Epoxy Oils From Plant Seeds[1]

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Abstract

Epoxy acids have been reported in seed oils from more than 60 species in 12 plant families. The discovery of 9,10-epoxyoctadec-12-ynoic and 9,10-epoxy-*trans*-3,*cis*-12-octadecadienoic acids brings to six the number of natural epoxy acids now known to occur in seed oils. These latest epoxy acids and 15,16-epoxy-*cis*-9,*cis*-12-octadecadienoic acid have been found in only one species each and at levels lower than 5% of the oil. Coronaric (9,10-epoxy-*cis*-12-octadecenoic) acid and 9,10-epoxystearic acid have been encountered in several seed oils, the first as much as 15% of the oil and the latter in only small amounts. Vernolic (12,13-epoxy-*cis*-9-octadecenoic) acid, which has been identified in numerous oils, is the only epoxy acid known to occur in seed oils at levels above 15%, and it may constitute as much as 75%. On the basis of data available to date, *Vernonia anthelmintica* appears to have the best potential for commercial production of an epoxy oil. Although one improved line has been selected, continued improvement is needed. Formation of epoxy acids in oilseeds during storage after harvest has been demonstrated, and may be partly responsible for the small amounts of epoxide detected in oils from a wide variety of seeds.

Introduction

In the 16 years since an epoxy acid was first found as a natural constituent of a seed oil, research has established that this type of acid is present in oils from more than 60 species of plants. Presumptive evidence indicates that minor amounts of such materials occur in many other species widely distributed throughout the plant kingdom. Surprisingly, these reports have all been derived from only three groups of workers: Gunstone at St. Andrews and his students; Hopkins and Chisholm at Ottawa; and the New Crops group with which the author is associated in the Northern Regional Research Laboratory at Peoria.

Natural epoxy oils have now achieved the status of being included in reference books on oils (1–3) and in reviews on unusual fatty acids (4,5). Krewson (6) reviewed the literature on epoxy seed oils in 1968. This report supplements Krewson's review, provides a comprehensive list of seeds in which epoxy oils have been found, and relates some interesting new developments.

Methods

Before the discovery of epoxy acids is reviewed, methods used to detect and determine these acids will be considered to lend perspective in evaluating test results. Some procedures are not specific; they may indicate a structural group without regard to the identity of the entire molecule, or they may be subject to interference by other types of structures. In practice, especially with oils containing small amounts of oxygenated acids, adequate supplementary tests are often not made and the composition reported may not be fully definitive.

Epoxy groups can sometimes be detected in oils by IR spectrometry and by paper and thin layer chromatography (TLC).

IR absorption bands at 11.8 and 12.1 μ, indicative of the epoxy structure, are not very strong (7). Accordingly, the convenient technique of spreading thin films on salt plates to measure absorption cannot be relied on unless the epoxy acid is present in large amounts. Conversion of the epoxide to the chlorhydrin, as in the quantitative procedure, increases sensitivity markedly.

Both paper chromatography and TLC of methyl esters prepared from oils detect epoxy esters and give some indication of the number and type of compounds in samples studied (8). TLC has been modified by use of picric acid to reveal the spots containing epoxy compounds (9). An orange spot clearly indicates an epoxy group even in admixture with other material that would confuse interpretation of a charred plate. When applied to the chromatography of oils, the picric acid reagent will detect triglycerides with one, two or three epoxy groups per molecule, even when free acids or other oxygenated glycerides are present.

An extensive review of methods for the quantitative determination of epoxy groups has been published by Dobinson et al. (10). Although their work is directed primarily toward analysis of synthetic epoxidized materials, applicability of the methods to natural oils and fatty acids is covered. They conclude that the most promising chemical procedure is Jay's method (11) in which HBr is released from tetraethyl ammonium bromide in the reaction mixture by addition of $HClO_4$ until all the epoxide has reacted. They point out, however, that further study is needed of the applicability of the method to a variety of materials. These authors also state that the titration with HBr in acetic acid, on which AOCS Method Cd 9–57 (1961) is based, is "very useful" and "has almost become a standard technique for use with epoxides of various sorts." The AOCS method is recognized as being subject to interference by cyclopropenoid acids and conjugated dienols (12). If however the titration is carried out at icebath temperature, epoxy groups react promptly, but cyclopropenoid (13) and conjugated dienolic (14) acids react so slowly that they do not interfere with the determination. There is no assurance that other nonepoxy compounds may not contribute to erroneous results. Because of this uncertainty, titration results are often expressed as hydrogen bromide equivalent (HBE) and calculated as epoxyoleic acid.

