Glyceride Structure of *Cardamine impatiens* L. Seed Oil

K. L. MIKOLAJCZAK, C. R. SMITH, JR., and **I. A. WOLFF,** Northern Regional Research Laboratory,[1] Peoria, Illinois 61604

ABSTRACT

A group of unusual triglycerides, in which one of the acyl groups is a vicinal dihydroxy acid with one of the hydroxyl groups acetylated, has been isolated from *Cardamine impatiens* L. (Cruciferae) seed oil. Hydrolysis of these triglycerides with castor bean lipase facilitated isolation and identification of a mixture of C_{18}, C_{20}, C_{22}, and C_{24} hydroxy acetoxy fatty acids. Pancreatic lipase hydrolysis data revealed that these monoacetylated dihydroxy acid residues are esterified exclusively with one of the α-positions of the glycerol moiety. The remaining acyl groups are comprised of ordinary C_{18} unsaturated acids (which occupy 98% of the β-position), palmitic acid, and C_{20}, C_{22}, and C_{24} monoenoic fatty acids.

INTRODUCTION

SEED OIL OF *Cardamine impatiens* L. has recently been reported to contain 25% of a mixture of saturated, vicinal, dihydroxy acids with chain lengths of 18, 20, 22, and 24 carbon atoms. Essentially all the diols have the *erythro* configuration (1). In a preliminary communication (2), we concluded that these dihydroxy acids, together with ordinary long-chain acids and acetic acid, were constituents of unusual triglyceride components which comprised 40% of *Cardamine* oil; however the exact nature of these components remained unclear.

This paper presents results which show that these unusual triglycerides have one or both of the structures shown in Fig. 1. These substances, which will be referred to as hydroxy triglycerides, differ from one another only in chain length and degree of unsaturation of the acyl groups; they migrate as a single spot in thin-layer chromatography (TLC) on Silica Gel G and have been treated as an entity throughout this investigation.

EXPERIMENTAL PROCEDURES AND RESULTS

General

Infrared (IR) analyses were done on 1% solutions in carbon disulfide, chloroform, or carbon tetrachloride (depending on the solubility of the sample) with a Perkin-Elmer Model 137 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained with a Varian A-60 spectrometer on deuteriochloroform solutions containing tetramethylsilane as the internal reference. Mass spectral data were determined with a Nuclide 1290-G spectrometer. TLC analyses were done on plates spread with 250 μ layers of Silica Gel G. *Cardamine impatiens* seed oil was obtained and analyzed as described previously (1).

Isolation and Analysis of Hydroxy Triglycerides

Hydroxy triglycerides were separated from normal triglycerides of *Cardamine* oil with a 2.3 × 30-cm column of 100–140 mesh Adsorbosil CAB (Applied Science Laboratories Inc.). These separations were done in batches of 1.5 g of oil with the solvent system ethyl ether-petroleum ether, 70:30 (v/v). Fractions showing hydroxyl absorption and acetate absorption in their IR spectra were combined (40% of whole oil) to give the hydroxy triglyceride fraction. This fraction was demonstrated to be a glycerol-based lipid by the method of Holla et al. (3), in which the glycerol moiety is converted to triacetin and identified by gas-liquid chromatography (GLC). NMR indicated that glycerol α-carbon (multiplet centered at 5.76τ), olefinic (4.63τ), acetate (7.93τ), and C-methyl protons (9.12τ) were present. The fraction did not react with periodate, was optically active $[\alpha]_D^{27.5C}$ + 1.4° (c = 10, $CHCl_3$, visual polarimeter), and contained 13.5% oxygen by direct measurement. Migration characteristics of this fraction on TLC can be seen in Fig. 2, Sample 4. Its position on the plate demonstrates that it is made up of triglycerides since it migrates slightly faster than distearin. If it were composed of diglycerides containing a dihydroxy fatty acid moiety with one hydroxyl group free, it would be considerably more polar than normal diglycerides.

Chemical Hydrolysis of Hydroxy Triglycerides

Hydrolysis of the hydroxy triglycerides by refluxing 2 hr with 1% H_2SO_4 in methanol yielded a mixture of ordinary fatty acid methyl esters (excluding acetic acid) and dihydroxy acid methyl esters in a 2:1 molar ratio. This ratio was determined by separating the mixture

[1]No. Utiliz. Res. Dev. Div., ARS, USDA.

