New 3,4-*seco*-Lupane-Type Triterpene Glycosides from *Acanthopanax senticosus* forma *inermis*

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Four new 3,4-*seco*-lupane-type triterpene glycosides (**1**−**4**) were isolated from the leaves of *Acanthopanax senticosus* forma *inermis*. The structures of **1**−**4** were established as 11α-hydroxy-3,4-*seco*-lup-4(23),20(30)-dien-3-oic acid methyl ester 28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside, designated as inermoside (**1**); 1-deoxychiisanoside (**2**); 24-hydroxychiisanoside (**3**); and 11-deoxyisochiisanoside (**4**) by ^{1}H−^{1}H COSY and ^{1}H−^{13}C COSY(HMBC, HMQC) methods and FABMS.

The root and stem barks of *Acanthopanax* species have been used as a tonic and prophylactic in oriental herbal medication from olden times. The leaves and roots of this species have been also taken as a health drink and drug in Korea. In the course of studies on the phytochemical constituents of *Acanthopanax* species, we previously reported the isolation of five lupane-triterpene glycosides from the leaves of *A. koreanum* and *A. trifoliatus*,[1−3] and 3,4-*seco*-lupane triterpenes from the leaves of *A. divaricatus* var. *albeofructus*.[4] Among the *Acanthopanax* species, *A. senticosus* forma *inermis* is predominantly cultivated for medicinal use in Korea. We report herein the isolation and structure determination of four new 3,4-*seco*-lupane triterpene glycosides (**1**−**4**, Chart 1) from the leaves of *A. senticosus* forma *inermis* (Araliaceae). Additionally, the known compounds chiisanoside, divaroside, 22α-hydroxychiisanoside, isochiisanoside, and isochiisanoside methyl ester were isolated and identified by comparison of their ^{1}H and ^{13}C NMR data with reported values.[5−9]

Results and Discussion

The methanolic extract of the leaves of *A. senticosus* forma *inermis* was passed through a Diaion-HP 20 column by elution with H_2O, 30% MeOH, 50% MeOH, 80% MeOH, and MeOH, successively. The 80% MeOH eluate was concentrated under reduced pressure to give a residue, which was subjected to various column chromatographies; that is, Si gel, Sephadex LH-20, and reversed-phase ODS, to give compounds **1**−**4**.

Compound **1**, white powder, had a molecular formula of $C_{49}H_{78}O_{19}$ as determined by elemental analysis, positive FABMS (*m*/*z* 994 $[M + Na]^+$), and its ^{13}C NMR spectrum. The ^{1}H NMR spectrum (in pyridine-d_5) showed signals for five tertiary methyl groups (*δ* 1.13, 1.20, 1.21, 1.69, 1.80), one secondary methyl group [*δ* 1.70 (d, *J* = 6.1 Hz)], three anomeric protons [*δ* 4.95 (d, *J* = 7.3 Hz), 5.84 (br s), 6.32 (d, *J* = 7.9 Hz)], two sets of exomethylene olefinic protons [*δ* 4.63 (1H, s), 4.81 (1H, s), 4.88 (1H, s), 4.97 (1H, s)], and one methoxyl group [*δ* 3.59 (3H, s)]. The ^{13}C NMR spectrum showed 49 signals, of which 31 were assigned to a triterpenoid moiety and 18 to a three-sugar moiety.

Alkaline hydrolysis of **1** gave two novel sapogenols, inermogenin (**1a**) and 1-deoxychiisanogenin (**1b**). Incubation of **1** with esterase, which was obtained from the leaves of *Acanthopanax* species by gel filtration, for 5 days at 37 °C, also afforded **1a** and **1b**. The molecular weights of **1a** and **1b** were estimated as 500 and 468, respectively, from their EIMS.

