

SEED OILS OF *PODOCARPUS NAGI* AND OTHER NINE SPECIES OF JAPANESE PLANTS

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This paper records the results of our study on the properties and components of the seed oils from the following species of Japanese plants.

1. *Podocarpus Nagi* Zoll et Moritzi. (Podocarpaceae)
2. *Viburnum erosum* Thunb. (Caprifoliaceae)
3. *Ligustrum japonicum* Thunb. (Oleaceae)
4. *Hibiscus mutabilis* L. (Malvaceae)
5. *Ilex integra* Thunb. (Aquifoliaceae)
6. *Ilex macropoda* Miq. (Aquifoliaceae)
7. *Ilex serrata* Thunb. var. *Sieboldii* Loesn. (Aquifoliaceae)
8. *Pourthiaea villosa* Dence. (Rosaceae)
9. *Smilax china* L. (Liliaceae)
10. *Smilax nipponica* Miq. (Liliaceae)

Oil contents of the seeds of *P. Nagi* and *L. japonicum* were reported to be 29.97 and 15.41%, respectively.¹⁾ Among seed oils from the Japanese plants listed above, the seed oil of *P. Nagi*²⁾ was reported to have a specific gravity (23°C) of 0.921 and smoke more intensely than rape oil for lighting purpose. Concerning seed oils of other nine species of Japanese plants, no literature has been known to us. However, seed oils from *V. dentatum*,³⁾ *V. americanum*,⁴⁾ *V. opulus*,⁴⁾ *V. lantana*,⁵⁾ and *V. prunifolium*⁵⁾ of the *Viburnum* genus, seed oils from *H. manihot*,⁶⁾ *H. cannabinus*,⁷⁾ *H. esculentus*,⁷⁾ *H. moscheutos*⁷⁾ and *H. sabdariffa*⁷⁾ of the

TABLE I. List of

Species	<i>P. Nagi</i>	<i>V. erosum</i>	<i>L. japonicum</i>	<i>H. mutabilis</i>
Growing place	Nara	Chikusa-ku, Nagoya	Chikusa-ku, Nagoya	Nishikamo-gun, Aichi-ken
Harvesting season	Middle Nov.	Middle Sept.- middle Oct.	Late Dec.- late Jan.	Middle Oct.
Weight of fruits (g.)	476	1,650	9,100	320
Weight of dried seeds (g.)	133	337	1,892	230
Average wt. of one dried seed (g.)	0.394	0.021	0.032	0.003
Ether-extract $\left\{ \begin{array}{l} \text{(g.)} \\ \text{(\%)} \end{array} \right.$	52.5 39.5	39.1 11.6	192.0 10.2	29.3 12.7
Hexane-soluble $\left\{ \begin{array}{l} \text{(g.)} \\ \text{oil (seed oil) (\%)} \end{array} \right.$	52.1 39.2	38.4 11.4	181.4 9.6	28.4 12.3

Notes: Fruits of *H. mutabilis* were weighed after removal of capsules. Percentage

Hibiscus genus, seed oils from *I. crenata*⁸⁾ and *I. paraguariensis*⁹⁾ of the Ilex genus, and wax from the root of *S. ornata*¹⁰⁾ of the Smilax genus have hitherto been reported in the literature.

1. Seeds used for extraction of oil

Regarding the seeds used for extraction of oil in this study, the growing place of parental plant, the harvesting season of fruit and the yield of seed oil are listed in Table 1. For the separation of seeds from fruits, whole fruits were smashed, a sufficient amount of water was added to the smashed material, and the mixture was rubbed until husks, pericarps and fleshy portions were removed from seeds. Seeds were dried at about 80°C in an electric oven, and the dried seeds were crushed to powders and then extracted with ether. To the ether-extract obtained was added about ten times its weight of hexane, and the insoluble matter was removed by filtration. The seed oil was recovered from the hexane solution by distilling off hexane.

2. Properties of oil

Characteristics of the seed oils obtained above are given in Table 2.

