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TRITERPENOID SAPONINS FROM *FATSIA JAPONICA*

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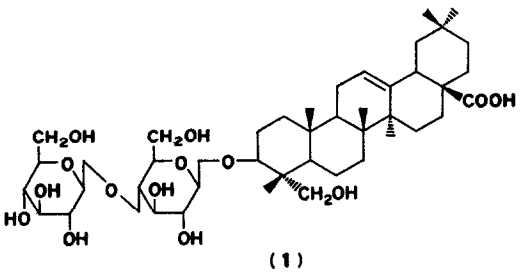
Key Word Index—*Fatsia japonica*; Araliaceae; triterpenoid saponins; 3-*O*-[β-D-glucopyranosyl(1→4)-β-D-glucopyranosyl]-hederagenin; 3-*O*-[β-D-glucopyranosyl(1→4)-α-L-arabinopyranosyl]-oleanolic acid; 3-*O*-[α-L-arabinopyranosyl]-hederagenin; 3-*O*-[β-D-glucopyranosyl]-hederagenin; 3-*O*-[β-D-glucopyranosyl(1→4)-α-L-arabinopyranosyl]-hederagenin; distribution of the saponins.

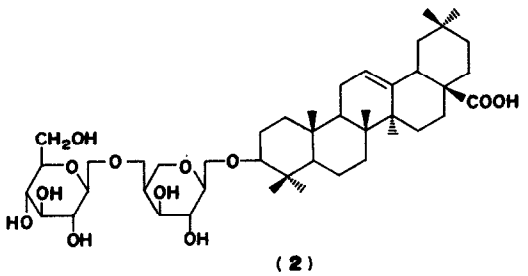
Abstract—Five triterpenoid saponins isolated from the flowers, the mature fruits and the leaves of *Fatsia japonica* were identified as 3-*O*-[β-D-glucopyranosyl(1→4)-β-D-glucopyranosyl]-hederagenin (**1**), 3-*O*-[β-D-glucopyranosyl-(1→4)-α-L-arabinopyranosyl]-oleanolic acid (**2**), 3-*O*-[α-L-arabinopyranosyl]-hederagenin (**3**), 3-*O*-[β-D-glucopyranosyl]-hederagenin (**4**) and 3-*O*-[β-D-glucopyranosyl(1→4)-α-L-arabinopyranosyl]-hederagenin (**5**). The saponins **1** and **2** are new, naturally occurring, triterpenoid saponins. The distribution of the five saponins in three parts of the plant was investigated. Saponins **2**, **3** and **5** were present in the flowers, saponins **1**, **3**, **4** and **5** were in the mature fruits and saponins **2**, **3**, **4** and **5** were in the leaves.

INTRODUCTION

Fatsia japonica (Japanese name: Yatsude), which is used in folk remedies, has been described to contain highly hemolytic and toxic constituents [1–3]. This plant blooms in the autumn and bears fruits in the winter which mature in the early summer of the next year. Recently it was reported that five triterpenoid saponins were isolated from the leaves of the plant, which were collected in the summer [4]. We have investigated the saponin constituents of the flowers collected in November, the mature fruits in June and the leaves in March, and isolated five triterpenoid saponins from these three parts. We report here evidence leading to the structures **1** and **2** for two of the saponins which have not previously been reported in nature.



(1)



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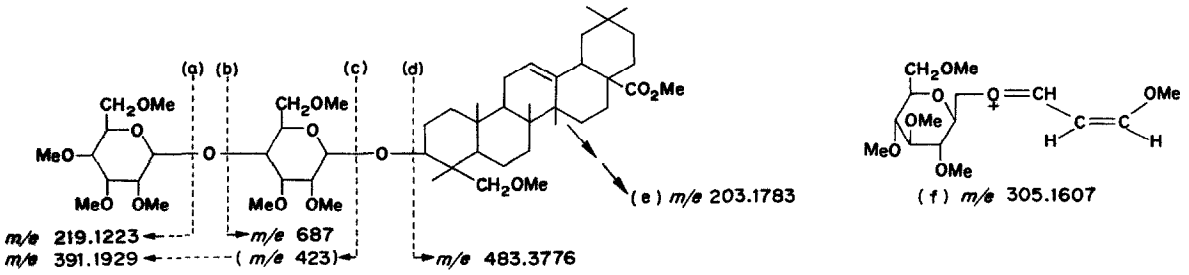
RESULTS AND DISCUSSION

