3.65 (3H), 3.67 (3H) (each s, -$COOCH_3$), 4.84 (1H, br s, C_{23}-Ha), 4.63 (1H×2, br s, C_{23}-Hb and C_{30}-Ha), 4.73 (1H, br s, C_{30}-Hb).

iv) Reduction of **15**: A solution of **15** (160 mg) and $LiAlH_4$ (100 mg) in anhydrous Et_2O (20 ml) was allowed to stand for 24 h at room temperature. The reaction mixture was acidified with 10% H_2SO_4 and extracted with Et_2O. The Et_2O layer was washed with H_2O, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue (148 mg) was crystallized from benzene to give **16** (84 mg). Colorless needles, mp 212—213 °C, $[\alpha]_D^{22}$ +45.3° (c=1.78, MeOH). *Anal.* Calcd for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.10; H, 11.58. IR ($CHCl_3$): 3400 (OH), 1640, 885 ($C=CH_2$) cm^{-1}. ^{1}H-NMR ($CDCl_3$, 100 MHz) δ: 0.80 (3H), 1.01 (3H), 1.07 (3H), 1.70 (3H×2) (each s, *tert*-CH_3), 3.56 (2H, t, J=8 Hz, C_3-H_2), 3.33 (1H, d, J=11 Hz, C_{28}-Ha), 3.80 (1H, d, J=11 Hz, C_{28}-Hb), 4.60 (1H×2, br s, C_{23}-Hb and C_{30}-Ha), 4.68 (1H, br s, C_{30}-Hb), 4.81 (1H, br s, C_{23}-Ha).

Modified Horeau's Method[7] for 7 (Determination of Chirality of C-1)——A solution of **7** (3 mg) and (±)-2-α-phenylbutyric acid anhydride (1.8 mg) in dry pyridine was allowed to stand in a sealed micro tube at room temperature for 20 h, then 12 μl of (+)-(*R*)-α-phenylethylamine was added. After standing for 30 min, then mixture was concentrated to dryness by blowing N_2 gas over it. The residue extracted with a small amount of EtOAc and the solution was subjected to GLC analysis (dual FID; carrier gas, N_2 2.1 kg/cm^2; column packed with 2% SE-30, 2 m×2.6 mm; isothermal 200 °C; injection and detector temperature, 250 °C). The relative proportions of the amides of (−)-(*R*)-and (+)-(*S*)-α-phenylbutyric acid were calculated from the areas of their peaks. Subtraction of the corresponding value from the reaction with cyclohexanol gave the decrement of the percentage area representing the (−)-(*R*)-acid: −5.9%.

Cleavage of Ester–Glycoside Linkage[8] of 1——A solution of **1** (100 mg), anhydrous LiI (100 mg) and 2,6-lutidine (5 ml) in anhydrous MeOH (5 ml) was refluxed for 15 h. The reaction mixture was deionized with Amberlite MB-3 resin and concentrated to dryness. The residue was chromatographed on Diaion HP-20 with H_2O to give methyl oligoglycoside (yield, 23 mg), which was identified by comparison of the ^{13}C-NMR spectrum with that of an authentic sample.[8]

Formation of 2 from 1——A solution of **1** (135 mg) in 3.5% HCl/H_2O–dioxane (1 : 1) was allowed to stand for 41 h at room temperature. The solution was neutralized with Amberlite MB-3 resin and then concentrated to dryness. The crude product was purified by silica gel column chromatography ($CHCl_3$–MeOH–H_2O (10 : 5 : 1)) to give an amorphous powder (yield, 84 mg), which was identified by comparison of physical constants and spectral data with those of **2**.

Methylation of 2——Compound **2** (37 mg) was treated with CH_2N_2 in Et_2O–MeOH to give a methyl ester (yield, 35 mg), which was identified by comparison of physical constants and spectral data with those of **3**.

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