SEED OILS FROM FIFTEEN SPECIES OF JAPANESE PLANTS

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(Received May 26, 1958)

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

1. *Viburnum odoratissimum* Ker. (Caprifoliaceae)
2. *Elaeagnus umbellata* Thunb. (Elaeagnaceae)
3. *Eurya japonica* Thunb. (Theaceae)
4. *Cleyera ochnacea* DC. (Theaceae)
5. *Ampelopsis heterophylla* Sieb. et Zucc. (Vitaceae)
6. *Cissus japonica* Willd. (Vitaceae)
7. *Parthenocissus tricuspidata* Planch. (Vitaceae)
8. *Acer Ginnala* Maxim. (Aceraceae)
9. *Acer trifidum* Hook. et Arn. (Aceraceae)
10. *Euphorbia heterophylla* L. (Euphorbiaceae)
11. *Amelanchier asiatica* Endl. (Rosaceae)
12. *Kadsura japonica* Dunal. (Magnoliaceae)
13. *Celtis sinensis* Pers. *var. japonica* Nakai. (Ulmaceae)
14. *Alnus firma* Sieb. et Zucc. (Betulaceae)
15. *Alnus multinervis* Call. (Betulaceae)

No literature has been known to us concerning seed oils from the Japanese plants listed above. However, seed oils from *V. dentatum*,[1] *V. americanum*,[2] *V. opulus*,[2] *V. lantana*,[3] *V. prunifolium*[3] and *V. erosum*[4] of the Viburnum genus, seed oil from *A. quinquefolia*[5] of the Ampelopsis genus, seed oil from *P. quinquefolia*[5] of the Parthenocissus genus, seed oils from *A. platanoides*,[6] *A. pseudoplatanus*,[6] *A. saccharinum*,[6] *A. truncatum*[6] and *A. campestre*[7] of the Acer genus, seed oils from a number of species of the Euphorbia genus,[8] seed oils from *C. occidentalis*[9] and *C. integrifolia*[9] of the Celtis genus, and bark waxes from *A. glutinosa*[10] and *A. viridis*[10] of the Alnus genus have been reported by previous authors. The seed oil from bilberry[11] which appears to be closely related to *Amelanchier asiatica* was also reported in the literature.

1. Seeds used for extraction of oil

Referring to the seeds used for extraction of oil in this study, the growing place of parental plant, date of the harvest of fruit and yield of seed oil are listed in Table 1. For the separation of seeds from fruits, the latter were smashed, a sufficient quantity of water was added to the smashed material, and the mixture was rubbed until seeds were separated from husks, pericarps and fleshy portions.

TABLE 1. List of Seed Samples

Species	Growing place	Date of harvest of fruits	Wt. of fruits (g)	Wt. of dried seeds (g)	Average wt. of one dried seed (g)	Ether-extract		Hexane-sol. oil	
						(g)	(%)	(g)	(%)
V. odoratissimum	Chikusa-ku, Nagoya	Early Oct., 1957	4,430	730	0.023	167.0	22.9	166.4	22.8
E. umbellata	Chikusa-ku, Nagoya	Late Dec., 1956	5,868	364	0.014	58.6	16.1	57.2	15.7
E. japonica	Chikusa-ku, Nagoya	Late Oct., 1957	815	55	0.0005	10.9	19.8	9.6	17.5
C. ochnacea	Chikusa-ku, Nagoya	Early Nov., 1956	1,070	105	0.003	29.3	27.9	28.1	26.8
A. heterophylla	Chikusa-ku, Nagoya	Middle Oct., 1956	1,620	182	0.023	39.4	21.6	39.1	21.5
C. japonica	Chikusa-ku, Nagoya	Early-middle Nov., 1956	875	60	0.012	12.7	21.1	12.5	20.8
P. tricuspidata	Higashi-ku, Nagoya	Middle Nov., 1957	410	149	0.020	34.1	22.9	34.0	22.8
*A. Ginnala**	Chikusa-ku, Nagoya	—	—	134	0.021	16.4	12.2	16.1	12.0
*A. trifidum**	Chikusa-ku, Nagoya	—	—	343	0.015	25.8	7.5	24.7	7.2
*E. heterophylla**	Ama-gun, Aichi-ken	Middle Sept.-Middle Nov., 1955	—	318	0.008	113.0	35.5	112.2	35.3
A. asiatica	Chikusa-ku, Nagoya	Middle Oct., 1956	1,315	144	0.008	20.8	14.4	18.6	12.9
K. japonica	Higashikamo-gun, Aichi-ken	Late Nov., 1956	2,150	228	0.017	52.1	22.9	49.0	21.5
C. sinensis	Higashikamo-gun, Aichi-ken	Late Nov., 1956	1,025	505	0.038	69.0	13.7	68.0	13.5
A. firma	Chikusa-ku, Nagoya	Late Dec., 1957	987	195	0.001	19.7	10.1	18.1	9.3
A. multinervis	Chikusa-ku, Nagoya	Early Oct., 1957	1,983	202	0.0006	10.8	5.3	7.5	3.7

