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Naturally occurring epoxy acids: I. detection and evaluation of epoxy fatty acids by paper, thin-layer, and gas-liquid chromatography*†

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SUMMARY

Chromatographic procedures for the detection and evaluation of oxygenated fatty acids are described. Emphasis has been placed on epoxy acids, but these methods promise to be of great value in studies of all classes of oxygenated acids. Paper chromatography of fatty acids and their esters has been developed for the examination of mixtures containing oxygenated derivatives. The method of adsorption chromatography on thin layers of silicic acid has been shown to be a powerful tool in studies of epoxy acids and hydroxy acids. Gas-liquid chromatography (GLC) of epoxy esters has been studied using both polar and nonpolar columns and has great utility in the detection and analysis of these compounds in mixtures. These methods have been applied to the examination of the epoxy components of six seed oils. Thin-layer and GLC proved particularly useful in this study and together demonstrated the presence of at least three distinct epoxy acids in each of these oils. Some conclusions as to the probable structures of these epoxy components are presented on the basis of their chromatographic characteristics in relation to known substances.

The first epoxy acid shown to occur naturally, *cis*-12:13-epoxyoctadec-9-enoic acid,[1] was discovered and characterized by Gunstone (1) in 1954. It comprised 72% of the mixed acids of *Vernonia anthelmintica* seed oil. Since then the same acid has proved to be a constituent of several other oils (2, 3, 4) and its isomer, *cis*-9:10-epoxyoctadec-12-enoic acid has been described as a component of *Chrysanthemum coronarium* seed oil (5). These two acids are obviously structurally related to linoleic acid. Epoxy acids similar structurally to linolenic acid and oleic acid have also been characterized. An example of the former, 15:16-epoxyoctadec-9,12-dienoic acid, occurs in *Camelina sativa* seed oil (6), and the latter, cis-9:10-epoxystearic acid comprises 28% of the mixed acids from the uredospores of a wheat stem rust (7) and 3% of the acids of *Tragopogon porrifolius* seed oil (8). Many other seed oils have now been shown to have epoxy components (9, 10, 11), not yet characterized, and a widespread distribution of these acids in nature is now becoming recognized.

The above-mentioned structural relationship between the epoxy acids thus far described and the common unsaturated acids poses the question of a more general relationship between the two classes of compounds. The possibility exists that the epoxy group is a precursor of unsaturation or a product of the metabolism of unsaturated acids. These compounds may therefore be of greater importance in lipid chemistry than previously imagined, and improved methods for their detection and evaluation in small amounts are desirable.

Most methods in general use for the detection and estimation of long-chain epoxy compounds depend on the uptake of hydrogen halide by the epoxy group. Measurement of hydrogen halide uptake can be done directly (12), potentiometrically (13), or by back

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[1] The epoxy ring structure is indicated by a colon, e.g., 12:13-epoxyoctadec-9-enoic acid, which is synonymous with 12,13-epoxyoctadec-9-enoic acid.

titration (14, 15, 16), and unless fairly large samples are available little reliance may be placed on results indicating much below 5% of epoxy component in a fat. In some cases much higher apparent epoxy values may be completely spurious (10, 11, 15). Of other methods which have been tried, polarography offers little promise (17) and colorimetry (18) has not yet been developed for long-chain epoxides. Proton magnetic resonance (19) is useful only with concentrations higher than 5%, and glycol determination by periodate oxidation, after splitting the epoxide group (20), is more tedious and less sensitive than the standard methods.

In view of the possible importance of epoxy compounds in lipid chemistry, more sensitive methods are required for their detection and measurement. This paper describes the development of paper, thin-layer, and gas-liquid chromatographic methods for this purpose.

EXPERIMENTAL

Materials. cis-12:13-Epoxyoleic acid was prepared from *Vernonia anthelmintica* mixed acids by partition between 80% methanol and hexane (20). A portion of this acid, which had oxirane oxygen 5.2% (theory 5.4%), was esterified by reaction with ethereal diazomethane.

