perature for several days 3.8 g. of crude crystalline material was deposited. This was chromatographed on a Florisil column using benzene as solvent. The benzene eluates gave 1.4 g. of a monohydroxy sapogenin. This material (0.2 g.) was refluxed for 30 min. in 5 ml. of acetic anhydride in pyridine (1:1). The solution was evaporated to dryness *in vacuo* and the residue recrystallized from methyl alcohol to give needles, m.p. 194–197°. These crystals were identified as yamogenin acetate by comparison of its infrared spectra with that of an authentic specimen. Elution with benzene-chloroform (1:1) and chloroform gave 1.8 g. of chiapagenin which, after recrystallization from methyl alcohol, melted at 249–251°, $[\alpha]_D^{24°}$ −126°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.25 H, 10.06.

Acetylation of 0.2 g. of chiapagenin, as in the previously described manner, and recrystallization of the product from methyl alcohol yielded 0.14 g. rectangular needles, m.p. 191–193°, $[\alpha]_D^{24°}$ −127°. The infrared spectrum shows strong bands at 1739 and 1245 cm.$^{-1}$ (acetate carbonyl and —C—O—C— stretching respectively) and bands at 988 and 920 (strong), 900 and 850 (weak) cm.$^{-1}$, all four bands attributed to the normal spiroketal system. Bands at 840 and 805 cm.$^{-1}$ are due to Δ^5 unsaturation.

Anal. Calcd. for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found: C, 72.65; H, 9.22.

Conversion of chiapagenin to rockogenin. A 0.272-g. sample of chiapagenin was dissolved in a solution of 100 ml. of a 5% solution of glacial acetic acid in ethyl alcohol to which was then added 0.272 g. of platinum oxide. The mixture was hydrogenated at 3 atm. for 21 hr. at room temperature. The platinum was filtered and all the solvent removed *in vacuo*. Infrared analysis showed the double bond had been saturated (absence of 805 and 840 cm.$^{-1}$ bands). The white amorphous looking material which resulted was dissolved in 47 ml. of absolute ethyl alcohol. Concentrated hydrochloric acid (7 ml.) was then added and the resulting solution was refluxed 48 hr. Solvent was removed *in vacuo* and the residue chromatographed on a Florisil column using benzene as solvent. Elution with chloroform gave a glass which on crystallization from acetone yielded 60 mg. of crystals. This crystalline fraction was acetylated in the usual manner and gave crystals from methyl alcohol, m.p. 200–203. The infrared spectrum was identical with that of authentic rockogenin diacetate.

Conversion of correllogenin to chiapagenin. A 0.33-g. sample of correllogenin acetate was dissolved in a mixture of 5 ml. of anhydrous ether and 5 ml. of anhydrous tetrahydrofuran in a 100 ml. two-necked round bottomed flask. The solution was added, with continued stirring, to a solution of liquid ammonia (25 ml.) containing 5 ml. of methyl alcohol. Lithium wire, 0.2 g., cut in small pieces, was added rapidly. After 5 min. 2.0 g. of ammonium chloride was added and the evaporation of ammonia was hastened by a warm water bath. Water (50 ml.) was then added and the aqueous slurry extracted twice with 20-ml. portions of methylene chloride. The solvents were evaporated and the residue acetylated, using the procedure previously described, to yield 0.21 g. of short rectangular needles from methyl alcohol, m.p. 191–193°, and an infrared spectrum identical with that of natural chiapagenin diacetate.

Acknowledgment. We gratefully acknowledge the assistance, in many botanical matters, of Dr. Bernice G. Schubert, Botanist of the Plant Introduction Section, Crops Research Division, U. S. Department of Agriculture, Beltsville, Maryland. We thank Miss Oksana Panasiuk for carbon and hydrogen analyses and Mr. Carl T. Leander, Jr., under the supervision of Dr. C. Roland Eddy, for the infrared spectra. We wish to express our deep appreciation to Dr. J. J. Willaman, formerly Chief of the Plant Products Laboratory, Eastern Utilization Research and Development Division, for his inspiring leadership and for his help in obtaining the tubers of *Dioscorea chiapasensis* (Matuda).

PHILADELPHIA, PA.

