SEED OILS FROM EIGHT SPECIES OF JAPANESE PLANTS; *MELOTHRIA JAPONICA, ARALIA ELATA, GILIBERTIA TRIFIDA, KALOPANAX INNOVANS, IDESIA POLYCARPA, STAPHYLEA BUMALDA, EUSCAPHIS JAPONICA* AND *PRUNUS SPINULOSA*

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This paper records the results of our study on the properties and components of the seed oils from the Japanese plants enumerated in the title. No literature has been known to us, which deals with these seed oils except those from *A. elata* and *G. trifida*. However, the seed oil from *S. pinnata*[1)2)], a species of the genus *Staphylea* like *S. Bumalda*, and seed oils from a number of species other than *P. spinulosa* of the genus *Prunus*[3)] have been reported by previous authors. Relating to seed oils of the family *Araliaceae*, the occurrence of petroselinic acid has already been confirmed in seed oils from *Hedera rhombea*[4)5)], *Fatsia japonica*[6)], *Panax Ginseng*[7)] and *A. elata* var. *canescens*[8)]. Recently it has been stated that the seed oil from *G. trifida*[9)] also appears to contain petroselinic acid. Furthermore, seed oils from *A. elata*[10)] and *A. cordata*[11)] have been reported to contain a solid unsaturated acid fraction of high melting point.

In this study, the presence of petroselinic acid in the seed oils from three Japanese species of the *Araliaceae*, *A. elata*, *G. trifida* and *K. innovans*, has been demonstrated. Although the occurrence of petroselinic acid in seed oils from various species of the *Umbelliferae* is known, it is noteworthy that this acid is widely distributed also in seed oils from the *Araliaceae*. It is also worthy of special mention in this study that elaeostearic acid has been separated and identified from the seed oil of *P. spinulosa* of the genus *Prunus*. Elaeostearic acid is known as a major component of the fatty acids of tung oil. It occurs also in several other oils. But the occurrence of this acid in a seed oil from the genus *Prunus* has hitherto been reported only for the oil of *P. mahaleb*[12)]. It appears to be of interest to study the distribution of elaeostearic acid in seed oils from other species of the genus *Prunus*.

1. Seeds used for extraction of oil

Regarding the seeds used for extraction of oil in this study, the name of the species of parental plants, the growing place, the date of the harvest of fruits and the yield of seed oil are shown in Table 1. Seeds were separated from fruits and dried in an infrared drying oven. The dried seeds were crushed and then extracted with ether. To the ether-extract was added about ten times its weight

TABLE 1. List of Seed Samples

Species	*Melothria japonica* Maxim.	*Aralia elata* Seem.	*Gilibertia trifida* Makino.
Family	*Cucurbitaceae*	*Araliaceae*	*Araliaceae*
Growing place	Atsuta-ku, Nagoya	Naga-gun, Mie-ken	Atsuta-ku, Nagoya
Date of harvest of fruits	Early Nov., 1957	Late Nov., 1955	Early Nov., 1957
Wt. of fruits (g)	1,875	224	2,030
Wt. of dried seeds (g)	215	28	470
Average wt. of one dried seed (g)	0.006	0.0008	0.020
Ether-extract (g)	65.5	7.8	136.2
Ether-extract (%)	30.5	27.9	29.0
Hexane-sol. oil (g)	64.1	7.2	133.5
Hexane-sol. oil (%)	29.8	25.7	28.4

Notes: Fruits of *S. Bumalda* and *E. japonica* are weighed after removal of capsules. Percentage yields of ether-extract and hexane-soluble oil are expressed on the basis of dried seeds.

TABLE 2. Properties of Oil

Oil		*M. japonica*	*A. elata*	*G. trifida*
Appearance at ordinary temperature		Yellowish brown	Dark green, with a large amount of solid	Yellowish brown with some solid
d_4^{20}		0.9203	0.9185	0.9157
n_D^{20}		1.4780	1.4728	1.4717
Acid value		1.7	4.0	1.9
Saponification value		185.2	191.4	191.5
Iodine value		146.6	96.2	92.2
Unsaponif. matter (%)		4.30	3.63	3.13
Fatty acids	n_D^{30}	1.4641	1.4603	1.4597
	Neutralization value	193.3	199.7	199.7
	Iodine value	151.7	99.5	93.2
	Acetyl value	7.1	2.2	0.3
	Ether-insol. bromide (%)	0	0	0

Notes: Iodine values are determined by the Wijs method. Acetyl values for the fatty acids are calculated from the values determined with the methyl esters of fatty acids. The fractions insoluble in cold methanol from the unsaponifiable matter of *M. japonica*, *G. trifida* and *K. innovans* oils show iodine values 160.5, 184.9 and 168.8, respectively.

