[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY[1]]

Lesquerolic Acid. A New Hydroxy Acid from *Lesquerella* Seed Oil[1a]

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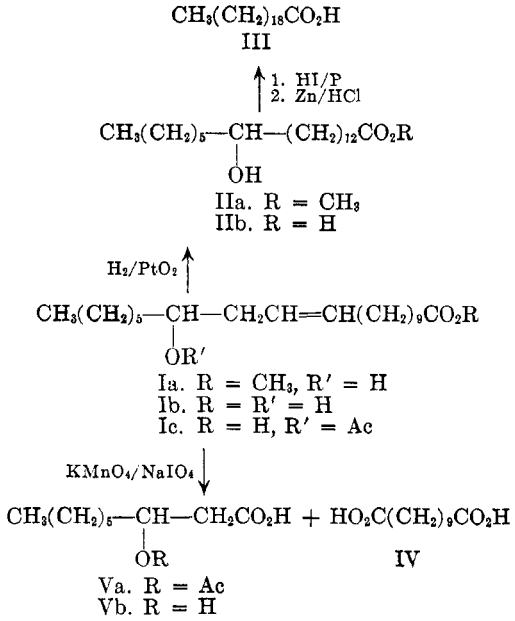
A major constituent of *Lesquerella lasiocarpa* seed oil is characterized as (+)-14-hydroxy-*cis*-11-eicosenoic acid. For convenience, it is called lesquerolic acid.

Lesquerella lasiocarpa seed oil contains an unknown hydroxy acid.[3] On the basis of gas chromatographic evidence, Miwa and co-workers suggested that it is a C_{20} hydroxy acid analogous to ricinoleic acid. Infrared spectra, gas chromatographic data and chemical determination of hydroxyl indicated the presence of substantial amounts of a hydroxy acid in *L. lasiocarpa* seed oil (40–45%) as well as in *L. lindheimerii* (51–72%). The present paper will describe the isolation and structural identification of this acid.

A sample of *Lesquerella* oil was converted to a mixture of methyl esters by treatment with 1% methanolic sulfuric acid. The esters wre fractionated by countercurrent distribution, using the solvent system acetonitrile-hexane.[4] After thirty transfers, the methyl ester (Ia) of the new acid, for which the name *lesquerolic acid* is proposed, was obtained as a distinct peak with its maximum at tube 11. Iodine number determination indicated the presence of one double bond. The infrared spectrum indicated that this double bond had a *cis* configuration (no absorption 10–11 μ). The stability of the hydroxyl towards dehydration in the presence of acid implied that it was neither allylic to the double bond nor α or β to the carboxyl group. Alkaline hydrolysis of methyl lesquerolate yielded the free acid (Ib), a weakly dextrorotatory liquid. On hydrogenation a saturated hydroxy acid (IIb), m.p. 83–84.5°, was obtained.

Dihydrolesquerolic acid, in the form of its methyl ester (IIa), was reduced by hydrogen iodide and phosphorus followed by zinc and hydrochloric acid.[5] The resulting product was identified unequivocally as eicosanoic acid (III) by mixed melting point determinations and by gas chromatographic analysis. A normal C_{20} skeleton for lesquerolic acid was thereby established.



Because of the small amount of material available, it was imperative that any method used for oxidative cleavage of lesquerolic acid give the minimum number of possible products. The Lemieux–von Rudloff permanganate-periodate oxidation method[6] appeared to be ideally suited. Wall and Serota[7] applied a modification of this procedure to a steroidal sapogenin without attacking a hydroxyl group. Lesquerolic acid (Ib), when oxidized by the permanganate-periodate method, yielded two main cleavage products. One of these was identified unequivocally as undecandioic acid (IV) by mixed melting point and by gas chromatographic analysis. This identification showed that the double bond was in the Δ^{11}-position, and that the hydroxyl was not located between the double bond and the carboxyl group. The other product, presumably a hydroxy acid, was not obtained in pure form. A ketonic oxidation product was found to be present to the extent of only 0.5%, but further degradation might be expected if a β-keto acid

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(2) Deceased Nov. 3, 1960.

