14. Brooke, D. G., and Smith, J. C., Chem. and Ind. (London), *1957*, 49.
15. Lewis, B. A., and Raphael, R. A., *ibid.*, *1957*, 50.
16. Brooke, D. G., and Smith, J. C., *ibid.*, *1957*, 1508.
17. Smith, C. R. Jr., Wilson, T. L., Melvin, E. H., and Wolff, I. A., J. Am. Chem. Soc., in press (1960).
18. Nystrom, R. F., and Brown, W. G., J. Am. Chem. Soc., *69*, 1197, 2548 (1947); *ibid.*, *70*, 3738 (1948).
19. Ligthelm, S. P., Chem. and Ind. (London), *1954*, 249.
20. Crombie, L., and Griffin, B. P., J. Chem. Soc., *1958*, 4435.
21. Brown, H. C., and Subba Rao, B. C., J. Am. Chem. Soc., *78*, 2582 (1956).
22. American Oil Chemists' Society, "Official and Tentative Methods," 2nd ed. (1958 revision), Method Cb-25.
23. Faure, P. K., Nature, *178*, 372 (1956).
24. McFarlane, J. J., Shenstone, F. S., and Vickery, J. R., *ibid.*, *179*, 830 (1957).
25. Craven, B., and Jeffrey, G. A., *ibid.*, *183*, 676 (1959).
26. Dijkstra, G., and Duin, H. J., *ibid.*, *176*, 71 (1955).
27. Krull, L., Fette Seifen Anstrichmittel, *61*, 223 (1959).
28. Morris, L. J., personal communication.

[Received December 1, 1959]

# Vicinally Unsaturated Hydroxy Acids in Seed Oils[1]

**L. J. MORRIS, R. T. HOLMAN, and K. FONTELL,[2] The Hormel Institute and Department of Physiological Chemistry, University of Minnesota, Austin, Minnesota**

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 HBr 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 HBr reagent (5). This acid is present in the seed oils of several species of *Dimorphotheca* and has been named dimorphecolic 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 HBr 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.

TABLE I

| | |
|---|---|
| $CH_3(CH_2)_4$ CH=CH (*trans*) CH=CH (*trans*) $CH(OH)$ $(CH_2)_7$ COOH | I |
| $CH_3(CH_2)_4$ CH=CH (*cis*) CH=CH (*trans*) $CH(OH)$ $(CH_2)_7$ COOH | II |
| $CH_3(CH_2)_4$ $CH(OH)$ CH=CH (*trans*) CH=CH (*cis*) $(CH_2)_7$ COOH | III |
| $CH_3(CH_2)_4$ $CH(OH)$ CH=CH (*trans*) CH=CH (*trans*) $(CH_2)_7$ COOH | IV |
| $CH_3(CH_2)_4$ CH=CH (*trans*) CH=CH (*trans*) CH=CH (*cis*) $(CH_2)_6$COOH (*trans*) | V |

## 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* (lalob), *Cosmos bipinnatus* (cosmos), and *Helianthus annuus* (sunflower) were investigated. The mixed acids of these oils were obtained by conventional alkaline hydrolysis 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$\mu$ 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 ¼-in. coiled copper column having, as its stationary phase, 15% L.A.C.-2R446[3] 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 $\beta$-ionization detector[4] was operated at 800 volts. Some studies were also carried out, using a 2 ft. x ¼-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.
[2] Permanent address: Åbo, Finland. Fulbright Scholar to the Hormel Institute, 1958–1960.

[3] Obtainable from Cambridge Instruments Company, Cambridge, Mass.
[4] Research Specialties, Richmond, Calif.

effluent gas through a short Teflon tube into methanol held at −70°C. After each fraction the tube was removed and rinsed with methanol.

## Spectrophotometric Studies

*Near Infrared.* Measurement of the absorption of oil samples at 2.795μ in the near infrared region, before and after treatment with anhydrous ethereal hydrogen chloride, provides a highly specific method for the detection and estimation of epoxides (1). The increase in associated hydroxyl absorption due to chlorohydrins, produced from epoxides, is measured, and there is no interference by other functional groups since no other group generates an hydroxyl function on treatment with hydrogen chloride.

