Densipolic Acid: a Unique Hydroxydienoid Acid from *Lesquerella densipila* Seed Oil[1a]

C. R. SMITH, JR.,[1b] T. L. WILSON,[1c] R. B. BATES,[2] AND C. R. SCHOLFIELD

Northern Regional Research Laboratory,[3] Peoria, Illinois

Received March 23, 1962

A new hydroxy fatty acid found as a major constituent of *Lesquerella densipila* seed oil glycerides, to be called densipolic acid, was shown to be 12-hydroxy-*cis*-9,*cis*-15-octadecadienoic acid. Structure Ia was deduced chemically by oxidative degradations and corroborated by n.m.r. spectra. An anomalous case of the von Rudloff permanganate–periodate oxidation was found in which β-hydroxyadipic acid (VIII) expected as one of the end products instead undergoes oxidative decomposition.

Seed oils of a number of species in the genus *Lesquerella* (fam. Cruciferae) have been examined at this laboratory. All contain high percentages of hydroxy fatty acids as glyceride substituents. A group of these, typified by *L. lasiocarpa*, have as their predominant hydroxy acid substituent (45–75%) lesquerolic acid,[4] which has been characterized as (+)-14-hydroxy-*cis*-11-eicosenoic acid.[5] A smaller group of *Lesquerella* species, typified by *L. densipila*, instead produce seed oils containing two C_{18}-hydroxy acids comprising *ca.* 50% of the total.[4] The less abundant of these two was presumed to be the familiar ricinoleic acid; it is accompanied in oils of this group by larger amounts of a new hydroxy acid for which the trivial name *densipolic acid* is suggested. Small amounts (*ca.* 2–3%) of a C_{16} hydroxy acid, apparently monoethenoid, are also found in *L. densipila* oil. This paper presents the structural determination of densipolic acid.

Free fatty acids obtained by conventional saponification of *Lesquerella densipila* oil were partitioned between acetonitrile and hexane[6,7] to obtain a concentrate of hydroxy acids suitable for further purification. Thirty-transfer countercurrent distributions of methyl esters of this concentrate, with either hexane–acetonitrile or hexane–85% methanol as the solvent system, resolved the three components only slightly, but did indicate certain separation trends. Hexane–acetonitrile tended to separate C_{18} monoene from methyl densipolate, but the C_{16} monoenoid ester moved closely with methyl densipolate. This behavior was analogous to that observed by Scholfield and co-workers[7] for palmitate, oleate, and linoleate esters. In contrast, the hexane–85% methanol system had somewhat less resolving power for the two C_{18} esters, but did resolve the C_{16} ester from methyl densipolate to some extent. Methyl densipolate of purity suitable for structural characterization (*ca.* 95%) was obtained by a 400-transfer countercurrent distribution with this latter solvent system.

The purified methyl densipolate (Ib) was obtained as a colorless liquid which consumed two moles of hydrogen. The infrared and ultraviolet spectra indicated that the carbon-carbon unsaturation was in the form of *cis* double bonds (no *trans* C=C absorption at 10.3 μ; no acetylenic absorption near 4.5 μ; no terminal C=C absorption near 11.2 μ[8]) and that there was no conjugation of any unsaturated centers (no ultraviolet absorption maxima above 220 mμ). Methyl densipolate appeared to be optically inactive in methanol.[9]

Treatment of methyl densipolate with lipoxidase[10] failed to produce appreciable conjugation of double bonds, as evidenced by the ultraviolet spectrum of the product. This enzymatic reaction is known to be specific for *cis,cis*-methylene-interrupted or "skipped" double bonds (—CH=CH—CH_2—CH=CH—)[11] so the presence of this grouping was not indicated. Even much more rigorous treatment of Ib with potassium *t*-butoxide[12] failed to force the double bonds into conjugation appreciably. Thus the presence of at least two methylene groups between these two unsaturated centers was established.[13]

(1) (a) To be presented at 142nd American Chemical Society Meeting, Atlantic City, New Jersey September 9–14, 1962; (b) Present address: Department of Chemistry, Western Michigan University, Kalamazoo, Michigan; (c) Present address: Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania.

(2) Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Illinois.

(3) One of the laboratories of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(4) K. L. Mikolajczak, F. R. Earle, and I. A. Wolff, *J. Am. Oil Chemists' Soc.*, **39**, 78 (1962).

(5) C. R. Smith, Jr., T. L. Wilson, T. K. Miwa, H. Zobel, R. L. Lohmar, and I. A. Wolff, *J. Org. Chem.*, **26**, 2903 (1961).

(6) E. M. Meade in "Progress in the Chemistry of Fats and Other Lipids," Vol. 4, ed. by R. T. Holman *et al.*, Pergamon Press, New York, 1957, p. 56.

(7) C. R. Scholfield, J. Nowakowska, and H. J. Dutton, *J. Am. Oil Chemists' Soc.*, **37**, 27 (1960).