cis-9:10-Epoxystearic acid (m.p. 57.5°-58°; reported 59.5°), *trans*-9:10-epoxystearic acid (m.p. 53°-54°; reported 55.5°), and *cis,cis*-9:10,12:13-diepoxystearic acid (m.p. 78°-78.5°; reported 79°) were prepared by the methods of Swern *et al.* (21, 22). The methyl esters were prepared by reaction with diazomethane in anhydrous ether solution. *threo*-12,13-Dihydroxyoleic acid (m.p. 50°-52°; reported 53°-54°) was derived from methyl epoxyoleate by reaction with acetic acid followed by hydrolysis (1) and the ester prepared with diazomethane. The mixed isomeric 12,-13-chlorohydroxyoleic and 13,12-chlorohydroxyoleic acids or esters were prepared from 12:13-epoxyoleic acid or ester by the action of anhydrous ethereal hydrogen chloride (14). 12-Hydroxyoleic acid (ricinoleic acid) was isolated from the mixed acids of castor oil by partition between 80% methanol and hexane (20). A portion of the acid was esterified with methanolic hydrogen chloride.

The esters of the oils which were studied by the methods described here were obtained by alkaline hydrolysis at room temperature, acidification, and esterification of the resultant acids with diazomethane. Prolonged contact of samples with mineral acids during acidification was avoided.

Paper Chromatography. Whatman No. 1 paper was siliconized by immersion in a solution of 4% of Dow Silicone No. 200[2] in ethyl ether. The procedures used have been described by Schlenk *et al.* (23, 24). Chromatograms were stained with iodine vapor to develop unsaturated components, or α-cyclodextrin and iodine for saturated components. The common solvent systems used in the study of fatty derivatives are aqueous acetic acid or aqueous peracetic acid mixtures. These are unsuitable for studies of oxygenated acids and esters because, with the usual proportions of acetic acid, epoxy compounds react and migrate to the solvent front along with any hydroxy components. Aqueous acetonitrile and aqueous tetrahydrofuran systems, however, have been found to be suitable for studies of these compounds.

Free acids tend to smear during chromatography with the acetonitrile systems, due to dimerization. This has been prevented by the addition of 2% acetic acid to the solvent, producing discrete spots. However, the tetrahydrofuran system used gave little smearing with free acids.

R_f values of standard acids and methyl esters, using aqueous acetonitrile and aqueous tetrahydrofuran systems, are listed in Table 1.

The acetonitrile systems are shown to be most suitable for studies of oxygenated derivatives since non-oxygenated saturated and unsaturated acids or esters migrate very little and can be readily distinguished from epoxy and hydroxy components. A mixture of monoepoxy, monohydroxy, diepoxy, and dihydroxy C_{18} acids or esters can be separated into the four components. Such a separation is not possible with the aqueous tetrahydrofuran systems where diepoxy and monohydroxy derivatives have similar R_f values. A consideration of time also favors the acetonitrile systems since a solvent front migration of 25 cm takes only 5 to 6 hours, whereas the tetrahydrofuran systems require more than 24 hours for the same migration.

The tetrahydrofuran systems give less separation between classes of compounds, so that careful measurement and comparison with standards is necessary in studies of unknown mixtures. However, this system may be useful as a confirmation of the results of the aqueous acetonitrile system.

Paper chromatography will detect less than 1% of epoxy component in a mixture and, if esters are prepared with radioactive diazomethane, the components

[2] Dow Corning Corporation, Chicago 1, Ill.

TABLE 1. R_f VALUES OF FATTY ACIDS AND THEIR METHYL ESTERS IN PAPER CHROMATOGRAPHY*

Compound	Esters		Acids	
	MeCN/H_2O 50/50	C_4H_8O/H_2O 75/25	MeCN/H_2O/AcOH 45/53/2	C_4H_8O/H_2O 80/20
$C_{16:1}$ + $C_{18:1}$	0.05 + 0.02	0.46 + 0.42	0.10 + 0.04	0.70 + 0.63
9:10-epoxy $C_{18:0}$	0.28	0.51	0.48	0.74
12:13-epoxy $C_{18:1}$	0.33	0.55	0.59	0.78
9:10,12:13-diepoxy $C_{18:0}$	0.61	0.67	0.84	0.95
12-hydroxy $C_{18:1}$	0.50	0.65	0.72	0.88
12,13-dihydroxy $C_{18:1}$	0.80	0.92	0.91	0.99
12,13-chlorohydroxy $C_{18:1}$ and 13,12-chlorohydroxy $C_{18:1}$	0.48	0.58	0.74	0.83

MeCN = acetonitrile; C_4H_8O = tetrahydrofuran; AcOH = acetic acid.
* Samples were approximately 5λ of 1% solutions, i.e., about 0.05 mg.

can be estimated quantitatively by the method of Mangold *et al.* (25).