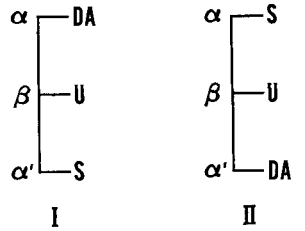

FIG. 1. Schematic representation of *Cardamine impatiens* triglyceride structures; D = dihydroxy acyl moiety, A = acetyl moiety, U = unsaturated C_{18} acyl moiety, and S = saturated or long chain ($>C_{18}$) acyl moiety.

into the two ester types on a 1.4 × 25-cm column of Adsorbosil CAB and weighing each fraction. Ordinary esters were eluted with chloroform and the dihydroxy esters with methanol-chloroform (5:95). TLC indicated that hydrolysis was complete. Alkaline hydrolysis in aqueous alcohols (e.g., 5% KOH in 80% aqueous methanol), followed by esterification with diazomethane, gave the same result. However, when the hydroxy triglycerides are refluxed 3 hr or more with alkali in absolute alcohol (e.g., 5% KOH in absolute methanol), the esterified products contain a small amount (less than 10%) of an artifact. This artifact was isolated by preparative TLC and yielded both ordinary and dihydroxy acids upon hydrolysis with acidic methanol, but its exact structure is not known.

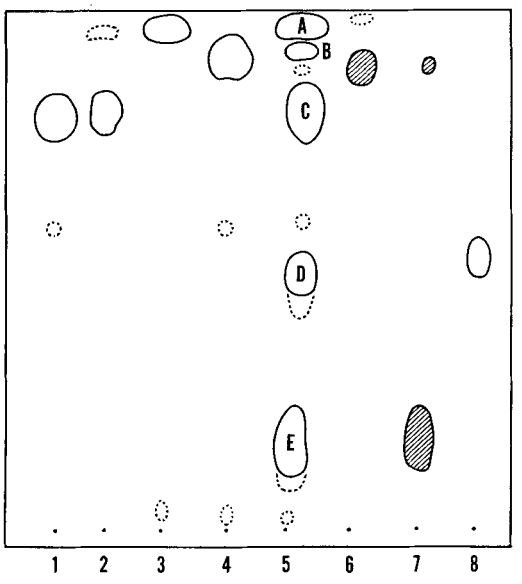

FIG. 2. Tracing of thin-layer chromatogram on Silica Gel G developed with methanol-chloroform (2:98). Spots were visualized with dichlorofluorescein and H_2SO_4 charring. The shaded spots did not char. Samples are: *1*, methyl esters of fraction DA; *2*, methyl 13(14)- and 14(13)-hydroxyacetoxydocosanoate; *3*, methyl oleate; *4*, *Cardamine* hydroxy triglycerides; *5*, methyl esters of pancreatic lipase hydrolysis products of *Cardamine* hydroxy triglycerides; *6*, distearin; *7*, monostearin; and *8*, methyl esters of *Cardamine* dihydroxy acids.

Enzymic Hydrolysis of Hydroxy Triglycerides

Castor bean lipase was isolated and purified according to the methods of Ory et al. (4,5). Under the selected conditions the enzyme did not hydrolyze methyl oleate or 9(10)-hydroxy-10(9)-propionoxystearic acid to any measurable extent. About 0.300 g of sample to be hydrolyzed was weighed into a 25 × 70-mm screwcap vial, and 0.020 g of purified enzyme, 0.5 ml of 0.1M Tris buffer (pH 8.0), and 1.5 ml of water were added. This mixture was acidified with 0.1M acetic acid to pH 4.3, and the vial was flushed with nitrogen and capped tightly. The contents were stirred vigorously for 3-5 hr at 25C. Hydrolysis of glyceryl ester linkages was complete after 3 hr; this point was established by the absence of monoglycerides and starting triglycerides from the products as indicated by TLC. These hydrolysis products, recovered by ether extraction, were converted to methyl esters (by treatment with diazomethane) to facilitate separation of the mixture of acetylated dihydroxy acid fragments (designated DA, Fig. 1) from the unsubstituted acyl fragments (S and U, Fig. 1).

