Fatty Acids: XXII[1] Partial Synthesis of Racemic Helenynolic Acid From Crepenynic Acid by a Possible Biosynthetic Route and the Discovery of *cis*-9,10-Epoxyoctadec-12-ynoic Acid in *Helichrysum bracteatum* Seed Oil[2]

H. B. S. CONACHER[3] and F. D. GUNSTONE, Chemistry Department, Purdie Building, The University of St. Andrews, St. Andrews, Scotland

ABSTRACT

Racemic helenynolic acid has been prepared from crepenynic acid by epoxidation followed by base-catalyzed rearrangement. This may be the pathway by which helenynolic acid is produced in *Helichrysum bracteatum* seed oil from the crepenynic acid also present. A reinvestigation of the epoxy acids in this oil has shown that *cis*-9,10-epoxyoctadec-12-ynoic acid accompanies the coronaric acid previously identified.

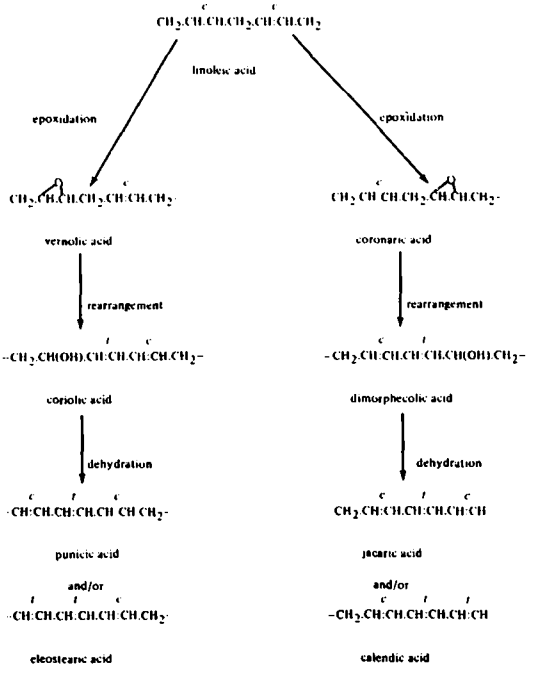
INTRODUCTION

The many long chain acids which have been discovered in recent years include a number of conjugated 9,11,13 and 8,10,12 octadecatrienoates, some hydroxy conjugated octadecadienoates, and several epoxy acids. It has been proposed recently that these are biogenetically related and that the conjugated trienoic acids are produced from the very common linoleic acid via epoxy monoenoic and hydroxy dienoic acids as in Scheme 1 (1,2).

There is no proof of this hypothesis but the smooth conversion of vernolic acid to coriolic acid has been demonstrated in vitro. This change occurs in high yield under mild conditions (0 C, 1 hr) under the influence of lithium diethylamide (2). Attention has been drawn to the correlation of absolute configuration which should exist between these natural acids and it has been suggested that other stereoisomers await discovery (2).

Helichrysum bracteatum seed oil is known to contain several unusual acids including (in addition to palmitic, stearic, oleic and linoleic) coronaric acid, hydroxy dienoic acids (including possibly α-dimorphecolic), crepenynic acid, and helenynolic acid, this last being a new hydroxy enynoic acid (3-5). (Following the designation of natural eleostearic acid (9*c*11*t*13*t*) as the α isomer and the all *trans* acid as the β-isomer, we use (14,15) the symbols α and β with coriolic and dimorphecolic acids to indicate the *cis,trans* and *trans,trans* isomers respectively). If α-dimorphecolic acid arises from linoleic acid via coronaric acid then it seems likely that helenynolic acid might be formed from crepenynic acid via an undiscovered epoxy acetylenic acid.

We have now shown that crepenynic acid readily furnishes racemic helenynolic acid by epoxidation and rearrangement and, encouraged by this, we have re-examined the epoxy acids of *Helichrysum bracteatum* seed oil and shown them to contain the hitherto unknown *cis*-9,10-epoxyoctadec-12-ynoic acid.



SCHEME 1

[1]Part XXI, Chem. Phys. Lipids 3:203 (1969).

[2]Presented at the AOCS Meeting, New York, October 1968.

[3]Present address: Food and Drug Directorate, Dept. of National Health and Welfare, Ottawa, Canada.

EXPERIMENTAL PROCEDURES

All solvents were dried and distilled before use. Petroleum refers to the fraction of bp 40-60 C.

TLC was carried out with thin layers of silica (0.3 mm for analytical purposes and 1.0 mm for preparative purposes). When required, silver nitrate (15%) was incorporated into the silica layers. The developing solvent was ether mixed with benzene or petroleum and these mixtures are indicated by symbols such as PE30 indicating a 70:30 mixture of petroleum and ether.

