Naturally Occurring Epoxy Oils[1]

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Abstract

Fatty acids containing oxirane functional groups have unexpectedly been found in wide distribution in seed oils, usually present as long-chain glycerides. Four such acids have been discovered: *cis*- and *trans*-9,10-epoxystearic acid; vernolic (*cis*-12,13-epoxyoleic) acid; coronaric acid, isomeric with vernolic acid but with the oxirane and double-bond functions in the reverse positions; and *cis*-15,16-epoxylinoleic acid. No single analytical tool has been universally successful in measuring the oxirane content of epoxides. This appears to be caused by the way in which the chemical reactivity of the three-membered cyclic ether is modified by molecular structure and by the presence of nearby substituents.

A survey of the published methods for the determination of oxirane functions, including mention of the nature of interfering substances, is given. Techniques used to overcome some of these difficulties are discussed. These procedures include the use of chromatographic and spectrophotometric procedures to solve some of the analytical problems which are encountered. Special consideration is given to vernolic acid and its glycerides since this is the most widely distributed and abundant epoxy acid found in seed oils to date.

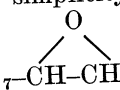
Introduction

STUDIES ON THE COMPOSITION of plants present the chemist with complex and often extremely elusive problems. Hundreds of components are present in a single plant, representing many classes of diversified organic compounds. Thousands of unique, oil-rich seeds have never been investigated, and certainly no plant has ever been completely analyzed. To pursue plant investigations, an intimate knowledge of natural products and of the methods used in the detection, separation, isolation, purification, and identification of the components is essential. Thorough familiarity with the use of the new and elegant techniques is also necessary, including all types of chromatography and various spectrophotometric methods such as infrared (IR), ultraviolet (UV), fluorometry, and both mass and nuclear magnetic resonance (NMR). Pertinent remarks by Wolff (98) concerning the composition and analysis of "seed lipids" appeared in a recent issue of Science.

Epoxy- Fatty Acids

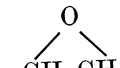
Fatty acids which contain the oxirane function have unexpectedly appeared in wide distribution in seed oils and are usually present as long-chain glycerides. In a current speculative article on the role of epoxy acids as intermediates in the biosynthesis of polyunsaturated fatty acids, Gunstone (21) mentions that epoxy acids have been reported in more than 40 species from 12 different plant families. These acids are related to the common 18-carbon unsaturated acids, oleic, linoleic and linolenic. At present four epoxy acids are known to occur as fatty acyl groups in natural oils (11a, 20, 22, 82, 83, 88, 95). The four acids by formula, without reference to optical rotation or configuration, are given.

First in simplicity is *cis*-9, 10-epoxystearic acid,



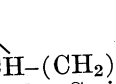
$CH_3(CH_2)_7$–CH–CH–$(CH_2)_7$–COOH, isolated in 1959 from *Tragopogon porrifolius* seed oil by Chisholm and Hopkins (11a) and by Tulloch and co-workers (88) from the oil of wheat-stem rust uredospores. The *trans*-isomer was found in orujo oil by Vioque et al. (95) in 1961.

Second, and the first natural epoxy acid to be discovered, is *cis*-12,13-epoxyoleic (vernolic) acid,



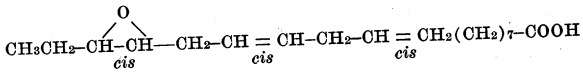
$CH_3(CH_2)_4$–CH–CH–CH_2–CH=CH–$(CH_2)_7$–COOH, by Gunstone (20) in 1954 from *Vernonia anthelmintica* seed oil.

Third is an isomer of vernolic acid, *cis*-9,10-epoxy-*cis*-12-octadecenoic, $CH_3(CH_2)_4CH=CH$–



CH_2CH–CH–$(CH_2)_7$–COOH, better known as coronaric acid, by Smith et al. (82,83) in 1959, from *Chrysanthemum coronarium* seed oil. In coronaric acid the oxirane oxygen and the double bond are in reverse positions compared with those of vernolic acid.

