Triterpenoid Saponins of Aquifoliaceous Plants. XI.[1] Ilexosides XLI—XLV from the Leaves of *Ilex rotunda* THUNB.[2]

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Five new saponins, ilexosides XLI—XLV, were isolated from fresh leaves of *Ilex rotunda*, and their structures were elucidated on the basis of chemical and physicochemical evidence. Ilexoside XLI is rotundic acid 28-*O*-α-D-glucopyranosyl (1→6)-β-D-glucopyranoside, ilexoside XLII is 23-oxorotungenic acid 28-*O*-β-D-glucopyranoside, ilexoside XLIII is 30-hydroxyrotundic acid 28-*O*-β-D-glucopyranoside, ilexoside XLIV is 3,28,bis-*O*-β-D-glucopyranosyl ilexosapogenin B (3-*O*-β-D-glucopyranosyl 23,30-dihydroxyursolic acid 28-*O*-β-D-glucopyranoside), and ilexoside XLV is 24-hydroxyrotundioic acid 28-*O*-β-D-glucopyranoside.

Keywords *Ilex rotunda*; Aquifoliaceae; ilexoside; α-glucoside; ilexosapogenin B; rotundic acid

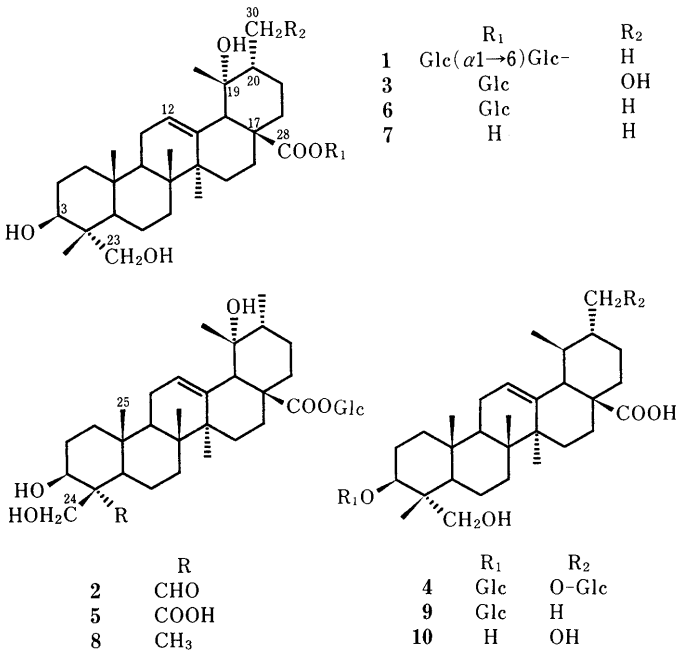
In a previous paper,[1] we have reported the isolation and structure determination of two triterpenes, ilexolic acids A and B, and three saponins, ilexosides XXXVIII—XL from the fresh leaves of *Ilex rotunda* THUNB. We now wish to report the isolation and structure determination of five additional new saponins from the leaves of the title plant.

The 70% EtOH extract of the dried leaves (4 kg) of *Ilex rotunda* THUNB., was subjected to Amberlite XAD-2 column chromatography to give a saponin fraction (165 g). Repeated separation of a part (45 g) of the saponin fraction by ordinary-phase (SiO_2) and reversed-phase octadecyl silica (ODS) column chromatography furnished ilexosides XLI (**1**, 0.025 g), XLII (**2**, 0.055 g), XLIII (**3**, 0.04 g), XLIV (**4**, 0.08 g), and XLV (**5**, 0.025 g).

Ilexoside XLI (**1**) was obtained as colorless needles and the relative molecular mass (M_r) was considered to be 812, as the deprotonated molecular ion was apparent at m/z 811 in its fast atom bombardment mass spectrum (FAB-MS). The molecular formula of this compound was confirmed as $C_{42}H_{68}O_{15}$ by elemental analysis. A carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectral comparison of **1** with pedunculoside (**6**), obtained in our previous investigation,[3] showed that **1** is also a glycoside of rotundic acid (**7**),[4] and varies structurally from **6** only in its saccharide moieties, though these sugar units are also affixed to the C-28 position.

