Fatty Acids of Kenaf Seed Oil[1, 2]

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A RECENT EXAMINATION of okra seed oil (*Hibiscus esculentus* L.) in this laboratory showed the presence of a small proportion of 12,13-epoxyoleic acid as a component of the glycerides (3). It was of interest to determine whether the same acid occurs in other *Malvaceae* seed oils.

A second objective was to isolate the epoxy acid as such. Identification of epoxyoleic acid in seed oils has been made hitherto by means of derivatives (2, 3, 4).

In preliminary tests of several species of *Malvaceae* a sample of kenaf seed (*Hibiscus cannabinus* L.) gave an oil with higher epoxy content than those of other species. It was therefore selected for study. Kenaf oil was examined by Bauman in 1926 (1). He reported palmitic, stearic, oleic, and linoleic as the fatty acid components. Lewy determined the constants of the oil and described its possible production and uses (6).

Experimental

Seed of *Hibiscus cannabinus* L., var. *Cubano* was obtained from Cuba. Its identity was confirmed by growing an herbarium specimen from the seed. The seed was ground in a Wiley mill (without decortication), the moisture content of the meal was determined, and the oil was extracted with petroleum ether. The oil content of the meal was 15.0% on a 10% moisture basis. Constants of the oil are given in Table I. A sample of seed from another source had a much lower content of oxirane oxygen.

TABLE I

Constants of Kenaf Seed Oil

Iodine value	100.2
Saponification value	192.1
Unsaponifiable matter, %	1.0
Acid value	1.3
Peroxide value	2.1
Acetyl value	12.3
Oxirane oxygen, %	0.38 [a]
Refractive index, $n^{25°/D}$	1.4717
Halphen test	positive

[a] Method of Swern (8).

Isolation of 12,13-Dihydroxyoleic Acid. A portion of the oil (115 g.) was acetylated and then saponified by heating with ethanolic KOH under nitrogen, thus converting the epoxy acid to dihydroxy acid (4). The total mixed acids were then partitioned by Gunstone's method (4). The methanol portion gave 9.9 g. of dark, viscous liquid, which, on recrystallization, yielded 4.7 g. of solid acid. After one further crystallization from acetone at −25° there were obtained 3.7 g. of threo-12,13-dihydroxyoleic acid, m.p. 52–54°. The melting point was unchanged in admixture with an authentic sample, the structure of which had been established previously (3). Omitting the acetylation of the oil but saponifying and partitioning as before did not yield any solid hydroxy acid. Thus the content of dihydroxy acid (free or as glycerides) in the oil was negligible.

Hydrogenation of the dihydroxyoleic acid gave threo-12,13-dihydroxystearic acid, m.p. 96.0–96.5° alone and mixed with an authentic sample.

Examination of the Methyl Esters. The fatty acids, freed from epoxy acid by acetylation and solvent partition as described above, were esterified with methanol. The esters contained no oxirane oxygen. A portion of the esters (50 g.) was distilled through a spinning band column at 0.5-mm. pressure with the results shown in Table II.

TABLE II

Distillation of Methyl Esters
(Freed of Epoxy Acid)

Fraction	Temp., °C. (0.5 mm.)	Weight (g.)	Chain length	Iodine value
1	112–123	0.9	......	18.3
2	123–125	7.8	C_{16}	6.2
3	125–128	1.3	C_{16}	11.1
4	128–135	1.3	......	66.1
5	135–142	4.8	C_{18}	129.3
6	142	13.1	C_{18}	135.8
7	142	13.1	C_{18}	130.6
8	142	2.9	C_{18}	97.9
R	Residue	4.6	......	......

It was estimated from the distillation curve that esters of C_{16} acids constituted 20% of the total sample. The remainder of the distillate consisted of esters of C_{18} acids.

The distilled fractions were examined individually, and the components were isolated by low-temperature crystallization or identified by chemical derivatives (Table III). The identity of each acid or its derivative was confirmed by a mixed melting point with an authentic sample. The distillation residue yielded a little solid acid melting about 71°, probably impure arachidic acid. The unsaturated portion of the residue could not be purified.

Isolation of 12,13-Epoxyoleic Acid. A portion of the freshly-extracted oil (145 g.) was saponified by allowing it to stand over-night at 25° with a solution of 50 g. of potassium hydroxide in 750 ml. of ethanol. The mixture was diluted with an equal volume of water, and the unsaponifiable matter was removed by extracting three times with petroleum ether. The remaining solution of soaps was diluted further and

TABLE III

Identification of Acids

Fraction	Acid	Identified as (acid):	Melting point, °C.
1,2	9-Hexadecenoic	Erythro-9,10-dihydroxypalmitic [a]	123.5–124
2	Palmitic	Palmitic	62–62.5 (Equiv. wt. 256.8)
6	Linoleic	Tetrahydroxystearic [a]	170–171
6	Oleic	Erythro-9,10-dihydroxystearic [a]	129–130
8	Stearic	Stearic	68.5–69 (Equiv. wt. 285.0)
Oil	12,13-Epoxyoleic	12,13-Epoxyoleic	29–30
		Threo-12,13-dihydroxyoleic [b]	52–54

[a] By reaction with alkaline K Mn O_4.
[b] By acetylation and hydrolysis.

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[1] Presented at the fall meeting, American Oil Chemists' Society, Cincinnati, O., September 30–October 2, 1957.

[2] Issued as N.R.C. No. 5053.

stirred vigorously while dilute hydrochloric acid was added slowly to liberate the fatty acids.