The molecular formula of inermogenin (**1a**) was determined as $C_{31}H_{48}O_5$ by elemental analysis, EIMS (*m*/*z* 500, $[M]^+$), and ^{13}C NMR spectra. The ^{1}H NMR spectrum of **1a** in pyridine-d_5 displayed five tertiary methyl, four olefinic, one oxygen-bearing, and one methoxyl signals. The ^{13}C NMR spectrum suggested the presence of two carboxyl groups, two disubstituted double bonds, one oxygen-bearing methine carbon, five methine carbons, nine methylene carbons, five methyl carbons, and one methoxyl carbon as listed in Table 1. The oxygen-bearing proton of **1a** appeared at *δ* 4.17 (ddd, $J_{11,12eq}$ = 4.6, $J_{11,12ax}$ = 10.7, $J_{11,9}$ = 10.7 Hz), coupled with H-9 [*δ* 1.97 (d, *J* = 10.7 Mz)], H-12*eq* [*δ* 2.42 (ddd, $J_{12eq,13}$= 4.1, $J_{12eq,11}$ = 4.6, J_{gem} = 12.6 Mz)], and H-12*ax* (*δ* 1.76, overlapped). These data indicate a 9−11-diaxial arrangement of the hydrogens and an equatorial hydroxyl group. On the basis of the above evidence and 2D NMR data, **1a** was determined to be 11α-hydroxy-3,4-*seco*-lup-4(23),20(30)-dien-3-oic acid methyl ester 28-oic acid, named inermogenin.

1-Deoxychiisanogenin (**1b**) revealed a molecular ion peak $[M]^+$ at *m*/*z* 468, and the ^{13}C NMR data were consistent with the molecular formula $C_{30}H_{44}O_4$, which corresponded to the aglycon of **2**. The ^{1}H NMR spectrum in pyridine-d_5 displayed signals for five tertiary methyl groups, four olefinic protons, and an oxygen-bearing proton. The ^{13}C NMR spectrum indicated the presence of two carbonyl groups, two disubstituted double bonds, one oxygen-bearing methine carbon, five methine carbons, nine methylene carbons, and five methyl carbons. In the ^{13}C and ^{1}H NMR spectra, signals due to C-11 and H-11 appeared at *δ* 75.5 and *δ* 4.54, respectively, which showed respective downfield shifts of 5.9 and 0.4 ppm, when compared with those of **1**. The *J*-value (1H, ddd, *J* = 9.4, 9.4, 9.4) of H-11 in **1b** was in good agreement with that of chiisanogenin.[9] Thus, **1b** was determined to be an artifact derived through ring closure between the free 3-carboxylic acid and the 11-hydroxyl group in **1a** during hydrolysis, and 2D NMR data confirmed the above assignment.

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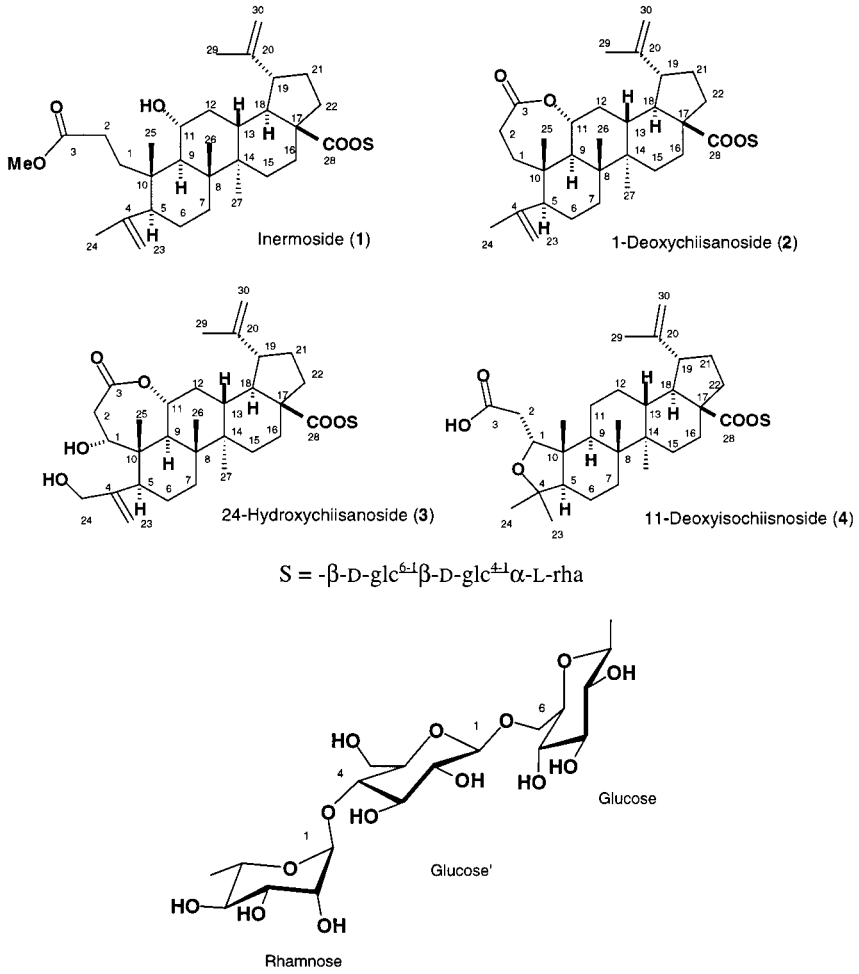
Chart 1