A comparison shows that a potentiometric collidine hydrochloride method is more precise than the HBr titration (15). Another modification (16) of an older method (17) involves determining excess HCl by addition of $KBrO_3$ and KI and titrating the liberated iodine.

Epoxy acids have been measured quantitatively in a number of oils by converting the epoxide to the chlorhydrin and determining the IR absorption at 2.795 μ before and after the conversion (18). Applying gaseous HCl directly to an oil solution has been proposed as the means of conversion (19).

Direct gas liquid chromatography (GLC) of epoxy oils is not fully satisfactory as a quantitative procedure. Significant amounts of the epoxy glycerides are

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often lost in the column (R. Kleiman, unpublished data). In the peaks that emerge, each epoxy group increases retention time about the same amount as two carbon atoms. The progression of peaks is much like that of an oil containing significant amounts of C_{20} acids and the relative amounts of triglycerides containing epoxy acids cannot be observed. Conversion of the epoxy acids to the 1,3-dioxolane derivative of cyclopentanone increases retention time of the epoxy glycerides enough to make them emerge after tristearin (C_{54}) and reveals the series of peaks related to epoxy glycerides (20).

Gas chromatography of methyl esters, however, is an effective method of analyses (14), though new columns sometimes yield distorted peaks for the epoxy esters until several samples have been injected (21). Preparation of esters either by alkaline transesterification or by saponification and treatment of acids with diazomethane maintains the epoxy group intact. The quicker Metcalfe method of making esters can be applied because it converts almost all the epoxy acid to derivatives, presumably the vic-methoxy, hydroxy isomers, which are eluted together as a single peak from nonpolar columns and can be measured readily (22).

Determination of the position of the epoxy group in the molecule has been facilitated by adaptation of the periodic acid technique (23) to micro samples (24,25). Cleavage of the molecule gives two aldehyde fragments that can be identified and quantitated by GLC. Ozonolysis of another portion of the sample followed by GLC of the products reveals the positions of double bonds (26).

In studies of the geometrical configuration of an epoxy acid, an ion exchange resin has been used for converting the epoxy group to a diol, which is then compared with known *threo*- and *erythro*-9,10-dihydroxystearates by TLC on Silica Gel G plates containing boric acid (27).

Discovery of Epoxy Acids

In 1954, Gunstone described his investigation of a literature claim that oil from *Vernonia anthelmintica* contained a long chain hydroxy acid. His finding that the unusual component was not a hydroxy acid but 12,13-epoxy-*cis*-9-octadecenoic acid (epoxyoleic or vernolic acid) was the first report of a natural epoxy acid in a seed oil (28). In a rather similar way, a literature reference to a probable long chain acid led Chisholm and Hopkins to discover in the seed oil of *Hibiscus esculentus* (okra) an epoxyoleic acid (29). In later work they showed that the epoxy acid present in several members of the family Malvaceae, including *Hibiscus,* was (−)-epoxyoleic, whereas that in *Vernonia* was the (+) form (30). Absolute configurations are 12*S*, 13*R* for the (+)-acid and 12*R*,13*S* for the (−)-acid (4,6).

In 1959, three new epoxy acids were announced. Smith and coworkers (31,32) reported the occurrence of *cis*-9,10-epoxy-*cis*-12-octadecenoic (coronaric) acid in seed oil from one sample of *Chrysanthemum coronarium,* but they noted that only a minor amount of HBE was present in another sample. Gunstone and Morris (33) found a small amount of *cis*-15,16-epoxy-*cis*-9,*cis*-12-octadecadienoic (epoxylinoleic) acid in oil from *Camelina sativa* and said that other epoxy acids were present. Chisholm and Hopkins (34) reported *cis*-9,10-epoxyoctadecanoic (epoxystearic) acid in *Tragopogon porrifolius* oil. The absolute stereochemistry of both coronaric and *cis*-9,10-epoxystearic acid is 9*R*,10*S* (4).

After a lapse of some 11 years, with no new epoxy acids reported, Conacher and Gunstone (35) found that the 14% of epoxy acids in *Helichrysum bracteatum* oil included, along with 10% of coronaric previously reported in the same accession (36), 3% of a new acid, *cis*-9,10-epoxyoctadec-12-ynoic acid and 1% of epoxystearic acid. Soon thereafter, Kleiman et al. (24) identified 9,10-epoxy-*trans*-3,*cis*-12-octadecadienoic acid in oil from one sample of *Stenachaenium macrocephalum.* Coronaric and epoxystearic acid and several hydroxy-conjugated dienolic acids also occur in this oil. Another sample of seed, however, provided oil with no more than 1% total HBE.

