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

RYOJI KASAI,^a KAZUHIRO MATSUMOTO,^a SHIGENORI TANIYASU,^a
OSAMU TANAKA,^{*,a} JEUNG-HEE KIM,^b
and DUG-RYONG HAHN^b

*Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine,^a
Kasumi, Minami-ku, Hiroshima 734, Japan and College of Pharmacy,
Chung-Ang University,^b 221 Huksuk-Dong,
Kwanak-ku, Seoul 151, Korea*

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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 $\rightarrow$ 4)- β -D-glucopyranosyl (1 $\rightarrow$ 6)- β -D-glucopyranoside of (1*R*)-1,11 α -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 (¹H- and ¹³C-NMR) spectra indicated the presence of one terminal methylene, two carbonyl and six methyl groups. On treatment with diazo-methane in ether, **4** afforded a dimethyl ester (**5**).

In the previous communication,¹⁾ it was reported that on acid hydrolysis with 1.5% H₂SO₄ in an aprotic solvent, a mixture of dichloromethane (CH₂Cl₂) 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 ¹H- and ¹³C-NMR spectrometry as shown in Chart 1. Further examination of the acid hydrolysis

Chart 1

of **1** disclosed that on acid treatment with 1.5% H₂SO₄ using 50% ethanol as a solvent, instead of CH₂Cl₂-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 ¹³C-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 ¹H- and ¹³C-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-Rha)Ac₉), 561 ((Glc-Rha)Ac₆) and 273 ((Rha)Ac₃). The ¹³C-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 mild acidic conditions.

Glycoside **3** was obtained as a white powder. The ¹H- and ¹³C-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 $\text{C}_5\text{D}_5\text{N}$ (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 CH ₃							14.5
-OCH ₂ CH 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 μ m, 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 Identification³⁾ 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 μ l of H_2O was treated with a solution of α -methylbenzylamine (9 mg) and $NaBH_3CN$ (0.6 mg) in 50 μ l 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 $\times$ 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, $[\alpha]_D^{25} + 7.7^\circ$ ($c=1.69$, 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} . 1H -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)Ac₉), 561 ((Glc-Rha)Ac₆), 273 (terminal-Rha-Ac₃). 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, $[\alpha]_D^{17} - 12.9^\circ$ ($c=1.01$, 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} . 1H -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)Ac₉, 561 ((Glc-Rha)Ac₆), 273 (terminal-Rha-Ac₃). 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, $[\alpha]_D^{17} - 10.5^\circ$ ($c=0.95$, 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} . 1H -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)Ac₁₀), 561 ((Glc-Rha)Ac₆), 273 (terminal-Rha-Ac₃). ^{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, $[\alpha]_D^{22} + 86.4^\circ$ ($c=0.66$, 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. 1H -NMR ($CDCl_3$, 270 MHz) δ : 0.91 (3H), 1.02 (3H), 1.08 (3H) (each s, *tert*-CH₃), 1.73 (3H, s, C_{24} -CH₃), 1.69

(3H, s, C₂₉-CH₃), 2.72 (1H, dd, $J=15, 8$ Hz, C₂-Ha), 2.93 (1H, d, $J=15$ Hz, C₂-Hb), 2.97 (1H, ddd, $J=11, 11, 4$ Hz, C₁₉-H), 3.57 (1H, d, $J=8$ Hz, C₁-H), 4.53 (1H, ddd, $J=8, 8, 8$ Hz, C₁₁-H), 4.83 (1H, brs, C₂₃-Ha), 4.86 (1H, brs, C₂₃-Hb), 4.64 (1H, brs, C₃₀-Ha), 4.76 (1H, brs, C₃₀-Hb). EI-MS m/z : 484 (M⁺), 469 (M⁺ - CH₃), 466 (M⁺ - H₂O), 41 (base peak, isopropenyl). ¹³C-NMR data are listed in Table I.