Thin-layer Chromatography. The separation of mixtures by adsorption chromatography on silica gel, in the form of a thin layer on a glass plate, has been developed by Stahl (26, 27). This very elegant method for the separation of different classes of compounds has been applied to the analysis of lipid materials by Mangold and Malins (28, 29). Details of the preparation of chromatoplates and of their use may be found in these references.

After elution of the chromatogram, the position of components can be determined by the methods of paper chromatography (24, 25). A more general method of detection, however, is applicable to thin-layer chromatograms and was used for most of this work. This consists of spraying the plate with 50% sulfuric acid and heating until all organic constituents are charred and appear as black spots. Moreover, the rate of appearance and the initial color of individual spots give qualitative information regarding the degree and type of unsaturation of the components. Thus conjugated triene or α-hydroxydiene components appear as brown spots immediately after spraying, and conjugated dienes produce brown spots shortly after heating. Nonconjugated unsaturated components appear as yellow spots after a longer heating period and have changed color to brown before yellow spots, due to saturated components, appear. Spots can also be made visible by their absorption or fluorescence in ultraviolet light after spraying the plate with a 0.2% solution of 2′,7′-dichlorofluorescein in ethanol.

When thin-layer chromatography was applied to the examination of long-chain esters, it was found that, by varying the polarity of the solvent, one class at a time could be made to migrate. For instance, 1% ethyl ether in petroleum ether (b.p. 35°-45°) caused only the common saturated and unsaturated esters to migrate, as a class, at an R_f value of about 0.4, all other components remaining at the starting point. The saturated and unsaturated esters migrated close to the solvent front when the proportion of ethyl ether was increased to 5%, and monoepoxy esters then moved at an R_f value of about 0.3. If 10% ethyl ether was used (Fig. 1), monoepoxy esters migrated at about R_f 0.7, monohydroxy esters moved at R_f 0.2, diepoxy stearate moved at R_f 0.1, and dihydroxy derivatives remained on the base line. Petroleum ether containing 20% ether caused the migration of these constituents at R_f values of 1.0, 0.5, 0.3, and 0.1, respectively.

With acids it was found to be impossible to move only one class at a time, as with esters. This is because the high polarity of the carboxyl groups of acids reduces the differences between classes. However, each class has a different R_f value and was separated from the others. Figure 1 demonstrates the migration of various fatty acids with a 10% ethyl ether-petroleum ether solvent containing 1% acetic acid to prevent smearing. The nonoxygenated acids migrated close to the solvent front, with monoepoxy acids near R_f 0.6 and diepoxystearic acid at 0.1. Monohydroxyoleic acid (ricinoleic acid) moved to an R_f value of about 0.3, and dihydroxyoleic acid remained at the starting point.