*Dimorphotheca* oil, as a 1% solution in carbon tetrachloride, was found to give a strong unassociated hydroxyl band at 2.762μ. After treatment with ethereal hydrogen chloride however this band was absent, demonstrating that an acid-catalyzed hydroxyl elimination had occurred. This appears to be accompanied by a partial substitution of chlorine for hydroxyl since a high epoxide value is obtained (1) by the hydrogen chloride method of Swern (11). This is similar to the bromine substitution postulated by Smith *et al.* (4).

It was found that several other oils, having similar absorption because of hydroxyl groups, lost part or all of this absorption after treatment with hydrogen chloride (Figure 1). This indicated the possible presence of dimorphecolic acid (I) or a similarly reactive hydroxy acid in these oils.

Smith and co-workers have shown that dimorphecolic acid comprises 55% of *Dimorphotheca* oil. A value for the decrease in specific absorptivity of this acid at 2.795μ after HCl treatment could thus be calculated. Hence the proportions of "dimorphecolic" acid in the other oils were obtained (Table II).

We found that all six oils contained small amounts of epoxy acids. The epoxy acid content of those oils with a high proportion of "dimorphecolic" acid (*Dimorphotheca, Artemisia,* and *Calliandra* oils) was estimated. Correction was made for the contributions of the adjacent strong maxima. The large alterations of absorption in the 2.8μ region made direct measurement impossible. The epoxy acid content of the other three oils, having smaller amounts of reactive hydroxy acids, could be measured directly. The epoxy values obtained for these oils by Smith *et al.*, using the Durbetaki method, are included in Table II, and it may be noted that the sum of epoxy acid and "dimorphecolic" acid values, as determined by our method, is close in each case to the "apparent" epoxy values.

*Ultraviolet.* Dimorphecolic acid contains a *trans-trans* conjugated diene structure, and therefore the concentration of this acid is measurable by ultraviolet spectrophotometry. The extinction coefficient of *Dimorphotheca* oil at 231 mμ in methanol solution was found to be 55.3% of that of a pure *trans-trans* octadecadienoic acid, and, allowing for the difference in molecular weight, this indicates a concentration of 58.4% of hydroxydienoic acid in the oil. The conjugated diene content of the other oils was similarly measured, and agreement between these values for "dimorphecolic" acid content and those obtained by the near infrared method is good (Table II).

As a further check the oils were each boiled with glacial acetic acid, a procedure which, according to Smith *et al.* (5), dehydrates dimorphecolic acid (I) to a conjugated trienoate (V). Ultraviolet absorption measurements after this treatment showed an increase in conjugated triene content in all samples, but complete conversion of hydroxy diene to triene was not achieved in any sample. It seems possible that acetylation or acetoxy substitution, analogous to the reaction with hydrogen chloride or bromide, may occur to prevent triene formation.

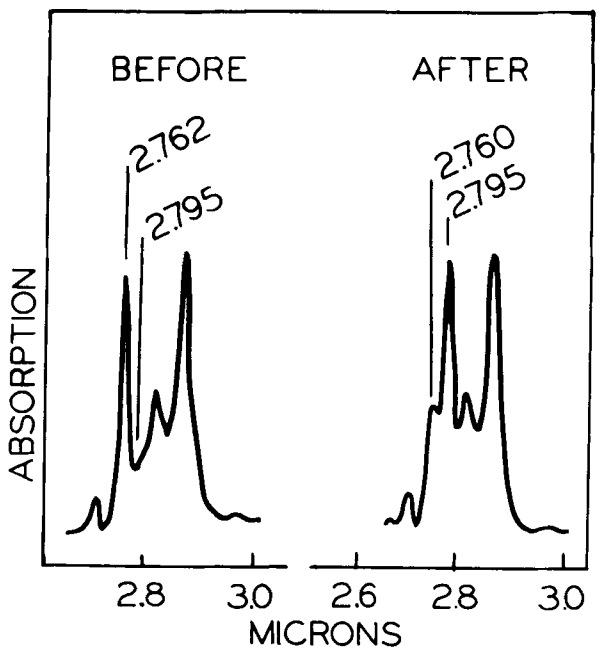


FIG. 1. Near infrared spectra of *Balanites aegyptica* seed oil before and after treatment with ethereal HCl reagent. Solutions are 3.0% in $CCl_4$.

