

lyst that he used was more active than the one employed in this study as tests in our laboratory had shown. Many of his runs were at a temperature lower than 115°C., the lowest temperature of the study. The catalyst used here was not appreciably active at such low temperatures. Investigations in which vigorous agitation is used and in which an effective low-temperature catalyst is employed are therefore still necessary to determine the variables of importance in the solvent type of hydrogenations at relatively low temperatures.

The use of a solvent in the reaction vessel was found to have a significant effect on the relationship between the hydrogenation rate and temperature. Without a solvent the maximum rate occurs well above 200°C. (1), but it appears to be appreciably less with solvents, *e.g.*, about 135–145°C. when isopropyl alcohol was used. The optimum temperature is undoubtedly controlled by the complex relationship of the true reaction-rate constants, the mass-transfer resistances, and the solubility of hydrogen in the liquid phase. Equilibrium solubilities of hydrogen in organic liquids are interesting and relatively unique since in several cases at least the solubility increases with increased temperature, *e.g.*, solubilities in triglycerides (1, 9) and in toluene (3). A better understanding of the solubility phenomenon would probably help explain the effect of temperature and pressure on the hydrogenation reaction. When a solvent is employed, the hydrogen solubility, no doubt, changes radically and may be responsible for the lower optimum temperatures found in this investigation.

The present results indicate that a commercial process might be practical in which the cottonseed oil

is hydrogenated while still in the solution obtained by the solvent extraction of the oil. The rates of hydrogenation are in the same order of magnitude even though the solvent diluted the oil and hence decreased the concentration of unsaturated bonds.

Acknowledgment

The Procter and Gamble Company donated the refined and bleached cottonseed oil and performed most of the analyses. H. K. Hawley of that company offered valuable advice at frequent intervals during the investigation.

Conclusions

Mixtures of cottonseed oil and hexane, isopropyl alcohol, or di-isopropyl ether were hydrogenated in a dead-end hydrogenator. The rates of hydrogenation of the cottonseed oil for solvent hydrogenation were somewhat less than conventional nonsolvent hydrogenations. The selectivity and *trans*-isomerization characteristics were essentially unchanged by the presence of the solvent.

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[Received September 15, 1959]

Differentiation of Hydrogen Bromide-Reactive Acids in Seed Oils

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A METHOD is presented for differentiating between epoxy acids and those without epoxy groups that react similarly in the Durbetaki titration of seed oils. Application of the technique to selected oils is discussed. The basis of the procedure is titration with hydrogen bromide before and after reduction of the acid with lithium aluminum hydride. A procedure is also presented for verifying one of the interfering nonepoxy-containing oils, dimorphecolic acid. The latter procedure is based on isolation of the acid by solvent partitioning and reduction to a crystalline derivative.

The discovery that an epoxy acid, vernolic acid, is the principal fatty acid in *Vernonia anthelmintica* seed oil led to a search, both at this laboratory (1, 2)

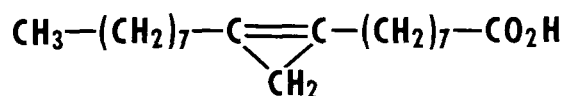
and elsewhere (3, 4, 5), for other natural sources of epoxy acids by examining numerous seed oils. Vernolic acid, shown by Gunstone (6) to be *cis*-12,13-epoxy-*cis*-9-octadecenoic acid, has also been found in seed oils of several other species, including *Vernonia colorata* (3), *Hibiscus esculentus* (3), *Hibiscus cannabinus* (4), and *Clarkia elegans* (7). In addition, three other naturally-occurring epoxy acids have been discovered in seed oils: 9,10-epoxystearic acid, found in *Tragopogon porrifolius* oil (5), coronaric acid, present in *Chrysanthemum coronarium* oil (7, 8) and 15,16-epoxylinoleic acid, present in *Camelina sativa* oil (9).

