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

The Epoxy Acids of *Chrysanthemum coronarium* and *Clarkia elegans* Seed Oils

C. R. SMITH, JR., M. O. BAGBY, R. L. LOHMAR, C. A. GLASS, AND I. A. WOLFF

Received August 26, 1959

Coronaric acid, the constituent epoxy acid of *Chrysanthemum coronarium* seed oil, is characterized as *cis*-9,10-epoxy-*cis*-12-octadecenoic acid. The epoxy acid of *Clarkia elegans* seed oil is identified as vernolic acid.

Vernolic acid, the principal fatty acid of *Vernonia anthelmintica* seed oil, was the first epoxy fatty acid found to occur naturally. Gunstone[2] proved its structure to be *cis*-12,13-epoxy-*cis*-9-octadecenoic acid. Vernolic acid was also found by Bharucha and Gunstone in the oil of *Cephalocroton cordofanus*,[3] and by Hopkins and Chisholm in oils of *Vernonia colorata*,[4] *Hibiscus esculentus* (okra),[4] and *Hibiscus cannabinus*.[5] The screening of a considerable number of previously uninvestigated species suggested that epoxy acids are constituents of numerous seed oils in the composite family.[6] The discovery of a second epoxy fatty acid as a constituent of a vegetable oil was reported in a preliminary communication from this laboratory.[7] This new epoxy acid, coronaric acid (I), was found in *Chrysanthemum coronarium* (garland chrysanthemum) seed oil and was tentatively identified as *cis*-9,10-epoxy-*cis*-12-octadecenoic acid. A third epoxy acid, 15,16-epoxy-9,12-octadecadienoic acid, was shown by Gunstone and Morris[8] to be a constituent of *Camelina sativa* seed oil. This paper will present conclusive evidence for the structure suggested tentatively for coronaric acid. It will also show that the epoxy acid in *Clarkia elegans* seed oil[9] is vernolic acid. The epoxy acids in both of these oils were accompanied by mixtures of fatty acids of the common types (see Experimental.)

Structural work on coronaric acid (Ia) was carried out on different samples of *Chrysanthemum coronarium* seed oil containing 0.15 to 0.85%[6] oxirane oxygen as determined by Durbetaki's procedure.[10] The epoxy acid was not isolated as such, but was converted by acetolysis and saponification to an unsaturated dihydroxy acid (II); the latter was isolated by solvent partitioning with aqueous methanol–petroleum ether. The procedure followed was that used by Gunstone in his work with *Vernonia anthelmintica* oil.[2] Chemical determination of oxirane oxygen in *Chrysanthemum coronarium* oil was in agreement with infrared evidence and indicated the presence of an epoxy group, but no appreciable hydroxyl. Therefore, II was undoubtedly derived from Ib.

The unsaturated dihydroxy acid (II), m.p. 57–58°, was shown by infrared spectrum and quantitative hydrogenation to have one *cis* double bond. Its melting point was markedly depressed by admixture with *threo*-12,13-dihydroxy-*cis*-9-octadecenoic acid (X) prepared from *Vernonia anthelmintica* seed oil. Hydrogenation of II produced saturated dihydroxy acid III, m.p. 93–93.5°, whose melting point was markedly depressed by admixture with *threo*-12,13-dihydroxyoctadecanoic acid (m.p. 96–97°). No depression of melting point was evident, however, on admixture with *threo*-9,10-dihydroxyoctadecanoic acid. Thus it was indicated that *C. coronarium* oil contains an epoxy acid which is different from, and isomeric with, vernolic acid.

The unsaturated dihydroxy acid, II, was subjected to periodate cleavage, using Gunstone's procedure for cleaving compound X. Two C_9 aldehydes, compound IV and V, resulted; these were separated by steam distillation and characterized as 2,4-dinitrophenylhydrazones. The derivative o IV had $\lambda_{max}^{C_2H_5OH}$ 375 mμ, strongly suggesting α,β-unsaturation.[11] The 2,4-dinitrophenylhydrazone of V was isolated as an ethyl ester and had $\lambda_{max}^{C_2H_5OH}$ 356 mμ. II was also cleaved by the permanganate-periodate procedure of Lemieux and von Rudloff.[12] A model experiment was first carried out with X; the nonvolatile acid obtained was nonandioic acid, showing that no double bond migration occurred in

(1) This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) F. D. Gunstone, *J. Chem. Soc.*, **1954**, 1611.