TABLE II

| Oil from | "Dimorphecolic" acid | | Epoxy acid as($C_{18}$') | |
|---|---|---|---|---|
| | by I.R. | by U.V. | by I.R. as chlorohydrin | by HBr titration |
| | % | % | % | % |
| *Compositae:* | | | | |
| *Dimorphotheca aurantiaca* | (55.0)[a] | 58.4 | 0.6[a] | 52.0 |
| *Artemisia absinthium* | 9.0[a] | 10.3 | 14.9[a] | 23.0 |
| *Cosmos bipinnatus* | 3.5 | 3.5 | 3.2 | 6.6 |
| *Helianthus annuus* | 2.0 | 2.0 | 1.9 | 3.1 |
| *Leguminosae:* | | | | |
| *Calliandra eriophylla* | 5.0[a] | 5.7 | 5.9[a] | 11.0 |
| *Zygophyllaceae:* | | | | |
| *Balanites aegyptica* | 4.0 | 4.1 | 3.5 | 7.9 |

[a]These values were estimated, making allowance for neighboring peaks.

## Chromatographic Studies

The good agreement between the values for "dimorphecolic" acid as measured by near infrared absorption, as a function of reactive hydroxyl, and by ultraviolet absorption, as a function of conjugated diene, is real for *Dimorphotheca oil,* from which a high concentration of dimorphecolic acid has been isolated. This agreement may be fortuitous for the other oil samples since part or all of the conjugated diene groups may not be adjacent to the hydroxyl groups, which may be activated toward the hydrogen halides by some other functional group. A single unsaturated bond, for instance, adjacent to the hydroxyl may suffice to activate it in this way. The experiments described below were performed to determine whether a dimorphecolic-type acid was present in these oils or

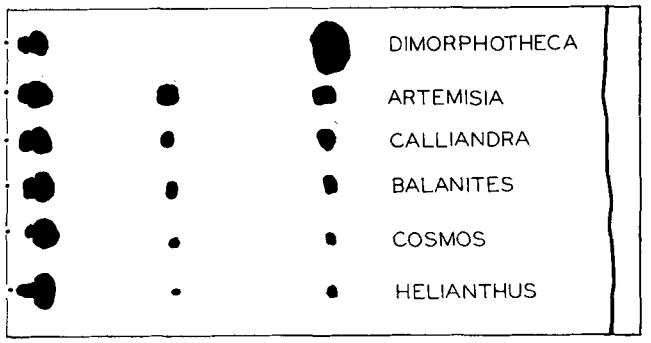


FIG. 2. Paper chromatographic separation of mixed esters, using acetonitrile-water (50/50) as solvent. Papers were developed with iodine vapors and reproduced by tracing the outlines of the spots.

whether some other activated hydroxy acid was present along with a conjugated dienoic acid.

*Paper Chromatography.* The mixed esters from the oils were separated by partition chromatography on siliconized paper (Figure 2), using aqueous acetonitrile (50/50, v/v). Each showed a spot, at an $R_f$ value of about 0.55, due to the ester of an hydroxy acid (7). In addition, all samples except that from *Dimorphotheca* oil showed a spot at about 0.30, due to an epoxy component (7), and spots, due to nonoxygenated constituents near the starting point. The chromatograms of the mixed acids of these oils developed with aqueous acetonitrile-acetic acid (48/50/2) gave a similar picture, all showing an hydroxylated component at an $R_f$ of about 0.9 and an epoxy constituent near 0.8 besides the common acids much nearer the base line. No other unusual components were demonstrated by this method, which confirmed the conclusions from spectral data.

*Thin-Layer Chromatography.* This versatile new method demonstrated that the constitution of all the oils, except *Dimorphotheca* oil, was more complex than was apparent from previous experiments. A solvent system of 10% ether in petroleum ether for the chromatography of esters, and the same system with an added 1% of acetic acid for the acids, showed a variety of epoxy constituents in the various samples of $R_f$ values of around 0.5. Suggestions as to the structures of these epoxy components are presented in another paper (7). Of more interest to this study was the separation of two hydroxy constituents, $R_f$ 0.1, from all samples except that derived from *Dimorphotheca* oil. More polar solvent systems, containing 20% ether, caused migration of epoxy components directly behind the normal unsubstituted acids or esters at the solvent front. The presence and separation of two hydroxy esters in five samples is clearly demonstrated in Figure 3. The lower spots migrate similarly to dimorphecolic ester and the more polar constituent of reduced linoleate hydroperoxide whereas the upper series corresponds, with regard to their $R_f$ values, to the less polar constituent of the reduced peroxide.