The bromination test on the fatty acids of each seed oil, except *P. Nagi* oil described later, gave no ether-insoluble bromides but hexane-insoluble bromides which, after recrystallization from 90% ethanol, had m.p. 113°-114°C in the case of the fatty acids of *I. macropoda* and *P. villosa* oils and m.p. 114°-115°C in other cases, and showed no depression of melting point when mixed with a specimen of tetrabromostearic acid (m.p. 114°-115°C) prepared from linoleic acid.

The fatty acids of *V. erosum* oil were separated into the solid and liquid acids by means of the lead salt ethanol method. The liquid acids were converted to their lithium salts, and the latter were recrystallized from aqueous ethanol (1:1). The recrystallized material was acidified with hydrochloric acid to give a fatty acid fraction of neutralization value 201.9 and iodine value 101.7. This fraction was heated for 30 min. at about 150°C in the presence of powdered selenium, and the product was recrystallized from 80% ethanol. The recrystallized product had m.p. 42.5°-43°C, neutralization value 197.1 and iodine value 92.1 and showed no depression of melting point on admixture with elaidic acid (m.p. 43.5°-44°C, neutralization value, calcd., 198.6 and iodine value, calcd., 89.9). The fatty acids of

Seed Samples

<i>I. integra</i>	<i>I. macropoda</i>	<i>I. serrata</i>	<i>P. villosa</i>	<i>S. china</i>	<i>S. nipponica</i>
Nishikamo-gun, Aichi-ken	Chikusa-ku, Nagoya	Chikusa-ku, Nagoya	Chikusa-ku, Nagoya	Fuwa-gun, Gifu-ken	Higashikomo- gun, Aichi-ken
Middle Oct.	Late Oct.	Middle Oct.	Middle Oct.- late Oct.	Early Oct.	Late Nov.
920	275	630	485	930	590
143	49	130	31	140	139
0.018	0.008	0.003	0.009	0.026	0.044
23.1	6.1	20.3	7.7	15.7	9.7
16.2	12.4	15.6	24.8	11.2	7.0
22.0	6.0	19.4	7.5	15.5	9.5
15.4	12.2	14.9	24.2	11.1	6.8

yields of ether-extract and hexane-soluble oil were expressed on the basis of dried seeds.

TABLE 2. Properties

Oil	<i>P. Nagi</i>	<i>V. erosum</i>	<i>L. japonicum</i>	<i>H. mutabilis</i>
Appearance at ordinary temp.	Yellowish orange, with a little solid	Greenish yellow	Dark yellowish orange, with some solid	Yellowish orange, with some solid
d_4^{20}	0.9228	0.9212	0.9261	0.9252
n_D^{20}	1.4774	1.4749	1.4760	1.4753
Acid value	1.2	1.7	3.9	3.7
Saponif. value	187.0	189.0	183.7	190.7
Iodine value	158.8	126.8	104.2	125.2
Unsaponifiable matter (%)	0.55	1.92	7.22	1.76
Fatty acids				
n_D^{20}	1.4648	1.4617	1.4591	1.4605
Neutralization value	195.4	197.0	194.7	201.0
Iodine value	166.6	132.0	103.9	129.7
Acetyl value	8.7	4.7	3.0	5.5

Notes: The iodine value was determined by the Wijs method. The determination value for the fatty acids was calculated from the observed data for the methyl esters.

H. mutabilis, *I. serrata* and *P. villosa* oils, similarly treated, gave elaidic acid fractions of m.p. 43°–44°C, 42.5°–43°C and 42.5°–44°C, respectively.

Each oil was saponified with a 1/5 N KOH solution in ethanol. The saponification product was diluted with ethanol and examined for its ultraviolet absorption spectrum. No absorption maximum at 233 m μ was observed for each of the oils, indicating the absence of diene-conjugated acids.

The *L. japonicum* oil contains a relatively large amount of unsaponifiable matter as shown in Table 2. The unsaponifiable matter was a mixture of crystalline solid and yellowish orange, viscous liquid. This was first separated into a fraction (I) difficultly soluble in hot methanol and a fraction soluble in hot methanol, and the latter was then fractionally crystallized from methanol to give the 1st crop (II) and the 2nd crop (III). The fraction I was a very viscous liquid at the ordinary temperature and showed iodine values varying from 138 to 178 depending on the amount of excess of the Wijs reagent used. The crop II, after recrystallization from benzene-ethanol, had m.p. 80°–82°C and consisted of saturated compounds free from sterol. The crop III, recrystallized from 95% ethanol, had m.p. 115°–120°C and was positive for the Liebermann-Burchard reaction.

