ethanolic phase with concentrated hydrochloric acid to pH 3, subsequent extraction with ethyl ether, and removal of the ether by distillation under reduced pressure in an atmosphere of carbon dioxide yielded 112.4 g. (52%) of crude fatty acids.

The methyl esters were prepared by refluxing a solution of 112.4 g. of the fatty acids in 250 ml. of absolute methanol, containing 5% sulfuric acid (by weight) for 6 hrs. Removal of the methanol by distillation *in vacuo* gave a residual oil, which was diluted with 300 ml. of water and neutralized with a 10% sodium bicarbonate solution. The neutral solution was extracted with ethyl ether, the ether extracts were washed with water and dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure, giving 105 g. of crude methylated fatty acids.

The crude methyl esters were fractionated in an electrically heated, 24-in. Stedman column equipped with a D. M. Smith still head. Five fractions were collected at temperatures between 155–184°C. at 2–3 mm. of pressure with a reflux ratio of 10:1. The iodine absorption number (Wijs method) (4), saponification equivalent (5), and refractive index ($n^{25/D}$) of each fraction were determined.

Each fraction of the distilled esters was saponified, and the fatty acids were separated by the lead salt-ether procedure (4). The saturated fatty acids were identified by their *p*-bromophenacyl-derivatives (5) and the unsaturated fatty acids by their hydroxy- (6) or bromo- (7) derivatives. The *p*-bromophenacyl esters were recrystallized from ethanol and the dihydroxy acids from ethyl acetate. Fractionation of the bromo-derivatives was accomplished from their different solubilities in petroleum ether, ethyl ether, and benzene. The tetrabromo acids were recrystallized from ethylene dichloride and the hexabromo acids from dioxane.

In corn pollen the saponifiable material was found to represent 52% of the ether extracts. The lipides of corn pollen thus contain large amounts (48%) of unsaponifiable materials; this value is much greater than has been reported for corn oil (8). Results of the fractionation of the methyl esters from the saponifiable material in corn pollen and the physical and chemical characteristics used in identifying the fatty acids are shown in Table I. Palmitic acid was identified in fractions 1, 2, and 3; linoleic (9:10-, 12:13-octadecadienoic) acid in fractions 2, 3, and 5; oleic (9:10-octadecenoic) acid in fractions 3 and 4; stearic acid in fractions 4 and 5; and linolenic (9:10-, 12:13-, and 15:16-octadecatrienoic) acid in fractions 4 and 5.

Summary

The methyl esters of the fatty acids of corn pollen were prepared and fractionated through a Stedman column. Palmitic, stearic, oleic, linoleic, and linolenic acids were identified by the melting points of the *p*-bromophenacyl esters of the saturated acids and the hydroxy and bromine addition compounds of the unsaturated acids.

REFERENCES

1. Anderson, R. J., J. Biol. Chem., *55*, 611 (1923).
2. Miyake, S., J. Biochem. (Japan), *2*, 27 (1922).
3. Lunden, R., Swensk Ken. Tidskr., *66*, 201 (1954).
4. "Official Methods of Analysis," 8th Ed., Association of Official Agricultural Chemists, Washington, D. C., 1955, pp. 460, 465, 469.
5. Shriner, R. L., and Fuson, R. C., "The Systematic Identification of Organic Compounds," New York, John Wiley and Sons Inc., 1948, pp. 134, 157, 223.
6. Lapworth, A., and Mottram, E. N., J. Chem. Soc., *127*, 1628 (1925).
7. McCutcheon, J. W., "Organic Syntheses," Coll. Vol. 3, New York, John Wiley and Sons Inc., 1955, pp. 527, 532.
8. Jamieson, G., "Vegetable Fats and Oils," New York, Reinhold Publishing Corporation, 1943, pp. 178, 345.