A. elata oil reported by Ueno and Matsushima[10] has d_4^{15} 0.9222, n_D^{20} 1.4716, acid value 3.7, saponif. value 192.5, iodine value 101.8 and unsaponif. matter 1.2%; its fatty acids have neutralization value 198.4 and iodine value 105.6. *G. trifida* oil reported by Kashimoto[9] has d_4^{30} 0.9137, n_D^{25} 1.4644, acid value 3.97, saponif. value 163.9, iodine value 89.52 and unsaponif. matter 2.36%.

Kalopanax innovans Miq.	*Idesia polycarpa* Maxim.	*Staphylea Bumalda* Sieb. et Zucc.	*Euscaphis japonica* Pax.	*Prunus spinulosa* Sieb. et Zucc.
Araliaceae	*Flacourtiaceae*	*Staphyleaceae*	*Staphyleaceae*	*Rosaceae*
Chikusa-ku, Nagoya	Hida-gun, Oita-ken	Higashikamo-gun, Aichi-ken	Higashikamo-gun, Aichi-ken	Chikusa-ku, Nagoya
Middle Nov., 1956	Middle Oct., 1957	Late Nov., 1956	Late Nov., 1956	Late Apr., 1957
520	832	80	137	605
155	198	72	110	328
0.013	0.002	0.025	0.051	0.058
41.9 27.0	54.5 27.5	17.3 24.0	11.6 10.6	53.2 16.2
40.5 26.1	54.4 27.5	17.1 23.8	10.8 9.8	45.1 13.8

K. innovans	*I. polycarpa*	*S. Bumalda*	*E. japonica*	*P. spinulosa*
Reddish brown, with a small amount of solid	Yellowish orange	Yellow	Yellow	Dark greenish brown
0.9182	0.9250	0.9249	0.9238	0.9393
1.4726	1.4785	1.4774	1.4747	1.5143
3.1	2.1	1.4	2.9	5.3
190.6	192.7	196.1	194.7	196.0
95.9	148.7	136.8	103.5	157.8
2.15	1.66	4.21	5.27	1.75
1.4600	1.4648	1.4639	1.4616	1.5022
199.3	199.8	203.9	202.7	198.3
97.9	153.7	142.4	107.6	159.2
2.3	8.9	5.7	—	—
0	0	5.7	0	0

of hexane, and the hexane-insoluble matter was removed by filtration. The seed oil was obtained from the filtrate by distilling off hexane.

2. Properties of oil

Characteristics of the seed oils and their fatty acids prepared in the usual way are given in Table 2.

i. Bromination test. The ether-insoluble bromide from the fatty acids of *S.*

Bumalda oil had m.p. 180.5°–181.5°C after recrystallization from benzene and showed no depression of melting point when mixed with a specimen of hexabromostearic acid prepared from linolenic acid. The ether-soluble bromide fraction obtained in the usual way was treated with hexane to give the hexane-insoluble bromide which showed m.p. 113.5°–114°C after recrystallization from 90% ethanol and was identified with tetrabromostearic acid derived from linoleic acid by the mixed melting point test.

Fatty acids from *M. japonica, A. elata, G. trifida, K. innovans, I. polycarpa* and *E. japonica* oils yielded no ether-insoluble bromide but hexane-insoluble bromide which, after recrystallization from 90% ethanol, showed the following melting points, alone and mixed with tetrabromostearic acid prepared from linoleic acid: 113°–114°C, 112.5°–113.5°C, 113°–114°C, 114°–115°C, 113°–114°C and 113°–114.5°C, respectively.