On acid hydrolysis, **1** gave D-glucose. The proton magnetic resonance (^{1}H-NMR) and ^{13}C-NMR spectra of **1** indicated the presence of one α-glucosyl unit [H-1: δ 5.44 (d, J=3.5 Hz): C-1 δ 100.5] and one esteric β-glucosyl unit [H-1: δ 6.24 (d, J=6.5 Hz: C-1 δ 95.6). A ^{13}C-NMR spectral comparison of **1** with **6** revealed a glycosylation shift[5] at the C-6′ position of the 28-*O*-glucosyl unit (+5.6 ppm from δ 62.4 to 68.0), implying that an α-glucopyranosyl group is joined to C-6-OH of the glucosyl unit. Therefore, **1** was formulated as rotundic acid 28-*O*-α-D-glucopyranosyl (1→6)-β-D-glucopyranoside.

Ilexoside XLII (**2**), $C_{36}H_{56}O_{11}$ was obtained as a white powder and the M_r was considered to be 664, as the deprotonated molecular ion was apparent at m/z 663 in the negative FAB-MS. Its ^{1}H-NMR spectrum showed the presence of the four tertiary methyl groups (δ 1.02, 1.18, 1.40, and 1.62), one secondary methyl group (δ 1.07, J=6.6 Hz), a vinyl proton (dd, J=4.0, 3.4 Hz), an isolated oxymethylene group (δ 4.48 and 4.80, each d, J=11.7 Hz)



	R₁	R₂
1	Glc(α1→6)Glc-	H
3	Glc	OH
6	Glc	H
7	H	H

	R
2	CHO
5	COOH
8	CH_3

	R₁	R₂
4	Glc	O-Glc
9	Glc	H
10	H	OH

Fig. 1

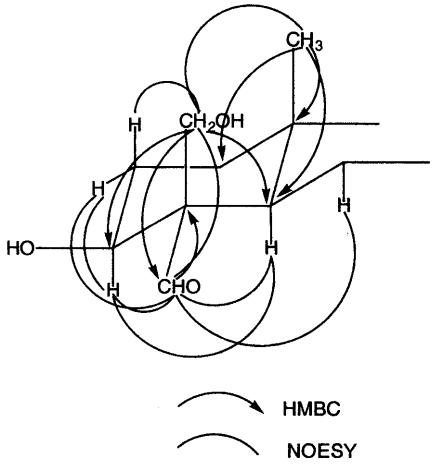


Fig. 2

and an aldehyde group (δ 10.30, s). The electron impact mass spectrum (EI-MS) of **2** showed characteristic ion peaks due to retro Diels–Alder fission in the C ring: *m/z* 238, 220 (238 − H_2O), 191 (238 − H_2O − CHO), and 189 (238 − H_2O − CH_2OH) due to the A/B rings, and *m/z* 264, 246 (264 − H_2O), and 201 (264 − H_2O − COOH) due to the D/E rings. These results indicate that the aglycone is an amyrin derivative having two hydroxyls and one aldehyde in the A/B rings and one esteric carboxyl and one hydroxyl in the D/E rings.[6,7]

Acid hydrolysis of **2** afforded D-glucose. A ^{13}C-NMR spectral comparison of **2** with ilexoside XXXV (**8**)[8] isolated from this plant, showed that **2** is differs structurally from **8** only its A ring, though the sugar unit is also affixed to the C-28 position. In the ^{13}C-NMR spectrum of **2**, the C-4 (δ 59.0) signal was shifted downfield by 15.8 ppm, while those of C-3 (δ 74.6) and C-5 (δ 50.9) were shifted upfield by 5.8 and 5.7 ppm, respectively, compared with those of **8**, indicating the aldehyde group to be at the C-4 position. Additional information on the functional groups in the A ring was obtained from a ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) experiment. In the HMBC experiment, the aldehyde proton at δ 10.30 (C_{23}-H) gave cross peaks with the quaternary carbon at δ 59.0 (C-4) and the methylene carbon at δ 61.0 (C-24). The methylene protons (C_{24}-H_2)