Acid hydrolysis of **1** afforded L-rhamnose and D-glucose as the sugar moieties. The identity of the single sugar chain and the sequence of the oligosaccharide chain were determined by DEPT, COSY, HMQC, and HMBC spectra. The β-anomeric configuration for the two glucose moieties were judged from their large $^3J_{H1,H2}$ coupling constants (7.9, 7.3 Hz). The ^{13}C NMR chemical shift of C-5 of rhamnose (δ 70.3) indicated β-orientation of the anomeric center.[10] Rhamnose was considered to be the terminal unit, as shown by the absence of a glycosylation shift in its ^{13}C NMR. From the HMBC spectrum, cross-peaks were observed for C-28 (δ 175.2) with H−G1 (δ 6.32), C−G6 (δ 69.4) with H−G′1 (δ 4.95), and C−G′4 (δ 78.6) with H−R1 (δ 5.84). Carbon signals due to this sugar moiety were superimposable on those of chiisanoside isolated from *A. chiisanensis*.[6,7] Consequently, **1** was determined to be 11α-hydroxy-3,4-*seco*-lup-4(23),20(30)-diene-3-carboxyl acid methyl ester 28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, named inermoside.

The second new compound, named 1-deoxychiisanoside (**2**), showed a quasimolecular ion peak $[M + Na]^+$ at *m/z* 962 in the positive FABMS, 16 mass units lower than that of chiisanoside,[5−9] and the ^{13}C NMR data were consistent with the molecular formula $C_{48}H_{74}O_{18}$. The ^{1}H NMR spectrum revealed signals for five tertiary methyl groups, a secondary methyl group, four olefinic protons on the disubstituted double bond, an oxygen-bearing proton, and three anomeric protons. The ^{13}C NMR spectrum of **2** closely resembled that of chiisanoside, except for signals due to the lactone-ring and B-ring moieties. In ^{13}C NMR data, the two methine carbon signals at δ 56.9 and 52.4 were shifted downfield by 7.3 and 8.9 ppm, respectively, and one quaternary carbon signal at δ 39.9 was shifted upfield by 2.4 ppm when compared with those of chiisanoside,[5−9] indicating no oxygen on C-1. The aglycon structure of **2** was the same as **1b** and was confirmed by 2D NMR data.

Acid hydrolysis of **2** gave L-rhamnose and D-glucose. The β-anomeric configurations of both glucoses and α-anomeric configuration of rhamnose were concluded from large $^3J_{H1,H2}$ coupling constants (8.6 and 7.9 Hz) of the glucoses, and the chemical shift at C-5 of rhamnose (δ 70.3).[10] The identity and sequence of the sugars were determined by 1D and 2D NMR spectra and were the same as in inermoside (**1**). Thus, the evidence led us to assign the structure of **2** as 1-deoxychiisanoside.

The positive FABMS of **3**, named 24-hydroxychiisanoside, showed the quasimolecular ion peak $[M + Na]^+$ at *m/z* 994, and the ^{13}C NMR data were consistent with the molecular formula $C_{48}H_{74}O_{20}$. The ^{1}H NMR spectrum showed signals indicating four tertiary methyl groups, one secondary methyl group, four olefinic protons on disubstituted double bonds, one hydroxymethyl group, two oxygen-bearing methine protons, and three anomeric protons. Signals of C-5 (δ 45.5) and C-23 (δ 111.3) shifted upfield 4.1 and 2.6 ppm, respectively, and that of C-4 (δ 152.7) shifted downfield 5.0 ppm, when compared with chiisanoside,[5−9] suggesting that C-24 was hydroxylated. This conclusion was supported by 2D NMR data; that is, oxymethylene proton signals (δ 4.44 and 4.65) were correlated to the olefinic carbon signal of C-23 (δ 111.3), and olefinic proton signals of H-23 (δ 5.20 and 5.60) also were

