Triterpenoid Saponins of Aquifoliaceous Plants. XII.[1] Ilexosides XLVI—LI from the Leaves of *Ilex rotunda* THUNB.[2]

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Six new saponins, ilexosides XLVI—LI, were isolated from fresh leaves of *Ilex rotunda*, and their structures were elucidated on the basis of chemical and physicochemical evidence. Ilexosides XLVI, XLVII, L and LI are 3,28-bisglycosides of ilexosapogenin A, spathodic acid, bredemolic acid, and siaresinolic acid, respectively. Ilexosides XLVIII and XLIX are 3,28-bisglycosides of hederagenin.

Keywords *Ilex rotunda*; ilexoside; Aquifoliaceae; triterpenoid saponin; ilexosapogenin A; bredemolic acid

In a previous paper,[1] we have reported the isolation and structure determination of five saponins, ilexosides XLI—XLV, from fresh leaves of *Ilex rotunda* THUNB. In the present paper, we report the isolation and structure determination of six new saponins from the leaves of the title plant.

The 70% EtOH extract of fresh 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/or reversed-phase (ODS) column chromatography furnished six new saponins, ilexosides XLVI (**1**, 0.08 g), XLVII (**2**, 0.03 g), XLVIII (**3**, 0.2 g), XLIX (**4**, 0.05 g), L (**5**, 0.03 g) and LI (**6**, 0.06 g).

Proton spin decoupling, ^1H–^1H correlation spectroscopy (^1H–^1H COSY), ^1H–^{13}C COSY, and ^1H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) experiments led to determination of the complete structures of **1**—**6**, inclusive of the sequence of the sugar moieties and the position of attachment of the sugar chains to the aglycone.

Ilexoside XLVI (**1**), $[\alpha]_D$ −0.6° (MeOH) was obtained as a white powder and deduced to have the molecular formula $C_{42}H_{66}O_{16}$ as a result of the observation of a deprotonated molecular ion peak at *m/z* 825 in the fast-atom bombardment mass spectrum (FAB-MS) and carbon numbers in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum. The electron impact mass spectrum (EI-MS) of **1** showed characteristic ion peaks due to retro Diels–Alder fission in the C ring: *m/z* 224, 206 (224 − H_2O), and 175 (224 − 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. Those results 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.[3,4] Acid hydrolysis of **1** afforded D-glucose and D-glucuronolactone as sugar components, and ilexosapogenin A (**1a**),[5] an aglycone of ilexoside XXXII (**7**).[5]

The proton magnetic resonance (^1H-NMR) spectrum of **1** showed the presence of two β-linked sugar units [H-1: δ 5.29 (d, *J* = 8.0 Hz), H-1: δ 6.36 (d, *J* = 8.0 Hz)]. Comparison of the ^{13}C-NMR spectrum of **1** with that of **7** showed that the chemical shift of C-28 in **1** was shifted by −3.6 ppm, indicating that the β-glucopyranosyl group is present at C-28-OH. The carbon signals due to sugar moieties are superimposable on those of ilexoside XXXI (**8**).[5] Hence, **1** was formulated as 3-*O*-β-D-glucuronopyranosyl, 28-*O*-β-D-glucopyranosyl ilexosapogenin A.

Ilexoside XLVII (**2**), $[\alpha]_D$ −11.3° (MeOH) was obtained as colorless needles and deduced to have the same molecular formula ($C_{42}H_{66}O_{16}$ [FAB-MS: 825 $(M-H)^-$] as **1**. The EI-MS of **2** 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. Compound **2** afforded D-glucose and D-glucuronolactone on acid hydrolysis. The ^1H-NMR spectrum of **2** showed the presence of the six tertiary methyl groups, one olefinic proton, and two β-linked sugar units [H-1: δ 5.14 (d, *J* = 7.5 Hz), H-1: δ 6.37 (d, *J* = 8.0 Hz)]. A ^{13}C-NMR spectral comparison of **2** with **1** showed that **2** differs structurally from **1** only in the configuration of its C-4 hydroxymethyl group. In the nuclear Overhauser enhancement spectroscopy (NOESY) experiment on **2**, NOEs were observed among the H-25 proton signal at δ 0.80 and both the proton signals of the hydroxymethyl group at δ 3.64 and 4.38, disclosing the hydroxymethyl group at C-4 to be β (axial). Hence, the aglycone of **2** was formulated as 3β,19α,24-trihydroxyolean-12-en-28-oic acid, namely spathodic acid (**8**).[6]