Saponin **1**, mp 235.5–236.5°, $[\alpha]_D^{25}$ +42.4° (*c* 0.453, C_5H_5N), isolated from the mature fruits, afforded on acid hydrolysis hederagenin and glucose. Exhaustive methylation of saponin **1** by Hakomori's method [5] gave the methylated product (**6**), which showed NMR signals for nine *O*-methyls (δ 3.28–3.62 ppm), two anomeric protons (δ 4.21 ppm, 1H, *d*, *J* 7Hz; 4.58 ppm, 1H, *d*, *J* 6Hz) and an olefinic proton (δ 5.25 ppm, 1H). The molecular ion peak was at *m/e* 922 ($C_{51}H_{86}O_{14}$). From the above results, it was apparent that saponin **1** was composed of two glucose units and hederagenin.

The linking of the two glucose units was determined to be 1→4 by such observations as the appearance of the *m/e* 305 peak (high resolution MS: 305.1607, $C_{14}H_{25}O_7$) due to fragment (f) [6] (Scheme 1) and the formation of 2,3,4,6-tetra-*O*-methylglucopyranose, 2,3,6-tri-*O*-methylglucopyranose and 23-mono-*O*-methyl hederagenin methyl ester (**7**) (mp 187–188° [7] and M^+ at *m/e* 500) on hydrolyzing the nona-*O*-methylate (**6**). The attachment of the 1→4 linked diglucopyranoside to the C-3 hydroxyl group of hederagenin was indicated by the MS peaks at *m/e* 391 (high resolution MS: 391.1929, $C_{18}H_{31}O_9$) and *m/e* 483 (high resolution MS: 483.3776, $C_{32}H_{51}O_3$) [8] and by the NMR spectral data (δ 3.28 and 3.60 ppm, 2H, AB-*d*, *J* 11Hz, $-CH_2OMe$; δ 3.35 ppm, 3H, *s*, $-CH_2OCH_3$; δ 3.63 ppm, 3H, *s*, $-COOCH_3$) of the methyl ester (**7**). Other fragments shown in Scheme 1 also support the structure of the methylate (**6**).

The β-1→4 glycosidic linkages were indicated by coupling constants of the anomeric protons in the NMR spectrum described above [9]. This was further confirmed, as shown in Table 1, by application of both Klyne's [10] and Hudson's [11] rules using the values of the molecular rotation of the compounds listed in Table 2. The structure of saponin **1** was thus established to be 3-*O*-[β-D-glucopyranosyl(1→4)-β-D-glucopyranosyl]-hederagenin.



Scheme 1. Mass spectral fragmentation patterns of **6**.

Saponin **2**, mp 259.5–260.0°, $[\alpha]_D^{25}$ +38.5° (*c* 1.430, C_5H_5N), isolated from the flowers, furnished on acid hydrolysis arabinose, glucose and oleanolic acid. The exhaustively methylated product (**8**) of the saponin **2** showed NMR signals of seven *O*-methyls (δ 3.34–3.62 ppm), two anomeric protons (δ 4.51 ppm, 1H, *d*, *J* 6Hz; 4.60 ppm, 1H, *d*, *J* 5Hz) and an olefinic proton (δ 5.30 ppm, 1H) and the molecular ion peak at *m/e* 848 ($C_{48}H_{80}O_{12}$). These results showed that the saponin **2** was composed of one molecule each of arabinose, glucose and oleanolic acid.

The linking of arabinose and glucose was determined to be 1→4 by the observation of the *m/e* 305 peak [6] (high resolution MS: 305.1576, $C_{14}H_{25}O_7$) and by the formation of 2,3,4,6-tetra-*O*-methylglucopyranose, 2,3-di-*O*-methylarabinopyranose and methyl oleanolate (mp 195–200°C [4] and M^+ at *m/e* 470) by acid hydrolysis of the hepta-*O*-methylate (**8**). Thus the disaccharide was attached to the C-3 hydroxyl group of oleanolic acid. Further, the MS peaks at *m/e* 453 (high resolution MS: 453.3712, $C_{31}H_{49}O_2$), *m/e* 262 (high resolution MS: 262.1910, $C_{17}H_{26}O_2$), *m/e* 219 (high resolution MS: 219.1220, $C_{10}H_{19}O_5$) and *m/e* 203 (high resolution MS: 203.1810, $C_{15}H_{23}$) supported the structure of the methylate (**8**).