The bromination of the fatty acids of *P. spinulosa* oil, carried out in the usual way, gave no ether-insoluble bromide, indicating that linolenic acid is absent or present only in an extremely minor amount in this oil. The total bromination product was somewhat unstable to heat, and the hexane-insoluble bromide could not be separated. Therefore, the fatty acids of this oil were dissolved in ten times their weight of hexane and brominated at −15°C. The insoluble bromide formed was once separated and refluxed with hexane. The solution was then allowed to cool at the room temperature, and the insoluble bromide formed was separated and treated with hexane as before. After repeating these treatments several times, the hexane-insoluble bromide eventually obtained had m.p. 113°–114°C. The melting point was not lowered on admixture with a specimen of tetrabromide, m.p. 113°-114°C, derived from elaeostearic scid.

ii. Petroselinic acid from *A. elata, G. trifida* and *K. innovans* oils. The fatty acids of *A. elata* oil were separated into the solid and liquid acids by the lead salt ethanol method. The solid acids had m.p. 25°–27°C and iodine value 73.9. Recrystallization of these solid acids from 60% ethanol gave a crystalline acid (*A*) which had m.p. 30°–31°C and showed no depression of melting point on admixture with petroselinic acid, m.p. 31°–32°C, prepared from *Fatsia japonica* oil. The elaidinization product obtained by treating the acid *A* with sodium nitrite and nitric acid (30%) showed m.p. 49°–50°C after recrystallization from 60% ethanol. The melting point was not lowered when mixed with a specimen of elaidinized petroselinic acid, m.p. 52°–53°C.

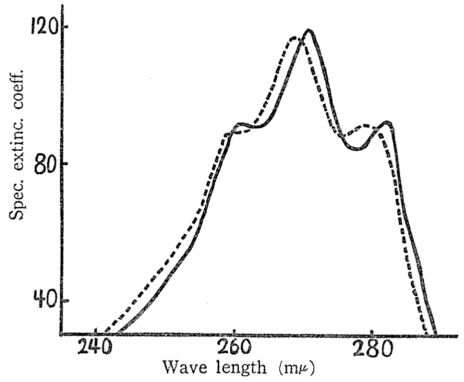
The solid acids were separated from *G. trifida* oil by the lead salt ethanol method; yield 53.0%, neutralization value 199.7 and iodine value 84.1. These solid acids were recrystallized from 60% ethanol giving a crystalline acid of m.p. 32°–33°C, neutralization value 199.2 and iodine value 88.3. This acid and its elaidinization product, m.p. 52°–52.5°C, were recognized as petroselinic acid and elaidinized petroselinic acid, respectively, by the mixed melting point test.

In the case of the fatty acids of *K. innovans* oil, the lead salt of solid acids was recrystallized several times from 90% ethanol. The solid acids liberated from the recrystallized lead salt gave a crystalline acid of m.p. 31°–32°C after purification by recrystallization. The elaidinization product showed m.p. 51°–52°C. These were identified with petroselinic acid and elaidinized petroselinic acid, respectively, by the mixed melting point test.

Infrared absorption measurements on the solid acids from *G. trifida* and *K.*

innovans oils indicated the absence of *trans* double bond suggesting the absence of preformed elaido-petroselinic acid in these oils.

iii. Conjugated unsaturated acids. Each seed oil, except *P. spinulosa* oil described later, was saponified with a 1/5 solution of potassium hydroxide in methanol. The saponification product was diluted with methanol and examined for its ultraviolet absorption spectra. *S. Bumalda* and *E. japonica* oils exhibited an absorption peak at 233 mμ. The contents of conjugated dienoic acids in these oils were calculated from the specific extinction coefficients at 233 mμ as 3.8% and 4.0%, respectively. In the case of the five oils other than the above mentioned two oils, no absorption maximum at 233 mμ was observed suggesting the absence of conjugated dienoic acids in these five oils.