In the HMBC experiment on **2**, the ester carbon signal at δ 177.2 (C-28) gave a cross peak with the anomeric proton signal (glucose) at δ 6.37, indicating that the glucopyranosyl unit is linked at C-28-OH. Further, an NOE was observed between the anomeric proton signal (glucuronic acid) at δ 5.14 and the signal of methine carbon bearing an oxygen atom at δ 3.60, establishing the existence of the glucuronosyl group at position 3 in the aglycone. Hence, **2** was formulated as 3-*O*-β-D-glucuronopyranosyl, 28-*O*-β-D-glucopyranosyl spathodic acid.

Ilexoside XLVIII (**3**), $[\alpha]_D$ +19.3° (MeOH), was obtained as colorless needles and deduced to have the molecular formula $C_{42}H_{66}O_{15}$ as a result of the observation of a deprotonated molecular ion peak at *m/z* 809 in the FAB-MS and carbon numbers in the ^{13}C-NMR spectrum. On acid hydrolysis, **3** afforded D-glucose and D-glucuronolactone as component sugars, and hederagenin (**3a**)[7] as the aglycone. The ^1H- and ^{13}C-NMR spectra indicated the presence of one β-glucosyl unit [H-1: δ 6.30 (d, *J* = 8.0 Hz), C-1: δ 95.8] and one β-glucuronosyl unit [H-1: δ 5.21 (d, *J* = 7.5 Hz), C-1: δ 106.3]. A ^{13}C-NMR spectral comparison of **3** with

3a showed that those sugar units are also affixed to the C-3 and C-28 positions. The carbon signals due to the sugar moieties of **3** were superimposable on those of **2**, indicating that the sugar moieties are the same. Therefore, **3** was formulated as 3-*O*-*β*-D-glucuronopyranosyl, 28-*O*-*β*-D-glucopyranosyl hederagenin.

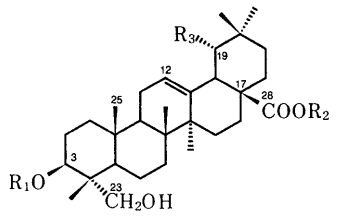
Ilexoside XLIX (**4**), $[\alpha]_D$ +18.9° (MeOH), $C_{48}H_{76}O_{20}$, was obtained as colorless needles. The negative FAB-MS of **4** revealed a quasi-molecular ion peak at *m/z* 971 $[M-H]^-$, *i.e.*, 162 mass units more than that of **3**. Acid hydrolysis of **4** afforded **3a**, and D-galactose, D-glucose and D-glucuronolactone as component sugars. The ^{1}H- and ^{13}C-NMR spectra indicated the presence of one *β*-galactosyl unit [H-1: *δ* 5.19 (d, *J*=6.5 Hz), C-1: *δ* 106.7], one *β*-glucosyl unit [H-1: *δ* 6.28 (d, *J*=8.0 Hz), C-1: *δ* 95.8], and one *β*-glucuronosyl unit [H-1: *δ* 5.29 (d, *J*=7.5 Hz), C-1: *δ* 104.4]. Comparison of the ^{13}C-NMR spectrum of **4** with that of **3** showed that the chemical shift C-2 of the glucuronosyl unit was shifted by +7.9 ppm, indicating the *β*-galactopyranosyl group is linked to C-2-OH of the glucuronosyl unit. Hence, **4** was formulated as 3-*O*-*β*-D-galactopyranosyl(1→2)-*β*-D-glucuronopyranosyl, 28-*O*-*β*-D-glucopyranosyl hederagenin.