With regard to the configuration of the glycosidic linkages it is generally observed that D- and L-sugars have β- and α-glycosidic linkages respectively [15]. On the basis of both the molecular rotations shown in Table 1 and the coupling constants of the anomeric proton signals of the methylate (**8**) [4,9], the glycosidic linkage of the L-arabinopyranose and the D-glucopyranose was confirmed to be α and β respectively. These facts indicated that the structure of saponin **2** was 3-*O*-[β-D-glucopyranosyl(1→4)-α-L-arabinopyranosyl]-oleanolic acid.

Bukharv *et al.* [16] isolated scabioside B, mp 210–212°, $[\alpha]_D^{20}$ − 7°, $[M]_D^{20}$ − 52°, from *Patrinia scabioside* and proposed the same structure as saponin **2**. The structure of scabioside B was elucidated only by application of Klyne's rule [10]. If the saponin was composed of L-arabinopyranose, D-glucopyranose and oleanolic acid and the L-arabinopyranose and the D-glucopyranose were linked α and β respectively, as in the structural formula described in their paper [16], then the molecular rotation of this saponin should be $[M]_D$ + 308.4° as calculated using their data and by application of both Klyne's [10] and Hudson's [11] rules. Accordingly it is likely that the structure of scabioside B is different from that of saponin **2**.

Saponin **3**, mp 238–240°, $[\alpha]_D^{25}$ +81.0° (*c* 0.88, C_5H_5N), and saponin **5**, mp 256.5–260°, $[\alpha]_D^{25}$ +39.9° (*c* 1.00, C_5H_5N), were identical with β_2- and α-fatsins respectively, which have been isolated from the leaves of *F. japonica* and identified as 3-*O*-[β-D-glucopyranosyl]-hederagenin and 3-*O*-[β-D-glucopyranosyl(1→4)-α-L-arabinopyranosyl]-hederagenin, respectively, by Tane-

Table 1. $[M]_D$ of saponins **1** and **2** and the sugars in the saponins and determination of the C-1 configuration of the sugars

Compounds	Obsd. $[M]_D$	Calcd. $[M]_D$	$[M]_D$ evald. for the sugars	Configuration at C-1 of the sugars
Saponin **1**	+337.3°	+258.1°		
Glucoses in **1**			−45.0°	β,β
Saponin **2**	+288.5°	+281.4°		
Arabinose in **2**			+35.5°	α
Glucose in **2**			−55.0°	β

Table 2. $[M]_D$ of glucoside, arabinoside, hederagenin and oleanic acid used for the calculations

Compounds	$[M]_D$	References
Methyl α-D-glucopyranoside	+304.58°	11
Methyl β-D-glucopyranoside	−62.08°	11
Methyl α-L-arabinopyranoside	+28.37°	12
Methyl β-L-arabinopyranoside	+402.62°	12
Hederagenin	+382.3°	13
Oleanolic acid	+315.12°	14

Table 3. The distribution of saponins **1**–**5** in the flowers, the mature fruits and the leaves of *F. japonica*

Saponins	% Composition of the saponins		
	Flowers	Fruits	Leaves
1	—	5.6	—
2	13.7	—	24.8
3	5.2	49.5	21.1
4	—	25.8	26.4
5	81.1	19.1	27.7

mura *et al.* [4]. In addition, these authors also isolated three other triterpenoid saponins, 3-*O*-[β-D-glucopyranosyl]-oleanolic acid (β_1-fatsin), the glucoside of oleanolic acid (β_3-fatsin) and the glycoside of echinocystic acid (β_4-fatsin), from the leaves of the plant collected in early summer [4]. However, we could not isolate these three saponins from the leaves, the flowers or the mature fruits, but instead isolated three other saponins **1, 2** and **4.** The first two saponins were the new triterpenoid saponins described above. The last saponin, **4**, mp 246–247°, $[\alpha]_D^{25}$ +47.4° (*c* 1.22, C_5H_5N), was identified as 3-*O*-[β-D-glucopyranosyl]-hederagenin. A hederagenin monoglucoside similar to this saponin has been isolated from *Aster tartaricus*, although the structure was not elucidated fully [17].