Table 1. ^{13}C NMR Data for Compounds **1**, **1a**, **1b**, **2**, **3**, and **4** in Pyridine-d_5[a]

C	1	1a	1b	2	3	4
aglycon						
1	37.5 t	37.6 t	39.5 t	39.5 t	70.9 d	85.4 t
2	30.0 t	30.1 t	30.2 t	30.2 t	38.3 t	38.3 t
3	174.9 s	175.3 s	175.7 s	175.7 s	172.9 s	174.9 s
4	148.3 s	148.4 s	147.3 s	147.4 s	152.7 s	81.0 s
5	51.3 d	51.3 d	56.9 d	56.9 d	45.5 d	56.2 d
6	25.1 t	25.2 t	25.4 t	25.3 t	26.6 t	18.8 t
7	33.7 t	34.0 t	32.6 t	32.4 t	32.1 t	34.4 t
8	40.0 s	40.1 s	41.7 s	41.7 s	41.7 s	42.2 s
9	45.8 d	45.9 d	52.5 d	52.4 d	44.3 d	42.6 d
10	42.3 s	42.3 s	40.0 s	39.9 s	44.3 s	47.7 s
11	69.6 d	69.8 d	75.5 d	75.2 d	75.4 d	23.7 t
12	37.9 t	38.1 t	33.5 t	33.3 t	33.4 t	25.5 t
13	37.3 d	37.6 d	35.2 d	35.1 d	35.2 d	38.5 d
14	42.1 s	43.1 s	42.1 s	42.0 s	42.2 s	41.6 s
15	30.1 t	30.3 t	29.7 t	29.5 t	29.6 t	30.4 t
16	32.1 t	32.8 t	32.6 t	32.1 t	32.8 t	32.3 t
17	57.0 s	56.7 s	56.4 s	56.8 s	56.8 s	56.9 s
18	49.4 d	49.4 d	49.8 d	49.7 d	49.6 d	49.7 d
19	47.1 d	47.5 d	47.9 d	47.6 d	47.6 d	47.4 d
20	150.4 s	150.9 s	150.6 s	150.1 s	150.0 s	150.8 s
21	30.9 t	31.3 t	31.1 t	30.7 t	30.7 t	30.8 t
22	36.6 t	37.4 t	37.3 t	36.8 t	36.7 t	36.9 t
23	113.9 t	114.0 t	114.2 t	114.1 t	111.3 t	24.7 q
24	23.7 q	23.8 q	23.7 q	23.7 q	67.2 t	32.7 q
25	20.8 q	20.9 q	18.8 q	18.9 q	18.7 q	19.2 q
26	17.3 q	17.4 q	17.6 q	17.7 q	18.1 q	17.0 q
27	14.7 q	14.8 q	13.8 q	13.8 q	13.8 q	14.8 q
28	175.2 s	178.9 s	178.7 s	175.0 s	175.1 s	174.5 s
29	19.5 q	19.6 q	19.0 q	18.9 q	18.9 q	19.4 q
30	110.2 t	110.1 t	110.7 t	110.7 t	110.7 t	110.1 t
CH_3O–	51.0 q					
C-28-*O*-inner GLC						
1	95.3 d			95.4 d	95.3 d	95.2 d
2	73.9 d			73.9 d	73.9 d	73.9 d
3	78.6 d			78.7 d	78.7 d	78.6 d
4	70.8 d			70.8 d	70.8 d	70.8 d
5	77.1 d			77.1 d	77.1 d	77.1 d
6	69.4 t			69.3 t	69.3 t	69.4 t
GLC′(4←1) rha						
1′	105.0 d			105.0 d	105.0 d	105.0 d
2′	75.2 d			75.2 d	75.2 d	75.2 d
3′	76.3 d			76.5 d	76.4 d	76.4 d
4′	78.3 d			78.4 d	78.3 d	78.2 d
5′	78.0 d			78.0 d	78.0 d	78.0 d
6′	61.3 t			61.3 t	61.3 t	61.3 t
terminal rha						
1	102.7 d			102.7 d	102.7 d	102.7 d
2	72.5 d			72.5 d	72.5 d	72.5 d
3	72.7 d			72.7 d	72.7 d	72.7 d
4	74.0 d			74.1 d	74.0 d	74.0 d
5	70.3 d			70.3 d	70.3 d	70.3 d
6	18.5 q			18.5 q	18.5 q	18.4 q

