Triterpenoid Saponins of Aquifoliaceous Plants. VIII.[1] Ilexosides XXIX—XXXII from the Leaves of *Ilex rotunda* THUNB.[2]

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From the fresh leaves of *Ilex rotunda*, were isolated four new saponins named ilexosides XXIX—XXXII, together with the known saponins pedunculoside, ziyu-glycoside I, suavissimoside F1 and chikusetsusaponin IVa. Their structures were established on the basis of spectral and chemical evidence.

Keywords *Ilex rotunda*; Aquifoliaceae; pentacyclic triterpene; ilexoside; ilexosapogenin A; rotundioic acid; siaresinolic acid; pomolic acid; pomolic acid 3-*O*-sulfate; ursane-type 3-*O*-sulfate

In a continuation of our chemical examination of Aquifoliaceous plants,[1] we have now investigated *Ilex rotunda* THUNB., which is distributed in Eastern Asia (Japan, China, Taiwan and Indonesia). The barks, leaves and roots have been used as a traditional Chinese remedy for scalds and burns, to control bleeding, and as a binding medicine.[3] In our previous studies with *Ilex integra*, we obtained four ursane-glycosides, ilexosides XXV—XXVIII.[1] The present paper reports the isolation and structure determination of four new saponins from *Ilex rotunda* along with the known saponins.

The 70% EtOH extract of the 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 (165 g) by column chromatography on silica gel furnished four new saponins, ilexosides XXIX (**1**, 0.6 g), XXX (**2**, 0.24 g), XXXI (**3**, 0.06 g), and XXXII (**4**, 0.03 g), besides pedunculoside (**5**, 0.8 g),[4] ziyu-glycoside I (**6**, 0.1 g),[5] suavissimoside F1 (**7**, 4.5 g)[6] and chikusetsusaponin IVa (**8**, 0.1 g).[7]

Ilexoside XXIX (**1**), $[\alpha]_D$ +13.7° (MeOH) was obtained as colorless needles with the molecular formula $C_{36}H_{57}NaO_{12}S$ as judged from the quasi-molecular ion at *m/z* 759 $[M+Na]^+$ in the positive fast-atom bombardment mass spectrum (FAB-MS) and the carbon numbers in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum. Compound **1** was treated with Amberlyst 15 to give the free sulfate (**1a**) $[\alpha]_D$ +15.0° (MeOH), $C_{36}H_{58}O_{12}S$ [positive FAB-MS: *m/z* 737 $[M+Na]^+$]. The presence of the sulfate function in **1** was suggested by a positive result in the potassium rhodizonate reagent test,[8] and the distinctive absorption (1230 cm^{-1}) in its infrared (IR) spectrum due to the S–O bond stretching vibration,[9] together with an esterified carboxylic group (1740 cm^{-1}). Alkaline hydrolysis of **1** furnished an aglycone (**1b**), $[\alpha]_D$ +30.8° (MeOH), $C_{30}H_{48}O_7S$ [negative FAB-MS *m/z* 551], which, on solvolysis[10] afforded pomolic acid (**1c**).[11,12] A ^{1}H- and ^{13}C-NMR spectral comparison of **1b** with **1c**, revealed an esterification shift[13] at position 3 in the aglycone [+1.05 ppm (H-3), +7.9 ppm (C-3)]. Therefore, in **1b**, O-3 should be sulfated.

Solvolysis of **1** afforded a desulfated derivative (**1d**). Compound **1d**, $C_{36}H_{58}O_9$, $[\alpha]_D$ +24.1° (MeOH) revealed a quasi-molecular ion peak at *m/z* 633 $[M-H]^-$ in the negative FAB-MS. Hydrolysis of **1d** with crude cellulase gave **1c**, while acid hydrolysis of **1d** liberated D-glucose. Comparison of ^{13}C-NMR spectrum of **1d** with that of **1c** showed a glycosylation shift[14] for the C-28 signal (−3.6 ppm), demonstrating that a β-glucopyranosyl group is located at C-28-OH. Therefore, **1d** was formulated as 28-*O*-β-D-glucopyranosyl pomolic acid. Hence, the structure of **1** was established as the 3-*O*-sulfate of 28-*O*-β-D-glucopyranosyl pomolic acid.