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Search for New Industrial Oils. I. Selected Oils from 24 Plant Families

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AN EXTENSIVE PROGRAM has been initiated in the U. S. Department of Agriculture (12) to search for new industrial raw materials among the many plants that have had little or no study of their chemical composition. Ideally such raw materials would fill a present or anticipated need and would not be in competition with presently grown crops, especially those now in surplus supply. Examples of preferred products from major plant constituents are cellulosic fibers for the paper industry, proteins for feed and industrial use, vegetable oils of special composition, and useful polysaccharides other than starch. Insecticides, alkaloids, waxes, essential oils, and many other constituents of potential value may also be found as the program develops.

In one phase of the screening research, seeds from many species have been analyzed for moisture, ash, protein, and oil and have been tested qualitatively for starch, tannin, and alkaloids. The protein, oil and starch analyses indicate major components; the remaining tests give supplementary information without any great increase in the time required for analysis. Limiting the variety of analyses performed initially permitted examination of an increased number of samples even though such limitation might result in incomplete identification of, or sometimes failure to find, components of special interest.

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Oil has a higher value per pound than protein or starch, the other common major components of seeds, and is used in many applications in modern industry. Uses change with the years. Present trends indicate increasing use of oils as sources of chemical intermediates that can be purified and processed into products of controlled quality. Desired oils for processing might provide either a high concentration of one of the common, well-known acids or a suitable concentration of a less-common or presently unknown acid having structure suitable for the preparation of useful derivatives. Examples of such structures include oxygenated acids (hydroxylated, ketonic, or epoxidized), unusual unsaturation (conjugated, acetylenic, or position isomers), branched or cyclic acids, and combinations of these structures.

Oils were prepared for characterization from species found in the preliminary screening to contain more than 20% seed oil. Among the first 621 seed samples analyzed, there were 119 such samples representing 92 species. Oils from 86 species have been analyzed by methods that give an adequate representation of the composition of many oils and detect unusual composition in many others. Composition of oils from only 21 of these species is reported in the reference works of Hilditch (6) and Eckey (4). The present work is minuscule when compared with the estimated total of 250,000 species of higher plants but is quite significant when compared with our present knowledge of the chemical composition of plants.

Results of studies on 42 species are presented in Table I. Selected seed oils from the *Compositae* family and oils having high iodine values are reported separately (2, 3). Oils from the *Umbelliferae* and *Cruciferae* require further study before characterization is sufficiently complete for publication.

Materials and Methods

Seed samples for the present study were obtained from numerous sources, including the repositories of the New Crops Research Branch, commercial seed suppliers, various State Agricultural Experiment Stations, and collections from wild plants.

In preparation for analysis for total oil and protein content, seeds were freed of foreign material, damaged seeds, and such low-oil material as glumes and shells by means appropriate to the samples. Often tedious hand-separation was required. Larger samples to provide approximately 4–10 g. of oil for characterization were cleaned in a similar manner but not always so thoroughly.

There are no standard methods of analysis for most of the seeds examined. Samples were usually ground in a 6-in. hammer mlll through a screen with 1/16-in. round perforations or in a small buhr mill. Nitrogen was determined by the A.O.C.S. procedure (1) except that the ammonia was received in boric acid. Oil was determined by a 6-hr. extraction with petroleum ether (boiling range 30°–60°C.) in the Butt apparatus, with a few samples being reground in a mortar during the extraction. The solvent was removed first under nitrogen, then in a vacuum oven at 70°C. Iodine values, refractive indices, polyunsaturated acids, and oxirane oxygen were determined by A.O.C.S. methods (1). Saponification value and hydroxyl content were determined by the micro methods of Van Etten (11) and Ogg, Porter, and Willits (10), respectively. A qualitative test for carbonyl was made with *m*-dinitrobenzene (5). Infrared spectra were obtained from films of oil on silver chloride plates. Gas chromatography of the methyl esters utilized a liquid phase of Apiezon L in a 2-ft. column at 230° to 240°C. These conditions permitted indication of the esters of acids up to C_{22} and C_{24} but did not permit separation of individual acids of the same chain-length. Identification of chain-length is based only on the time required for elution. Results are calculated as methyl ester in the recovered esters rather than as acids in the original oil. Only esters moving at rates differing from those of the esters of the common C_{16} and C_{18} acids are reported in Table I.

