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HEXANE SOLUBLE NON-VOLATILES IN FLOWERING TO FRUITING STAGES OF *FATSIA JAPONICA*

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Key Word Index—*Fatsia japonica*; Araliaceae; flowering; fruiting; fatty acids; fatty acid methyl esters; triglycerides; sterols; steryl and β-amyrenyl esters; distribution of the compounds.

Abstract—The *n*-hexane soluble non-volatile fraction of the acetone extracts from the flower buds, the flowers and the immature and the mature fruits of *Fatsia japonica* were all found to contain fatty acids, fatty acid methyl esters, squalene, β-amyrin and sterols. At all the stages between budding to the mature fruit, the major fatty acids were palmitic and linoleic acids and the major phytosterol was stigmasterol. In addition steryl and β-amyrenyl esters were found in the flowers and the immature and the mature fruits, but these esters were not present in the flower buds. Sitosteryl ester was the major constituent of the steryl ester fraction in the fruiting stages. Phytol was found in only the flowering stage and triglycerides in only the mature fruits. The variations in the lipid constituents is discussed in relation to the stages from budding to the mature fruit.

INTRODUCTION

Fatsia japonica (Japanese name: Yatsude) bears the flower buds in the early autumn and the fruits in the winter. The fruits mature in the early summer of the next year. This plant has been reported to contain highly hemolytic and toxic constituents [1–3]. There have also been reports on the saponins found in the leaves [1–5], the flowers [5] and the roots [3] and on steam-distillates of the leaves [6]. However, there has been no work on the *n*-hexane soluble non-volatiles of the flower buds, the flowers and the fruits. Previously we reported [7] the lipid constituents of the *n*-hexane soluble, non-volatile fraction of the leaves; the constituents were phytyl palmitate, phytyl linoleate, stigmasterol, phytol, *n*-alkanes and fatty acids.

We have now examined the lipid constituents of the *n*-hexane soluble non-volatile fraction of the flower buds, the flowers and the immature and the mature fruits of *F. japonica* to determine the variations in the lipid constituents at these four stages and to make comparisons of the constituents with those found in the leaves [7].

RESULTS AND DISCUSSION

The flower buds in October, the flowers in November, the immature fruits in December, the mature fruits in June of the next year and the leaves in March [7] were collected and each part was immersed in acetone to extract its lipid constituents. The acetone extract was concentrated to dryness to give a viscous oily substance, which was then extracted with *n*-hexane. This *n*-hexane soluble part was treated in the usual way to give acidic and neutral fractions. The lipid constituents of these two fractions at each stage from the budding to the maturing fruit were examined.

All the *n*-hexane soluble fractions obtained from the four stages were found to contain fatty acids, fatty acid methyl esters, squalene, β-amyrin and sterols. In addition to these compounds, steryl and β-amyrenyl esters were found in the flowers and the immature and the mature fruits, but these esters were not present in the flower buds. Phytol and triglycerides occurred in the flowers and the mature fruits. Table 1 shows the quantities of the lipid constituents in the budding to the maturing stages of the plant. The fatty acid compositions of the acid and the ester fractions and the sterol compositions of the free and the esterified sterol fractions in the budding to the maturing stages are shown in Tables 2 and 3, respectively.

Generalizations could not be drawn due to the variations of the total quantities of free fatty acids in the budding to the maturing stages (Table 1) but is was clear that (a) the proportion of oleic acid in the free fatty acid fractions increased gradually with the progression from the budding to the maturing stage, (b) the major fatty acid of the acid fractions was linoleic acid in the flower buds and the immature and the mature fruits and palmitic acid in the flowers and (c) the proportion of free linolenic acid was small in the flower buds, the flowers and the mature fruits, but it was appreciable in the immature fruits and and the leaves [7], as shown in Table 2. On the other hand, the quantities of fatty acid methyl esters decreased gradually from the flowering to the maturing stage (Table 1). The fatty acid pattern of the fatty acid methyl esters was very similar to the pattern of the free fatty acids at the four stages of development (Table 2). Triglycerides occurred in only the mature fruits. The fatty acid pattern of triglycerides was different from that of the free fatty acids present in the mature fruits. One notable feature was that oleic acid was the major fatty acid (up to 54%). In the flowers and the immature and the mature fruits, the fatty acid pattern of the steryl and β-amyrenyl esters was different to that of the free fatty acids and the fatty acid methyl esters. The most abundant fatty acid of the steryl and β-amyrenyl esters was palmitic acid throughout the

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Table 1. The quantities of the lipid constituents in the budding to the fruiting stages of *F. japonica*

Constituents	Quantities of the constituents in the stages*				
	Flower buds	Flowers	Immature fruits	Mature fruits	Leaves [7]
Fatty acids	0.0021	0.0310	0.0010	0.0336	0.0019
Fatty acid methyl esters	0.0494	0.0044	0.0017	0.0015	—
Triglycerides	—	—	—	0.0215	—
Squalene	trace†	trace†	trace†	trace†	trace†
β-Amyrin	trace†	trace†	trace†	trace†	trace†
Sterols	0.0016	0.0044	0.0001	trace†	0.0012
β-Amyrenyl esters / Steryl esters	—	0.0002	0.0001	0.0005	—
Phytol	—	0.0010	—	—	0.0012
Phytyl palmitate	—	—	—	—	0.0013
Phytyl linoleate	—	—	—	—	0.0031
n-Alkanes	—	—	—	—	0.0001