TABLE I. ^{13}C-NMR Spectral Data for Compounds **1**—**6**, **8**—**10** (Pyridine-d_5, δ-Values)

C	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[a]	**6**[a]	**8**[a]	**9**[a]	**10**[b]
1	38.9	38.6	39.0	38.9	39.3	39.0	39.0	39.0	38.9
2	27.7	27.9	27.8	26.0	28.5	27.7	28.5	26.0	27.7
3	73.6	74.6	73.8	82.4	77.1	73.7	80.4	82.3	73.5
4	42.9	59.0	42.9	43.5	58.0	42.9	43.2	43.5	42.8
5	48.6	50.9	48.6	48.1	52.7	48.7	56.6	47.7	48.6
6	18.8	21.4	18.9	18.4	22.7	18.9	19.4	18.3	18.6
7	33.2	33.3	33.4	33.3	33.9	33.3	34.0	33.0	33.3
8	40.6	40.7	40.6	40.1	40.9	40.6	40.7	40.2	40.0
9	47.8	47.6	47.9	47.7	48.3	47.9	48.0	47.6	48.0
10	37.2	36.7	37.3	36.9	37.0	37.3	37.2	36.8	37.1
11	24.1	24.2	24.2	23.8	24.4	24.2	24.4	23.7	23.7
12	128.4	128.1	128.8	124.4	128.2	128.5	128.4	126.1	125.5
13	139.3	139.3	138.8	136.5	139.3	139.3	139.3	138.4	135.5
14	42.1	42.1	42.2	42.7	42.2	42.2	42.1	42.5	42.5
15	29.3	29.2	29.3	28.8	29.3	29.3	29.3	28.7	28.5
16	26.0	26.1	26.3	25.1	26.1	26.2	26.2	24.6	25.0
17	48.7	48.6	48.6	48.1	48.6	48.7	48.7	48.1	48.0
18	54.3	54.5	54.4	53.7	54.5	54.5	54.5	53.3	51.7
19	72.6	72.6	74.0	34.7	72.7	72.7	72.8	39.3[c]	33.9
20	42.0	42.1	48.2	45.1	42.0	42.2	42.2	39.1[c]	47.5
21	26.7	26.7	22.4	26.0	26.8	26.8	26.8	30.7	25.7
22	37.7	37.7	37.7	37.3	37.7	37.8	37.8	36.8	37.4
23	67.8	208.8	68.2	64.5	62.4	68.0	23.7	64.7	68.1
24	13.1	61.0	13.1	13.9	179.6	13.2	64.7	13.6	13.1
25	16.1	15.8	16.2	16.4	16.3	16.2	16.3	16.3	16.1
26	17.5	17.3	17.6	17.7	17.2	17.6	17.4	17.6[d]	17.5
27	24.6	24.4	24.2	24.1	24.5	24.6	24.6	23.7	23.9
28	177.1	176.9	177.0	180.8	177.0	177.1	177.0	176.1	180.0
29	26.9	27.0	27.5	17.4	27.1	27.1	27.2	17.7[d]	17.2
30	16.7	16.7	64.9	73.7	16.8	16.8	16.8	21.2	65.2
3-*O*-Glc									
1				105.9				105.9	
2				75.8				75.9	
3				78.6				78.7	
4				71.8				71.8	
5				78.6				78.4	
6				62.9				62.9	
28-*O*-Glc(inner) or 30-*O*-Glc									
1	95.6	95.8	95.9	105.0	95.9	95.9	95.9		
2	73.8	74.0	74.0	75.2	74.1	74.1	74.1		
3	78.9	79.0	79.0	78.6	79.0	79.0	79.0		
4	71.7	71.3	71.3	71.7	71.3	71.3	71.3		
5	77.1	79.3	79.3	78.2	79.3	79.3	79.3		
6	68.1	62.4	62.4	63.0	62.2	62.4	62.4		
28-*O*-Glc(terminal)									
1	100.5								
2	74.1								
3	75.5								
4	72.0								
5	74.0								
6	62.6								

a) 100 MHz, *b*) 125 MHz. *c*, *d*) Assignments may be interchanged in each column.

at δ 4.48 and 4.80 further showed cross peaks with the methine carbon at δ 50.9 (C-5) and with carbon bearing an oxygen atom at δ 74.6 (C-3) (Fig. 2).

