429. *Fatty Acids. Part VI.* The Oxygenated Acid present in* Camelina sativa *(Crantz.) Seed Oil.*

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Evidence is presented for the occurrence of 15 : 16-epoxylinoleic acid in *C. sativa* seed oil.

CAMELINE oil, also known as dodder oil, German sesame oil, Leindotteröl, and Rullöl, is obtained from the seeds of *Camelina sativa* (Crantz.), syn. *Myagrum sativum* (Crantz.). This plant is widely dispersed through Europe and small amounts of the seed oil enter commerce. Recent chemical investigations of the oil have been reported by Swedish [1] and German workers [2,3] who in general agree about its composition. The saturated acids are accompanied by monoethenoid, linoleic, and linolenic acid, and as with other oils of the Cruciferae the monoethenoid acids include those of the C_{16}, C_{18}, C_{20}, and C_{22} series. von Mikusch also draws attention to the presence of an oxygenated acid which we have now further examined.

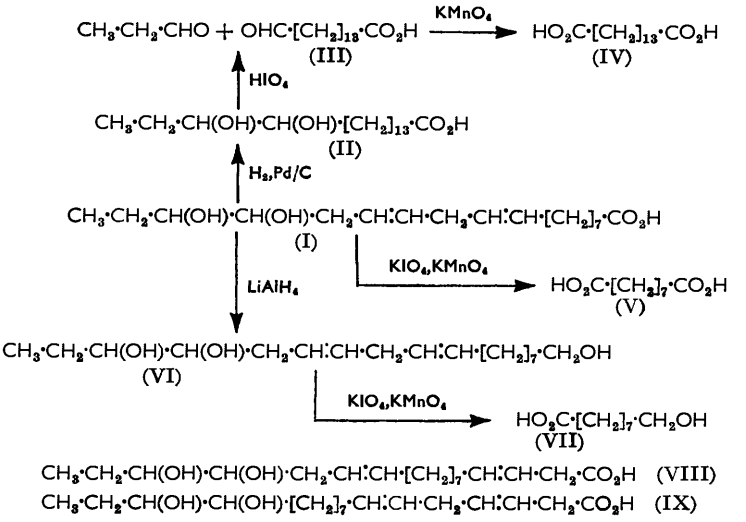
The oil was treated with acetic acid to hydrolyse any epoxide and, from the acids resulting on hydrolysis, concentrates of a dihydroxy-acid were isolated by urea-complex formation, partition, and chromatography. There was evidence of other hydroxy-acids but attention was directed mainly to the dihydroxy-acid obtained as a 55% and as a 75% concentrate, designated concentrate A and B respectively.

Concentrate A in the presence of palladium–charcoal took up 2 mols. of hydrogen and furnished a dihydroxystearic acid (II) (42% yield) different from the known 9 : 10- and 12 : 13-*threo*-dihydroxystearic acids of similar melting point. Concentrate B gave the same product (58%). Periodate oxidation of the hydrogenation product occurred smoothly and gave propionaldehyde and 14-formyltetradecanoic acid (III). The latter was recognised, after oxidation, as pentadecanedioic acid (IV) by comparison with an authentic sample prepared by Arndt–Eistert bishomologation [4] of tridecanedioic acid, itself obtained by oxidation of erucic acid. The hydrogenation product must therefore be the hitherto unknown (+)-15 : 16-dihydroxystearic acid (II) and in view of its melting point is probably a *threo*-isomer [cf. the values given by Huber [5] for the (±)-7 : 8- to -12 : 13-di-

* Part V, *J.*, 1957, 487.

[1] Holmberg and Sellmann, *Svensk Kem. Tidskr.*, 1952, **64**, 270; *Chem. Abs.*, 1953, **47**, 2514.
[2] von Mikusch, *Farbe und Lack*, 1952, **58**, 402; *Deutsche Farben-Zeitschrift*, 1952, 391.
[3] von Mikusch and Dylla, *J. Amer. Oil Chemists' Soc.*, 1954, **31**, 114.
[4] Cf. Walker, *J.*, 1940, 1304.
[5] Huber, *J. Amer. Chem. Soc.*, 1951, **73**, 2730.

hydroxystearic acids). The relation between the melting point of this acid and of its methyl and ethyl esters (96°, 90°, and 74° respectively) differs somewhat from those in the corresponding (±)-9 : 10- (95°, 71°, and 60°) and (—)-12 : 13-series (95°, 68°, and 62°).