Notes: Percentage yields of ether-extract and hexane-soluble oil are expressed on the basis of dried seeds. * These are received as seeds.

The seeds thus separated were dried in an electric oven at about 80°C, and the dried seeds were crushed to powders and then extracted with ether. The ether-extract was treated with about ten times its weight of hexane, and the insoluble matter was removed. The hexane solution, after removal of hexane, yielded seed oil.

2. Properties of oil

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

Oleic acid from *V. odoratissimum* oil.—The fatty acids of *V. odoratissimum* 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 dilute hydrochloric acid to give a fatty acid fraction of neutralization value 199.8 and iodine value 92.8. This fraction was heated for 1 hr. 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°-43°C and showed no depression of melting point on admixture with elaidic acid (m.p. 43.5°-44°C).

Bromination test.—Ether-insoluble bromides from the fatty acids of *E. umbellata*, *A. Ginnala*, *A. trifidum* and *E. heterophylla* oils showed m.p 179.5°-180°C, 180°-181°C, 178°-179.5°C and 180.5°-181.5°C, respectively, after recrystallization from benzene. The melting points were not lowered when admixed with a pure specimen of hexabromostearic acid prepared from linolenic acid. Ether-soluble bromide fractions obtained from these oils after removal of ether-insoluble bromides were treated with hexane to give hexane-insoluble bromides which showed m.p. 113°-114°C (m.p. 112.5°-113.5°C in the case of *A. trifidum* oil) after recrystallization from 90% ethanol and were identified with tetrabromostearic acid derived from linoleic acid by the mixed melting point test. Fatty acids from *V. odoratissimum*, *E. japonica*, *C. ochnacea*, *A. heterophylla*, *C. japonica*, *P. tricuspidata*, *A. asiatica*, *K. japonica*, *C. sinensis var. japonica*, *A. firma* and *A. multinervis* oils yielded no ether-insoluble bromides but hexane-insoluble bromides which showed the following melting points, respectively, after recrystallization from 90% ethanol and no depression of melting points on admixture with tetrabromostearic acid prepared from linoleic acid: 113°-114.5°C, 114°-115°C, 114°-115°C, 112.5°-114.5°C, 112.5°-114.5°C, 113.5°-115°C, 114°-115°C, 114.5°-115°C, 113°-113.5°C, 114°-115°C and 114°-115°C.

Conjugated unsaturated acids.—In order to know whether conjugated unsaturated acids are present in the seed oils listed in Table 2, ultraviolet absorptions were measured in *n*-heptane for *E. umbellata*, *A. Ginnala*, *A. trifidum* and *C. sinensis var. japonica* oils. No absorption maximum at 233 mμ was observed for all these oils indicating the absence of conjugated diethenoid acid. A very small absorption peak at 268 mμ was observed for *E. umbellata* and *A. trifidum* oils suggesting the presence of an extremely minor amount, say less than 0.1%, of conjugated triethenoid acid in these oils. As for eleven oils other than the above mentioned four oils, they were saponified with a 1/5 N KOH solution in methanol, and the soap solutions diluted with methanol were submitted to the ultraviolet absorption measurements. No absorption maximum at 233 mμ was observed for all oils indicating the absence of conjugated diethenoid acid. A very