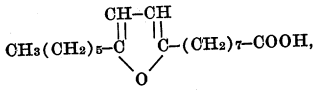
And fourth is *cis*-15,16-epoxylinoleic acid,



CH_3CH_2–CH–CH—CH_2–CH = CH–CH_2–CH = $CH_2(CH_2)_7$–COOH (*cis*, *cis*, *cis*)

discovered by Gunstone and Morris (22) in 1959, isolated from *Camelina sativa* seed oil.

It is probably only a matter of time until plant sources of the two remaining epoxy acids, corresponding to epoxidation at the 9- and/or 12-olefinic bond of linolenic acid, will be discovered. With respect to 18-carbon seed-oil acids, it is possible that epoxy functions could be present in other positions. Recently Morris et al. (61) announced the presence of a unique 18-carbon furanoid fatty acid,



$CH_3(CH_2)_5$–C(CH)–C(CH)–$(CH_2)_7$–COOH, (O)

as of possible occurrence in the seed oil of *Exocarpus cupressiformis*. The acid could be an artifact from some other unique fatty acid as yet not characterized (61a); when more *Exocarpus* seed becomes available, these investigators hope to obtain unequivocal confirmation that this furanoid acid is a natural product. The existence, in some seed oils, of other than 18-carbon epoxy fatty acids and of epoxides containing more than one epoxy function cannot be ruled out. Sometimes two or more of the four known epoxy acids occur in the same seed oil (60, 64, 65, 86).

Other types of epoxides have been located in plant leaves, stems, flowers, and roots. Some of these are antitumor agents. Kupchan et al. (10,44–46) are among those active in this field. One compound illustrative of this unusual epoxide type is euparotin acetate,

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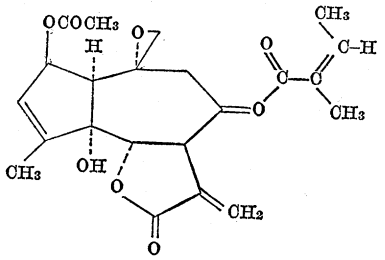
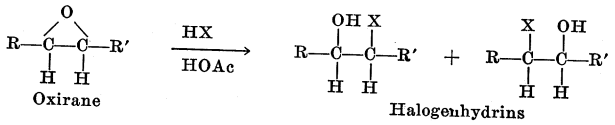


obtained from *Eupatorium rotundifolium* (46). Another unique natural epoxide is 1α, 2α-epoxyscillirosidine, obtained from *Homeria glauca*, which causes "tulip poisoning" in South African livestock (16). Mono- and diepoxides of carotenoids have been found in plants; a variety has been reported present (11b, 100) in the peel and pulp of citrus. Recently spiro-epoxides from *Fusaria* and *Hyphomycetes* have been described (54b). The occurrence of all *trans* (−)-14,15-epoxygeranylgeraniol in *Pterodon pubescens* has been established and its prophylactic activity against infection by *Schistosoma mansoni* demonstrated. Epoxides of various types, as yet not discovered, may also be present in seed oils, especially in the unsaponifiable fractions.

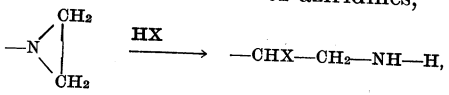
Determination of Oxirane Oxygen

At this point it seems expedient briefly to outline methods of epoxide analysis in use before elaborating upon the composition of any epoxy seed oil in particular. In 1930 Nicolet and Poulter (62) reported that the epoxy group in 9,10-epoxystearic acid could be measured quantitatively by treating this compound with an excess of HCl in dry ether and titrating the excess with KOH. In 1947 Swern et al. (85) tested this method on a variety of compounds with the oxirane group at different positions in the molecule, concluding that this hydrohalogenation, with slight modification, was quite specific for compounds containing oxirane oxygen and that other related compounds, such as peroxides, did not interfere; α,β-unsaturated carbonyl compounds were claimed to absorb HCl. Swern considered the reaction as one of ring opening and halogen addition as illustrated



in a recent review article on oxirane determinations by Maerker (48). King (32,33) later made several modifications of the hydrohalogenation procedure.