These experiments suggest that the acid probably contains two double bonds. The low absorption at 234 mμ before isomerisation by alkali and the greater value after isomerisation are indicative of the pentadiene system $-CH{:}CH{\cdot}CH_2{\cdot}CH{:}CH-$ familiar among natural unsaturated acids.[6] The extinction ($E^{1\%}_{1\,cm.}$) estimated to be 590 for the pure acid by extrapolation of values reported on p. 2130, is slightly lower than might have been expected from known octadecadienoic acids even after allowance for the increased molecular weight. Absence of absorption in the 10 μ region, associated with *trans*-olefinic groups, suggests that both unsaturated centres must have the *cis*-configuration.

The position of these unsaturated centres has been determined by oxidative degradation by the permanganate–periodate procedure of von Rudloff.[7] The unsaturated acid itself gave azelaic acid (V) which accords with structure (I) but would also result from structures (VIII) and (IX). The last two are less likely in view of the ultraviolet spectrum after isomerisation by alkali and would be novel for unsaturated acids of lipid origin.

A decision between these possibilities is made by labelling the carboxyl group and then oxidising in such a way as to retain the label in one of the oxidation products. This is generally done by esterification, or more satisfactorily, by extending the chain-length of the molecule by the Arndt–Eistert reaction.[8] However, in this investigation, the unsaturated acid (I) was reduced by lithium aluminium hydride to the unsaturated alcohol (VI), and this was oxidised by von Rudloff's method to 9-hydroxynonanoic acid, thereby confirming structure (I). The reaction was tested with oleyl alcohol which also provided a reference sample of the hydroxynonanoic acid.

These experiments show that concentrates of 15 : 16-dihydroxylinoleic acid are obtainable from cameline oil but do not indicate whether the compound is present as a glyceride in the original oil or as an epoxy-glyceride, or whether it is an artefact produced by oxidation, presumably of linolenic acid. It is considered to occur as an epoxy-glyceride for the

[6] Pitt and Morton, " Progress in the Chemistry of Fats and Other Lipids," Pergamon Press, London, 1957, Vol. IV, p. 228.

[7] von Rudloff, *J. Amer. Oil Chemists' Soc.*, 1956, **33**, 126; *Canad. J. Chem.*, 1955, **33**, 1714; 1956, **34**, 1413; Lemieux and von Rudloff, *ibid.*, 1955, **33**, 1701, 1710; Jones and Stolp, *J. Amer. Oil Chemists' Soc.*, 1958, **35**, 71.

[8] Whitcutt and Sutton, *Biochem. J.*, 1956, **63**, 469.

following reasons. (i) The 15 : 16-dihydroxystearic acid (II) shows a small but real optical activity whereas products resulting from atmospheric oxidation would be optically inactive. The dihydroxylinoleic acid (I) is also optically active but since this is not pure it cannot be used as a basis for argument. (ii) Gold [9] has recently concluded that autoxidation of *cis*-olefins affords *cis*- and *trans*-epoxides and thence *threo*- and *erythro*-glycols, whilst *trans*-olefins give only *trans*-epoxides and thence *erythro*-glycols. The higher-melting and less soluble *erythro*-glycols are more easily isolated and since only a *threo*-glycol was obtained in the present work it is unlikely to have resulted by autoxidation. (iii) Evidence in favour of the presence of an epoxide in the original oil is based on the infrared spectra of the neutral oil and of the mixed esters derived therefrom by acid-hydrolysis (which would open any epoxide ring). The spectra of cameline oil and its ester were compared with those of olive oil and its ester (as a control containing no epoxide), and with those of *Vernonia anthelmintica* seed oil and its ester (as a control containing 67% of epoxyoleic glycerides [10]). The region 2·6—3·3 μ was scanned since hydroxyl groups show an absorption band at 2·8 μ attributed to the O–H stretching vibration.[11] Olive oil showed slight absorption between 2·8 and 2·9 μ, possibly owing to autoxidation as the sample was not very fresh; the derived methyl esters showed an almost identical curve. With vernonia oil, on the other hand, there was very little absorption in this region, but very strong absorption with the methyl esters owing to the liberated glycol group. Cameline oil showed slight absorption in the 2·8—2·9 μ region but a very much larger absorption after conversion into the esters. This is taken as evidence that the diol group is formed at this stage, presumably from epoxides. Incidentally this provides a more sensitive method of detecting small amounts of epoxides than the quantitative methods involving reaction with excess of halogen acid.