(3) K. E. Bharucha and F. D. Gunstone, *J. Sci. Food Agr.*, **7**, 606 (1956).

(4) M. J. Chisholm and C. Y. Hopkins, *Can. J. Chem.*, **35**, 358 (1957).

(5) C. Y. Hopkins and M. J. Chisholm, *J. Am. Oil Chemists' Soc.*, **36**, 95 (1959).

(6) F. R. Earle, Q. Jones, and I. A. Wolff, presentation before the 32nd Fall Meeting, American Oil Chemists' Society, Chicago, Oct. 20–22, 1958.

(7) C. R. Smith, K. F. Koch, and I. A. Wolff, *Chem. & Ind. (London)*, **1959**, 259.

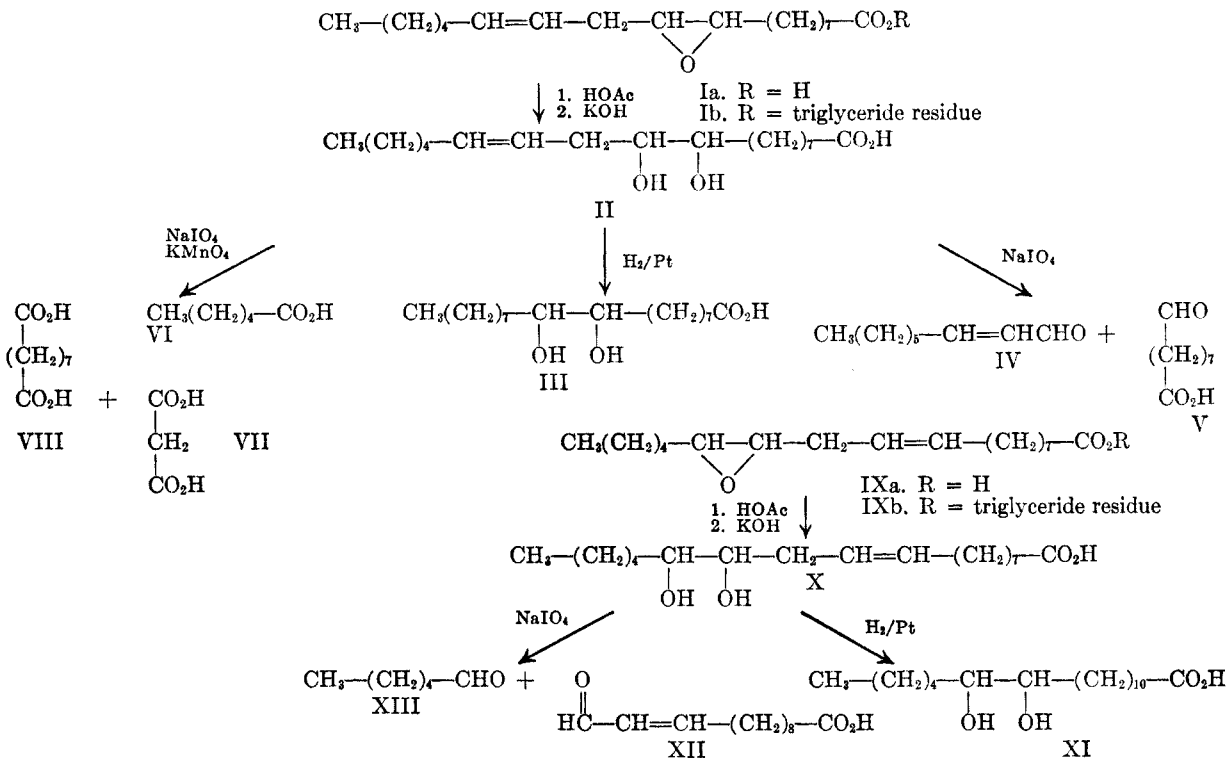
(8) F. D. Gunstone and L. J. Morris, *J. Chem. Soc.*, **1959**, 2127.