(8) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed., John Wiley and Sons, Inc., New York, 1958, pp. 34, 58.

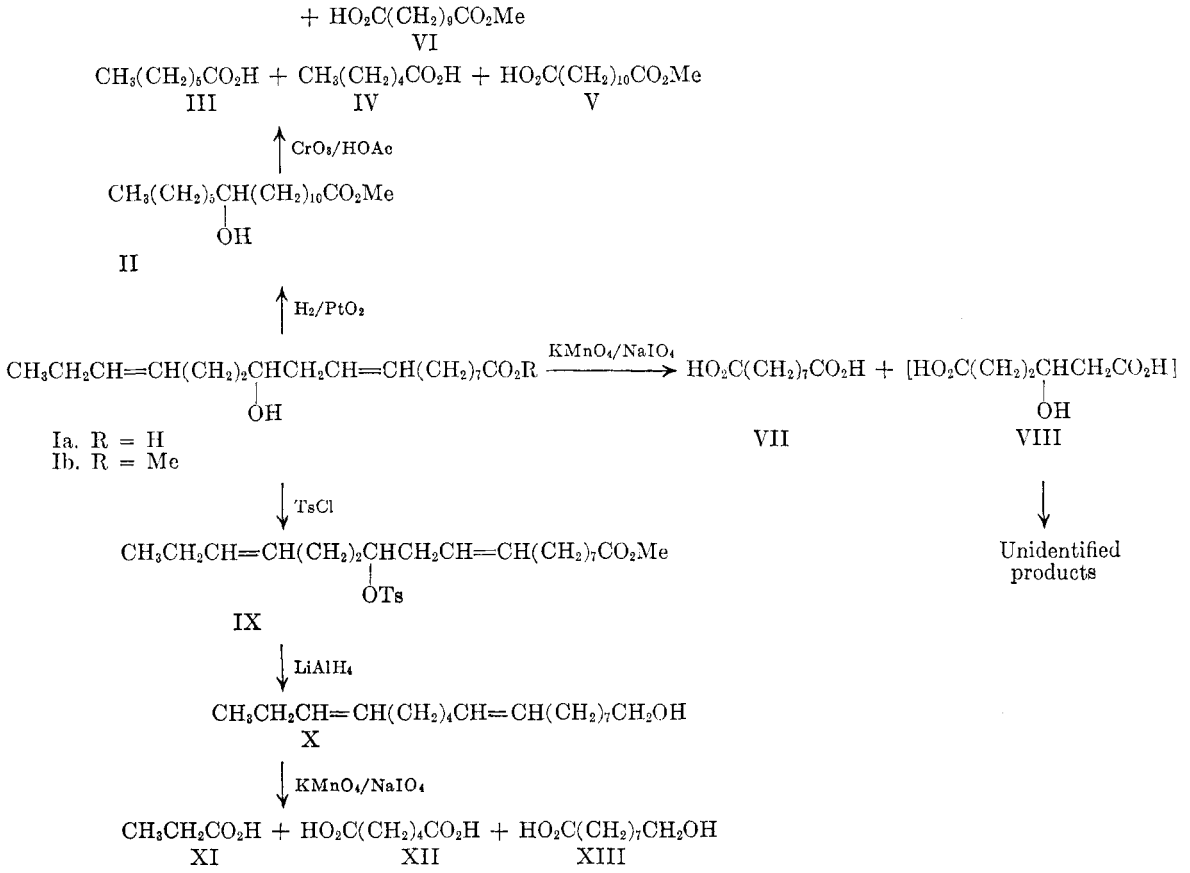
(9) Since it has an asymmetric carbon, it is very unlikely that methyl densipolate is truly optically inactive, *i.e.*, a racemic mixture. We did not have sufficient sample to permit additional polarimeter readings at higher concentrations, in different solvents, or "neat."

(10) J. MacGee, *Anal. Chem.*, **31**, 298 (1959).

(11) R. T. Holman in "Food Enzymes," ed. by H. W. Schultz, Avi Publishing Co., Westport, Conn., 1960, p. 77.

(12) H. B. White and F. Quackenbush, *J. Am. Oil Chemists' Soc.*, **36**, 653 (1959).

(13) B. M. A. de Surville, D. E. A. Rivett, and D. A. Sutton, *J. Chem. Soc.*, 3304 (1957).