Isolation and Analysis of Fraction DA

Fraction DA was separated from other fragments by chromatographing the methyl ester mixture on a 1.4 × 30-cm column of 100–140 mesh Adsorbosil CAB. At the start of the run the solvent was 20% ethyl ether in petroleum ether (bp 30-60C). The ethyl ether content was increased in increments of 10% until the most polar materials were finally eluted with 50% ethyl ether in petroleum ether. Normal fatty acid methyl esters recovered by this procedure totaled 64 mole %, and the acetylated dihydroxy acid (DA) methyl ester fraction accounted for the remaining 36 mole % [calculated from the weight of each fraction isolated and the known composition reported previously (1)]. Unacetylated dihydroxy acid esters were not observed. Each ester fraction gave only one spot on TLC. The hydroxyl and acetate absorption observed in the IR spectrum of the hydroxy glycerides was retained in esterified fraction DA.

The NMR spectrum of fraction DA methyl esters revealed equivalent numbers of methoxyl

(6.33τ), acetate (7.93τ), and C-methyl (9.12τ) protons. No olefinic proton signal was observed. Elemental analyses indicated that esterified DA contained 18.4% oxygen (by difference). The calculated oxygen content is 18.5% by assuming a mixture of hydroxy acetates with the amounts of the various chain-length homologs found previously (1).

An additional sample of fraction DA was isolated (yield, 30% of theoretical) as the mixed free acids by column chromatography on Adsorbosil CAB. Ethyl ether-petroleum ether was the eluting solvent, and the ethyl ether content was increased gradually in increments of 5%. Excessive tailing caused a low yield, but a sample of fraction DA containing ca. 90% of the four homologs was obtained and was shown to have a neutral equivalent of 475, indicating monomeric substances.

Chemical Hydrolysis of Fraction DA

Refluxing 0.073 g of the methyl esters of DA with 25 ml of 2% sulfuric acid in methanol for 3 hr under nitrogen yielded 0.065 g of dihydroxy acid methyl esters. Analysis of the product by GLC under conditions described previously (1) revealed that only a trace of ordinary fatty acid esters was present (methyl acetate was volatilized in the work-up process). A parallel experiment in which DA was refluxed with 5% potassium hydroxide in 80% aqueous methanol for 2 hr and the resulting products were esterified with diazomethane, produced the same results. The absence of ordinary esters was confirmed by TLC. By this method it was also demonstrated that both hydrolyses were complete.

Synthesis of Methyl 13(14)-Acetoxy-14(13)-Hydroxydocosanoate

Erucic (*cis*-13-docosenoic) acid (0.410 g) in 3 ml of chloroform was treated at room temperature dropwise with a solution of 0.420 g of *m*-chloroperbenzoic acid (6) in 5 ml of chloroform. After the mixture was stirred magnetically for 1.25 hr, excess peracid was destroyed by addition of a 5% solution of sodium sulfite. The chloroform layer was withdrawn and washed successively with sodium bicarbonate solution and water. Removal of the chloroform and crystallization of the crude product from warm petroleum ether yielded 0.330 g of *cis*-13,14-epoxydocosanoic acid, mp 62-63C [lit. value, 63.5C (7)]. The epoxide was converted to methyl 13(14)-acetoxy-14(13)-hydroxydocosanoate by refluxing for 2 hr with glacial acetic acid, followed by treatment with diazomethane.

Identification of Fraction DA by GLC, TLC, and IR

Analysis of the synthetic methyl acetoxyhydroxydocosanoate and of the methyl esters of fraction DA by GLC was carried out on a 60 × 0.3-cm O.D. column packed with 3% OV-1[2] on 60-80 mesh Gas-Chrom Q (Applied Science Laboratories Inc.). The column temperature was programmed at 4C/min from the starting temperature of 145C; helium was the carrier gas. An F&M Model 810 chromatograph with a hydrogen flame detector was used. Methyl acetoxyhydroxydocosanoate emerging from the column at 197C. Fraction DA methyl esters gave four peaks, the major one emerged also at 197C. The next largest peak emerged at 208C and was attributed to the C_{24} hydroxyacetate. Two minor peaks emerging at 173C and 185C were attributed to the C_{18} and C_{20} hydroxyacetates respectively. On TLC, fraction DA methyl esters and synthetic methyl acetoxyhydroxydocosanoate each gave a major spot of R_f 0.81 as indicated in Samples 1 and 2 respectively (Fig. 2). In addition, the IR spectrum of the synthetic material was identical to that of fraction DA methyl esters.