In 1956 Durbetaki (13) proposed the use of hydrogen bromide in glacial acetic acid as a hydrohalogenating agent, and in 1964 this procedure became the AOCS Tentative Method, Cd 9–63, "Oxirane Oxygen" (4,19). For use in this method the HBr can be purchased commercially as a 30% to 32% solution in glacial acetic acid and can be diluted to the required 0.1 *N* with glacial acetic acid. This HBr solution must be standardized frequently; "Tham," tris(hydroxymethyl) aminomethane, is a suitable reagent for this purpose. It has been found (35) that a small amount of hydroquinone (0.02 molar) in the HBr-acetic acid reagent has a stabilizing effect in the prevention of free bromine side reactions. Jay (31) has found that the problem of HBr storage is avoided in a useful and important variation of the Durbetaki method. In Jay's method HBr is prepared *in situ* by treatment of a quaternary ammonium halide with standard perchloric acid. He also uses this method for the determination of aziridines,



which, if present, interfere with epoxide analysis.

Many modifications of the hydrohalogen procedure have been offered because of the recognition that substances other than epoxides, capable of adsorbing HBr, may be present. Durbetaki claimed no interference from hydroperoxides, and Magne (52) mentions that cyclopropyl, olefinic, or conjugated olefinic groups do not interfere but that conjugated hydroxydiolefins do. Haeberer and Maerker (23) have stated that alkali metal carboxylic acid salts of long-chain fatty acids (soaps) can also be determined by titration with HBr and hence, if present, will interfere with oxirane analysis. Krull (43) has claimed interference by glycerine (14% water), crotonaldehyde, mesityl oxide, amines, and urea in the hydrohalogenation procedure but none with the use of his argentometric method. The presence of some compounds in seed oils may cause more subtle errors, such as epoxides, which absorb less than theoretical quantities of hydrogen halide, for example, one claimed by McConnell and Moore (55), 3,4-epoxy-2,2,4-trimethylpentylisobutyrate.

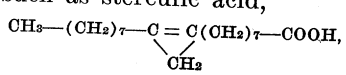
It is also possible that stereoisomers of epoxides may be present, such as the *syn*- and *anti*-diepoxides of methyl 9,10: 12,13-diepoxystearates, obtained by Maerker et al. (51) in the epoxidation of methyl linoleate. The well-known solid isomer melting at 32C, representing about 60% of the product, titrates completely with HBr, but the newly-discovered liquid isomer is measured incompletely by HBr titration.

It has recently been observed in this laboratory (C. F. Krewson and O. T. Chortyk, 1967, unpublished data) that nicotine and related alkaloids, such as nornicotine and anabasine, absorb HBr. This problem in connection with tobacco leaf studies is receiving further attention.

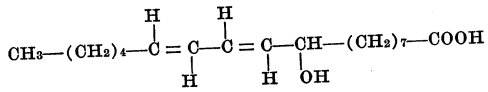
Bentz and associates (3a-c) have recently introduced a colorimetric method for oxirane determination which makes use of picric acid addition to epoxides; they claimed no interference from cyclopropene, conjugated dienols, or α,β-unsaturated carbonyls. Hassen and Lea (25) have checked this picric acid method on epoxy seed oils and have obtained satisfactory agreement by preparing methyl esters and using the alcoholysis procedure of Barford et al. (2). Their resulting methyl esters were analyzed by gas liquid chromatography (GLC) with the method of Herb et al. (26). Mallah et al. (54a) have also checked this method against hydrohalogenation and GLC procedures and found it to be simple and sensitive with reproducible results on malvaceous seed oils.

Recently Urbanski and Kainz (89) have reported on an oxirane oxygen method with 2,4-dinitrobenzenesulfonic acid as reagent.

As early as 1960 Smith et al. (84) reported that at least two fatty acid types in seed oils interfered in the hydrohalogenation procedure: a) cyclopropenoid acids such as sterculic acid,



which is obtained in abundance from *Sterculia foetida* seed oil, and b) conjugated hydroxydiolefins such as dimorphecolic acid,



which is 9-hydroxy-*trans,trans*-10,12-octadecadienoic acid found in *Dimorphotheca aurantiaca* seed oil. These investigators (84) described a method of differentiation between epoxy acids and acids of these types, basing their procedure mainly upon titration with hydrogen bromide before and after reduction with lithium aluminum hydride.