(9) F. R. Earle, E. H. Melvin, L. H. Mason, C. H. Van-Etten, and I. A. Wolff, *J. Am. Oil Chemists' Soc.*, **36**, 304 (1959).

(10) A. J. Durbetaki, *Anal. Chem.*, **28**, 2000 (1956).

(11) A. E. Gillam and E. S. Stern, *Introduction to Electronic Absorption Spectroscopy in Organic Chemistry*, Edward Arnold, London, 1954, p. 706.

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



this oxidation and that the same method presumably could be used with II. Permanganate-periodate oxidation of II yielded hexanoic and nonandioic acids, which were separated by steam distillation and characterized as *p*-bromophenacyl esters. The middle fragment, malonic acid, was not isolated. These degradations clearly established the structure of coronaric acid (I) as *cis*-9,10-epoxy-*cis*-12-octadecenoic acid. They also showed that when II was oxidized with periodate under acidic conditions, its double bond migrated into conjugation, following the reaction observed by Gunstone in the case of X.[2]

The assignment of a *cis* configuration to the 9,10-epoxy group of coronaric acid (Ia) followed from the fact that a dihydroxy acid of the *threo*-series was produced by acetolysis of the epoxide ring followed by alkaline hydrolysis of the resulting 9,10-dihydroxy monoacetate.[13-16] However, Bharucha and Gunstone[14] found that X obtained from *Vernonia* oil was optically active and that the saturated dihydroxy acid derived from X apparently was optically active also. They reasoned that derivation of an optically active glycol from IXb resulted from formation of unequal amounts of the two isomers obtainable by opening the oxirane ring. Coronaric acid is probably also optically active, although we did not have sufficient material to check this point experimentally. The specimen used for determining the mixed melting point with III was DL-*threo*-9,10-dihydroxyoctadecanoic acid prepared by epoxidation of oleic acid; it had a melting point very similar to that of III. It may be noted that McGhie and coworkers[16] resolved DL-*threo*-9,10-dihydroxyoctadecanoic acid into its enantiomorphic forms, and found that these optical isomers had m.p. 99.5° and $[\alpha]_D \pm 23°$.

The DL form of II was apparently reported by McKay and Bader,[17] who prepared a compound which they termed "*cis*-9,10-dihydroxy-12-octadecenoic acid." In view of configurational reassignments by Swern,[13] however, this acid should be considered a *threo*-glycol. McKay and Bader obtained it only as an oil.

A sample of *Clarkia elegans* oil containing 0.54% oxirane oxygen[9] was obtained for isolation work. The infrared spectrum of this oil indicated no free hydroxyl. The epoxy acid was converted to an unsaturated dihydroxy acid and thus isolated; the latter was shown by mixed melting point determinations, hydrogenation, and oxidative cleavage to be identical with X isolated from *Veronia anthelmintica* oil by Gunstone's procedure.[2] The mother liquors from crystallization of X that was isolated from *Clarkia elegans* were carefully chromatographed, but no evidence for the presence of a dihydroxy acid other than X was found. The substituent epoxy acid of *Clarkia elegans* seed oil therefore is vernolic acid.

(13) D. Swern, *J. Am. Chem. Soc.*, **70**, 1235 (1948).
(14) K. E. Bharucha and F. D. Gunstone, *J. Chem. Soc.*, **1956**, 1611.
(15) W. J. Gensler and H. N. Schlein, *J. Am. Chem. Soc.*, **78**, 169 (1956).
(16) J. F. McGhie, W. A. Ross, and D. J. Polton, *Chem. & Ind. (London)*, **1956**, 353.
(17) A. F. McKay and A. R. Bader, *J. Org. Chem.*, **13**, 75 (1948).

Vernolic acid, coronaric acid, and the 15,16-epoxylinoleic acid in *Camelina sativa* oil have a striking feature in common: All three may be considered common fatty acids of the type having methylene-interrupted unsaturation, but with one double bond replaced by an epoxide ring. It appears possible that these epoxy acids may be derived biogenetically from linoleic or linolenic acid through the action of unknown epoxidases which preferentially attack one or the other of the available double bonds.[17a] Epoxy acids may be of rather wide occurrence in the plant kingdom, having now been found in seed oils of genera distributed among several plant families—the Compositae (includes *Vernonia* and *Chrysanthemum*), Euphorbiaceae (*Cephalocroton*), Malvaceae (*Hibiscus*), Cruciferae (*Camelina*), and now in one of the Onagraceae (*Clarkia*).