Conversion of Fraction DA to α-Ketols

A 0.116-g sample of fraction DA methyl esters in 5 ml of dry acetone was treated for 5 min at room temperature with 0.4 ml of Jones reagent (8). The reaction mixture was diluted with water; the oxidized products were recovered by ether extraction and were transesterified by refluxing with 2% sulfuric acid in methanol. IR analysis of the resultant α-hydroxy ketones (1%, CCl_4) indicated hydroxyl (3450 cm^{-1}), ester carbonyl (1725 cm^{-1}), and ketone absorption (1700 cm^{-1}). The intensity of the ketone band nearly equalled that of the ester carbonyl band. Synthetic hydroxyacetoxydocosanoate was similarly converted to the corresponding α-ketol.

We had no success in cleaning these α-ketols with periodate although other workers have done this with C_{18} fatty acid derivatives (9). A similar cleavage reaction utilizing lead tetraacetate (10) was carried out on the α-ketols. Although cleavage was complete, over-oxidation led to the isolation of carboxylic acids as the only products.

Mass Spectra of Fraction DA and Hydroxyacetoxydocosanoate

The revealing portions of the fragmentation pattern for methyl hydroxyacetoxydocosanoate

[2]OV-1 is a silicone stationary phase with a high percentage of phenyl groups. It is similar to SE-30 in separating characteristics but superior to SE-30 in thermal stability.

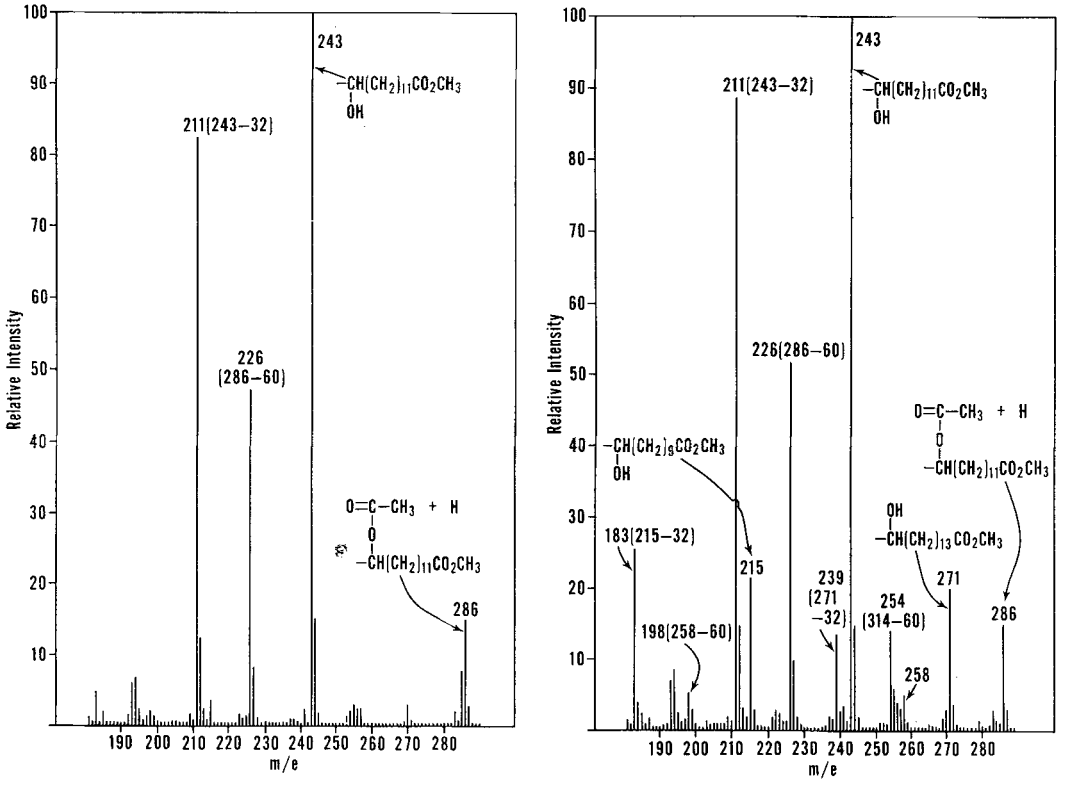

FIG. 3. Portions of mass spectra of methyl 13(14)- and 14(13)-dihydroxyacetoxydocosanoate (left), and methyl esters of *Cardamine* fraction DA (right).