The Durbetaki method for determination of oxirane oxygen (10, 11) is used routinely at this laboratory as a screening method for detecting epoxy acids in seed oils. It is based on the opening of the oxirane ring to form a bromohydrin:

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A sample, dissolved in benzene, is titrated with hydrogen bromide-acetic acid solution to the crystal violet end-point. The procedure is admirably suited to a screening program because of its simplicity and rapidity but is not specific for epoxides. At least two fatty acids that occur in certain seed oils, sterculic acid and dimorphecolic acid, interfere with the Durbetaki determination of epoxides. Although they are not epoxy acids, they consume up to a mole of hydrogen bromide sufficiently rapidly to be mistaken for such. Sterculic acid, discovered in *Sterculia foetida* seed oil, contains a cyclopropene ring (12, 13, 14, 15, 16). The double bond in this strained three-membered ring is



Sterculic Acid

highly reactive; presumably it adds hydrogen bromide very rapidly in the benzene-acetic acid medium. Dimorphecolic acid, found in *Dimorphothecca aurantiaca* seed oil, was recently shown to be 9-hydroxy-*trans*,*trans*-10,12-octadecadienoic acid (17). The reaction of this acid with hydrogen bromide in acetic acid has not been fully studied, but apparently it involves both acid-catalyzed elimination of hydroxyl and replacement of hydroxyl with bromine.

As a confirmatory test to help differentiate epoxy acids from interfering materials which respond to the hydrogen bromide titration, we applied lithium aluminum hydride (LAH) reduction. This reagent reduces epoxides to monohydroxy compounds (18). Whether an epoxy acid is in the free state or in combined form as a triglyceride, the epoxy group is reduced to a monohydroxy derivative by LAH. The carboxylic acid or ester group is concurrently reduced to a primary alcohol group (18). This reduction product is unreactive towards hydrogen bromide-acetic acid under conditions of the Durbetaki titration. In contrast, the hydrogen bromide-reactive groupings of dimorphecolic and sterculic acids are unaffected by LAH. Nunn showed that this reagent reduces sterculic acid to a primary alcohol in which the cyclopropene ring is retained intact (12). Dimorphecolic acid is reduced to 10,12-octadecadiene-1,9-diol, which consumes the hydrogen bromide reagent in the same manner as the parent acid (17).

If the hydrogen bromide uptake of an oil is substantially undiminished by LAH reduction, epoxy acids cannot be present in more than trace amounts. Conversely, substantially complete destruction of the reactive component indicates the presence of epoxy acids. One should be cautious however about assuming that their presence is proved thereby. Other acids containing the same functional groups as dimorphecolic and sterculic acids will no doubt interfere similarly in the Durbetaki titration by appearing to be epoxy acids. For example, 8-hydroxyximenynic acid has a structure rather similar to that of dimorphecolic acid (19, 20) although it has one acetylenic bond. Its LAH reduction product is isomeric with that obtained

from dimorphecolic acid and certainly must have similar reactivity.

Lithium Aluminum Hydride Reduction Method

To determine the percentage of epoxy acid in an oil, a portion was reduced with LAH, then titrated with hydrogen bromide-acetic acid according to the Durbetaki method. The result was compared with that obtained for the untreated oil. The method used for the reductions, based on procedures of Nystrom and Brown (18), was as follows. Approximately 3 g. of LAH were placed in a round-bottomed flask, fitted with condenser and drying tubes. Anhydrous ether (100 ml.) was added. The oil sample (*ca.* 1 g.) was dissolved in anhydrous ether and added dropwise into the flask. Reflux was maintained for 2 hrs. The reaction mixture was then cooled in an ice-water bath, and 50 ml. of ethyl acetate were added slowly to destroy excess hydride. The step was followed by addition of 100 ml. of 10% aqueous sodium hydroxide to decompose the complexes. The resulting mixture was transferred to a separatory funnel. The layers were shaken together and separated. The aqueous layer was returned to the funnel and re-extracted with ether. The combined ether layers were washed with water and dried over sodium sulfate. The dried solution was evaporated on a steam bath under nitrogen. Recoveries of 90% or better were obtained. The method can be adapted to reduction of smaller samples. An alternative reduction method, using sodium borohydride-aluminum chloride in diglyme solution (21), was applied to *Vernonia anthelmintica* oil with good results.