Discussion

The number of oils containing unusual acids or giving evidence of reacting abnormally in the determination of polyunsaturated acids offers hope that new oils of useful composition will be found in our screening program.

The concentrations of conjugated trienoic acid in oils from *Valeriana officinalis* [43][3] and from *Momordica balsamina* [15] are sufficiently high to warrant further study and evaluation of the oils although an attempt to increase the concentration of these unsaturated acids by breeding appears desirable. Similarly the apparent linoleic acid content of the oils from *Broussonetia papyrifera* [24] and *Macleaya cordata* var. *japonica* [27] is high enough to indicate possible usefulness of the oils in applications depending on the reactions of linoleic acid. Five additional oils containing 65 to 69% of apparent linoleic acid might also be suitable for direct use. Oils from *Sanguisorba minor* [36] and from the four members of the borage family [3–6] contain sufficient apparent linolenic acid to suggest that they might serve as drying oils.

Oils indicated as having major amounts of acids not classified by the analyses performed include those from *Limnanthes douglasii* [22], in which practically all the acids move more slowly than linolenic acid in the chromatographic column, and from *Thalictrum polycarpum* [35], which contains some component that interferes seriously with the determination of polyunsaturated acids. Negative values for saturated acids in oils from *Polemonium caeruleum* [31] and *Penstemon spectabilis* [41] are obviously incorrect and may indicate interference by unknown materials or a fortuitous accumulation of errors. The high percentage of monoene in *Sterculia foetida* [42] is probably mostly sterculic acid (6). The slow component in oil from *Ceiba acuminata* [2] may also be sterculic acid because the oil, like *Sterculia* oil, gives a positive Halphen reaction and shows absorption in the infrared at 9.9 μ.

Additional evidence of unusual constituents in oils is provided by a plot of refractive index against iodine value (Figure 1). The regression equation,

$$\text{I.V.} = 8555.559\ \eta^{40/D} - 12425.928,$$

was obtained by the method of least squares from data on oils from 70 species of plant seeds, including 36 of the 42 species in Table I. Oils were omitted from the calculation if they were reported in the literature to contain unusual acids or if they deviated widely from the 70 species comprising the main body of the present data. The equation is in excellent

[3] Figures in brackets refer to the species as listed in Table I.