The saturated hydroxy ester (II) obtained by hydrogenating methyl densipolate had m.p. 56.0–56.5°; no depression was observed on admixture with authentic methyl D-12-hydroxyoctadecanoate.[14] This saturated ester (II) was cleaved oxidatively by chromium trioxide in acetic acid.[15] The cleavage products obtained, hexanoic and heptanoic acids in approximately equal amounts together with half esters of undecanedioic and dodecanedioic acids in nearly equal amounts, placed the hydroxyl at C-12 on a normal C_{18} skeleton. Chain branching, both in II and in cleavage products III, IV, V, and VI, could be excluded by gas chromatographic analyses because acids with branched chains have distinctly shorter retention times than their straight-chain isomers.[16,17]

Von Rudloff's permanganate–periodate oxidation procedures[18] were applied to densipolic acid in an effort to locate its double bonds. Hydroxy acid concentrates containing *ca.* 59% Ia were used in these oxidations. Nonanedioic acid (VII) was invariably obtained as one product in amounts that left no doubt that densipolic acid must have one double bond in the 9,10-position, and that the other must be located farther along the chain. Positions 11,12 and 12,13 were excluded by the location of the hydroxyl as well as by the attempted isomerizations; the 13,14-position was also excluded because of the stability of the hydroxyl to acid-catalyzed dehydration which ruled out the possibility that it could be allylic. The terminal 17,18-position had already been ruled out on infrared spectral grounds. Thus there were three possible locations for the remaining double bond of densipolic acid—the 14,15-, 15,16-, and 16,17-positions. It was tempting to place it at the biogenetically attractive 15,16-position, but there was no chemical evidence to justify such an assignment yet. Despite repeated attempts, no fragment was found among the permanganate–periodate cleavage products corresponding to the expected β-hydroxy dicarboxylic acid (VIII) or to any lactone formed from it. A similar result was obtained when the hydroxyl was protected by acetylation before oxidation.

Efforts were then directed towards reductive removal of the hydroxyl. The feasibility of accomplishing this by lithium aluminum hydride reduction of the *p*-toluenesulfonate (tosylate)[19,20] of Ib

(14) K. Serck-Hannsen, *Chem. and Ind.* (London), 1554 (1958).
(15) G. D. Meakins and R. Swindells, *J. Chem. Soc.*, 1044 (1959).
(16) J. C. Hawke, R. P. Hansen, and F. B. Shorland, *J. Chromatog.*, **2**, 547 (1959).
(17) R. A. Landowne and S. R. Lipsky, *Biochim. Biophys. Acta*, **47**, 589 (1961).
(18) (a) R. U. Lemieux and E. von Rudloff, *Can. J. Chem.*, **33**, 1710 (1955); (b) E. von Rudloff, *J. Am. Oil Chemists' Soc.*, **33**, 126 (1956); (c) E. von Rudloff, *Can. J. Chem.*, **34**, 1413 (1956).
(19) (a) H. Schmid and P. Karrer, *Helv. Chim. Acta*, **32**, 1371 (1949); (b) P. Karrer, H. Asmis, K. N. Sareen, and P. Schyzer, *ibid.*, **34**, 1022 (1951).
(20) J. Strating and H. J. Backer, *Rec. trav. chim.*, **69**, 638 (1950).

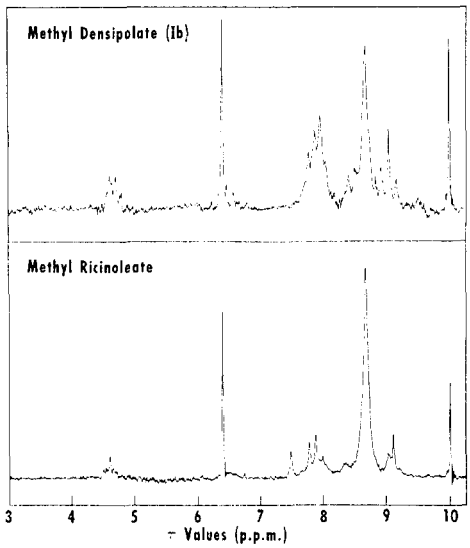


Fig. 1.—N.m.r. spectra of methyl densipolate and methyl ricinoleate.

was explored in experiments with methyl ricinoleate. The tosylates of ricinoleic acid or its esters were not found in the literature. We nevertheless obtained the tosylate of methyl ricinoleate by conventional methods without difficulty. The product obtained upon lithium aluminum hydride reduction of this tosylate was oxidized by permanganate–periodate in 60% *t*-butyl alcohol.[18e] The resulting products included (in addition to nonanoic acid) 9-hydroxynonanoic and 3-hydroxynonanoic acids in a 3:1 ratio, indicating that the reduction had proceeded in the desired sense to yield a methylene group to the extent of 75% and that the product of the competing reaction to regenerate the hydroxyl formed about 25% of the product. This result is in general accord with results of Karrer and coworkers[19b] on secondary alcohols. Permanganate–periodate oxidation of pure oleyl alcohol in 60% *t*-butyl alcohol proceeded smoothly to yield nonanoic and 9-hydroxynonanoic acids. Purified methyl densipolate (Ib) was tosylated without difficulty; the product (IX) was reduced with lithium aluminum hydride to dienol X in about 64% yield; no diol was detected among the by-products. X was cleaved by permanganate–periodate to propionic, adipic, and 9-hydroxynonanoic acids accompanied by only small amounts of by-products or contaminants. These degradation products indicated densipolic acid to be 12-hydroxy-*cis*-9,-*cis*-15-octadecadienoic acid (Ia).