Results obtained by applying this method to a number of oils having an appreciable content of "apparent epoxy acid" are listed in Table I. Nearly complete reduction of the "apparent epoxy acid" was observed for oils of several genera in the *Compositae*. The outstanding exception was *Dimorphothecca aurantiaca*, previously shown to contain a high percentage of dimorphecolic acid (17). *Sterculia foetida* oil, known to be rich in sterculic acid, was unaffected by reduction. It seems probable that the sample of *Brachychiton*, another member of the *Sterculiaceae*, also produces sterculic or a similar acid. In seed oils of the *Malvaceae* there is evidence for acids that are similar to sterculic acid. Some of these oils give a positive Halphen test (22, 23), a color reaction used to detect the cyclopropene ring. McFarlane and co-workers obtained evidence for a C_{18} -cyclopropenoid acid, malvalic acid, in oils of two *Malva* species (24). The C_{18} -structure was recently confirmed by Craven and Jeffrey (25). A cyclopropenoid acid (bombacic acid) from another source (kapok seed oil, family *Bombacaceae*) was reported earlier by Dijkstra and Duin (26). They tentatively assigned a C_{18} -formula to bombacic acid. The work of Hopkins and Chisholm points to the probable occurrence of epoxy and cyclopropenoid acids together in certain malvaceous oils. Oils of *Hibiscus esculentus* and *H. cannabinus*, in which they found epoxy acids, gave positive Halphen tests (3, 4). Their observations afford a likely explanation for the substantial but incomplete reduction of "apparent epoxy acid" of *H. moscheutos* oil. The apparent epoxide content of *H. syriacus* oil is probably caused only by a cyclopropenoid acid. Incomplete reduction of hydrogen bromide-reactive components in the seed oils of *Arctotis*, *Artemisia*, *Cer-*

TABLE I
 Lithium Aluminum Hydride Reduction of Various Seed Oils

Species	Family	Apparent Epoxy Acid Content ^a		Acid presumed present
		Original oil ^b	After LiAlH ₄ reduction	
<i>Veronica anthelmintica</i>	Compositae	71.8	0.3	Epoxy (confirmed by isolation [6])
<i>Chrysanthemum coronarium</i>	Compositae	14.7	0.4	Epoxy (confirmed by isolation [7, 8])
<i>Cynara cardunculus</i>	Compositae	{ 11.9 12.4 }	{ 0.8 1.9 }	Epoxy
<i>Artemisia absinthium</i>	Compositae	23.0	7.2	Epoxy + dimorphecolic-type
<i>Haliopsis pitcheriana</i>	Compositae	13.5	0.3	Epoxy
<i>Arctotis grandis</i>	Compositae	7.0	3.0	Epoxy (+ some interfering acid?)
<i>Dimorphothecca aurantiaca</i>	Compositae	51.5	55.0	Dimorphecolic (confirmed by isolation [17])
<i>Cercidium floridum</i>	Leguminosae	10.0	2.8	Epoxy (+ some interfering acid?)
<i>Calliandra eriophylla</i>	Leguminosae	18.0	3.8	Epoxy (+ some interfering acid?)
<i>Clarkia elegans</i>	Onagraceae	10.0	0.2	Epoxy (confirmed by isolation [7])
<i>Sterculia foetida</i>	Sterculiaceae	44.1	46.5	Sterculic (confirmed by isolation [12])
<i>Brachyhiton acerifolius</i>	Sterculiaceae	11.0	10.5	Sterculic-type
<i>Hibiscus syriacus</i>	Malvaceae	21.0	24.0	Sterculic-type
<i>Hibiscus moscheutos</i>	Malvaceae	15.0	10.4	Sterculic-type + epoxy
<i>Daucus carota</i>	Umbelliferae	{ 20.0 7.6 }	{ ca. 0 0.2 }	Epoxy

^a Calculated as percentage cis-epoxy acid found by the Durbetaki method (10, 11).

^b Some values in this column are from reference (1); others are from unpublished data of F. R. Earle and co-workers.

cidium, and *Calliandra* is presumptive evidence for the presence of interfering nonepoxy acids in these oils. The probable presence of a dimorphecolic type of acid in *Artemisia absinthium* seed oil was pointed out in a previous report from this laboratory (2).

After completing this work, we became aware of recent efforts of other investigators to find more definitive means of analyzing for epoxy acids. Krull (27) has used argentometric back-titration after refluxing the epoxide with collidine hydrochloride. Morris (28) has used spectral examination of the chlorohydrins. Results obtained by Morris in applying his method are presented in a paper published simultaneously with ours.