[a] δ in ppm, 500 MHz; multiplicities were deduced from a DEPT experiment.

correlated to the oxygen-bearing methylene carbon signal of C-24 (δ 67.2) in the HMBC spectrum. The sugar moiety of **3** was again the same as that of **1** on the basis of sugar analysis and 1D and 2D NMR data. Thus, the structure of **3** was assigned as 24-hydroxychiisanoside.

Compound **4**, named 11-deoxyisochiisanoside, showed a quasimolecular ion peak $[M + Na]^+$ at *m/z* 980 (positive FABMS), 16 mass units less than that of isochiisanoside.[8,9] Its molecular formula was assigned as $C_{48}H_{76}O_{19}$ from the ^{13}C NMR data. The ^1H NMR spectrum revealed signals for six tertiary methyl groups, a secondary methyl group, two olefinic protons on a disubstituted double bond, an oxygen-bearing proton, and three anomeric protons. The ^{13}C NMR spectrum indicated that one signal of an oxygen-bearing methine carbon was replaced by a methylene carbon when compared with isochiisanoside.[8,9] The ^{13}C NMR signals for C-9 and C-12 (δ 42.6 and 25.5) shifted upfield 6.3 and 11.2 ppm, respectively, and those of C-8, C-10, and C-13 shifted downfield 0.3, 0.9, and 1.0 ppm, respectively, when compared with those of isochiisanoside, indicating C-11 was no longer oxygen bearing. 2D NMR data confirmed the above structure assignment for the aglycon. The sugar moiety of **4** was identical to that of **1** by sugar analysis and 1D and 2D NMR data. These results led us to assign the structure of **4** as 1-deoxyisochiisanoside.

The 3,4-*seco*-lupane triterpene glycosides are characteristic of the *Acanthopanax* genus. Chiisanoside was isolated from *A. chiisanensis* as the first 3,4-*seco*-lupane triterpene glycoside in 1985.[6] There are few reports for 3,4-*seco*-lupane triterpenoid outside of the gennus *Acanthopanax* other than the following: *Dacryodes edulis*, *Euphorbia boteria*. and *Canarium* species.[11,12] Several *Acanthopanax* species (*A. divaricatus*, *A. chiisanensis*, *A. sesilliflofus*, *A. pedunculus*, etc.) contain the same 3,4-*seco*-lupane triterpenes: chiisanoside, isochiisanoside, divaroside, and so forth.[5–9] However, *Acanthopanax* species do not always include these components; some genera (*A. hypoleucus*, *A. sieboldiaus*, and *A. spinosus*, for example) contain only oleanane triterpenes,[13–15] and others (*A. koreanum* and *A. trifoliatus*) contain only normal-lupane triterpene glycosides.[1–3] These data may be useful for the chemotaxonomy of *Acanthopanax* species.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 polarimeter. Melting points (uncorrected) were measured using a Yanagimoto micromelting point apparatus. ^1H and ^{13}C NMR spectra were run on a JEOL α-500 spectrophotometer (500 MHz) in pyridine-d_5 with TMS as an internal standard. FABMS were obtained in a glycerol matrix in positive-ion mode on a JEOL JMS-DX300 and JMX-DX 303HF, and EIMS were obtained on a JEOL JMS-01SG and JMS-DX303HF. Elemental analysis was determined on a YANACO MT-5S elemental analyzer. Si gel 60 (0.040−0.063 mm, Merck), Sephadex LH-20 (25−100 μm, Pharmacia Fine Chemical), and Chromatorex ODS (30−50 μm, Fujisilysia Chemical Ltd.) were used for column chromatography. TLC was performed on precoated Si gel 60 F_{254} (Merck) and RP_{18} F_{254S} (Merck), and the spots were detected by spraying with 20% alcoholic H_2SO_4, followed by heating. GLC was performed on a HP5890A gas chromatograph with a flame-ionization detector.

