or hydroxy-groups in the chain increased solubility. Phenylstearic acid is a mixture of several position isomers, and perhaps partly for this reason the salts of α-sulfophenylstearic acid show increased solubility. Substitution of the phenyl group in the hydrophobic chain did not effect much change in c.m.c. Disodium α-sulfophenylstearate has good detergent and foaming properties in hard water.

The substitution of two chlorine atoms increased both solubility and c.m.c. In Table I disodium 9,10-dichloro-α-sulfostearate has the best foaming properties.

Substitution of two hydrophilic hydroxyl groups in the hydrophobic chain markedly increased solubility and c.m.c. Wetting and detergent properties were poor, but foaming properties were moderately good although all three properties were measured at concentrations below the c.m.c.

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Epoxy Acid in Seed Oils of *Malvaceae* and Preparation of (+) *threo*-12,13-Dihydroxyoleic Acid [1,2]

C. Y. HOPKINS and MARY J. CHISHOLM, National Research Council (Canada), Ottawa, Canada

Seed oils of six species of *Malvaceae*, representing four genera, were found to contain *cis*-12,13-epoxyoleic acid in amounts estimated at 1.5–7% of the total fatty acids. Acetolysis of the oils gave the corresponding dihydroxyoleic acid, which was shown to be predominantly a *dextro*-rotatory form of *threo*-12,13-dihydroxyoleic acid. It was obtained optically pure, and its structure was confirmed by orthodox methods. The hydrogenation product, (+)-*threo*-12,13-dihydroxystearic acid, was also obtained optically pure and characterized. The best yield of dihydroxyoleic acid was obtained from the seed oil of *Malope trifida*.

Samples of oil from four other species of *Malvaceae* had a very low or negligible content of epoxy acid.

IT WAS SHOWN in earlier work that *cis*-12,13-epoxyoleic acid occurs in the glycerides of okra seed oil and kenaf seed oil in amounts up to 5% of the total fatty acids (1,2). The present work deals with the examination of other seed oils of the *Malvaceae* family and the detection of epoxy acid in some of them.

The oxirane oxygen content of each oil was determined and calculated as epoxyoleic acid. Oils having an apparent epoxyoleic acid content of more than 3% were acetylated, saponified, and partitioned by solvents to isolate the resulting *threo*-12,13-dihydroxyoleic acid. Isolation and identification of this acid confirmed the presence of *cis*-12,13-epoxyoleic acid in the oil and gave a further indication of the amount.

Attempts to convert the epoxy acid to monohydroxy acid by hydrogenating the entire oil and then to isolate the monohydroxy acid by solvent partition were not successful. It was not possible to obtain a pure hydroxy acid by this treatment, perhaps because of the formation of position isomers or because of the fact that the partition ratio for separating the acids is less favorable for mono- than for dihydroxy acids.

Dextro-rotatory Dihydroxyoleic Acid. Gunstone found that the oil of *Vernonia anthelmintica*, when treated by acetolysis, gave *levo*-rotatory *threo*-12,13-dihydroxyoleic acid (3). It is now shown that oils of the *Malvaceae* species, treated in the same way, give a *dextro*-rotatory form of the same acid. Preparation of pure (+)- and (−)-*threo*-12,13-dihydroxyoleic acids was reported recently in a preliminary communication from this laboratory (4). The (+) form was prepared from *Malope trifida* and the (−) form from *Vernonia colorata*. Their specific rotations were equal but of opposite sign. In both cases the natural epoxy acid is presumed to be optically active, but conversion to the dihydroxy acid is only partially stereospecific, resulting in a mixture of two enantiomers in which one predominates. The enantiomer present in excess was isolated by fractional crystallization. All of the *Malvaceae* oils which yielded *threo*-12,13-dihydroxyoleic acid in the present work gave the (+) isomer.

Hydrogenation of (+)-*threo*-12,13-dihydroxyoleic acid gave (+)-*threo*-12,13-dihydroxystearic acid. Since these two acids are new compounds, it was necessary to prove their structure; and this was done by analysis and by identification of the degradation products, as follows. The unsaturated acid, shown by analysis to be $C_{18}H_{34}O_4$, absorbed one mole of hydro-

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[2] Issued as N.R.C. 6042.