—— : *P. spinulosa* oil

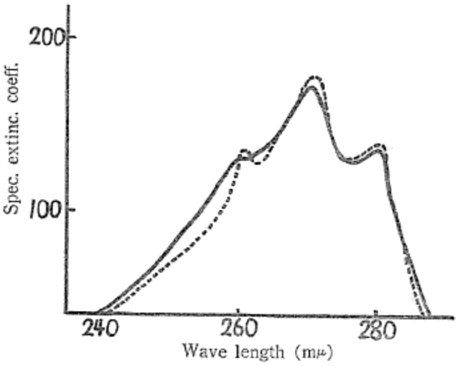
----- : Alkali-isomerized fatty acids from *P. spinulosa* oil

FIG. 1. Ultraviolet absorption curves for *P. spinulosa* oil and alkali-isomerized fatty acids from *P. spinulosa* oil

iv. Elaeostearic acid from the seed oil of *P. spinulosa.* The fatty acids from the seed oil of *P. spinulosa* are a yellowish brown solid of m.p. 30°–34°C. They are prone to undergo some change and form a brown viscous product during their storage in hexane. They had a very high refractive index, n_D^{30} 1.5022. Their iodine value determined by the Wijs method was found to be 159.2 after 2 hours' reaction period and 170.4 after 24 hours' reaction period. The ultraviolet absorption measured in heptane for *P. spinulosa* oil exhibited absorption peaks at 261 mμ, 270.5 mμ and 282 mμ with the specific extinction coefficients of 92.2, 121.1 and 94.0, respectively (Fig. 1).

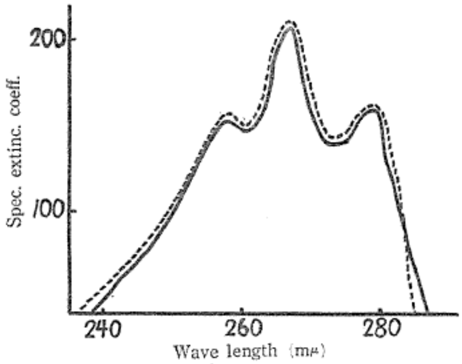
The mixed fatty acids (6.8 g) were separated into three fractions by the lithium salt acetone method. The yield, the melting point and the refractive index for each fraction are as follows: (I) 1.0 g, m.p. 36°–37°C, n_D^{30} 1.4986; (II) 4.2 g, m.p. 35°–36°C, n_D^{30} 1.5020; (III) 1.4 g, dark brown viscous liquid, n_D^{30} 1.5042. The fraction II was recrystallized from 80% ethanol, and the recrystallized material was treated with hexane in order to remove the hexane-insoluble matter. The crystalline acid (*B*) obtained from the hexane solution after removal of hexane had m.p. 46.5°–47.5°C. No depression of melting point was observed on admixture with a specimen of elaeostearic acid, m.p. 47°–48°C, prepared from tung oil.

When a solution of the acid *B* in hexane with the addition of a bit of iodine was allowed to stand over a night, a viscous oily substance (polymerization product) separated from the solution. This was removed by filtration, and the clear filtrate gave a crystalline acid (*C*) of m.p. 69.5°–70°C after removal of hexane. The melting point was not lowered on admixture with β-elaeostearic acid, m.p. 70°–70.5°C. Ultraviolet absorption values for the crystalline acids, *B* and *C*, along with α- and β-elaeostearic acids were measured in ethanol. The acid *B* exhibited absorption peaks at 260 mμ, 270 mμ and 280 mμ with the specific extinction coefficients of 132.7, 173.2 and 136.8, respectively, while α-elaeostearic acid showed the corresponding values of 134.1, 177.2 and 138.6, respectively (Fig. 2). The acid *C*



——: Crystalline acid *B* from *P. spinulosa* oil
-----: α-Elaeostearic acid

FIG. 2. Ultraviolet absorption curves for the crystalline acid *B* from *P. spinulosa* oil and α-elaeostearic acid



——: Crystalline acid *C* from *P. spinulosa* oil
-----: β-Elaeostearic acid

FIG. 3. Ultraviolet absorption curves for the crystalline acid *C* from *P. spinulosa* oil and β-elaeostearic acid

exhibited absorption peaks at 258 mμ, 267 mμ and 279 mμ with the specific extinction coefficients of 153.1, 206.9 and 160.7, respectively, while β-elaeostearic acid showed the corresponding values of 156.4, 211.2 and 163.0, respectively (Fig. 3).

The content of elaeostearic acid in *P. spinulosa* oil is estimated at about 70% from the ultraviolet absorption data for this oil.

3. Fatty acid composition

The fatty acid composition for each oil was approximately estimated in the following way.