A Pye 104 was used for GLC. It was fitted with a column (5 ft x 1/4 in.) packed with Gas Chrom Z (70-80 mesh) coated with diethylene glycol succinate (DEGS 20%) or Apiezon L (ApL 5%) and normally operated at 190 C or 210 C, respectively. In the following account symbols such as DEGS, 20.0 indicate a carbon number (equivalent chain length) of 20.0 on a DEGS column.

Infrared spectra were run on Perkin Elmer spectrophotometers (137,237, or 261) using thin films in sodium chloride discs or 1% solutions (carbon disulphide) in 1 mm path-length cells. Ultraviolet spectra were recorded in methanol solution with a Unicam SP/800 spectrophotometer. A Perkin Elmer R10 spectrometer (60 Mc/sec) was used to record NMR spectra on 15% solutions in carbon tetrachloride.

Methylation was carried out with boron trifluoride-methanol (6) and von Rudloff oxidation as recommended by Tulloch and Craig (7).

Conversion of Methyl Crepenynate to Racemic Methyl Helenynolate

Afzelia cuanzensis seed oil (2.36 g) was refluxed for 15 min with methanolic sodium methoxide (25 ml, 0.1 N) and the methyl esters (2.28 g) recovered by petroleum extraction of the acidified reaction mixture. The esters contained about 40% of methyl crepenynate (DEGS, 21.6) and this was isolated by preparative silver ion TLC (PE25). GLC showed it to be pure, apart from methyl linoleate (3%). Methyl crepenynate (285 mg, 1 mmole) reacted overnight with an ethereal solution of monoperphthalic acid (5 ml, 2.2 mmole) and the monoepoxide (202 mg; DEGS, 26.0) was readily separated from unreacted crepenynate (24 mg) and diepoxide (10 mg) by preparative TLC (PE30).

This epoxy ester (240 mg) was also prepared (more conveniently) directly from *Afzelia* esters (1.0 g) by reaction with a 10-fold excess of monoperphthalic acid. Preparative TLC (PE30) gave a mixture of epoxystearate and epoxyoctadecynoate which was then separated by preparative silver ion TLC.

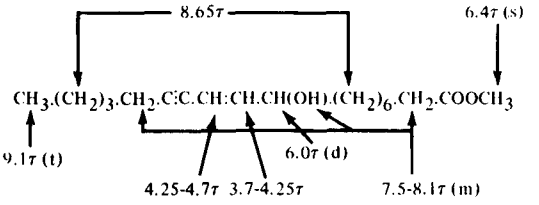
In a nitrogen atmosphere, diethylamine (0.4 ml) in anhydrous ether (10 ml) was slowly added, dropwise, to an ice cold solution of phenyl-lithium (8) (5 ml, 4 mmole) in anhydrous ether (10 ml). After 10 min, epoxy ester (250 mg) in anhydrous ether (10 ml) was added, and after stirring for 1 hr at 0 C, the reaction product (270 mg) was recovered by ether extraction. This was separated by preparative TLC (BE25) into four main fractions: epoxy ester (20 mg, 9%), hydroxy ester (120 mg, 53%) and (presumably) epoxy amide (28 mg, 12%) and hydroxy amide (60 mg, 26%).

Proof of Structure

The major product of the rearrangement reaction was shown to be methyl 9-hydroxyoctadec-*trans*-10-en-12-ynoate on the following evidence:

(a) Compared with methyl 9-hydroxyoctadeca-*trans*-10, *cis*-12-dienoate the rearrangement product had a slightly lower R_f value on silica (PE40) and a slightly higher value on silica impregnated with silver nitrate (BE25). After conversion to its trimethysilyl ether with hexamethyldisilazane and trimethylchlorosilane it gave a single peak on GLC (DEGS, 23.4).

(b) The ester had strong absorption bands at 3595, 1730 and 950 cm^{-1} in its IR spectrum. Its UV spectrum showed an absorption peak at 228 mμ ($E^{1\%}_{1cm}$ 600) and an inflexion at 238 mμ ($E^{1\%}_{1cm}$ 510). The NMR spectrum is summarized:



(c) The rearrangement product (20 mg), hydrogenated in methanol solution (5 ml) with palladium-charcoal (10%, 20 mg), gave a mixture of methyl hydroxy- and oxostearates (DEGS, 25.9 and 24.9 respectively). Dissolved in acetic acid (2 ml), this mixture (15 mg) was stirred at room temperature for 2 hr with a solution of chromium trioxide (120 mg) in acetic acid (2 ml). Thereafter the mixture was diluted with water (25 ml), treated with sulfur dioxide to destroy excess of oxidant, and extracted with petroleum (2 x 10 ml). After esterification, GLC showed the presence of methyl octanedioate and nonanedioate.