In recent years, epoxy acids prepared chemically have attained a position of industrial importance. Consequently, natural sources of such acids may be of considerable future significance.

EXPERIMENTAL[18]

Isolation of threo-9,10-dihydroxy-12-octadecenoic acid (II) *from Chrysanthemum coronarium oil.* Coarsely ground seeds of *Chrysanthemum coronarium* were extracted overnight in a Soxhlet apparatus with 30–60° petroleum ether. 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. Part of the isolation work was carried out on oil containing 0.85% oxirane-oxygen;[10] oil containing only 0.15% oxirane-oxygen was used for the rest.[19] (These percentages corresponded to a content of 2.8 to 15.8% monoepoxy C_{18} acids.) The infrared spectrum of the oil showed a low-intensity maximum at 11.85 μ (epoxy), but no appreciable hydroxyl content. Acids accompanying the epoxy acid in this oil were apparently predominantly of the common types: 11% saturated, 24% monoethenoid, and 59% nonconjugated diethenoid.[6]

Acid-free *C. coronarium* oil (32.5 g.) was refluxed with glacial acetic acid (300 ml.) for 3 hr. under an atmosphere of nitrogen. Most of the acetic acid was removed by distillation; the residue was diluted with water, then extracted with ether. The oil obtained by evaporation of the ether extract (29.6 g.) had a negligible oxirane-oxygen content,[10] and was saponified by refluxing with 2*N* ethanolic potassium hydroxide (150 ml.) under nitrogen for 30 min. The resulting mixture was diluted with water and extracted with ether to remove unsaponifiables. The alkaline liquor, on acidification and extraction with ether, yielded 27.1 g. of free acids.

Free acids thus obtained (22.0 g.) were fractionated by solvent partitioning between aqueous methanol and petroleum ether following Gunstone's scheme.[2] The combined methanol fractions yielded 0.815 g. of dihydroxy acid concentrate; additional concentrate was prepared similarly. A 2.85-g. portion of such concentrate was purified by recrystallization from acetone (28 ml.) at −45°. A yellow solid (1.91 g.) was obtained which was similarly recrystallized from 19 ml. of acetone. A 1.52-g. yield of II, m.p. 53–57°, resulted. On admixture with *threo*-12,13-dihydroxy-9-octadecenoic acid (X) a melting point of 42–52° was observed. The specimen of X (lit.[2] m.p. 53–54°) used in this mixed melting point determination was prepared in this laboratory from *Vernonia anthelmintica* seed oil, following Gunstone's procedure. The infrared spectrum of II showed absorption at 2.95 μ (medium intensity); no *trans* C=C absorption.

Anal. Calcd. for $C_{18}H_{34}O_4$: C, 68.7; H, 10.9. Found: C, 68.6; H, 10.6; absorbs 0.95 mol. of hydrogen (*cf.* following paragraph for conditions).

Hydrogenation of threo-9,10-dihydroxy-12-octadecenoic acid (II). A 0.364-g. portion of II was hydrogenated at atmospheric pressure and room temperature, using platinum oxide catalyst in ethanol solution. Crude III was obtained having m.p. 83–91°. Recrystallization from chloroform yielded 0.245 g. of III, m.p. 93–93.5°; no depression of melting point was observed on admixture with authentic *threo*-9,10-dihydroxyoctadecanoic acid (lit.[20] m.p. 93–94°). A marked depression of melting point (83–93°) was evident, however, on admixture with *threo*-12,13-dihydroxyoctadecanoic acid (XI) (lit.[20] m.p. 95–96°). The specimen of XI used for mixed melting point determination was prepared in this laboratory by hydrogenation of X and had m.p. 96–97°.