Ilexoside XXX (**2**), $C_{36}H_{56}O_{11}$, was obtained as colorless needles. A deprotonated ion peak at *m/z* 663 $[M-H]^-$ in the negative FAB-MS showed that the relative molecular mass (M_r) was 664, *i.e.*, 14 mass units more than **5**. The electron impact mass spectrum (EI-MS) of **2** showed characteristic peaks at *m/z* 238 and 264 due to retro Diels–Alder fission, which suggested the occurrence of one hydroxy group and one carboxy group in both the A/B and D/E rings on the amyrin skeleton.[15,16] Compound **1** afforded D-glucose on acid hydrolysis and its ^{1}H-NMR spectrum indicated the presence of one β-glucosyl unit [H-1: δ 6.29 (d, J=8.0 Hz)]. Alkaline hydrolysis of **2** afforded an aglycone (**2a**), $[\alpha]_D$ +53.9° (MeOH), $C_{30}H_{46}O_6$ [negative FAB-MS *m/z* 501], which was identified as rotundioic acid,[17] previously isolated from the fruits of this plants, on the basis of physical data. In the ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence (HMBC) experiment on **2**, the ester carbon signal at δ 177.0 (C-28) gave a cross peak with the anomeric proton signal (glucose) at δ 6.29 and with H-18 at δ 2.93, indicating that the glucopyranosyl group is located at position 28 in the aglycone. Accordingly, **2** was formulated as 28-*O*-β-D-glucopyranosyl rotundioic acid.

Ilexoside XXXI (**3**), $[\alpha]_D$ −2.0° (MeOH), was obtained as an amorphous powder and the M_r was proposed to be 810 as the deprotonated molecular ion was apparent at *m/z* 809 in its FAB-MS, and the formula of this compound was confirmed as $C_{42}H_{66}O_{15}$ by elemental analysis. Comparison of the ^{13}C-NMR spectrum of **3** with that of ilexoside XV (**9**) obtained in our previous investigation,[18] showed that **3** is also a glycoside of siaresinolic acid (**10**),[19] and differs structurally from **9** only in its saccharide moieties, though these sugar units are also affixed to the C-3 and C-28 positions. The sugar units obtained after acid hydrolysis of **3** were identified by HPLC as D-glucose and D-gluculonolactone. The ^{1}H-NMR spectrum of **3** indicated the presence of two β-linked sugar units [H-1: δ 5.03 (d, J=7.5 Hz) and H-1: δ 6.35 (d, J=8.0 Hz)]. Compound **3** provided methyl siaresinolate (**3a**) on methanolysis.

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In the HMBC experiment on **3**, the ester carbon signal at δ 177.3 (C-28) gave a cross peak with the anomeric proton signal (glucose) at δ 6.35, and the signal of the methine carbon bearing an oxygen atom at δ 89.2 showed a cross peak with the anomeric proton signal (glucuronic acid) at δ 5.03, establishing the existence of the glucuronyl group at position 3 and the glucosyl group at position 28 in the aglycone. Therefore, **3** was formulated as 3-*O*-β-D-glucuronopyranosyl-28-*O*-β-D-glucopyranosyl siaresinolic acid.