The chlorohydrins from 12:13-epoxyoleic acid and

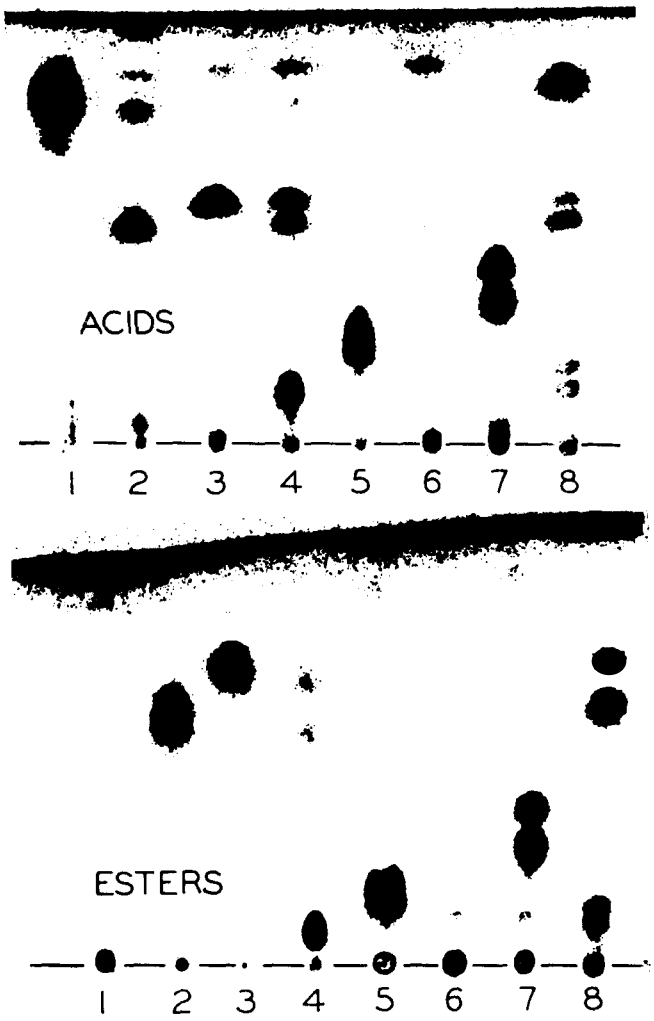

FIG. 1. Thin-layer silicic-acid chromatography of known compounds. Solvent used for esters was 10% ethyl ether in petroleum ether (b.p. 35°-45°) and for acids was the same with added 1% of acetic acid. Spots were developed by heat, after spraying with 50% sulfuric acid, and reproduced by photocopying. 1. Palmitoleic and oleic; 2. *cis*-9:10-epoxystearic; 3. *cis*-12:13-epoxyoleic; 4. *cis,cis*-9:10,12:13-diepoxystearic plus two mono-epoxy-impurities; 5. 12-hydroxyoleic; 6. *threo*-12,13-dihydroxyoleic; 7. *threo*-12,13-chlorohydroxyoleic and *threo*-13,12-chlorohydroxyoleic; 8. *Artemisia absinthium* mixed acids and mixed esters.

ester were shown to be intermediate in migrating speed between the corresponding epoxy and hydroxy derivatives. This is probably caused by intramolecular hydrogen bonding between the hydroxy and chloro groups, reducing the polarity of the hydroxy group. The two possible isomers were clearly separated on both plates (Fig. 1.) Considerations of structure in relation to hydrogen bonding suggest that the 13-chloro-12-hydroxyoleic derivative may have the higher R_f value.

It is also noteworthy that the two mono-epoxidized linoleic acids and esters, present as impurities in the diepoxystearic samples, were clearly separated. The upper spot is identical with that of the 12:13-epoxyoleic derivative and the lower spot therefore represents the 9:10-epoxyoctadec-12-enoic compound.

It should be noted here that the 9:10-epoxyoctadec-12-enoic acid and ester have the same R_f values, respectively, as 9:10-epoxystearic acid and ester. These compounds are inseparable by this method but can be distinguished by other methods (*vide infra*).

This ability to separate very similar compounds makes thin-layer chromatography a more useful method, in many cases, than paper chromatography, which does not separate the two pairs of isomeric compounds described above. As a further example of this difference in resolving power, the esters from *Artemisia absinthium* seed oil, when separated by paper chromatography (30), showed one spot corresponding to an epoxy ester and one corresponding to a monohydroxy ester. Thin-layer chromatography, however, demonstrated the presence of two epoxy esters, two monohydroxy esters, and at least one dihydroxy derivative (Fig. 1). The sensitivity of thin-layer chromatography is such that about 0.1% of an epoxy component can be detected in a 1 mg sample of mixed acids or esters, as shown with *Dimorphotheca aurantiaca* esters in Figure 3. This oil contains approximately 0.6% of epoxy acids (11) yet three epoxy components are clearly visible. Radioactive esters, prepared with carbon-labeled diazomethane, can be separated by thin-layer chromatography and a quantitative estimation of epoxy and other oxygenated components obtained by the method of Mangold (31).

Gas-liquid Chromatography. Three liquid phases were used to evaluate this method for studies of epoxy compounds: The nonpolar Apiezon L grease,[3] and the polar LAC-2-R446[4] and Craig[5] polyesters, coated on acid and alkali washed Celite® in copper tubing. Further details are given under Figure 3 and Table 3. Detection was by β-ray ionization using either Research Specialties Company Model 600-2 or Barber-Coleman Model 10. Samples were injected as 1% solutions in methyl hexanoate or acetone in 5 to 10λ amounts.

[3] Registered: Metropolitan-Vickers Electrical Co., Ltd., England.

[4] Diethylene glycol adipate and pentaerythritol adipate polyesters, obtainable from Cambridge Instrument Co., Cambridge, Mass.