Harris et al. (24) have overcome cyclopropenoid interference by modifying the Durbetaki procedure: epoxy compounds can be selectively titrated at 3C; continuation of the titration at 55C gives an estimate of the cyclopropenoid fatty acids which titrate rapidly at the higher temperature. Hopkins (28) has successfully used NMR in the determination of such cyclopropenoid acids as sterculic acid.

In view of the possible addition of HBr to groups other than epoxides in seed oils, the term HBr-equivalent (HBE) seems more appropriate than oxirane oxygen. As early as 1960 (15d) and in numerous subsequent reports, investigators at the Northern Regional Research Laboratory have referred to HBr-uptake (14,15) as HBE. Wilson et al. (97) define hydrogen bromide equivalent as the moles of HBr consumed per mole of C_{18}-epoxy acid (equivalent weight 296.5) times 100.

Morris et al. (58) adapted a wide range of isolation methods to the separation of epoxy components from seed oils. They (59) applied GLC and thin-layer chromatography (TLC), ultraviolet (UV), and infrared (IR) spectrophotometry for the detection of small amounts of hydroxy-substances interfering in epoxy determinations; they (60) were also able to detect and estimate small quantities of epoxy compounds in large amounts of hydroxy-fatty acids by use of near IR spectra. They (60) were the first to apply a combination of TLC and GLC techniques in the detection and estimation of epoxy components in seed oils and to *Vernonia* seed oil in particular. They obtained their mixed acids from *V. anthelmintica* by techniques similar to those used by the Hopkins and Smith groups. However Morris et al. converted their mixed acids with diazomethane to methyl esters for GLC analysis. Barford et al. (2) prepared methyl esters from *V. anthelmintica* seed oil directly for GLC analysis, without oxirane destruction, by alcoholysis of the oil with potassium methylate as the catalyst. They obtained methyl vernolate of 93.6% purity in 92% yield, following one crystallization at −60C. Miwa et al. (56) and Herb et al. (26,27) have used GLC techniques for analysis of the methyl esters which were prepared from *V. anthelmintica, E. lagascae,* and other seed oils.

A number of investigators (1,26,73,90,91) have reported that oxygenated compounds can be chromatographed quantitatively on silicon rubber columns. Herb et al. (26) prepared a column by using silicone polymer SE-30 as the stationary phase and found that, when methyl vernolate was chromatographed, a single symmetrical peak appeared which had an area comparable with that of esters of the normal fatty acids; but unfortunately the saturated and unsaturated esters of the same chain-length were not well separated on this stationary phase. In order to obtain a complete analysis of an epoxy-bearing seed oil, it was also necessary to chromatograph the sample of methyl esters on a polyester, ethylene glycol succinate (EGS) column as well as on the SE-30 column and to calculate the composition. Column analyses are given in Table I. Data on Method A were obtained with the SE-30 (temperature-programmed) and those in Method B with the EGS column by using correction factors as described (26). Their percentages of methyl epoxyoleate compared favorably with that of 75.1% obtained by HBr titration. Position isomers, such as coronaric and vernolic acid methyl esters, are, of course, not distinguishable by this procedure.

12,13-Epoxyoleic Acid

Source Materials. Attention will be devoted mainly to compositional studies on 12,13-epoxyoleic acid in its *dextro*-rotatory form and its naturally occurring derivatives since this acid appears to be the most widely distributed and abundant of the epoxy fatty acids known to occur in plants.

The *Vernonia anthelmintica* and *Euphorbia lagascae* have been the chief epoxy seed oil source materials available for major studies. The oil and various parts of *V. anthelmintica* have been used for medicinal purposes for centuries (35). There are an estimated 400–600 species (18,47) of the genus *Vernonia* to which *anthelmintica* belongs. Epoxy-bearing oils have been found in other *Vernonia* species and certainly will be located in others although some species examined have had little or none present. Rao (68) has reported on a number of interesting nonfatty compounds in *Vernonia cinnerea.* In addition to the previously reported epoxy acid, *cis* (+) epoxyoleic acid (30a-c), Toubiana and Gaudemar (87) recently described the presence of a sesquiterpenoid ester in *Vernonia colorata.*

Chief seed supplies for initial chemical and enzymatic studies (15d,20,34,35,38,39,41,42,77–79,82) on *V. anthelmintica* have been received from India and Pakistan; seed supplies for studies (15–1,38,40,63,76) on *E. lagascae* have been received from Spain. White et al. (96a-e) have reported regularly on growing experiments in this country over a wide area. Seed for a pilot-plant study (37) on *V. anthelmintica* extraction was of domestic origin. In a private communication for this publication White (96f) has reviewed the present situation concerning the growing of these plants as follows.

