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TRITERPENOID SAPONINS FROM THE FLOWER BUDS OF *FATSIA JAPONICA*

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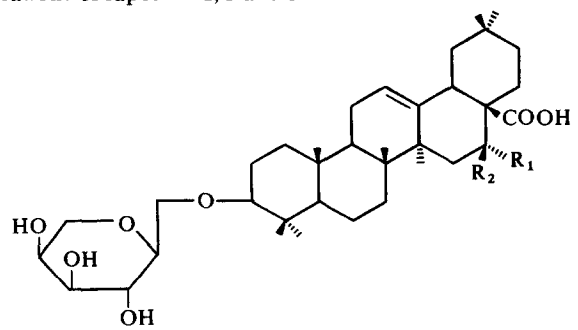
Key Word Index—*Fatsia japonica*; Araliaceae; flower buds; triterpenoid saponins; 3-*O*-[α-L-arabinosyl]-echinocystic acid; 3-*O*-[α-L-arabinosyl]-16-epiechinocystic acid; 3-*O*-[α-L-arabinosyl]-oleanolic acid.

Abstract—Six triterpenoid saponins isolated from the flower buds of *Fatsia japonica* were identified as 3-*O*-[β-D-glucopyranosyl(1 → 4)-α-L-arabinopyranosyl]-oleanolic acid, 3-*O*-[α-L-arabinopyranosyl]-hederagenin, 3-*O*-[β-D-glucopyranosyl(1 → 4)-α-L-arabinopyranosyl]-hederagenin, 3-*O*-[α-L-arabinopyranosyl]-echinocystic acid, 3-*O*-[α-L-arabinopyranosyl]-16-epiechinocystic acid and 3-*O*-[α-L-arabinopyranosyl]-oleanolic acid. Of these saponins, three are new.

INTRODUCTION

Fatsia japonica (Japanese name: Yatsude) has been described to contain highly hemolytic and toxic constituents [1–3]. This plant bears the flower buds in the early autumn, blooms in the autumn and bears fruits in the winter which mature in the early summer of the next year. Takamura *et al.* [4] have reported five triterpenoid saponins from the leaves of the plant, which were collected in the summer. In addition, Gabadadze *et al.* [5] isolated seven triterpenoid saponins from this plant cultivated in USSR. However, their structures are still not completely elucidated. Independently, we have isolated five triterpenoid saponins from the flowers, the mature fruits and the leaves of this plant. These triterpenoid saponins 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**) [6]. We have now investigated the saponin constituents of the flower buds and isolated six triterpenoid saponins, **2**, **3**, **5**, **6**, **7** and **8**. We report here evidence leading to structures **6**, **7** and **8** for three of the saponins which have not previously been reported in nature and the identifications of saponins **2**, **3** and **5**.



(**6**) R_1 = OH, R_2 = H
(**7**) R_1 = H, R_2 = OH
(**8**) R_1 = R_2 = H

RESULTS AND DISCUSSION

Saponin **6**, mp 222–224°, $[\alpha]_D^{25}$ + 42.1° (MeOH; *c* 0.24), afforded echinocystic acid and arabinose on hydrolysis with 2% H_2SO_4 for 6 hr. Exhaustive methylation of saponin **6** by Hakomori's method [7] gave penta-*O*-methylate (48.8%) (**9**) and tetra-*O*-methylate (51.2%) (**10**). The methylate (**9**) showed PMR signals for penta-*O*-methyls (δ 3.31–3.60 ppm), the 16β-H (δ 3.96 ppm, 1H, *m*), an anomeric proton (δ 4.25 ppm, 1H, *d*, *J* = 6 Hz) and an olefinic proton (δ 5.33 ppm, 1H, *m*). The molecular ion peak was at *m/e* 674 ($C_{40}H_{66}O_8$). From the above results, it was apparent that saponin **6** was composed of one molecule each of arabinose and echinocystic acid. The methylate (**9**) furnished 2,3,4-tri-*O*-methylarabinopyranose and methyl 16-mono-*O*-methyl echinocystate (**11**) on hydrolysis with 10% methanolic HCl for 6 hr. That the arabinopyranose was linked with echinocystic acid through the C-3 hydroxyl group was clear from PMR spectral data (δ 3.31 ppm, 3H, *s*, $>C_{(16)}HOC\underline{H}_3$; 3.60 ppm, 3H, *s*, $-COOC\underline{H}_3$; 3.96 ppm, 1H, *br*, $>C_{(16)}\underline{H}OMe$) of the methyl ester (**11**). Further, the MS peaks at *m/e* 483 ($C_{32}H_{51}O_3$), 278 ($C_{17}H_{26}O_3$), 277 ($C_{17}H_{25}O_3$), 260 ($C_{17}H_{24}O_2$), 245 ($C_{16}H_{21}O_2$), 201 ($C_{15}H_{21}$), 175 ($C_8H_{15}O_4$), 143 ($C_7H_{11}O_3$) and 101 ($C_5H_9O_2$) supported the structure of the methylate (**9**).