Compound 4: A white powder, $[\alpha]_D^{20} + 49.3^\circ$ ($c=0.89$, MeOH). Anal. Calcd for C₃₀H₄₆O₆ · 1/2H₂O: C, 70.42; H, 9.26. Found: C, 70.49; H, 9.31. IR (CHCl₃): 3400 (OH), 1695 (COOH), 1640, 890 (C=CH₂) cm⁻¹. ¹H-NMR ((CD₃)₂CO, 270 MHz) δ : 0.99 (3H), 1.08 (3H), 1.09 (3H), 1.19 (3H), 1.24 (3H) (each s, *tert*-CH₃), 1.72 (3H, s, C₂₉-CH₃), 3.10 (1H, dd, $J=14, 3$ Hz, C₂-Ha), 2.29 (1H, dd, $J=11, 14$ Hz, C₂-Hb), 3.05 (1H, ddd, $J=11, 11, 3$ Hz, C₁₉-H), 4.25 (1H, dd, $J=11, 3$ Hz, C₁-H), 3.89 (1H, ddd, $J=11, 11, 5$ Hz, C₁₁-H), 4.60 (1H, brs, C₃₀-Ha), 4.73 (1H, brs, C₃₀-Hb). EI-MS m/z : 502 (M⁺), 487 (M⁺ - CH₃), 41 (base peak, isopropenyl). ¹³C-NMR data are given in Table I.

Methylation of 4 and 7—Compound 7 (76 mg) was methylated with CH₂N₂ in Et₂O, 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, $[\alpha]_D^{20} + 84.0^\circ$ ($c=0.5$, MeOH). IR (CHCl₃): 3400 (OH), 1720 (COOR), 1640, 890 (C=CH₂) cm⁻¹. ¹H-NMR (CDCl₃, 100 MHz) δ : 0.90 (3H), 1.01 (3H), 1.06 (3H) (each s, *tert*-CH₃), 1.74 (3H, s, C₂₄-CH₃), 1.69 (3H, s, C₂₉-CH₃), 3.58 (1H, d, $J=8$ Hz, C₁-H), 3.67 (3H, s, -COOCH₃), 4.85 (2H, brs, C₂₃-H₂), 4.63 (1H, brs, C₃₀-Ha), 4.76 (1H, brs, C₃₀-Hb).

Compound 5: A white powder, $[\alpha]_D^{19} + 44.2^\circ$ ($c=0.65$, MeOH). IR (CHCl₃): 3400 (OH), 1720 (COOR), 1640, 890 (C=CH₂) cm⁻¹. ¹H-NMR ((CD₃)₂CO, 270 MHz) δ : 0.96 (3H), 1.04 (3H), 1.09 (3H), 1.17 (3H), 1.28 (3H) (each s, *tert*-CH₃), 1.71 (3H, s, C₂₉-CH₃), 3.57 (3H, s, -COOCH₃), 3.65 (3H, s, -COOCH₃), 4.23 (1H, dd, $J=11, 3$ Hz, C₁-H), 4.26 (1H, brs, C₃₀-Ha), 4.75 (1H, brs, C₃₀-Hb).

Acid Hydrolysis of 1—i) Formation of **6**: Concentrated H₂SO₄ (0.3 ml) and CH₂Cl₂ (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 H₂O and then the organic layer was dried and evaporated. The residue was chromatographed on a silica gel column (CHCl₃-MeOH (10:1)) to give **6** (yield, 6 mg). A white powder, $[\alpha]_D^{15} + 32.6^\circ$ ($c=0.46$, MeOH). IR (CHCl₃): 1730 (COOR), 1690 (COOH), 1640, 870 (C=CH₂) cm⁻¹. ¹H-NMR (CDCl₃, 400 MHz) δ : 0.96 (3H), 1.02 (3H), 1.11 (3H), 1.18 (3H), 1.25 (3H) (each s, *tert*-CH₃), 1.69 (3H, s, C₂₉-CH₃), 2.34 (1H, dd, $J=11, 14$ Hz, C₂-Ha), 3.09 (1H, dd, $J=14, 3$ Hz, C₂-Hb), 3.00 (1H, ddd, $J=11, 11, 4$ Hz, C₁₁-H), 4.31 (1H, dd, $J=11, 3$ Hz, C₁-H), 3.94 (1H, ddd, $J=11, 11, 5$ Hz, C₁₁-H), 4.64 (1H, brs, C₃₀-Ha), 4.76 (1H, brs, C₃₀-Hb). High-resolution MS m/z : 484.3166 (M⁺, Calcd for C₃₀H₄₄O₅: 484.3189). ¹³C-NMR data are given in Table I.

ii) Formation of **8**: A solution of **1** (90 mg) in 1.5 % H₂SO₄/H₂O-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 (CHCl₃-MeOH-H₂O (10:5:1)) to give **8** (yield, 13 mg). A white powder, $[\alpha]_D^{19} + 40.3^\circ$ ($c=0.90$, MeOH). IR (CHCl₃): 1720 (COOR), 1690 (COOH), 1640, 870 (C=CH₂) cm⁻¹. ¹H-NMR (C₅D₅N, 100 MHz) δ : 1.11 (3H, t, $J=6$ Hz, CH₃CH₂-), 1.12 (3H), 1.16 (3H), 1.32 (3H), 1.40 (3H) (each s, *tert*-CH₃), 1.76 (3H, s, C₂₉-CH₃), 3.84 (2H, q, $J=6$ Hz, CH₃CH₂O-), 4.16 (1H, dd, $J=12, 6$ Hz, C₁-H), 4.68 (1H, brs, C₃₀-Ha), 4.84 (1H, brs, C₃₀-Hb). ¹³C-NMR data are given in Table I.