The mixed acids were partitioned between petroleum ether and aqueous methanol (4 vols. CH_3OH:1 vol. H_2O). Heating was avoided at all stages. The solvent was removed from the product at reduced pressure; the temperature was held below 30°. The methanol portion yielded 5.4 g. of crude epoxyoleic acid. This material was crystallized three times from acetone (at $-70°$, $-60°$, and $-40°$) to give 0.25 g. of pure acid, which solidified after standing for some time and then melted at 29–30°. Calculated for epoxyoleic acid, $C_{18}H_{32}O_3$:C, 72.93; H, 10.88. Found: C, 72.89; H, 10.59. The Halphen test was negative.

On acetylation and saponification this acid gave the same product, threo-12,13-dihydroxyoleic acid, as had been obtained by treating the oil directly. The procedure was as follows. The epoxyoleic acid was acetylated by allowing it to stand for 4 hrs. with glacial acetic acid and then refluxing the mixture for 1½ hrs. The excess acetic acid was removed at 40–50° under reduced pressure. The residue was treated with 0.5 N ethanolic KOH, allowed to stand overnight, and then refluxed for one hour. On diluting with water and acidifying with HCl, the product precipitated as an oil. It was dissolved in a mixture of petroleum ether and ethyl ether (3:1) and cooled to $-20°$, giving crystals which melted at 47–50°. After two further crystallizations the substance melted at 52–54°. The melting point was unchanged in admixture with an authentic sample of threo-12,13-dihydroxyoleic acid of m.p. 53–54°. The original acid is therefore *cis*-12,13-epoxyoleic acid since the ring opening by acetylation is accompanied by inversion (4, 7).

Composition of the Total Fatty Acids. A sample of the mixed fatty acids was subjected to alkali isomerization and examined for diene and triene acids by ultraviolet absorption. The content of diene acid determined in this way was 41.2% of the total acids. The content of triene acid was negligible. The proportions of the various acids, including the epoxy acid, were calculated from the distillation data, the amounts of each acid isolated, and the analytical data, and are shown in Table IV.

TABLE IV
Estimated Fatty Acid Composition
(Percentage of Total Fatty Acids by Weight)

Acid	%	Acid	%
9-Hexadecenoic	1	Stearic	2
Palmitic	19	Arachidic	Trace
Linoleic	41	12,13-Epoxyoleic	5
Oleic	30	Undetermined	2

Discussion

Epoxy Acid. The main sample of seed, var. *Cubano,* gave an oil having an appreciable content of 12,13-epoxyoleic acid in its glycerides. Analytical determination of epoxide in the oil indicated a content of 7% of epoxyoleic acid, based on the total fatty acids.

However, when converted to dihydroxyoleic acid, isolated as such, and purified, the yield was equivalent to 3.6% of epoxyoleic acid. Allowing for losses during partitioning and crystallization, it is estimated that the original content of epoxyoleic acid was not less than 5% of the total fatty acids. This compares with 3% found in the best sample of okra seed oil.

The epoxyoleic acid was isolated in small yield by saponifying the oil in the cold and partitioning the acids, keeping the solutions at room temperature throughout. The acid was difficult to crystallize and was unstable, as shown by a fall in melting point on standing.

TABLE V
Estimated Fatty Acid Composition of *Malvaceae* Oils

	Percentage of total acids by wt.		
	Kenaf (present work)	Cotton (5)	Okra (3)
Myristic	....	1	....
Hexadecenoic	1	2	1
Palmitic	19	23	29
Linoleic	41	48	39
Oleic	30	23	23
Stearic	2	1	2
Arachidic	Trace	1	Trace
Epoxyoleic	5	....	3

Other Fatty Acids. In addition to the ordinary fatty acids, previously reported by Bauman (1), 9-hexadecenoic acid was identified in our sample, and evidence of a little arachidic acid was noted. Myristic, eicosenoic, and trienoic acids were not detected. The content of palmitic acid is noticeably lower than in okra oil. With the exception of the content of epoxyoleic acid, the fatty acid composition of this sample of kenaf oil resembles that of cottonseed oil (Table V).

Summary

Freshly extracted oil from a sample of kenaf seed, var. *Cubano,* was found to contain an appreciable amount of epoxy acid as glyceride; *cis*-12,13-epoxyoleic acid was isolated. Its identity was confirmed by analysis and by conversion to threo-12,13-dihydroxyoleic acid. The remaining fatty acids were esterified, distilled, and identified by the usual procedures. The percentage composition of the fatty acids was estimated from the data as follows: 9-hexadecenoic 1, palmitic 19, linoleic 41, oleic 30, stearic 2, arachidic (trace), 12,13-epoxyoleic 5, undetermined 2.

REFERENCES

1. Bauman, M. R., Chem. Abs. *23*, *3117 (1929)*.
2. Bharucha, K. E., and Gunstone, F. D., J. Sci. Fd. Agric., *7*, 606–609 (1956).
3. Chisholm, M. J., and Hopkins, C. Y., Can. J. Chem., *35*, 358–364 (1957).
4. Gunstone, F. D., J. Chem. Soc., 1611–1616 (1954).
5. Hilditch, T. P., and Maddison, L., J. Soc. Chem. Ind., *59*, 162–168T (1940).
6. Lewy, Mario, J. Am. Oil Chemists' Soc., *24*, 3–5 (1947).
7. Swern, Daniel, J. Amer. Chem. Soc., *70*, 1235–1240 (1948).
8. Swern, Daniel, Findley, T. W., Billen, G. N., and Scanlan, J. T. Anal. Chem., *19*, 414–415 (1947).

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