The stereochemistry of C-4 of **2** was determined by a nuclear Overhauser enhancement spectroscopy (NOESY) experiment (Fig. 2). The cross peaks among C_{23}-H–C_3-H, –C_5-H, –C_6-αH and –C_{24}-H_2, and among C_{24}-H_2–C_2-βH and –C_{25}-H_3 in the NOESY experiment, indicated the aldehyde group to be α and the hydroxymethyl group to be β. Hence, the aglycone of **2** was formulated as 3β,19α,24β-trihydroxyurs-12-en-23-oxo-28-oic acid (23-oxorotungenic acid); this compound has not been reported before. Accordingly, **2** was formulated as 3β,19α,24-trihydroxyurs-12-en-23-oxo-28-oic acid (23-oxorotungenic acid) 28-*O*-β-D-glucopyranoside.

Ilexoside XLIII (**3**), $C_{36}H_{58}O_{11}$ was obtained as a white powder. The FAB-MS of **3** revealed a quasi-molecular ion peak at *m/z* 665 $[M-H]^-$, 16 mass units more than that of **6**. On acid hydrolysis, **3** afforded D-glucose. A ^{13}C-NMR spectral comparison of **3** with **6** showed that **3** differs structurally from **6** only its E ring, though the sugar unit is also affixed to the C-28 position. In comparing the ^{13}C-NMR spectrum of **3** and **6**, hydroxylation shifts were observed at C-19 (+1.3 ppm), C-20 (+6.0 ppm), C-21 (−4.4 ppm) and C-30 (+48.1 ppm). Therefore, the C-30 of **3** must be hydroxylated. The stereochemistry of C-20 of **3** was elucidated by a NOESY experiment. The cross peaks among C_{18}-H–C_{12}-H, –C_{20}-H, –C_{22}-βH, and –C_{29}-H_3 in the NOESY experiment indicated the hydroxymethyl group to be α. Hence, the aglycone of **3** was formulated as 3β,19α,23,30-tetrahydroxyurs-12-en-28-oic acid (30-hydroxyrotundic acid); this compound has not been reported before. Accordingly, **3** was formulated as 3β,19α,23,30-tetrahydroxyurs-12-en-28-oic acid (30-hydroxyrotundic acid) 28-*O*-β-D-glucopyranoside.