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(6)(a) R. U. Lemieux and E. von Rudloff, *Can. J. Chem.*, **33**, 1701 (1955); (b) E. von Rudloff, *J. Am. Oil Chemists' Soc.*, **33**, 126 (1956).

(7) M. E. Wall and S. Serota, *J. Org. Chem.*, **24**, 741 (1959).

were formed.[8] *O*-Acetylricinoleic acid was oxidized smoothly with permanganate-periodate,[9] but less favorable results were obtained with *O*-acetyllesquerolic acid (Ic), which oxidized much slower, probably because of its lower solubility in the oxidation medium. The acetoxy acid fragment (Va) was hydrolyzed to a hydroxy acid (Vb) which was shown by mixed melting point determinations and by X-ray diffraction to be identical with D-3-hydroxynonanoic acid prepared by degradation of ricinoleic acid. This evidence placed the hydroxyl of lesquerolic acid at C-14, in a position β to the double bond.

Infrared evidence also indicated the hydroxyl of lesquerolic acid was β to the double bond. Recent work has shown that in proper steric environments, there are hydrogen-bonding interactions between hydroxyl groups and π-electrons of various unsaturated centers, including olefinic linkages.[10] Morris[11] has found that α-, β-, and γ-hydroxyolefins in the fatty acid series can be differentiated on the basis of their near infrared spectra. Apparently the β-hydroxyolefin provides an environment such that both free and hydrogen-bonded hydroxyl peaks are observed as a doublet; the free peak (2.761 μ) is slightly stronger than the hydrogen-bonded one (2.788 μ). The near infrared spectrum of methyl lesquerolate is very similar to that of methyl ricinoleate, but differs distinctly from those of methyl esters of dimorphecolic acid (an α-hydroxydiene[12]) and of 9-hydroxy-*cis*-12-octadecenoic acid (a γ-hydroxyolefin). Methyl dimorphecolate has a single, sharp hydroxyl peak located at 2.755 μ. The γ-hydroxyolefin mentioned has a sharp peak at 2.755 μ, with a much smaller one at 2.802 μ.[11]

On the basis of these two lines of evidence lesquerolic acid can be assigned structure Ib, that of (+)-14-hydroxy-*cis*-11-eicosenoic acid. It may be regarded as a C_{20} analog of ricinoleic acid. Since its optical rotation has the same sign as that of ricinoleic acid, it seems highly probably that the new acid has the same absolute configuration (D)[13] as does ricinoleic acid.[14] From the biogenetic standpoint, it is of interest that the position of the double bond in lesquerolic acid is the same as that in the commonest C_{20} monoethenoid acid of plant origin, 11-eicosenoic acid, which has been demonstrated to occur in the same plant family (Cruciferae) to which *Lesquerella* belongs.

EXPERIMENTAL[15]

Preparation of methyl lesquerolate (Ia). Coarsely ground seeds of *Lesquerella lasiocarpa* (40.1 g.) were extracted overnight in a Soxhlet apparatus 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. A yield of 10.7 g. of oil was obtained; this was transesterified by refluxing 2 hr. in 1% methanolic sulfuric acid. The esters were isolated by diluting with water and extracting repeatedly with ether. Combined ether extracts were washed with 5% potassium carbonate and dried over sodium sulfate. On evaporation, 10.0 g. of mixed methyl esters was obtained.