TABLE I

Analytical Data on Seeds and Derived Oils

Source	Common name[a]	Seed analysis[b]		Iodine value	Saponification value	Refractive index $\eta^{40/D}$	Fatty acid content of oil								Infrared bands or groups
		Oil content % DB	Protein content N x 6.25 % DB				Nonconjugated triene, as linolenic %	Nonconjugated diene, as linoleic %	Monoene, as oleic %	Conjugated triene %	Conjugated diene %	Saturated %	Oxirane oxygen, as epoxyoleic	Gas chromatog., slow component %	
Araliaceae															
1. *Aralia spinosa*	Devil's walkingstick	46.3	17.5	96	180	1.4614	0.1	14	88	0	0	3.6	0	....	9–10 μ
Bombacaceae															
2. *Ceiba acuminata*	Ceiba	32.0	31.2	89	190	1.4622	.5	23	51	0	0	21	2	8	9.9 μ
Boraginaceae															
3. *Anchusa capensis*	Cape bugloss	29.0	19.4	157	194	1.4693	24[c]	28	28	0	0	11	0	....	Usual[d]
4. *Anchusa italica*	Italian bugloss	21.4	16.4	134	180	1.4665	12	40	32	0	0	12	0	....	Usual
5. *Borago officinalis*	Borage	38.3	20.9	139	190	1.4680	19	35	26	0	0	16	0	....	Usual
6. *Cynaglossum amabile*	Chinese houndstongue	22.6	17.6	126	177	1.4673	13[e]	24	42	0	0	14	1	....	Usual
Buxaceae															
7. *Buxus sempervirens*	Common box	41.6	31.4	124	180	1.4663	1.4	46	42	0	0	6.5	0	0	Usual
Campanulaceae															
8. *Lobelia erinus*	Edging lobelia	46.7	21.9	141	186	1.4685	.6	69	17	0	0	9.4	0	0	Usual
Capparidaceae															
9. *Cleome pungens*	Spider flower	33.0	19.8	113	185	1.4646	4.0	41	32	0	0	19	0	....	Usual
Celastraceae															
10. *Euonymus alatus*	Winged euonymus	44.4	20.5	96	264	1.4649	2.9	34	25	0	2.4	32	1	0	7.3; 9.5 μ
Cucurbitaceae															
11. *Cucurbita pepo*	Pumpkin	46.2	39.3	119	186	1.4664	0	51	30	0	0	15	1	....	Usual
12. *Luffa acutangula*	Vegetable sponge	23.5	27.5	115	194	1.4656	0	51	21	.6	0	24	2	....	Usual
13. *Marah gilensis*	Bigroot	57.1	27.8	114	184	1.4644	.6	46	33	0	0	16	0	....	Usual
14. *Marah macrocarpa*	Bigroot	54.4	29.4	115	186	1.4656	.5	50	24	.2	0	21	0	....	Usual
15. *Momordica balsamina*	Balsam apple	39.9	29.5	139	189	1.5017	(0)[g]	(0)[g]	....	50	0	....	0	64	Trichosanic
Cyperaceae															
16. *Cyperus esculentus*[f]	Chufa	27.4	6.1	78	192	1.4606	.2	11	64	0	0	20	....	....	8.9 μ
Dipsacaceae															
17. *Scabiosa atropurpurea*	Sweets scabious	25.4	34.8	102	176	1.4650	.3	34	35	.2	3.6	22	8	....	Usual
Labiatae															
18. *Stachys lanata*	Woolly betony	31.9	20.4	142	189	1.4679	.6	65	24	0	0	5.1	0	....	Usual
Leguminosae															
19. *Acacia willardiana*	Willard acacia	21.3	35.2	87	187	1.4633	.4	37	18	0	1.2	38	2	....	Usual
Liliaceae															
20. *Cordyline australis*	Giant dracena	42.3	19.9	144	185	1.4688	.3	67	23	.2	0	4.7	0	....	Usual
21. *Yucca elata*	Soaptree yucca	29.1	20.6	128	188	1.4679	0	52	32	0	2.8	8.8	6	....	Usual
Limnanthaceae															
22. *Limnanthes douglasii*	Douglas meadowfoam	24.5	24.7	87	168	1.4628	1.1	2	(89)[g]	0	0	(3.5)[g]	0	94	Usual
Malvaceae															
23. *Gossypium hirsutum*	Cotton	38.4	37.1	106	190	1.4648	.1	47	22	0	.9	26	2	....	Usual
Moraceae															
24. *Broussonetia papyrifera*	Paper mulberry	30.1	18.5	144	203	1.4685	.7	71	14	0	0	9.5	0	0	Usual
Onagraceae															
25. *Oenothera lamarckiana*	Lamarck evening primrose	26.1	17.2	153	196	1.4703	8.3	62	18	0	.6	6.2	1	....	Usual
26. *Clarkia elegans*	Rose clarkia	35.5	28.7	131	184	1.4687	1.3	57	20	.1	3.8	14	10	8	Usual
Papaveraceae															
27. *Macleaya cordata* var. *japonica*	Pink plume poppy	40.7	17.6	142	184	1.4682	.3	70	16	0	0	9.2	....	....	Usual
28. *Dicentra ochraleuca*	Cream bleedingheart	30.9	13.6	124	190	1.4664	.5	55	25	0	0	15	0	....	Usual
29. *Papaver rhoeas*	Corn poppy	47.6	21.8	143	187	1.4675	2.5	67	16	0	0	9.6	0	0	Usual
Polemoniaceae															
30. *Cobaea scandens*	Cup-and-saucer vine	22.1	18.2	88	182	1.4621	.4	23	51	0	0	22	0	0	8.9 μ
31. *Polemonium caeruleum*	Jacob's ladder	26.1	23.9	150	190	1.4709	3.0	60	(33)[g]	1.3	0	(−1.8)[g]	....	....	Usual
Ranunculaceae															
32. *Anemone coronaria*	Poppy anemone	20.7	26.0	135	186	1.4666	.7	62	17	0	2.6	13	6	....	Usual
33. *Nigella hispanica* var. *damascena*	Love-in-a-mist	46.0	23.8	122	190	1.4677	0	44	48	0	0	4.2	3	5	Usual
34. *Nigella hispanica* var. *damascena*	Love-in-a-mist	42.7	25.7	130	188	1.4690	0	56	27	2.0	0	11	2	4	Usual
35. *Thalictrum polycarpum*	Sierra meadowrue	33.1	24.5	170	189	1.4720	1.0	56	(73)[g]	0	0	(−35)[g]	....	....	*trans*
Rosaceae															
36. *Sanguisorba minor*	Small burnet	20.3	13.5	167	187	1.4713	29	38	19	0	0	8.8	0	....	Usual
Scrophulariaceae															
37. *Chelone barbata*	Turtlehead	24.2	18.9	124	181	1.4741	1.4	51	32	0	0	11	2	....	Carbonyl
38. *Digitalis purpurea*	Common foxglove	36.1	16.8	145	192	1.4691	7.1	58	23	0	0	7.1	0	....	Usual
39. *Linaria maroccana*	Morocco toadflax	43.1	21.1	142	193	1.4759	.7	66	22	0	0	6.7	0	....	Usual
40. *Penstemon murraya* x *grandiflora*	.....	25.8	19.7	129	164	1.4685	.9	47	45	0	0	2.5	0	0	Usual
41. *Penstemon spectabilis*	Showy penstemon	30.2	11.5	120	162	1.4733	0	32	(68)[g]	0	0	(−4.9)[g]	1	....	Carbonyl
Sterculiaceae															
42. *Sterculia foetida*	Hazel sterculia	51.5	20.5	84	184	1.4649	0	9.4	74	0	0	12	44	56	9.9 μ
Valerianaceae															
43. *Valeriana officinalis*	Common valerian	30.0	19.9	156	181	1.4970	(0)[g]	(31)[g]	....	42	0	....	0	50	eleostearic