[5] Butanediol-succinate polyester (32).

Mixtures of straight-chain saturated fatty esters with pure samples of methyl *cis*-12:13-epoxyoleate, *cis*-9:10-epoxystearate and *trans*-9:10-epoxystearate were injected to determine the retention characteristics of each column. The results are presented as semilogarithmic graphs in Figure 2.

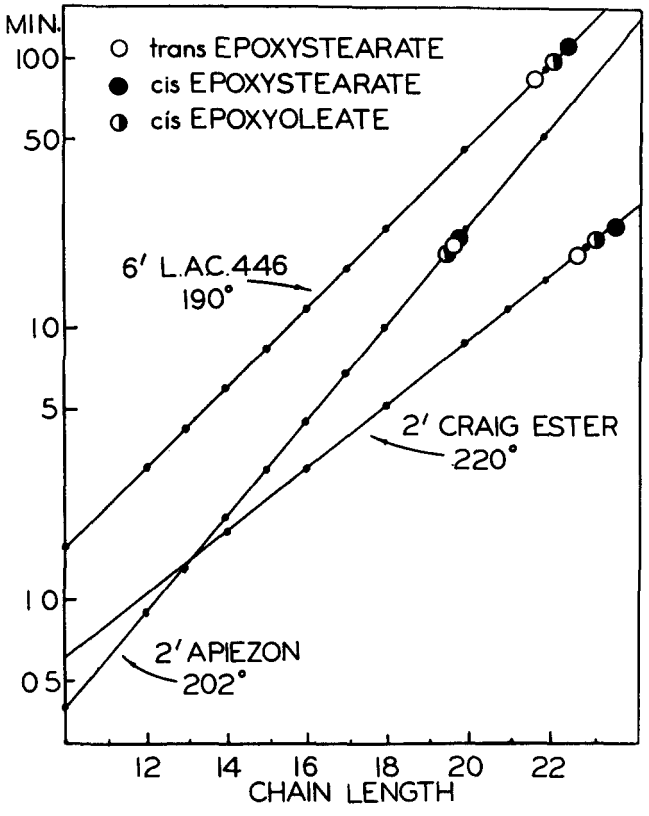

FIG. 2. The relation between the logarithm of the retention time and the chain length for some normal saturated acid methyl esters and methyl *cis*-12:13-epoxyoleate; methyl *cis*-9:10-epoxystearate and methyl *trans*-9:10-epoxystearate. Conditions: 1. LAC-2-R446, 16 g (16.5%) on 80 to 100 mesh acid and alkali-washed Celite®. Column 6′ × ¼″ at 190° and flow rate 62 ml/minute. 2. Craig polyester, 6 g (20%) on 80 to 100 mesh acid and alkali-washed Celite®. Column 2′ × ¼″ at 220° and flow rate 67 ml/minute. 3. Apiezon L, 5 g (17%) on 60 to 100 mesh acid and alkali-washed Celite®. Column 2′ × ¼″ at 202° and flow rate 71 ml/minute. Flow rates calculated at S.T.P.

On the polyester columns these epoxy esters emerged with "carbon numbers" of 22-23 and 23-24, respectively, and the order of emergence was *trans*-epoxystearate, *cis*-epoxyoleate and *cis*-epoxystearate. On the nonpolar column, however, they had a lower retention time and emerged with "carbon numbers" 19-20, *cis*-epoxyoleate having the shortest retention time followed by *trans*- and *cis*-epoxystearates. None of these columns separated the individual epoxy esters completely and mixtures appeared as composite peaks. However, a longer nonpolar column, 4-foot, Apiezon L, operated under similar conditions, clearly separated *cis*-epoxyoleate from *cis*-epoxystearate (*vide infra*) and longer polyester columns may similarly improve separation.

We have recently described the alteration, during gas-liquid chromatography (GLC), of several hydroxy and other acids (33) and the possibility of alteration of epoxy esters was not overlooked. No change in composition of epoxy esters was noted. Epoxyoleate, for example, was collected after GLC and chromatographed alongside the original ester on a thin-layer plate. Both samples had the same R_f value.

The sensitivity of detection of methyl epoxyoleate in admixture with C_{16} and C_{18} esters was found to be of the order of 0.1% if sufficient sample was injected.

