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STRUCTURES OF 3,28-*O*-BISGLYCOSIDIC TRITERPENOID SAPONINS OF *FATSIA JAPONICA*

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Key Word Index—*Fatsia japonica*; Araliaceae; mature fruits; 3,28-*O*-bisglycosidic triterpenoid saponins.

Abstract—Four novel 3,28-*O*-bisglycosidic triterpenoid saponins were isolated from the mature fruits of *F. japonica*. They were characterized as the 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosides of 3-*O*-α-L-arabinopyranosyl echinocystic acid, 3-*O*-α-L-arabinopyranosyl hederagenin, 3-*O*-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl oleanolic acid and 3-*O*-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl hederagenin respectively.

INTRODUCTION

Fatsia japonica (Japanese name: Yatsude) has been reported to contain highly haemolytic and toxic constituents [1–3]. In previous studies, we isolated eight saponins from the flower buds, the flowers, the mature fruits and the leaves of the plant [4, 5], and showed them all to be 3-*O*-glycosidic triterpenoid saponins. In a continuation of these studies, we have now isolated four kinds of more polar saponins than the above eight saponins from the mature fruits of the plant and have shown them to be 3,28-*O*-bisglycosides of the triterpenic acids echinocystic acid, oleanolic acid and hederagenin.

RESULTS AND DISCUSSION

The saponins **1**–**4** were extracted from mature fruits by the methods described in the Experimental. They are numbered in order of increasing polarity on TLC.

Saponin **2** on acid hydrolysis furnished hederagenin, rhamnose, arabinose and glucose. The mole ratio of the sugars was 1:1:2 (GLC). On alkaline hydrolysis saponin **2** gave hederagenin 3-*O*-α-L-arabinopyranoside (**5**) [4, 6]. From these results and from a comparison of the ^{13}C NMR spectrum of saponin **2** with those of hederagenin methyl ester (**9**) and saponin **5** (Table 1), it was clear that C-1 of an arabinose molecule (δ_C 106.3) was linked by an α-glycosidic linkage to the C-3 hydroxyl group of hederagenin. It also appeared that there was an oligosaccharide moiety composed of two glucose molecules and a rhamnose molecule linked to the C-28 carboxyl group of the aglycone by β-glycosidic linkage through C-1 of the glucose (δ_C 95.5), to which in turn another glucose molecule (δ_C 104.5) and a rhamnose molecule (δ_C 102.6) were linked by β- and α-glycosidic linkages, respectively [8–10]. This was confirmed by analysing the anomeric proton signals in the ^1H NMR spectrum, the fragmentation patterns in the MS and the acid hydrolysis products of an exhaustively methylated product (**10**) of saponin **2** as follows. Exhaustive methylation of saponin **2** by Hakomori's method [11] gave a methylated product (**10**), which showed ^1H NMR signals of four anomeric protons assignable to an anomer proton of arabinose (δ 4.17, 1H, *d*, J = 5 Hz), glucose (δ 4.28, 1H, *d*, J = 7 Hz), rhamnose (δ 4.98, 1H, *s*) [12–14] and ester glycosidic glucose (δ 5.40, 1H, *d*, J = 7 Hz) [12], respectively. The coupling constants indicated that the glycosidic linkages of rhamnose and arabinose were α and those of two glucoses were all β [15]. The MS of **10** contained fragment ions at m/z 175 and 189 due to terminal permethyl arabinose and rhamnose units without the glycosidic oxygen [13], respectively. Methanolysis of **10** gave 23-mono-*O*-methyl hederagenin (**11**), methyl 2,3,4-tri-*O*-methylrhamnopyranoside, methyl 2,3,4-tri-*O*-methylarabinopyranoside and methyl 2,3,6-tri-*O*-methylglucopyranoside. On reduction with $LiAlH_4$, **10** gave two products, **12** and **13**. **12** gave an MS peak at m/z 175 due to a terminal permethyl arabinose unit without the glycosidic oxygen, ^1H NMR signals due to an anomeric proton of arabinose (δ 4.18, 1H, *d*, J = 6 Hz) and an IR absorption band due to a hydroxyl group. Methanolysis of **12** gave 23-methoxyolean-12-ene-3,28-diol and methyl 2,3,4-tri-*O*-methylarabinopyranoside. These results indicate that **12** was 23-methoxyolean-12-en-28-ol 3-*O*-α-L-2,3,4-tri-*O*-methylarabinopyranoside. On the other hand, **13** gave ^1H NMR signals due to two anomeric protons (δ 4.28, 1H, *d*, J = 8 Hz; δ 4.93, 1H, *s*) and an IR absorption band due to a hydroxyl group. The diacetate (**14**) of the reduction product (**13**) gave the high resolution MS spectral fragmentation pattern shown in Scheme 1. Thus, **13** was 2,3,4-tri-*O*-methylrhamnopyranosyl-(1 → 4)-2,3,6-tri-*O*-methylglucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methylsorbitol. Since it is a general rule that the glycosidic linkages of sugars in the D- and L-series are β and α, respectively [13, 14, 16], the above results indicated that saponin **2** is the 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside of saponin **5**. Consequently, saponin **2** was defined as 3-*O*-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside.