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

Unique Fatty Acids from *Limnanthes douglasii* Seed Oil: The C_{20}- and C_{22}-Monoenes[2]

C. R. SMITH, JR., M. O. BAGBY, T. K. MIWA, R. L. LOHMAR, AND I. A. WOLFF

Received February 25, 1960

The principal fatty acids of *Limnanthes douglasii* seed oil are shown to include two previously unknown components: *cis*-5-eicosenoic (65%) and *cis*-5-docosenoic acid (7%). The oil also contains *cis*-13-docosenoic (erucic) acid (13%) and 10% of an unknown C_{22}-acid.

Limnanthes douglasii or meadow-foam (fam. Limnanthaceae) is an annual herb native to coastal California and presently cultivated as an ornamental.[3] A recent paper from this laboratory[4] indicated that the seed oil of this species is highly unusual in containing 94% of fatty acids longer than C-18. The present paper will report isolation and characterization of three of the four principal fatty acids of *Limnanthes douglasii* seed oil.

Isolation of pure acids. Gas chromatographic analysis of the methyl esters of the mixed acids from *Limnanthes* oil indicated that the principal components were a C_{20}-monoene, a C_{22}-monoene

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

(2) Presented before the Division of Organic Chemistry, 137th Meeting, American Chemical Society, Cleveland, Ohio, April 5–14, 1960.

(3) L. Abrams, *Illustrated Flora of the Pacific States*, Vol. III, Stanford University Press, Stanford, Calif., 1951, p 46.

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

TABLE I

GAS CHROMATOGRAPHIC ANALYSIS OF METHYL ESTERS OF *Limnanthes douglasii* SEED OIL AND VARIOUS DERIVED FRACTIONS

Percent Acid

Type of Acid	Original Oil	Hydrogenated Oil	Conc. B	Countercurrent Dist. Run I Trans. 310	Countercurrent Dist. Run I Trans. 340	Countercurrent Dist. Run II Trans. 640
C_{10}-sat.	—	tr	tr			
C_{12}-sat.	tr	tr	tr			
C_{14}-sat.	tr	0.1	0.1			
C_{16}-sat.	0.4	0.5	0.1	—	—	
C_{16}-monoene	0.3	—	—			
C_{18}-sat.	0.3	1.7	0.2	—	—	
C_{18}-monoene	1.7	tr	0.4			
C_{18}-diene	0.7	—	—	—	—	
C_{18}-triene	tr	—	—			
C_{20}-sat.	1.0	65	0.7	1.2	0.1	1.1
C_{20}-monoene	65	0.5	66	37.6	97.2	
C_{20}-unknown	0.3	0.3	—		—	
C_{21}-monoene (?)	—	—	tr	0.9		0.4
C_{22}-sat.	—	31	—	—	—	
C_{22}-unknown	10	—	2	—	0.4	
C_{22}-monoene	20	—	30	60.3	2.3	98.5
C_{24}-unknown	<1	—	—			

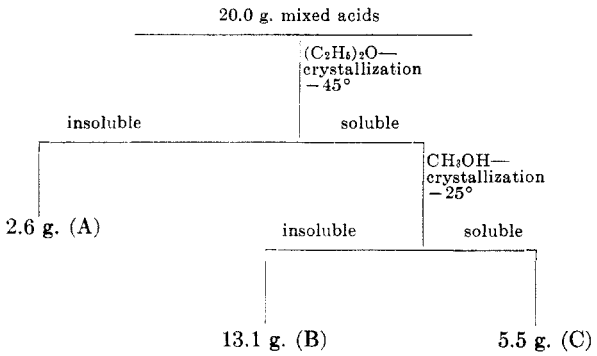

Fig. 1. Low-temperature crystallization of mixed fatty acids of *Limnanthes douglasii* seed oil

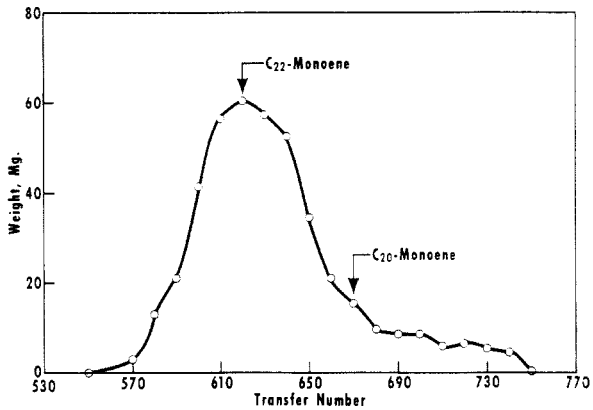

Fig. 2. Countercurrent distribution of methyl esters of *Limnanthes douglasii* seed oil fractions (Run I)