Ilexoside XL (**5**), $[\alpha]_D$ +5.3° (MeOH) was obtained as

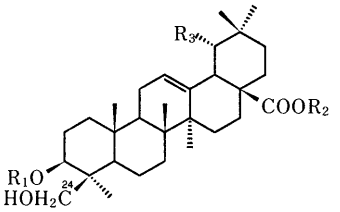
TABLE I. ^{13}C-NMR Spectral Data for Compounds **1**, **1a**, **2**—**6**, **6a**, and **7** (Pyridine-d_5, *δ*-Values)

Carbon	1	1a	2	3	4	5	6	6a	7
1	38.5	38.6	38.9	38.8	38.8	38.5	38.7	38.8	38.5
2	26.1	27.7	26.8	26.1	26.1	26.8	26.8	28.1	26.1
3	82.1	73.3	89.1	81.8	82.4	89.0	89.6	78.2	82.1
4	43.6	42.9	44.4	43.6	43.6	44.4	39.8	39.3	43.6
5	48.4	48.6	56.2	47.6	48.2	56.1	56.1	56.0	48.4
6	18.4	18.7	19.1	18.3	18.3	18.9	18.9	18.9	18.4
7	32.9	33.0	33.6	32.7	32.7	32.6	33.3	33.2	33.0
8	40.3	40.0	40.2	40.1	40.4	39.9	40.4	40.0	40.1
9	47.7	48.4	48.1	48.2	47.7	47.9	48.4	47.3	47.7
10	37.1	37.4	36.8	37.0	36.9	36.7	37.2	37.5	37.1
11	24.2	24.2	24.3	23.5	23.6	23.4	24.3	24.1	24.2
12	123.4	123.4	124.3	123.0	122.6	122.9	123.4	123.3	123.4
13	144.4	144.9	144.7	144.3	144.3	144.1	144.5	144.3	144.9
14	42.1	42.1	42.1	42.3	42.3	42.2	42.3	42.1	42.2
15	28.0	28.4	28.9	28.4	28.4	28.3	29.1[a]	29.0	28.4
16	29.1	29.2	29.1	24.0	24.0	24.0	28.1	28.1	29.2
17	46.5	46.1	46.5	47.1	47.2	47.0	46.7	46.4	46.1
18	44.6	44.8	44.6	41.8	41.9	41.8	44.8	44.6	44.8
19	81.0	81.2	81.0	46.3	46.3	46.2	81.2	81.0	81.2
20	35.6	35.7	35.6	30.9	30.8	30.8	35.7	35.6	35.8
21	29.0	29.2	29.2	34.1	34.1	34.0	29.3[a]	29.0	29.2
22	33.1	33.6	33.1	32.9	32.9	33.4	33.4	33.2	33.7
23	64.5	67.8	23.4	64.4	64.3	23.4	28.4	28.2	64.5
24	13.6	13.0	63.2	13.8	13.7	63.3	16.8	16.5	13.6
25	16.1	15.8	15.2	16.3	16.2	15.4	15.7	15.5	16.0
26	17.7	17.5	17.4	17.7	17.7	17.4	17.8	17.4	17.6
27	24.7	24.8	24.7	26.3	26.3	26.1	24.9	24.7	24.9
28	177.3	181.0	177.2	176.7	176.8	176.5	177.6	178.7	180.9
29	28.8	28.9	28.8	33.3	33.2	33.2	29.0	28.8	28.9
30	25.0	24.8	24.9	23.8	23.8	23.7	25.1	24.9	24.9
$COOCH_3$								51.7	
3-*O*-GlcA									
1′	106.3		106.5	106.3	104.4	106.5	105.5		106.3
2′	75.5		75.4	75.5	83.4	75.4	83.8		75.1
3′	78.0[a]		78.1[a]	78.1	77.4[a]	78.0[a]	77.2[b]		78.0[a]
4′	73.5		73.5	73.5	73.0	73.5	73.3		73.5
5′	78.1[a]		78.2[a]	78.1	77.8	78.1[a]	77.8		78.1[a]
6′	172.9		172.7	172.8	172.6	172.9	173.3		172.8
2′-*O*-Gal									
1″					106.7		107.1		
2″					74.4		74.6		
3″					75.0		75.0		
4″					69.6		69.5		
5″					77.2[a]		77.1[b]		
6″					61.5		61.4		
28-*O*-Glc									
1‴	95.9		95.9	95.8	95.8	95.8	96.0		
2‴	74.1		74.2	74.1	74.1	74.2	74.1		
3‴	79.0		79.0	78.8	78.8	78.9	78.8		
4‴	71.1		71.0	71.1	71.1	71.1	71.1		
5‴	79.3		79.4	79.4	79.4	79.4	79.4		
6‴	62.2		62.2	62.2	62.2	62.2	62.2		

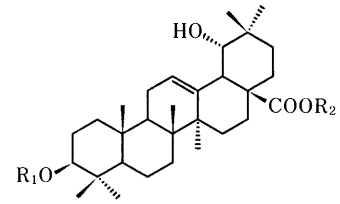
a, b) Assignments may be interchanged in each column.