The nuclear magnetic resonance (n.m.r.) spectrum of methyl densipolate (Ib; see Fig. 1) was obtained and is fully in accord with the structure proposed on chemical grounds. An n.m.r. spectrum of methyl ricinoleate[21] was obtained in the same way for comparison (Fig. 1). The observed peaks for methyl densipolate (with τ units,[22] numbers of protons calculated from relative areas, and assignments based on structure Ib) were at 4.5–4.8 (four protons on C-9, C-10, C-15, and C-16); 6.4–6.8 (four protons on C-12 and —OCH_3), 7.5–8.2 (eleven protons on C-2 C-8, C-11, C-14, C-17, and —OH), 8.3–8.9 (twelve protons on C-3, C-7, and C-13), and 8.9–9.3 (three protons, on C-18). The triplet for the C-18 methyl protons (central peak at 9.05 τ, J = 7 c.p.s.) is particularly important in that it clearly shows the presence of a 15,16-double bond, since it consists of three relatively sharp peaks, as in the spectrum of methyl linolenate, instead of a sharp one flanked by two broad ones.[23] The n.m.r. spectrum would place the other double bond somewhere between the C-4 and C-11 positions from the relative area of the peaks from 7.5–8.2 τ. The hydroxyl could not be allylic because of the absence of absorption in the 5–6-τ region. In contrast to their n.m.r. spectra, the infrared spectra of Ib and methyl ricinoleate were practically indistinguishable.

The failure of the permanganate–periodate oxidation of Ia to follow a straightforward course was most unexpected, since fragments containing isolated secondary hydroxyls have been obtained as products by this oxidation method from other long-chain hydroxy acids.[5,24] Furthermore, a hydroxynonanoic acid was present in the reaction mixtures under discussion. Succinic acid also is known to be stable as a product of this oxidation.[25] Despite these precedents, it appears that β-hydroxyadipic acid (VIII), if formed as expected as a fragment in the permanganate–periodate cleavage of densipolic acid, suffers oxidative decomposition into other fragments (*e.g.* malonic acid) that are not readily detected.

The C_{18}-hydroxy monoethenoid acid accompanying densipolic acid was not isolated in a pure form and was not characterized rigorously. Products from permanganate–periodate oxidation of the mixed hydroxy acid concentrates did, however, afford presumptive evidence that they contain the familiar ricinoleic acid. The C_{16} hydroxy acid in *L. densipila* oil was concentrated in tubes 145–165 of the 400-tube countercurrent distribution, but was not obtained very pure. No attempt was made to characterize it chemically.

Densipolic acid apparently has not been reported in the literature either as a natural or a synthetic substance. It follows certain obvious biogenetic trends in location of the double bonds and hydroxyl. Just as ricinoleic acid might be derived formally from linoleic acid by addition of the elements of water to the 12,13-double bond, densipolic acid might be regarded as similarly derived from

(21) C. Y. Hopkins and H. J. Bernstein, *Can. J. Chem.*, **37**, 775 (1959).

(22) G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).

(23) W. H. Storey, Jr., *J. Am. Oil Chemists' Soc.*, **37**, 676 (1960).

(24) P. A. J. Gorin, J. F. T. Spencer, and A. P. Tulloch, *Can. J. Chem.*, **39**, 846 (1961).

(25) M. O. Bagby, C. R. Smith, K. L. Mikolajczak, and I. A. Wolff, *Biochemistry*, in press.

linolenic (9,12,15-octadecatrienoic) acid. The new acid is rather unusual among natural fatty acids in having a hydroxyl situated between two double bonds. It is isomeric with another hydroxydienoid fatty acid reported recently, dimorphecolic (9 - hydroxy - *trans,trans*-10,12-octadecadienoic) acid,[26] but completely different in the relative positions of the double bonds and hydroxyl. Although the absolute configuration of densipolic acid remains to be determined, the apparent identity of II with methyl D-12-hydroxyoctadecanoate suggests tentatively that densipolic has the D-configuration in common with ricinoleic acid.[14]

Experimental

Infrared spectra were determined with an Infracord Model 137[27] spectrophotometer, on 1% carbon tetrachloride solutions unless otherwise specified; n.m.r. spectra were determined with a Varian A-60 spectrophotometer on 10% carbon tetrachloride solutions containing 0.5% tetramethylsilane. Ultraviolet spectra were determined in ethanol solution with a Beckman DU spectrophotometer. Melting points were determined with a Fisher–Johns block and are uncorrected. Gas chromatographic analyses were carried out as described by Miwa and co-workers[28]; equivalent chain length values for methyl esters and some other compounds not given in their paper are in Table I. Mixtures of free acids (including some half acid, half esters) were analyzed in several cases, under similar operating conditions. The free acids were not assigned equivalent chain lengths, but were identified simply by comparing retention times of observed peaks with those found for a standard mixture.