A 3.40-g. portion of the mixed methyl esters was subjected to a 30-transfer countercurrent distribution in a Craig-Post apparatus. The solvent system used was acetonitrile-hexane.[4] Methyl lesquerolate formed a distinct peak in tubes 2–18 comprising *ca.* 49% of the starting material. The products obtained from tubes 6–14, shown by gas chromatographic analyses to be 90 to 97% pure, were pooled for structural studies. The infrared spectrum showed maxima at 2.740, 2.770 μ (doublet[16]), 5.72 μ[17]; no maximum at 10–11 μ.[17] The near infrared spectrum of methyl ricinoleate determined on the same instrument showed a doublet at 2.740 and 2.770 μ.[16] In contrast, methyl esters of *Strophanthus courmontii* seed oil containing 9-hydroxy-12-octadecenoic acid[18] had peaks at 2.755 μ (strong) and 2.802 μ (weak).[11] The ultraviolet spectrum of Ia showed no absorption maxima.[19]

Anal. Calcd. for $C_{21}H_{40}O_3$ (one C=C): Wijs iodine value, 74.7. Found: 77.8.

Preparation of lesquerolic acid (Ib). Purified methyl lesquerolate (1.11 g.) was refluxed 1 hr. with 0.8*N* ethanolic potassium hydroxide. This material was worked up in the usual manner and 0.90 g. of lesquerolic acid was obtained as an oil, $[\alpha]_D^{22} + 6 \pm 1°$ (*c* 1.6, chloroform).[20]

Hydrogenation of lesquerolic acid. Lesquerolic acid (0.311 g.)

(8) C. F. Huebner, S. R. Ames, and E. C. Bubl, *J. Am. Chem. Soc.*, **68**, 1621 (1946).

(9) O. S. Privett and E. C. Nickell, *Fette Seifen Anstrichmittel*, **61**, 842 (1959).

(10) P. von R. Schleyer, C. Wintner, D. S. Trifan, and R. Bacskai, *Tetrahedron Letters*, No. 14, 1 (1959); E. J. Moriconi *et al.*, *J. Am. Chem. Soc.*, **81**, 6472 (1959); D. S. Trifan, R. Bacskai, P. v. R. Schleyer, and C. Wintner, presented before the Division of Organic Chemistry, 135th Meeting, American Chemical Society, Boston, Mass., April 5–10, 1959; P. v. R. Schleyer, D. S. Trifan, and R. Bacskai, *J. Am. Chem. Soc.*, **80**, 6691 (1958) and references cited therein; C. H. DuPuy and P. R. Story, *Tetrahedron Letters*, No. 6, 20 (1959); H. M. Fales and W. C. Wildman, presented before the Division of Organic Chemistry, 138th Meeting, American Chemical Society, New York, N. Y., Sept. 11–16, 1960; R. West, *J. Am. Chem. Soc.*, **81**, 1614 (1959).

(11) Personal communication from Dr. L. J. Morris, Hormel Institute, Austin, Minn.

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

(13) K. Serck-Hanssen, *Chem. & Ind. (London)*, 1554 (1958).

(14) J. A. Mills and W. Klyne in *Progress in Stereochemistry*, Vol. 1, ed. by W. Klyne, Butterworths Scientific Publications, London, 1954, p. 205; J. H. Brewster, *J. Am. Chem. Soc.*, **81**, 5475 (1959).

(15) Melting points were determined with a Fisher-Johns block and are uncorrected. The mention of trade names or products does not constitute endorsement by the Department of Agriculture over those not named. Gas chromatographic analyses were carried out on methyl esters as described in Ref. 3.

(16) Determined with a Perkin-Elmer model 21 spectrophotometer, using lithium fluoride optics and dilute (*ca.* 0.3%) solutions of the sample in carbon tetrachloride.

(17) Determined as films on silver chloride plates with a Perkin-Elmer model 21 spectrophotometer using sodium chloride optics.

(18) F. D. Gunstone, *J. Chem. Soc.*, 1274 (1952); *J. Sci. Food Agr.*, **3**, 129 (1953).

(19) Determined with a Beckman model DU spectrophotometer in ethanol solution.