[a] Mostly from "Standardized Plant Names," H. P. Kelsey and W. A. Dayton, editors, 2nd ed., 1942. J. Horace McFarland Company, Harrisburg, Pa. [b] Analysis on materials as cleaned. [c] Contains 4.4% tetraene. [d] Absorption similar to soybean, cottonseed, and linseed oils. [e] Contains 2.1% tetraene. [f] Tuber. [g] Obviously incorrect results in parentheses indicate inapplicability of the analytical method.

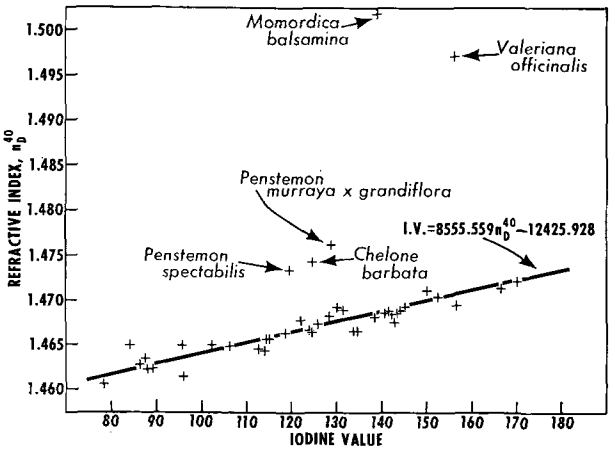

FIG. 1. Relation between iodine value and refractive index for oils from 42 plant species from 24 families.

agreement with lines reported for soybean oil (8) and linseed oil (7, 9, 13).