Reductive Lactone Ring Cleavage⁴⁾ of 7—A solution of **7** (40 mg), NaBH₄ (5.8 mg) and AlCl₃ (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 CHCl₃. The organic layer was washed with H₂O, dried and evaporated to dryness. The residue was purified by silica gel column chromatography (CHCl₃-MeOH (10:1)) to give **17** (yield, 18 mg). A white powder, $[\alpha]_D^{16} + 40.0^\circ$ ($c=0.30$, MeOH). ¹H-NMR (CDCl₃, 270 MHz) δ : 1.01 (3H), 1.03 (3H), 1.31 (3H) (each s, *tert*-CH₃), 1.84 (3H, s, C₂₄-CH₃), 1.70 (3H, s, C₂₉-CH₃), 3.00 (1H, ddd, $J=11, 11, 4$ Hz, C₁₉-H), 3.97 (1H, ddd, $J=11, 11, 6$ Hz, C₁₁-H), 4.76 (1H × 2, brs, C₂₃-Ha and C₂₃-Hb), 4.88 (1H, brs, C₃₀-Hb), 4.63 (1H, brs, C₃₀-Ha). High-resolution MS m/z : 488.3512 (M⁺, Calcd for C₃₀H₄₈O₅: 488.3502).

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 $\text{NH}_2\text{OH} \cdot \text{HCl}$ (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 3N 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 ($\text{CHCl}_3\text{-MeOH}$ (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, $[\alpha]_D^{24} + 15.2^\circ$ ($c = 3.0$, MeOH). IR (CHCl_3): 1720 (COOCH_3), 1635, 890 ($\text{C}=\text{CH}_2$) cm^{-1} . $^1\text{H-NMR}$ (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, $-\text{COOCH}_3$), 4.84 (1H, brs, $\text{C}_{23}\text{-Ha}$), 4.63 (1H $\times$ 2, brs, $\text{C}_{23}\text{-Hb}$ and $\text{C}_{30}\text{-Ha}$), 4.73 (1H, brs, $\text{C}_{30}\text{-Hb}$).

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 $^\circ\text{C}$, $[\alpha]_D^{22} + 45.3^\circ$ ($c = 1.78$, MeOH). Anal. Calcd for $\text{C}_{30}\text{H}_{50}\text{O}_2$: C, 81.39; H, 11.38. Found: C, 81.10; H, 11.58. IR (CHCl_3): 3400 (OH), 1640, 885 ($\text{C}=\text{CH}_2$) cm^{-1} . $^1\text{H-NMR}$ (CDCl_3 , 100 MHz) δ : 0.80 (3H), 1.01 (3H), 1.07 (3H), 1.70 (3H $\times$ 2) (each s, *tert*- CH_3), 3.56 (2H, t, $J = 8$ Hz, $\text{C}_3\text{-H}_2$), 3.33 (1H, d, $J = 11$ Hz, $\text{C}_{28}\text{-Ha}$), 3.80 (1H, d, $J = 11$ Hz, $\text{C}_{28}\text{-Hb}$), 4.60 (1H $\times$ 2, brs, $\text{C}_{23}\text{-Hb}$ and $\text{C}_{30}\text{-Ha}$), 4.68 (1H, brs, $\text{C}_{30}\text{-Hb}$), 4.81 (1H, brs, $\text{C}_{23}\text{-Ha}$).

Modified Horeau's Method⁷⁾ for 7 (Determination of Chirality of C-1)—A solution of **7** (3 mg) and ($\pm$)-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 $\times$ 2.6 mm; isothermal 200 $^\circ\text{C}$; injection and detector temperature, 250 $^\circ\text{C}$). 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 Linkage⁸⁾ 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 $^{13}\text{C-NMR}$ spectrum with that of an authentic sample.⁸⁾

Formation of 2 from 1—A solution of **1** (135 mg) in 3.5% $\text{HCl/H}_2\text{O}$ –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 ($\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ (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 $\text{Et}_2\text{O-MeOH}$ 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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