Periodate oxidation of II. A 1.01-g. portion of II dissolved in 50 ml. of ethanol was combined with 0.8 g. of sodium periodate in 38 ml. of 1*N* sulfuric acid. The resulting solution was kept at 40° for 15 min., then chilled, diluted with 100 ml. of water, and extracted with ethyl ether four times. The combined ether extracts were dried over sodium sulfate; most of the ether was removed by distillation. The residual liquid, which contained considerable ethanol, was diluted with water to give Solution A, which was extracted repeatedly with petroleum ether to remove the volatile aldehyde. The combined petroleum ether extracts were distilled to remove most of the volatile solvent and the residue was steam-distilled. The cloudy distillate was extracted four times with ethyl ether (combined, dried ether extracts = Solution C).

Solution A, having been previously extracted with petroleum ether, was extracted repeatedly after steam distillation with ethyl ether (combined extracts = Solution B).

2,4-Dinitrophenylhydrazone of volatile aldehyde (IV). Solution C was distilled to remove the volatile solvent. A 2,4-dinitrophenylhydrazone of IV was prepared from the residue according to the procedure of Shriner, Fuson, and Curtin.[21] The crude product obtained was 0.584 g. of orange solid, m.p. 84–92°. After two recrystallizations from ethanol, the substance had m.p. 119–121° (lit.[22] m.p. for 2-nonenal-2,4-dinitrophenylhydrazone, 124–124.5°). The visible spectrum showed λ_{max} 375 mμ (ϵ 28,650; conjugated carbonyl derivative[11]).

(17a) NOTE ADDED IN PROOF: Since this paper was submitted, *cis*-9,10-epoxyoctadecanoic acid has been found in *Tragopogon porrifolius* seed oil [M. J. Chisholm and C. Y. Hopkins, *Chem. and Ind. London*, 1154 (1959)] and also in wheat stem rust lipids [A. P. Tulloch, B. M. Craig, and G. A. Ledingham, *Can. J. Microbiol.*, **5**, 485 (1959)]. This saturated epoxy acid is related to oleic acid in the same manner that the unsaturated ones are related to linoleic or linolenic acid.

(18) Melting points were determined with a Fisher-Johns block and are uncorrected. Infrared spectra were measured with a Perkin-Elmer model 21 rock salt spectrophotometer. Visible spectra were determined in ethanol solution with a Beckman DU spectrophotometer. The mention of trade names or products does not constitute an endorsement by the Department of Agriculture over those not named.

(19) There is considerable variability among samples in the oxirane-oxygen content of the oil of *C. coronarium*. Seed from a source other than used in this investigation had a negligible oxirane-oxygen content.

(20) W. F. Huber, *J. Am. Chem. Soc.*, **73**, 2731 (1951).

(21) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, *Systematic Identification of Organic Compounds*, 4th Ed., John Wiley & Sons, New York, 1956.

(22) C. J. Martin, A. I. Shepartz, and B. F. Daubert, *J. Am. Chem. Soc.*, **70**, 2601 (1948).

Anal. Calcd. for $C_{15}H_{20}N_4O_4$: C, 56.2; H, 6.3; N, 17.5. Found: C, 56.2; H, 6.1; N, 17.7.

2,4-Dinitrophenylhydrazone of nonvolatile aldehyde (V). Solution B was distilled to remove most of the solvent; 0.377 g. of crude aldehyde (V) was obtained as a light-colored oil. This oil was steam-distilled to remove any volatile impurities. The residual aqueous liquor was saturated with sodium chloride and extracted repeatedly with ethyl ether. The combined ether extracts were dried over sodium sulfate; 0.271 g. of aldehydoacid (V) was obtained. A 2,4-dinitrophenylhydrazone was prepared from V according to the procedure of Shriner, Fuson, and Curtin.[21] A crude yield (0.323 g.) of poorly crystalline yellow solid was obtained as an ethyl ester, m.p. 47–52°, and was recrystallized from ethanol. A sample having m.p. 54–56° was obtained (lit.[23] m.p. 63–64°); its spectra showed λ_{max} 356 (ϵ 18,320; no conjugation[11]) and an infrared maximum at 5.73 μ (ester).

Anal. Calcd. for $C_{17}H_{24}N_4O_6$: C, 53.7; H, 6.4; N, 14.7. Found: C, 54.0; H, 6.4; N, 15.1.