Quantitative results were obtained by the use of added epoxy ester as an internal standard. Areas under curves were determined by cutting out peaks and weighing the paper. The results obtained using this method to determine the epoxyoleate content of some standard mixtures and of the mixed esters from *Vernonia anthelmintica* seed oil are shown in Table 2. The epoxyoleate values obtained agree well with the known values.

The method of internal normalization of the areas under curves was not suitable for mixtures containing epoxy esters. The β-ionization detector, under the conditions used, was found to give a lower response with epoxy esters than with unoxygenated esters.

TABLE 2. QUANTITATIVE ESTIMATION OF EPOXYOLEATE BY GAS-LIQUID CHROMATOGRAPHY

Sample*	Epoxyoleate	
	Actual	Estimated
	per cent	*per cent*
Standard mixture 1	2.0†	1.95
Standard mixture 2	5.0†	5.0
Standard mixture 3	10.0†	9.7
Vernonia mixed esters	71.9‡	73.0

* The standard mixtures 1, 2, and 3 consisted of methyl epoxyoleate in the amounts shown, made up with a mixture of methyl oleate and methyl palmitate (1:9 by weight).

† By weight.

‡ By chemical analysis (1).

Applications. The methods described above were applied in a study of the epoxy components of the seed oils of *Dimorphotheca aurantiaca, Artemisia absinthium, Calliandra eriophylla, Balanites aegyptica, Cosmos bipinnatus,* and *Helianthus annuus.* All these oils give epoxy values by the Durbetaki and Swern methods which are higher than the true epoxy content (30). We have shown them to contain isomers of dimorphecolic acid (9-hydroxy-*trans-trans*-10,12-octadecadienoic acid) and these acids are responsible for the errors in conventional epoxide determinations (10). However, we have shown that all these oils contain epoxy constituents (11) and their presence is confirmed by the methods described in this paper.

Paper chromatography of the esters derived from these seed oils (see Reference 30 for illustration) showed the presence of an epoxy constituent in every sample except that from *Dimorphotheca* oil. Thin-layer chromatography of the acids or esters from these oils presented a more complete and more informative picture of their epoxy constituents. A chromatogram of the esters from the oils run alongside known epoxy esters is shown in Figure 3.

Artemisia and *Calliandra* esters both show two well-defined spots produced by epoxy constituents. These correspond, in R_f values, to the two mono-epoxidized linoleate isomers which were present as impurities in the standard sample of diepoxystearate. The upper spot in this standard is shown to be the 12:13-epoxyoctadec-9-enoate isomer by comparison with the authentic sample of that ester, and the lower spot then is 9:10-epoxyoctadec-12-enoate. The major epoxy constituent of *Artemisia, Calliandra, Cosmos,* and *Helianthus* corresponds to this 9:10-epoxy ester and all demonstrate an upper spot corresponding to the 12:13-epoxyoleate. *Balanites* esters show their main epoxy constituent to be the 12:13-epoxyoleate with a small trace of the more polar isomer. The *Dimorphotheca* sample, however, besides showing both these components, has its main epoxy constituent at an even higher R_f value.

The R_f values of 9:10-epoxystearate, 12:13-epoxyoleate and this last compound increase linearly (0.43, 0.51, and 0.59). This suggests that it could be 15:16-epoxylinoleate, which has already been identified as a constituent of the seed oil of *Camelina sativa* (6). However, the spot on the thin-layer chromatogram of *Dimorphotheca* esters representing this component appeared as quickly after spraying with H_2SO_4, as that produced by the diene-hydroxy constituent, indicating the presence of a system of conjugated double bonds. That this spot of the thin-layer chromatogram of *Dimorphotheca* esters represents two epoxy components, one of which is novel, is indicated by GLC and other studies recorded below.