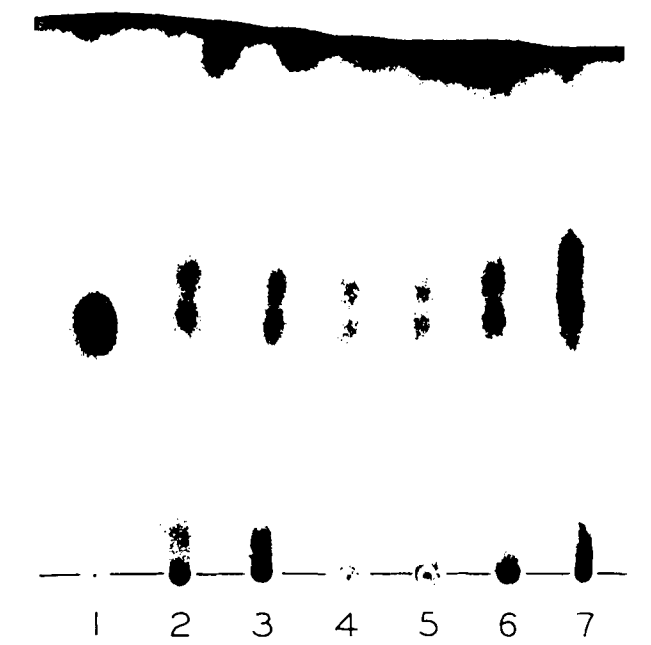


FIG. 3. Thin-layer chromatographic separation of mixed esters using ether, petroleum ether (20,80) as solvent, Plate was developed by heat, after spraying with 50% $H_2SO_4$, and reproduced by contact printing. 1. *Dimorphotheca*, 2. *Artemisia*, 3. *Calliandra*, 4. *Balanites*, 5. *Cosmos*, 6. *Helianthus*, 7. Reduced linoleate hydroperoxides.

The reduced linoleate hydroperoxide sample used was a mixture of four compounds, the *cis-trans* and *trans-trans* isomers of 9-hydroxy-10,12-octadecadienoate (II, I,) and of 13-hydroxy-9,11-octadecadienoate (III, IV). The spots on the thin-layer chromatograms of this standard sample were more diffuse and ran into each other more than those of the oil samples, indicating the greater complexity of the standard, but a separation into two main spots was apparent. Whether this gross separation was of *cis-trans* from *trans-trans* isomers or of 9-hydroxy from 13-hydroxy compounds however was not apparent from these chromatograms alone.

Moreover neither of the hydroxy esters of the five samples showing two spots was necessarily either dimorphecolate or an isomer of it, although this seemed probable, since it is very possible that other hydroxy compounds may have the some adsorption characteristics in the solvent systems used. Gas-liquid chromatographic studies were therefore undertaken to determine if these hydroxy esters were indeed isomers of methyl dimorphecolate.

*Gas-Liquid Chromatography.* In a recent publication (12) we have shown that several hydroxy compounds are dehydrated during GLC. Methyl dimorphecolate (I), for instance, was found to alter to a mixture of conjugated trienoates (V). *alpha*-Hydroxy-monoenes emerged as conjugated dienes, but hydroxy compounds not activated by adjacent unsaturation were stable during GLC.

Analyses of the mixed esters from each of the oils, on both polar and nonpolar columns, gave two peaks at relative retention times corresponding to a mixed *cis-trans* trienoate and to an *all-trans* trienoate, respectively (12), in addition to peaks due to epoxy and other components. This demonstrated that at least one isomer of dimorphecolic acid was present in each of the five oils.

Separation and isolation of the two hydroxy constituents from the mixed esters of each oil was accomplished by thin-layer chromatography (10). Approximately 25 mg. of mixed esters were separated on each plate, and spots were made visible by spraying with 2',7'-dichlorofluorescein and viewing under ultraviolet light. After scraping off the spots, the adsorbed hydroxy esters were eluted with ether and the resultant solutions were concentrated. These hy-

droxy esters were then examined by GLC on the polyester column. Both hydroxy components from each oil gave peaks identical with those from dimorphecolate, and ultraviolet spectra of the collected GLC eluents confirmed the mixed *cis-trans* triene structure of the first peak constituent and the *all-trans* triene structure of the other.