Saponin **4**, on acid hydroysis under the same conditions as saponin **2**, gave hederagenin, rhamnose, arabinose and

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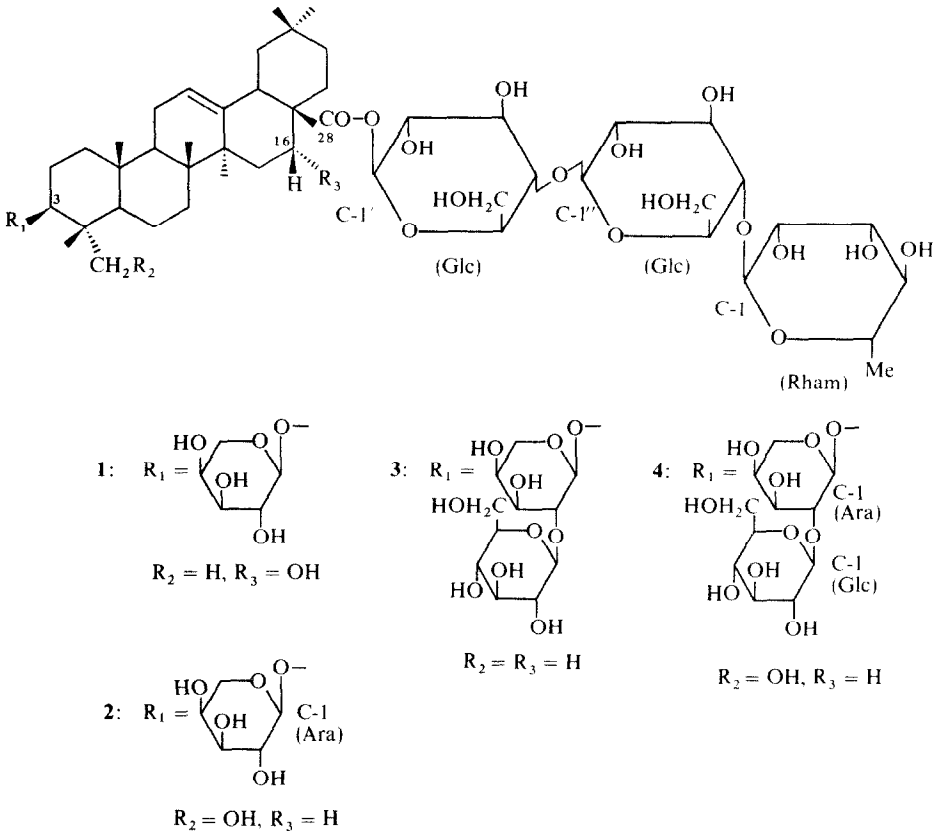
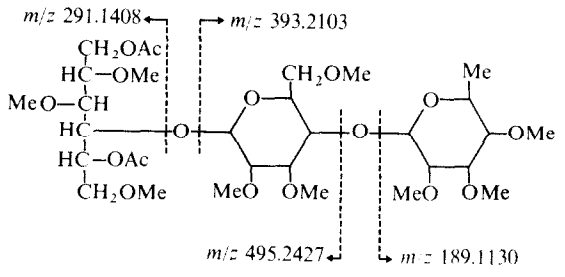