This is now the third epoxy-acid to be reported in seed fats: 12 : 13-epoxyoleic acid (*Vernonia anthelmintica*,[10] *V. colorata*,[12] *Cephalocroton cordofanus*,[13] and *Hibiscus esculentus* [12]), 12 : 13-epoxylinoleic acid (*Camelina sativa*), and 9 : 10-epoxyoctadec-12-enoic acid (*Chrysanthemum coronarium* [14]). Four different plant families are included in this list and it seems that epoxy-acids are more widely distributed than has been recognised hitherto. Epoxyoleic and epoxylinoleic acids form a series and it is interesting to speculate on the possible natural occurrence of 9 : 10-epoxystearic acid which would complete the series. This would give rise to 9 : 10-dihydroxystearic acid and it is known that such an acid is present in castor oil. There is, however, no evidence that this is formed from an epoxide precursor [15] and, in addition, this natural dihydroxy-acid is of the *erythro*-series which would be derived, if at all, from the *trans*-epoxide whereas the natural epoxy-acids are *cis*-isomers, forming the *threo*-glycols.

Experimental

Fuller experimental details of some procedures are given in earlier papers by one of us.[10,13,16] Glycol values [16] are expressed as percentage of dihydroxylinoleic acid, except where otherwise stated.

Preparation of a Concentrate of the Unsaturated Dihydroxy-acid.—Cameline oil (32·5%) was obtained by extracting the crushed seeds with light petroleum (b. p. 40—60°). The oil (500 g.) was refluxed with acetic acid (2 l.) for 8 hr. (to hydrolyse any epoxides) and after removal of the acetic acid the mixed fatty acids were liberated by alkaline hydrolysis in the usual manner.

The mixed acids (471 g.), acetylated by treatment with boiling acetic anhydride, were dissolved in methanol (500 ml.) and added during half an hour to a hot mixture of urea (1·4 kg.)

[9] Gold, *J.*, 1958, 934.
[10] Gunstone, *J.*, 1954, 1611.
[11] Ahlers and McTaggart, *Analyst*, 1954, **79**, 70.
[12] Chisholm and Hopkins, *Canad. J. Chem.*, 1957, **35**, 358.
[13] Bharucha and Gunstone, *J. Sci. Food Agric.*, 1956, **7**, 606.
[14] Smith, Koch, and Wolff, *Chem. and Ind.*, 1959, 259.
[15] Gunstone and Sykes, unpublished observation.
[16] Bharucha and Gunstone, *J. Sci. Food Agric.*, 1955, **6**, 373.

and methanol (1·8 l.). The mixture was kept at room temperature overnight, then filtered, and the filter-cake washed with cold methanol–urea solution. The acids (95·5 g.) which did not form a complex were removed from the filtrate by ether-extraction after dilution with water. (In a similar experiment, where the acetylated methyl esters were used, this fraction was shown to contain practically all the acetylated material.)

Hydroxy-compounds were further concentrated by partition between 80% methanol and light petroleum,[16] and the material in the methanol extract was hydrolysed and freed from unsaponifiable material, leaving the concentrate A (10·69 g., 2·3% of the mixed acids; glycol value 55%) (the petroleum extract contained 17·6% of the total acids by wt.).

After repetition of the partition, samples of the aqueous methanol extract were further purified by chromatography. In a typical experiment the concentrate (4·17 g.), dissolved in a little benzene, was applied to the top of a column of silica gel (300 g.; 65 × 2·5 cm., activated at 200°/5 mm. for 1 hr.), and fractions were removed with benzene (i), 1 : 3 ether–benzene (ii), 3 : 1 ether–benzene (iii), and 1 : 9 methanol–ether (iv). Fraction (iii), rechromatographed (100 g.; 25 × 2·5 cm.), gave fractions with 1 : 3 ether–benzene (iii*a*), 3 : 1 ether–benzene (iii*b*), 1 : 9 methanol–ether (iii*c*), and 3 : 7 methanol–ether (iii*d*). Fractions iii*b* and *c* formed concentrate B (0·34% of the mixed acids). Further description of these fractions is summarised:

	Concentrate A	i	ii	iii	iv	iii*a*	iii*b*	iii*c*	iii*d*
Wt. (g.)	—	0·74	0·84	1·85	0·72	0·99	0·35	0·35	0·63
Glycol value (%)	55	0	24	64	94	48	74	76	—
$E^{1\%}_{1\,cm.}$ before isomern....	139	—	94	—	35	93	62	53	—
$E^{1\%}_{1\,cm.}$ after isomern. ...	473	—	247	—	121	368	489	482	—

Fraction iii*b* had $[\alpha]_D^{20}$ + 6·15° (2·6% ethanolic solution, 1 dm. tube). Despite the high glycol value of fraction iv this material differed from the other fractions in its behaviour on alkali-isomerisation and on hydrogenation and was not further investigated.

