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New Triterpene Saponins from *Ilex pubescens*

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Novel triterpenoid saponins, ilexsaponins B_1, B_2 and B_3, have been isolated from the roots of *Ilex pubescens*. Degradative and spectroscopic studies have established their structures as shown in formulae **5**, **6**, and **7**. Ilexsaponin B_3 was found to possess an activity against experimental hypercholesteremia in mice.

Keywords——*Ilex pubescens*; Aquifoliaceae; triterpene; saponin; antihypercholesteremia; ^{13}C-NMR

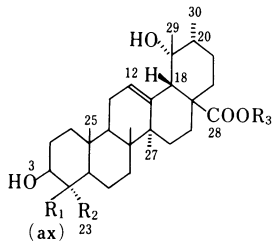
In China, "mao-dong-qing" (毛冬青), the root of *Ilex pubescens* HOOK. *et* ARN. (Aquifoliaceae), is widely used for the treatment of cardiovascular diseases, hypercholesteremia, *etc.*[1] In a preliminary screening test for the antihypercholesteremic action of several crude drugs, this plant showed significant activity. For the purpose of identifying the active principle, the chemical constituents of mao-dong-qing were investigated. This plant is known to contain several simple phenolics, *i.e.* 3,4-dihydroxyacetophenone, hydroquinone, scopoletin, esculetin, homovanillic acid and glaberide I, a natural degradation product of syringaresinol.[2] From a closely related species, *I. cornuta*, two glycosides of pomolic acid (**3**),[3] and from *I. rotunda*, oleanolic acid and rotundic acid (**4**)[4] have been isolated.

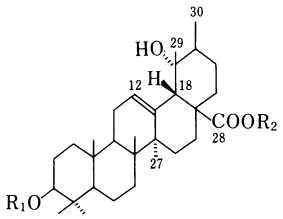
During the course of our investigations on the chemical constituents of *Ilex pubescens*, we have isolated and elucidated the structure of a triterpene, ilexgenin A (**1**), and its glucoside, ilexsaponin A_1 (**2**) from the EtOAc-soluble fraction of the MeOH extract of the roots.[5] In the present study we report the isolation and the structural elucidation of three new triterpene glycosides having a new aglycone, from the BuOH-soluble fraction of the MeOH extract of the roots. One of the compounds showed antihypercholesteremic activity.

Mao-dong-qing, the dried roots of *Ilex pubescens* were first extracted with benzene, followed by MeOH. The MeOH extract was suspended in water, then extracted with benzene, EtOAc, 1-BuOH–H_2O successively, and the antihypercholesteremic activity of each fraction was assayed. The BuOH-soluble fraction showed significant activity.

From the EtOAc fraction, compounds **1** and **2** were isolated by reversed-phase and normal-phase column chromatography.[5] Neither of them showed antihypercholesteremic activity.

From the active BuOH-soluble fraction, in addition to compound **1**, three new compounds, ilexsaponins B_1 (**5**), B_2 (**6**), and B_3 (**7**), were isolated by chromatography on Diaion HP-20, followed by silica gel chromatography. By the carbon-13 nuclear magnetic





	R_1	R_2	R_3
1	COOH	CH_3	H
2	COOH	CH_3	Glc
3	CH_3	CH_3	H
3a	CH_3	CH_3	CH_3
4	CH_3	CH_2OH	H
9	CH_2OH	CH_2OH	H

	R_1	R_2
5	–Xyl^2-Glc	H
6	–Xyl^2-Glc^2-Rha	H
7	–Xyl^2-Glc	Glc
8	H	H
8a	H	CH_3
10	–Xyl	H

Xyl: β-D-xylopyranosyl
Glc: β-D-glucopyranosyl
Rha: α-L-rhamnopyranosyl

TABLE I. ^{13}C-NMR Spectral Data for Genin Moieties of Ilexsaponins B_1 (**5**), B_2 (**6**) and B_3 (**7**) and Related Compounds