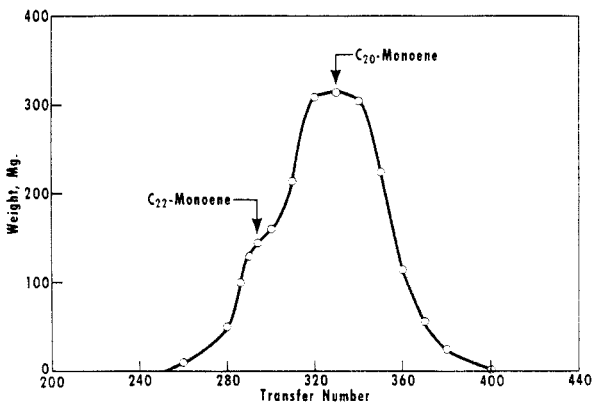

Fig. 3. Countercurrent distribution of methyl esters of *Limnanthes douglasii* seed oil fractions (Run II)

and a C_{22}-"unknown" (see Table I). When the mixed acids were hydrogenated, these unsaturated components were converted to eicosanoic (arachidic) and docosanoic (behenic) acids (Table I). Concentrates of the monoenes (A and B) and of the unknown (C) were obtained by a sequence of low-temperature crystallizations (Fig. 1 and Table I) and by countercurrent distributions (Figs. 2 and 3). Concentrate B from the low-temperature crystallization scheme was shown by gas chromatography to contain 66% C_{20}-monoene and 30% C_{22}-monoene (Table I). A portion of this concentrate B was subjected to countercurrent distribution (Run I) in a 200-tube automatic Craig-Post apparatus. The solvent system acetonitrile-hexane[5] was used and a total of 440 transfers were made by making use of an automatic fraction collector in which two transfers per tube were collected. This treatment effected only a partial resolution of the C_{20}- and C_{22}-monoene components (Fig. 2 and Table I); the C_{22}-monoene appeared as a shoulder on the peak formed by the slower-moving C_{20}-monoene. A concentrate of C_{20}-monoene suitable for structural determination was obtained by pooling material from transfers 342 to 390, having 90 to 97% purity. A C_{22}-monoene concentrate was obtained by pool-

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

ing material from appropriate tubes and was further fractionated by countercurrent distribution Run II (Fig. 3 and Table I). A small amount of C_{22}-monoene of *ca.* 98% purity was obtained by combining peak fractions from Run II. The presence of a single double bond in acids from these fractions was confirmed by quantitative hydrogenation or iodine value.

Characterization of monoethenoid acids. The purified C_{20}-monoene was subjected to oxidative cleavage by both the permanganate-periodate method[6] and the older permanganate–acetic acid method.[7] Efforts were concentrated on application of the permanganate–acetic acid technique because of solubility difficulties encountered with the permanganate-periodate method. When separation of the degradation products by steam distillation was attempted in the usual manner, there was obtained in the distillate a very slightly steam-volatile acid, which tended to solidify in the condenser and which unexpectedly proved to be pentadecanoic acid. The identity of this fragment was established by mixed melting point determinations on the free acid and on the *p*-bromophenacyl ester, and also by gas chromatographic analysis. The nonvolatile dicarboxylic acid fragment from the C_{20}-monoene was proved to be glutaric acid. These cleavage products, together with the infrared spectrum (no maxima in the 10–11 μ region), clearly established the structure of the C_{20}-monoene as the previously unknown *cis*-5-eicosenoic acid.