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 [8]. The α-glycosidic linkage was indicated by coupling constants of the anomeric proton in the PMR spectrum described above [9]. This was further confirmed, as shown in Table 1, by application of both Klyne's [10, 11] and Hudson's [12] rules using the values of the molecular rotation of the compounds listed in Table 2. The structure of saponin **6** was thus established to be 3-*O*-[α-L-arabinopyranosyl]-echinocystic acid.

The tetra-*O*-methylate (**10**) gave 2,3,4-tri-*O*-methylarabinopyranose and methyl echinocystate on acid

Table 1. $[M]_D$ of saponins **6**, **7** and **8** 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 of the sugars at C-1
Saponin **6**	+230.0°	+163.8°		
Ara in **6**			+94.5°	α
Saponin **7**	+279.9°	+302.1°		
Ara in **7**			+6.1°	α
Saponin **8**	+312.2°	+343.5°		
Ara in **8**			−2.9°	α

hydrolysis under the same condition as described above. This fact showed that the hydroxyl group located at C-16 is difficult to methylate and therefore it must be axial 16α-hydroxyl [13].

Saponin **7**, mp 211–216°, $[\alpha]_D^{25}$ +46.3° (MeOH; *c* 0.96), furnished a triterpenic acid and arabinose on acid hydrolysis under the same condition as saponin **6**. Saponin **7** was more polar than saponin **6** on TLC (Merck GF_{254}) with $CHCl_3$–MeOH (9:1). The methyl ester (**13**) of the triterpenic acid was found to be identical with methyl echinocystate (**12**) derived from saponin **6** on the basis of mp, mmp, co-TLC and PMR. Furthermore, identity of each methyl diketone [18, 19] produced from the echinocystate **12** and the methyl ester **13**, respectively, on oxidation with the Jones reagent [20] was established by comparison of mmp, co-GLC and PMR. These results demonstrate that the 16β-hydroxyl group of saponin **7** was epimerized to the stable 16α-hydroxyl group when saponin **7** was subjected to acid hydrolysis. From these facts, it became apparent that saponin **7** is an epimer of saponin **6** with respect to the C-16 hydroxyl group in the aglycone moiety.

Exhaustive methylation of saponin **7** by Hakomori's method [7] gave penta-*O*-methylate (78.2%) (**14**) and tetra-*O*-methylate (21.8%) (**15**). The methylate (**14**) revealed PMR signals for penta-*O*-methyls (δ 3.32–3.63 ppm), the 16α-H (δ 3.96 ppm, 1H, *m*), an anomeric proton (δ 4.25 ppm, 1H, *d*, J = 6 Hz) and an olefinic proton (δ 5.33 ppm, 1H, *m*) and exhibited the molecular ion peak at *m/e* 674 ($C_{40}H_{66}O_8$). These results showed that saponin **7** was composed of one molecule each of arabinose and 16-epiechinocystic acid. The methylate (**14**) gave 2,3,4-tri-*O*-methylarabinopyranose and methyl 16-mono-*O*-methyl echinocystate (**16**) on hydrolysis under the same condition as the methylate **9**. The PMR spectral data (δ 3.30 ppm, 3H, *s*, $>C_{(16)}HOC\underline{H}_3$; 3.60 ppm, 3H, *s*, $-COOC\underline{H}_3$; 3.98 ppm, 1H, *br*, $>C_{(16)}\underline{H}OMe$) of the methyl ester (**16**) revealed that the arabinopyranose was attached to the C-3 hydroxyl group of 16-epiechinocystic acid. On the basis of both the molecular rotation shown in Table 1 and the coupling constants of the anomeric proton signals of the methylate (**14**), the glycosidic linkage of the L-arabinopyranose was confirmed to be α. These results showed that the structure of saponin **7** was 3-*O*-[α-L-arabinopyranosyl]-16-epiechinocystic acid.