"An intensive plant-improvement program on *Vernonia anthelmintica* at Purdue University, under an Agricultural Research Service Contract of the U. S. Department of Agriculture, is resulting in improved lines through selection activity. The more promising lines are shorter, somewhat earlier, and more determinate in flowering. Unfortunately, good seed-retention has not been obtained. Through the use of white-flowered selections, it has been found that some out-crossing does occur, but *V. anthelmintica* is nevertheless highly self-fertile. Crosses are being made between different genotypes, but progeny from

TABLE I
GLC Analysis of *Vernonia anthelmintica* Seed Oil

Components	Method A	Method B
	%	%
16:0	2.52	1.94
18:0	1.37	1.40
18:1	1.88	2.05
18:2	8.10	7.86
18:3	0.26	0.30
18:1 Epoxy	76.7	76.4
Other fatty acids	1.35	2.31
Unsaponified	7.76	7.76

these have not been evaluated. The use of improved lines is essential to the commercialization of the crop; therefore cultural tests have been limited. Cultural practices will have to be determined or refined for the improved lines. In general, the crop prospect for *Vernonia* appears good, but considerable effort will be required to reach the goal of crop status.

"The crop potential of *Euphorbia lagascae* would be good if seed retention and disease (and nematode) resistance were obtainable. This species may have a slightly greater range of adaptation since oil content and oil quality of *Vernonia* are adversely affected in northern latitudes. Good data on yield are not available because of shattering and disease problems. A breeding program on *E. lagascae* seemingly will be necessary, and additional accessions are needed to provide a broader base of germ plasm."

As indicated in the reports by White et al. (96a-e), other plant species are undergoing evaluation as possible epoxy acid sources. These are species of *Cephalaria* and *Crepis,* but most of these are too low in oil and/or epoxy content to make them promising.

Structural Considerations. Vidyarthi and Mallya (93), as early as 1939, were the first to report the presence of a new acid in *V. anthelmintica* seed oil; they reported this to be a hydroxy-acid isomeric with ricinoleic (12-hydroxyoleic) acid. Vidyarthi (94) supplied the name "vernolic" to the new acid. It was not until 1954 that Gunstone (20) discovered their mistake and pointed out that vernolic acid contained an epoxy- not the hydroxy-function. By thorough degradative studies he concluded that vernolic acid was 12,13-epoxyoctadec-9-enoic (12,13-epoxyoleic) acid.

Briefly, Gunstone obtained his oil from *V. anthelmintica* seed (yield 26.9%, with a free fatty acid content as epoxyoleic acid of 26.4%). He extracted the ground seed by Soxhlet procedure with the use of low-boiling petroleum ether. His quantitative determination of oxirane oxygen indicated that the epoxy functional group was present in high proportion. For structure proof, because of the reactivity of the epoxy compound, he converted the vernolic acid to the acetoxyhydroxy-acid with acetic acid, then hydrolyzed this acid with KOH to the corresponding dihydroxy-acid. He partitioned the dihydroxyoleic acid concentrate between petroleum ether and aqueous methanol. The specimen from the methanol portion was almost certainly the *cis*-isomer; after isomerization, the higher-melting *trans*-isomer was isolated. Hydrogenation of the mixed acids gave dihydroxystearic acid. When treated with periodate, this acid gave a volatile aldehyde, not isolated, and a solid aldehydo-acid ($C_{12}H_{22}O_3$), which Gunstone concluded must have been 11-formylundecanoic acid because, on oxidation, it gave dodecanedioic acid. This acid had not been previously known although its methyl ester had been described.