	R_1	R_2	R_3
1	-GlcA	-Glc	-OH
1a	-H	-H	-OH
3	-GlcA	-Glc	-H
3a	-H	-H	-H
4	-GlcA^{2}Gal	-Glc	-H
7	-GlcA	-H	-OH



	R_1	R_2	R_3
2	-GlcA	-Glc	-OH
5	-GlcA	-Glc	-H
8	-H	-H	-OH
9	-H	-H	-H



	R_1	R_2
6	-GlcA^{2}Gal	-Glc
6a	-H	-CH_3

GlcA : β-D-glucuronopyranosyl
Glc : β-D-glucopyranosyl
Gal : β-D-galactopyranosyl

Fig. 1

colorless needles and deduced to have the same molecular formula $C_{42}H_{66}O_{15}$ as **3**, as a result of the observation of a deprotonated molecular ion at *m/z* 809 in the FAB-MS and carbon numbers in the ^{13}C-NMR spectrum. The EI-MS of **5** showed ion peaks at *m/z* 248, 224, 206 (224 − H_2O), 203 (248 − COOH) and 175 (264 − H_2O − CH_2OH), indicating that the aglycone is an amyrin derivative having two hydroxyls in the A/B rings and one esteric carboxyl in the D/E rings. Acid hydrolysis of **5** afforded D-glucose and D-glucuronolactone as component sugars. The ^{1}H- and ^{13}C-NMR spectra indicated the presence of one β-glucosyl unit [H-1: δ 6.30 (d, *J*=7.0 Hz), C-1: δ 95.8] and one β-glucuronosyl unit [H-1: δ 5.13 (d, *J*=7.5 Hz), C-1: δ 106.5].

A ^{13}C-NMR spectral comparison of **5** with **3** showed that **5** was differs structurally from **3** only in the configuration of its C-4 hydroxymethyl group. In the NOESY experiment on **5**, NOEs were observed between the H-25 proton signal at δ 0.76 and both the proton signals of the hydroxymethyl group at δ 3.62 and 4.35, disclosing the hydroxymethyl group at C-4 to be β (axial). Hence, the aglycone of **5** was formulated as 3β,24-dihydroxyolean-12-en-28-oic acid, namely bredemolic acid (**9**).[8,9] Therefore, **5** was formulated as 3-*O*-β-D-glucuronopyranosyl, 28-*O*-β-D-glucopyranosyl bredemolic acid.

Ilexocide LI (**6**), obtained as colorless needles, had the same molecular formula $C_{48}H_{76}O_{20}$ [FAB-MS: 971 $(M-H)^-$] as **4**. The EI-MS of **6** showed characteristic peaks at *m/z* 208, 190 (208 − H_2O), 175 (208 − H_2O − CH_3), and 264, 246 (264 − H_2O), 201 (264 − H_2O − COOH) due to retro Diels–Alder fission, which suggested the occurrence of one hydroxyl group in the A/B rings, and one hydroxyl group and one carboxyl group in the D/E rings on the amyrin skeleton. Compound **6** afforded D-glucose and D-galactose and D-glucuronolactone on acid hydrolysis. The ^{1}H- and ^{13}C-NMR spectrum of **6** showed the presence of one β-galactosyl unit [H-1: δ 5.02 (d, *J*=7.5 Hz), C-1: δ 107.1], one β-glucosyl unit [H-1: δ 6.36 (d, *J*=7.0 Hz), C-1: δ 96.0], and one β-glucuronosyl unit [H-1: δ 5.24 (d, *J*=8.0 Hz), C-1: δ 105.5].