TABLE I

EQUIVALENT CHAIN LENGTH[28] VALUES USED IN IDENTIFYING VARIOUS COMPONENTS IN GAS CHROMATOGRAPHIC ANALYSIS

	—Stationary Phase—	
Component	Apiezon L	LAC-2-R-446
Methyl 3-hydroxynonanoate	10.4	14.1
Methyl 9-hydroxynonanoate	11.8	17.4
Oleyl alcohol	17.7	...
9,15-Octadecadiene-1-ol	17.5	...
Methyl 12-hydroxyoctadecanoate	19.8	24.6
Methyl ricinoleate[a]	19.4	24.7
Methyl densipolate	19.3	25.2
Methyl hydroxyhexadecenoate[a]	17.4	22.7
Methyl stearate[a]	18.0	18.0

[a] Values given by Miwa and co-workers[28] and included here to facilitate comparisons.

Preparation of Hydroxy Acid Concentrate *of Lesquerella densipila* **Seed Oil.**—Coarsely ground seeds of *Lesquerella densipila* (26.7 g.) were extracted overnight with petroleum ether (b.p. 30–60°). The bulk of the solvent was evaporated on a steam bath under a nitrogen atmosphere and the remainder was removed *in vacuo* with a rotating evaporator. The oil obtained (7.1 g.) was saponified by refluxing with 165 ml. of 1.2 *N* ethanolic potassium hydroxide under nitrogen. Unsaponifiables were removed by extracting the acid soap solution with petroleum ether–ethyl ether (1:1). Free acids (6.5 g.) were obtained by acidification with hydrochloric acid and extraction with ethyl ether. The composition of these acids (based on gas chromatographic analysis of their methyl esters prepared with diazomethane) was 38.4% densipolic, 19.6% "ricinoleic," and 2.6% C_{16}-hydroxymonoenoic acid; other components were present in approximately the same amounts as reported previously.[4]

A 6.4-g. portion of the free acids was dissolved in 70 ml. of hexane-saturated acetonitrile[6,7]; The resulting solution was extracted with (4 × 35 ml.) portions of acetonitrile-saturated hexane. Upon evaporation, the acetonitrile phase yielded 3.1 g. containing a total of 89.0% hydroxy acids.

Isolation of Methyl Densipolate (I) by Countercurrent Distribution.—Countercurrent distribution of methyl esters of the hydroxy acid concentrate prepared with diazomethane (0.74 g.) was carried out in a 30-tube Post apparatus using as the solvent system hexane–acetonitrile,[7] 40 ml. per tube of each solvent mutually saturated. Products from the individual tubes after evaporating solvents *in vacuo* were analyzed by gas chromatography. The peak for methyl densipolate was found at tube 7; for methyl "ricinoleate," at tube 10; for the C_{16} hydroxy ester, at tube 7. A distribution was carried out similarly on 0.17 g. of this same concentrate, except that the solvent system hexane–85% aqueous methanol was used. After thirty transfers with the latter solvent system, the peak for methyl densipolate was found at tube 9; for methyl "ricinoleate," at tube 11; and for the C_{16}-hydroxy ester, at tube 7.

A 400-transfer distribution was carried out on a 3.54-g. portion of methyl esters with a 200-tube Post apparatus, using as the solvent system mutually saturated hexane and 85% aqueous methanol. A 40-ml. portion of lower phase was placed in each of the 200 tubes. The methyl esters to be distributed were divided evenly between the first two tubes. The automatic operation of the instrument introduced 40 ml. of equilibrated upper phase to tube 0 at every transfer stage. As hexane upper layers progressed past tube 200, they were decanted into a receptacle in which they were pooled. Upper phases from transfers 201–250 and 251–300 were combined in this manner. The major peak was found at tube 191; methyl densipolate, a colorless liquid *ca.* 95% pure according to gas chromatographic analyses, was obtained from tubes 180–199 (*ca.* 1.06 g.). The peak for the C_{16} hydroxymonoene (*ca.* 58 to 69% pure) was at about tube 145. Because all the hydroxy esters moved more rapidly than anticipated on the basis of preliminary experiments, the methyl "ricinoleate" was found in pooled transfers 201–300, mixed with densipolate. Properties of methyl densipolate were determined on material from tubes 190–200. Infrared maxima included 5.73 μ (ester), 2.75 μ (OH), but none at 10.0–11.5 μ; there was no selective ultraviolet absorption; the n.m.r. spectrum is in Fig. 1; $[\alpha]^{27}D$ 0 ± 1° (*c* 3.7, methanol).

Anal. Calcd. for $C_{19}H_{34}O_3$: C, 73.5; H, 11.0; OMe, 10.0. Found: C, 73.1; H, 10.9; OMe, 10.6; absorbs 1.9 moles hydrogen per mole.