Another portion of the dihydroxyoleic acid Gunstone oxidized with periodate, producing a volatile aldehyde which proved to be that of n-hexanal because its 2,4-dinitrophenylhydrazone (DNP) gave the same analysis and mp as the DNP of n-hexanal. The other product of this oxidation of dihydroxyoleic acid was an unsaturated aldehydo-acid ($C_{12}H_{20}O_3$), which readily gave a deep orange DNP. This compound was formulated as 11-formylundec-10-enoic acid since it was oxidized to sebacic acid. Another portion of the dihydroxyoleic concentrate was oxidized with $KMnO_4$ in acetic acid (a double-bond oxidation). The n-hexanoic and azelaic acids were obtained in good yields. Thus Gunstone's classical degradative studies indicated that the dihydroxy-acid he prepared from vernolic acid was 12,13-dihydroxyoctadec-9-enoic and the original must have been 12,13-epoxyoleic (vernolic) acid.

He was concerned about the appearance however of 11-formylundec-10-enoic acid in the periodate oxidation because of the movement of double bonds into conjugation under his mild oxidative conditions; but that is what happened, a fact which has been confirmed in this laboratory by Maerker (personal communication). Recently Maerker et al. (49,50) have published data on a more direct method of periodate cleavage by using methyl vernolate. They have been able to distinguish between the relative quantities of methyl vernolate and methyl coronarate which are present in monoepoxidized methyl linoleate.

The first absolute optical configuration of a naturally occurring epoxy fatty acid, that of vernolic, has been unequivocally established by the elegant work of Morris and Wharry (57a,b) They established vernolic acid to be *cis*-12-D,13-D-epoxy-*cis*-9-octadecenoic acid. The key to this work was dependent upon the investigations of Serck-Hanssen (80), who proved that ricinoleic, (+)-12-hydroxyoleic acid, had the D-configuration, that is, (R) according to the Cahn-Ingold-Prelog system (6). From their studies Morris and Wharry conclude that the (−)-epoxy oleic acid from the Malvaceae seed oils must have been the *L*-configuration. Also it became evident that the enzymatic hydration of vernolic acid in crushed, incubated *Vernonia* seed (77–79) and in crushed *Euphorbia* seed (76) must have given the opposite enantiomer to that which predominated in the chemical acetolysis product prepared from these oils. Enzymatic hydration therefore gave the dextrorotatory L-12,D-13-dihydroxyoleic acid. Thus Morris and Wharry (57b) stated that "the enzymatic attack must have been at the 12-position, resulting in inversion there, and the oxygen of the D-13-hydroxyl groups is presumably the oxygen from the original epoxide groups."

By similar studies Powell et al. (65) have confirmed the absolute configuration of vernolic acid and have established for the first time the absolute configuration of the two 9,10-epoxy acids, coronaric as *cis*-9-L,10-L-epoxy-*cis*-12-octadecenoic acid, and *cis*-9,10-epoxystearic as *cis*-9-L-10-L-epoxyoctadecanoic acid. Complete details of both of these fine studies are beyond the limits of this review.

It is rather remarkable that *cis*-12,13-epoxyoleic acid occurs naturally in both of its optically active forms. The (+)-acid (vernolic), which gives rise to predominately (−)-*threo*-12,13-dihydroxyoleic acid by acetolysis, is present in the seed oils of a number of species of the *Compositae, Dipsacacea Euphorbiacae, Onagracea* and *Valerianaceae* families (86) whereas the (−)-epoxy acid, which by similar cleavage gives predominately the (+)-dihydroxyoleic acid enantiometer, is a constituent of the seed oils of several Malvaceae (29a,b,30a).

Vernoloyl groups occur in both enantiomeric forms (29a,b,30a-c,77,79,86) and epoxystearoyl groups as both *cis* (88) and *trans* (95) geometric isomers. The (+)-vernoloyl (*cis*-12,13-epoxy-*cis*-9-octadecenoyl) groups in *V. anthelmintica* are present almost exclusively as trivernolin (34–36). It is therefore of interest to determine the identity and the distribution of epoxyacyl groups in new, naturally occurring epoxy

oils, especially where there is indication of significant quantities of epoxides present.