Preparation and Characterisation of (+)-15 : 16-*Dihydroxystearic Acid.*—Concentrate A (2·55 g.), at room temperature in the presence of 5% palladium–charcoal, took up 2 mols. of hydrogen. The product (2·43 g.), after a number of crystallisations from ethyl acetate and extractions with light petroleum (b. p. 60—80°), gave (+)-15 : 16-*dihydroxystearic acid* (1·02 g., 42%), m. p. 96—97°, $[\alpha]_D^{20}$ +3·3° (in 4% ethanolic solution; 2 dm. tube) (Found: C, 68·1; H, 11·6%; equiv., 320, 319, 312. $C_{18}H_{36}O_4$ requires C, 68·3; H, 11·5%; equiv., 316). The acid was converted by the Fischer–Speier procedure into the *methyl*, m. p. 89·5—90·5° (Found: C, 69·0; H, 11·5. $C_{19}H_{38}O_4$ requires C, 69·1; H, 11·6%), and *ethyl ester*, m. p. 74—74·5° (Found: C, 69·5; H, 11·5. $C_{20}H_{40}O_4$ requires C, 69·7; H, 11·7%).

A portion of concentrate B (130 mg.), similarly hydrogenated, gave the same product (75 mg.), m. p. 92—95° (58%; 77% based on the glycol value).

The glycol value (97% calc. as dihydroxystearic acid) of this acid (*ca.* 80 mg.) was determined and the oxidation fragments were recovered. The solution was diluted with water, and the white precipitate was treated in aqueous acetone with excess of permanganate solution. The resulting mixture was decolorised by sulphur dioxide and diluted, a solid separating (17 mg.), m. p. 96—109°. This m. p., raised to 107—111° by crystallisation from ethyl acetate and from nitromethane, was depressed (92—102°) with a crude but authentic sample of tridecanedioic acid (m. p. 105—114°) but remained unchanged (107—112°) with pentadecanedioic acid (m. p. 111—113°).

The solution remaining after removal of the above aldehydo-acid was steam-distilled (50 ml.) into 2 : 4-dinitrophenylhydrazine reagent. Next day this mixture which contained some orange precipitate was thoroughly extracted with chloroform, and the chloroform solution concentrated to 25 ml. and added to a 4 : 1 bentonite–kieselguhr column [17] (7·5 g.) which was then eluted with chloroform. An orange oil (15 mg.) was followed by a solid (45 mg.; m. p. 125—139°) which after several crystallisations from ethanol melted at 154—155° and was unchanged when mixed with a sample of propanal dinitrophenylhydrazone. The identity of these two was further proved by paper chromatography with two solvent systems.[18,19]

[17] Elvidge and Whalley, *Chem. and Ind.*, 1955, 589.
[18] Rice, Keller, and Kirchner, *Analyt. Chem.*, 1951, **23**, 194.
[19] Buyske, Owen, Wilder, and Hobbs, *ibid.*, 1956, **28**, 910.

Characterisation of (+)-*Dihydroxylinoleic Acid.*—The stock oxidising agent was a mixture of potassium periodate (22·43 g., 0·0975 mole) and potassium permanganate (0·395 g., 0·0025 mole) in 1 l. of aqueous solution which had to be warmed before use to effect complete dissolution of the periodate.

(a) *Oxidation of dihydroxylinoleic acid.* Concentrate B (78 mg.) was oxidised in an aqueous solution (300 ml.) containing *tert.*-butyl alcohol (90 ml.), the periodate–permanganate reagent (60 ml.), and enough potassium carbonate to give a pH of 8—9. The reaction was stopped after 8 hr. by addition of hydrochloric acid and enough sodium metabisulphite to convert all periodate, iodate, and iodine into iodide. The decolorised solution was basified, the butyl alcohol was distilled off under reduced pressure, and the remaining solution acidified and continuously extracted with ether. The extract was triturated with light petroleum (b. p. 40—60°): the soluble portion (7·1 mg.) was not identified, but the insoluble fraction (54 mg.) was azelaic acid (m. p. 100—104° after crystallisation from water, raised to 102—106° when mixed with an authentic sample).