Plant Material. Leaves of *A. senticosus* Harms forma *inermis* Harms were collected at Kwang-neung, Kyungkee-do Province, Korea, in July 1998, and botanically identified by Prof. Chang-Soo Yook in the Department of Pharmacognosy in Kyung-Hee University. A voucher specimen has been deposited at the Herbarium of the college of Pharmacy, Kyung-Hee University.

Extraction and Isolation. Dried leaves (600 g) of *A. senticosus* forma *inermis* were extracted repeatedly with hot MeOH. After the removal of the solvent by evaporation, the MeOH extract (34.4 g) was dissolved in H_2O and passed through a Diaion-HP 20 column, which was eluted with H_2O, 30% MeOH, 50% MeOH, 80% MeOH, and MeOH, succesively. The 80% MeOH eluate was concentrated (13.4 g) and chromatographed on Si gel with $CHCl_3$−MeOH−H_2O (9:2:0.1→8:2:0.2) to give 9 fractions (I−IX): I (1.34 g), II (0.38 g), III, IV, V (4.96 g), VI (1.0 g), VII (1.8 g), VIII (0.73 g), and IX (0.30 g). Fractions III−V (4.96 g) were evaporated to dryness in vacuo, and the residue was dissolved in MeOH and passed through Sephadex LH-20 with MeOH as the elution solvent. The eluents were evaporated to dryness in vacuo to give a residue, which was recrystallized from MeOH−H_2O to yield chiisanoside[5] (1.8 g). The filtrate was chromatographed on Chromatorex ODS, using gradient elution from 60% MeOH to 90% MeOH, to give **1** (70.5 mg), **2** (20.7 mg), and **3** (28.7 mg).

Fraction VIII (0.73 g) was also chromatographed on Chromatorex ODS (from 60% MeOH to 100% MeOH) to give **4** (32.5 mg).

Inermoside (1): white power; $[\alpha]^{25}_D$ −15.0° (*c* 0.52, MeOH); FABMS (positive-ion mode) *m/z* 994 $[M + Na]^+$; ^{1}H NMR (pyridine-d_5) δ 1.13 (3H, s, H-27), 1.20 (3H, s, H-25), 1.21 (3H, s, H-26), 1.69 (3H, s, H-29), 1.70 (3H, d, *J* = 6.1 Hz, Rha H-6), 1.80 (3H, s, H-24), 1.93 (1H, d, *J* = 10.4 Hz, H-9), 2.34 (1H, ddd, *J* = 3.3, 4.3, 12.8 Hz, H-12a), 2.88 (1H, ddd, *J* = 3.3, 12.2, 12.2 Hz, H-13), 3.37 (1H, ddd, *J* = 4.5, 10.4, 10.9 Hz, H-19), 3.59 (3H, s, CH_3O−), 4.17 (1H, overlapped, H-11), 4.63 (1H, br s, H-30*a*), 4.8 (1H, br s, H-30*b*), 4.88 (1H, br s, H-23*a*), 4.95 (1H, d, *J* = 7.3 Hz, outer Glc H-1), 4.97 (1H, br s, H-23*b*), 5.84 (1H, s, Rha H-1), 6.32 (1H, d, *J* = 7.9 Hz, inner Glc H-1); ^{13}C NMR, see Table 1.

Inemogenin (1a): white solid; mp 120−125°; $[\alpha]^{25}_D$ +21.7° (*c* 0.32, MeOH); ^{1}H NMR (pyridine-d_5) δ 1.15 (3H, s, H-27), 1.19 (3H, s, H-25), 1.21 (3H, s, H-26), 1.76 (3H, s, H-29), 1.81 (3H, s, H-28), 1.97 (1H, d, *J* = 10.7 Hz, H-9), 2.42 (1H, ddd, *J* = 4.1, 4.6, 12.6 Hz, H-12b), 2.97 (1H, ddd, *J* = 3.6, 12.6, 12.6 Hz, H-13), 3.53 (1H, ddd, *J* = 4.9, 11.0, 11.0 Hz, H-19), 3.60 (3H, s, CH_3O−), 4.17 (1H, ddd, *J* = 4.6, 10.7, 10.7 Hz, H-11), 4.67 (1H, br s, H-30*a*), 4.89 (1H, br s, H-30*b*), 4.89 (1H, br s, H-23*a*), 4.98 (1H, br s, H-23*b*); ^{13}C NMR, see Table 1; EIMS *m/z* 500 $[M]^+$ (4), 483 $[M - OH]^+$ (17), 469 $[M - OCH_3]^+$ (64), 424 (33), 423 (32), 341 (28), 252 (28), 187 (38), 119 (47), 107 (61), 95 (57), 81 (68), 69 (70), 55 (100); *anal*. C 69.99%, H 9.29%, calcd for $C_{31}H_{48}O_5 \cdot 2H_2O$, C 69.36%, H 9.77%.