Periodate-permanganate oxidation of threo-12,13-dihydroxy-9-octadecenoic acid (X). The general procedure of Lemieux and von Rudloff[12] was followed. A 0.314-g. portion (1 mmol.) of X, 0.416 g. (3 mmol.) of potassium carbonate, 2.60 g. of sodium periodate (12 mmol.), and 0.02 g. (0.13 mmol.) of potassium permanganate were combined in 400 ml. of water. The mixture was allowed to stand 24 hr. at room temperature with occasional stirring; the suspended solid gradually disappeared during this time. The mixture was acidified with 10% sulfuric acid and extracted with ether. Evaporation of the ether after drying over sodium sulfate yielded 0.163 g. of mixed acids. This mixture was steam-distilled. After the distillation, the aqueous residue was extracted with ether. On evaporation, the dried ether extract yielded 0.089 g. of acid, m.p. 97–104°. Recrystallization from petroleum ether–chloroform yielded 0.060 g., m.p. 105–106°. No depression of melting point was observed on admixture with authentic nonandioic acid. The other cleavage products were not characterized.

Periodate-permanganate oxidation of II. The oxidation of II was carried out in the same manner as that of X. A 0.302-g. portion of II was oxidized; 0.291 g. of mixed acids were obtained. On steam-distilling this, 0.053 g. of volatile acid (VI) was obtained from the distillate and 0.167 g. of nonvolatile acid (VII), m.p. 96–103°, from the residual liquor. Recrystallization of the latter from ethyl acetate–petroleum ether produced a sample having m.p. 103.5–105°; no depression of melting point was observed on admixture with authentic nonandioic acid (VIII). The *bis-p*-bromophenacyl ester of the dicarboxylic acid was prepared, m.p. 129–130.5°; mixed m.p. with the authentic derivative of nonandioic acid, 130–131.5°.[21]

The steam-volatile acid was dissolved in 4.2 ml. of 0.1*N* sodium hydroxide. The resulting solution was made slightly acidic by addition of 0.15 ml. of 0.1*N* hydrochloric acid, and was refluxed 1 hr. with 5 ml. of 95% ethanol and 0.114 g. of *p*-bromophenacyl bromide. Most of the ethanol was removed under reduced pressure, and the residue was extracted with ether. The ether extracts were dried over sodium sulfate and evaporated, yielding 0.111 g. of crude *p*-bromophenacyl ester, m.p. 47–59°. Two recrystallizations from 80% ethanol and two from hexane yielded a sample with m.p. 67–69°. The mixed melting point of this material with authentic *p*-bromophenacyl hexanoate (m.p. 70.5–71°) was 68.5–71°. On admixture with *p*-bromophenacyl heptanoate (m.p. 69–70°), the melting point was depressed to 61–64°.

Isolation of threo-12,13-dihydroxy-9-octadecenoic acid (X) *from Clarkia elegans oil.* Coarsely ground seeds of *Clarkia elegans* were extracted overnight in a Soxhlet apparatus with 30–60° petroleum ether. A 36.7% yield of oil was obtained containing 0.73% oxirane-oxygen.[9] Acids accompanying the epoxy acid in this oil were apparently predominantly of the common types: 14% saturated, 20% monoethenoid, and 57% nonconjugated diethenoid.[9] Its infrared spectrum showed no appreciable hydroxyl content. A 91.6-g. portion of acid-free oil was treated with acetic acid and saponified as described for *C. coronarium*. After acetolysis, the oil had a negligible oxirane-oxygen content. A yield of 83.9 g. of free acids and 1.1 g. of unsaponifiable matter was obtained. The dihydroxy acids were concentrated, following Gunstone's solvent partitioning scheme.[2] A dihydroxy acid concentrate of 7.9 g. was obtained. Fraction D[2] (5.7 g.) was recrystallized twice from acetone (4 ml.) at −40°. A solid (0.101 g.), m.p. 49–51.5°, was obtained; further recrystallization increased the m.p. to 50–52°. No depression of melting point was observed on admixture with authentic X prepared from *Vernonia anthelmintica* oil. The infrared spectrum of the solid, m.p. 49–51.5°, indicated no *trans*-unsaturation. Mother liquor from the recrystallization of the dihydroxy acid concentrate was evaporated and the residue chromatographed on neutral alumina, collecting numerous fractions. No evidence was found for a dihydroxy acid other than X.