TABLE 2. Properties of Oils

Oil	Appearance at ordinary temp.	d_4^{20}	n_D^{20}	Acid value	Saponification V.	Iodine V.	Unsaponifiable matter (%)	Fatty acids				
								n_D^{30}	Neutralization V.	Iodine V.	Acetyl V.	Ether-insol. bromide (%)
V. odoratissimum	Greenish brown	0.9153	1.4704	0.72	193.1	95.0	1.49	1.4580	200.7	98.2	2.8	0
E. umbellata	Dark reddish orange with some solid	0.9251	1.4755	3.5	192.4	125.2	2.37	1.4623	200.9	129.2	4.0	8.6
E. japonica	Dark green	0.9223	1.4739	6.2	191.1	129.6	3.51	1.4628	198.3	135.4	3.8	0
C. ochnacea	Dark yellowish orange	0.9235	1.4742	1.1	191.0	134.7	3.35	1.4638	201.9	140.4	4.0	0
A. heterophylla	Reddish brown	0.9219	1.4756	2.0	193.7	136.6	0.87	1.4618	201.6	142.7	9.9	0
C. japonica	Light yellow	0.9216	1.4761	0.81	193.7	142.8	0.83	1.4637	199.6	147.1	—	0
P. tricuspidata	Dark brown	0.9236	1.4773	1.5	192.0	142.7	1.71	1.4631	203.1	149.8	4.4	0
A. Ginnala	Brown	0.9176	1.4748	2.7	177.7	121.1	2.75	1.4611	188.8	125.3	4.3	3.6
A. trifidum	Green with some solid	0.9123	1.4723	7.3	180.7	109.1	2.72	1.4605	190.1	113.6	4.5	2.7
E. heterophylla	Yellow	0.9303	1.4827	1.4	196.5	197.3	0.93	1.4693	203.6	208.5	17.4	54.2
A. asiatica	Yellowish orange	0.9218	1.4728	5.1	190.5	104.8	3.06	1.4611	200.1	110.5	5.5	0
K. japonica	Yellowish orange	0.9361	1.4820	4.2	181.3	155.0	7.62	—	198.1	138.7	6.3	0
C. sinensis	Light yellow with a little solid	0.9293	1.4785	1.5	191.3	148.5	2.03	1.4643	200.2	152.7	3.8	0
A. firma	Dark green with a little solid	0.9327	1.4786	9.2	186.1	147.1	8.63	1.4651	195.4	154.7	2.2	0
A. multinervis	Dark green with some solid	0.9357	1.4783	11.0	181.7	143.7	8.39	1.4649	190.7	150.6	2.0	0

Notes: Iodine values were determined by the Wijs method. Acetyl values for the fatty acids were calculated from the values determined with methyl esters of fatty acids.

small absorption peak at 268 mμ was observed in the case of *C. japonica*, *P. tricuspidata* and *E. heterophylla* oils suggesting the presence of an extremely minor amount, say less than 0.1%, of conjugated triethenoid acid in these oils.

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 min. with a current of nitrogen, and the specific extinction coefficients of alkali-isomerized fatty acids at 233 mμ, 262 mμ, 268 mμ and 274 mμ were measured. The contents of linoleic and linolenic acids in total fatty acids were calculated from the absorption data by assuming diethenoid and triethenoid acid components to consist exclusively of linoleic and linolenic acids, respectively. The contents of oleic and saturated acids were then calculated from the iodine value of total fatty acids and the contents of linoleic and linolenic acids by assuming oleic acid as the sole component of monoethenoid acids. For the sake of comparison, the saturated methyl esters in the total methyl esters were determined for each oil except *K. japonica* oil by the permanganate oxidation in acetone, and the content of saturated acids in total fatty acids were calculated from the observed data. The fatty acids from *K. japonica* oil were analyzed for the content of solid acids by the lead salt ethanol method, and the content of saturated acids was calculated therefrom by assuming unsaturated components in solid acids to be solely oleic acid. The results are shown in Table 3.