and for the methyl esters of fraction DA are depicted in Fig. 3. The pattern shown in Fig. 3A for the synthetic methyl hydroxyacetoxydocosanoate is characterized by peaks due to both isomers (i.e., 13-hydroxy-14-acetoxy- and 14-hydroxy-13-acetoxydocosanoate) since both should be present in equal amounts. Cleavage between carbons 13 and 14 of the 13-hydroxy isomer gives the base peak at m/e = 243. Further fragmentation (loss of CH_3OH) of the 243 peak and subsequent rearrangement, probably to the ketene (11), gives the peak observed at m/e = 211. Similarly cleavage of the 13-acetoxy isomer yields two principal fragments, m/e = 286 and m/e =226. Probably the 226 peak arises by loss of acetic acid from the m/e = 286 peak. It could also be derived from the fragment appearing at m/e = 243 by loss of –OH, but this cleavage does not seem to occur appreciably in hydroxy acids (11).

The hydroxyacetoxy methyl esters from *Cardamine* oil give much the same fragmentation pattern (Fig. 3B). The four peaks are present in about the same proportion and are attributable to the C_{22} hydroxyacetate, the major constituent of the mixture. Fragmentation of the C_{24} isomer is consistent with that of the C_{22} and gives peaks at m/e = 314 (not shown), 271, 254, and 239. The 314 peak is caused by a fragment comparable with that causing the 286 peak but with two additional methylene groups. A similar pattern results from the C_{20} isomer, with peaks at m/e = 258, 215, 198, and 183. These data indicate that the acetate functions in this glyceride are approximately equally distributed between the vicinal hydroxyl groups of each dihydroxy acid.

Pancreatic Lipase Hydrolysis of Hydroxy Triglycerides

This reaction procedure was based on the semimicro method developed by Luddy et al. (12). To a 0.200-g sample of the *Cardamine* hydroxy triglycerides in a 25 × 70-mm vial was added 0.060 g of pig pancreatic lipase, EC 3.1.1.3, (Nutritional Biochemicals Corporation, Cleveland, O.), 3 ml of 1M Tris buffer (at pH 8.0), 0.3 ml of 22% calcium chloride solution, and 1 ml of 0.1% sodium cholate solution. A Teflon-coated magnetic stirring bar was placed in the vial, the contents were flushed thoroughly with nitrogen, and the vial was tightly

capped. After the vial was placed in a water bath maintained at 35-40C, the contents were agitated vigorously by a magnetic stirrer for 15 min. The hydrolysis products were isolated by ether extraction of the acidified reaction mixture; the ether extract was washed with water, concentrated to 5 ml, and reacted with diazomethane. TLC of the esterified mixture on Silica Gel G gave the chromatogram reproduced in Fig. 2, Sample 5.

Pure fractions of the hydrolyzed normal acids (as methyl esters) and of the monoglycerides, spots A and E respectively (Fig. 2, Sample 5), were obtained by column chromatography on Adsorbosil CAB. The ester mixture in petroleum ether (soluble with warming) was applied to the column, and the solvent was changed to chloroform after all the normal esters had been eluted with petroleum ether. Chloroform eluted the material contained in spot C (methyl esters of DA), but early fractions were contaminated with residual hydroxy triglycerides (spot B), and the final fractions contained some material from spot D (diglycerides containing one normal acid and one acetylated dihydroxy acid).

GLC analysis of the trimethylsilyl ethers of the materials in spot D demonstrated that unacylated dihydroxy acid methyl esters were not present. Most of the monoglyceride portion (spot E) was obtained pure with 2% methanol in chloroform although it was eluted from the column slowly. Methyl esters of the monoglyceride acyl groups were prepared by acid-catalyzed transesterification. Analysis of these esters by IR and TLC established that neither hydroxylated nor acetylated material was present.

Methyl esters of normal acids from the pancreatic lipase hydrolysis mixture were analyzed by GLC as described previously (1), and the resulting area percentages were: $C_{14:0}$, 0.3%; $C_{16:0}$, 9.7%; $C_{18:0}$, 1.8%; $C_{18:1}$, 4.4%; $C_{18:2}$, 5.2%; $C_{18:3}$, 2.0%; $C_{20:0}$, 2.1%; $C_{20:1}$, 18.3%; $C_{20:2}$, 1.8%; $C_{22:0}$, 1.6%; $C_{22:1}$, 45.2%; $C_{22:2}$, 1.0%; $C_{24:0}$, trace; $C_{24:1}$, 6.0%; and unidentified, 0.6%. The esters derived from the monoglyceride fraction were: $C_{16:0}$, 0.6%; $C_{18:1}$, 24.0%; $C_{18:2}$, 62.2%; $C_{18:3}$, 12.0%; and unidentified, 1.2%.