Associated with the elaborate screening program in progress at the Northern Regional Research Laboratory (15a-m), Tallent et al. (86) have recently reported on the identification and distribution of the epoxyacyl groups in the 10 seed oils found to contain the largest quantities of epoxyacyl groups along with *V. anthelmintica* seed oil for comparison. The (+)-vernoloyl was the only epoxyacyl group found in these oils. Their intradistribution studies (86) revealed a general preference of the (+)-vernoloyl groups for the β-position of triglyceride molecules. Intraglyceride distribution of (+)-vernoloyl groups was studied in three oils and found not to agree with predictions based on either 1,2,3-random or 1,3-random-2-random distribution. A striking exception to the general intraglyceride distribution pattern was discovered in the monoepoxy triglyceride fraction from *E. lagascae seed* oil. The vernoloyl groups are more concentrated in the α- than in the β-position.

Unique Facts About Seed Oils and Meals

Gunstone did not actually isolate 12,13-epoxyoleic (vernolic) acid. Smith et al. (82) first isolated it from *V. anthelmintica* in 1959. However Hopkins and Chisholm (29a,b) had obtained the *levo*-12,13-epoxyoleic acid from *Hibiscus cannabinus* seed oil, which they first reported in 1957. Hopkins and Chisholm were able to saponify their oil, as was Smith et al. with *Vernonia* oil, by alcoholic KOH at room temperatures. It is now well known that epoxide oils will withstand normal saponification at reflux temperatures. Following saponification, the unsaponifiables were removed in the usual manner with petroleum ether, and the soaps were converted to free fatty acids with dilute HCl. Partitioning procedures were used to separate epoxy from nonoxygenated fatty acids. Crude vernolic acid was purified by repeated crystallizations.

Techniques used for the production of *V. anthelmintica* seed oil depend entirely upon the oxygenated component (or components) which are desired. In early seed extractions none of the investigators were aware of the reactivity, or the unique behavior, of the *Vernonia* enzyme systems once the seed was crushed. The author's free fatty acid values for *Vernonia* seed oils have varied from 0.8 to 50.2% (39,77). This variation has depended upon the seed accession, the method of handling, and the time lapse between grinding and extracting the seed. In leisurely grinding of *Vernonia* seed, followed by the usual Soxhlet extraction, the FFA content will vary from about 15 to 30%. An extract with a high content will contain a variety of oxygenated compounds, vernolic acid, 1,3-divernolin, and trivernolin (12,28, 34,35) and, depending upon the amount of moisture present, (+)-*threo*-12,13-dihydroxyoleic acid. The latter is a product of enzymatic hydration. As much as 9.3% of the weight of *Vernonia* oil present in the seed has been the 12,13-dihydroxyoleic acid, under forced conditions of hydration described (77–79). Sampugna et al. (74) have concluded that, since a preponderance of 2-monoglyceride was produced by the action of pancreatic lipase on trivernolin (from *Vernonia* oil), the structure of the triglyceride is not solely responsible for this secondary position specificity.

Euphorbia seed undergoes the same behavior pattern, but the enzymatic activity (40,76) is not as rapid. This activity may well be the pattern of other epoxy-bearing seeds, differing only in degree. In fact, in a recent report (65) on the composition of *Xeranthemum annum* seed oil, extracted by Soxhlet procedure, a FFA content of 17% was obtained. If this type of lipolysis of epoxy glycerides, which may also be accompanied by hydration to dihydroxy acids, is to be avoided, then one of several procedures which have been previously described (35,39,42) must be used. The enzyme systems may be deactivated by heat (autoclaving preferred) before grinding, or the use of rapid extraction techniques (preferred) may be used: a) the seed is disintegrated in the solvent or b) it is extracted as rapidly as possible immediately after flaking.

The epoxy compounds, vernolic acid, 1,2,-divernolin, and trivernolin have been obtained chromatographically pure by a combination of procedures described by Krewson and Luddy (36).

Optimum conditions have been developed (39,41,42) for the production of *Vernonia* seed oil with trivernolin as the chief epoxy component or, if preferable, for the direct production of trivernolin (96–98% pure) in 50–60% yields based upon the oil. The rapid-extraction procedure (35,39,41,42) has been used for this purpose. High-quality trivernolin was readily obtained from the original petroleum ether-extracting solvent by crystallization at temperature below −8C but not lower than −20C. The process has successfully been carried through pilot-plant stages in a small, continuous soybean-extraction plant with only minor changes in equipment (37).