Attempted Isomerizations of Methyl Densipolate. (a) **Lipoxidase Method.**—A solution of 35.4 γ of hydroxy acid concentrate (58.8% densipolic acid) was treated with lipoxidase according to the procedure of MacGee.[10] Conjugated diene absorbance (235 mμ) observed, even after 30 min., corresponded to a concentration of only 3.9%. An isomerization of 19.5 γ of linoleic acid run in parallel as a control had absorbance indicating 99.5% conversion to conjugated diene after 5 min.

(b) **Potassium *t*-Butoxide Method.**—Methyl densipolate (95% pure) in parallel with methyl linoleate was treated with potassium *t*-butoxide reagent, in general according to the method of White and Quackenbush.[12] The quantities were scaled down to one-tenth those used in the published procedure. The calculated conversion of linoleate to a conjugated diene was 53.9%; for methyl densipolate, 4.2%.

(26) C. R. Smith, T. L. Wilson, E. H. Melvin, and I. A. Wolff, *J. Am. Chem. Soc.*, **82**, 1417 (1960).

(27) The mention of trade names or products does not constitute endorsement by the Department of Agriculture over those not named.

(28) T. K. Miwa, K. L. Mikolajczak, F. R. Earle, and I. A. Wolff, *Anal. Chem.*, **32**, 1739 (1960).

Hydrogenation of Methyl Densipolate (I).—Methyl densipolate (98.5% pure, 0.069 g.) was hydrogenated 0.5 hr. in ethanol with platinum oxide catalyst at room temperature, atmospheric pressure. The crude product (II) obtained upon filtration to remove the catalyst and evaporation of solvent had m.p. 45–53°; the bulk of this was reserved for oxidative cleavage without further purification. A small portion (0.013 g.) was recrystallized twice from petroleum ether; the product had m.p. 56.0–56.5°; mixed melting point with authentic methyl D-12-hydroxyoctadecanoate[29] (m.p. 56.5–57.0°) was 56.5–57.0° (lit., m.p. for L-isomer 56.8–57.1).[14]

Chromium Trioxide Oxidation of II.[15]—An 0.056-g. portion of II was dissolved in 3 ml. of glacial acetic acid. To this was added, dropwise and with continuous stirring, a solution of 0.35 g. of chromium trioxide, 2.5 ml. of acetic acid, and 0.3 ml. of water. The mixture was stirred 2 hr. then diluted with ice water and extracted repeatedly with 1:1 petroleum ether–ethyl ether. The combined extracts were dried over sodium sulfate. The solvent was distilled cautiously, using a short fractionating column, to minimize loss of volatile acids. The cleavage products were examined by gas chromatography both in the form of free acids and as the corresponding methyl esters prepared with diazomethane. The main short-chain ($<C_9$) components found among the free acids were hexanoic (IV) (40.7%) and heptanoic (III) (38.3%); small amounts of the C_5 and C_4 homologs were also present. The gas chromatograms of the ester preparation showed a considerable number of components, mostly relatively fast-moving (equivalent chain lengths shorter than C_{10}). Aside from these, the main constituents found were methyl esters of undecanedioic and dodecanedioic acids in nearly equal amounts.

Permanganate–Periodate Oxidation of Hydroxy Acid Concentrate.[18b]—An 0.065-g. portion of concentrate of hydroxy acids from *L. densipila* oil (Table I) was stirred 4 hr. at room temperature with 0.25 g. of potassium carbonate, 0.54 g. of sodium periodate, and 0.011 g. of potassium permanganate in 45 ml. of water. The reaction was terminated by addition of excess sodium bisulfite. The mixture was then acidified with sulfuric acid and extracted six times with ethyl ether. Combined ether extracts were dried with sodium sulfate and evaporated *in vacuo* at temperatures < 40°. This product was partially fractionated by repeated trituration with petroleum ether; the supernatant portions of solvent were decanted, combined, and evaporated. The insoluble (0.040 g.) and soluble portions were esterified with diazomethane and analyzed by gas chromatographic analysis. The petroleum ether-insoluble portion contained 84.8% nonanedioic acid (VII) and 7.5% of 3-hydroxynonanoic acid (or a component having very similar equivalent chain lengths).[28] There were small amounts of unidentified components in both fractions, but none in amounts that would correspond to VIII or a γ-lactone derived from it. Products from two other oxidations carried out in similar fashion also lacked such a component. A similar result was also obtained when the hydroxyl groups in the hydroxy acid concentrate were protected by acetylation.

