SEED OILS OF THREE JAPANESE SPECIES OF CELASTRACEAE, *EUONYMUS JAPONICA, EUONYMUS ALATA* AND *EUONYMUS SIEBOLDIANA*

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The seed oil of *Euonymus europea*, a species of Celastraceae, was reported 50 years ago by Kochs[1] to have a saponification value of 230.1 and a Reichert-Meissl value of 35.3 and contain acetic and benzoic acids as its lower acid components. Later, Ferencz[2] also reported S.V. 235–237 and R.M.V. 32.0 for this oil. The oil from the seeds of *Celastrus scandens*, collected in Kentucky, was found by Barkenbus and Krewson[3] to have a remarkably high saponification value and contain acetic, formic and caproic acids as its lower acid components. The oil from the seeds of *Celastrus paniculatus* of India was reported by Kumaraswamy and Manjunath[4] to contain acetic and benzoic acids besides higher fatty acids. Gunde and Hilditch[5] also found the seed and aril (fruit-coat) oils of *C. paniculatus* to contain volatile acids; 6.0% of formic acid, 1.8% of acetic acid and 2.0% of benzoic acid in the total fatty acids of seed oil. The results of their study showed further that these volatile acids are most likely to be present in the form of esters other than glycerides, the alcohol component of which apparently has four hydroxyl groups. Cattaneo and co-workers[6] identified acetic and benzoic acids in the volatile acid components of the seed oil of *Maytenus disticha* of Argentina. This oil yielded glycerol in an amount of 9.8%, which is smaller than the amount obtainable on the assumption that the fatty acids are present wholly as triglycerides.

In a previous study by the authors,[7] the oil extracted from the whole seeds, including aril, of *Euonymus japonica* was found to have a remarkably high S.V. of 264.5 and contain a considerable amount of water-soluble acids besides insoluble fatty acids which consist of saturated acids (about 18%), oleic acid and linolic acid together with a minor amount of linolenic acid. The water-soluble acids were not closely studied, but they were considered to contain some non-volatile and water-soluble acids besides volatile acids. At that time, the authors presumed these non-volatile and water-soluble acids to be some pigments of acid nature which might have been extracted from the reddish orange aril. However, the authors have subsequently extracted the oil from the seeds freed from the aril portion, and found that this oil also has an exceedingly high saponification value and contains a considerable amount of water-soluble acids. Hence, it became evident that water-soluble acids in this oil have nothing to do with pigments. Since the literatures cited above show that the seed oils of Celastraceae hitherto reported contain peculiar lower acids such as acetic and benzoic acids, the authors have now examined the seed oils of three Japanese species of Celastraceae, *Euonymus japonica*, *Euonymus alata* and *Euonymus Sieboldiana*, with a particular reference to their

lower acids.

The results of the present study indicate that all of these three Japanese oils contain lower acids such as acetic and benzoic acids. The oil of *Euonymus europea* is said to be used for exterminating noxious insects, while the oil of *Celastrus paniculatus* is used for treatment of rheumatic diseases and for other medicinal purposes in India. The fruits of *Euonymus japonica* is said to be used in some districts for exterminating vermins in the hair. Such medicinal effect may be thought to be closely related to the presence of peculiar lower acids, especially benzoic acid, in seed oils of Celastraceae, and it appears to be worth studying the utilization of these oils for medicinal, antiseptic and similar purposes.

1. Seeds of three species of Celastraceae used for extraction of oil

The name of species and the growing place of the plants, the date of harvest of the seeds and some data on the yield of oil are shown in Table 1. The orange or red aril in which the true seeds are shrouded is difficultly removable when the seeds are raw. But after drying the whole seeds in an oven at 80°–90°C, the aril was easily removed by rubbing the dried whole seeds by the hand. The dried seeds freed from the aril were ground to fine meals, and the oil was extracted with ether. The ether-extract was then treated with about tenfold hexane, the hexane-insoluble matter was removed by filtration, and the hexane-soluble oil was obtained after distilling off hexane from the hexane solution.

TABLE 1. Seeds for Oil Extraction

Species of plant	*Euonymus japonica*	*Euonymus alata*	*Euonymus Sieboldiana*
Growing place	Chikusa-ku, Nagoya	Higashi Kamo-gun, Aichi-ken	Kita-ku, Nagoya
Date of harvest	Middle Dec., 1955	Late Oct., 1955	Middle-Late Nov., 1955
Raw seed including aril (g)	127	306	250
Dried whole seed (g)	66	160	190
Dried seed freed from aril (g)	41	134	137
Ether-extract (g)	11.9	61.0	50.5
Ether-extract (%)*	29.0	45.5	36.9
Hexane-soluble oil (g)	11.9	55.7	49.0
Hexane-soluble oil (%)*	29.0	41.6	35.8

* Expressed on the basis of dried seed freed from aril.