TABLE 3. Fatty Acid Composition Obtained by

Fatty acids	<i>V. erosum</i>	<i>L. japonicum</i>	<i>H. mutabilis</i>	<i>I. integra</i>
Specific extinc. coeff. at 233 m μ	49.58	24.40	54.04	46.35
Linoleic acid (%).....	53.8	26.5	58.7	50.3
Oleic acid (%).....	38.5	62.2	26.0	41.5
Saturated acids (%)...	7.7	11.3	15.3	8.2

of Oil

<i>I. integra</i>	<i>I. macropoda</i>	<i>I. serrata</i>	<i>P. villosa</i>	<i>S. china</i>	<i>S. nipponica</i>
Yellowish orange	Yellow	Dark yellowish orange	Dark yellowish orange with some solid	Yellow, with a little solid	Yellowish orange
0.9193	0.9221	0.9225	0.9223	0.9175	0.9212
1.4737	1.4760	1.4759	1.4742	1.4728	1.4730
0.8	2.0	4.1	3.4	1.8	2.0
190.3	190.2	190.1	189.5	191.6	192.2
122.5	140.2	136.4	114.8	110.2	116.8
0.85	1.26	1.50	2.27	1.01	1.31
1.4605	1.4625	1.4622	1.4596	1.4593	1.4597
201.3	198.3	199.6	196.1	199.6	201.4
128.4	146.0	142.2	118.3	114.3	121.5
1.7	2.0	1.8	7.3	3.4	3.1

of acetyl value was carried out with the methyl esters of fatty acids, and the acetyl

3. Fatty acid composition

The fatty acid composition for each oil, except *P. Nagi* oil, was approximately estimated in the following way.

The fatty acids were isomerized under the condition of 6.5% KOH-ethylene glycol, 180°C and 25 min. with a current of nitrogen, and the specific extinction coefficient of the alkali-isomerized fatty acids at 233 m μ was measured. The linoleic acid content of the fatty acids was calculated from the specific extinction coefficient at 233 m μ .¹¹⁾ The contents of oleic and saturated acids were then calculated from the iodine value of the fatty acids and the linoleic acid content.

For the sake of comparison, the fatty acids from *V. erosum*, *L. japonicum* and *H. mutabilis* oils were analyzed for the content of solid acids by the lead salt ethanol method, and the content of saturated acids was calculated by assuming the unsaturated acids contained in the solid acids to consist wholly of oleic acid. The contents of oleic and linoleic acids were then calculated from the iodine value of the fatty acids and the content of saturated acids.

The results are shown in Tables 3 and 4. Comparing the figures obtained by

the Ultraviolet Spectrophotometric Method

<i>I. macropoda</i>	<i>I. serrata</i>	<i>P. villosa</i>	<i>S. china</i>	<i>S. nipponica</i>
63.04	58.77	38.48	35.99	44.94
68.4	63.8	41.8	39.1	48.8
24.6	29.7	47.4	48.4	36.8
7.0	6.5	10.8	12.5	14.4

the spectrophotometric method and those obtained by the determination of solid acids, both figures agree fairly well with each other except the figures for the oleic acid content of the fatty acids of *V. erosum* oil.

TABLE 4. Fatty Acid Composition Obtained by the Determination of Solid Acids

Fatty acids	<i>V. erosum</i>	<i>L. japonicum</i>	<i>H. mutabilis</i>
Linoleic acid (%).....	52.4	25.9	58.2
Oleic acid (%).....	41.2	63.4	27.0
Saturated acids (%)..	6.4	10.7	14.8
Solid acids (%).....	7.5	11.4	16.4
m.p. (°C).....	49-50.5	53-54	52.5-53
Iodine value	13.5	5.7	8.7