Ilexoside XXXII (**4**), $[\alpha]_D$ +2.2° (MeOH) was obtained as colorless needles and deduced to have the molecular formula $C_{36}H_{56}O_{11}$, from the deprotonated molecular ion at *m/z* 663 in its negative FAB-MS and carbon numbers in the ^{13}C-NMR spectrum. On acid hydrolysis, **4** afforded D-gluculonolactone and a sapogenol (**4a**), colorless needles, mp 278—280 °C, $[\alpha]_D$ +46.1° (MeOH), $C_{30}H_{48}O_5$. The EI-MS of **4a** showed a molecular ion peak at *m/z* 488 and other characteristic peaks at *m/z* 224 and 264 due to retro Diels–Alder fission, which suggested the occurrence of two hydroxy groups in the A/B rings, and one hydroxy group and one carboxy group in the D/E rings on the amyrin skeleton. A ^{13}C-NMR spectral comparison of **4a** with **10** showed that **4a** differes structurally from **10** only in its C-4 substituent: a hydroxymethylene group in **4a** instead of a methyl group in **10**. In the nuclear Overhauser enhancement spectroscopy (NOESY) experiment on **4a**, an NOE was observed between the H-25 signal (δ 1.10) and H-24 signal (δ 1.16), indicating the hydroxymethyl group to be α. Hence, **4a** was formulated as 3β,19α,23-trihydroxyolean-12-en-28-oic acid and named ilexosapogenin A. Comparison of the ^{13}C-NMR spectrum of **4** with that of **4a** showed that the signal of C-3 in **4** was shifted by +8.8 ppm, indicating that the β-glucuronopyranosyl group is linked to the C-3-OH. Hence, **4** was formulated as 3-*O*-β-D-glucuronopyranosyl 3β,19α,23-trihydroxyolean-12-en-28-oic acid (3-*O*-β-D-glucuronopyranosyl ilexosapogenin A).

TABLE I. ^{13}C-NMR Spectral Data for Compounds **1**, **1b—d**, **2**, **2a**, **3**, **4**, **4a** and **9** (Pyridine-d_5, δ-Values)

C	1	1b	1c	1d	2	2a	3	4	4a	9
1	38.8	38.8	38.9	39.2	39.1	39.2	38.5	38.5	38.6	38.7
2	24.9	28.6	28.0	28.2	27.6	27.8	26.7	26.1	27.7	26.7
3	85.6	86.1	78.2	78.3	75.5	75.6	89.2	82.1	73.3	88.8
4	38.9	38.6	39.3	39.5	54.4	54.5	39.6	43.6	42.9	39.7
5	56.3	56.2	55.8	56.0	51.9	52.1	55.9	48.4	48.6	56.0
6	19.0	18.8	18.9	19.1	21.8	21.9	18.7	18.4	18.7	18.8
7	33.6	33.5	33.6	33.7	33.3	33.5	33.1	33.0	33.0	33.1
8	40.6	40.3	40.3	40.7	40.9	40.8	40.2	40.1	40.0	40.3
9	47.7	47.6	47.7	47.9	48.0	48.1	48.2	47.7	48.4	48.4
10	37.2	37.1	37.3	37.5	36.8	36.9	37.1	37.1	37.4	37.2
11	24.1	24.0	24.0	24.1	24.1	24.2	24.1	24.2	24.2	24.2
12	128.5	127.8	128.1	128.5	128.2	128.0	123.4	123.4	123.4	123.4
13	139.3	140.0	139.9	139.3	139.2	140.0	144.4	144.9	144.9	144.4
14	42.2	42.4	42.1	42.2	42.0	42.2	42.1	42.2	42.1	42.2
15	29.3	29.4	29.2	29.3	29.2	29.4	28.9	28.4	28.4	29.0
16	26.2	26.5	26.6	26.2	26.1	26.4	29.1	29.2	29.2	28.0
17	48.8	48.4	48.2	48.9	48.6	48.4	46.5	46.1	46.1	46.5
18	54.5	54.7	54.5	54.5	54.4	54.7	44.6	44.8	44.8	44.7
19	72.8	72.9	72.7	72.7	72.6	72.7	81.0	81.2	81.2	81.1
20	42.2	42.2	42.3	42.2	42.0	42.5	35.6	35.8	35.7	35.6
21	26.8	27.0	27.0	26.8	26.7	27.0	29.2	29.2	29.2	29.1
22	37.8	38.6	37.4	37.8	37.6	38.6	33.2	33.7	33.6	33.2
23	28.8	28.6	28.7	28.9	180.6	180.7	28.2	64.5	67.8	28.2
24	17.1	17.0	16.7	16.8	12.1	12.3	16.9	13.6	13.0	16.9
25	15.7	15.5	15.5	15.8	16.1	16.1	15.5	16.0	15.8	15.6
26	17.5	17.3	17.1	17.6	17.3	17.3	17.6	17.6	17.5	17.6
27	24.8	24.8	24.6	24.7	24.5	24.8	24.7	24.9	24.8	24.7
28	177.2	180.5	180.6	177.0	177.0	180.7	177.3	180.9	181.0	177.3
29	27.2	27.2	26.8	27.1	26.9	27.2	28.8	28.9	28.9	28.8
30	16.8	16.9	16.4	16.6	16.7	16.9	25.0	24.9	24.8	25.0
glcA										
1							107.1	106.3		
2							75.5	75.5		
3							78.1[a]	78.1[a]		
4							73.5	73.5		
5							78.0[a]	78.0[a]		
6							172.8	172.8		
glc										
1	95.9			95.9	95.8		95.9			
2	74.0			74.1	73.8		74.2			
3	79.1[a]			79.3[a]	79.1[a]		79.3[b]			
4	71.4			71.3	71.1		71.1			
5	78.7[a]			79.0[a]	78.6[a]		79.0[b]			
6	62.5			62.4	62.2		62.2			