We compared the distribution of saponins in the flowers, the mature fruits and the leaves of *F. japonica.* The results (Table 3) indicate that the distribution of the five saponins, **1, 2, 3, 4** and **5**, differs in the three parts of the plant. The saponin **1** was present in only the mature fruits, which also contained the saponins **3, 4,** and **5.** A major saponin constituent of the flowers was **5,** which was accompanied with a fairly high quantity of the saponin **2** and a smaller amount of the saponin **3.** Saponins of the leaves were composed of nearly equal amounts of the saponins **2, 3, 4** and **5.**

EXPERIMENTAL

IR spectra were taken in KBr pellets or in a CCl_4 soln. NMR spectra were measured in a $CDCl_3$ soln using TMS as internal standard. MS were taken with a direct inlet system at 70 eV.

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); *n*-BuOH–EtOH–H_2O (8:2:1) (solvent D); *n*-BuOH–HOAc–H_2O (4:1:2) (solvent E); EtOAc–HOAc–H_2O (9:2:1) (solvent F); EtOAc–C_5H_5N–HOAc–H_2O (5:5:1:3) (solvent G).

Isolation of saponins from the mature fruits. The mature fruits of *Fatsia japonica* Decne et Planch were extracted with Me_2CO. The Me_2CO soln, after concn at red pres, was defatted with *n*-hexane and evaporated to dryness (12 g). The residue was separated into four fractions by chromatography on a Si gel column (400 g, 3 × 100 cm, Merck 200 mesh) with $CHCl_3$–MeOH mixture with MeOH increasing from 0 to 40%. Each of the fractions was purified further by PLC on Si gel (Merck GF_{254}, 0.75 mm thick) with $CHCl_3$–MeOH (4:1) to give saponins **1** (0.15 g), **3** (1.325 g), **4** (0.690 g), and **5** (0.510 g.) The saponins **1, 4,** and **5** were crystallized from MeOH and the saponin **3** from MeOH–HOAc–H_2O.

Hydrolysis of **1.** **1** (50 mg) was refluxed with 2% H_2SO_4 for 6 hr. After addition of H_2O and filtration, the ppt (35 mg) was methylated with CH_2N_2, crystallized from MeOH and identified as hederagenin methyl ester (mp, mmp, co-TLC, NMR, IR and MS). The aq. mother liquor was neutralized with Amberlite IR-45 (OH^-) and subjected to PC with solvents A and B, and the presence of glucose was established.

Exhaustive methylation of **1** *and hydrolysis of the methylate.* Following the method developed by Hakomori [5], a mixture of **1** (146 mg) and DMSO (24 ml) was stirred for 1 hr at room temp. under N_2. NaH (500 mg) was added to the soln, which was further stirred under N_2 for 1.5 hr. An excess of CH_3I (2.6 ml) was then added to the soln with stirring during a period of 4.5 hr. After the reaction mixture was poured into ice-cold H_2O (300 ml), the methylated product was extracted with $CHCl_3$. The $CHCl_3$ soln was washed with H_2O and evaporated *in vacuo.* The residue was dissolved in EtOAc (200 ml). The EtOAc soln was washed with H_2O (300 ml × 4) to remove a trace of DMSO and on evaporation of the solvent gave the nona-*O*-methylate (104 mg) (**6**) as a syrup. A part (70 mg) of the syrup **6** was hydrolyzed with 10% methanolic HCl (10 ml) for 6 hr and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and dried over Na_2SO_4. Removal of the solvent gave 23-mono-*O*-methyl hederagenin methyl ester **7** (35 mg), which was identified by direct comparison (mp, mmp, co-TLC, IR, NMR and MS) with a known sample. The aq. layer was neutralized with Amberlite IR-45 (OH^-) and conc *in vacuo.* PC and co-PC analyses of the residue with solvents C, D and E showed the presence of 2,3,4,6-tetra-*O*-methylglucopyranose and 2,3,6-tri-*O*-methylglucopyranose.