Ilexoside XLIV (**4**) was obtained as colorless needles and its M_r was considered to be 812, as the deprotonated molecular ion was apparent at *m/z* 811 in its FAB-MS, and the elemental formula of this compound was confirmed as by elemental analysis. On acid hydrolysis, **4** afforded D-glucose. The ^{1}H-NMR spectrum of **4** showed the presence of four tertiary methyl groups (δ 0.89, 0.96, 1.01, and 1.13), one secondary methyl group (δ 0.98, d, J=6.4 Hz), a vinyl proton (δ 5.44, br t), and two β-glucosyl units [H-1: δ 4.87 (d, J=7.5 Hz); H-1: δ 5.13 (d, J=7.8 Hz)]. A ^{13}C-NMR spectral cmparison of **4** with ilexoside XXV (**9**),[9] obtained from *I. integra*, showed that **4** is also a glycoside of a 23-hydroxyursolic acid analogue that differs structurally from **9** only in its E ring. Cellulase treatment of **4** afforded the aglycone (**10**), $C_{30}H_{48}O_5$. The ^{13}C-NMR spectrum of **10** showed 30 carbon signals, *i.e.* CH_3- ×5, –CH_2- ×9, CH- ×5, C ×5, –CH-O ×1, –CH_2-O ×2, C=CH ×1, CO ×1. The EI-MS of **10** showed ion peaks at *m/z* 264, 246 (264−H_2O), 224, 206 (224−H_2O), 201 (264−H_2O−COOH) and 175 (264−H_2O−CH_2OH), which indicate that the aglycone is an amyrin derivative having two hydroxyls in the A/B rings and one esteric carboxyl and one hydroxyl in the D/E rings. The ^{1}H–^{1}H-correlation spectroscopy (COSY) and homonuclear Hartman–Hahn (HOHAHA) experiments on **4** revealed an isolated spin system [H-18–19(29)–20(30)–21–22], indicating that a hydroxy group is present at C-30. The cross peak between C_{18}-H (δ 2.62, d, J= 11.4 Hz) and C_{20}-H (δ 1.37, m) in the NOESY experiment indicated the absolute configuration at C-20 to be 20*R*. Therefore, **4** was represented as 3β,23,30-trihydroxyurs-12-en-28-oic acid, and we named it ilexosapogenin B. The sugar sequence of **4** was determined by HMBC and NOESY experiments. The HMBC spectrum of **4** showed long-range correlations between C_1-H (δ 5.13) of the glucose and C-3 (δ 82.4) of the aglycone, and between C_1-H (δ 4.87) of glucose and C-30 (δ 64.7). Moreover, NOESY correlations were found between C_1-H (δ 5.13) of glucose and C_3-H (δ 4.26) of the aglycone, and between C_1-H (δ 4.87) of glucose and C_{30}-H_2 (δ 3.86, 4.00), indicating that β-glucopyranosyl units are located at C-3-OH and C-30-OH. Therefore, **4** was formulated as 3-*O*-β-D-glucopyranosyl ilexosapogenin B 30-*O*-β-D-glucopyranoside.

Ilexoside XLV (**5**), $C_{36}H_{56}O_{12}$ was obtained as colorless needles. The FAB-MS of **5** revealed a quasi-molecular ion peak at *m/z* 679 $[M-H]^-$, 16 mass units more than that of **2**. On acid hydrolysis, **5** afforded D-glucose. The fragment ions at *m/z* 254, 236 (254−H_2O) and 191 (254−H_2O−COOH) in the EI-MS of **5** suggested the presence of one carboxyl group in the A/B rings. A ^{13}C-NMR spectral comparison of **5** with **2** showed that **5** differs structurally from **2** only in its A ring, though the sugar unit is also affixed to the C-28 position. In the ^{13}C-NMR spectrum of **5**, the C-4 (δ 58.0) signal was shifted downfield by 14.8 ppm, while those of C-3 (δ 77.1) and C-5 (δ 52.7) were shifted upfield by 3.3 and 3.9 ppm, respectively, compared with those of **8**, indicating the carboxyl group to be at the C-4 position. The stereochemistry of C-4 of **5** was determined by the NOESY experiment. The cross peaks between C_{24}-H_2 (δ 4.54 and 4.83) and C_{25}-H_3 (δ 1.09) in the NOESY experiment indicated the hydroxymethyl group to be β and the carboxyl group to be α. Hence, the aglycone of **5** was formulated as 3β,19α,24β-trihydroxyurs-12-en-23,28-dioic acid (24-oxorotundioic acid); this compound has not been reported before. Accordingly, **5** was formulated as 3β,19α,24-trihydroxyurs-12-en-23,28-dioic acid (24-oxorotundioic acid) 28-*O*-β-D-glucopyranoside.

Experimental

Melting points were measured with a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were taken on a JASCO DIP-140 digital polarimeter. ^{1}H- (400 or 600 MHz) and ^{13}C- (100 or 125 MHz) NMR spectra were recorded on a JEOL GX-400 or Varian UNITY600 spectrometer in pyridine-d_5 solution using tetramethylsilane as an internal standard. Chemical shifts are given in δ (ppm) and coupling constants (J values) are given in hertz (Hz). The following abbreviations are used: s=singlet, d=doublet, t=triplet, m=multiplet and br=broad. The EI-MS and FAB-MS were measured with a JEOL JMS-PX303 mass spectrometer. High-performance liquid chromatography (HPLC) was carried out with a Waters ALC/GPC 244 instrument. For column chromatography, Silica gel 60 (230—400 mesh, Merck) was used. TLC, precoated Silica gel 60F-254 (Merck) was used.