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

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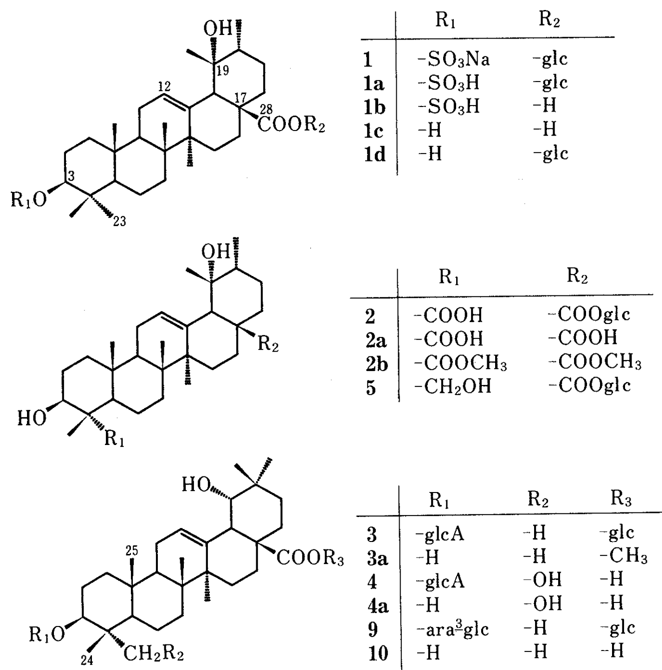

	R_1	R_2
1	$-SO_3Na$	-glc
1a	$-SO_3H$	-glc
1b	$-SO_3H$	-H
1c	-H	-H
1d	-H	-glc

	R_1	R_2
2	-COOH	-COOglc
2a	-COOH	-COOH
2b	$-COOCH_3$	$-COOCH_3$
5	$-CH_2OH$	-COOglc

	R_1	R_2	R_3
3	-glcA	-H	-glc
3a	-H	-H	$-CH_3$
4	-glcA	-OH	-H
4a	-H	-OH	-H
9	$-ara^{3}glc$	-H	-glc
10	-H	-H	-H

Fig. 1

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) in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet and br = broad. The EI and FAB-MS spectra were measured with a JEOL JMS-PX303 mass spectrometer. 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 (Merck) was used.

Extraction and Isolation of Compounds 1—4 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 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) to give **1** (0.6 g), **2** (0.24 g), **3** (0.06 g), **4** (0.03 g), **5** (0.8 g), **6** (0.1 g), **7** (4.5 g), and **8** (0.1 g).