Each of the five oils, other than *Dimorphotheca*, was thus shown to contain two dimorphecolic-type acids. Whether these isomers were *cis-trans*, *trans-trans*, or positional analogues was still not known since the isomerization of conjugated trienes during GLC (12) would produce the same pattern from all isomers.

## Structural Considerations

As mentioned earlier, although the mixture of reduced linoleate hydroperoxides was separated into two fractions by thin-layer chromatography, it was impossible to decide whether this was separation of *cis-trans* from *trans-trans* analogues or of 9-hydroxy from 13-hydroxy isomers. Thin-layer chromatography of *cis-trans* and *trans-trans* dienoates showed a slight difference in $R_f$ values of the two, but a mixture was not separated and gave only a more diffuse spot. The same result was observed with *cis-trans-trans* and *trans-trans-trans* trienoates. The isomer having a *cis*-double bond had a slightly higher $R_f$ value than the other but was not separated from the mixture. It seems therefore that thin-layer chromatographic separation of the hydroxy-dienoic compounds is by positional rather than by *cis-trans* isomerism. This is somewhat similar to the thin-layer separation of 9,10-epoxyoctadec-12-enoate from 12,13-epoxyoctadec-9-enoate (7) and of 9-hydroxyoctadec-12-enoate from 12-hydroxyoctadec-9-enoate (Figure 4). The derivatives having an oxygen-containing functional group located between the ester group and the unsaturated center are more polar than those having both ester group and double bond on the same side relative to this group. The 9-hydroxy-10,12-dienoate (I or II) then is the more polar isomer while the most logical choice of structure for the isomer represented by the upper spot is 13-hydroxy-9,11-octadecadienoate (III or IV).

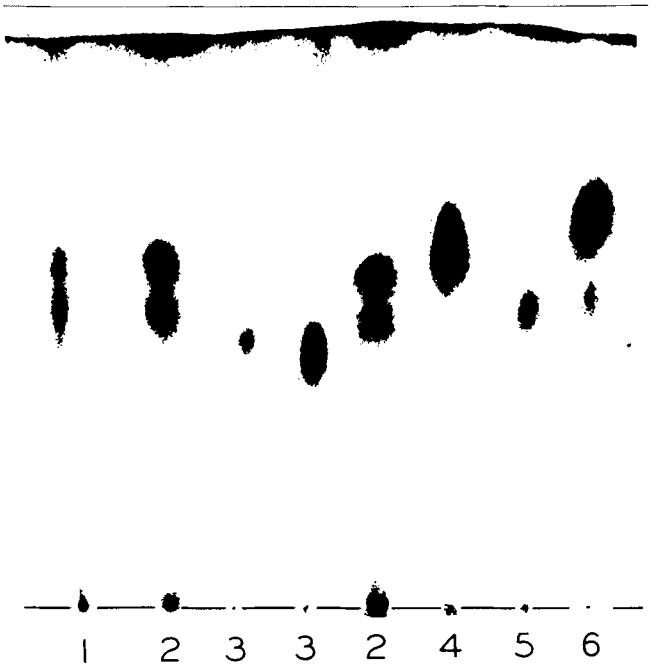


FIG. 4. Treatment the same as in Figure 3. 1. Reduced linoleate hydroperoxides, 2. *Artemisia*, 3. *Dimorphotheca*, 4. 12-Hydroxyoctadec-9-enoate, 5. 9-hydroxyoctadec-12-enoate, 6. Mixture of 4 and 5.

Having shown that these oils probably contain both 9-hydroxy and 13-hydroxy isomers, it was necessary to determine the configurations of the double bond systems. The individual esters of the hydroxy acids of *Artemisia* oil were separated and isolated by thin-layer chromatography, and their infrared spectra were determined. Both showed strong absorption at 10.13 and 10.51$\mu$. The ratio of the absorbance at 10.13 to that at 10.51$\mu$ was 1.08 and 1.20 for the less polar and more polar constituents, respectively. This showed that both isomers have a *cis-trans* or *trans-cis* diene structure and are uncontaminated with *trans-trans* isomers (13). The isomer with an absorbance ratio of 1.08 has apparently been isolated in a purer form than the standard *cis-trans* diene of Chipault and Hawkins (13), whose lowest absorbance ratio was 1.198. It may be however that the presence of an adjacent hydroxyl group causes a difference in this ratio. Thus both hydroxy constituents of *Artemisia* oil are different from the dimorphecolic acid from *Dimorphotheca* oil, the *trans-trans* diene structure of which was confirmed by the procedures described above. The adsorption characteristics of the hydroxy constituents of the five oils containing two of these components are identical. The apparent identity of the more polar constituents with dimorphecolate (Figure 3) was shown to be false when a smaller sample of *Dimorphotheca* esters was chromatographed alongside the esters of *Artemisia* (Figure 4). The more polar nature of the component from *Dimorphotheca* oil, due to the *trans-trans* diene system, is apparent.