Table 2. $[M]_D$ of arabinopyranosides and echinocystic, 16-epiechinocystic and oleanolic acids used for the calculations

Compounds	$[M]_D$	References
Methyl α-L-arabinopyranoside	+28.37°	14
Methyl β-L-arabinopyranoside	+402.62°	14
Echinocystic acid	+135.46°	15
16-Epiechinocystic acid	+273.76°	16
Oleanolic acid	+315.12°	17

Table 3 The distribution of the saponins in the flower buds, flowers, fruits and leaves of *F. japonica*

Saponins	% Composition of the saponins			
	Flower buds	Flowers [6]	Fruits [6]	Leaves [6]
1	—	-	5.6	—
2	24.3	13.7	—	24.8
3	20.6	5.2	49.5	21.1
4	—	- -	25.8	26.4
5	28.2	81.1	19.1	27.7
6	10.7	—	—	- -
7	13.7	-	- -	- -
8	2.5	—	- -	—

The tetra-*O*-methylate (**15**) afforded 2,3,4-tri-*O*-methylarabinopyranose and methyl echinocystate on hydrolysis under the same condition as **9**. This result indicated that the formation of the tetra-*O*-methylate (**15**) is caused by incomplete methylation of saponin **7**, which seems to be due to the steric hindrance around the C-16 hydroxyl group in the aglycone moiety of saponin **7**.

Saponin **8**, mp 230–235°, $[\alpha]_D^{25}$ +53.1° (MeOH; *c* 2.00), afforded oleanolic acid and arabinose on hydrolysis under the same condition as saponin **6**. Exhaustive methylation of saponin **8** by Hakomori's method [7] gave tetra-*O*-methylate (**17**). The methylate **17** showed PMR signals for tetra-*O*-methyls (δ 3.34–3.62 ppm), an anomeric proton (δ 4.25 ppm, 1H, *d*, J = 6 Hz) and an olefinic proton (δ 5.25 ppm, 1H, *m*) and the MS spectral molecular ion peak at *m/e* 644 ($C_{39}H_{64}O_7$). These results showed that saponin **8** was composed of one molecule each of arabinose and oleanolic acid. The methylate **17** gave 2,3,4-tri-*O*-methylarabinopyranose and methyl oleanolate (**18**) on hydrolysis with 10% methanolic HCl for 6 hr. Thus the arabinopyranose was found to be linked to the C-3 hydroxyl group of oleanolic acid. Further, the MS peaks at *m/e* 453 ($C_{31}H_{49}O_2$), 393 ($C_{29}H_{45}$), 262 ($C_{17}H_{26}O_2$), 203 ($C_{15}H_{23}$), 175 ($C_8H_{15}O_4$) and 143 ($C_7H_{11}O_3$) supported the structure of the methyl ester (**17**). The α-glycosidic linkage was established by observation of coupling constants of the anomeric proton in the PMR spectrum of the methylate **17** and by application of both Klyne's [10, 11] and Hudson's [12] rules (Table 1). These facts demonstrated the structure of saponin **8** to be 3-*O*-[α-L-arabinopyranosyl]-oleanolic acid.

We examined the composition of the saponins **1**–**8** in the flower buds and compared the distribution of the saponins in the flower buds, the flowers, the fruits and the leaves of *F. japonica*. The results (Table 3) indicated that the distribution of the eight saponins differ in the four parts of this plant. The most interesting feature was that various monoarabinosides, such as **3**, **6**, **7** and **8**, were contained in the flower buds. The last three saponins were present in only the flower buds and not in the flowers, the fruits and the leaves. The major saponins of the flower buds were **2**, **3** and **5**, which were accom-

panied with fairly large quantities of **6** and **7** and a smaller amount of **8**. Neither the flower buds nor the flowers contained **1** and **4**. The distribution of the saponins **1**–**5** in the flowers, the fruits and the leaves was discussed in our previous paper [6].

EXPERIMENTAL

IR spectra were taken in KBr pellets or in CCl_4 soln. PMR spectra were obtained in a $CDCl_3$ soln using TMS as int. stand. MS analyses were performed with a direct inlet system, ionizing at the order of 70 eV. FID and a glass column (2 m × 3 mm) packed with OV-17 (2%) on Chromosorb W (80–100 mesh) was employed for GLC analyses. The samples of methyl-16-mono-*O*-methyl echinocystate and methyl diketoechinocystate were analyzed at 310° on OV-17. 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).

Isolation of saponins from the flower buds. The flower buds of *Fatsia japonica* Decne et Planch, collected in October in Hiroshima City of Japan, were extracted in Me_2CO for 3 months at room temp. The Me_2CO soln, after concn at red. press., was defatted with *n*-hexane and evapd to dryness to give an Me_2CO extract. This extract (7 g) was separated into six fractions by chromatography on a Si gel column (400 g, 4 × 100 cm, Merck 200 mesh) with $CHCl_3$–MeOH (MeOH increasing 0 to 40%). Each of the fractions was purified further by PLC on Si gel (Merck GF_{254}, 0.75 mm) with $CHCl_3$–MeOH (9:1) to give saponins **3** (449 mg), **6** (234 mg), **7** (298 mg) and **8** (55 mg) and further with $CHCl_3$–MeOH (4:1) to give saponins **2** (530 mg) and **5** (582 mg). The saponin **3** was crystallized from MeOH–HOAc–H_2O and saponins **6**, **7** and **8** from MeOH–H_2O and saponins **2** and **5** from MeOH.