TABLE I

Epoxy Acid in Oils of *Hibiscus* Species

Species	Common name	Oil in seed, %	Oxirane oxygen, %	Epoxyoleic acid, calcd. %	Yield of dihydroxy oleic acid, %	Melting point of (+) isomer, °C.
H. abelmoschus L.	Ambrette	17.5	0.24	4.4	2.0	60.5–61.5
H. moscheutos L.	Rose mallow	11.2	0.16	3.0	0.6	61.0–61.5
H. syriacus L.	Rose of Sharon	20.6	0.16	3.0	<0.5	59.5–60.5
H. esculentus L.	Okra	16.9	0.21	3.9	2.5	60.5–61.5

gen (one double bond) and had infrared absorption at 3560 cm.$^{-1}$ (hydroxyl). Cleavage by periodate-permanganate gave hexanoic and azelaic acids hence the points of cleavage were at the 9,10 and 12,13 linkages. On hydrogenation it gave a dihydroxystearic acid, the melting point (96.5–97°) of which indicated a *threo* configuration. Determination of its α-glycol value confirmed the presence of adjacent hydroxyl groups. Periodate cleavage of the saturated acid gave hexanoic and dodecanedioic acids, showing that the hydroxyls are at the 12,13 position and that the acid is therefore (+)-*threo*-12,13-dihydroxystearic acid.

The double bond in the unsaturated acid must have been in the 9,10 position since this was the point of cleavage when oxidized. Its infrared spectrum showed no *trans*-absorption hence the double bond has the *cis* configuration and the acid is (+)-*threo*-12,13-dihydroxyoleic acid. When pure, it melted at 61–61.5°, and its specific rotation was +18.9°. The mixtures of the (+) and (−) forms obtained previously from okra and kenaf oils melted at about 52–54°, and their specific rotation was about +6°. After isolation of the pure (+) enantiomer by crystallization, the residual mixture of (+) and (−) acids in the mother liquors had a specific rotation of less than 1°.

All of the dihydroxyoleic acids of m.p. 60–61.5° obtained from the *Malvaceae* species described herein were found to be identical by mixed melting-point determinations. As shown earlier for kenaf and okra oils, the epoxy acid present in these oils is *cis*-12,13-epoxyoleic acid.

Epoxy Acid Content of the Oils. Results of the examination of oils of the genus *Hibiscus* are shown in Table I. Some analytical data on these oils are given in the earlier literature, but there has been no report of oxygenated acids except in the publications from this laboratory (1,2). *Hibiscus syriacus* had the highest oil content of the group, but its epoxy content was low and it gave a large proportion of other methanol-soluble material, possibly formed from malvalic acid. *H. abelmoschus* oil yielded dihydroxyoleic acid readily.

Data for seed oils from other genera of *Malvaceae* are given in Table II. Of those with an appreciable epoxy content *Malope* oil gave dihydroxyoleic acid most readily and in the best yield. *Malva* and *Lavatera* oils, on acetolysis, produced a large methanol-soluble fraction, but much of it was evidently not dihydroxyoleic acid.

TABLE II

Tests for Epoxy Acid in Other *Malvaceae* Oils

Species	Oxirane oxygen, %	Epoxyoleic acid, calcd., %	Yield of dihydroxy-oleic acid, %	Melting point of (+)isomer, °C.
Malva moschata L.	0.40	7.4	2.1	59.5–60.5
Lavatera trimestris L.	0.32	5.8	1.5	60.5–61.5
Althaea rosea Cav.	0.08	1.5	....	....
Sidalcea hybridum Gray	0.27	5.0	....	....
Abutilon theophrasti Medic.	0.04	0.7	....	....
Malope trifida Cav.	0.48	8.8	4.0	61.0–61.5
Thespesia populnea Soland.	0.02	0.4	none	....
Gossypium hirsutum L.	0.07	1.3	....	....

Thespesia populnea oil was subjected to acetolysis, and the resulting acids were partitioned in the usual way but did not yield any dihydroxy acid. This result confirms the low analytical figure for oxirane oxygen (0.02%). *Sidalcea hybrida* oil appeared to have an appreciable content of epoxy acid, but the amount of seed available was insufficient for further study.

Specimens of oil from *Althaea rosea, Abutilon theophrasti,* and *Gossypium hirsutum* had very low oxirane oxygen content and were not examined further.

Analytical constants for some of these oils are reported in the literature, but apparently none has been published for *Malva moschata, Sidalcea hybridum,* and *Malope trifida.*

The actual content of epoxyoleic acid in the oils is believed to lie between the amount calculated from the oxirane oxygen analysis and the amount actually isolated as dihydroxyoleic acid. Allowing for losses in partitioning and recrystallizing, it is estimated that the content of epoxyoleic acid, based on the total fatty acids, was as follows: *Hibiscus abelmoschus* 3%, *H. moscheutos* 1.5%, *H. syriacus* 1.5%, *H. esculentus* 3%, *Malva moschata* 3%, *Lavatera trimestris* 3%, and *Malope trifida* 7%.