(b) *Preparation of dihydroxylinoleyl alcohol.* A solution of the concentrate B (224 mg.) in anhydrous ether was added to lithium aluminium hydride (100 mg.) in the same solvent at such a rate that steady refluxing occurred. After 48 hr. at room temperature the mixture was decomposed with 4N-sulphuric acid and extracted with ether. The extract, when washed with carbonate solution and with water and evaporated, afforded the desired alcohol (195 mg., 91%).

(c) *Oxidation of dihydroxylinoleyl alcohol.* This alcohol (75 mg.), oxidised by permanganate–periodate, gave a crude product (51 mg.). The portion (36 mg.) insoluble in ice-cold light petroleum gave 9-hydroxynonanoic acid (15 mg., 35%), m. p. 40—46° (from ethyl acetate–light petroleum). This m. p. was raised to 46—49° (lit.,[20] 53°) and was then undepressed when mixed with an authentic sample. The *p*-bromophenacyl ester had m. p. 75—78°, slightly raised when mixed with an authentic sample.

(d) *Oxidation of oleyl alcohol.* A similar oxidation of oleyl alcohol (268 mg.) gave nonanoic acid (93%), characterised as its *p*-bromophenacyl ester, and 9-hydroxynonanoic acid [94%; m. p. 35—46°, raised to 45—51° (lit. 53°) when crystallised once from ether–light petroleum; *p*-bromophenacyl ester, m. p. 78—79°].

Infrared Studies.—(*a*) The spectrum of fraction iii*a* (48% diol) obtained with a Grubb-Parsons GS2A double-beam grating instrument showed no evidence of appreciable absorption in the 10·3 μ region.

(*b*) Olive, cameline, and vernonia oils were neutralised by passing a chloroform solution of each through a column of alumina. The corresponding mixed methyl esters were prepared by (i) acid hydrolysis with acetic acid, (ii) alkaline hydrolysis and recovery of the mixed acids, and (iii) esterification with anhydrous methanolic hydrogen chloride. Spectra in the region of 2·6—3·3 μ were measured for thin films of these oils and their esters.

Preparation of Tridecanedioic Acid.—Mixed acids (76 g.) of rape oil, crystallised from ethanol (200 ml.) and water (70 ml.) at 0°, gave a fraction of fairly pure erucic acid (9·3 g.; m. p. 31—34°, lit. 33°; I val. 73·3, theor. 75·0). This was oxidised by potassium permanganate–acetic acid,[10] and tridecanedioic acid separated from the aqueous solution after removal of the nonanoic acid. After one crystallisation from ethyl acetate (m. p. 106—111°, lit., 113°) it was considered pure enough for further reaction.

Preparation of Pentadecanedioic Acid.—Tridecanedioic acid (2·5 g.) was refluxed with pure thionyl chloride (10 ml.) for 2 hr., then volatile material was removed and the residual diacid chloride (2·85 g.) in pure ether (20 ml.) was added to excess of ethereal diazomethane. Next day the excess of diazomethane was distilled off with ether, and the solution concentrated to 20 ml. The bisdiazo-ketone (1·43 g.) which separated on cooling was dissolved in anhydrous methanol (20 ml.) in a conical flask fitted with a dropping funnel and connected to a gas-measuring burette. To this solution a 10% solution of silver benzoate in triethylamine (5 ml.) was added in 30 min. and the mixture was stirred for a further hour. During this time nitrogen evolution corresponded to 90% reaction. The solution was boiled and filtered, and the solvent removed from the filtrate. The residue, dissolved in a little ether and washed with a little alkali and water, gave crude dimethyl pentadecanedioate (2·16 g., 32% overall; m. p. *ca.* 40°, lit. 43°). This was hydrolysed by aqueous alcoholic potassium hydroxide; the crude acid (1·8 g.), purified

[20] Lycan and Adams, *J. Amer. Chem. Soc.*, 1929, **51**, 625.

by crystallisation from ethyl acetate before and after vacuum-sublimation at 200°, had m. p. 111—113° (lit.,[21] 115°).

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[21] Chuit, *Helv. Chim. Acta*, 1926, **9**, 264.