Ilexoside XXIX (**1**): Colorless needles from MeOH, mp 204—206 °C, $[\alpha]_D$ +13.7° (c=4.6, MeOH). FAB-MS m/z: 759 [(M+Na)$^+$]. ^{13}C-NMR: Table I.

Ilexoside XXX (**2**): Colorless needles from EtOH, mp 214—215 °C, $[\alpha]_D$ +26.9° (c=3.2, MeOH). FAB-MS m/z: 663 [(M−H)$^-$]. *Anal.* Calcd for $C_{36}H_{56}O_{11}\cdot 2H_2O$: C, 61.70; H, 8.63. Found: C, 61.90; H, 8.22. ^{1}H-NMR δ: 1.04, 1.22, 1.40, 1.65, 1.66 (3H each, s, *tert*-$CH_3\times 5$), 1.06 (3H, d, J=6.0 Hz), 2.93 (1H, s, H-18), 4.66 (1H, dd, J=11.0, 5.5 Hz, H-3), 5.57 (1H, br t, H-12), 6.29 (1H, d, J=8.0 Hz, H-1 of esteric glc). ^{13}C-NMR: Table I.

Ilexoside XXXI (**3**): A white powder, $[\alpha]_D$ −2.0° (c=3.1, MeOH). FAB-MS m/z: 809 [(M−H)$^-$]. *Anal.* Calcd for $C_{42}H_{66}O_{15}\cdot 2H_2O$: C, 59.56; H, 8.33. Found: C, 59.40; H, 8.51. ^{1}H-NMR δ: 0.86, 0.99, 1.01, 1.13, 1.16, 1.31, 1.67 (3H each, s, *tert*-$CH_3\times 7$), 3.37 (1H, dd, J=11.0, 4.5 Hz, H-3), 3.52 (1H, br s, H-18), 3.58 (1H, d, J=3.0 Hz, H-19), 5.03 (1H, d, J=7.5 Hz, H-1 of glcA), 5.50 (1H, br t, H-12), 6.35 (1H, d, J=8.0 Hz, H-1 of esteric glc). ^{13}C-NMR: Table I.

Ilexoside XXXII (**4**): Colorless needles from MeOH, mp 218—220 °C, $[\alpha]_D$ +2.2° (c=1.8, MeOH). FAB-MS m/z: 663 [(M−H)$^-$]. *Anal.* Calcd for $C_{36}H_{56}O_{11}\cdot 3H_2O$: C, 60.15; H, 8.69. Found: C, 60.01; H, 8.75. ^{1}H-NMR δ: 0.93, 0.96, 1.06, 1.12, 1.20, 1.64 (3H each, s, *tert*-$CH_3\times 6$), 3.60 (2H, br s, H-18 and H-19), 3.72, 4.36 (each 1H, d, J=11.0, H_2-23), 4.33 (1H, dd, J=11.0, 4.5 Hz, H-3), 5.30 (1H, d, J=8.0 Hz, H-1 of glcA), 5.54 (1H, br t, H-12). ^{13}C-NMR: Table I.

Free Sulfate (**1a**): A white powder, $[\alpha]_D$ +15.0° (c=1.5, MeOH). FAB-MS m/z: 737 [(M+Na)$^+$]. ^{1}H-NMR δ: 0.86, 0.97, 1.03, 1.37, 1.47, 1.68 (3H each, s, *tert*-$CH_3\times 6$), 1.14 (3H, d, J=6.5 Hz), 3.00 (1H, s, H-18), 4.50 (1H, dd, J=11.0, 4.0 Hz, H-3), 5.60 (1H, br t, H-12), 6.04 (1H, dd, J=9.5, 8.5 Hz, H-2 of glc), 6.27 (1H, d, J=8.5 Hz, H-1 of esteric glc).