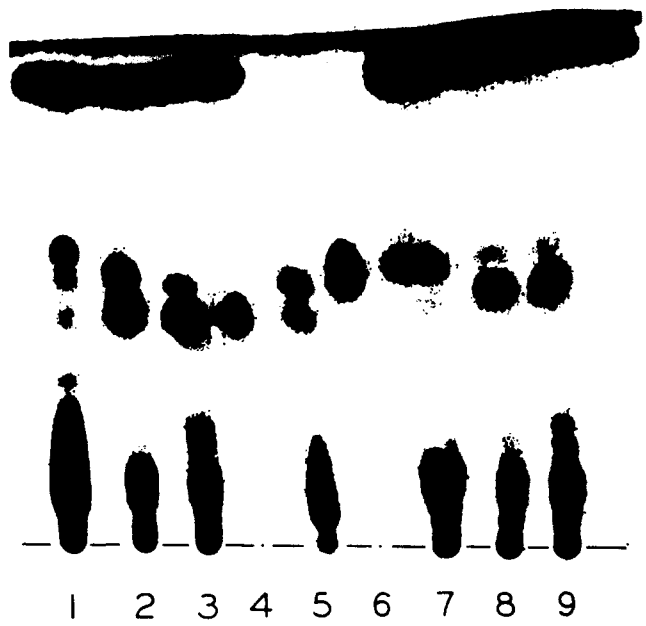

FIG. 3. Thin-layer silicic-acid chromatography of mixed esters from seed oils and standard epoxy esters. Solvent was 5% ethyl ether in petroleum ether (b.p. 35°-45°). Spots were developed by heat, after spraying with 50% sulfuric acid, and reproduced by photocopying. 1. *Dimorphotheca;* 2. *Artemisia;* 3. *Calliandra;* 4. *cis*-9:10-epoxystearate; 5. *cis,cis*-9:10,12:13-diepoxystearate and monoepoxy isomers; 6. *cis*-12:13-epoxyoleate; 7. *Balanites;* 8. *Cosmos;* 9. *Helianthus.*

It is obvious from Figures 1 and 3 that 9:10-epoxystearate and 9:10-epoxyoctadec-12-enoate have very similar migration characteristics on thin-layer chromatography. Consequently, it cannot be decided from R_f values alone whether the natural epoxy esters with the same R_f values as these two compounds are saturated or unsaturated. The observation that sulfuric acid develops color faster with more unsaturated constituents was used to determine if the natural epoxy components were saturated or unsaturated. It was found that as heating progressed, the spots produced by 12:13-epoxyoleate, its 9:10-epoxy isomer and the corresponding natural epoxy esters all appeared some time before the spots produced by mono- and diepoxystearates became visible. The conclusion that the natural epoxy esters from the oils were unsaturated was confirmed by developing a chromatoplate with iodine vapors, after spraying with a 1% solution of α-cyclodextrin. Only the spots produced by epoxystearate and diepoxystearate appeared white; all others became dark, showing unsaturation. These results prove that the lower of the two "epoxyoleate" spots, in each case,

contains an unsaturated epoxy ester, probably 9:10-epoxyoctadec-12-enoate. They do not, however, exclude the possibility that 9:10-epoxystearate may also be present in each sample as another component of this spot. GLC studies resolved this question (*vide infra*) and showed that both saturated and unsaturated components were represented in the lower spot from each of these oils.

Although not obvious from the reproduction used as Figure 3, the original chromatoplates demonstrated trace components in all samples corresponding to the "epoxylinoleate" spot of *Dimorphotheca*. A trace component with an even higher R_f value than this ester was present in all samples but no conclusions as to its structure have been reached.

Gas chromatographic analysis of the separate epoxy components from each sample was carried out on a nonpolar column. Epoxy constituents were first separated from the mixed ester samples by thin-layer chromatography using 10% ethyl ether in light petroleum as solvent. The spots produced by individual epoxy components were made visible under ultraviolet light by spraying with dichlorofluorescein, were scraped from the plate, and eluted from the adsorbant with ether (29). By this method individual components were isolated in amounts up to several milligrams. The resulting solutions were concentrated, analyzed by GLC, and the results are given in Table 3. The GLC column used here was unable to separate 9:10-epoxyoctadec-12-enoate from 12:13-epoxyoctadec-9-enoate but readily separated 9:10-epoxystearate from these isomers. Table 3 shows that all six oils contain two monounsaturated epoxy isomers, probably the two mentioned above, and a saturated epoxy acid. In addition, the components from the main epoxy ester spot from *Dimorphotheca* oil, on GLC, gave a minor peak with the carbon number to be expected for 15:16-epoxylinoleate and a pronounced peak emerging later

