Fatty Acid Composition of Maturing *Vernonia anthelmintica* (L.) Willd. Seeds. Dihydroxyoleic Acid--A Possible Precursor of Epoxyoleic Acid[1]

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Abstract

Quantitative measurements of epoxyoleic acid and co-occurring fatty acids in maturing *Vernonia anthelmintica* (L.) Willd. seeds were made to elucidate partially the mode of epoxyoleic biosynthesis. Free (+) *threo*-12,13-dihydroxy-*cis*-9-octadecenoic acid was the major component at an intermediate maturation stage, and is believed to be dehydrated to free epoxyoleic acid before incorporation into glycerides.

Introduction

THE PRINCIPAL constituent (*ca.* 70%) in the oil extracted from mature *Vernonia anthelmintica* (L.) Willd. (ironweed) seeds has been shown to be *cis*- 12,13-epoxy-*cis*-9-octadecenoic acid (6), commonly called epoxyoleic or vernolic acid. To elucidate the mode of biosynthesis of epoxyoleic acid and its quantitative relationship to co-occurring fatty acids, compositional studies were made on the oils extracted at different stages of seed maturation. Oxygenated fatty acids occurring only in immature seeds were sought as probable precursors to epoxyoleic acid. Oleic and linoleic acid concentrations were also examined for trends which would provide some bases for the assumption that either may be the substrate for epoxidation. "Free" and "glyceride" fatty acid fractions were analyzed separately to determine the state in which the fatty acids existed when undergoing epoxidation.

Experimental

Seed Preparation. Vernonia anthelmintica (ironweed) plants were grown in a controlled environment, and seeds were collected at 3-day intervals, beginning 12 days after initiation of flowering and ending after 36 days when seeds were considered mature. All seeds were stored at −20C immediately after harvesting to minimize chemical changes.

Preliminary Seed and Oil Analyses. The weight per thousand seeds was determined for each sample, and then the seeds were ground in a 6-in. hammer mill for moisture determination and oil extraction, each according to procedures recommended by the AOCS (11). The extracted oil was analyzed for epoxyoleic acid content by HBr titration (4), for free fatty acid content by aqueous ethanolic KOH titration, and for total fatty acid content by saponification (17).

Separation of Free Fatty Acids. Isolation of free fatty acids and preparation of methyl esters from glyceride (or any esterified) fatty acids were carried out in a single process. Because the oxirane ring of epoxyoleic acid is subject to chemical changes by acid catalysts, methanolic sodium methoxide was used. The equivalency ratio of sodium methoxide to total fatty acids was 1.0, calculated from the saponification value of the oil.

The oil (0.95 g, 1 mmole of triglyceride), sodium methoxide (0.069 g, 3 mg atoms of sodium in 6 g of methanolic solution), anhydrous methanol (50 ml) and anhydrous ethyl ether (5–20 ml, depending on solubility of the oil in methanol) were shaken gently in a ₸, 250-ml, round-bottom flask at room temperature (23–26C) for 4 hr. The stopper was lubricated with methanol and fastened to the flask to prevent loss of sample.

The solution was concentrated under vacuum to approximately 5 ml and transferred to a 125 ml separatory funnel with 25 ml of ACS ethyl ether. The solid sodium derivatives remaining in the flask were dissolved in 5 ml of water and slowly transferred to the separatory funnel, as a layer. Upon standing for 1 min the solid sodium derivatives dissolved into the aqueous phase, which was then withdrawn. The round-bottomed flask was rinsed with 10 ml of ethyl ether and 5 ml of water, and the heterogeneous rinse was transferred to the separatory funnel. After gently swirling the aqueous phase was withdrawn. The reaction flask was rinsed twice again in this manner; after each transfer the separatory funnel was shaken with increasing vigor. After the four withdrawals, the system was free from emulsification even with vigorous shaking. The ether phase was further washed with five 10-ml portions of water, and the combined aqueous phase was acidified with 31 ml of 0.10 N HCl and extracted with five 20-ml portions of ethyl ether. The latter ether extract was concentrated by vacuum distillation of the ether and methanol. The residue was dissolved in 20 ml of ethyl ether and washed five times with 5 ml of 0.5 N K_2CO_3 and three times with 5 ml of water. The combined aqueous extracts contained the unesterified free fatty acids. The ether phase was combined with the previous ether phase and concentrated by vacuum distillation under nitrogen. Traces of water were removed by distillation at 40C (bath) and 15–20 mm for 1 hr. From the weight of recovered methyl esters, the yield of glyceride fatty acids was calculated after composition of the methyl ester was determined by gas-liquid chromatography (GLC).