* Expressed as the weight per cent of the constituents for the weight of the fresh plant materials.
† These were detected by co-TLC and co-GLC, respectively.

flowering and the maturing stages, but this fatty acid decreased with the fruiting, as shown in Table 2. These results indicate that in all the stages concerned the free fatty acids and the fatty acid methyl esters are in a state of simple equilibrium, but the fatty acid patterns of the triglycerides and steryl and β-amyrenyl esters are independent of the fatty acid patterns of the free fatty acids and the fatty acid methyl esters.

A considerable amount of free sterol was present at the flowering stage, but the quantity of sterol diminished in the fruiting stages (Table 1). The major free sterol was stigmasterol in all the stages (Table 3). The proportion of free stigmasterol gradually decreased with the progression of the stages from budding to fruiting, but sitosterol increased with the fruiting. Cholesterol was not present in the flower buds, but it was found in the flowers and the immature and the mature fruits in traces to small amounts. On the other hand, the sterol compositions of steryl esters were markedly different in the flowers and the immature and the mature fruits (Table 3). In the following stage, the major sterol of the steryl esters was stigmasterol as found in the free sterols. With the changes from the flowering to the maturing fruit the proportion of of sitosterol in the esterified sterols increased and reached a maximum at the maturing stage. A considerable amount of esterified cholesterol was present in the immature fruits, but it diminished in the maturing stage. Thus, it was found that the free and the esterified sterols are not in an equilibrium state [8, 9].

Squalene and β-amyrin occurred in only trace amounts in the budding to the fruiting stages. Appreciable amounts of the triterpenoid saponins have been found in the flowers, the mature fruits and the leaves of *F. japonica* [4, 5]. Accordingly, squalene and β-amyrin may be rapidly transformed to the triterpenoids [10–13], followed by conversion into the triterpenoid saponins [14]. The other interesting feature in the budding to the fruiting stages was the absence of *n*-alkanes, phytyl palmitate and phytyl linoleate which occur in the leaves [7]. However, phytol did occur in the free form in the flowers.

Table 3. Sterol compositions of free and esterified sterols in the budding to the fruiting stages of *F. japonica*

	% Composition of sterols*					
	Cholesterol		Stigmasterol		Sitosterol	
	F	E	F	E	F	E
Flower buds	—	—	94.9	—	5.1	—
Flowers	0.5	---	94.7	96.3	4.8	3.7
Immature fruits	trace	19.8	86.9	13.1	13.1	67.1
Mature fruits	2.5	2.7	73.8	11.3	23.7	86.0

* F and E denote free and esterified sterols, respectively.

EXPERIMENTAL

IR spectra were taken in KBr pellets, CCl_4 soln or as liquid films. 60 MHz NMR spectra were measured in a $CDCl_3$ soln using TMS as internal standard. MS analyses were performed with a direct inlet system at 70 eV. GLC employed FID and a glass column (2.0 m × 3 mm) packed with OV-17 (2%), OV-101 (2%) or DEGS (15%) on Chromosorb W (80–100 mesh). Fatty acid methyl esters were analyzed at 195° on DEGS,

Table 2. Fatty acid compositions of the free acids and

	Flower buds % Composition of fatty acids						Flowers % Composition of fatty acids					
	16:0	18:0	18:1	18:2	18:3	unidentified	16:0	18:0	18:1	18:2	18:3	unidentified
Free fatty acids	31.8	0.6	0.6	57.6	9.4	—	74.4	2.0	2.9	17.2	1.8	1.7
Fatty acid methyl esters	24.2	0.1	0.2	66.5	9.0	—	69.8	3.1	4.1	21.1	1.9	—
Triglycerides	—	—	—	—	—	—	—	—	—	—	—	—
Steryl and β-amyrenyl esters	—	—	—	—	—	—	82.9	1.4	6.1	1.8	1.2	6.6

sterols at 310° on OV-17 and at 280° on OV-101, squalene and β-amyrin at 300° on OV-17 and glycerol at 100° on OV-17, respectively. Retention times were compared to those of authentic samples. The relative retention times of the methyl esters of palmitic, stearic, oleic, linoleic and linolenic acids were 1.00, 1.78, 2.11, 2.69 and 3.61, respectively and those of cholesterol, stigmasterol and sitosterol were 1.00, 1.25 and 1.45, respectively.

Materials. The flower buds (9.7 kg) in October, the flowers (9.0 kg) in November, the immature fruits (0.69 kg) in December, the mature fruits (12.5 kg) in June of the next year and the leaves (14.0 kg) in March [7] were collected in Hiroshima City, Japan.