Majundar (53) has reported that brassicasterol and stigmasterol are present in the unsaponifiable material of *Vernonia* oil. This unsaponifiable material amounts to 6–8% (35) of the weight of the oil.

Chalvardjian et al. (8) have stated that the administration of *Vernonia* oil to weanling rats for 28 days at a 10% level showed no adverse effect upon gross or microscopic anatomy. However, on two different samples of *Vernonia* oil in experiments performed at different times (5), rats refused to eat a ration containing 10% of the oil and grew poorly, losing weight (primarily because of reduced feed intake) in contrast to rats which were fed the same basic diet with 10% corn oil that produced weight gain. In India the oil has been used as an anthelmintic for hookworm (7) and as a remedy for leucoderma (17). It is a drying oil.

Amino acid analysis of *Vernonia* meal made by Van Etten et al. (92) indicated that the methionine and lysine content were limiting factors for its use as the only source of protein for animal feeding. Booth (5) observed a normal rate of growth when autoclaved *Vernonia* meal was fed to rats at a 20% dietary level for 90 days in a diet containing other protein supplements. Because of its high fiber content the meal may ultimately prove to be a more suitable feed for sheep and cattle than for swine or poultry. Booth's results (5) in feeding to five weanling male rats, unheated *Vernonia* pericarp and kernel meal fractions for 49 days suggested that pancreatic enlargement (0.54 vs. 0.74 g) was produced by the raw-kernel fraction. Since growth was also markedly inhibited (154 vs. 72 g), it was tempting to implicate a trypsin inhibitor which in raw soybean meal does cause similar effects in rats.

A *Vernonia* kernel fraction was sent to J. J. Rackis

of the Oilseed Crops Laboratory of the Northern Regional Research Laboratory, USDA, Peoria, Ill., for trypsin inhibitor assay. No trypsin inhibitor activity was detected (personal communication, 1957). The *Vernonia* kernel extract fraction actually had 10% more proteolytic activity compared with the standard solution of trypsin in the assay method of Wu and Scheraga (99). This method measures both activities mentioned. The extracts for trypsin inhibitor assay were obtained by extracting raw meal with water and using a water-to-meal ratio of 10:1, followed by a second extraction with a ratio of 5:1. Extractions were made at both pH 7.0 and 9.0. Dr. Rackis' results indicated that the *Vernonia* kernel fraction may contain quite active papain-like protease. He thinks it may still be possible that Vernonia contains a trypsin inhibitor since rat-feeding tests showed pancreatic hypertropy. With soybeans Dr. Rackis' group has concluded that trypsin inhibitors account for 30–50% of the rat growth-inhibitory capacity of raw meal and for nearly 100% of the pancreatic hypertropic effect (66).

Euphorbia lagascae seed is an especially attractive source of epoxyl oil (15–1,38,40,63,76). This seed contains 39–50% oil, and the oil has a 60–70% epoxide composition expressed as vernolic acid, which is present chiefly in the form of mixed glycerides that contain only about 18–25% trivernolin. *Euphorbia* oil has caused fatalities in both frogs and rats when injected (63). This has been confirmed with *Euphorbia* oil in both mice and rats (5). However rats injected with *Vernonia* and corn oil survived (5). It would appear that *Euphorbia* oil contains a toxic factor (not related to the epoxy group) which is not present in *Vernonia* oil. Rats fed a 10% *Euphorbia* oil diet also lost weight. In preliminary studies with a limited quantity of *Euphorbia* meal, Booth (5) found no harmful effects in the general appearance or in the weight gain of rats which were fed the meal at 20% dietary levels for 28 days.

Practical application tests have been made in this laboratory on *Vernonia* products and their derivatives and an *Euphorbia* oil and epoxidized *Euphorbia* oil (38,69–72, and unpublished data). These studies have been chiefly in the field of plastics. Also stability data of *Vernonia* products have been reported (75a,b).

In conclusion, the discovery of many more new naturally occurring epoxy oils may be anticipated since this field of natural products is certainly in its infancy.

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