Extraction and Isolation of Compounds 1—5 Fresh leaves (4 kg) of *Ilex rotunda* were extracted with 70% EtOH and the EtOH extract, obtained after removal of the solvent under reduced pressure, was applied to an Amberlite XAD-2 column and eluted with MeOH. A part (45 g) of the crude saponins (165 g) obtained by evaporation of the MeOH eluate was repeatedly chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (25:2:0.1—25:8:0.5) and $CHCl_3$–MeOH–

EtOAc–H_2O (2:2:4:1), and purified by HPLC (ODS, 30—35% CH_3CN) to give **1** (0.025 g), **2** (0.055 g), **3** (0.04 g), **4** (0.08 g) and **5** (0.025 g).

Ilexoside XLI (1) Colorless needles from MeOH, mp 198—200 °C, $[\alpha]_D^{22}$ +47.0° (c=0.6, MeOH). FAB-MS m/z: 811 $[(M-H)^-]$, 649 $[(M-H-Glc)^-]$, 487 $[(M-H-2Glc)^-]$. *Anal.* Calcd for $C_{42}H_{68}O_{15}\cdot H_2O$: C, 60.71; H, 8.49. Found: C, 60.60; H, 8.31. ^{1}H-NMR (400 MHz) δ: 1.07, 1.08, 1.24, 1.43, 1.65 (3H each, s, *tert*-$CH_3\times5$), 1.04 (3H, d, J=6.5 Hz), 2.48 (1H, dt, J=14.0, 3.0 Hz, H-15β), 2.96 (1H, s, H-18), 3.02 (1H, dt, J=14.0, 3.0 Hz, H-16α), 3.70, 4.17 (each 1H, d, J=10.5 Hz, H_2-23), 5.44 (1H, d, J=3.5 Hz, H-1 of Glc), 5.57 (1H, br t, H-12), 6.24 (1H, d, J=6.5 Hz, H-1 of Glc). ^{13}C-NMR: Table I.

Ilexoside XLII (2) A white powder, $[\alpha]_D^{22}$ +30.8° (c=2.4, MeOH). FAB-MS m/z: 663 $[(M-H)^-]$, 501 $[(M-H-Glc)^-]$. *Anal.* Calcd for $C_{36}H_{56}O_{11}\cdot 2H_2O$: C, 61.69; H, 8.63. Found: C, 61.50; H, 8.70. ^{1}H-NMR (600 MHz) δ: 1.02, 1.18, 1.40, 1.62 (3H each, s, *tert*-$CH_3\times4$), 1.07 (3H, d, J=6.6 Hz), 2.42 (1H, dt, J=14.0, 3.0 Hz, H-15β), 2.92 (1H, br s, H-18), 3.07 (1H, dt, J=14.0, 3.0 Hz, H-16α), 4.06 (1H, m, H-5 of Glc), 4.22 (1H, dd, J=8.0, 8.8 Hz, H-2 of Glc), 4.31 (1H, t, J=8.8 Hz, H-3 of Glc), 4.37 (1H, dd, J=9.0, 8.8 Hz, H-4 of Glc), 4.39 (1H, dd, J=11.0, 4.5 Hz, H-3 of aglycone), 4.41 (1H, dd, J=12.0, 2.7 Hz, H-6 of Glc), 4.48, 4.80 (1H, d, J=11.7 Hz, H_2-24 of aglycone), 5.54 (1H, dd, J=4.0, 3.4 Hz, H-12), 6.28 (1H, d, J=8.0 Hz, H-1 of Glc), 10.30 (1H, s, H-23). ^{13}C-NMR: Table I.