(20) Ricinoleic acid has $[\alpha]_D^{29}$ +5° (*c* 1.2, chloroform).

dissolved in 20 ml. ethanol was hydrogenated at 1 atm. 0.5 hr. with platinum oxide catalyst. After filtration and evaporation, 0.306 g. was obtained, m.p. 67–73°. After one recrystallization from hexane and one from hexane-chloroform, 0.128 g. of 14-hydroxyeicosanoic acid was obtained, m.p. 83.5–85.0°.

Anal. Calcd. for $C_{20}H_{40}O_3$: C, 73.1; H, 12.3 Found: C, 73.4; H, 12.3.

Conversion of methyl lesquerolate to eicosanoic acid[5] (IIa). Methyl lesquerolate (0.204 g.) dissolved in 20 ml. ethanol was hydrogenated at 1 atm. 0.5 hr. with platinum oxide catalyst. After filtration and evaporation, the dihydro ester (IIa) was obtained, m.p. 54–58°. This material was recrystallized from hexane and 0.122 g. was obtained, m.p. 56–59°.

Anal. Calcd. for $C_{21}H_{42}O_3$: OMe, 9.1. Found, 9.4.

Dihydro ester (IIa; 0.084 g.) was refluxed 17 hr. with 0.035 g. of red phosphorus and 3 ml. of hydriodic acid (sp. gr. 1.7). The mixture was diluted with water and extracted repeatedly with ether. Combined ether extracts were washed with 5% sodium metabisulfite, then dried over sodium sulfate. Upon evaporation, 0.101 g. of clear oil was obtained; this was reduced by heating at reflux 4 hr. with 0.20 g. of granular zinc, 5 ml. of methanol and 1 ml. of concd. hydrochloric acid. The mixture was then diluted with water and extracted repeatedly with ether. After drying and evaporation, there was obtained 0.085 g. of solid ester, m.p. 40–43°. Gas chromatographic analysis indicated this material to be 90.9% methyl eicosanoate. This ester was saponified as described and 0.052 g., m.p. 66–73°, was obtained. Recrystallization from methanol produced 0.019 g., m.p. and mixed m.p. with authentic eicosanoic acid, 74–75°.

Permanganate-periodate oxidation of lesquerolic acid[6] (Ib). Lesquerolic acid (0.093 g., 0.285 mmole) and 0.118 g. of potassium carbonate were dissolved in 60 ml. of water. To this mixture was added a solution of 0.478 g. of sodium periodate and 1.0 ml. of 0.057*M* potassium permanganate in 60 ml. of water. The mixture was stirred at room temperature for 24 hr., then reduced with sodium metabisulfite, acidified with hydrochloric acid, and extracted with ether. Ether extracts after drying and evaporation yielded a semisolid mixture which was fractionated by triturating repeatedly with petroleum ether (b.p. 30–60°). The insoluble residue, 0.051 g., had m.p. 96–105°. By recrystallizing this from chloroform-hexane, 0.023 g. was obtained, m.p. 105–108°; mixed m.p. with authentic undecanedioic acid (m.p. 107–109°), 107–109°. The methyl ester was prepared by treatment with diazomethane in ether solution. Gas chromatographic analysis verified the identity of this product as undecanedioic acid. Gas chromatographic analysis of methyl esters of the petroleum ether-soluble acids indicated the presence of a C_9-hydroxy acid, together with small amounts of undecanedioic acid and unidentified impurities.

A parallel oxidation of ricinoleic acid was carried out under similar conditions. The resulting petroleum ether-soluble fraction was found by gas chromatographic analysis to contain 91% C_9-hydroxy acid, 3.5% nonandioic acid, and 5.8% unidentified components. Colorimetric analysis for carbonyl oxygen[21] indicated 0.5% keto acid (calculated as C_9-acid).