## Conclusions

The constituents of six vegetable oils, which are responsible for interference in the Durbetaki method of epoxide determination, are acids containing an $\alpha\beta,\gamma\delta$-unsaturated hydroxyl structure. Some confirmation has been found that the structure of the hydroxy acid of *Dimorphotheca* oil is 9-hydroxy-*trans-trans*-10,12-octadecadienoic acid. Two isomers of this acid, containing *cis-trans* diene unsaturation, have been shown to occur in each of the other five oils studied and, although determinations of the positions of the functional groups in these acids have not been carried out, it is considered that they are 9-hydroxy-10,12- and 13-hydroxy-9,11-octadecadienoic acids, respectively. The specific assignment of the *cis* and *trans* double bonds relative to the hydroxy groups has not been made, but if these acids are products of the metabolism of linoleic acid, possibly secondary products from lipoxidase oxidation, it is likely that they are 9-hydroxy-*trans*-10-*cis*-12-octadecadienoate (II) and 13-hydroxy-*cis*-9-*trans*-11-octadecadienoate (III), respectively.

It is suggested that, if the proposed structures of these acids are confirmed and if trivial names are necessary, the 9-hydroxy-*trans-cis*-dienoic acid (II) be called $\alpha$-dimorphecolic acid, the dimorphecolic acid of Smith *et al.* (I) be called $\beta$-dimorphecolic acid, analogous to the nomenclature of the elaeostearic and kamlolenic acids, and the 13-hydroxy-*cis-trans*-isomer (III) be named $\alpha$-artemisic acid.

It is noteworthy that these acids are constituents of the members of three families: *Compositae (Dimorphotheca, Artemisia, Cosmos,* and *Helianthus),* the *Leguminosae (Calliandra),* and the *Zygophyllaceae (Balanites).* This indicates that the distribution of these acids in nature may be widespread and that they may have some metabolic significance.

The tremendous potential of the method of thin-layer chromatography is demonstrated in this work since, without its aid, the detection and differentiation of two hydroxy constituents in five of the oils could not readily have been accomplished.

Our results confirm and amplify some of the findings of Smith *et al.* (4). It is considered that the method of detection of this type of acid by near infrared spectrophotometry, as described in this paper, is more specific and more sensitive than the chemical methods outlined by them. However the requirement of expensive specialized equipment may make it less desirable to some laboratories. The complementary use of GLC, thin-layer chromatography, and ultraviolet and infrared spectrophotometry provided a means for detecting and evaluating small amounts of the hydroxy substances which interfere in the determination of epoxy acids.


## Summary

The interference of certain unsaturated hydroxy acids in the Durbetaki method of epoxide determination has been demonstrated. The concentrations of these constituents were determined concurrently with those of epoxy components by measurement of the near infrared spectra of samples before and after treatment with anhydrous ethereal hydrogen chloride. The individual hydroxy esters were separated and isolated from samples of mixed esters by thin-layer chromatography. GLC of these esters resulted in their alteration to conjugated trienoates and gave proof of their conjugated diene hydroxyl structure. Thin-layer chromatographic and infrared studies verified the *trans-trans* diene unsaturation of the acid from *Dimorphotheca aurantiaca* oil and showed that the other hydroxy compounds examined have a *cis-trans* diene system. These data suggest that the seed oils of *Artemisia absinthium, Calliandra eriophylla, Balanites aegyptica, Cosmos bipinnatus, and Helianthus annuus* contain 9-hydroxy-*trans*-10-*cis*-12- and 13-hydroxy-*cis*-9-*trans*-11-octadecadienoic acids.



## Acknowledgment

The authors gratefully acknowledge the cooperation of C. R. Smith Jr., and F. R. Earle of the Northern Regional Laboratory, U. S. Department of Agriculture, Peoria, Ill., in providing us with the seed oils used in this investigation and in making some of their results available to us.