glucose. The mole ratio of these monosaccharides was found to be 1:1:3 (GLC). Alkaline hydrolysis of saponin **4** gave hederagenin 3-*O*-*β*-D-glucopyranosyl-(1 → 2)-*α*-L-arabinopyranoside (**6**) [4, 6, 7]. Comparison of the ^{13}C NMR chemical shifts with those of saponins **2** and **6** and hederagenin methyl ester (**9**) (Table 1), suggested that saponin **4** was a derivative of saponin **2** which was glucosidated at C-2 of arabinose by a *β*-glycosidic linkage. Exhaustive methylation of saponin **4** furnished a methylated product (**15**), which exhibited ^1H NMR signals of five anomeric protons assignable to an anomer proton of arabinose (*δ* 4.17, 1H, *d*, *J* = 5 Hz), glucose (*δ* 4.26 and 4.30, 1H, *d*, *J* = 7 Hz, respectively), rhamnose (*δ* 4.95, 1H, *s*) [12–14] and ester glycosidic glucose (*δ* 5.37, 1H, *d*, *J* = 8 Hz) [12], respectively. The MS of **15** contained fragment ions at *m/z* 189 and 219 due to terminal permethyl rhamnose and glucose units without the glycosidic oxygen, respectively. These data suggested that saponin **4** contained the same oligosaccharide moiety as that of saponin **2** linked in the same way to the C-28 carboxyl group of hederagenin. This was confirmed by the formation of 23-mono-*O*-methyl hederagenin, methyl 2,3,4-tri-*O*-methylrhamnopyranoside, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 3,4-di-*O*-methylarabinopyranoside and methyl 2,3,6-tri-*O*-methylglucopyranoside from the methylated product (**15**) on acid hydrolysis. Reduction of **15** with $LiAlH_4$ gave two products, **13** and **16**. Methanolysis of **16** gave 23-methoxyolean-12-ene-3,28-diol, methyl 2,3,4,6-tetra-*O*-methylglycopyranoside and methyl 3,4-di-*O*-methylarabinopyranoside. This indicated that saponin **4** was 28-*O*-*α*-L-rhamnopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-glucopyranoside of saponin **6**. The structure of saponin **4** was thus shown to be 3-*O*-*β*-D-glucopyranosyl-(1 → 2)-*α*-L-arabinopyranosyl hederagenin 28-*O*-*α*-L-rhamnopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 4)-*β*-D-glucopyranoside.



Scheme 1. Mass spectral fragmentation pattern of **14**.

Saponin **1** furnished echinocystic acid, rhamnose, arabinose and glucose on acid hydrolysis under the same conditions as saponin **2**. The mole ratio of these sugars was found to be 1:1:2 (GLC). Alkaline hydrolysis of saponin **1** in the same manner as saponin **2** produced echnocystic acid 3-*O*-*α*-L-arabinopyranoside (**7**) [5]. These observations suggested that one molecule of L-arabinose was linked by an *α*-glycosidic linkage to the C-3 hydroxyl group of echinocystic acid, while the same oligosaccharide moiety as that of saponin **2** was bound by a *β*-ester glycosidic linkage to the C-28 carboxyl group of the aglycone. This was demonstrated by methanolysis and reduction of the methylate (**17**) derived from saponin **1** on exhaustive methylation according to Kuhn's method [17]. Methanolysis of **17** under the same conditions as **10** gave 16-mono-*O*-methyl echinocystic

Table 1. ^{13}C NMR data for saponins **2**, **4**, **5**, **6** and compound **9***

Carbon		2	4	5	6	9
	1	38.8	38.8	38.6	38.6	38.7
	2	26.0	26.1	26.1	26.1	27.4
	3	82.2	82.4	82.1	82.3	73.7
	4	43.4	43.5	43.3	43.4	42.6
	5	47.5	47.5	47.5	47.8	48.7
	6	18.3	18.3	18.1	18.2	18.5
	7	33.2	33.1	33.2	33.3	32.7
	8	39.9	39.9	39.8	39.7	39.6
	9	47.9	47.9	38.1	48.0	47.9
	10	37.0	37.0	36.9	36.9	37.1
	11	23.6	23.7	23.7	23.7	23.7
	12	122.5	122.7	122.5	122.5	122.9
	13	144.2	144.2	144.9	144.9	144.1
	14	42.1	42.1	42.1	42.1	41.9
	15	28.3	28.2	28.3	28.3	28.0
	16	23.0	23.0	23.7	23.7	23.3
	17	47.0	47.1	46.5	46.6	46.9
	18	41.7	41.8	42.1	42.1	41.7
	19	46.2	46.2	46.5	46.6	46.0
	20	30.7	30.7	30.9	30.9	30.7
	21	34.1	33.7	34.2	34.2	33.9
	22	32.7	32.8	33.2	33.0	32.7
	23	64.6	64.6	64.4	64.8	68.4
	24	13.5	13.3	13.5	13.3	12.8
	25	16.2	16.2	16.0	16.0	15.9
	26	17.5	17.6	17.4	17.4	17.1
	27	26.0	26.1	26.1	26.1	26.1
	28	176.7	176.8	180.3	180.2	177.9
	29	33.2	32.2	33.2	33.3	33.1
	30	23.6	23.7	23.7	23.7	23.7
	—O—Me					51.4
Ara	C-1	106.3	103.9	106.3	103.8	
Rham	C-1	102.6	102.8			
Glc	C-1		105.5		105.6	
Glc	C-1′	95.5	95.5			
Glc	C-1″	104.5	104.6			