Permanganate-periodate oxidation of O-acetylricinoleic acid.[9] Ricinoleic acid (1.55 g.) was stirred overnight at room temperature with 15 ml. of acetic anhydride and 5 ml. of pyridine. The mixture was diluted with water and the *p*H of the solution was adjusted so as to be slightly acid, then extracted with ether. From the combined dried ether extracts was obtained 1.39 g. of the acetylated acid; its infrared spectrum showed maxima at 5.73 μ (ester), 5.83 μ (acid), and no OH absorption.[22]

O-Acetylricinoleic acid (1.20 g.) was stirred 21 hr. in an aqueous solution containing 5.96 g. of sodium periodate, 1.44 g. of potassium carbonate, and 0.077 g. potassium permanganate. The mixture was worked up as described; 1.30 g. of semisolid product was obtained. This product was fractionated by repeated extraction with petroleum ether (b.p. 30–60°). The extract yielded 0.665 g. of oil; the residue was 0.491 g. of white crystals, m.p. 104–106° (nonanedioic acid).

A 0.132-g. portion of the petroleum ether-soluble fraction (see above) was stirred 2 hr. at room temperature with 5 ml. of 0.8*N* ethanolic potassium hydroxide. This mixture was worked up in the usual manner, avoiding prolonged exposure to mineral acid; 0.094 g. of crystalline D-3-hydroxynonanionic acid was obtained, m.p. 37–43°. By two recrystallizations from petroleum ether, 0.015 g. was obtained, m.p. 43–46° (lit., m.p. 49–50°,[23] 51.1–51.6°[13]).

Permanganate-periodate oxidation of O-acetyllesquerolic acid (Ic). Lesquerolic acid (Ib; 0.40 g.) was treated 3 days with 5 ml. pyridine and 5 ml. of acetic anhydride. The mixture was then chilled, diluted with water, acidified with hydrochloric acid, and extracted with petroleum ether. The combined extracts were dried with sodium sulfate and yielded 0.36 g. of *O*-acetyllesquerolic acid.

The 0.36-g. portion of Ic was subjected to permanganate-periodate oxidation essentially as described for *O*-acetylricinoleic acid. After 20 hr., the reaction mixture was worked up in the usual manner. An oily product was obtained, 0.43 g.; a portion of this was withdrawn, saponified and methylated, and was found by gas chromatographic analysis to contain a considerable proportion of unoxidized starting material. Consequently, the remainder of this oily product (0.23 g.) was subjected to 3 days' additional oxidation under similar conditions. From this second oxidation was obtained 0.22 g. of semisolid product; this material was fractionated by trituration with ice-cold petroleum ether. An insoluble fraction (0.098 g.) and a soluble one (0.111 g.) were obtained. The soluble fraction was saponified by stirring 3 hr. at room temperature with 0.8*N* ethanolic potassium hydroxide. The mixture was worked up in the usual way and 0.105 g. of oil was obtained. Efforts to crystallize this product were fruitless; when this mixture was analyzed by gas chromatography (in the form of methyl esters prepared with diazomethane), it was found to contain 69% hydroxynonanoic acid, 22% undecanedioic acid, and minor amounts of unidentified fragments. The hydroxynonanoic acid was purified in the form of its methyl ester by preparative gas chromatography, using a Burrell Kromatog K-5 instrument and a column packed with Resoflex LAC-2-R-446 supported by Johns-Manville Celite 545. The operating temperature was 197°. Several runs were made, condensing the hydroxy ester in a capillary tube. Combined condensates amounted to 5 mg.; this material was saponified to yield acid having m.p. 42–45°, undepressed when mixed with authentic D-3-hydroxynonanoic acid (m.p. 43–46°). This degradation product was also shown by X-ray diffraction to be identical with a known specimen of D-3-hydroxynonanoic acid.

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(21) Determined by the general method of Henick, Benca, and Mitchell [*cf. J. Am. Oil Chemists' Soc.*, 31, 88 (1954)].

(22) Determined with a Baird model KM-1 spectrophotometer, using *ca.* 3% solution of the sample in chloroform.

(23) St. E. Brady, *J. Am. Chem. Soc.*, 61, 3464 (1939). Considerably higher melting points (*ca.* 58–59°) have been reported for the DL-acid (*cf.* Ref. 13 and references cited therein).