We are also grateful to P. R. Edmondson and H. Dinsmore, of the Department of Medicine of this university, for performing infrared analyses, on the micro scale, of some of our products.


REFERENCES


1. Morris, L. J., and Holman, R. T., J. Lipid Res., in press.
2. Durbetaki, A. J., Anal. Chem., *28*, 2000 (1956).
3. American Oil Chemists' Society, "Official and Tentative Methods," 2nd ed. (1958 revision), Method Cd 9-57.
4. Smith, C. R. Jr., Burnett, M. C., Wilson, T. L., Lohmar, R. L., and Wolff, I. A., J. Am. Oil Chemists' Soc., *37*, 320 (1960).
5. Smith, C. R. Jr., Wilson, T. L., Melvin, E. H., and Wolff, I. A., J. Am. Chem. Soc., *82*, 1417 (1960).
6. Schlenk, H., Gellerman, J. L., Tillotson, J. A., and Mangold, H. K., J. Am. Oil Chemists' Soc., *34*, 377 (1957).
7. Morris, L. J., Holman, R. T., and Fontell, K., J. Lipid Res., in press.
8. Stahl, E., Pharmazie, *11*, 633 (1956); Chemiker-Ztg., *82*, 323 (1958).
9. Mangold, H. K., and Malins, D. C., J. Am. Oil Chemists' Soc., in press.
10. Malins, D. C., and Mangold, H. K., J. Lipid Res., in press.
11. Swern, Daniel, Findley, T. W., Billen, G. N., and Scanlan, J. T., Anal. Chem., *19*, 414 (1947).
12. Morris, L. J., Holman, R. T., and Fontell, K., J. Lipid Res., in press.
13. Chipault, J. R., and Hawkins, J. M., J. Am. Oil Chemists' Soc., *36*, 535 (1959).


[Received December 28, 1959]

# Report of the Uniform Methods Committee, 1959-60

THE MEETING of the Uniform Methods Committee of the American Oil Chemists' Society was held at 2 p.m., April 5, 1960, in the Baker Hotel. The meeting was attended by six members of the committee. (S. E. Tierney had resigned.) E. W. Sallee, editor of Methods, was present as a member *ex officio.* Guests present were R. H. Dreyer, Edward Handschumaker, E. R. Hahn, J. C. Harris, R. A. Marmor, L. D. Metcalfe, and W. E. Link.

The following matters were discussed, and the indicated decisions were made:

**1. Fat Analysis Committee, V. C. Mehlenbacher, chairman**

a) *Hydroxyl Value. Tentative Method Cd 13-60.* This new method was proposed for adoption as tentative. With some minor revisions and with the data on its precision to be calculated and given for each of the products studied in the collaborative work, the method is recommended for adoption as tentative.

b) *Soap in Oil. Tentative Method Cc 15-60.* This new method was proposed for adoption as tentative. It was developed and checked by a subcommittee under its chairman, P. W. Morgan. Precision data have been obtained. It is recommended for adoption as tentative.

c) *Nonvolatiles (Solids). Tentative Method Ka 14-60.* This new method was proposed for adoption as tentative. It is for the determination of nonvolatiles (solids) in solutions of natural and synthetic drying oils in organic solvents. It was developed by the drying oils subcommittee, K. E. Holt, chairman. Precision data have been obtained. This method is recommended for adoption as tentative.

d) *Consistency-Penetration Method. Tentative Method Cc 16-60.* This new method was proposed for adoption as tentative. The procedure is an adaptation of one of the A.S.T.M. methods, made by the consistency subcommittee, N. W. Ziels, chairman. With the precision to be calculated as coefficients of variation, the method is recommended for adoption as tentative.

e) *Solid Fat Index. Tentative Method Cd 10-57.* It was proposed that Note 5 be revised to read as follows:

"*Note 5*—The liquid thermal expansion is basic for calculating the solid fat index. It must therefore be accurate. Repeated analyses have shown values of 0.83 to 0.85 ml./kg. to be normal for cottonseed oil, soybean oil, lard, and tallow. Lauric acid oils, such as coconut, have values of 0.85 to 0.87 ml./kg. If determined values are abnormal, the analyses should be repeated. Standard thermal expansions may be applied in routine determinations where results are used within an organization, provided they are checked periodically by actual measurement."