*Signals were assigned by comparison with reported data [8–10].

acid, methyl 2,3,4-tri-*O*-methylrhamnopyranoside, methyl 2,3,4-tri-*O*-methylarabinopyranoside and methyl 2,3,6-tri-*O*-methylglucopyranoside. On reduction with $LiAlH_4$, **17** gave the sorbital derivative **13** and a product (**18**), which on methanolysis furnished 16-mono-*O*-methyllolean-12-ene-3,28-diol and methyl 2,3,4-tri-*O*-methylarabinopyranoside. These facts indicated that saponin **1** was 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside of saponin **7**. The structure of saponin **1** was thus established as 3-*O*-α-L-arabinopyranosyl echinocystic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside.

Saponin **3** afforded oleanolic acid, rhamnose, arabinose and glucose on acid hydrolysis. GLC analysis indicated the mole ratio of these sugars to be 1:1:3. Alkaline hydrolysis of saponin **3** gave oleanolic acid 3-*O*-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside (**8**) [4, 7]. These observations suggested that a disaccharide moiety composed of β-D-glucopyranosyl-(1 → 2)-L-arabinopyranose was linked by an α-glycosidic linkage to the C-3 hydroxyl group of oleanolic acid, while the same trisaccharide moiety as that in saponin **2** was linked by a β-ester glycosidic linkage to the C-28 carboxyl group of the aglycone. In the same way as described above, this was confirmed by the formation of oleanolic acid, methyl 2,3,4-tri-*O*-methylrhamnopyranoside, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 3,4-di-*O*-methylarabinopyranoside and methyl 2,3,6-tri-*O*-methylglucopyranoside from a methylated product (**19**) of saponin **3** by methanolysis, as well as the formation of the tri-*O*-methylsorbitol derivative (**13**) and olean-12-en-28-ol 3-*O*-β-D-2,3,4,6-tetra-*O*-methylglucopyranosyl-(1 → 2)-α-L-3,4-di-*O*-methylarabinopyranoside (**20**) from **19** on reduction with $LiAlH_4$. The olean-12-en-28-ol methylglycoside was identified from the olean-12-ene-3,28-diol, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 3,4-di-*O*-methylarabinopyranoside formed on methanolysis. These facts indicated that saponin **3** was 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-

(1 → 4)-β-D-glucopyranoside of saponin **8**. Thus, the structure of saponin **3** was elucidated as 3-*O*-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranoside.

The new saponins (**1**–**4**) from the mature fruits of *F. japonica* are 3,28-*O*-bisglycosides of oleanolic acid, echinocystic acid and hederagenin. The C-3 hydroxyl group of the aglycone in each of the saponins is glycosidated with arabinose, which, in saponins **3** and **4**, is β-glucosidated at the 2-position. The C-28 carboxyl group of each aglycone in these four saponins is linked by a β-glycosidic linkage to C-1 of glucose, to which, in turn, another glucose and rhamnose are bound by β- and α-glycosidic linkages, respectively, at their 1- and 4-positions.

EXPERIMENTAL

Mps are uncorr. NMR spectra were measured, with TMS as int. standard, at 60 and 90 MHz for ^1H ($CDCl_3$) and 22.6 MHz for ^{13}C (C_5D_5N). MS were recorded by direct inlet at 70 eV ionization. Analytical HPLC were run on a stainless steel column (4.6 mm × 1 m) packed with Si ODS (TSK GEL-LS 410) using H_2O–MeCN (1:19 v/v) as the mobile phase. The column effluent was monitored at 222 nm. GLC (FID) was carried out on a glass column (3 mm × 2 m) packed with 2% OV-17 and 15% butane-1,4-diol succinate on Chromosorb W (80–100 mesh). Exhaustively acetylated sugars were analysed at 190°, methyl oleanolate at 330° and methyl 16-mono-*O*-methylechinocystate at 310° on OV-17, respectively, and exhaustively or partially methylated sugars at 175° on butane-1,4-diol succinate. Analytical TLC (0.25 mm) and prep. TLC (0.75 mm) were carried out on Si gel (Merck 60 GF 254). The compounds were visualized as coloured spots by spraying with HNO_3–H_2SO_4 (1:19) and then heating. For PC analyses of sugars, Toyo Roshi filter paper (No. 51) and the following solvents were employed: H_2O–PhOH (1:5) (solvent A); *n*-BuOH–C_5H_5N–H_2O (6:4:3) (solvent B), *n*-BuOH–C_5H_5N–H_2O (6:2:1) (solvent C).