Extraction and isolation. Each part of *Fatsia japonica* Decne et Planch was immersed in Me_2CO for 3 months at room temp. to extract the constituents. The acetone soln, after concn at red. pres., was extracted with *n*-hexane. The *n*-hexane extract was treated with 5% $NaHCO_3$ and then 5% NaOH and separted into two acidic fractions and a neutral fraction. As the two acidic fractions exhibited the same behaviour on TLC, these fractions were combined. The combined acidic fraction was methylated with CH_2N_2 and then subjected to co-GLC with authentic samples. The neutral fraction was separated by column-chromatography on Si gel (Merck 200 mesh) with a *n*-hexane–EtOAc mixture with EtOAc increasing 0 to 40%. Each component was purified further by means of PLC, $AgNO_3$-impregnated PLC or continuously developing PLC.

Identification of compounds. Squalene ($C_{30}H_{50}$), after purification by PLC on Si gel (Merck GF_{254}, 0.25 mm thick) with *n*-hexane, was identified by co-GLC with an authentic sample. β-Amyrin ($C_{30}H_{50}O$) was purified by PLC on Si gel impregnated with 10% $AgNO_3$ using C_6H_6–EtOAc–MeOH (90:9:1) [15] and then subjected to co-GLC with an authentic sample. Stigmasterol ($C_{29}H_{48}O$), mp 166–167° (crystallized from MeOH); $[\alpha]_D^{25}$ −41.7° ($CHCl_3$; *c* 0.420) [16]; IR ν_{max}^{KBr} cm^{-1}: 3400 (O—H), 1620 (C=C), 969 (*trans* —CH=CH—), 837 (>C=CH—); NMR: δ 5.06 (2H, *t*, *J* = 3 Hz, *trans* —CH=CH—), 5.37 ppm (1H, *br*, >C=CH—); MS *m/e*: 412 (M^+), 55 (base), was purified by PLC on a Si gel (Merck GF_{254}, 0.25 mm thick) impregnated with 10% $AgNO_3$ using *n*-hexane–EtOAc (4:1) and identified by mp, mmp, IR, NMR, MS and co-GLC comparisons with an authentic sample. Phytol ($C_{20}H_{40}O$), $[\alpha]_D^{25}$ +0.89° ($CHCl_3$; *c* 0.563); IR ν_{max}^{liquid} cm^{-1}: 3420 (O—H), 1610 (C=C); NMR: δ 1.67 (3H, *s*, >C=CCH$_3$), 4.18 (2H, *d*, *J* = 7 Hz, =CH—CH$_2$—O—), 5.43 ppm (1H, *t*, *J* = 7 Hz, >C=CH—CH_2—O—); MS *m/e*: 296 (M^+), 71 (base), was purified by continuously developing PLC on 10% $AgNO_3$-Si gel with *n*-hexane–EtOAc (49:1) and identified by comparisons of IR, NMR and MS with a known sample [17, 18]. Steryl esters and β-amyrenyl esters, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2910, 2835, 1455 (—CH_2—, Me—), 1745, 1175 (ester —CO—O—), 1640 (C=C), 1371, 1362 (>CMe_2), could not be separated from each other even by means of continuously developing PLC on 10% $AgNO_3$-Si gel. A mixture of the ester was submitted to saponification with 5% methanolic KOH for 1.5 hr to give a mixture of cholesterol, stigmasterol, sitosterol and β-amyrin (identified by co-TLC and co-GLC) (Table 3) and a mixture of palmitic, stearic, oleic, linoleic and linolenic acids (identified by co-GLC of the methylated acids) (Table 2). Thus, the mixture was judged to be composed of steryl esters and β-amyrenyl esters. Triglycerides, IR ν_{max}^{liquid} cm^{-1}: 1737, 1150 (ester —CO—O—), 1645 (C=C); NMR: δ 1.97 (*m*, —CH=CH—CH$_2$—CH_2—), 2.29 (*t*, *J* = 7 Hz, —CO—CH$_2$—CH_2—), 4.16 or 4.23 (*d*, *J* = 3 Hz, >CH—CH$_2$—O—CO—), 5.33 ppm (*m*, —CH_2—CH—CH_2—; —CH_2—CH=CH—CH_2—), were characterized by furnishing glycerol (identified by co-GLC) as an alcohol and palmitic, stearic, oleic and linoleic acids (identified by co-GLC of the methylated acids) as an acid (Table 2), after saponification with 5% methanolic KOH for 1.5 hr.

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esters in the budding to the fruiting stages of *F. japonica*

Immature fruits % Composition of fatty acids						Mature fruits % Composition of fatty acids					
16:0	18:0	18:1	18:2	18:3	unidentified	16:0	18:0	18:1	18:2	18:3	unidentified
19.6	0.4	17.6	40.1	18.7	3.6	27.4	0.9	29.6	30.5	5.4	6.2
17.9	0.6	9.6	45.8	22.7	3.4	28.5	0.6	16.4	39.3	11.6	3.6
—	—	—	—	—	—	24.1	0.6	54.7	20.6	—	—
74.4	1.6	12.2	10.1	—	1.7	58.0	1.3	13.7	22.2	1.4	3.4