Identification of **3.** **3** was hydrolyzed in the same way as **1** and shown to contain hederagenin and arabinose. Exhaustive methylation of **3** by Hakomori's method [5] yielded the methylate, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1721 (ester); NMR: δ 3.30–3.60 (15H, $-OCH_3$ × 5), 4.17 (1H, *d*, *J* 6Hz, anomeric H), 5.30 (1H, *br*, >C=C<u>H</u>–). Hydrolysis of this methylate by the same method as **1** gave 23-mono-*O*-methyl hederagenin methyl ester (mp, mmp, co-TLC and NMR) and 2,3,4-tri-*O*-methylarabinopyranose (co-PC).

Identification of **4.** On hydrolysis of **4** in the same way as **1**, **4** was established to be composed of hederagenin and glucose. This was confirmed further by the formation of 23-mono-*O*-methyl hederagenin methyl ester (mp, mmp, and NMR) and 2,3,4,6-tetra-*O*-methylglucopyranose (PC) on hydrolyzing the exhaustively methylated product, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1721 (ester); NMR: δ 3.29–3.60 (18H, $-OCH_3$ × 6), 4.18 (1H, *d*, *J* 7Hz, anomeric H), 5.28 ppm (1H, *br*, >C=C<u>H</u>–).

Identification of **5.** **5** was hydrolyzed to hederagenin, arabinose and glucose. Hydrolysis of the methylated derivative, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1721 (ester); NMR: δ 3.33–3.62 (24H, $-OCH_3$ × 8), 4.37 (1H, *d*, *J* 6Hz, anomeric H), 4.56 (1H, *d*, *J* 7Hz, anomeric H), 5.30 ppm (1H, *br*, >C=C<u>H</u>–); MS *m/e*: 703 ($M^+-C_8H_{15}O_4$), 483, 451, 305, 262, 219, 203, 187 (base), gave 23-mono-*O*-methyl hederagenin methyl ester (mp, mmp and NMR), 2,3,4,6-tetra-*O*-methylglucopyranose and 2,3-di-*O*-methylarabinopyranose (co-PC).

Isolation of saponins from the flowers. The flowers were extracted with Me_2CO. The Me_2CO soln, after concn, was defatted with *n*-hexane and evaporated to dryness (25 g). The residue was separated, by PLC on Si gel with $CHCl_3$–MeOH (4:1), into three fractions, which gave saponins **2** (1.05 g), **3** (400 mg) and **5** (6.2 g) respectively. The saponins **2** and **5** were crystallized from MeOH and the saponin **3** from MeOH–HOAc–H_2O.

In the same way as described above, **3** and **5** were identical to those isolated from the mature fruits.

Hydrolysis of **2.** **2** (45 mg) was refluxed with 2% H_2SO_4 for 6 hr. After addition of H_2O and filtration, the ppt. (31 mg) was methylated with CH_2N_2. The methylate was crystallized from MeOH and identified as methyl oleanolate (mp, mmp, co-TLC, NMR and IR). The aq. mother liquor, after neutralization with Amberlite IR-45 (OH^-), was tested by co-PC with solvents A and B, and the presence of glucose and arabinose was established.

Exhaustive methylation of **2** *and hydrolysis of the methylate.* Exhaustive methylation of **2** (136 mg) in the same way as

in the case of **1** gave penta-*O*-methylate (98 mg), which on hydrolysis in the same manner as above produced methyl oleanolate (55 mg), 2,3,4,6-tetra-*O*-methylglycopyranose and 2,3-di-*O*-methylarabinopyranose. These methylated sugars were identified by means of co-PC with solvents D, F and G.

Isolation of saponins from the leaves. The leaves were extracted with Me_2CO. The Me_2CO soln, after concn at red pres, was defatted with *n*-hexane and evaporated to dryness (7 g). The residue was separated into four fractions by chromatography on a Si gel column (350 g, Merck 200 mesh) with $CHCl_3$–MeOH with MeOH increasing from 0 to 40%. Each of the fractions was further purified by PLC on Si gel with $CHCl_3$–MeOH (4:1) to give saponins **2** (406 mg), **3** (346 mg), **4** (434 mg) and **5** (455 mg). Each saponin was crystallized in a similar manner as above. Compound **2** was identical to that isolated from the flowers and **3**, **4** and **5** were identical to the corresponding compounds isolated from the mature fruits.

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