Isolation of saponins from the mature fruits. The mature fruits (12.52 kg) of *Fatsia japonica* Decne et Planch were collected in May and immersed in Me_2CO. The Me_2CO soln, after concn *in vacuo* and defatting with hexane, was separated into an aq. layer and ppts. The aq. layer was extracted with *n*-BuOH. The *n*-BuOH extract, after concn *in vacuo*, was separated into four saponin fractions by prep. TLC with $CHCl_3$–MeOH–H_2O (25:17:3). Each of fractions was further purified by repeated prep. TLC using the same solvent to give saponins **1** (33 mg, R_f 0.57), **2** (315 mg, R_f 0.49), **3** (69 mg, R_f 0.43) and **4** (122 mg, R_f 0.37), which were all crystallized from MeOH–H_2O.

Acid and alkali hydrolyses of saponin **2**. Saponin **2** (85 mg), mp 184–187° (dec.); $[\alpha]_D^{25}$ + 5.3° (10% H_2O–MeOH; *c* 1.05); IR ν_{max}^{Nujol} cm^{-1}: 3395 (OH), 1732 (—CO—O—), was refluxed with 2% H_2SO_4 (10 ml) for 6 hr. After addition of H_2O and filtration, the ppt. (40 mg) was methylated with CH_2N_2, crystallized from MeOH, and identified as hederagenin methyl ester (**9**) (co-TLC, mp, mmp, ^1H NMR, IR and MS) [4, 5]. The mother liquor was neutralized with Amberlite IR-45 (OH^-) and the presence of arabinose, rhamnose and glucose established by PC with solvents A, B and C. The sugar portion was evapd to dryness and acetylated with Ac_2O–pyridine to give tetra-*O*-acetylrhamnose, tetra-*O*-acetylarabinose and penta-*O*-acetylglucose, whose mole ratio was found to be 1:1:2 by GLC using as references authentic samples prepared in the ratio of 1:1:1, 1:1:2 and 1:1:3.

Saponin **2** (70 mg) was refluxed with 0.4% NaOH (7 ml) in dioxane (5 ml) for 3 hr. The reaction mixture was extracted with *n*-BuOH. Removal of the solvent from the *n*-BuOH soln gave a residue (38 mg), a part (10 mg) of which was further hydrolysed with 2% H_2SO_4 under the same conditions as saponin **2** to give hederagenin (identified by mp, mmp and co-TLC of its methyl ester (**9**)) and arabinose (co-PC). Another part of the residue was recrystallized from MeOH–H_2O–HOAc and identified as hederagenin-3-*O*-α-L-arabinopyranoside (**5**) (mp, mmp, co-TLC and co-HPLC) [4].

Exhaustive methylation of saponin **2** *and methanolysis of the methylate* (**10**) *produced.* Following the method developed by Hakomori [11], saponin **2** (160 mg) in DMSO (15 ml) was added to a mixture of NaH (750 mg) and DMSO (15 ml). An excess of MeI (3.5 ml) was added to the soln with stirring over a period of 4 hr. The reaction mixture was poured into ice-cold H_2O (300 ml), and the methylated product extracted with EtOAc. Removal of the solvent from the EtOAc soln, after washing with H_2O (300 ml × 3), gave **10** (155 mg), IR ν_{max}^{film} cm^{-1}: 1734 (—CO—O—); ^1H NMR: δ 3.37–3.73 (39 H, —OMe × 13), 5.33 (1 H, *m*, >C=CH—), as a syrup.

A portion of **10** (64 mg) was hydrolysed with dry 10% HCl–MeOH (10 ml) for 4.5 hr. The reaction mixture was neutralized with Ag_2CO_3, filtered and evapd to dryness. The residue was recrystallized from MeOH to give 23-mono-*O*-methyl hederagenin (**11**) (17 mg), which was converted to its methyl ester with CH_2N_2 and then identified by comparison with an authentic sample (mp, mmp, co-TLC, IR and ^1H NMR). TLC and GLC analyses of the combined mother liquors of recrystallization indicated the existence of three kinds of methylated monosaccharide, which were identified as methyl 2,3,4-tri-*O*-methylarabinopyranoside, methyl 2,3,4-tri-*O*-methylrhamnopyranoside and methyl 2,3,6-tri-*O*-methylglucopyranoside (co-TLC and co-GLC with authentic samples).