Compound **6** provided methyl siaresinolate (**6a**)[10] on methanolysis. A ^{13}C-NMR spectral comparison of **6** with **6a** showed that those sugar units are also affixed to the C-3 and C-28 positions. The carbon signals due to the sugar moieties of **6** were superimposable on those of **4**, indicating that the sugar moieties are the same. Hence, **6** was formulated as 3-*O*-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl, 28-*O*-β-D-glucopyranosyl siaresinolic acid.

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 MHz) and ^{13}C- (100 MHz) NMR spectra were recorded on a JEOL GX-400 spectrometer in pyridine-d_5 solution using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ (ppm) and coupling constants (*J* values) are given in 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. For TLC, precoated Silica gel 60F-254 plates (Merck) was used.

Isolation of Compounds 1—6 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 passed through 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 chromatogaphed 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 further purified by HPLC (ODS, 30—35% CH_3CN) to give **1** (0.08 g), **2** (0.03 g), **3** (0.2 g), **4** (0.05 g), **5** (0.03 g) and **6** (0.06 g).

Ilexoside XLVI (1) A white powder, $[\alpha]_D^{22}$ −0.6° (*c*=3.2, MeOH). FAB-MS *m/z*: 825 $[(M-H)^-]$. EI-MS *m/z* (%): 470 (5), 442 (22), 370 (8), 264 (25), 246 (54), 224 (15), 218 (33), 206 (31), 201 (50), 175 (46), 146 (100). *Anal.* Calcd for $C_{42}H_{66}O_{16}\cdot 2H_2O$: C, 58.45; H, 8.18. Found: C, 58.30; H, 8.25. ^{1}H-NMR δ: 0.95, 0.97, 0.98, 1.15, 1.15, 1.60 (3H each, s, *tert*-CH_3 × 6), 3.51 (1H, br s, H-18), 3.55 (1H, d, *J*=2.0 Hz, H-19), 5.29 (1H, d, *J*=8.0 Hz, H-1 of GlcA), 5.49 (1H, br t, H-12), 6.36 (1H, d, *J*=8.0 Hz, H-1 of esteric Glc). ^{13}C-NMR: Table I.

Ilexoside XLVII (2) Colorless needles from MeOH, mp 238—239 °C, $[\alpha]_D^{22}$ −11.3° (*c*=0.7, MeOH). FAB-MS *m/z*: 825 $[(M-H)^-]$. EI-MS *m/z* (%): 470 (4), 442 (20), 370 (8), 264 (28), 246 (50), 224 (12), 218 (35), 206 (35), 201 (45), 175 (41), 146 (100). *Anal.* Calcd for $C_{42}H_{66}O_{16}\cdot 3H_2O$: C, 57.26; H, 8.24. Found: C, 57.10; H, 8.50. ^{1}H-NMR δ: 0.80, 0.98, 1.11, 1.15, 1.53, 1.66 (3H each, s, *tert*-CH_3 × 6), 2.39 (1H, ddd, *J*=11.0, 11.0, 3.0 Hz, H-15β), 2.85 (1H, ddd, *J*=11.0, 11.0, 3.0 Hz, H-16α), 3.52 (1H, br s, H-18), 3.58 (1H, d, *J*=2.5 Hz, H-19), 3.60 (1H, dd, *J*=11.0, 4.5 Hz, H-3), 3.64, 4.38 (each, 1H, d, *J*=11.5 Hz, H_2-24), 5.14 (1H, d, *J*=7.5 Hz, H-1 of GlcA), 5.47 (1H, br t, H-12), 6.37 (1H, d, *J*=8.0 Hz, H-1 of esteric Glc). ^{13}C-NMR: Table I.

Ilexoside XLVIII (3) Colorless needles from MeOH, mp 200—201 °C, $[\alpha]_D^{22}$ +19.3° (*c*=7.2, MeOH). FAB-MS *m/z*: 809 $[(M-H)^-]$. EI-MS *m/z* (%): 454 (4), 410 (25), 248 (30), 224 (10), 206 (33), 203 (40), 175 (48), 146 (100). *Anal.* Calcd for $C_{42}H_{66}O_{15}\cdot H_2O$: C, 60.85; H, 8.29. Found: C, 60.60; H, 8.40. ^{1}H-NMR δ: 0.87, 0.89, 0.92, 0.94, 1.12, 1.22 (3H each, s, *tert*-CH_3 × 6), 5.21 (1H, d, *J*=7.5 Hz, H-1 of GlcA), 5.42 (1H, br t, H-12), 6.30 (1H, d, *J*=8.0 Hz, H-1 of esteric Glc). ^{13}C-NMR: Table I.