(d) von Rudloff oxidation gave hexanoic and nonanedioic acids.

(e) The hydroxy ester (43 mg), dissolved in ether (3 ml), was added to a suspension of lithium aluminium hydride (200 mg) in anhydrous ether (2 ml), and the mixture refluxed for 2 hr. The recovered product (37 mg) was purified by preparative TLC (PE60) and the major component (33 mg) was shown to be an allene by its IR absorption at 1950 cm^{-1}.

(f) Refluxed with methanolic hydrogen chloride (7 ml, 0.1 N) and isolated by preparative TLC (PE30), the hydroxy ester (24 mg) furnished an ether (IR absorption at 1080 and 1100 cm^{-1}) as major product (18 mg). GLC (DEGS, 25.2) indicated that some minor components were also present.

(g) The hydroxy ester (40 mg) was stirred with potassium azodicarboxylate (1.2 g) in anhydrous methanol (3 ml) and a mixture of methanol-acetic acid-water (1:1:1) was slowly added, dropwise, until the yellow color disappeared. The product (34 mg) was divided into three fractions by preparative silver ion TLC (BE15): H1; 17 mg; 63%; DEGS, (TMSi derivative) 23.4; IR absorption at 3595 and 950 cm^{-1} unreacted hydroxy enyne: H2; 3 mg; 11%; DEGS, (TMSi derivative) 20.8; IR absorption at 3595, 980 and 945 cm^{-1}; hydroxy *cis,trans* diene: H3; 7 mg; 26%; DEGS, (TMSi derivative) 21.6; IR absorption at 3595 cm^{-1}, hydroxy yne. Von Rudloff oxidation of H1 and of H2 gave hexanoic and nonanedioic acids; oxidation of H3 gave hexanoic acid and (presumably) the lactone of 4-hydroxydodecanedioic acid (IR absorption at 1770 and 1730 cm^{-1}; DEGS, 29.0; ApL, 17.2). Oxidation of methyl 9-hydroxyoctadec-12-enoate gave products with the same chromatographic and spectroscopic properties.

Isolation of an Acetylenic Epoxy Acid From Helichrysum bracteatum Seed Oil

Helichrysum bracteatum seeds (9.8 g) extracted with petroleum gave a yellow oil (2.02 g) which was shaken at room temperature overnight with anhydrous methanolic sodium methoxide (25 ml, 0.1%). The methyl esters (1.67 g) were recovered and separated into four fractions by preparative TLC (PE30): A, 1.01 g, 68%; B, 0.21 g, 14%; C, 0.19 g, 13%; D, 0.08 g, 5%. Fraction B, with the same R_f value as methyl 12,13-epoxyoleate showed only three peaks in its GLC: X; 6%; DEGS, 24.0: Y; 69%; DEGS, 24.6: Z; 25%; DEGS, 26.0. Attempts to separate these by silver ion TLC were unsuccessful. Fraction B (200 mg) was therefore treated overnight at room temperature with excess of monoperphthalic acid in ether (5 ml, 2.2 mole). The product was separated by preparative TLC (PE30) into monoepoxide (56 mg) and diepoxide (121 mg). The monoepoxide contained only two components [DEGS, 24.0 (20%) and 26.0 (80%)] and these were separated by preparative silver ion chromatography (PE30) into an upper (11 mg) and lower (42 mg) band.

Proof of Structure

Compound Z was shown to be identical with the methyl *cis*-9,10-epoxyoctadec-12-ynoate previously obtained by epoxidation of methyl crepenynate.

(a) The natural and synthetic esters were identical in their behavior on TLC (PE30), GLC (DEGS, 26.0; ApL, 19.1) and in their infrared and NMR spectra (no signal for olefinic proton, broad multiplet centered on 7.27τ for epoxy ring protons).

(b) The natural ester (20 mg) was subjected to acetolysis (9) by reaction first with acetic acid (2 ml) and then with aqueous methanolic (1:4) sodium hydroxide (5 ml, 8%). After recovery (18 mg), part of the product (10 mg) was hydrogenated with palladium-charcoal. Von Rudloff oxidation furnished hexanoic and nonanedioic acids from the nonhydrogenated ester and nonanoic and nonanedioic acids from the reduced product.

The acetylenic dihydroxy ester, the saturated dihydroxy ester, and methyl *threo*-9,10-dihydroxystearate showed identical behavior on a TLC plate impregnated with boric acid (5%, PE50) (10).