Tosylation of Methyl Ricinoleate.—An 0.55-g. portion of methyl ricinoleate (1.77 mmoles) was tosylated essentially according to the procedure of Tipson,[30] except that a considerable excess of *p*-toluenesulfonyl chloride (tosyl chloride) was used. The product (0.71 g.) was obtained as a viscous oil; its infrared spectrum had maxima not found in the starting material that included 7.35 μ (strong, sharp), 8.55 and 8.63 μ (strong, sharp doublet), 9.13 μ (med., sharp), 11.08 μ (strong, broad) and 12.25 μ (med.); but no OH absorption (2.5–3.0 μ).[31] The first three of these maxima have been recorded as characteristic of sulfonates.[32]

Lithium Aluminum Hydride Reduction of Methyl Ricinoleate Tosylate.[19,20]—A portion of methyl ricinoleate tosylate (0.71 g.), dissolved in 250 ml. of anhydrous ether, was reduced with 2.6 g. of lithium aluminum hydride. The reagent and substrate were brought into contact by continually extracting the former from a Soxhlet thimble. The mixture was refluxed overnight. Excess hydride was destroyed by gradual addition of ethyl acetate. Hydrochloric acid (0.6 *N*) was added to dissolve inorganic matter. The ether and aqueous layers were shaken together, then separated and appropriately re-extracted. Combined ether layers, upon drying with sodium sulfate and evaporation, yielded 0.35 g. of product. The infrared spectrum of the product indicated OH (2.73 μ); characteristic tosylate peaks were absent. The presence of a carbonyl-containing by-product was indicated in this spectrum.

Permanganate–Periodate Oxidation of Oleyl Alcohol.[18a]—An 0.25-g. portion of oleyl alcohol[33] was dissolved in 90 ml. of *t*-butyl alcohol. Potassium carbonate (3.0 g.) and 151 ml. of permanganate–periodate stock solution (prepared according to von Rudloff[18c]) were added, and the mixture was stirred overnight. The excess reagents were reduced with sodium bisulfite, and the mixture was acidified with hydrochloric acid. The mixture was extracted repeatedly with ethyl ether; additional water was added to ensure separation of layers. The organic layer was dried with sodium sulfate, and the solvent was evaporated *in vacuo*, using a rotating evaporator. The product (0.34 g.) was analyzed by gas chromatography both in the form of free acids and as methyl esters (prepared by esterifying the acids with 1% methanolic sulfuric acid). Nonanoic acid constituted 95.9% of the short-chain components (C_9 or shorter) found in the free acids. The methyl esters contained 20.4% nonanoate and 77.3% 9-hydroxynonanoate (XIII); some methyl nonanoate was presumed to have been lost by volatilization in preparing the esters. The infrared spectrum of these methyl esters showed very strong OH absorption (2.73 μ), and indicated that little, if any, oxidation of the primary hydroxyl occurred.

Permanganate–Periodate Oxidation of Reduction Product from Methyl Ricinoleate Tosylate.—The oxidation of this reduction product (in effect, a crude preparation of oleyl alcohol) was carried out essentially as described in the preceding section. The product was analyzed by gas chromatography both in the form of free acids and as methyl esters. The main short-chain component (70.2%) found among the free acids was nonanoic acid; it was accompanied by smaller amounts of C_8, C_7, and C_6 homologs. The methyl esters contained 56.5% 9-hydroxynonanoate and 14.1% 3-hydroxynonanoate in addition to 13.5% nonanoate (some loss of this last ester by volatilization was expected) and smaller amounts of unidentified components.

Tosylation of Methyl Densipolate (Ib).—Tosylation of Ib (0.37 g.) was carried out essentially as described for methyl ricinoleate. A yield of 0.27 g. of the tosylate (IX) was obtained which had an infrared spectrum similar to that of methyl ricinoleate tosylate.

Lithium Aluminum Hydride Reduction of IX.—An 0.26-g. portion of tosylate (IX) was dissolved in 20 ml. of anhydrous tetrahydrofuran. This solution was added dropwise to 100 ml. of tetrahydrofuran in which 1.5 g. of lithium aluminum hydride was suspended. The mixture was refluxed overnight. Excess hydride was destroyed by gradual addition of ethyl acetate, then of ethanol. Sufficient 0.6 *N* hydrochloric acid was added to dissolve precipitated inorganic matter. The organic and aqueous layers were separated and appro-

(29) The 99.8% pure grade sold by Applied Science Laboratories, State College, Pa., and prepared by hydrogenation of methyl ricinoleate.

(30) R. S. Tipson, *J. Org. Chem.*, **9**, 235 (1944).

(31) Determined as a liquid film on sodium chloride disks.

(32) Ref. 8, p. 364.

(33) A 96% pure product sold by Applied Science Laboratories, State College, Pennsylvania.

priately re-extracted. The combined organic layers were dried over sodium sulfate and evaporated to yield 0.19 g. of reduction product having an infrared spectrum similar to the corresponding reduction product of methyl ricinoleate; gas chromatographic analysis indicated 64.3% 9,15-octadecadiene-1-ol (X) accompanied by unidentified products.