Identification of Interfering Acids

The Halphen color reaction of sterculic-type acids has already been mentioned. Examination of the infrared and ultraviolet spectra of oils containing interfering acids will aid in identifying these constituents. For example, the cyclopropene ring has a characteristic infrared maximum at 9.92 μ (23). Dimorphecolic acid, as well as any of its homologs or positional isomers that may occur in oils under examination, will give rise to infrared maxima at 2.94 μ (hydroxyl) and 10.09 μ (*trans,trans*-conjugated diene) (17). The ultraviolet absorption maximum at 231 $m\mu$, characteristic of conjugated dienes, will also be evident. The 1-hydroxy-2,4-diene system of dimorphecolic acid may be confirmed by boiling the oil under study with glacial acetic acid for 2 hrs. and by determining the ultraviolet spectrum of the product. Dienoid absorption will be replaced by the triplet maximum characteristic of a conjugated triene (259, 268, 279 $m\mu$).

If confirmation of dimorphecolic acid by a chemical method is desired, it may be isolated by solvent partitioning of the mixed free acids. Dimorphecolic acid is semisolid at room temperature but may be reduced by LAH to 10,12-octadecadiene-1,9-diol. This diol, though low-melting (46–47°), serves as a satisfactory derivative for determination of melting point and mixed melting-point. The procedure applied in this laboratory to several oils was as follows.

A portion of oil (ca. 1.3 g.) apparently containing dimorphecolic acid was saponified by stirring 3 hrs. at room temperature with 0.8 N ethanolic potassium hydroxide. The free fatty acids were isolated in the conventional manner, avoiding prolonged exposure to

mineral acid; yields of 75–85% of mixed acids were obtained. Free fatty acids (ca. 1 g.) were dissolved in 10 ml. of acetonitrile saturated with hexane. The resulting solution was extracted with (5 × 10 ml.) portions of hexane saturated with acetonitrile. The acetonitrile layer was evaporated and yielded 75–81% of the theoretically expected dimorphecolic acid. Further purification of this acid will be described in a later publication. The material thus obtained was satisfactory however for LAH reduction, which was carried out as described previously for methyl dimorphecolate (17). Mixed melting point determinations were carried out with specimens of authentic 10,12-octadecadiene-1,9-diol.

After the discovery of dimorphecolic acid in *Dimorphothecca aurantiaca* seed oil, it became of interest to determine whether this acid also occurred in seeds of several other *Dimorphothecca* species that became available. The results obtained by our isolation method confirmed the presence of dimorphecolic acid in all of these, including *D. annua*, *D. calendulacea*, *D. sinuata*, *D. ecklonis*, and *Dimorphothecca aurantiaca* var. "tetra-goliath." Screening analyses had indicated these to contain as much dimorphecolic or similar acid as *D. aurantiaca*.

Acknowledgment

The authors thank Quentin Jones, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, for his cooperation in obtaining seeds used in these studies.

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[Received December 1, 1959]

Vicinally Unsaturated Hydroxy Acids in Seed Oils¹

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A METHOD for the detection and estimation of epoxy components of oils, based on near infrared spectrophotometry, has recently been developed in this laboratory (1). In the course of this work we examined several seed oils which gave appreciable, apparent epoxy values by the hydrogen bromide titration method of Durbetaki (2,3). We found that all these oils contained at least a trace of epoxy constituents, as measured by near infrared spectra, but not to the extent indicated by IBr titration. In a paper published simultaneously with this (4) Smith and co-workers describe a method of differentiating between epoxy acids and some other acids which lead to spurious epoxide values by the Durbetaki method. They have determined the structure of an hydroxy acid, 9-hydroxy-*trans-trans*-10,12-octadecadienoic acid (I, Table I), which reacts with the IBr reagent (5). This acid is present in the seed oils of several species of *Dimorphotheca* and has been named dimorpheolic acid.

The present contribution records studies, by the physical methods of chromatography and spectrophotometry, of six seed oils which show a discrepancy in epoxy values as measured by IBr titration and by near infrared spectra. By these methods we were able to show the presence of epoxy acids in these oils along with certain unsaturated hydroxy acids which give rise to this discrepancy. In some respects this publication and that of Smith *et al.* (4) are complementary.