The purified C_{22}-monoene was also cleaved by permanganate-acetic acid oxidation and was shown to be a mixture of two isomers: *cis*-5-docosenoic, another previously unknown acid, in combination with the familiar *cis*-13-docosenoic (erucic) acid. The monobasic acid fragments from the C_{22}-monoene were separated from the dibasic acids by solvent partitioning. Gas chromatographic analysis of the resulting monobasic acid fraction indicated that the principal fragments were nonanoic and heptadecanoic acids in a ratio of about 2:1. Several other fragments were present in small amounts; the work of Begemann and coworkers[7] indicated that the permanganate–acetic acid method suffers from the drawback of formation of secondary degradation products. The nonanoic acid fragment was isolated from the monobasic acid fraction by steam distillation and characterized as a *p*-bromophenacyl ester. The heptadecanoic acid fragment was crystallized from the nonvolatile residue after steam distillation and characterized by mixed melting point determination. A tridecanedioic acid fragment, corresponding to nonanoic acid was isolated from the dibasic acid concentrate and identified by mixed melting point. A glutaric acid fragment, corresponding to heptadecanoic acid, was not isolated in a state of sufficient purity for characterization.

Isolation and partial purification of the C_{22}-unknown have also been accomplished. An account of this work and of structural studies now in progress on this acid will be given in a later paper.

DISCUSSION

The *cis*-5-eicosenoic acid found in *Limnanthes* oil appears to be unique. No other monoethenoid fatty acids with Δ^5-double bonds appear to have been recorded as triglyceride constituents in either the vegetable or animal kingdoms.[8] Two other monoethenoid C_{20}-acids in glycerides have been reported: the 9- and 11-isomers. The occurrence of *cis*-11-eicosenoic acid in a number of vegetable and marine animal oils is well established.[9] In contrast, 9-eicosenoic acid has thus far been found only in animal oils.[10] The melting point of *cis*-5-eicosenoic acid (26–27°) is about four degrees higher than melting points reported for the 9- and 11-isomers.[9b,11] 5-Docosenoic acid is also unique; C_{22}-monoenes in the fatty acid series previously reported as occurring naturally are the 11- and 13-isomers.[9a]

Correlations made by Lovern[12] and by Klenk and Debuch[13] have called attention to certain biogenetic patterns in unsaturated fatty acids. From their observations may be drawn the empirical generalization that double bonds in these acids tend to occur in positions $3n$ carbon atoms removed from one end of the chain (n being a small integer). Thus $n = 3$ for oleic and erucic acids and $n = 4$ for petroselinic acid. 5-Eicosenoic acid conforms to this pattern in the sense of having the integral value $n = 5$, counting the methyl group as C_1. In addition, it is significant that there are natural polyunsaturated C_{20}-fatty acids having

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

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

(8) Human hair oil has been reported to contain several unusual monounsaturated *free* fatty acids, including a 5-tetradecenoic acid [*cf.* A. W. Weitkamp, A. M. Smiljanic, and S. Rothman, *J. Am. Chem. Soc.*, **69**, 1936 (1947)]. Note Added in Proof: A recent communication states that human fecal lipids contain several unusual C_{18}-monoethenoid acids, including 5-octadecenoic acid [*cf.* A. T. James, J. P. W. Webb, and T. D. Kellock, *Biochem. J.*, 74, 21P (1960)].

(9) (a) T. P. Hilditch, *Chemical Constitution of Natural Fats*, 3rd Ed., John Wiley & Sons, New York, 1956; (b) C. Y. Hopkins, M. J. Chisholm, and J. Harris, *Can. J. Res.*, **27B**, 35 (1949), and subsequent papers by Hopkins and Chisholm.

(10) Y. Toyama and T. Tsuchiya, *J. Soc. Chem. Ind., Japan*, **37**, Suppl. binding 14 (1934); *Chem. Abstr.*, **28**, 2208 (1934); W. Bergmann, S. M. Creighton, and W. M. Stokes, *J. Org. Chem.*, **21**, 721 (1956).

(11) B. W. Broughton, R. E. Bowman, and D. E. Ames, *J. Chem. Soc.*, 671 (1952).

(12) J. A. Lovern, *J. Sci. Food Agr.*, **9**, 773 (1958).

(13) E. Klenk and H. Debuch in *Annual Reviews of Biochemistry*, Vol. 28, ed. by J. M. Luck *et al.*, Annual Reviews, Inc., Palo Alto, Calif., 1959, p. 39.