(c) Base-catalyzed isomerization (lithium diethylamide) of the natural ester (17 mg) gave a product (27 mg) from which hydroxy ester (7 mg) was isolated by preparative TLC (PE45). This had an ultraviolet spectrum (λ max 228 mμ, $E^{1\%}_{1cm}$ 500; 238 mμ $E^{1\%}_{1cm}$ 430) and an IR spectrum (absorption at 3595 and 950 cm^{-1}) identical with those obtained from the product of a similar reaction on the synthetic epoxy ester.

Component X was shown to be methyl *cis*-9,10-epoxystearate. After acetolysis and hydrolysis it gave methyl dihydroxystearate which was oxidized to nonanoic and nonanedioic acids and which behaved in the same way as authentic methyl *threo*-9,10-dihydroxystearate on thin layer plates treated with boric acid.

Examination of Dimorphotheca pluvialis ringens and D. aurantiaca Seed Oils

Seeds were extracted with petroleum and the oils converted to methyl esters by overnight reaction with cold dilute sodium methoxide. Epoxy esters were then isolated by TLC (PE30)

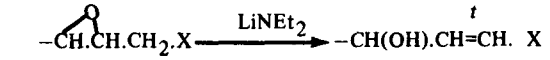
and examined further by GLC, silver ion TLC, and IR spectroscopy.

The esters from *D. pluvialis ringens* seed oil gave an epoxide fraction of only 1%. This was epoxystearate (10%) and epoxyoctadecenoate (90%) but the latter showed no significant absorption in the 900-1000 cm^{-1} region of its IR spectrum.

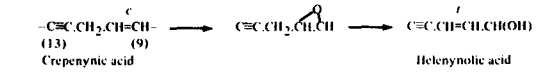
D. aurantiaca seed oils also gave about 1% of epoxy esters comprising saturated (15%) and unsaturated (85%) components. The latter showed no evidence of *trans* saturation in its IR spectrum.

DISCUSSION

We have already shown (2) that suitably constructed epoxides (1) rearrange, under the influence of lithium diethylamide, to the *trans* enol (2). Under the conditions examined this occurs only when the CH_2 group α to the epoxide is activated by some adjacent group X. Reaction proceeds smoothly when X is a double bond and not at all when X is a



saturated polymethylene chain. It was of interest to know whether this reaction would occur when X is a triple bond since this would make possible a partial synthesis of racemic helenynolic acid from crepenynic acid and provide (perhaps) a chemical analogy for a possible biosynthetic pathway.



Using *Afzelia cuanzensis* (11) as a source of crepenynic acid the desired epoxy acid was prepared by epoxidation with monoperphthalic acid. Rearrangement occurred at 0 C in 1 hr and a hydroxy ester (53%) was isolated by TLC. As with the olefinic compounds already examined the yield of ester is reduced by a competitive reaction leading to hydroxy *N,N*-diethylamide (20%). The IR and UV spectra of the hydroxy ester showed the presence of a conjugated *trans*-enyne system and a hydroxyl group. The NMR spectrum was the same as that reported for natural helenynolic ester (4). The hydroxyl group was attached to C_9 since oxidation of the perhydro hydroxy ester gave octanedioic and nonanedioic esters. Since von Rudloff oxidation gave hexanoic and nonanedioic acids the hydroxy enyne system must lie between C_9 and C_{13}. The synthetic ester was reduced by lithium aluminium hydride to an allene and etherified, rather than dehydrated, with methanolic hydrogen chloride as described by Powell et al. (4) for the natural acid. Unsaturation was shown to be 10-en-12-yne by partial reduction with di-imide (generated from azo dicarboxylic acid) which furnished, among other products, a hydroxy ynoic acid oxidized to hexanoic acid and (probably) the lactone of 4-hydroxy-dodecanedioic acid. An authentic sample of methyl 9-hydroxyoctadec-12-enoic acid gave the same oxidation products.

If helenynolic acid is produced naturally from crepenynic acid by an enzyme-catalyzed rearrangement of the epoxide it seemed possible that the epoxide of crepenynic acid might occur in *Helichrysum bracteatum* seed oil. The epoxy esters in this oil were readily isolated by TLC, and GLC showed the presence of three components which, from their carbon numbers, could be saturated, olefinic, and acetylenic epoxy esters. Attempts to separate these by silver ion chromatography were not successful but this difficulty was overcome by treating the natural epoxy esters with excess of monoperphthalic acid. The olefinic epoxide was converted to a diepoxide but the saturated and acetylenic epoxides were unchanged. After separation from the diepoxide the two monoepoxides could be separated by silver ion TLC.