DISCUSSION

The experimental work shows conclusively that the unusual hydroxy triglycerides of *Cardamine impatiens* oil have acetylated dihydroxy acids attached to one of the α-positions of glycerol; saturated and long-chain ($>C_{18}$) ordinary acids attached to the other α-position; and oleic, linoleic, and linolenic acids esterified at the β-position. As far as we know, kamala seed oil (13), ergot lipids (14), and *Sapium sebiferum* seed oil (15) are the only previous examples of glycerides containing acylated hydroxy acid moieties. Estolides or polyestolides, in which a chain of two or more hydroxy acids is built up (13), were not observed in *Cardamine* triglycerides as shown by TLC data, and this conclusion is supported by the 2:1 molar ratio of ordinary acids to dihydroxy acids.

Other workers (14,16) have encountered difficulty in hydrolyzing esters of secondary hydroxyl groups by standard methods. However, we experienced no difficulty in removing the acetate group described in this work by either acid or base hydrolysis. Its removal is probably facilitated considerably by the neighboring group effect of the hydroxyl group on the adjacent carbon atom (17).

Preparation of α-ketols from the corresponding hydroxy acetoxy compounds involves an hydrolysis step (to remove acetate) after the hydroxyl group has been oxidized to a keto group. This hydrolysis with either acid or base may cause equilibration (18,19) between the two isomeric forms of the resulting α-hydroxy ketones. Therefore the use of these α-ketols in cleavage reactions designed to pinpoint the original location of the acetate function may lead to erroneous results. In any event, the failure of periodate to cleave the α-ketols described here is surprising since Cramp et al. (9) used this method to cleave a C_{18} α-ketol. The C_{22} and C_{24} chain-lengths of our compounds cause a solubility problem, and the nature of the fat globule surface may be such that periodate is unable to effect cleavage.

A brief consideration of the question of possible acyl migration in the acetylated dihydroxy acids is pertinent to this work. Acyl migration in glycerides was first observed by Fischer (20) and since has been shown to be a common occurrence in partial glycerides (21) and in acetylated carbohydrates (22). The generally accepted mechanism for these migrations involves a cyclic intermediate (20,23,24). Mass spectral data for these hydroxy acetates from *Cardamine* oil lead to the conclusion that the acetate group is distributed approximately equally between the vicinal hydroxyl groups. This conclusion is disturbing because natural biosynthetic processes tend to be selective, and one might expect to find preferential acetylation. This preferential acylation may actually exist but equilibration by heat or electron im-

pact, or both, may have occurred and thus given the observed spectra.

Little is known concerning the preference of hydroxy acids for the various glycerol positions. However saturated acids and acids longer than C_{18} are usually found on the glycerol α-positions in plant lipids where they have a clear-cut choice of position. Therefore the marked preference of the acetylated dihydroxy acids for the glycerol α-positions is not surprising since the dihydroxy acid residues are saturated and almost all have more than 18 carbon atoms. In contrast, estolide moieties in ergot lipids (14), although not directly comparable to our acetylated acids, seem to exhibit no clear-cut preference for either the α- or β-positions of glycerol.

Recent work has shown that linoleic acid is the direct precursor of ricinoleic acid in ergot oil (25). The speculation is made (14) that the biosynthesis of ricinoleic acid and its acylation (in ergot lipids) may occur directly from linoleic acid which is already in triglyceride form. Such a mechanism could possibly be operative in *Cardamine* triglycerides as well. Cruciferae seed oils generally contain erucic (*cis*-13-docosenoic) acid, and many have small amounts of nervonic (*cis*-15-tetracosenoic) acid as well (26,27). Since these long-chain monoenes would normally be attached to the α-position of glycerol, it is conceivable that the dihydroxy acids could be derived by hydroxylation of the corresponding monoenes after the triglyceride structure has been elaborated. Epoxy acids might serve as immediate precursors of the dihydroxy acids, or they might be subject to ring opening by acetate, which would give the hydroxy acetoxy acids directly. This last choice may be even more attractive when one considers that no diacetoxy derivatives were observed and that the acetylation step appears analogous to the chemical acetolysis of an epoxy group in that both hydroxy acetates are formed in equal amounts.