4. Separation of a new eicosatrienoic acid from *P. Nagi* oil

The fatty acids (14 g.) of this oil were dissolved in ten times their weight of ether and brominated at -10°C . Bromine substitution did not take place at all, since no evolution of hydrogen bromide was observed during the bromination. After bromination, the insoluble bromides (about 2 g.) formed were separated and recrystallized successively from benzene and carbon tetrachloride. The recrystallized material had m.p. 152° - 152.5°C and Br-content 60.93% (calcd. for $\text{C}_{20}\text{H}_{34}\text{O}_2\text{Br}_6$, 61.01%). The ether-soluble bromides recovered from the ether solution were fractionated using hexane and 90% ethanol to give a bromide fraction which had m.p. 113° - 114°C and Br-content 53.18% (calcd. for $\text{C}_{18}\text{H}_{32}\text{O}_2\text{Br}_4$, 53.27%) and showed no depression of melting point on admixture with tetrabromostearic acid (m.p. 114° - 115°C) prepared from linoleic acid.

In another experiment, the fatty acids (26 g.) were dissolved in ten times their weight of acetone, the solution was cooled at -25°C , and the solid acids formed were separated by filtration. The fatty acids remaining in the filtrate were recovered and then dissolved in five times their weight of hexane. The hexane solution was cooled at -25°C and the solid acids formed were separated. The united solid acids weighed about 1 g. and had m.p. 52° - 53.5°C . The liquid acids (A) recovered from the hexane filtrate were brominated in ether, and the insoluble bromides formed were separated and recrystallized successively from benzene and carbon tetrachloride to give a bromide fraction (1.2 g.) of m.p. 152° - 152.5°C . This was debrominated with zinc and hydrochloric acid in methanol to give a methyl ester fraction of n_D^{20} 1.4714 and saponification value 176.3 (calcd. for $\text{C}_{21}\text{H}_{36}\text{O}_2$, 175.1). Saponification of this methyl ester gave a fatty acid fraction (B) of n_D^{30} 1.4757, neutralization value 179.5 (calcd. for $\text{C}_{20}\text{H}_{34}\text{O}_2$, 183.1) and iodine value 235.2 (calcd. for $\text{C}_{20}\text{H}_{34}\text{O}_2$, 248.4). This acid B was a liquid with some solid at the ordinary temperature. Hydrogenation of this acid gave a saturated acid which, after recrystallization from ethanol, had m.p. 74.5° - 75°C and neutralization value 177.1 and showed no depression of melting point on admixture with arachidic acid (m.p. 75°C and neutralization value, calcd., 179.6).

It is concluded from the foregoing results that the fatty acid B is an eicosatrienoic acid. Infrared absorption measurements (Figs. 1 and 2) indicated that while the methyl esters of the liquid acids A before bromination contained no

trans ethylenic bond, the methyl ester of the fatty acid *B* obtained by debromination contained *trans* ethylenic bond. Hence, the *cis* ethylenic bonds of the eicosatrienoic acid occurring in *P. Nagi* oil are considered to undergo partially isomerization in the course of bromination-debromination resulting in the formation of *trans* ethylenic bonds. The infrared absorption spectrum of the methyl ester of the fatty acid *B* indicated also the absence of triple bond and terminal double bond. The ultraviolet absorption spectrum of this acid indicated the absence of conjugated double bond. Although this acid is a triethenoid acid, this acid unlike linolenic acid is difficultly isomerizable with alkali under the condition of 6.5% KOH-ethylene glycol, 180°C and 25 min., yielding diene-conjugated acid ($k_{233} = 82.50$) without forming triene-conjugated acid (Fig. 3). Also the total fatty acids of *P. Nagi* oil yielded only

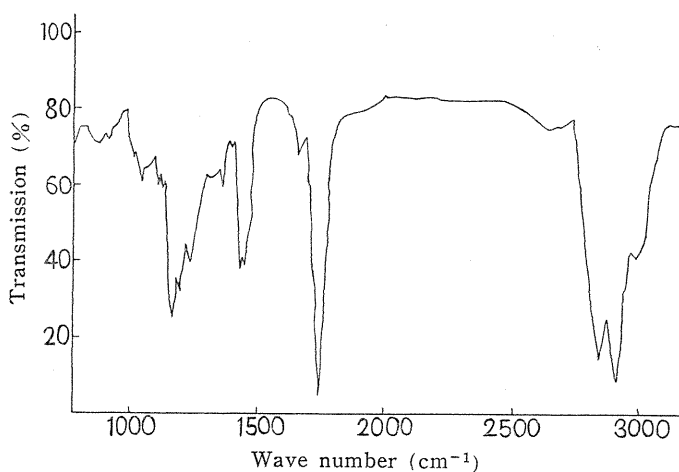


FIG. 1. Infrared absorption spectrum of the methyl esters of the fatty acids *A*.