TABLE 3. Fatty Acid Composition

Oil	Fatty acids (%)				
	I				II
	Linolenic	Linoleic	Oleic	Saturated	Saturated
V. odoratissimum	0.4	20.7	66.3	12.6	14.3
E. umbellata	12.3	35.8	34.1	17.8	16.4
E. japonica	—	59.1	31.6	9.3	8.3
C. ochnacea	—	63.9	27.5	8.6	6.3
A. heterophylla	—	73.3	11.1	15.6	16.4
C. japonica	—	78.1	6.3	15.6	17.0
P. tricuspidata	0.2	82.0	1.0	16.8	15.1
A. Ginnala	6.7	38.7	40.9	13.7	13.6
A. trifidum	5.3	36.4	36.8	21.5	23.0
E. heterophylla	58.2	25.7	3.0	13.1	11.2
A. asiatica	—	32.8	56.8	10.4	10.9
K. japonica	—	69.6	14.0	16.4	14.3
C. sinensis	—	81.0	6.8	12.2	12.6
A. firma	—	76.8	17.5	5.7	6.4
A. multinervis	—	72.3	21.9	5.8	7.2

Notes: The fatty acid composition given in I was calculated from the absorption values of alkali-isomerized fatty acids. The saturated acid content given in II, except in the case of *K. japonica* oil, was calculated from the content of saturated methyl esters in the total methyl esters determined by the permanganate oxidation in acetone. The saturated acid content in the case of *K. japonica* oil was calculated from the solid acid content determined by the lead salt ethanol method. The blanks in the linolenic acid content denote the absence or presence only in an amount less than 0.1%.

4. Unsaponifiable matter

The unsaponifiable matter from each oil, except *K. japonica* oil, was a mixture of crystalline solid and viscous liquid. On treating the unsaponifiable matter with hot methanol, insoluble and soluble fractions were separated. The fraction insoluble in hot methanol was a crystalline solid and negative for the Liebermann-Burchard reaction for sterol. Recrystallization of the fraction soluble in hot methanol from methanol gave a crystalline substance which showed the Liebermann-Burchard reaction. Sterol fractions from unsaponifiable matter of *V. odoratissimum*, *A. heterophylla*, *P. tricuspidata*, *E. heterophylla* and *A. firma* oils were obtained in a relatively high yield and showed m.p. 133°–135°C, m.p. 128°–132°C, m.p. 133°–135°C, m.p. 129°–132°C and m.p. 114°–118°C, respectively. The unsaponifiable matter from *K. japonica* oil was a viscous liquid at the ordinary temperature and had n_D^{30} 1.5100 and varying iodine values ranging from 198 to 210 depending on the amount of excess of the Wijs reagent used. It was almost completely soluble in cold methanol without yielding crystalline solid.

Summary

1. Seed oils from *Viburnum odoratissimum*, *Elaeagnus umbellata*, *Eurya japonica*, *Cleyera ochnacea*, *Ampelopsis heterophylla*, *Cissus japonica*, *Parthenocissus tricuspidata*, *Acer Ginnala*, *Acer trifidum*, *Euphorbia heterophylla*, *Amelanchier asiatica*, *Kadsura japonica*, *Celtis sinensis var. japonica*, *Alnus firma* and *Alnus multinervis* were analyzed for their characteristics. The bromination test on the fatty acids indicated the presence of linoleic acid in each oil and linolenic acid in *E. umbellata*, *A. Ginnala*, *A. trifidum* and *E. heterophylla* oils.

2. Assuming the fatty acids of each oil to consist of saturated, oleic and linoleic acids with or without linolenic acid, the fatty acid composition for each oil was approximately estimated by the ultraviolet absorption measurements of alkali-isomerized fatty acids. For comparison, the saturated acid content in total fatty acids was estimated from the results obtained by the determination of saturated methyl esters in total methyl esters by the permanganate oxidation in acetone or by the determination of solid acids in total fatty acids by the lead salt ethanol method.

3. Crystalline components of unsaponifiable matter from each oil were separated into a non-sterol fraction insoluble in hot methanol and a sterol fraction soluble in hot methanol. However, the unsaponifiable matter from *K. japonica* oil was almost completely soluble in cold methanol without yielding crystalline solid.

The authors are indebted to the Higashiyama Botanical Garden, Nogoya, for the supply of a part of seeds used in this study and also to S. Ito and H. Kambe for their help in laboratory work.

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