Δ^5-double bonds, *e.g.* the nutritionally essential 5,8,11,14-eicosatetraenoic (arachidonic) acid. Certain exceptions to this widespread biogenetic scheme are known, including 11-octadecenoic (vaccenic) acid and the Δ^4-unsaturated acids of the Lauraceae.[12] 5-Docosenoic acid, in common with these exceptions, does not conform to any biogenetic pattern that is now apparent.

EXPERIMENTAL

General methods. The gas chromatographic analyses were carried out with a Burrell Kromatog K-5 instrument.[14] The columns were U-shaped glass tubings 1.25 to 2.75 m. in length and $^1/_8$- to $^1/_4$-inch inner diameter, packed with LAC-2-R-446 (a polyester of diethylene glycol-pentaerythritol and adipic acid) or Apiezon L (a hydrocarbon grease) supported by Johns-Manville Celite 445. The carrier gas was helium and the operating temperature ranged from 185 to 250°, depending on the sample. For quantitative determination of composition, the areas under peaks were measured by the instrument's automatic integrator. Mixtures of acids were analyzed in the form of their methyl esters.

Except where otherwise noted, methyl esters were prepared by refluxing the desired mixture of acids 1 hr. in excess 1% sulfuric acid in methanol. Esters were isolated by ether extraction in the usual way; unchanged acids were removed by washing the ethereal solutions of esters with 5% potassium carbonate. When required for characterization work, fractionated esters were saponified by refluxing 1 hr. with 0.8*N* ethanolic potassium hydroxide.

Preparation of mixed fatty acids. Coarsely ground seeds of *Limnanthes douglasii* 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. The infrared spectrum of this oil showed no *trans* C=C adsorption (10.3 μ).

A 37.6-g. portion of *Limnanthes* oil was refluxed 30 min. with 240 ml. of 2*N* ethanolic potassium hydroxide under a nitrogen atmosphere. The resulting solution was diluted with water and extracted with ether. Combined ether extracts were, in turn, extracted by water which was combined with the original alkaline liquor. Free fatty acids (35.0 g.) were obtained by acidification of the alkaline liquor and extraction with ether. A sample of methyl esters was prepared for gas chromatographic analysis by esterification with diazomethane (see Table I).

A portion of these mixed fatty acids were hydrogenated in ethanol with platinum oxide catalyst at room temperature and atmospheric pressure. The saturated acids, as well as the methyl esters prepared from them, were white crystalline solids (see Table I).

Low-temperature crystallizations (Fig. 1). Mixed fatty acids (20.0 g.) were dissolved in 440 ml. of ether and the solution was cooled slowly to −45°. After standing 2 hr., the filtrate was removed with a filter stick. The solid was redissolved in 117 ml. and cooled to −46° for 2 hr., yielding 2.551 g. of crystals (fraction A), m.p. 25–26.5°, iodine number 77. Gas chromatographic analysis of similarly prepared material indicated 90% C_{20}-monoene and 6% C_{22}-monoene.

Combined mother liquors from crystallizations of fraction A were evaporated and the resulting residue was crystallized from 360 ml. of methanol; 14.5 g. of solid was obtained after keeping the solution at −25° for 2 hr. This product was similarly crystallized from 360 ml. of methanol, and a yield of 13.1 g. of solid (fraction B) resulted, iodine value 89 (see Table I for gas chromatographic analysis of a similarly prepared fraction).

The combined mother liquors from crystallizations of fraction B yielded 5.5 g. of liquid (fraction C), iodine number 125. According to gas chromatographic analysis, a similarly prepared fraction contained 37% C_{20}-monoene, 10% C_{22}-monoene, and 40% C_{22}-"unknown."

Fractionation by countercurrent distribution. (a) *Run I. Isolation of C_{20}-monoene.* Fraction B was esterified as described under Methods. Methyl esters thus obtained (10.0 g.) were subjected to a 440 transfer countercurrent distribution in a 200-tube Craig-Post apparatus. The solvent system used was mutually saturated acetonitrile and hexane[5] (8 to 1); 40 ml. of acetonitrile was placed in each of the 200 tubes. The methyl esters to be distributed were divided evenly among the first five tubes. The automatic operation of the instrument introduced 5 ml. of equilibrated hexane to tube 0 at each transfer stage. As hexane upper layers progressed past tube 200, they were decanted into an automatic fraction collector, combining two transfers per tube, and successively collected until 240 upper phases had been withdrawn. The weight curve obtained by evaporating solvent from contents of the various tubes is indicated in Fig. 2. Gas chromatographic analyses of selected tubes are indicated in Table I. On the basis of these analyses, contents of tubes 342–378 and 312–340 were combined and saponified to afford C_{20}-monoene for structural elucidation. Combined acids from transfers 342–378 had an iodine value of 83.0; calculated for one double bond, 81.8. A portion of combined acids from transfers 342–378 was hydrogenated in ethanol with platinic oxide catalyst at room temperature and atmospheric pressure. The product obtained by filtration and evaporation melted at 74° without recrystallization; the mixed melting point with authentic eicosanoic (arachidic) acid was 74–75°. Material from transfers 264–310, richest in C_{22}-monoene, was combined.