Detection of Sulfate Group in 1 Compound **1** (2 mg) was refluxed with 2 N hydrochloric acid for 2 h and then extracted with 1-BuOH. The water layer was evaporated to dryness under a vacuum. The residue was subjected to paper chromatography [Whatman No. 1, with MeOH–1-BuOH–H_2O (3:1:1)]. After drying in air, the paper was sprayed with a solution of $BaCl_2$ (100 mg/50 ml in 70% MeOH) and dried again. The paper was then sprayed with a solution of potassium rhodizonate (10 mg/50 ml in 50% MeOH) to develop the light yellow color.

Alkaline Hydrolysis of 1 Compound **1** (50 mg) was heated in 50% EtOH with 5% KOH (5 ml) for 6 h at 80 °C. The solution was passed through Amberlite XAD-2 and eluted with MeOH. The residue of the MeOH solution was passed through Sephadex LH 20 (MeOH) to give **1b** (15 mg). Compound **1b**, colorless needles from MeOH, mp >350 °C, $[\alpha]_D$ +30.8° (c=0.8, MeOH). FAB-MS m/z: 551 [(M−H)$^-$]. ^{1}H-NMR δ: 0.81, 0.94, 1.03, 1.33, 1.47, 1.70 (3H each, s, *tert*-$CH_3\times 6$), 1.14 (3H, d, J=6.5 Hz), 3.08 (1H, s, H-18), 4.50 (1H, dd, J=11.5, 4.0 Hz, H-3), 5.57 (1H, br t, H-12). ^{13}C-NMR: Table I.

Solvolysis of 1b A solution of **1b** (20 mg) in pyridine (1.5 ml) and dioxane (0.4 ml) was heated for 5 h at 80 °C. After evaporation to dryness the residue was suspended in H_2O and extracted with 1-BuOH. The residue of the 1-BuOH solution was passed through Sephadex LH 20 (MeOH) to give pomolic acid (**1c**) (12 mg). Colorless needles from MeOH, mp 298—300 °C, $[\alpha]_D$ +55.1° (c=0.7, THF). IR (KBr) ν_{max}: 3400 (br, OH), 1690 (C=O), 1045, 1025. FAB-MS m/z: 473 [(M+H)$^+$, $C_{30}H_{48}O_4$: 472], 495 [(M+Na)$^+$, $C_{30}H_{48}O_4$: 472]. EI-MS m/z: 472 (M$^+$), 454 (M−H_2O)$^+$, 410 (M$^+$−H_2O−CO_2), 264, 246, 208, 201, 190, 175. ^{1}H-NMR δ: 0.92, 1.04, 1.11, 1.24, 1.47, 1.73 (3H each, s, *tert*-$CH_3\times 6$), 1.13 (3H, d, J=6.5 Hz, 30-CH_3), 3.05 (1H, br s, H-18), 3.45 (1H, dd, J=10.4, 5.5 Hz, H-3), 5.62 (1H, br t, H-12). ^{13}C-NMR: Table I.

Solvolysis of 1 A solution of **1** (30 mg) in pyridine (1.5 ml) and dioxane (0.4 ml) was heated for 5 h at 80°C. After evaporation to dryness the residue was suspended in H_2O and extracted with 1-BuOH. The residue of the 1-BuOH solution was passed through Sephadex LH 20 (MeOH) to give **1d** (20 mg). Compound **1d**, colorless needles from MeOH, mp 226—228 °C, $[\alpha]_D$ +24.1° (c=1.9, MeOH). FAB-MS m/z: 633 [(M−H)$^-$]. ^{1}H-NMR δ: 0.96, 1.05, 1.21, 1.23, 1.44, 1.70 (3H each, s, *tert*-$CH_3\times 6$), 1.09 (3H, d, J=6.5 Hz), 2.94 (1H, s, H-18), 3.45 (1H, dd, J=10.0, 6.0 Hz, H-3), 5.59 (1H, br t, H-12), 6.28 (1H, d, J=8.5 Hz, H-1 of esteric glc). ^{13}C-NMR: Table I.