Reduction of **10** *with* $LiAlH_4$. **10** (100 mg) in dry THF (10 ml) containing a suspension of $LiAlH_4$ (50 mg) was refluxed for 3 hr. The reaction mixture was treated with H_2O under cooling to decompose excess $LiAlH_4$ and then acidified with 5% HCl to dissolve the ppt. The soln obtained was concd at red. pres. and extracted with $CHCl_3$. Removal of the solvent from the $CHCl_3$ soln, after washing with H_2O, gave a residue which was separated into **12** (46 mg) and **13** (11 mg) by prep. TLC with $CHCl_3$–MeOH (9:1).

23-Methoxyolean-12-en-28-ol 3-*O*-α-L-2,3,4-tri-*O*-methylarabinopyranoside (**12**) (30 mg), IR ν_{max}^{film} cm^{-1}: 3495 (OH); ^1H NMR: δ 3.31–3.56 (12 H, —OMe × 4), 4.18 (1 H, *d*, *J* = 6 Hz, anomeric H), 5.17 (1 H, *m*, >C=CH—); MS *m/z* (rel. int.): 455 (18), 234 (22), 203 (100), 175 (40), was hydrolysed with dry 10% HCl–MeOH (10 ml) to give methyl 2,3,4-tri-*O*-methylarabinopyranoside (co-TLC and co-GLC) and 23-methoxyolean-12-ene-3,28-diol (17 mg), IR ν_{max}^{film} cm^{-1}: 3458 (OH), 1655 (C=C); ^1H NMR: δ 3.34 (3 H, *s*, —OMe), 5.19 (1 H, *m*, >C=CH—); MS *m/z* (rel. int.): 472 (M^+, 5), 454 ($M^+ - H_2O$, 2), 237 (10), 234 (29), 203 (100). This diol was identified by comparison with an authentic sample prepared from 23-mono-*O*-methyl hederagenin [4].

2,3,4-Tri-*O*-methylrhamnopyranosyl-(1 → 4)-2,3,6-tri-*O*-methylglucopyranosyl-(1 → 4)-2,3,6-tri-*O*-methylsorbitol (**13**) (8 mg), IR ν_{max}^{film} cm^{-1}: 3641 (OH); ^1H NMR: δ 1.27 (3H, *d*, *J* = 6 Hz, >CH—Me), 3.37–3.57 (27H, —OMe × 9), 4.28 (1H, *d*, *J* = 8 Hz, anomeric H), 4.93 (1H, *s*, anomeric H), was acetylated with Ac_2O–pyridine to give diacetate **14** (6 mg), IR ν_{max}^{film} cm^{-1}: 1733 (—OCOMe); ^1H NMR: δ 1.27 (3H, *d*, *J* = 6 Hz, >CH—Me), 2.08 (6H, *s*, —OCOMe × 2), 3.36–3.56 (27H, —OMe × 9), 4.21 (1 H, *d*, *J* = 8 Hz, anomeric H), 4.27 (2 H, *d*, *J* = 7 Hz, >CH—CH_2—OAc), 4.96 (1H, *s*, anomeric H), 5.17 (1H, *m*, >CH—OAc); MS *m/z* (rel. int.): 495.2427 (calc. for $C_{22}H_{39}O_{12}$: 495.2439, 1), 463.2172 (calc. for $C_{21}H_{35}O_{11}$: 463.2176, 4), 393.2103 (calc. for $C_{18}H_{33}O_9$: 393.2122, 1),

291.1408 (calc. for $C_{13}H_{23}O_7$: 291.1442, 33), 189.1130 (calc. for $C_9H_{17}O_4$: 189.1126, 31).

Acid and alkali hydrolyses of saponin **4**. On acid hydrolysis under the same conditions as saponin **2**, saponin **4** (18 mg), mp 180–183° (dec.); $[\alpha]_D^{25}$ + 34.9° (10% H_2O–MeOH; *c* 0.22); IR ν_{max}^{Nujol} cm^{-1}: 3377 (OH), 1736 (—CO—O—), furnished hederagenin (identified by direct comparison of mp, mmp, co-TLC and ^1H NMR of its methyl ester with a known sample), rhamnose, arabinose and glucose (co-GLC of their acetate and co-PC). The mole ratio of rhamnose, arabinose and glucose was determined to be 1:1:3 in the same way as saponin **2**. Alkaline hydrolysis of saponin **4** (17 mg) under the same conditions as saponin **2** gave a crystalline mass (6 mg), which was identified as hederagenin 3-*O*-β-D-glucopyranosyl - (1 → 2) - α - L - arabinopyranoside (**6**) on the basis of mp, mmp, co-TLC and co-HPLC [5–7] and the formation of hederagenin, arabinose and glucose on acid hydrolysis.