Free fatty acids were recovered from the aqueous phase, their yield was determined and methyl esters were prepared by the diazomethane method (1).

Fatty Acid Composition by GLC. A Burrell Kromo-Tog K-5 gas chromatograph equipped with thermal conductivity cells was used to analyze the methyl ester derivatives. Details of experimental conditions and identification procedures have been reported earlier (12). Composition based on methyl esters was corrected to the fatty acid basis before tabulation.

Characterization of 12,13-Dihydroxyoleic Acid. The major component in the 21-day oil, believed to be the

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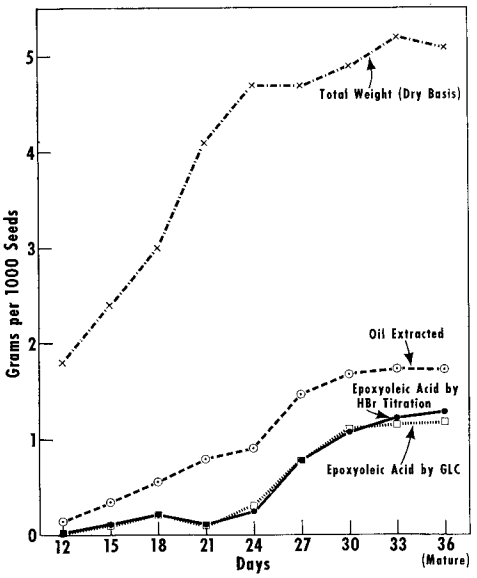


FIG. 1. Weight of maturing *Vernonia anthelmintica* seed and its oil and epoxyoleic acid content.

immediate precursor of epoxyoleic acid, was characterized by gas chromatography using equivalent chain lengths (ECL) (12) and by comparison with a synthetic standard of 12,13-dihydroxyoleic acid (2). Infrared absorption spectra were obtained for methyl esters of the 21-day free, 33-day free, and 33-day glyceride fatty acid fractions to study the correlation between IR and GLC analyses for the concentration of the hydroxy constituent.

TABLE I

Free and Total Fatty Acids in Maturing *Vernonia anthelmintica* Seed (Milliequivalents per gram of oil)

Sample identified by day	Free fatty acids		Total fatty acids	
	By titration	By isolation and GLC analysis	By saponification	By isolation and GLC analysis
12	1.8	2.3	....	3.2
15	2.2	2.2	3.2	3.1
18	1.9	1.8	3.1	3.0
21	2.0	2.1	3.1	3.2
24	1.9	1.8	....	3.1
27	1.7	1.6	3.0	3.1
30	1.6	1.7	3.1	3.1
33	1.1	1.2	3.0	3.3
36 (Mature)	1.1	0.7	....	3.3

Isolation of the 12,13-dihydroxyoleic acid was accomplished by separating the free from the glyceride fatty acids in the 21-day oil sample (0.1850 g) according to the procedure given above, dissolving the free fatty acids (0.1240 g) in 25 ml of distilled acetonitrile, removing the nonpolar fatty acids (0.0514 g) with seven 10-ml portions of *n*-hexane and finally recrystallizing, three times, the dihydroxyoleic acid from analytical grade acetone cooled to −74C by solid carbon dioxide in ethanol. (Yield = 0.0302 g).