For clarity the structures of some of the acids discussed are shown in Table I and referred to in the text by Roman numerals.

Materials and Methods

The mixed acids and esters derived from the seed oils of *Dimorphotheca aurantiaca* (Cape marigold), *Artemisia absinthium* (wormwood), *Calliandra eriophylla* (fairy dusters), *Balanites aegyptica* (lalo), *Cosmos bipinnatus* (cosmos), and *Helianthus annuus* (sunflower) were investigated. The mixed acids of these oils were obtained by conventional alkaline hy-

TABLE I

$\text{CH}_3(\text{CH}_2)_4 \underset{\text{trans}}{\text{CH}=\text{CH}} \underset{\text{trans}}{\text{CH}=\text{CH}} \text{CH}(\text{OH})(\text{CH}_2)_7 \text{COOH}$	I
$\text{CH}_3(\text{CH}_2)_4 \underset{\text{cis}}{\text{CH}=\text{CH}} \underset{\text{trans}}{\text{CH}=\text{CH}} \text{CH}(\text{OH})(\text{CH}_2)_7 \text{COOH}$	II
$\text{CH}_3(\text{CH}_2)_4 \text{CH}(\text{OH}) \underset{\text{trans}}{\text{CH}=\text{CH}} \underset{\text{cis}}{\text{CH}=\text{CH}}(\text{CH}_2)_7 \text{COOH}$	III
$\text{CH}_3(\text{CH}_2)_4 \text{CH}(\text{OH}) \underset{\text{trans}}{\text{CH}=\text{CH}} \underset{\text{trans}}{\text{CH}=\text{CH}}(\text{CH}_2)_7 \text{COOH}$	IV
$\text{CH}_3(\text{CH}_2)_4 \underset{\text{trans}}{\text{CH}=\text{CH}} \underset{\text{trans}}{\text{CH}=\text{CH}} \underset{\text{trans}}{\text{CH}=\text{CH}}(\text{CH}_2)_6 \text{COOH}$	V

drolysis at room temperature; prolonged contact with mineral acids after acidification was avoided. Esters were prepared from the acids with diazomethane.

Ultraviolet and near infrared spectral data were obtained with a Beckman DK2 Recording Spectrophotometer. Methanol was the solvent used in the ultraviolet range, and solutions of 3.0 or 1.0% in carbon tetrachloride were used for near infrared studies. Silica cells of 1-cm. path length were used throughout. Infrared spectra in the 2–15 μ region were determined by using a Perkin-Elmer 12C Spectrophotometer with Model 81 microscope and a Reeder thermocouple.

Paper chromatography was carried out on siliconized Whatman No. 1 paper (6) with solvent systems of aqueous acetonitrile for esters and of aqueous acetonitrile and acetic acid for acids (7).

Silicic acid was coated as a thin layer on glass plates by the procedure of Stahl (8). The acid and ester mixtures were chromatographed and individual components were isolated, using methods and solvents already described by Mangold and Malins (9,10,7).

Gas-liquid chromatography (GLC) was carried out on a 6 ft. x 1/4-in. coiled copper column having, as its stationary phase, 15% L.A.C.-2R446³ polyester resin coated on Celite, 80–100 mesh. The column was held at 196°C., and the flash heater and detector at 250°C. Argon, as carrier gas, flowed at a rate of 60 c.c./min. under a head pressure of 25 p.s.i. The β -ionization detector⁴ was operated at 800 volts. Some studies were also carried out, using a 2 ft. x 1/4-in. straight copper column, containing 20% Apiezon M hydrocarbon grease on Celite, 80–100. This column was run at 204°C. with flash heater and detector at 240°C., and argon, at 9 p.s.i. pressure flowing at 60 cc./min.

Fractions were collected after GLC by passing the

¹ Supported by grants from The Hormel Foundation and the National Institutes of Health (Research Grant No. H-3559), and presented in part at the 33rd fall meeting, American Oil Chemists' Society, Los Angeles, Calif., September 28–30, 1959.

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³ Obtainable from Cambridge Instruments Company, Cambridge, Mass.

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