Exhaustive methylation of saponin **4** *and methanolysis of the methylated product* (**15**). Exhaustive methylation of saponin **4** (75 mg) in the same manner as saponin **2** gave a methylated product (**15**) (20 mg); IR ν_{max}^{film} cm^{-1}: 1741 (—CO—O—); ^1H NMR: δ 3.32–3.61 (48H, —OMe × 16), 5.30 (1H, *m*, >C=CH—). Hydrolysis of the methylate (**15**) (10 mg) under the same conditions as **10** afforded 23-mono-*O*-methyl hederagenin, which was converted to its methyl ester with CH_2N_2 and then identified with a known sample (mp, mmp, co-TLC, IR and ^1H NMR), and four kinds of methylated monosaccharide, which were identified as methyl 2,3,4-tri-*O*-methylrhamnopyranoside, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 3,4-di-*O*-methylarabinopyranoside and methyl 2,3,6-tri-*O*-methylglucopyranoside (co-TLC and co-GLC with authentic samples).

Reduction of the methylate (**15**) *with* $LiAlH_4$. Reduction of **15** (10 mg) with $LiAlH_4$ in the same way as **10** gave **13** (4 mg) (identified by co-TLC and ^1H NMR of its acetate) and **16** (4 mg). Compound **16** on acid hydrolysis under the same conditions as **10** gave 23-methoxyolean-12-ene-3,28-diol (identified by direct comparison of co-TLC, IR and ^1H NMR with a known sample) and two kinds of methylated monosaccharide, which were identified as methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 3,4-di-*O*-methylarabinopyranoside (co-TLC and co-GLC).

Acid and alkali hydrolyses of saponin **1**. Acid hydrolysis of saponin **1** (8 mg), mp 210–215° (dec.), $[\alpha]_D^{25}$ + 16.6° (10% H_2O–MeOH; *c* 0.30), IR ν_{max}^{Nujol} cm^{-1}: 3412 (OH), 1739 (—CO—O—), in the same manner as saponin **2** gave a crystalline mass (3 mg), which on methylation with CH_2N_2 as usual yielded a methylated product. This methylate was identified as methyl echinocystate (identified by mp, mmp and co-TLC) [5]. The aq. mother liquor was neutralized, concd and subjected to GLC (their acetate) and PC analyses to characterize rhamnose, arabinose and glucose. The mole ratio of these sugars was determined to be 1:1:2 in the same manner as saponin **2**. Alkaline hydrolysis of saponin **1** (5 mg) in the same way as saponin **2** gave a crystalline mass (2 mg), which was identified as echinocystic acid 3-*O*-α-L-arabinopyranoside (**7**) (co-TLC and co-HPLC with a known sample [5]). This was further confirmed by the formation of echinocystic acid and arabinose on acid hydrolysis under the same conditions as saponin **2**.

Exhaustive methylation of saponin **1** *and methanolysis of the methylate* (**17**) *produced*. Following Kuhn's method [17], saponin **1** (4 mg) dissolved in DMF (3 ml) was methylated with MeI (1 ml) in the presence of Ag_2O (30 mg) at room temp. for 90 hr with stirring in the dark. A filtrate of the reaction mixture was again methylated with MeI (2 ml) and Ag_2O (70 mg) in the same manner as above. A filtrate of the second reaction mixture was diluted with H_2O to give a ppt., which was dissolved by adding solid KCN. The soln was extracted with $CHCl_3$. Removal of the solvent from the $CHCl_3$ soln gave a methylated product (**17**) (2 mg). **17** (1 mg) was subjected to acid hydrolysis with dry 10% HCl–MeOH (1 ml) for 6 hr. The reaction mixture was treated with Ag_2CO_3, filtered and evapd to dryness. The residue obtained was separated into 16-mono-*O*-methyl echinocystic acid (identified by direct comparison of its methyl ester with a known sample [5]) and a sugar portion composed of three kinds of methylated monosaccharide. The methylated monosaccharides were identified as methyl 2,3,4-tri-*O*-methylrhamnopyranoside, methyl 2,3,4-tri-*O*-methylarabinopyranoside and methyl 2,3,6-tri-*O*-methylglucopyranoside (co-TLC and co-GLC).