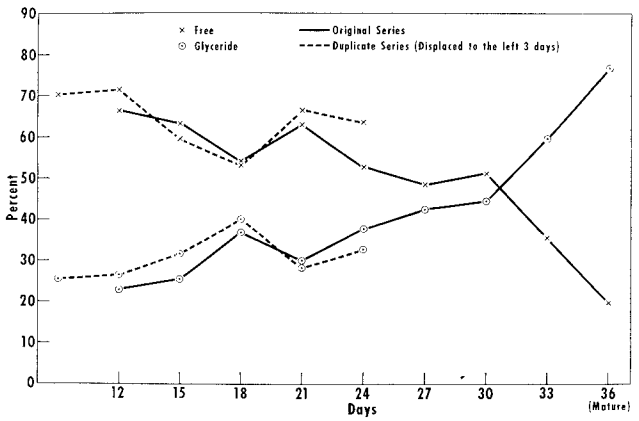


FIG. 2. Percentage of free and glyceride fatty acid fractions in maturing *Vernonia anthelmintica* seed oil.

Melting point (Fisher-Johns melting point apparatus) and specific rotation (Rudolph polarimeter) determinations were followed by partial catalytic hydrogenation (13) of the monoenoic acid. In order to establish the positions of the unsaturation and the two hydroxyls in a single step, hydrogen was added semiquantitatively aiming to convert approximately 75% of the monoenoic to the saturated acid. The mixture of dihydroxyoleic and dihydroxystearic acids was subjected to permanganate-periodate oxidative cleavage (18). The C_9 and C_{12} saturated dicarboxylic acids were recovered, and their ratio was determined by gas chromatographic analysis of the methyl esters, prepared by reacting the acids with diazomethane (1).

Partial Identification of Unknowns. Six unknowns were found in the gas chromatograms of the fatty acid methyl esters, as shown in Table II. Evaluation of the chromatograms suggested the possibility that five of the six may be bound as Coenzyme A thioesters in the extracted oil as discussed in the Results section. UV spectrophotometric analysis was carried out on the 15-day oil to acquire additional evidence for the presence of Coenzyme A (5,10). The same oil sample was also tested qualitatively for sulfur by sodium fusion and lead acetate addition (15).

Results

Preliminary Seed and Oil Analyses. The dry weight per thousand seeds at different stages of maturation is shown in Figure 1, as well as the amount of oil extracted. Although growth rate was highest between 18 and 21 days, maximum rate of oil production was observed at 24 to 27 days, coinciding with the maximum rate of increase of epoxyoleic acid; little change in total dry weight of the seed occurred during this period. From 21 days on to maturity the curves for oil and epoxyoleic acid are almost parallel and indicate that the oil increase is primarly due to epoxyoleic acid. Oil content increases only slightly from 30 days on to maturity.

A comparison between the analyses for epoxyoleic acid content by HBr titration of the oil and by GLC analysis of total methyl esters is shown in Figure 1. Generally the agreement between the two is good. The largest discrepancy is in the mature sample, 1.28 vs 1.18 g per 1,000 seed kernels. In the HBr titration the mean deviation of duplicate runs was 6 parts per 1,000 (ranging from 1 to 15 parts per 1,000).

Free and total fatty acid contents, determined by direct analysis of the oil are given in Table I. The mean deviation of duplicate runs in the free fatty acid titration analysis was 9 parts per 1,000 (ranging from 0–13 parts per 1,000), and that for total fatty acid by saponification was 7 parts per 1,000 (ranging from 0–17 parts per 1,000). For comparative purposes, values obtained from the isolated free acid and glyceride fractions are also given in Table I. The values were calculated by assigning molecular weights to the components found by GLC analysis after partial identification was made on the unknowns. Discussion of the unknowns is presented in a later section.

Separation of Free from Glyceride Fatty Acids. The relationship between free fatty acids and glycerides is shown in Figure 2. The broken lines represent

a second series run a year later to evaluate the reproducibility of the experimental results. Since maturation of seeds in the second series appeared to lag when results were compared, the curve was displaced 3 days to the left.