Anal. Calcd. for $C_{18}H_{34}O_4$: Neut. equiv., 314. Found: Neut. equiv., 313; absorbs 1.0 mol. of hydrogen.

Hydrogenation of threo-12,13-dihydroxy-9-octadecenoic acid (X). The dihydroxy acid (X) from *Clarkia* oil was hydrogenated as described for II. Crude XI was obtained, m.p. 90–95°, and was recrystallized from ethyl acetate. A sample, m.p. 94–96.5°, was obtained; no depression of melting point was observed on admixture with X (prepared from authentic IX from *Vernonia anthelmintica* seed oil), m.p. 96–97°.

Periodate oxidation of X. The dihydroxy acid (X) from *Clarkia* oil (0.803 g.) was subjected to periodate cleavage and separated in much the same manner as with II.

A yield of 0.642 g. of nonvolatile aldehyde (XII) was obtained; 0.413 g. of this was converted to a 2,4-dinitrophenylhydrazone by the method of Shriner, Fuson, and Curtin.[21] Orange crystals (0.625 g.) of a crude derivative were obtained; the melting point of this material after recrystallization from ethanol was 63–68.5°. By a combination of recrystallizations from ethanol and chromatography on alumina (eluting with benzene), 0.096 g. of the derivative was obtained, m.p. 81–83° (lit.[2] m.p. for 11-formyl-10-undecenoic acid 2,4-dinitrophenylhydrazone, 81.5–83°); λ_{max} 375 mμ (ϵ 26,290; conjugated carbonyl derivative[11]).

The steam-volatile aldehyde (XIII) obtained was similarly converted to a 2,4-dinitrophenylhydrazone (0.235 g.), m.p. 84–93°, and similarly purified; 0.053 g., m.p. 102.5–104°, was obtained. No depression of melting point was observed on admixture with authentic *n*-hexanal-2,4-dinitrophenylhydrazone (lit.[21] m.p. 104°). The identity of XIII with *n*-hexanal was confirmed by paper chromatography, using the solvent system heptane/methanol.[24] The observed R_f value was 0.80 for the 2,4-dinitrophenylhydrazones of both XIII and authentic *n*-hexanal when run simultaneously.

Permanganate oxidation of X. Oxidation of X (0.636 g.) was carried out with potassium permanganate in acetic acid solution, essentially as described by Begemann and coworkers.[25] After completion of the oxidation, most of the acetic acid was removed by distillation. The residue was dissolved in dilute sulfuric acid and decolorized with sodium sulfite. The resulting aqueous solution was extracted with ether and dried over sodium sulfate. The acids obtained by evaporation of solvent were separated by steam distillation; 0.101 g. of steam-volatile and 0.417 g. of crude nonvolatile acid were obtained. The latter was recrystallized from water and then from petroleum ether–ethyl acetate; 0.055 g., m.p. 102–105° resulted. This melting point was undepressed on admixture with authentic nonandioic acid. The nonvolatile

(23) J. T. Scanlan and D. Swern, *J. Am. Chem. Soc.*, **62**, 2305 (1940).

(24) D. F. Meigh, *Nature*, **170**, 579 (1952).

(25) P. H. Begemann, I. G. Keppler, and H. A. Boekenoogen, *Rec. trav. chim.*, **69**, 439 (1950).

acid was also characterized as a *p*-bromophenacyl ester, prepared as described by Shriner, Fuson, and Curtin.[21] A 0.091-g. portion of the acid yielded 0.043 g. of *p*-bromophenacyl ester, m.p. 129–131°, undepressed on admixture with authentic *p*-bromophenacyl nonandioate. The volatile acid (0.101 g.) was similarly converted to a *p*-bromophenacyl ester, 0.011 g. after two recrystallizations from 50% ethanol, m.p. 68–70°, undepressed on admixture with authentic *p*-bromophenacyl hexanoate. The malonic acid fragment (VII) was not isolated.

Acknowledgment. The authors wish to thank Mrs. Clara McGrew for microanalyses; Dr. E. J. Dufek for furnishing a sample of *threo*-9,10-dihydroxyoctadecanoic acid; and Dr. Quentin Jones, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, for his cooperation in obtaining seeds.