Ilexoside XLIII (3) A white powder, $[\alpha]_D^{22}$ +9.5° (c=2.1, MeOH). FAB-MS m/z: 665 $[(M-H)^-]$, 503 $[(M-H-Glc)^-]$. *Anal.* Calcd for $C_{36}H_{58}O_{11}\cdot H_2O$: C, 63.32; H, 8.56. Found: C, 63.20; H, 8.50. ^{1}H-NMR (600 MHz) δ: 1.04, 1.08, 1.24, 1.59, 1.65 (3H each, s, *tert*-$CH_3\times5$), 2.48 (1H, dt, J=13.5, 3.5 Hz, H-15β), 2.96 (1H, br s, H-18), 3.16 (1H, dt, J=14.0, 3.0 Hz, H-16α), 3.71 and 4.17 (1H, each, d, J=10.5 Hz), 3.99 (1H, dd, J=11.0, 2.5 Hz, H-30 of aglycone), 4.06 (1H, m, H-5 of Glc), 4.19 (1H, dd, J=11.0, 4.5 Hz, H-3 of aglycone), 4.24 (1H, dd, J=8.8, 8.8 Hz, H-2 of Glc), 4.28 (1H, dd, J=11.0, 5.0 Hz, H-30 of aglycone), 4.32 (1H, dd, J=9.0, 8.8 Hz, H-3 of Glc), 4.38 (1H, dd, J=9.3, 9.0 Hz, H-4 of Glc), 4.42 (1H, dd, J=12.0, 4.0 Hz, H-6 of Glc), 4.48 (1H, dd, J=12.0, 2.5 Hz, H-6 of Glc), 5.59 (1H, dd, J=4.0, 3.5 Hz, H-12), 6.33 (1H, d, J=8.3 Hz, H-1 of Glc). ^{13}C-NMR: Table I.

Ilexoside XLIV (4) Colorless needles from MeOH, mp 225—227 °C, $[\alpha]_D^{22}$ +5.8° (c=4.3, MeOH). FAB-MS m/z: 811 $[(M-H)^-]$, 649 $[(M-H-Glc)^-]$, 487 $[(M-H-2Glc)^-]$. *Anal.* Calcd for $C_{42}H_{68}O_{15}\cdot H_2O$: C, 60.71; H, 8.49. Found: C, 60.55; H, 8.33. ^{1}H-NMR (600 MHz) δ: 0.89, 0.96, 1.01, 1.13 (3H each, s, *tert*-$CH_3\times4$), 0.98 (3H, d, J=6.5 Hz), 2.62 (1H, d, J=11.4 Hz, H-18), 3.70 and 4.33 (1H each, d, J=11.0 Hz, H_2-23), 3.86 (1H, dd, J=9.4, 3.5 Hz, H-30), 3.90 (1H, m, H-5 of 3-Glc), 4.00 (1H, dd, J=9.4, 5.0 Hz, H-30), 4.05 (1H, m, H-5 of 30-Glc), 4.04 (1H, dd, J=8.8, 8.5 Hz, H-2 of 3-Glc), 4.06 (1H, dd, J=8.8, 7.5 Hz, H-2 of 30-Glc), 4.18 (1H, dd, J=8.8, 8.8 Hz, H-3 of Glc), 4.23 (1H, dd, J=9.3, 8.8 Hz, H-4 of 3-Glc), 4.25 (1H, dd, J=8.8, 9.2 Hz, H-4 of 30-Glc), 4.26 (1H, dd, J=11.0, 4.5 Hz, H-3 of aglycone), 4.29 (1H, dd, J=8.8, 8.8 Hz, H-3 of 30-Glc), 4.38 (1H, dd, J=11.7, 5.2 Hz, H-6 of 3-Glc), 4.42 (1H, dd, J=12.0, 5.5 Hz, H-6 of 30-Glc), 4.52 (1H, dd, J=11.7, 2.2 Hz, H-6 of 3-Glc), 4.60 (1H, dd, J=12.0, 2.2 Hz, H-6 of 30-Glc), 4.87 (1H, d, J=7.5 Hz, H-1 of 30-Glc), 5.13 (1H, d, J=7.8 Hz, H-1 of 3-Glc), 5.44 (1H, br t, H-12). ^{13}C-NMR: Table I.