	3a[a)]	**8a**[a)]	**8**	**5**	**6**	**7**	**10**
C-1	38.7	38.4	39.0	38.8	38.8	38.8	38.7
C-2	27.4	27.1	28.1	27.0	27.0	26.8	27.0
C-3	79.0	79.0	78.2	88.9	89.7	88.8	88.7
C-4	38.5	38.7	39.4	39.5	39.7	39.6	39.5
C-5	55.2	55.1	55.9	55.9	56.0	55.9	55.9
C-6	18.4	18.4	19.0	18.6	18.6	18.7	18.6
C-7	32.8	32.7	33.6	33.5	33.5	33.5	33.5
C-8	39.9	39.8	40.3	40.2	40.2	40.3	40.2
C-9	47.2	47.3	47.8	47.7	47.7	47.7	47.7
C-10	36.9	37.0	37.4	36.9	37.0	36.9	37.0
C-11	23.7	23.9	24.9	24.8	24.9	24.6	24.8
C-12	129.1	128.5	127.3	127.2	127.3	127.5	127.2
C-13	138.0	137.7	139.5	139.5	139.5	138.8	139.4
C-14	41.1	41.1	42.2	42.0	42.1	42.1	42.1
C-15	28.1	28.1	29.3	29.2	29.2	29.1	29.2
C-16	25.5	26.1	27.1	26.6	26.6	26.7	26.7
C-17	47.9	47.4	48.0	47.8	47.9	48.3	47.9
C-18	53.2	46.6	47.4	47.3	47.4	47.1	47.4
C-19	73.1	74.0	73.4	73.4	73.4	73.4	73.4
C-20	41.1	41.3	43.1	42.8	43.0	42.6	42.9
C-21	26.0	23.6	24.0	23.9	23.9	23.8	24.0
C-22	37.4	31.2	32.4	32.3	32.5	31.7	32.4
C-23	28.1	28.1	28.8	28.0	28.4	28.1	28.2
C-24	15.2	15.2	15.6	15.5	15.4	15.6	15.5
C-25	15.5	15.6	16.2	16.1	16.1	16.0	16.1
C-26	16.6	16.7	17.3	17.2	17.3	17.4	17.3
C-27	24.5	24.5	24.4	24.3	24.4	24.3	24.4
C-28	178.3	178.4	180.7	180.7	180.7	177.1	180.9
C-29	27.2	30.1	29.9	29.8	29.8	29.7	29.8
C-30	16.1	15.8	16.6	16.6	16.7	16.7	16.9
$COO\underline{CH_3}$	51.5	51.6					

δ relative to TMS in C_6D_5N (except **3a** and **8a**). *a*) Measured in $CDCl_3$; data for **3a** are cited from ref. 7.

TABLE II. ^{13}C-NMR Spectral Data for Sugar Moieties of Ilexsaponins B_1 (**5**), B_2 (**6**) and B_3 (**7**) and the Prosapogenin (**10**)

	10	**5**	**6**	**7**
3-*O*-Sugar				
Xyl-1	107.6	105.7	105.9	105.8
Xyl-2	75.4	82.8	79.0	83.0
Xyl-3	78.5	77.8	77.8	77.5
Xyl-4	71.2	71.5	71.3	71.5
Xyl-5	67.0	66.5	66.6	66.7
Glc-1		105.8	102.3	105.8
Glc-2		76.8	79.4	76.9
Glc-3		78.1	79.0	78.3
Glc-4		70.8	72.3	70.9
Glc-5		77.8	78.5	77.9
Glc-6		62.6	63.3	62.6
Rha-1			102.0	
Rha-2			72.6	
Rha-3			72.6	
Rha-4			74.3	
Rha-5			69.5	
Rha-6			18.9	
28-*O*-Sugar				
Glc-1				95.7
Glc-2				74.0
Glc-3				79.1
Glc-4				70.9
Glc-5				78.8
Glc-6				62.1

δ relative to TMS in C_6D_5N.

resonance (^{13}C-NMR) analysis, **5**, **6**, and **7** were proved to have the same aglycone (Tables I and II).

Structure of the Aglycone (8)

Enzymic hydrolysis of **5** afforded the aglycone (**8**), $C_{30}H_{48}O_4$. The ^{13}C-NMR spectrum of **8** showed 30 carbon signals, *i.e.* $CH_3-\times 7$, $-CH_2-\times 9$, $>CH-\times 4$, $>C<\times 5$, $>CH-O\times 1$, $>C-O\times 1$, $>C=CH-\times 1$, $CO\times 1$. Its infrared (IR) spectrum indicated the presence of –OH (3400 cm^{-1}), and –COO (1690 cm^{-1}) groups. The electron impact mass spectra (EIMS) of **8** revealed the fragment peak at *m/z* 264, which is characteristic of retro-Diels–Alder cleavage of olean-12-ene or urs-12-en-28-oic acid having a hydroxy group on the D or E ring. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum in pyridine-d_5 showed a 1H singlet signal at δ 3.30 (1H, s), assignable to 18-H of 19-hydroxylated ursane. The spectroscopic data for the **8**-methyl ester (**8a**) were compared with those of methyl pomolate (**3a**).[6] The EIMS of **8a** and **3a** were nearly identical, as were those of **8** and **3** (Table III).