(b) *Run II. Isolation of C_{22}-monoene.* Material from transfers 264–310, Run I, was subjected to a second distribution in the same manner, except that the material was recycled so that a total of 770 transfers were made. The weight curve obtained is given in Fig. 3. Gas chromatographic analysis of the peak tube (transfer 640) is shown in Table I. Material from transfers 576–640 was combined and saponified for structural elucidation. These combined acids (0.0472 g.), when hydrogenated quantitatively with platinum oxide in ethanol (room temperature, 1 atm.), absorbed 0.85 mole of hydrogen. The hydrogenated product, 0.0393 g., melted at 71–78°. On recrystallization from ether-hexane, 0.016 g. was obtained, m.p. 78.5–79.5°; the mixed melting point with authentic docosanoic (behenic) acid was 78.5–79.5°.

Permanganate-periodate oxidation of C_{20}-monoene.[6] A 0.063-g. portion of C_{20}-monoene (97% pure) and 0.083 g. of potassium carbonate were dissolved in 40 ml. of water. To this was added 1.0 ml. of 0.04*M* potassium permanganate and 0.334 g. of sodium periodate in 40 ml. of water. The mixture was stirred 24 hr., then reduced with sodium metabisulfite, acidified with hydrochloric acid, and extracted with ether. The mixed acids obtained by this oxidation were esterified without fractionation and analyzed by gas chromatography. Pentadecanoic acid constituted 80% of the total monocarboxylic acids found. As the only dicarboxylic acid found was glutaric acid, it seems likely that the shorter chain monocarboxylic acids were secondary degradation products.

Permanganate-acetic acid oxidation of C_{20}-monoene.[7] C_{20}-monoene concentrate (1.09 g.) was dissolved in 20 ml. of purified acetic acid. Finely ground potassium permanganate (8 g.) was added in portions over a period of several hours, during which the mixture was stirred continuously. The temperature did not exceed 40°. The mixture was diluted with water after standing overnight, reduced with sodium metabisulfite, and acidified with hydrochloric acid. The strongly acid solution was extracted repeatedly with ether. The ether was largely removed from the combined extracts

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

by distillation and the residue steam distilled 5 hr. During this time, a solid acid distilled over very slowly and incompletely. Undistilled acids were recovered from the aqueous liquor by extraction with ether and by evaporation of combined extracts. The residue thus obtained was triturated repeatedly with petroleum ether (b.p. 30–60°). The soluble portion was filtered to remove traces of suspended insoluble matter (dicarboxylic acid fragments). Upon evaporation, the petroleum ether extracts yielded 0.431 g. of monocarboxylic acid; an additional 0.182 g. was obtained from the steam distillate by extraction with ether. This cleavage fragment (0.034 g.) was recrystallized from aqueous methanol; 0.019 g. was obtained, m.p. 48–49°[15]; there was no depression on admixture with authentic pentadecanoic acid (m.p. 48–51°). A 0.123-g. portion was converted to the *p*-bromophenacyl ester. A yield of 0.116 g. of product was obtained, m.p. 68–72°; this yielded on recrystallization from aqueous ethanol 0.076 g., m.p. 75–76°; there was no depression on admixture with authentic *p*-bromophenacyl pentadecanoate (m.p. 75–77°).

The acidic aqueous liquors from the original oxidation mixture and those from the steam distillation were combined and extracted continuously with ether. A crude dicarboxylic acid fragment (0.172 g.) was obtained from the extract. Upon recrystallizing this from chloroform, 0.074 g. was obtained, m.p. 90–94°. Three additional recrystallizations from chloroform or chloroform-benzene had little effect on the melting point, but there was no depression when mixed with authentic glutaric acid (m.p. 96–97°). The identity of this fragment as glutaric acid was confirmed by x-ray diffraction.