All of the oils listed in Tables I and II give the Halphen test and so presumably contain malvalic acid (5) or a related cyclopropene acid in varying amounts. When these oils are acetylated, the malvalic acid appears to form an oxygenated acid which is soluble in methanol. If there is a substantial amount of this material, as in *H. syriacus,* it renders difficult the isolation of the accompanying dihydroxyoleic acid.

Experimental

Most of the seeds were purchased from commercial sources and were of the current year's stock. *Althaea rosea* was collected from a local garden. *Abutilon theophrasti* was kindly supplied by Earl Hammond, Iowa State University, and *Thespesia populnea* by the Inspector-General of Forests, French West Africa.

The seeds were ground and extracted in a Soxhlet apparatus with petroleum ether. Removal of the solvent from the oil was carried out under nitrogen. Oil content was calculated on a 10% moisture basis. Iodine values were determined by the Wijs method and oxirane oxygen by the HCl-ether method of Swern (6).

The procedure of acetolysis of the oils and solvent partition of the mixed acids was similar to that of Gunstone (3). In a typical experiment 50 g. of *Malope trifida* oil yielded 5.0 g. of crude hydroxy acid from the methanol extract. After two recrystallizations from acetone at −25° it melted at 52–54°. A portion (0.64 g.) was crystallized twice from a mixture of petroleum ether: ethyl ether (3:1) and yielded 0.18 g. of (+)-*threo*-12,13-dihydroxyoleic acid, m.p.[3] 61–61.5° $[\alpha]_D^{24}$ + 18.9° (c, 10.0). The opti-

[3] Melting points were determined with short-stem thermometers and do not require emergent stem correction.

cal rotation was measured in absolute ethanol in a 1-dm. tube. Calculated for $C_{18}H_{34}O_4$: C, 68.75; H, 10.89; neutralization equivalent, 314.4. Found, C, 68.63; H, 11.26; neutr. equiv. 314.5. The acid absorbed one mole of hydrogen.

A portion was submitted to oxidative fission by permanganate-periodate (7), using sufficient reagent to split the chain at the double bond and at the α-glycol grouping. At the end of the reaction the acidic products were extracted with ether, and the ether solution was made alkaline and evaporated to dryness. Methanol containing excess dry hydrogen chloride was added and refluxed gently. The mixture was poured into water, and the methyl esters were extracted with cyclohexane, washed with a little aqueous sodium bicarbonate, and dried. The cyclohexane solution was submitted to gas chromatography at 80°, 130°, and 175°. Peaks corresponding to hexanoate and azelate were observed. The emergence times were identical with those of reference samples of pure methyl hexanoate and azelate. There were no other peaks. The points of fission were therefore 9,10, which gives azelaic acid, and 12,13, which gives hexanoic acid.

A further portion of the dihydroxyoleic acid was hydrogenated with palladium catalyst at 25°, giving (+)-*threo*-12,13-dihydroxystearic acid, m.p. 96.5–97°, $[\alpha]_D^{24}$ + 23.8° (c, 3.0). Calculated for $C_{18}H_{36}O_4$: C, 68.31; H, 11.47. Found: C, 68.41; H, 11.55. The α-glycol value, determined by the Method of Bharucha and Gunstone (8) and calculated as dihydroxystearic acid was 98.7%.

The dihydroxystearic acid was oxidized by permanganate-periodate. Upon acidifying the reaction mixture, a crystalline precipitate formed, m.p. 125–126°. It was identified as 1,12-dodecanedioic acid by mixed melting-point with an authentic sample. The acids in the filtrate were esterified and submitted to gas chromatography at 80° and 180°, giving peaks corresponding to hexanoate and dodecanedioate. This confirms the identity of the hydrogenated acid as (+)-*threo*-12,13-dihydroxystearic, hence the acid obtained from the seed oil is (+)-*threo*-12,13-dihydroxy oleic acid.

The identity of the hydrogenated acid was confirmed in another way. Equal amounts of (+)-*threo*-12,13-dihydroxystearic acid (from *Malope*) and (−)-*threo*-12,13-dihydroxystearic acid [from *Vernonia* (4)] were mixed and crystallized from methanol. The resulting (±) form melted at 96.5–97°, and this melting-point was not lowered by admixture with an authentic sample of (±)-*threo*-12,13-dihydroxystearic acid.

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