TABLE 3. CARBON NUMBERS* (GAS-LIQUID CHROMATOGRAPHY)† OF EPOXY COMPONENTS ISOLATED FROM THIN-LAYER CHROMATOGRAMS

Oil	Epoxy Ester Spot‡	9:10 ep. $C_{18:0}$	9:10 ep. $C_{18:1}$	12:13 ep. $C_{18:1}$	15:16 ep. $C_{18:2}$	Other	Ratio§
Dimorphotheca	1	19.8	19.5				1:3
	2			19.4			
	3				19.1	20.0	1:17
Artemisia	1	19.8	19.5				1:12.5
	2			19.4			
Calliandra	1	19.7	19.4				1:12
	2	19.7		19.4			1:7
Balanites	1	19.9	19.5				1:23
	2			19.4			
Cosmos	1	19.9	19.5				1:0.9
	2			19.4			
Helianthus	1	19.8	19.4				1:7
	2			19.4			
Standards:							
ep. $C_{18:0}$		19.9					
12:13 ep. $C_{18:1}$				19.4			
ep. $C_{18:1}$ + ep. $C_{18:0}$		19.8		19.4			
impure diep. $C_{18:0}$			19.4			21.1	

* Methyl palmitate, under these conditions, had a retention time of 16 minutes.
† Column was 4′ × ¼″ packed with 60 to 100 mesh celite (acid and alkali washed) coated with Apiezon L grease (11 g, 17%). Column temperature was 200° and flow rate 54 ml/minute (S.T.P.).
‡ Isolated from thin-layer chromatograms. Numbering of epoxy ester is from the bottom up (cf. Fig. 3).
§ Ratio of GLC peak areas of earlier to later emerging components.

than epoxystearate. The corresponding trace components of the other five oils were not examined but may be similar.

Although structural studies by degradation have not been carried out, we consider that all of these oils contain *cis*-9:10-epoxystearic acid, *cis*-9:10-epoxyoctadec-12-enoic acid, and *cis*-12:13-epoxyoctadec-9-enoic acid in varying proportions. In addition, *Dimorphotheca* oil, and possibly each of the others, contains a trace of 15:16-epoxyoctadec-9,12-dienoic acid and a novel epoxy acid of unknown constitution. This last epoxy acid constitutes about 0.3% of the acids of *Dimorphotheca* oil. Its ester, isolated by thin-layer chromatography, showed a conjugated diene absorption band at 231 mμ in the ultraviolet spectrum, a *trans,trans* conjugated diene band at 10.07 μ and possible epoxy bands at 11.44 and 11.90 μ in the infrared spectrum. Reaction with ethereal HCl altered the ester, the single product appearing on a thin-layer chromatogram lower than the pair of chlorohydrins from epoxyoleate. Boiling with acetic acid, followed by hydrolysis and esterification also altered the ester and resulted in a spot in the position of a dihydroxy acid. These results are explicable if an epoxy-conjugated-diene structure is present. Assuming some relationship with the main component acid, 9-hydroxy-*trans,trans*-10,12-octadecadienoic acid (34, 10), it seems possible that this unknown acid of *Dimorphotheca* oil is 8:9-epoxyoctadec-*trans-trans*-10,12-dienoic acid.

CONCLUSIONS

Reversed-phase partition chromatography on paper, thin-layer adsorption chromatography on silicic acid, and GLC are methods eminently suitable for the study and analysis of fatty materials containing oxygenated acids. Although emphasis has been placed on studies of epoxy compounds, the chromatographic properties of certain hydroxy compounds on paper and thin-layer plates have been described and these methods are as useful for hydroxy acids as for epoxy acids.

Thin-layer chromatography is preferable to the older techniques of paper chromatography for studies of these oxygenated derivatives. It is far more sensitive, requires less material, and readily resolves pairs of compounds which are inseparable on paper. The potential of thin-layer chromatography is amply demonstrated in the studies of seed oils recorded here and elsewhere (30). The possibility of isolating individual components from a thin-layer chromatogram in milligram amounts and carrying out gas chromatographic, ultraviolet, and infrared spectral studies on these components makes this technique doubly important.

GLC is also a powerful tool for the detection and estimation of epoxy components in seed oils. We have shown that the use of this technique in conjunction with thin-layer chromatography gives a very powerful method for studies of epoxy esters. Thin-layer chromatography, for example, will readily separate 12:13-epoxyoleate and its 9:10-epoxy isomer but will not separate the latter from 9:10-epoxystearate. GLC, on the other hand, will not separate these unsaturated epoxy isomers but will readily separate (on Apiezon L) either of these from epoxystearate. Use of both methods therefore enables all three components to be detected in a mixture.

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