Enzymatic Hydrolysis of 1d A solution of **1d** (30 mg) and crude cellulase (30 mg, Sigma) in EtOH–H_2O (1:9) and 0.01 M NaH_2PO_4 buffer (pH 4.0) (2 ml each) was incubated for 72 h at 37 °C. After cooling, the reaction mixture was concentrated to dryness. The residue was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (25:4:0.5) to give pomolic acid (**1c**) (18 mg).

Alkaline Hydrolysis of 2 Compound **2** (200 mg) was stirred in 50% EtOH with 5% KOH (10 ml) for 12 h at 80 °C. The solution was passed through Amberlite XAD-2 and eluted with MeOH. The residue of the MeOH solution was passed through Sephadex LH 20 (MeOH) to give the aglycone (**2a**) (120 mg). Compound **2a**, colorless needles from MeOH, mp >300 °C, $[\alpha]_D$ +53.9° (c=1.3, MeOH). FAB-MS m/z: 501 [(M−H)$^-$]. ^{1}H-NMR δ: 0.99, 1.10, 1.46, 1.64, 1.69 (3H each, s, *tert*-$CH_3\times 5$), 1.13 (3H, d, J=6.5 Hz), 3.04 (1H, s, H-18), 4.67 (1H, dd, J=8.0, 8.0 Hz, H-3), 5.62 (1H, br t, H-12). ^{13}C-NMR: Table I. The above data were consistent with the literature data[14] on rotundioic acid.

Methylation of 2a Compound **2a** (100 mg) in ether was treated with CH_2N_2–Et_2O to give a dimethyl ester (**2b**, 100 mg). Compound **2b**, a white powder, $[\alpha]_D$ +44.5° (c=2.4, $CHCl_3$). FAB-MS m/z: 529 [(M−H)$^-$]. ^{1}H-NMR δ: 0.86, 0.96, 1.34, 1.52, 1.61 (3H each, s, *tert*-$CH_3\times 5$), 1.10 (3H, d, J=6.5 Hz), 2.82 (1H, s, H-18), 4.44 (1H, dd, J=8.0, 8.0 Hz, H-3), 5.49 (1H, br t, H-12).

Methanolysis of 3 A solution of **3** (50 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 (25 mg) was recrystallized from MeOH to give methyl siaresinolate (**3a**) (20 mg). Compound **3a**, mp 184—186 °C, $[\alpha]_D$ +46.0° (c=1.0, $CHCl_3$). EI-MS m/z: 486 (M)$^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.67, 0.77, 0.90, 0.95, 0.96, 1.00, 1.24 (3H each, s, *tert*-$CH_3\times 7$), 3.20 (1H, dd,

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$J=11.0$, 4.0 Hz, H-3), 3.10 (1H, br s, H-18), 3.33 (1H, d, $J=3.0$ Hz, H-19), 3.62 (3H, s, COOMe), 5.46 (1H, br t, H-12).

Acid Hydrolysis of 4 A solution of **4** (50 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 of the MeOH solution was passed through Sephadex LH 20 (MeOH) to give ilexosapogenin A (**4a**) (25 mg). Compound **4a**, colorless granules from MeOH, mp 278—280 °C, $[\alpha]_D$ +46.1° ($c=0.8$, 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, 68.65; H, 9.77. ^{1}H-NMR δ: 1.10, 1.16, 1.19, 1.22, 1.29, 1.71 (3H each, s, *tert*-$CH_3 \times 6$), 3.73 (2H, br s, H-18, H-19), 3.82, 4.28 (each 1H, d, $J=10.5$ Hz, H_2-23), 4.31 (1H, dd, $J=8.7$, 7.5 Hz, H-3), 5.67 (1H, br t, H-12). ^{13}C-NMR: Table I.

Identification of Component Sugars of 1—4 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 RI detection (Waters 410) and chiral detection (Shodex OR-1), respectively, in HPLC (Shodex RSpak DC-613, 75% CH_3CN, 1 ml/min, 70 °C) by comparison with authentic sugars (10 mmol each D-glc and D-glucuronolactone). These sugars gave the following peaks: D-(+)-glucuronolactone; 2.40 min, D-(+)-glc; 7.38 min.

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