2. Properties of oils

Properties of the hexane-soluble oils in Table 1 are recorded in Table 2. The fatty acids in Table 2 were prepared in the following way: the oil was saponified in the usual way, the unsaponifiable matter was extracted from the soap solution with ether, and the soap solution was then acidified with dilute sulfuric acid. The fatty acids liberated were taken up with ether, and the ether solution was washed with water. After the wash water had become free from sulfate ion, the washing was repeated three to four times more. Although the last wash water was still faintly acid to methyl orange, a further washing was ceased, and the fatty acids were obtained by distilling ether from the ether solution.

The ether-insoluble bromide from the fatty acids of *E. Sieboldiana* melted at 179°–180°C, and was identified with hexabromostearic acid by the mixed melting point

TABLE 2. Properties of Oils

Oil	*Euonymus japonica*	*Euonymus alata*	*Euonymus Sieboldiana*
Appearance at ordinary temp.	Yellow liquid	Orange yellow liq. with a little solid	Yellow liquid
d_4^{20}	0.9692	0.9579	0.9485
n_D^{20}	—	1.4751	1.4726
Acid V.	2.4	2.7	1.8
Saponif. V.	287.7	270.1	256.5
Iodine V. (Wijs)	71.3	87.6	75.8
Unsap. M. (%)	2.06	0.87	1.27
Fatty acids			
n_D^{20}	—	1.4610	1.4600
Neutr. V.	211.3	218.7	214.8
Iodine V.	103.2	97.2	88.8
Ether-insol. bromide (%)	—	Extremely small quantity	3.8
Saturated esters in the methyl esters of fatty acids (%)	—	21.9	20.7

test. On treating the ether-soluble bromides from the fatty acids of *E. alata* and *E. Sieboldiana* with hexane, relatively large amounts of hexane-insoluble bromides were separated. Recrystallization of hexane-insoluble bromides from 90% alcohol gave crystalline bromides of m.p. 114.5°–115°C which were identified with tetrabromostearic acid by the mixed melting point test.

Saturated esters in the methyl esters of fatty acids were determined by permanganate oxidation in acetone.

3. Examination of lower acids

(i) Oil of *Euonymus japonica*.—A specimen of oil extracted from the seeds collected in Kita-ku, Nagoya in late January, 1956 was used for the examination of lower acids. This oil (25.9 g, S.V. 291.9) was saponified with a 2 N alcoholic solution of potassium hydroxide, and after the completion of saponification, the alcohol was distilled off. The soap was decomposed with 600 cc of a dilute solution of sulfuric acid, and the resulting mixture was distilled, giving 550 cc of an aqueous distillate. The insoluble fatty acids (17.9 g) obtained from the distillation residue by using ether had Neutr. V. 202.2. The aqueous distillate required 3.17 g of potassium hydroxide for neutralization. The neutralized distillate was evaporated, the residue was acidified with 40 cc of a 10% solution of sulfuric acid, and the mixture was extracted with 800 cc of ether. The ether solution was washed twice with a little water, and the ether was distilled, leaving a residue consisting of lower acids (A). The distilled ether (B) was accompanied with some volatile acids which required 0.234 g of potassium hydroxide for neutralization.

The lower acids (A) were a mixture of crystalline solid and liquid. This mixture was dissolved in hot water, leaving a small amount of oily substance undissolved. The aqueous solution was separated by filtration. On cooling the solution, fine needles separated which had m.p. 121.5°–122.5°C and Neutr. V. 463.3 (calcd. for benzoic acid, 459.4) after one recrystallization. On warming a solution of this substance with a few drops of dilute solution of hydrogen peroxide and ferric chloride, a violet color was developed. Thus, this substance was identified with benzoic acid.

The mother liquor of crystallization of benzoic acid was neutralized with potassium hydroxide, and the solution was evaporated to dryness. The potassium salts of lower acids obtained as the evaporation residue were converted, through free acids and then ethyl esters, to the corresponding hydroxamic acids, and the latter were subjected to the paper chromatography with the results shown in Table 3. Hydroxamic acids derived from acetic, benzoic and caproic acids were separated and identified. While ethyl acetate was used as developer in the experiments shown in Table 3, another series of experiments were made using butanol saturated with water as developer. In this case, the R_f values for hydroxamic acids from butyric and benzoic acids were found to lie in close proximity to each other so that it was difficult to determine which of the two acids is present in the sample under examination.