Ilexoside XLV (5) Colorless needles from MeOH, mp 267—269 °C, $[\alpha]_D^{22}$ +24.7° (c=1.2, MeOH). FAB-MS m/z: 679 $[(M-H)^-]$, 517 $[(M-H-Glc)^-]$. *Anal.* Calcd for $C_{36}H_{56}O_{12}\cdot 2H_2O$: C, 60.66; H, 7.92. Found: C, 60.40; H, 8.00. ^{1}H-NMR (400 MHz) δ: 1.08 (3H, d, J=6.5 Hz), 1.09, 1.19, 1.43, 1.63 (3H each, s, *tert*-$CH_3\times4$), 2.42 (1H, dt, J=14.0, 3.0 Hz, H-15β), 2.93 (1H, s, H-18), 3.06 (1H, dt, J=14.0, 3.0 Hz, H-16α), 4.08 (1H, m, H-5 of Glc), 4.23 (1H, dd, J=8.0, 8.0 Hz, H-2 of Glc), 4.3 (2H, m, H-3 and H-4 of Glc), 4.40 (1H, dd, J=12.0, 4.0 Hz, H-6 of Glc), 4.50 (1H, dd, J=12.0, 2.5 Hz, H-6 of Glc), 4.54, 4.83 (each 1H, d, J=10.5 Hz, H_2-23), 4.74 (1H, dd, J=11.0, 4.5 Hz, H-3 of aglycone), 5.57 (1H, br t, H-12), 6.26 (1H, d, J=8.0 Hz, H-1 of Glc). ^{13}C-NMR: Table I.

Enzymatic Hydrolysis of 4 Compound **4** (15 mg) was taken up in a mixture of EtOH–H_2O (1:9) and 0.01 M NaH_2PO_4 buffer (pH 4.0), then 3 ml each, incubated with crude cellulase (40 mg, Sigma) for two weeks at 37 °C and worked-up as usual. The crude genin was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (25:4:0.1) giving ilexosapogenin B (**10**, 5 mg), colorless needles mp 252—254 °C, $[\alpha]_D^{22}$ +4.3° (c=0.5, MeOH). FAB-MS m/z: 487 $(M-H)^-$. *Anal.* Calcd for $C_{30}H_{48}O_5$: C, 73.73; H, 9.90. Found: C, 73.60; H, 10.01. IR (KBr): λ_{max} 3450 (br, OH), 1690 (C=O), 1045, 1025 (C–O) cm^{-1}. EI-MS m/z: 488 (M^+), 470, 452, 442, 424, 264, 246, 224, 206, 201, 175. ^{1}H-NMR δ: 0.97, 1.05, 1.09, 1.20 (3H×4, s, 24, 25, 26, 27-CH_3), 1.15 (3H, d, J=6.5 Hz, 29-CH_3), 2.75 (1H, d, J=11.7 Hz, H-18), 3.72, 4.18 (each, 1H, d, J=10.2 Hz, H_2-23), 3.90 (1H, dd, J=10.7, 6.0 Hz, H-30), 3.98 (1H, dd, J=10.7, 3.0 Hz, H-30), 4.20 (1H, dd, J=12.0, 5.0 Hz, H-3), 5.53 (1H, dd, J=3.7, 3.6 Hz, H-12). ^{13}C-NMR: Table I.

Identification of Component Sugars of 1—5 A solution of each compound (2—3 mg) in 5% H_2SO_4 in 50% EtOH was heated at 100 °C for 3 h. The reaction mixture was diluted with water, neutralized with Amberlite IR-45 and concentrated *in vacuo* to dryness. The form (D or L) of each sugar was determined by using HPLC (Shodex RSpak DC-613, 75% CH_3CN, 1 ml/min, 70 °C) with refractive index (RI) detection (Waters 410) and chiral detection (Shodex OR-1), in comparison with authentic sugars (10 mmol of each D-Glc and L-Glc). These sugars gave the following peaks: D-(+)-Glc; 7.38 min; L-(−)-Glc; 7.36 min.

References and Notes

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