The glutaric acid fragment was obtained in a purer form when the C_{20}-monoene was subjected to permanganate-periodate cleavage by the general procedure of Lemieux and von Rudloff.[6] Crude acid (0.056 g.), m.p. 86–90°, was recrystallized once from chloroform; a specimen was obtained having m.p. 94–95°. There was no depression of melting point on admixture with authentic glutaric acid. The permanganate-periodate method was abandoned, however, because of the limited solubility of the acid in the oxidizing medium.

Pure cis-5-eicosenoic acid. A 0.110-g. portion of acid, obtained by saponification of combined transfers 342–378, m.p. 26–27° (*ca.* 97% pure 5-eicosenoic acid—see Table I), was recrystallized twice from aqueous methanol without observable change in melting point. Its infrared spectrum showed no *trans* C=C absorption (10.3 μ).[16]

Anal. Calcd. for $C_{20}H_{38}O_2$: C, 77.4; H, 12.3. Found: C, 77.3; H, 12.6.

Permanganate-acetic acid oxidation of C_{22}-monoene. C_{22}-monoene concentrate (0.99 g.) was oxidized essentially as described for the C_{20}-monoene. Combined ether extracts from the bisulfite-reduced, acidified oxidation medium were concentrated so as to remove most of the solvent. The residue was dissolved in 30 ml. of methanol and 3 ml. of water. The resulting solution was extracted four times with 30-ml. portions of petroleum ether (b.p. 30–60°). The combined petroleum ether extracts were dried over sodium sulfate and evaporated, yielding 0.280 g. of semisolid, a concentrate of monocarboxylic acids. Upon evaporation, the aqueous methanol yielded 0.602 g. of dicarboxylic acids as a waxy solid.

Gas chromatographic analysis of the monocarboxylic acid concentrate indicated two principal fragments: nonanoic acid (27.8%) and heptadecanoic acid (21.9%). A portion (0.280 g.) of this material was separated by steam distillation. Nonanoic acid (0.067 g.) obtained by ether extraction of the distillate was converted to a *p*-bromophenacyl ester. The crude yield was 0.020 g., m.p. 50–54°. After two recrystallizations from aqueous ethanol and one from hexane, this derivative melted at 60–63°; the melting point was undepressed on admixture with authentic *p*-bromophenacyl nonanoate.

The nonvolatile monocarboxylic acid (0.110 g.) was obtained as a semisolid by petroleum ether extraction of the aqueous residue from steam distillation. After three recrystallizations from methanol, this acid melted at 54–58°, undepressed on admixture with authentic heptadecanoic acid (m.p. 56–59°). A considerable depression (m.p. 48–62°) was observed on admixture with palmitic acid (m.p. 62°).

The dicarboxylic acid concentrate obtained by cleavage of the C_{22}-monoene was dissolved in 10 ml. of chloroform. This solution was extracted repeatedly with 10-ml. portions of water, dried with sodium sulfate, and evaporated. A solid (0.111 g.), m.p. 77–95°, was obtained. This material was recrystallized twice from chloroform-hexane and once from aqueous ethanol; a sample was obtained, m.p. 110–111°; there was no depression on admixture with authentic tridecanedioic (brassylic) acid (m.p. 111–112°).

Combined acidified aqueous extracts from chloroform-water partitioning were extracted with ether. The product thus obtained, presumably containing glutaric acid, was not satisfactorily characterized.

Acknowledgments. The authors thank Mr. C. R. Scholfield and Dr. H. J. Dutton for assistance with countercurrent distributions; Mr. Henry Zobel for X-ray diffraction analysis of glutaric acid; Mr. C. A. Glass for infrared spectra; Mrs. Clara McGrew for microanalyses; and Dr. Quentin Jones, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, for making available generous quantities of *Limnanthes douglasii* seed.

PEORIA, ILL.

(15) Melting points were determined by a Fisher-Johns block and are uncorrected.

(16) Infrared spectra were measured as films on silver chloride plates with a Perkin-Elmer model 21 rock salt spectrophotometer.