Occurrence

Known epoxy oils are listed in Table I. Vernolic acid is the major epoxy acid in all those that contain more than 15% HBE, and certainly occurs in many of those containing smaller amounts. For some of the oils, the number and identity of the epoxy components have not been established; oils in which epoxy acids other than vernolic have been found are indicated by footnotes to the Table.

At this time only four plant families are known to have species that produce seed oils with more than 15% epoxy acids. The family Compositae is represented by 38 such species. The preponderance of *Vernonia* species probably reflects two facts: (a) the discovery species was of this genus, so close relatives were investigated; and (b) special emphasis has been placed on collecting wild *Vernonia* species in Africa. Most of the African species tested are rich in vernolic acid but few, if any, of the American species have significant amounts (44).

A comparable collection effort on the genus *Crepis* would add numerous species to the five listed. This genus produces crepenynic (*cis*-9-octadec-12-ynoic) acid as well as vernolic acid, and the species so far studied fall into three categories with respect to the amounts of these two acids in their seed oils. One group of species is rich in vernolic acid, another rich in crepenynic acid, and a third group is intermediate in composition. The species constituting these groups show agreement with a botanical sub-classification of *Crepis* into sections based on other criteria. Thus the species high in crepenynic acid are all in the same section. *C. occidentalis* and *C. intermedia* are both in another section and both contain intermediate amounts of the two acids under discussion. The three species (*C. aurea, C. biennis* and *C. vesicaria*) with oils highest in vernolic acid belong to three other sections in the genus (40).

In the family Dipsacaceae, only oils from the genus *Cephalaria* have been definitely shown to contain vernolic acid, and the concentration is not high. Three other genera of the family have HBE up to 10%, but the identity of the components has not been established (F. R. Earle, unpublished data). Three species of the family Euphorbiaceae produce rather large amounts of vernolic acid, whereas only one is known among the Valerianaceae. Although few samples of Valerianaceae have been examined, numerous Euphorbiaceae have no more than about 1% HBE.

The epoxy acid found in the next highest amount in seed oils is coronaric acid. It makes up some 14% of the oil from one sample of *Chrysanthemum coronarium* (32) and 10% of one sample from *Helichrysum bracteatum* (35,36). Both species have also produced oil samples with less than 3% total HBE, but the cause of this variability has not been fully explained (see section on 9,10 Epoxy Acids). Coronaric acid

TABLE I

Plant Species Reported to Produce Epoxy Acids in Seed Oils

Species	HBE,[a] %	Reference No.
Compositae		
Arctotis grandis	4	31,37
Artemisia absinthium	15[b]	8,18,31,37
Aster alpinus	2[c]	38
Carlina corymbosa	8	39
Chrysanthemum coronarium	14[c]	31,32,37
Cosmos bipinnatus	3[b]	8,18,37
Crepis aurea	54	14,40
Crepis biennis	68	14,40
Crepis intermedia	35	14,40
Crepis occidentalis	18–30	14,40
Crepis vesicaria	47	14,40
Cynara cardunculus	1	12,18,37
Dimorphotheca sinuata	0.6[b]	8,18
Erlangea tomentosa	52	41
Helianthus annuus	0–10[b]	8,18,37,42
Helichrysum bracteatum	14[b]	35,43,36
Heliopsis pitcheriana	1	12,18,37
Heliopsis scabra zinniaeflora	0.2	18,43
Schlectendalia luzulaeflora	46	(Unpublished)
Stenachaenium macrocephalum	6[b]	24
Stokesia laevis	74	(Unpublished)
Tragopogon porrifolius	3[d]	34
Vernonia abyssinica	58–68	44
Vernonia adoensis	43–66	44
Vernonia afromontana	35–75	44
Vernonia amygdalina	50	45
Vernonia anthelmintica	72	8,14,18,28,37,46
Vernonia cinerea	19	45
Vernonia colorata	14	29,30
Vernonia filipendula	38	44
Vernonia gerberiformis	52	44
Vernonia hymenolepis	46–68	44
Vernonia incompta	48	44
Vernonia lasiopus	70–73	44
Vernonia nigritiana	5	45
Vernonia pauciflora	68	44
Vernonia stenolepis	53	44
Xeranthemum annuum	13[b]	47
Cruciferae		
Camelina sativa	1[e]	33
Cucurbitaceae		
Apodanthera undulata	0.7	18,37
Dipsacaceae		
Cephalaria joppica	32	14
Cephalaria leucantha	28	14
Cephalaria transylvania	22	14
Euphorbiaceae		
Cephalocroton cordofanus	62	18,48
Cephalocroton peuschelii	72	49
Euphorbia lagascae	57–64	14,50,51
Leguminosae		
Calliandra eriophylla	6[b]	8,12,18,37
Cercidium floridum	7	12,37
Malvaceae		
Abutilon theophrasti	0.7	52
Althea rosea	2	52
Gossypium hirsutum	1	52
Hibiscus abelmoschus	4	52
Hibiscus cannabinus	5	18,53
Hibiscus esculentus	3	29,52
Hibiscus moscheutos	3	12,52
Hibiscus syriacus	3	52
Lavatera trimestris	6	52
Malope trifida	9	30,52
Malva moschata	7	52
Sidalcea hybridum	5	52
Thespezia populnea	0.4	52
Olacaceae		
Ongokea gore	1[d]	54
Onagraceae		
Clarkia elegens	10	31,37
Sterculiaceae		
Brachychiton acerifolius	0.2	18
Valerianaceae		
Valerianella radiata	30	14,43
Zygophyllaceae		
Balanites aegyptica	4[b]	8,18,37