The ^{13}C-NMR spectrum of **8a** was very similar to that of **3a**,[7] and A, B and C ring carbon signals of both compounds were essentially the same, though some carbon signals in rings D and E were significantly different (Table I). Among them, the carbon signals assignable to C-18 and C-22 of **8a** were shifted to high field by 6.6 and 6.2 ppm, respectively, from the corresponding carbon signals of **3a**. This can be explained in terms of the γ-effect of the 30-β-(axial) methyl group in place of α-(equatorial) methyl in **3a**. To confirm this, *J* and nuclear Overhauser effect (NOE) correlated 2-dimensional ^{1}H-NMR spectra of **8a** and acid (**4**) were measured at 400 MHz. Assignments of these spectra were mainly based on the reference

TABLE III. MS Data for **3**, **3a**, **8** and **8a**

3[a]	**8**	**3a**[a]	**8a**
472 (M^+, 9%)	472 (M^+, 6%)	486 (M^+, 8%)	486 (M^+, 10%)
454 ($M^+ - H_2O$, 12%)	454 ($M^+ - H_2O$, 9%)	468 ($M^+ - H_2O$, 9%)	468 ($M^+ - H_2O$, 7%)
439 ($M^+ - H_2O - CH_3$, 8%)	439 ($M^+ - H_2O - CH_3$, 3%)	453 ($M^+ - H_2O - CH_3$, 5%)	453 ($M^+ - H_2O - CH_3$, 3%)
264 (16%)	264 (6%)	426 ($M^+ - HCOOCH_3$, 21%)	426 ($M^+ - HCOOCH_3$, 20%)
207 (40%)	207 (33%)	278 (8%)	278 (5%)
146 (100%)	146 (100%)	207 (31%)	207 (29%)
		179 (100%)	179 (100%)

a) Data are cited from ref. 6.

data for clethric acid (**9**).[7] However, the original assignments of the C-27 and C-29 methyl groups should be reversed, since, in the NMR experiments on the stereochemically similar compound **4**, on irradiation of 18-H (δ 2.60), NOE was observed at δ 1.21 (29-Me), not at δ 1.27 (27-Me). With this change, the stereochemistry of **4** was reasonably explained. In the ^{1}H-NMR spectrum of **8a**, NOE was observed between 12-H (δ 5.32) and 18-H (δ 2.82), and irradiation of 18-H gave NOE at 29-CH_3 (δ 1.16) and 30-CH_3 (δ 0.99). On irradiation of 29-CH_3, NOE was observed at 12-H, 18-H and 30-CH_3. Therefore, it was concluded that all of these atoms or functional groups have the same configuration, β. It follows that **8** can be formulated as (30*S*)-3β,19α-dihydroxyurs-12-en-28-oic acid, and we named it ilexgenin B.

Structures of Ilexsaponins B_1 (5), B_2 (6) and B_3 (7)

In the ^{13}C-NMR spectrum of **5**, two sets of monosaccharide signals were observed, and the aglycone carbon signals of the B, C, D and E rings were nearly identical to those of ilexgenin B (**8**). A significantly different chemical shift was observed at C-3, which was shifted downfield by 10.7 ppm on going from **8** to **5** (Tables II and III). Acid hydrolysis of **5** afforded xylose and glucose, identified by gas liquid chromatography (GLC) analysis. By ^{13}C- and ^{1}H-NMR analyses, these sugars were identified as β-xylopyranose (anomeric H, δ 4.82, d, J= 6.3 Hz) and β-glucopyranose (anomeric H, δ 5.36, d, J=7.6 Hz). The absolute configurations of these sugars were assumed to be both D, by analogy with the glycosylation shift of holothurins, 2-quinovosylxylosides.[8,9] Gas chromatography-mass spectrometry (GC-MS) analysis of the acetates of the acid hydrolysis products of permethylated **5** revealed 2-linked xylopyranose and terminal glucopyranose. The EIMS of the acetate gave a peak at m/z 331 [glucose$(Ac)_4$]$^+$ and 547 [glucose$(Ac)_4$ – xylose$(Ac)_2$]$^+$. It follows that **5** can be formulated as the 3-*O*-β-D-glucopyranosyl(1→2)-β-D-xylopyranoside of **8**.