The two points farthest from the line in Figure 1 represent oils from *Momordica balsamina* [15] and *Valeriana officinalis* [43], both of which as already mentioned were found to contain conjugated triene. The three other points that deviate distinctly represent oils which give a positive qualitative test for carbonyl (5). However infrared absorption shows carbonyl only in oils from *Chelone barbata* [37] and *Penstemon spectabilis* [41].

The many indications of small amounts of apparent linolenic acid cannot be accepted as conclusive evidence until confirmed by independent methods. Although precautions were taken to prevent oxidation during preparation of the oil, they may not have been adequate; the traces of triene may be artifacts derived from oxidized acids. Similarly the numerous occurrences of low percentages of conjugated acids require confirmation although their presence probably will have little effect on industrial uses of the oils.

Analyses for oxirane oxygen gave results indicating small amounts in many oils and a large amount in *Sterculia* oil. The result for *Sterculia* oil is spurious; presumably the cyclopropene ring adds HBr. The occurrence in other oils requires confirmation.

Many of the oils exhibit light absorption in the infrared region characteristic of hydroxyl, but there is no differentiation between fatty acid hydroxyl and glyceryl hydroxyl. In only three oils, *Anemone coronaria* [32], *Nigella hispanica* [33], and *Penstemon spectabilis* [41], did chemical analysis confirm the infrared indication. The quantities found, equivalent to 5 to 11% of ricinoleic acid, are of interest primarily as a guide to plant families that may contain larger amounts of hydroxylated compounds.

The unusually high saponification value of *Euonymous alatus* [10] is in agreement with reports on oils from other members of the *Celastraceae* family shown to contain esters of formic, acetic, and benzoic acids (4). These acids are not indicated by any other of the tests applied. Failure to detect them by gas chromatography may indicate that they were lost in the preparation of the methyl esters.

The indication of nonconjugated tetraene in *Anchusa capensis* [3] and *Cynoglossum amabile* [6] is of no immediate industrial importance but may constitute the first report of such acids in vegetable oils.

Summary

The group of analyses used in this preliminary screening of oils has proved capable of indicating many seed species that contain oils of unusual or unknown composition. Some of the oils are characterized sufficiently to suggest probable commercial uses; others give no evidence of properties that would lead to their use while present commercial oils are in adequate supply. Still other oils are shown to have unknown composition, which must be determined before their potential value can be judged. The study as yet contains too few species to generalize about the relationship between botanical classification and oil composition. It does however provide numerous leads in the search for oils of industrial value.

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REFERENCES

1. American Oil Chemists' Society. "Official and Tentative Methods of Analysis," Chicago, 1957.
2. Earle, F. R., McGuire, T. A., Mallan, Jean, Bagby, M. O., and Wolff, I. A., presented at the 49th annual meeting, American Oil Chemists' Society, Memphis, Tenn., April 21–23, 1958.
3. Earle, F. R., Wolff, I. A., and Jones, Q., presented at the 32nd fall meeting, American Oil Chemists' Society, Chicago, Ill., October 20–22, 1958.
4. Eckey, E. W., "Vegetable Fats and Oils," New York, Reinhold Publishing Corporation Inc., 1954.
5. Esposito, G. G., and Swann, M. H., Anal. Chem., *29*, 1861–1862 (1957).
6. Hilditch, T. P., "The Chemical Constitution of Natural Fats," New York, John Wiley and Sons Inc., 1956.
7. Hopper, T. H., and Nesbitt, L. L., Oil and Soap, *14*, 34–36 (1937).
8. Majors, K. R., and Milner, R. T., Oil and Soap, *16*, 228–231 (1939).
9. deMello, L. M., and Castro, D. R., Agron. Lusitana, *18*, 61-73 (1956).
10. Ogg, C. L., Porter, W. L., and Willits, C. O., Ind. Eng. Chem., Anal. Ed., *17*, 394–397 (1945).
11. Van Etten, C. H., Anal. Chem., *23*, 1697–1698 (1951).
12. Wolff, I. A., and Jones, Q., Chemurgic Digest, *17*(9), 4–8 (1958).
13. Zeleny, L., and Coleman, D. A., U. S. Dept. Agr. Tech. Bull. 554 (1937).

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