[a] Hydrogen bromide equivalent, calculated as vernolic acid. Known or believed to be vernolic acid in all species with 15% or more and in some with smaller amounts. Identity of other minor acids indicated where known.

[b] Mixed acids.

[c] Coronaric acid.

[d] *Cis*-9,10-epoxystearic acid.

[e] 15,16-Epoxylinoleic acid.

also has been reported in oils from *Helianthus annuus* (8,42), *Stenachaenium macrocephalum* (24), *Dimorphotheca sinuata* (8), *Artemisia absinthium* (8), *Calliandra eriophylla* (8), *Balanites aegyptica* (8), *Cosmos bipinnatus* (8), *Xeranthemum annuum* (47) and *Aster alpinus* (38).

Cis-9,10-epoxystearic acid was found in 8 of the 11 oils containing coronaric acid, and in oils from *Ongokea gore* (54) and from *Tragopogon porrifolius*, the first known source (34). The amounts in seed oils have been very small, but in a nonseed source, the lipids of wheat stem rust (*Puccinia grandis*), epoxystearic makes up 27% of the total lipid (55,56). Another nonseed source is lycopodium powder (*L. complanatum*) (57). *Trans*-9,10-epoxystearic acid has been reported only from orujo, or sulfur olive, oil (58).

The other known epoxy acids have been definitely identified only in the oil where they were originally characterized.

The wide distribution of small amounts (up to 15%) of HBE (37,43) suggests that epoxy acids are present in many oils; further work would be required to confirm that epoxy acids are indeed there. Sometimes nonepoxy compounds probably account for the HBE. In the family Malvaceae and its close relatives, for example, cyclopropenoid acids are expected to be the major part of the material reacting with HBr unless the titration is carried out in the cold. In the family Umbelliferae, TLC plates sprayed with picric acid reagent give positive tests on some samples, but no epoxy acids have been found. Presumably the constituents responsible are not in the glyceride oils but in the essential oils, which are typically important components in species of this family. In two families, Compositae and Coriariaceae, large amounts of HBE may indicate acids like dimorphecolic (9-hydroxy-*trans*-10,*trans*-12-octadecadienoic) or coriolic (13-hydroxy-*cis*-9,*trans*-11-octadecadienoic) if the titration is carried out at or above room temperature (12,14). In oils with low amounts of HBE (up to 15%), the co-occurrence of two or more epoxy acids and acids of the coriolic type has been shown in enough species and families (8,18,24,33,35,36,42,47) to demonstrate that such mixtures are not uncommon. Therefore, any characterization studies of acids in low-HBE oils should include precautions to identify, or at least test for, the presence of multiple minor components of the oxygenated acid group.

Crop Prospects

Studies of potential usefulness of natural epoxy oils have been limited to those from *Vernonia anthelmintica* and *Euphorbia lagascae*. Although their oxirane content is low in comparison with commercially epoxidized oils, the natural oils and some derivatives prepared from them have definite usefulness as plasticizers and stabilizers for polyvinyl chloride (59, 60).