On enzymic hydrolysis, ilexsaponin B_2 (**6**) afforded the aglycone (**8**), the prosapogenin (**10**), and a saponin (**5**). In the ^{13}C-NMR spectrum of **6**, three sets of monosaccharide signals were observed, and the aglycone signals are identical with those of **5**. The structure of the oligosaccharide at the C-3-*O*-position was determined as above. Xylose, glucose, and rhamnose were detected by GLC, and the form of the sugars was deduced from the ^{13}C-NMR (Table II) and ^{1}H-NMR spectra. In the ^{1}H-NMR spectrum of **6**, anomeric signals of the sugars appear at δ 4.91 (1H, d, J=5.4 Hz), δ 5.83 (1H, d, J=7.1 Hz), δ 6.41 (1H, br s) for xylose, glucose and rhamnose, respectively, confirming them to be β-xylopyranose, β-glucopyranose and α-rhamnopyranose. The GC-MS analysis revealed 2-linked xylopyranose, 2-linked glucopyranose, and terminal rhamnopyranose. In addition, the EIMS of the acetate of **6** showed peaks at m/z 273 [rhamnose$(Ac)_3$]$^+$ and 561 [rhamnose$(Ac)_3$–glucose$(Ac)_3$]$^+$. It follows that **6** can be formulated as the 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-xylopyranoside of **8**.

The ^{13}C-NMR spectrum of **7** is very similar to that of **5**. The difference is that in the spectrum of **7**, the C-28 signal was shifted upfield by 3.6 ppm and a set of esterified β-glucopyranosyl signals was observed. In the ^{1}H-NMR spectrum, three anomeric signals were observed at δ 4.82 (1H, distorted d by virtual coupling), δ 5.39 (1H, d, $J=6.3$ Hz) and δ 6.37 (1H, d, $J=6.8$ Hz). Selective ester glycoside cleavage reaction[10] of **7** afforded ilexsaponin B_1 (**5**) and methyl glucopyranoside. It follows that **7** can be formulated as the 28-*O*-β-D-glucopyranoside of **5**.

Among the ilexsaponins isolated from *Ilex pubescens*, only ilexsaponin B_3 (**7**) showed the antihypercholesteremic activity. The value of inhibition by **7** was 68% of the treated control at the oral dose of 300 mg/kg.

Experimental

General Procedure——NMR spectra were taken at 25 °C using tetramethylsilane (TMS) as an internal standard; ^{13}C-NMR at 25.15 MHz and ^{1}H-NMR at 100 MHz unless otherwise stated. EIMS were taken at 75 eV. Melting points were taken on a micro hot stage and are uncorrected. Acid hydrolysis of each glycoside was carried out in the usual way, and the resulting monosaccharides were identified by GC as their trimethylsilyl ethers.

Plant Materials——A sample of "Mao-dong-qing," the root of *Ilex pubescens* HOOK. *et* ARN., was purchased from Mikuni Co., Ltd. (Osaka), in 1983.

Bioassay for Anti-hypercholesteremic Activity——The activity was examined in male dd-k mice (weighing *ca.* 23 g) which were fed on "fat emulsion" (olive oil, 32.6%; cholesterol, 2.5%; sucrose, 42.0%; sodium cholate, 1.8%, water, 21.1%). Just after, and 7 h after the administration of fat emulsion, samples to be tested were administered orally in the form of a suspension in Gumi Arabicum. At 17 h after the last feeding, the animals were anesthetized with ether, and the blood samples for cholesterol determination were collected from the abdominal aorta. The blood samples were centrifuged at 3000 ***g***, and the total cholesterol content of the blood serum was measured by the cholesterol oxidase–*p*-chlorophenol method, using the Cholesterol C II-Test Wako (Wako Pure Chemical Co., Ltd.). As positive control drugs, Niceritol and Nicomol were used.

Extraction of Triterpene and Glycosides——Dried roots (4.7 kg) were crushed and extracted with benzene (20 l × 2), the residue was extracted with MeOH (20 l × 4) and the MeOH extract was evaporated to dryness (414 g). A suspension of the resulting extract in H_2O was washed with benzene, then extracted with EtOAc and BuOH/H_2O successively to give the extracts, 103 and 256 g, respectively.