At the earliest stage of maturation free fatty acids predominate. As the seed matures, the free fatty acid percentage decreases as the amount of glycerides increases. This trend was reversed temporarily between the 18th and the 21st day when the rate of glyceride formation decreased. In the original series the net increase in weight of free fatty acids per thousand seeds during the 18- to 21-day period was 6 times greater than the increase in glyceride fatty acids, whereas, between the 15th and 18th day, glycerides were favored 1.5 times. With further active production of oil, the ratio of free fatty acids to glycerides gradually approached 1. After 30 days, when active oil production had ceased, the free fatty acids were rapidly incorporated into glycerides.

Fatty Acid Composition by GLC. The free and glyceride fatty acid composition for each sample is listed in Table II. The values represent percentage of acids in the whole oil, converted from the methyl ester composition of each fraction determined by GLC analysis.

Gas chromatographic identification of each acid is listed by its ECL (12). Factors for converting percentage in oil to gram per thousand seeds are given at the bottom of Table II.

At the earliest stage of maturation free oleic and linoleic acids are the major components, at 21 days, free dihydroxyoleic, and from 27 days on to maturity, free and glyceride epoxyoleic constitute the majority of the acids.

The relationship among free and glyceride epoxyoleic and free dihydroxyoleic acids is shown in Figure 3. Between 12 and 18 days, the free epoxyoleic acid increased rapidly, dropped to less than 3% of the total oil at 21 days, then increased substantially until 30 days. The glyceride epoxyoleic acid increased slowly in the early stages, remained unchanged between 18 and 21 days and increased, along with the free epoxyoleic, between 21 and 30 days. From this point to maturity the increase in glyceride epoxyoleic was approximately equal to the decrease in free epoxyoleic, indicating the incorporation of free epoxyoleic acid into glycerides.

Dihydroxyoleic acid, which was found only in the free fatty acid fractionation, increased at a rate equal to the glyceride epoxyoleic between 12 and 18 days. Between 18 and 21 days, dihydroxyoleic increased rapidly, mainly at the expense of free epoxyoleic. Dihydroxyoleic remained a major component while the seed actively produced oil, but when the seed matured and active oil production ceased, it disappeared completely. These data suggest that biosynthesis of epoxyoleic acid takes place in the free fatty acid state by dehydration of dihydroxyoleic acid and is followed by esterification to glyceryl epoxyoleate.

Characterization of (+) threo-12,13-Dihydroxy-cis-9-octadecenoic Acid. The gas chromatographic ECL of the dihydroxy acid were found to be identical to those of the synthetic standard—20.8 in Apiezon L grease and 29.6 in Resoflex 446 polyester. Infrared absorption analysis showed a strong absorption for OH stretching at 2.87 μ and a weak one for C-OH bending at 9.45 μ for the 21-day free fatty acid fraction, very weak ones at 2.87, and none at 9.45 μ for both 33-day samples.

TABLE II

Fatty Acids in Oils from Maturing *Vernonia anthelmintica* Seed (% by GLC)