The epoxyoctadecenoate (about 10% of total esters) was not examined further and is presumably the methyl coronarate already reported. The saturated epoxide (1%) was shown to be methyl *cis*-9,10-epoxystearate and the acetylenic epoxide (3%) was identified as methyl *cis*-9,10-epoxyoctadec-12-ynoate and was thus identical with epoxidized crepenynic acid.

The natural and synthetic acids were identical in their chromatographic (TLC and GLC) and spectrosopic (IR, NMR) behavior. The natural epoxy ester, by acetolysis, hydrolysis and re-esterification, gave an acetylenic dihydroxy ester, some of which was hydrogenated to a saturated dihydroxy ester. Oxidation of the unsaturated and saturated dihydroxy esters gave hexanoic and nonanedioic acids and nonanoic and nonanedioic acids respectively showing the position of the epoxide [C_9-C_{10}] and of the unsaturated center (Δ^{12}). Since the saturated and unsaturated dihydroxy esters run with methyl *threo*-9,10-dihydroxystearate on boric acid impregnated TLC plates (10) all three must be *threo* diols and the epoxide from which two of them were derived must be *cis*. Finally, base-

catalyzed rearrangement gives a product identical with that previously obtained from synthetic epoxidized crepenynic ester. This product is presumably the same as natural helenynolic ester.

Since methyl helenynolate has the 9D configuration we expect the new acetylenic epoxy acid to have the 9D, 10D configuration and, by analogy, we predict that coronaric acid and the hydroxy dienoic acid also present in this seed oil may be 9D, 10D-epoxyoctadec-*cis*-12-enoic acid and 9D-hydroxyoctadeca-*trans*-10, *cis*-12-dienoic acid (α-dimorphecolic). This coronaric acid would then be the stereoisomer of the 9L, 10L isomer recognized in *Xeranthemum annuum* seed oil (12).

β-Dimorphecolic acid (9-hydroxyoctadeca-*trans*-10, *trans*-12-dienoic acid) can be fitted into our biogenetic proposals (1,2) either by postulating a *cis,trans* stereomutation at some stage or by starting with the 9*c*,12*t* or 9*t*,12*t* stereoisomer of linoleic acid. Morris and Marshall (13) have recently shown that *Dimorphotheca aurantiaca* seed oil contains both β-dimorphecolic acid and the 9*c*,12*t* isomer of linoleic acid. Encouraged by our success with *Helichrysum bracteatum* seed oil we examined the seed oils of *D. aurantiaca* and *D. pluvialis ringens* to see if these contained any epoxy *trans* monoenoic acids. Both contained about 1% of epoxy esters which were mainly olefinic but we could find no infrared evidence of *trans* unsaturation.

ACKNOWLEDGMENT

The *Helichrysum* seeds used in this investigation were supplied by I. A. Wolff (Peoria). A research studentship was awarded to H. B. S. Conacher by the Science Research Council.

REFERENCES

1. Gunstone, F. D., Chem. Ind. (London) *1966*:1551.
2. Conacher, H. B. S., and F. D. Gunstone, Chem. Phys. Lipids 3:191 (1969).
3. Powell, R. G., C. R. Smith, Jr. and I. A. Wolff, JAOCS 42:165 (1965).
4. Powell, R. G., C. R. Smith, Jr., C. A. Glass and I. A. Wolff, J. Org. Chem. 30:610 (1965).
5. Cymerman Craig, J., S. K. Roy, R. G. Powell and C. R. Smith, Jr., Ibid. 30:4342 (1965).
6. Metcalfe, L. D., and A. A. Schmitz, Anal. Chem. 33:363 (1961).
7. Tulloch, A. P., and B. M. Craig, JAOCS 41:322 (1964).
8. Cope, A. C., and B. D. Tiffany, J. Am. Chem. Soc. 73:4158 (1951).
9. Gunstone, F. D., J. Chem. Soc. 1954:1611.
10. Morris, L. J., Chem. Ind. (London) 1962:1238.
11. Gunstone, F. D., D. Kilcast, R. G. Powell and G. M. Taylor, Chem. Commun. 1967:295.
12. Powell, R. G., C. R. Smith, Jr. and I. A. Wolff, Lipids 2:172 (1967).
13. Morris, L. J., and M. O. Marshall, Chem. Ind. (London) 1966:1493.
14. Morris, L. J., R. T. Holman and K. Fontell, JAOCS 37:323 (1960).
15. Tallent, W. H., J. Harris, I. A. Wolff and R. E. Lundin, Tetrahedron Letters 1966:4329.

[Received April 10, 1969]