A portion of the BuOH extract (32 g) was chromatographed on a column of Diaion HP-20 (Mitsubishi Chemical Ind. Co.) and eluted with 15% MeOH, 30% MeOH, MeOH and $CHCl_3$–MeOH (1 : 1), successively. The MeOH eluate was divided into two fractions before and after the appearance of a yellow band. This Diaion-chromatography was repeated 8 times to separate all the BuOH extract, affording 171 g of crude glycoside fraction as the second MeOH eluate. The glycoside fraction (156 g) was chromatographed on silica gel using the $CHCl_3$–MeOH–H_2O system, and divided into 31 fractions. From fractions 4 and 5 ($CHCl_3$–MeOH–H_2O, 15 : 3 : 0.2 eluate), compound **1** (3.1 g) was obtained. From fractions 10 and 11 ($CHCl_3$–MeOH–H_2O, 15 : 4 : 0.4 eluate), **5** (6.7 g) was obtained as colorless crystals. Fraction 15 ($CHCl_3$–MeOH–H_2O, 15 : 6 : 1 eluate) was chromatographed on ODS-Gel using MeOH–H_2O gradient to give **7** (610 mg, from 70% MeOH) and **6** (480 mg, from 60% EtOH).

Ilexsaponin B_1 (5)——mp 246—248 °C (MeOH), $[\alpha]_D^{20}$ −9.6° ($c=1.01$, pyridine). *Anal.* Calcd for $C_{41}H_{66}O_{13}\cdot 2H_2O$: C, 61.32; H, 8.79. Found: C, 61.32; H, 8.79. IR ν_{max}^{Nujol} cm^{-1}: 3300, 1690. EIMS of acetate[11] *m/z*: 331 $[Glc(Ac)_4]^+$, 547 $[Glc(Ac)_4-Xyl(Ac)_2]^+$. ^{1}H-NMR (in pyridine-d_5) δ: 0.90, 1.09, 1.10, 1.26, 1.44, 1.75 (3H × 6, s, 23, 24, 25, 26, 27 and 29-CH_3). 1.13 (3H, d, $J=7.3$ Hz, 30-CH_3), 3.29 (1H, s, 18-H), 4.82 (1H, d, $J=6.3$ Hz, Xyl-1-H), 5.05 (1H, s, 19α-OH), 5.36 (1H, d, $J=7.6$ Hz, Glc-1-H), 5.56 (1H, br t, 12-H). ^{13}C-NMR see Tables I and II.

Enzymic Hydrolysis of Ilexsaponin B_1 (5)——A few drops of toluene were added to a solution of **5** (370 mg) and crude hesperidinase (300 mg, Tanabe Pharm. Ind. Co., Ltd., Osaka) in McIlvaine buffer (pH 4.0, 200 ml). The mixture was incubated at 38 °C for 90 h and then extracted with $CHCl_3$ and BuOH saturated with water, successively. Each organic layer was washed with water and concentrated to dryness, affording 85 and 171 mg of residue, respectively. Both fractions were chromatographed on silica gel, affording the aglycone **8** (58 and 37 mg, respectively), and prosapogenin **10** (6 and 57 mg, respectively).

Ilexgenin B (8)——mp 276—279 °C (MeOH), $[\alpha]_D^{20}$ +28.1° ($c=0.48$, pyridine). *Anal.* Calcd for $C_{30}H_{48}O_4\cdot 1/2H_2O$: C, 74.80; H, 10.25. Found: C, 74.98; H, 10.47. High-resolution MS *m/z*: 472.3529, Calcd for $C_{30}H_{48}O_4$, 472.3553. EIMS see Table I. IR ν_{max}^{Nujol} cm^{-1}: 3450, 1690. ^{1}H-NMR (in pyridine-d_5) δ: 0.93, 1.04, 1.11, 1.24, 1.47, 1.76 (3H, s × 6), 1.14 (3H, d, $J=6.4$ Hz, 30-CH_3), 3.30 (1H, s, 18-H), 5.14 (1H, br s, 19-OH), 5.58 (1H, br s, 12-H). ^{13}C-NMR see Table I.

Methylation of 8——**8** (27 mg) was methylated with diazomethane to afford **8a** (9 mg) mp 119—123 °C from

MeOH, $[\alpha]_D^{20}$ +28.1° (c=0.48, $CHCl_3$). High-resolution MS m/z: 486.3709, Calcd for $C_{31}H_{50}O_4$, 486.3709. ^{1}H-NMR (in $CDCl_3$, 400 MHz) δ: 0.67, 0.78, 0.90, 0.98, 1.16, 1.23 (each 3H, s, 26-CH_3, 25-CH_3, 23-CH_3, 24-CH_3, 29-CH_3, 27-CH_3, respectively), 0.99 (3H, d, J=6 Hz, 30-CH_3), 2.82 (1H, s, 18-H), 3.22 (1H, dd, J=4, 10 Hz, 3αH), 3.62 (3H, s, -COOMe), 5.32 (1H, t, J=3 Hz, 12-H). ^{13}C-NMR see Table I.