Fatty acid	Equivalent chain length		12 Days		15 Days		18 Days		21 Days		24 Days		27 Days		30 Days		33 Days		36 (Mature)	
	Apiezon L	Resoflex 446	Free	Glyceride	Free	Glyceride	Free	Glyceride	Free	Glyceride	Free	Glyceride	Free	Glyceride	Free	Glyceride	Free	Glyceride	Free	Glyceride
Shorter than C_{16}	.....	.....	.2	.1	.....	.1	.1	.2	.4	.....	.....	.....	.....	.....	.....	.1	.....	.1	.....	.....
Palmitic	16.0	16.0	8.6	2.4	6.0	2.1	3.7	3.1	6.4	3.1	3.5	2.9	1.6	1.7	1.4	1.3	1.0	1.7	.5	1.9
Stearic	18.0	18.0	2.1	.5	1.8	.4	1.1	.8	2.7	.7	1.6	1.1	.7	.7	.5	.4	.4	1.0	.1	.7
Oleic	17.7	18.4	14.5	3.7	10.3	2.7	5.1	3.2	6.7	2.5	4.3	2.5	1.7	2.0	1.7	1.5	1.4	1.8	.7	1.7
Linoleic	17.6	19.0	25.5	7.8	19.0	7.5	9.1	10.3	15.3	12.4	7.8	8.3	4.6	6.1	4.0	4.7	2.7	6.1	1.7	6.8
Epoxyoleic	19.2	23.4	9.1	1.7	21.0	5.8	22.1	14.4	2.7	9.0	14.2	19.2	28.2	24.7	38.5	27.5	29.2	36.9	16.6	51.5
Dihydroxyoleic	20.8	29.6	5.7	.....	4.9	.....	12.1	.....	26.5	.....	19.0	.....	9.9	.....	3.5	.....	.2	.....	.....	.....
Palmito—unknown	16.6	16.2	.....	1.0	.....	.6	.....	.3	.....	.1	.....	.....	.....	.4	.....	.4	.....	.4	.....	.3
Stearo—unknown	18.6	18.2	.....	.2	.....	.....	.....	.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Oleo—unknown	18.3	18.6	.....	.7	.1	.9	.....	.3	.....	.1	.....	.2	.....	.3	.....	.3	.....	.6	.....	.6
Linoleo—unknown	18.2	19.2	.....	3.3	.1	2.3	.....	.8	.....	.6	.....	.8	.....	.9	.....	1.1	.....	1.2	.....	.9
Epoxyoleo—unknown	19.8	23.6	.....	.4	.....	1.7	.....	1.7	.....	.....	.....	1.1	.....	3.7	.....	5.2	.....	7.3	.2	9.3
Hydroxy—unknown (Linolenic?)	20.0	(?)	.3	.....	.1	.....	.6	.....	2.1	.....	1.9	.....	1.0	.....	.5	.....	.1	.....	.....	.....
TOTAL	17.6	19.8	66.3	21.8	63.3	24.1	53.9	35.2	62.8	28.5	52.6	36.1	48.4	40.5	51.2	42.5	35.5	57.1	19.8	73.7
Factors for conversion to gram per 1,000 seeds....			0.13×10^{-2}		0.34×10^{-2}		0.55×10^{-2}		0.79×10^{-2}		0.91×10^{-2}		1.47×10^{-2}		1.68×10^{-2}		1.74×10^{-2}		1.73×10^{-2}	

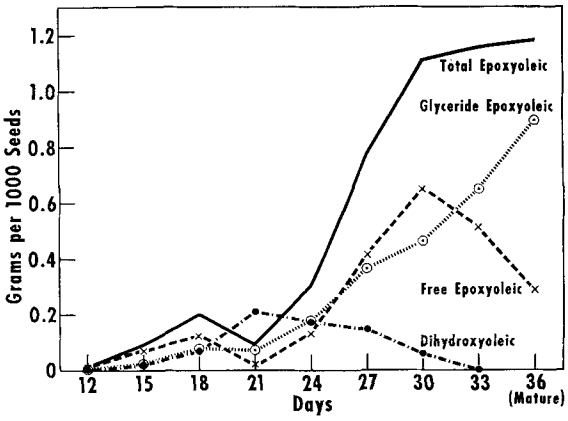


FIG. 3. Epoxyoleic and dihydroxyoleic acid content of maturing *Vernonia anthelmintica* seeds. (Analysis by gas-liquid chromatography.)

The isolated dihydroxyoleic acid melted at 55–57C, which agrees with the *threo*-12,13-dihydroxy-*cis*-9-octadecenoic acid reported previously (2,3). The specific rotation $[\alpha]_D^{25} = +20$ (ethanol, c = 0.302) also agrees with the dextrorotatory *threo* form reported earlier (3).