Many factors will influence the selection of species for commercial epoxy oil production. Among these (the order of listing does not indicate relative importance) will be percentage of epoxy acid in the oil, oil content of the seed, seed yield per acre, agronomic characteristics of the species, nonepoxy constituents in the oil, physical properties of the oil and composition of the seed meal.

At present, *Vernonia anthelmintica* and *Euphorbia lagascae* seem to be the best prospects. *Cephalocroton* is a shrub and probably could not be grown and harvested effectively. *Valerianella* and *Cephalaria* are too low in epoxy acids. The *Crepis* species tested are not very productive and lose their seeds to the wind (heads much like dandelions). The other varieties of *Vernonia, Erlangea tomentosa* and *Stokesia laevis* have not been under agronomic study long enough for evaluation. Criticism of species here is not intended to suggest that they could not be developed by breeding to become practical commercial crops; *V. anthelmintica* and *E. lagascae* seem better species to try at this time.

Euphorbia lagascae seed has about 45% oil, which contains 57–64% vernolic acid (51). The plant is an annual or winter annual and has shown good growth in a number of locations. Unfortunately, as the seed mature they dehisce vigorously and are lost in a manner reminiscent of the related castor bean. The best recovered yields have been about 850 kg/ha (G. A. White, unpublished data).

Vernonia anthelmintica seed has about 25% oil, which contains about 70% vernolic acid. This species, too, is an annual. A breeding program (61) has been successful in developing a line that matures its seed more nearly all at the same time, holds its seed above the main leafy mass of the plant and to some degree retains its seed for harvest if the weather is good. Further improvement is needed.

Vernonia oil has perhaps one unique advantage in its distribution of epoxy acid within the triglycerides: a much greater proportion exists as trivernolin than in other oils (14,62). Recovery of trivernolin from the oil (14,63) provides a higher concentration of epoxy acids than in any other natural oil so far discovered. However, more than 15% of the vernolic acid remains as mono- and divernoloyl triglycerides. In these, as in oil from *Euphorbia lagascae* (14) and *Erlangea tomentosa* (41), vernolic acid in the monoepoxy glycerides is primarily in the 1 or 3 position. Addition of a second vernoloyl moiety occurs at the 2 position (63). In oils from *Crepis aurea* and *Cephalaria joppica,* the pattern is distinctly different: in monoepoxy glycerides the vernolic acid is primarily at the 2 position (14). Causes for the difference in intraglyceride distribution of vernoloyl moieties between the two groups are unknown.

9,10-Epoxy Acids

Although current information indicates strongly that any epoxy seed oil for industrial use will be rich in vernolic acid, recent work justifies increased interest in the other epoxy acids. All but one of the known minor epoxy acids have the epoxy group at the 9,10-position. Although they have been found in only a few oils, these few constitute a very high proportion of the oils specifically examined for small amounts of epoxy acids. Obviously these acids are widespread. The existence of 15,16-epoxylinoleic acid alerts us to the probability that other acids may be found with epoxy groups in unusual positions.

The amount of HBE occurring in different samples of the same species varies markedly. In *Chrysanthemum coronarium* (31,32), *Helichrysum bracteatum* (35,36,43) and *Stenachaenium macrocephalum* (24), the difference in HBE has been related in part to epoxy acids. Mikolajczak et al. (42) found an increase in HBE from 2.9–4.2% to 7.1–9.6% in oils from sunflower seeds stored at 40 F for about 10 years and identified 9,10-epoxystearic and coronaric acids among the oxygenated acids. Fresh samples of the same lines were obtained and found to have only 1.0–1.2%. Later they caused the increase to occur in a shorter time by holding seed samples at an elevated temperature (64). Such post-harvest synthesis may explain the difference in the *Stenachaenium* samples mentioned, because the richer sample was held for two years before analysis.

The prevalence of *cis*-9,10-epoxy groups in these acids that have been formed after harvest of the seed suggests specific enzyme action, but no studies have as yet been made in this phase of epoxy acid formation. Studies of the reactions of epoxy acids and their interrelationships with other acids in seed oils (e.g., 35) may lead to an explanation of chemical steps involved and may be useful in work on enzyme mechanisms. Possibly, information obtained on the formation of 9,10-epoxystearic acid by wheat stem rust (65) may be of value also in the study of seeds.

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