PEORIA, ILL.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, NEW YORK UNIVERSITY]

Some Neutral Components of Cigarette Smoke[1]

ALVIN I. KOSAK AND JAMES S. SWINEHART[2]

Received September 11, 1959

The paraffin wax fraction of cigarette smoke has been shown to contain the fifteen normal alkanes from docosane to hexatriacontane and branched alkanes having between 21 and 32 carbon atoms, inclusive. About one third of this paraffin mixture consists of hentriacontane and tritriacontane. Also present in the neutral fraction of smoke are squalene, isosqualene, stigmasterol, β-sitosterol, and probably γ-sitosterol.

Carcinogenicity tests on mice[3] of fractions of cigarette smoke indicated that the fractions designated as K and M[4] were the most active. We have previously described, in part, the chemical composition of these materials; the present paper reports our further studies on the neutral components of M and related fractions.

An earlier paper from our laboratory[4] had presented indicative proof of the presence of hentriacontane and tritriacontane in the smoke of blended American cigarettes. A later report by Cuzin *et. al.*[5] stated that no evidence was found for the presence in smoke (or tobacco leaf) of alkanes of more than 32 carbons.[6] A reinvestigation of our material was accordingly undertaken. A purified paraffin mixture of M'[4] was prepared as before and its x-ray diffractogram was compared with those of synthetic samples of hentriacontane, tritriacontane, and pentriacontane; the respective values for the long-spacings were 42.7 Å, 41.8 Å, 44.1 Å, and 46.7 Å.[7] These results confirmed those obtained earlier by us in that the value for the wax from smoke fell between those of the C_{31} and C_{33} hydrocarbons; we were later able to obtain a mass spectrometric analysis which clearly showed this wax to be composed largely of equal amounts of the C_{31} and C_{33} *normal* alkanes, some C_{32} homolog, and small amounts of other alkanes (Table I).

TABLE I

MASS SPECTRA OF PARAFFIN MIXTURE FROM M'

No. of Carbons	% Normal	% Branched
30	2	
31	38	1
32	14	
33	39	1
34	5	

To obtain a broader analysis of the higher-alkane distribution in cigarette smoke, a sample of MM[4] was exhaustively extracted with concentrated

(1) Portions of this paper were presented at the Seventh International Cancer Congress, London, July 1958 and at the Meeting-in-Miniature of the New York Section of the American Chemical Society, April 1959; *cf.* also A. I. Kosak and J. S. Swinehart, *Chem. and Ind. (London)*, 1007 (1958).

(2) Abstracted from a part of the Ph.D. thesis of J.S.S., New York University, April 1959.

(3) W. E. Smith, N. Nelson, L. Orris, and A. I. Kosak, unpublished data.

(4) A. I. Kosak, J. S. Swinehart, and D. Taber, *J. Natl. Cancer Inst.*, **17**, 375 (1956).

(5) J. L. Cuzin, L. V. Thoi, and M. S. Morec, paper presented at the second International Tobacco Science Congress, Brussels, June 1958.

(6) The x-ray diffraction data listed by these workers for their highest molecular weight fraction was the same as that which we had reported and the difference is one of interpretation. They ascribed their *d*-value of 42.7 Å to dotriacontane. We felt that the *d*-value of this magnitude more probably represented a mixture of *odd*-numbered alkanes for four reasons: first, the paraffins of other plant waxes consist principally of this class of alkanes; second, a mixture of normal alkanes will have the same long-spacing as that of a single hydrocarbon whose molecular weight equals that of the average of the mixture, if the hydrocarbons of the mixture do not differ from each other by more than four carbon atoms [S. H. Piper, A. C. Chibnall, S. J. Hopkins, A. Pollard, J. A. B. Smith, and E. F. Williams, *Biochem. J.*, **25**, 2072 (1931)]; third, the "blurred" diffraction lines (Piper *et al.*, *loc. cit.*); fourth, evidence for the occurrence of tritriacontane in tobacco leaf [A. C. Chibnall, S. H. Piper, A. Pollard, E. F. Williams, and P. N. Sahai, *Biochem. J.*, **28**, 2189 (1934)].

(7) The values for the synthetic materials are in good agreement with those reported by D. R. Kreger in J. Bouman, *Selected Topics in X-Ray Crystallography*, Amsterdam, 1951, p. 316.