Partial hydrogenation of the dihydroxyoleic acid was found to be in the range of 86–91% instead of the intended 75% and the dicarboxylic acids recovered (94%) after oxidative cleavage were found by GLC to be nonanedioic 7% and dodecanedioic 93%, proving the hydroxyls to be at the C_{12} and C_{13} positions and the double bond, at the C_9, C_{10} position.

Partial Identification of Other Components. Five of the six unknowns listed in Table II were similar in several respects and were found primarily in the glyceride fraction, thereby suggesting their occurrence as esters or thioesters. All showed minima in their percentage composition curves at 21 days, indicating an intimate relationship with biosynthesis of the free fatty acids since greatest fluctuation in the content of free fatty acids occurred at 21 days. According to gas chromatography all five showed the same deviation from their related common fatty acids; namely, increased ECL and 0.6 and 0.2 in Apiezon L and Resoflex 446, respectively. Generally, increases in ECL greater in Apiezon L than in Resoflex 446 indicate a modification that resulted in an increased boiling point rather than an increased polarity by oxygenation. Increasing the number of isolated double bonds ordinarily leads to a decreased ECL in Apiezon L and an increased ECL in Resoflex 446. However, an α,β-desaturation may lead to an increased boiling point with negligible increase in effective olefinic polarity. For example, crotonic acid boils 25C higher than butyric acid (189 vs 165) (8) and the difference between their methyl esters is 18C (120.7 vs 102.3). Also 2-hexenoic acid boils 12–15C higher than hexanoic acid.

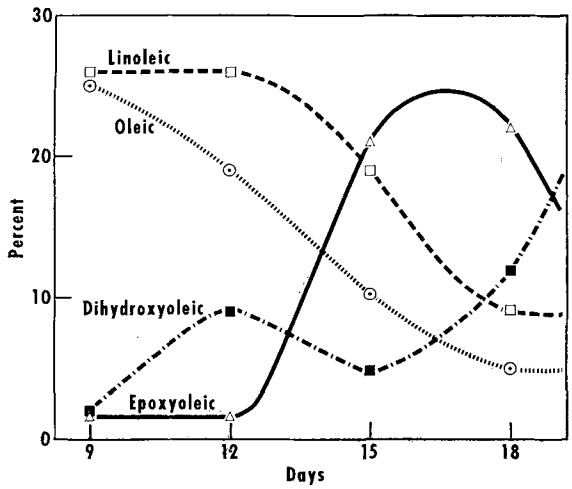


FIG. 4. Free fatty acids in immature *Vernonia anthelmintica* seed oil.

The speculation that these five unknowns have α,β-unsaturation and are present in the oil as Coenzyme A derivatives (7,14,16) has some credibility. They were present almost exclusively as esters. (An epoxyoleo-unknown was found in the free fatty acid fraction at the later stages of maturation.) The ultraviolet absorption curve of the 15-day oil in ethyl ether showed absorption at 238 mμ, indicating a thioester (10), and a shoulder at about 260–270 mμ, which suggests the presence of an adenine nucleotide. The 33-day glyceride fraction converted to methyl esters and free from thioesters showed peaks at 225 and 228, either one of which may be due to absorption by an α,β-unsaturated ester (10). Reduction of the 15-day oil by sodium fusion followed by addition of lead acetate produced black crystals of lead sulfide, which were insoluble in cold 6 N nitric acid but soluble when heated.

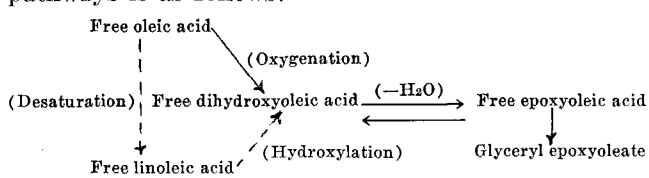
The sixth or hydroxy-unknown occurred in an identical manner as did dihydroxyoleic acid. It was found only in the free fatty acid fraction, went through its maximum content at 21 days, disappeared after 33 days, and behaved gas chromatographically as a highly polar compound.