TABLE 3. Paper Chromatography of Lower Acids from the Oil of *Euonymus japonica*

Hydroxamic acid	R_f value for pure substance	R_f value for the sample under examination
Acetic acid ·····	0.13	0.12
Butyric acid ·····	0.56	
Benzoic acid ·····	0.83	0.82
Caproic acid ·····	0.92	0.92

Notes: Ascending method; filter paper: Toyo Filter Paper No. 50; developer: ethyl acetate; room temperature: 8°–15°C; chromogenic reagent: a 10% alcoholic solution of ferric chloride.

(ii) Oil of *Euonymus alata*.—The oil (31.2 g) recorded in Table 2 was treated in nearly same way as described for the oil of *E. japonica*, and the aqueous distillate and the insoluble fatty acids (24.9 g, Neutr. V. 211.1) were separated. The aqueous distillate required 2.14 g of potassium hydroxide for neutralization. From the neutralized distillate, the lower acids (A) and the distilled ether (B) were obtained in the same way as described for the oil of *E. japonica*. The distilled ether (B) containing volatile acids required 0.228 g of potassium hydroxide for neutralization. From the lower acids (A), benzoic acid (m.p. 121.5°–122.5°C, Neutr. V. 462.5) was separated. The lower acids contained in the distilled ether (B) and the mother liquor of crystallization of benzoic acid were converted to the corresponding hydroxamic acids, and the latter were subjected to the paper chromatography. Hydroxamic acids derived from acetic, butyric, benzoic and caproic acids were separated and identified using ethyl acetate as developer, as shown in Table 4. In another series of experiments, in which butanol saturated with water was used as developer, the lowest R_f value obtained corresponded to the R_f value for hydroxamic acid derived from formic acid, while it was difficult to resolve hydroxamic acids derived from butyric and benzoic acids.

(iii) Oil of *Euonymus Sieboldiana*.—The oil (32.5 g) recorded in Table 2 gave the aqueous distillate containing lower acids in an amount equivalent to 2.13 g of potassium hydroxide and the insoluble fatty acids (24.9 g) having Neutr. V. 211.9. From the aqueous distillate, the lower acids (A) and the distilled ether (B) were obtained. The distilled ether (B) containing volatile acids required 0.130 g of potassium hydroxide for neutralization. From the lower acids (A), benzoic acid (m.p.

121.5°–122.5°C, Neutr. V. 460.0) was separated. The lower acids contained in the mother liquor of crystallization of benzoic acid and the distilled ether (B) were converted to the corresponding hydroxamic acids, and the latter were subjected to the paper chromatography (Table 4) with the same results as obtained for the oil of *E. alata*.

TABLE 4. Paper Chromatography of Lower Acids from the Oils of *Euonymus alata* and *Euonymus Sieboldiana*

Hydroxamic acid	R_f value for pure substance	R_f value for the sample under examination	
		E. alata	*E. Sieboldiana*
Acetic acid ·····	0.05	0.06	0.06
Butyric acid ·····	0.41	0.40	0.39
Benzoic acid ·····	0.61	0.64	0.62
Caproic acid ·····	0.76	0.77	0.79

Notes: Ascending method; filter paper: Toyo Filter Paper No. 50; developer: ethyl acetate; room temperature: 21°–22°C; chromogenic reagent: a 10% alcoholic solution of ferric chloride.

Summary

1. The fatty acids of seed oils of *Euonymus japonica*, *Euonymus alata* and *Euonymus Sieboldiana* contain a considerable amount of lower acids besides insoluble fatty acids. Like the insoluble fatty acids of seed oil of *E. japonica* reported in a previous study by the authors, the insoluble fatty acids of seed oils of *E. alata* and *E. Sieboldiana* consist of saturated acids (roughly 20%), oleic acid and linolic acid together with a minor amount of linolenic acid.

2. Among the lower acids, benzoic acid has been separated and identified from each of the three oils. Paper chromatography of the hydroxamic acids prepared from the lower acids indicated the presence of acetic and caproic acids besides benzoic acid in the oil of *E. japonica* and the presence of formic, acetic, butyric and caproic acids besides benzoic acids in the oils of *E. alata* and *E. Sieboldiana*.

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