Ilexoside XLIX (4) A white powder, $[\alpha]_D^{22}$ +18.9° (*c*=1.1, MeOH). FAB-MS *m/z*: $[(M-H)^-]$. EI-MS *m/z* (%): 454 (2), 410 (20), 248 (35), 224 (15), 206 (28), 203 (50), 175 (40), 146 (100). *Anal.* Calcd for $C_{48}H_{76}O_{20}\cdot 3H_2O$: C, 56.13; H, 8.05. Found: C, 56.00; H, 8.16. ^{1}H-NMR δ: 0.83, 0.85, 0.88, 1.02, 1.08, 1.19 (3H each, s, *tert*-CH_3 × 6), 5.19 (1H, d, *J*=6.5 Hz, H-1 of Gal), 5.29 (1H, d, *J*=7.5 Hz, H-1 of GlcA), 5.39 (1H, br t, H-12), 6.28 (1H, d, *J*=8.0 Hz, H-1 of esteric Glc). ^{13}C-NMR: Table I.

Ilexoside L (5) Colorless needles from MeOH, mp 250—252 °C, $[\alpha]_D^{22}$ +5.3° (*c*=7.9, MeOH). FAB-MS *m/z*: 809 $[(M-H)^-]$. EI-MS *m/z* (%): 454 (3), 410 (18), 248 (36), 224 (16), 206 (28), 203 (42), 175 (52), 146 (100).

Anal. Calcd for $C_{42}H_{66}O_{15}\cdot 2H_2O$: C, 59.56; H, 8.33. Found: C, 59.43; H, 8.55. ^{1}H-NMR δ: 0.76, 0.88, 0.93, 1.06, 1.29, 1.53 (3H each, s, *tert*-$CH_3\times 6$), 3.60 (1H, dd, $J=11.0$, 4.0 Hz, H-3), 3.62, 4.35 (each, 1H, d, $J=11.0$ Hz, H_2-24), 5.13 (1H, d, $J=7.5$ Hz, H-1 of 3-GlcA), 5.42 (1H, br t, H-12), 6.30 (1H, d, $J=7.0$ Hz, H-1 of esteric Glc).

Ilexoside LI (6) Colorless needles from MeOH, mp 207—209 °C, $[\alpha]_D^{22}$ −1.3° ($c=0.8$, MeOH). FAB-MS *m/z*: 971 $[(M-H)^-]$. EI-MS *m/z* (%): 454 (6), 410 (25), 264 (3), 246 (87), 233 (74), 224 (10), 207 (60), 201 (100), 190 (70), 175 (32), 146 (100). *Anal.* Calcd for $C_{48}H_{76}O_{20}\cdot 3H_2O$: C, 56.13; H, 8.05. Found: C, 56.02; H, 8.26. ^{1}H-NMR δ: 0.86, 0.99, 1.13, 1.13, 1.17, 1.31, 1.67 (3H each, s, *tert*-$CH_3\times 7$), 2.36 (1H, ddd, $J=11.0$, 11.0, 3.0 Hz, H-15β), 2.87 (1H, ddd, $J=11.0$, 11.0, 3.0 Hz, H-16α), 3.53 (1H, br s, H-18), 3.59 (1H, d, $J=2.5$ Hz, H-19), 5.02 (1H, d, $J=7.5$ Hz, H-1 of Gal), 5.24 (1H, d, $J=8.0$ Hz, H-1 of GlcA), 5.49 (1H, br t, H-12), 6.36 (1H, d, $J=7.0$ Hz, H-1 of esteric Glc). ^{13}C-NMR: Table I.