Tentative conclusions given in our preliminary report (2) that a dicarboxylic acid and an alkali-stable linkage were present in *Cardamine* hydroxy glycerides have now been shown to be erroneous. Interpretation of the original NMR data for fraction DA was based on the assumption that ordinary fatty acids were an integral part of DA when, in fact, they were impurities. An unidentified component (V) found earlier (2) was probably not a material containing alkali-stable linkages but more likely was an artifact formed by base in nonaqueous solvents in what appears to be a transesterification reaction.

ACKNOWLEDGMENTS

Seeds were supplied by Q. Jones, Crops Research Division, USDA, Beltsville, Md. GLC analyses by J. W. Hagemann; mass spectra and interpretation by W. K. Rohwedder; NMR by C. A. Glass and L. W. Tjarks; and elemental analyses by Mrs. B. R. Heaton.

REFERENCES

1. Mikolajczak. K. L., C. R. Smith Jr. and I. A. Wolff, JAOCS *42*, 939-941 (1965).
2. Mikolajczak, K. L., C. R. Smith Jr. and I. A. Wolff, Lipids *1*, 289-290 (1966).
3. Holla, K. S., L. A. Horrocks and D. G. Cornwell, J. Lipid Res. *5*, 263-265 (1964).
4. Ory, R. L., A. J. St. Angelo and A. M. Altschul, Ibid. *1*, 208-213 (1960).
5. Ory, R. L., A. J. St. Angelo and A. M. Altschul, Ibid. *3*, 99-105 (1962).
6. Schwartz, N. N., and J. H. Blumbergs, J. Org. Chem. *29*, 1976-1979 (1964).
7. Julietti, F. J., J. F. McGhie, B. L. Rao, W. A. Ross and W. A. Cramp, J. Chem. Soc. 4514-4521 (1960).
8. Bowden. K., I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, Ibid. 39-45 (1946).
9. Cramp, W. A., F. J. Julietti. J. F. McGhie, B. L. Rao and W. A. Ross, Ibid. 4257-4263 (1960).
10. Osman, S. M., and M. Ahmed, Fette Seifen Anstrichmittel *68*, 600-602 (1966).
11. Ryhage, R., and E. Stenhagen, J. Lipid Res. *1*, 361-390 (1960).
12. Luddy, F. E., R. A. Barford, S. F. Herb, P. Magidman and R. W. Riemenschneider, JAOCS *41*, 693-696 (1964).
13. Achaya, K. T., and J. S. Aggarwal, Chem. Ind. (London) 1616-1617 (1962).
14. Morris, L. J., and S. W. Hall, Lipids *1*, 188-196 (1966).
15. Sprecher, H. W., R. Maier, M. Barber and R. T. Holman, Biochemistry *4*, 1856-1863 (1965).
16. Jacobsen, M., M. Beroza and W. A. Jones, J. Am. Chem. Soc. *83*, 4819-4824 (1961).
17. Bruice, T. C., and T. H. Fife, Tetrahedron Lett. 263-266 (1961).
18. Elks, J., G. H. Phillips, T. Walker and L. J. Wyman, J. Chem. Soc. 4330-4344 (1956).
19. King, G., Ibid. 1788-1792 (1936).
20. Fischer, E., Chem. Ber. *53*, 1621-1633 (1920).
21. Mattson, F. H., and R. A. Volpenhein, J. Lipid Res. *3*, 281-296 (1962).
22. Bonner, W. A., J. Org. Chem. *24*, 1388-1390 (1959).
23. Doerschuk, A. P., J. Am. Chem. Soc. *74*, 4202-4203 (1952).
24. Boschan, R., and S. Winstein, Ibid. *78*, 4921-4925 (1956).
25. Morris, L. J., S. W. Hall and A. T. James, Biochem. J. *100*, 29C-30C (1966).
26. Mikolajczak, K. L., T. K. Miwa, F. R. Earle, I. A. Wolff and Q. Jones, JAOCS *38*, 678-681 (1961).
27. Miller, R. W., F. R. Earle, I. A. Wolff and Q. Jones, Ibid. *42*, 817-821 (1965).

[Received Nov. 14, 1967]