The fatty acids were isomerized under the condition of 6.5–6.6% KOH-ethylene glycol, 180°C and 25 minutes with a current of nitrogen, and the specific extinction coefficients of isomerized fatty acids at 233 mμ and 268 mμ were measured. The contents of linoleic and linolenic acids in the total fatty acids were calculated from the absorption data by assuming diethenoid and triethenoid components to consist exclusively of linoleic and linolenic acids, respectively. The contents of monoethenoid and saturated acids were then calculated from the iodine value of the total fatty acids by assuming monoethenoid acids to consist exclusively of C_{18}-acids (oleic and petroselinic acids). However these calculations are not applicable to the fatty acids of *P. spinulosa* oil that contain a considerable amount of elaeostearic acid. The ultraviolet absorption curve for the alkali-isomerized fatty acids of *P. spinulosa* oil is shown in Fig. 1.

For the sake of comparison, the saturated methyl esters in the methyl esters of total fatty acids were determined for each oil except *A. elata* oil by the permanganate oxidation in acetone, and the content of saturated acids in the total fatty acids was calculated from the observed data. The contents of linoleic and C_{18}-monoethenoid acids were then calculated for five oils containing no linolenic acid on the basis of the iodine value of the total fatty acids. The results are shown in Table 3.

TABLE 3. Fatty Acid Composition

	Fatty acids (%)	*M. japonica*	*A. elata*	*G. trifida*	*K. innovans*	*I. polycarpa*	*S. Bumalda*	*E. japonica*	*P. spinulosa*
I	Linolenic	0	0	0	0	0	18.7	0	—
	Linoleic	79.0	18.1	10.5	17.2	83.0	39.7	29.5	—
	Octadecenoic	9.6	74.2	82.5	74.3	3.8	21.5	60.3	—
	Saturated	11.4	7.7	7.0	8.5	13.2	20.1	10.2	—
II	Linoleic	80.4	—	10.8	17.2	82.7	—	32.2	—
	Octadecenoic	6.8	—	81.9	74.2	4.4	—	54.8	—
	Saturated	12.8	—	7.3	8.6	12.9	21.4	13.0	10.7

Notes: The fatty acid composition given in I is calculated from the absorption values of alkali-isomerized fatty acids. The fatty acid composition given in II is calculated on the basis of the content of saturated acids estimated by the permanganate oxidation of the total methyl esters. In the case of *S. Bumalda* and *E. japonica* oils, the figures for linoleic acid include those for preformed conjugated diethenoid acids, 4.0% and 4.2%, respectively.

4. Unsaponifiable matter

The unsaponifiable matter from each oil was a mixture of crystalline solid and viscous liquid. On treating the unsaponifiable matter with hot methanol, the insoluble and soluble fractions were separated. The fraction soluble in hot methanol was recrystallized from methanol giving crystalline substances for which the Liebermann-Burchard reaction was positive. The melting points of these sterol fractions from *M. japonica, G. trifida, K. innovans, I. polycarpa, S. Bumalda, E. japonica* and *P. spinulosa* oils were 120°–132°C, 144.5°–147°C, 144°–148°C, 134.5°–136°C, 133°–135°C, 130°–133°C and 124.5°–126°C, respectively.

Summary

1. Seed oils from *Melothria japonica, Aralia elata, Gilibertia trifida, Kalopanax innovans, Idesia polycarpa, Staphylea Bumalda, Euscaphis japonica* and *Prunus spinulosa* were analyzed for their characteristics. The bromination test on the fatty acids indicated the presence of linoleic acid in each oil except *P. spinulosa* oil, linolenic acid in *S. Bumalda* oil and elaeostearic acid in *P. spinulosa* oil. The presence of petroselinic acid in *A. elata, G. trifida* and *K. innovans* oils was demonstrated. This acid was separated and identified in each case. *P. spinulosa* oil was found to contain a large amount of conjugated acid component. This conjugated acid was identified with elaeostearic acid.

2. Assuming the fatty acids of each oil, except *P. spinulosa* oil, to consist of saturated, C_{18}-monoethenoid and linoleic acids with or without linolenic acid, the fatty acid composition was approximately estimated by the ultraviolet absorption measurements of alkali-isomerized fatty acids. For comparison, the saturated acids in the total fatty acids of each oil, except *A. elata* oil, were estimated by the permanganate oxidation of the methyl esters of the total fatty acids.

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