1-Deoxychiisanogenin (1b): colorless needles; mp 253−256°; $[\alpha]^{25}_D$ +59.4° (*c* 0.19, MeOH); ^{1}H NMR (pyridine-d_5) δ 0.94 (3H, s, H-27), 1.02 (3H, s, H-25), 1.07 (3H, s, H-26), 1.68 (3H, s, H-29), 1.72 (3H, s, H-24), 1.83 (1H, d, *J* = 9.4 Hz, H-9), 2.88 (1H, ddd, *J* = 4.3, 12.2, 12.2 Hz, H-13), 3.48 (1H, ddd, *J* = 4.1, 10.7, 10.7 Hz, H-19), 4.54 (1H, ddd, *J* = 9.4, 9.4, 9.4 Hz, H-11), 4.67 (1H, br s, H-30*a*), 4.74 (1H, br s, H-30*b*), 4.90 (1H, br s, H-23*a*), 4.95 (1H, br s, H-23*b*); ^{13}C NMR, see Table 1; EIMS *m/z* 468 $[M]^+$ (3), 483 $[M - OH]^+$ (53), 423 (31), 422 (30), 341 (24), 252 (100), 187 (28), 119 (29), 107 (40), 95 (31), 81 (34), 69 (25), 55 (38); *anal*. C 72.71%, H 9.27%, calcd for $C_{30}H_{44}O_4 \cdot 3/2H_2O$, C 72.69%, H 9.56%.

Deoxychiisanoside (2): white power; $[\alpha]^{25}_D$ −3.0° (*c* 0.36, MeOH); FABMS (positive-ion mode) *m/z*: 962 $[M + Na]^+$; ^{1}H NMR (pyridine-d_5) δ 1.01 (3H, s, H-27), 1.04 (3H, s, H-25), 1.04 (3H, s, H-26), 1.66 (3H, s, H-29), 1.70 (3H, d, *J* = 6.7 Hz, Rha H-6),1.77 (3H, s, H-24), 1.78 (1H, d, *J* = 9.2 Hz, H-9), 2.78 (1H, ddd, *J* = 4.5, 13.0, 13.0 Hz, H-13), 3.48 (1H, ddd, *J* = 4.3, 9.5, 9.5 Hz, H-19), 4.54 (1H, overlapped, H-11), 4.61 (1H, br s, H-30*a*), 4.74 (1H, br s, H-23*a*), 4.87 (1H, br s, H-30*b*), 4.90 (1H, br s, H-23*b*) 4.94 (1H, d, *J* = 7.9 Hz, outer Glc H-1), 5.84 (1H, s, Rha H-1), 6.34 (1H, d, *J* = 8.6 Hz, inner Glc H-1);^{13}C NMR, see Table 1.

24-Hydroxychiisanoside (3): white power; $[\alpha]^{25}_D$ −5.2° (*c* 0.50, MeOH); FABMS (positive-ion mode) *m/z*: 994 $[M + Na]^+$; ^{1}H NMR (pyridine-d_5) δ 1.01 (3H, s, H-27), 1.10 (3H, s, H-25), 1.14 (3H, s, H-26), 1.65 (3H, s, H-29), 1.70 (3H, d, *J* = 6.1 Hz, Rha H-6), 2.46 (1H, ddd, *J* = 3.5, 13.3, 13.3 Hz, H-12a), 2.70 (1H, d, *J* = 9.8 Hz, H-9), 3.06 (1H, d, *J* = 14.6 Hz, H-2b), 3.37 (1H, ddd, *J* = 4.9, 11.0, 11.0 Hz, H-19), 3.69 (1H, d, *J* = 7.9 Hz, H-1), 4.33 (1H, overlapped, H-11), 4.44 (1H, d, *J* = 14.0 Hz, H-24a), 4.61 (1H, br s, H-30*a*), 4.65 (1H, d, *J* = 14.0 Hz, H-24b), 4.87 (1H, br s, H-30*b*), 4.95 (1H, d, *J* = 7.9 Hz, outer Glc H-1), 5.20 (1H, overlapped, H-23*a*), 5.60 (1H, br s, H-23*b*), 5.85 (1H, s, Rha H-1), 6.34 (1H, d, *J* = 8.6 Hz, inner Glc H-1); ^{13}C NMR, see Table 1.