Acid Hydrolysis of 1 A solution of **1** (40 mg) in 5% H_2SO_4 in 50% EtOH (3 ml) was heated at 100 °C for 2 h. The solution was passed through Amberlite XAD-2 and eluted with MeOH. The residue obtained from the eluate was passed through Sephadex LH 20 (MeOH) to give ilexosapogenin A (**1a**) (20 mg). Compound **1a**, colorless needles from MeOH, mp 278—280 °C, $[\alpha]_D^{22}$ +46.5° ($c=1.0$, MeOH). FAB-MS *m/z*: 487 $[(M-H)^-]$. *Anal.* Calcd for $C_{30}H_{48}O_5\cdot 2H_2O$: C, 68.67; H, 9.99. Found: C, 68.60; H, 10.01. ^{1}H-NMR δ: 1.10, 1.17, 1.19, 1.22, 1.29, 1.72 (3H each, s, *tert*-$CH_3\times 6$), 3.73 (2H, br s, H-18, H-19), 3.82, 4.29 (each 1H, d, $J=10.5$, H_2-23), 4.30 (1H, dd, $J=8.5$, 7.5 Hz, H-3), 5.67 (1H, br t, H-12). ^{13}C-NMR: Table I.

Acid Hydrolysis of 3 A solution of **3** (30 mg) in 5% H_2SO_4 in 50% EtOH (3 ml) was treated in the same way as described for **1** to give **3a** (15 mg), which was identified as hederagenin by EI-MS, NMR and HPLC comparison with an authentic sample.

Acid Hydrolysis of 4 A solution of **4** (15 mg) in 5% H_2SO_4 in 50% EtOH (3 ml) was treated in the same way as described for **1** to give **4a** (5 mg), which was identified as hederagenin by EI-MS, NMR and HPLC comparison with an authentic sample.

Methanolysis of 6 A solution of **6** (40 mg) in 1 N HCl–MeOH (2 ml) was heated at 70 °C for 2 h. After work-up in a usual manner, the crude aglycone (15 mg) was recrystallized from MeOH to give methyl siaresinolate (**6a**, 10 mg). Compound **6a**, mp 185—186 °C, $[\alpha]_D^{22}$ +44.0° ($c=1.0$, $CHCl_3$). EI-MS *m/z*: 486 $(M)^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.66, 0.77, 0.91, 0.95, 0.96, 1.00, 1.24 (3H each, s, *tert*-$CH_3\times 7$), 3.21 (1H, dd, $J=11.0$, 4.0 Hz, H-3), 3.10 (1H, br s, H-18), 3.34 (1H, d, $J=3.0$ Hz, H-19), 3.61 (3H, s, COOMe), 5.46 (1H, br t, H-12). ^{13}C-NMR: Table I.

Identification of Component Sugars of 1—6 A solution of each compound (3—4 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 refraction index (RI) detection (Waters 410) and chiral detection (Shodex OR-1) in comparison with authentic sugars (10 mmol each D-glucose, D-galactose and D-glucuronolactone). These sugars gave the following peaks: D-(+)-glucuronolactone; 2.40 min, D-(+)-glucose; 7.38 min, D-(+)-galactose; 8.00 min.

References and Notes

1) Part XI: K. Amimoto, K. Yoshikawa, and S. Arihara, *Chem. Pharm. Bull.*, **41**, 39 (1993).
2) Those studies have been orally presented at the 108th Annual Meeting of the Pharmaceutical Society of Japan, Hiroshima, April 1988.
3) J. Karliner and C. Djerassi, *J. Org. Chem.*, **31**, 1945 (1966).
4) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 26, Holden-Day, San Francisco, 1987, p. 225.
5) Part VIII: K. Amimoto, K. Yoshikawa and S. Arihara, *Chem. Pharm. Bull.*, **40**, 3138 (1992).
6) S. Ngoela, B. Nyasse, E. Tsamo, B. L. Sondengam, and J. D. Connolly, *Phytochemistry*, **29**, 3959 (1990).
7) T. Takemoto, S. Arihara, K. Yoshikawa, K. Kusumoto, I. Yano, and T. Hayashi, *Yakugaku Zasshi*, **104**, 246 (1984).
8) R. Tschesche and A. K. S. Gupta, *Chem. Ber.*, **93**, 1903 (1960).
9) S. K. Singh, V. J. Tripathi, and R. H. Singh, *Phytochemistry*, **29**, 3360 (1990).
10) R. T. Aplin, W. H. Hui, C. T. Ho, and C. W. Yee, *J. Chem. Soc.*, (*C*), **1971**, 1067.