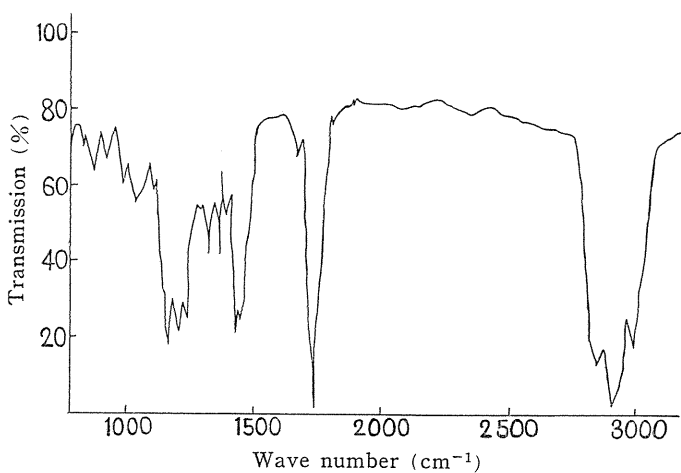


FIG. 2. Infrared absorption spectrum of the methyl ester of the fatty acid *B*.

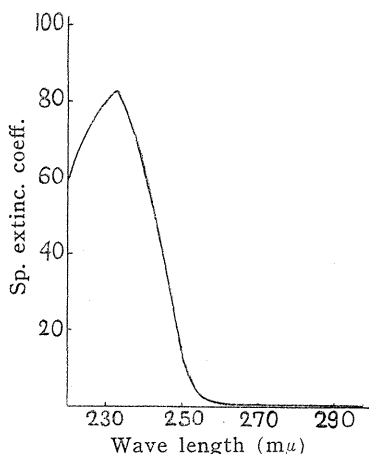


FIG. 3. Ultraviolet absorption spectrum of the alkali-isomerized product of the fatty acid B.

diene-conjugation without triene-conjugation by alkali-isomerization under the usual condition. This is a characteristic feature of the eicosatrienoic acid of *P. Nagi* oil, and the locations of ethylenic linkings in this acid appear to differ widely from those of linolenic acid. The structure of this acid is now being studied. The authors propose the name podocarpic acid for this acid.

Summary

1. Seed oils from *Podocarpus Nagi*, *Viburnum erosum*, *Ligustrum japonicum*, *Hibiscus mutabilis*, *Ilex integra*, *Ilex macro-poda*, *Ilex serrata var. Sieboldii*, *Pourthiaea villosa*, *Smilax china* and *Smilax nipponica* were analyzed for their characteristics.

The bromination test on the fatty acids of each oil indicated the presence of linoleic acid and the absence of linolenic acid. The presence of oleic acid in the fatty acids of *V. erosum*, *H. mutabilis*, *I. serrata* and *P. villosa* oils was demonstrated by separating elaidic acid from the elaidinized product of liquid fatty acid fraction. The unsaponifiable matter from *L. japonicum* oil contained unsaturated viscous oily compound, saturated crystalline compound and sterol.

2. The fatty acid composition for each oil except *P. Nagi* oil was approximately estimated by the ultraviolet absorption measurement of the alkali-isomerized fatty acids. Further the fatty acid composition for *V. erosum*, *L. japonicum* and *H. mutabilis* oils was approximately estimated based on the results of the determination of solid acids by the lead salt ethanol method.

3. The polyethenoid acids of *P. Nagi* oil were found to contain a new eicosatrienoic acid, named podocarpic acid by the authors, besides linoleic acid. This acid contained neither triple bond nor conjugated double bond. Unlike linolenic acid, this acid yielded diene-conjugated acid but no triene-conjugated acid by the alkali-isomerization under the usual condition.

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