Discussion

Biosynthesis of Epoxyoleic Acid. Oleic and linoleic acids were the major constituents in the early stages of maturation. At the earliest stage (the 12-day oil of the second series), free oleic (25%) and free linoleic (26%) outweighed the free dihydroxyoleic (2%) and epoxyoleic (1.5%) fourteen-fold. As the seed matured and the epoxyoleic acid content increased, the weight of total linoleic acid remained fairly constant; whereas, the oleic acid content decreased to about half of that found at the earliest stage. This decrease represents actual conversion of oleic to another component.

When the free oleic, linoleic, dihydroxyoleic and epoxyoleic percentage composition of the 12- and 15-day oils from the second series were taken as 9- and 12-day oils and plotted along with the 15- and 18-day oils from the original series, an interesting correlation among these four acids was revealed. The plots in Figure 4 suggest the oxygenation of oleic acid to dihydroxyoleic which, in turn, is dehydrated to epoxyoleic acid. Possibly in the earliest stages of maturation the seed is deficient in the enzyme system that oxygenates oleic or linoleic acid; between 18 and 21 days, the seed produces this system rapidly and in sufficient quantity to stock a large amount of dihydroxyoleic acid. Other enzymes having major functions are the dehydrating and esterifying systems.

A simplified diagram of the proposed biosynthetic pathways is as follows:



Recently, Krewson *et al.* (9) reported that grinding

mature *Vernonia anthelmintica* seeds triggers enzyme activity that releases free fatty acids from the triglycerides. We observed similar results when the ground mature seeds were allowed to stand at room temperature (ca. 23C) for ¼, ½, 4, and 24 hr before extraction. Titration of the recovered oils showed a progressive increase in milliequivalents of acid per gram of oil; namely, 0.11, 0.17, 0.43, and 1.1. However, no hydroxy acids were found in either the free or glyceride fatty acid fraction from a sample of mature seed which was ground, allowed to stand 24 hr at room temperature, extracted with petroleum ether and the oil stored in the refrigerator at ca. −7C for 8 months. Acidulation during the recovery of free epoxyoleic acid did not induce any hydration of the oxirane ring, even when the epoxyoleic acid content was as high as 82%.

On the other hand, in the immature seed the free fatty acid content did not vary at all when the 15-day seeds were ground and handled as described above. In addition, one portion was ground within the extracting solvent to eliminate any interval between grinding and extracting. All five samples had identical acidity; i.e., 2.2 milliequivalents of acid per gram of oil. This would indicate that the free acids are present in the immature seed intrinsically. Furthermore, in the sample with zero-delay between grinding and extracting, dihydroxyoleic acid was present in the free fatty acid fraction to an appreciable extent, thus providing evidence for the occurrence of free dihydroxyoleic acid in the immature seed.