11-Deoxyisochiisanoside (4): white power; $[\alpha]^{25}_D$ −14.0° (*c* 0.50, MeOH); FABMS (positive-ion mode) *m/z*: 980 $[M + Na]^+$; ^{1}H NMR (pyridine-d_5) δ 1.05 (3H, s, H-27), 1.05 (3H, s, H-25), 1.11 (3H, s, H-26), 1.11 (3H, s, H-23), 1.37 (3H, s, H-24), 1.67 (3H, d, *J* = 6.7 Hz, Rha H-6), 1.70 (3H, s, H-29), 1.78 (1H, dd, *J* = 1.8, 12.8 Hz, H-9), 2.07 (1H, ddd, *J* = 2.4, 13.7, 13.7 Hz, H-15a), 2.79 (1H, dd, *J* = 10.4, 13.8 Hz, H-2a), 3.33 (1H, ddd, *J* = 4.7, 10.7, 10.7 Hz, H-19), 4.42 (1H, dd, *J* = 3.7, 10.4 Hz, H-1), 4.60 (1H, br s, H-30*a*), 4.85 (1H, br s, H-30*b*), 4.91 (1H, d, *J* = 7.3 Hz, outer Glc H-1), 5.80 (1H, s, Rha H-1), 6.32 (1H, d, *J* = 8.5 Hz, inner Glc H-1); ^{13}C NMR, see Table 1.

Alkaline Hydrolysis of 1. Compound **1** (30 mg) was hydrolyzed with 2 mL of 3% KOH in MeOH for 1 h at 80 °C, followed by neutralization with 5% HCl in MeOH. After the removal of solvent by evaporation, the residue was purified using Diaion HP-20P column chromatography (30%MeOH→MeOH) and Si gel column chromatography (hexane−ethyl acetate = 3:1→2:1) to give **1a** (3 mg) and **1b** (2 mg).

Separation and Partial Purification of the Esterase. Fresh leaves (10 g) of *A. divaricatus* were intensively ground with 100 mL of 50 mM sodium phosphate buffer (pH 7.0) and centrifuged at 1000 rpm for 30 min. The supernatant was fractionated with 70% ammonium sulfate followed by centrifugation at 12 000 rpm for 1 h. The resulting pellet was suspended in 3 mL of 50 mM sodium phosphate buffer (pH 7.0), being further purified by Sephacryl S-300 HR gel filtration column chromatography using the same buffer as the eluent. The esterase activity appeared in the void volume, the early part of the chromatography. This desalted esterase was used for cleavage of ester derivatives.

Enzymatic Hydrolysis of 1. A solution of **1** (30 mg) and the esterase was incubated at 37 °C for 5 days. The reaction mixture was extracted with ethyl acetate, concentrated to dryness, and purified by column chromatography on Si gel (hexane−ethyl acetate 3:1→2:1) to give **1a** (5 mg) and **1b** (4 mg).

Acid Hydrolysis of 1−4. Each compound (3 mg) was hydrolyzed with 2 mL of 2 N HCl in H_2O for 4 h at 80 °C, followed by neutralization with 2 N NaOH in H_2O and extraction with $CHCl_3$. The H_2O layer of each reaction mixture was concentrated to dryness in vacuo. The residue was dissolved in dry pyridine, L-cystaine methyl ester hydrochloride was added, and this mixture was heated for 2 h at 60 °C and then concentrated under a N_2 stream. Trimethylsilylimidazole was added to the residues, and the mixtures were heated for 1 h at 60 °C. After concentrating the solvent under N_2, the residue was extracted with *n*-hexane and H_2O, the organic layer was injected into a gas chromatograph: OV-17 (0.32 mm × 30 cm); detector, FID; column temperature, 230 °C; carrier gas, He. D-Glucose and L-rhamnose (molar ratio 2:1) were detected in each case (**1**−**4**).

References and Notes

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