Reduction of **17** *with* $LiAlH_4$. Reduction of **17** (1 mg) with $LiAlH_4$ under the same conditions as **10** gave **13** (identified by co-TLC) and **18**. Acid hydrolysis of **18** furnished methyl 2,3,4-tri-*O*-methylarabinopyranoside (co-TLC and co-GLC) and 16-mono-*O*-methyl olean-12-ene-3,28-diol, MS *m/z* (rel. int.): 472 (M^+, 24), 457 (M^+ − Me, 8), 454 ($M^+ - H_2O$, 4), 264 (88), 233 (80), 207 (24), 201 (100) (identified by comparison (co-TLC and MS) with an authentic sample prepared from methyl 16-mono-*O*-methyl echinocystate [5]).

Acid and alkali hydrolyses of saponin **3**. On acid hydrolysis in the same way as saponin **2**, saponin **3** (20 mg), mp 190–194° (dec.). $[\alpha]_D^{25}$ + 40.7° (10% H_2O–MeOH; *c* 0.34), IR ν_{max}^{Nujol} cm^{-1}: 3341 (OH), 1738 (—CO—O—), gave oleanolic acid (identified by mp, mmp, co-TLC and co-GLC), rhamnose, arabinose and glucose (co-GLC (as acetates) and co-PC). The mole ratio of these sugars was determined to be 1:1:3 in the same manner as saponin **2**. Saponin **3** (8 mg) on alkali hydrolysis under the same conditions as saponin **2** gave oleanolic acid 3-*O*-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside (**8**) (mp, mmp, co-TLC and co-HPLC [4, 6, 7]).

Exhaustive methylation of saponin **3** *and methanolysis of the methylate* (**19**) *produced*. Exhaustive methylation of saponin **3** (31 mg) in the same way as saponin **2** gave **19** (6 mg). Methanolysis of **19** (3 mg) under the same conditions as **10** furnished oleanolic acid (identified by mp, mmp, co-TLC and co-GLC) and four kinds of methylated monosaccharide. The methylated monosaccharides were identified as methyl 2,3,4-tri-*O*-methylrhamnopyranoside, methyl 3,4-di-*O*-methylarabinopyranoside, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 2,3,6-tri-*O*-methylglucopyranoside (co-TLC and co-GLC).

Reduction of **19** *with* $LiAlH_4$. Reduction of **19** (3 mg) in the same way as **10** gave **13** (identified by co-TLC of its acetate) and **20**. Methanolysis of **20** furnished olean-12-ene-3,28-diol (identified by co-TLC) and two kinds of methylated monosaccharide, which were identified as methyl 2,3,4,6-tetra-*O*-methylglucopyranoside and methyl 3,4,-di-*O*-methylarabinopyranoside (co-TLC and co-GLC).

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REFERENCES

1. Ohta, K. (1924) *Keio Igaku* **3**, 1111.
2. Ohta, K. (1924) *Keiō Igaku* **4**, 157.

3. Kotake, M., Taguchi, K. and Okamoto, T. (1933) *Rikagaku Kenkyusho Hōkoku* **12,** 590.
4. Aoki, T., Tanio, Y. and Suga, T. (1976) *Phytochemistry* **15,** 781.
5. Aoki, T. and Suga, T. (1978) *Phytochemistry* **17,** 771.
6. Tanemura, T. and Takamura, K. (1975) *Yakugaku Zasshi* **95,** 1.
7. Tomimori, T. and Kizu, H. (1979) *Yakugaku Zasshi* **99,** 92.
8. Tori, K., Seo, S., Shimaoka, A. and Tomita, Y. (1974) *Tetrahedron Letters* 4227.
9. Seo, S., Tomita, Y., Tori, K. and Yoshimura, Y. (1978) *J. Am. Chem. Soc.* **100,** 3331.
10. Sakamoto, I., Yamasaki, K. and Tanaka, O. (1977) *Chem. Pharm. Bull.* **25,** 3437.
11. Hakomori, S. (1964) *J. Biochem. (Tokyo)* **55,** 205.
12. Kondo, N., Marumoto, Y. and Shoji, J. (1971) *Chem. Pharm. Bull.* **19,** 1103.
13. Higuchi, R. and Kawasaki, T. (1972) *Chem. Pharm. Bull.* **20,** 2143.
14. Kumekawa, Y., Itokawa, H. and Fujita, M. (1974) *Chem. Pharm. Bull.* **22,** 2294.
15. Capon, B. and Thacker, D. (1964) *Proc. Chem. Soc.* 369.
16. Hariharon, H. and Rangaswami, S. (1970) *Phytochemistry* **9,** 409.
17. Kuhn, R. (1955) *Angew. Chem.* **67,** 32.