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REFERENCES

1. Arndt, F., in "Organic Syntheses," Coll. Vol. II, A. H. Blatt, ed., John Wiley and Sons, Inc., New York, N.Y., 1943, pp. 165–6.
2. Bharucha, K. E., and F. D. Gunstone, J. Chem. Soc. *1956*, 1611–9.
3. Chisholm, M. J., and C. Y. Hopkins, Chem. and Ind. (London) *1960*, 1134–5.
4. Durbetaki, A. J., Anal. Chem. *28*, 2000–1 (1956).
5. Green, D. E., and D. M. Gibson, in "Metabolic Pathways," Vol. 1, D. M. Greenberg, ed., Academic Press Inc., New York, N.Y., 1960, p. 308.
6. Gunstone, F. D., J. Chem. Soc. *1954*, 1611–6.
7. Haurowitz, F., "Progress in Biochemistry since 1949," Interscience Publishers, Inc., New York, N.Y., 1959, p. 137.
8. "Handbook of Chemistry and Physics," C. D. Hodgman, ch. ed., 42nd ed., Chemical Rubber Publ. Co., Cleveland, Ohio, *1960–1961*.
9. Krewson, C. F., J. S. Ard, and R. W. Riemenschneider, JAOCS *39*, 334–40 (1962).
10. Lynen, F., and S. Ochoa, Biochim. Biophys. Acta. *12*, 299–314 (1953).
11. Mehlenbacher, V. C., T. H. Hopper, and E. M. Sallee, "AOCS Official and Tentative Methods," rev. to 1958, AOCS, Chicago, Ill.
12. Miwa, T. K., K. L. Mikolajczak,, F. R. Earle, and I. A. Wolff, Anal. Chem. *32*, 1739–42 (1960).
13. Ogg, C. L., and F. J. Cooper, *Ibid. 21*, 1400–2 (1949).
14. Seubert, W., and F. Lynen, J. Am. Chem. Soc. *75*, 2787–8 (1953).
15. Shriner, R. L., and R. C. Fuson, "The Systematic Identification of Organic Compounds," 3rd ed., John Wiley and Sons, Inc., New York, N.Y., 1948, pp. 52–3.
16. Stern, J. R., A. del Campillo, and I. Raw, J. Biol. Chem. *218*, 972–83 (1956).
17. Van Etten, C. H., Anal. Chem. *23*, 1697–8 (1951).
18. Von Rudloff, E., JAOCS *33*, 126–8 (1956).

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Epoxy Resins from Fatty Esters Derived from Cyclohexane and Epoxycyclohexane[1]

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Abstract

The magnitude of the physical properties of resins obtained by the phthalic anhydride cure of non-terminal epoxy monomers, prepared from fatty derivatives, was shown to be dependent on the distribution of the epoxy groups in the monomer.

The monomers were prepared by epoxidizing a series of unsaturated esters, selected to test the effect of the addition of a single symmetrically disposed epoxide group. Oleoyl and linoleoyl esters of tetrahydrophthalyl alcohol, as well as dioleyl and dilinoleyl tetrahydrophthalate, were epoxidized and cured with phthalic anhydride (Group A). For comparison, epoxy monomers were prepared from oleoyl and linoleoyl esters of hexahydrophthalyl alcohol, as well as dioleyl and dilinoleyl hexahydrophthalate (Group B). When these epoxides were cured with phthalic anhydride, it was found that the resins derived from Group A had heat distortion temperatures which were approximately 55–65C higher than those from Group B.

Introduction

IN THE DEVELOPMENT of epoxy resins, considerable research has been centered on glycidyl ethers of bifunctional phenols such as bisphenol A. Dearborn et al. (1) and Wynstra (2) have reported on the effect of varying the structure of the glycidyl ether on the physical properties of the resins.

In contrast with glycidyl resins, much less has been reported on the preparation of epoxy resins from non-terminal epoxides. However, during recent years, investigations concerning the preparation of epoxy resins from animal fat derivatives have been carried out at this laboratory and elsewhere. As a result of this work, it has been shown that resins can be prepared from epoxidized fatty glycerides, and that fat-based epoxy monomers can be used to modify or even replace commercially available epoxy monomers, such as the diglycidyl ether of bisphenol A (3,4).

It has been further demonstrated that variation of the chemical structure of the monomers prepared from fat-derived chemicals, leads to changes in the physical properties of the epoxy resins prepared from them. Correlations have been made between the chemical structure of epoxidized fatty esters and the properties of resins derived therefrom by curing with phthalic anhydride (5,6). It has been shown that linoleic acid derivatives produced epoxy resins exhibiting higher heat distortion temperatures than those of resins prepared from corresponding oleic acid intermediates. This was believed caused by the increased number of epoxide groups obtainable from linoleic acid derivatives, as well as by a more favorable distribution of these linkages within the monomer molecule.

The purpose of the present research was to study

[1] Presented in part at the AOCS meeting in New Orleans, La., 1962, It is V in the series "Epoxy Resins from Fats."
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