Prosapogenin (10)——^{13}C-NMR see Tables I and II.

Ilexsaponin B_2 (6)——mp 240—243 °C (EtOH), $[\alpha]_D^{20}$ −15.3° (c=1.20, pyridine). *Anal.* Calcd for $C_{47}H_{76}O_{17}\cdot 5/2H_2O$: C, 58.91; H, 8.35. Found: C, 58.94; H, 8.35. IR ν_{max}^{Nujol} cm^{-1}: 3300, 1685. EIMS of acetate[11] m/z: 273 $[Rha(Ac)_3]^+$, 561 $[Rha(Ac)_3-Glc(Ac)_3]^+$. ^{1}H-NMR (in pyridine-d_5) δ: 0.86, 1.08, 1.35, 1.43, 1.76 (3H×6, s), 1.12 (3H, d, J=9.0 Hz, 30-CH_3), 1.79 (3H, d, J=6.1 Hz, Rha-6-H), 3.30 (1H, s, 18-H), 4.91 (1H, d, J=5.4 Hz, Xyl-1H), 5.08 (1H, s, 19α-OH), 5.56 (1H, br t, 12-H), 5.83 (1H, d, J=7.1 Hz, Glc-1-H), 6.41 (1H, br s, Rha-1-H). ^{13}C-NMR see Tables I and II.

Enzymic Hydrolysis of Ilexaponin B_2 (6)——A few drops of toluene were added to a solution of **6** (100 mg) and crude hesperidinase (98 mg, Tanabe Pharm. Ind. Co., Ltd., Osaka) in McIlvaine buffer (pH 4.0, 100 ml). The mixture was incubated at 37 °C for 48 h and then extracted with $CHCl_3$ and BuOH saturated with water, successively. Each organic layer was washed with water and concentrated to dryness, affording 3 and 68 mg of residue, respectively. The latter was chromatographed on silica gel, affording the aglycone **8** (19 mg), the prosapogenin **10** (4 mg) and ilexsaponin B_1 (**5**) (11 mg).

Ilexsaponin B_3 (7)——A white powder, $[\alpha]_D^{20}$ +10.0° (c=0.40, MeOH). *Anal.* Calcd for $C_{47}H_{76}O_{18}\cdot 5/2H_2O$: C, 57.95; H, 8.38. Found C, 57.98; H, 8.31. IR ν_{max}^{Nujol} cm^{-1}: 3300, 1720. EIMS of peracetate[11] m/z: 331 $[Glc(Ac)_4]^+$, 547 $[Glc(Ac)_4-Xyl(Ac)_2]^+$. ^{1}H-NMR (in pyridine-d_5) δ: 0.95, 1.13, 1.21, 1.26, 1.41, 1.72 (3H×6, s), 0.99 (3H, d, J=8.8 Hz, C-30-Me), 3.20 (1H, s, 18-H), 4.82 (1H, distorted d by virtual coupling, Xyl-1-H), 5.19 (1H, s, 19α-OH), 5.39 (1H, d, J=6.3 Hz, 3-O-Glc-1-H), 5.56 (1H, br t, 12-H), 6.37 (1H, d, J=6.8 Hz, 28-O-Glc-1-H). ^{13}C-NMR see Tables I and II.

Selective Hydrolysis of Ester Sugar of Ilexsaponin B_3 (7)——A solution of **7** (51 mg) and anhydrous LiI (40 mg) in 2,6-lutidine (3 ml) and anhydrous MeOH (2 ml) was refluxed under N_2 gas for 13 h. After addition of H_2O (1 ml), the reaction mixture was deionized by passing it through a column of Amberlite MB-3 and then chromatographed on silica gel to give ilexsaponin B_1 (**5**, 20 mg) and methyl glucopyranoside (9 mg) by elution with chloroform–methanol–H_2O (150 : 25 : 1).

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References and Notes

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11) A saponin (*ca.* 1 mg) was heated with 5 drops of pyridine and 3 drops of Ac_2O in a sealed tube at 100 °C for 1 h. The reaction mixture was concentrated to dryness by blowing N_2 gas over it at room temperature. The residue was directly used for MS measurement.