Hydrolysis of **6**. **6** (50 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, crystallized from MeOH–H_2O and identified as methyl echinocystate (mp, PMR, IR and MS) [14, 18, 19, 21]. The aq. mother liquor was neutralized with Amberlite IR-45 (OH^-) and subjected to PC with solvents A and B, and the presence of arabinose in the mother liquor was established.

Exhaustive methylation of **6** *and hydrolysis of the methylether.* Following the method developed by Hakomori, a mixture of NaH (800 mg) and DMSO (22 ml) was stirred for 1 hr at room temp. under N_2, and **6** (180 mg) dissolved in DMSO (22 ml) was added to the mixture. After the soln was stirred for 1 hr under N_2, an excess of MeI (5 ml) was added to the soln with stirring during a period of 4 hr. The reaction mixture was poured into ice-cold H_2O (300 ml), and then the methylated product was extracted with EtOAc. The EtOAc soln was washed with H_2O (300 ml × 3) to remove a trace of DMSO and gave on evapn of the solvent penta-*O*-methylate (84 mg) (**9**) and tetra-*O*-methylate (88 mg) (**10**) as a syrup, respectively. A portion (54 mg) of **9** was hydrolysed with 10% methanolic HCl (10 ml) for 6 hr and extracted with $CHCl_3$. The $CHCl_3$ layer washed with H_2O and dried over Na_2SO_4 gave on removal of the solvent methyl 16-mono-*O*-methyl echinocystate (20 mg) (**11**) (identified by direct comparison of mp, IR, PMR and MS) and 2,3,4-tri-*O*-methylarabinopyranose (identified by co-PC with solvents C, D and E).

Hydrolysis of **7**. On hydrolysis under the same condition as **6**, **7** (50 mg) furnished arabinose (co-PC) and echinocystic acid (28 mg), which after methylation with CH_2N_2 was identified by direct comparison (mp, mmp and PMR) with a known sample.

Exhaustive methylation of **7** *and hydrolysis of the produced methylate.* Exhaustive methylation of **7** (70 mg) in the same way as **6** gave penta-*O*-methylate (54 mg) (**14**) and tetra-*O*-methylate (15 mg) (**15**). On hydrolysis in the same way as 9, the penta-*O*-methylate (40 mg) (**14**) gave methyl 16-mono-*O*-methyl echinocystate (20 mg) (identified by direct comparison of mp, mmp, co-GLC and PMR) and 2,3,4-tri-*O*-methylarabinopyranose (co-PC).

Jones oxidation of **12** *and* **13**. The oxidation was carried out by titrating a stirred soln of **12** (64 mg) in Me_2CO (2 ml) at 20° with the Jones reagent (0.8 ml) [20]. The product was purified on a Si gel plate (20 × 20 cm, Merck GF_{254}, 0·75 mm) by using *n*-hexane–EtOAc (4:1) to give methyl diketoechinocystate (43 mg), mp 158–160°; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3400 (no OH), 1696 (C=O); NMR: δ 0.89, 0.90, 0.93, 1.06, 1.08 and 1.20 ppm (—C<u>H</u>$_3$ × 7), 3.63 ppm (3H, *s*, —COOC<u>H</u>$_3$), 5.53 ppm (1H, *m*, >C=C<u>H</u>—); MS *m/e*: 482 (M^+), 464, 423 (base), 276, 217, 216 and 203 [22]. **13** was oxidized to methyl diketones under the same conditions as **12**. The identity of each methyl diketone produced was confirmed by direct comparisons of mp, mmp, co-GLC and PMR with a known sample.

Hydrolysis of **8**. Hydrolysis of **8** (25 mg) in the same way as **6** afforded arabinose (co-PC) and oleanolic acid (15 mg), which was identified by direct comparison (mp, mmp and PMR) of its methyl ester with a known sample.

Exhaustive methylation of **8** *and hydrolysis of the produced methylate.* Exhaustive methylation of **8** (30 mg) by Hakomori's method [7] furnished tetra-*O*-methylate (21 mg) (**17**), which on hydrolysis in the same way as **9** gave methyl oleanolate (**18**) (mp, mmp and PMR) and 2,3,4-tri-*O*-methylarabinopyranose (co-PC).

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