Permanganate–Periodate Oxidation of X.—The oxidation of X was carried out essentially as described for oleyl alcohol. Gas chromatographic analyses of the isolated cleavage products were carried out both as free acids and as methyl esters (prepared by esterifying the acids with 1% methanolic sulfuric acid). Propionic acid (XI) was the only short-chain acid ($< C_9$) detected in appreciable amount among the free acids. The methyl esters of adipic acid (XII; 35–37%) and 9-hydroxynonanoic acid (XIII; 55–58%) were the main constituents detected in the ester preparation; the balance was comprised by trace amounts of various unidentified components.

Acknowledgment.—The authors thank K. L. Mikolajczak, J. W. Hagemann, and Karen O'Connor for gas chromatographic analyses; M. O. Bagby and R. O. Butterfield for assistance with countercurrent distribution; Mrs. Clara McGrew for microanalyses; D. H. Johnson, Department of Chemistry, University of Illinois, Urbana, Illinois, for n.m.r. spectra; and Dr. Quentin Jones, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, for making *Lesquerella densipila* seed available to us.

Zwitterion Structure and Acylative Ring-Opening Reactions of 2-Aminothiazoline-4-carboxylic Acid

OSCAR GAWRON, JOSEPH FERNANDO, JOHN KEIL, AND T. J. WEISMANN

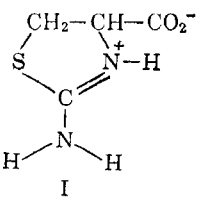
Department of Chemistry, Duquesne University, and Gulf Research & Development Company, Pittsburgh 19, Pennsylvania

Received April 30, 1962

pK_A values (pK_1 2.03, pK_2 8.48), variation of optical rotation with pH and other physical properties indicate that 2-aminothiazoline-4-carboxylic acid is a zwitterion in solution and in the solid state and that pK_2 can be ascribed to ionization from the ring nitrogen. Acylation of 2-aminothiazoline-4-carboxylic acid in aqueous medium is accompanied by ring opening. With acetic anhydride N′,S-diacetyl-N-carbamylcysteine is obtained and with benzoyl chloride S-benzoyl-N-carbamylcysteine and 2-imino-3-benzoylthiazolidine-4-carboxylic acid were obtained. Structures of the acylated products were proven by chemical methods and by interpretation of n.m.r. data.

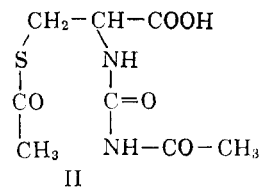
In connection with previous work on the kinetics of the cystine–cyanide reaction[1] and on the anti-acetylcholinesterase activity of thiazoline derivatives,[2] the zwitterion structure and acylative ring opening reactions of 2-aminothiazoline-4-carboxylic acid[3,4] have been investigated.

2-Aminothiazoline-4-carboxylic acid is a product of the reaction of cyanide with cystine[3,4] and is also a naturally occurring material.[5] As obtained from the reaction of cyanide with L-cystine, 2-aminothiazoline-4-carboxylic acid decomposes at 232–234° and the high decomposition temperature plus the presence of a basic ring system and a carboxyl group alpha to a ring nitrogen is indicative of a zwitterion structure. Evidence from titration studies, infrared spectra, optical rotation as a function of pH and n.m.r. studies corroborates a zwitterion structure which may be written as I.

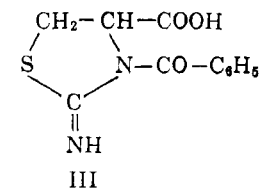


I

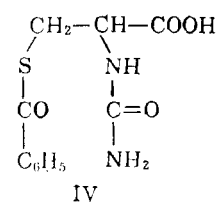
On acylation with acetic anhydride in aqueous medium, 2-aminothiazoline-4-carboxylic acid yields N′,S-diacetyl-N-carbamylcysteine (II) and on acylation with benzoyl chloride in aqueous medium, 3-benzoyl-2-iminothiazolidine-4-carboxylic acid (III) and S-benzoyl-N-carbamylcysteine (IV) are obtained. Acetylation of 2-aminothiazoline-4-carboxylic acid with acetic anhydride in aqueous solution has previously been reported[5,6] to yield 3-acetyl-2-iminothiazolidine-4-carboxylic acid, m.p. 179–180°. In our work the only compound that could be isolated from the aqueous acetylation was the diacetyl derivative II. Since this compound



II



III



IV

(1) O. Gawron and J. Fernando, *J. Am. Chem. Soc.*, **83**, 2906 (1961).
(2) O. Gawron and J. Keil, *Arch. Biochem. Biophys.*, **89**, 293 (1960).
(3) A. Schoberl and R. Hamm, *Ber.*, **81**, 210 (1948).
(4) A. Schoberl, M. Kawohl, and R. Hamm, *ibid.*, **84**, 571 (1951).
(5) J. L. Wood and S. L. Cooley, *J. Biol. Chem.*, **218**, 449 (1956).
(6) J. L. Wood, personal communication.