Hydrogenolysis of 1,1,3-Triacetoxypropane.—A 100-g. sample of 1,1,3-triacetoxypropane with three teaspoonfuls of Raney nickel was treated with hydrogen at 150° and 1500 p.s.i. for four hours. Distillation gave 40 g. (55% yield) of 1,3-diacetoxypropane, b.p. 88–90° (10 mm.), $n^{20}D$ 1.4206. The infrared absorption spectrum was essentially identical with the derivatives from hydrogenation of 1,3-diacetoxypropene or acetylation of 1,3-propanediol.

Reaction of 3,3-Diacetoxypropene with Potassium Cyanide.—A mixture of 0.5 mole (79 g.) of 3,3-diacetoxypropene and 2 moles (130 g.) of potassium cyanide was heated to about 110° when an exothermic reaction set in. The heating was stopped and the mixture was stirred well with occasional cooling to maintain the temperature at 110–120°. After the reaction subsided, the mixture was heated on the steam-cone for an hour. It was then cooled and extracted with chloroform. After evaporation of the chloroform under reduced pressure, the residue was distilled from a Claisen flask, yielding 32 g. of 3,3-diacetoxypropene and 17 g. (22.5% conversion and 38% yield) of a liquid which may be α-acetoxyglutaronitrile, b.p. 120–125° (0.3–0.5 mm.), $n^{20}D$ 1.4418, d^{20}_4 1.130.

Anal. Calcd. for $C_7H_8N_2O_2$: C, 55.29; H, 5.30; N, 18.4. Found: C, 55.19, 55.44; H, 5.37, 5.45; N, 18.2, 18.2.

The infrared absorption spectrum had bands indicative of nitrile (4.45 μ) and ester (5.68 μ) groups, but gave no indication of olefinic double bond. Since there is no double bond to give a vinyl acetate (5.67 μ) and no *gem* diacetoxy groups (5.68 μ), the acetoxy and one of the nitrile groups are indicated to be on the same carbon atom (acrolein cyanohydrin acetate, 5.68 μ).

Reaction of 1,3-Diacetoxypropene with Potassium Cyanide.—A mixture of 0.5 mole (79 g.) of 1,3-diacetoxypropene and 1 mole (65 g.) of potassium cyanide was heated to 110°. An exothermic reaction ensued. To control the reaction the mixture was stirred and cooled in an ice-bath. When the mixture had cooled to room temperature, it was extracted with chloroform. After evaporation of the chloroform, the residue was distilled, yielding 11.3 g. of liquid which may be α-acetoxyglutaronitrile, b.p. 112–114° (0.12 mm.), $n^{20}D$ 1.4440. This material had an infrared absorption spectrum essentially identical with the product from the above reaction.

EMERYVILLE, CALIFORNIA RECEIVED MARCH 12, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION, TEXAS AGRICULTURAL AND MECHANICAL COLLEGE SYSTEM]

Displacement Analysis of Lipids. VI. Separation of Unsaturated Acids[1a]

BY RALPH T. HOLMAN[1b] AND WILLIAM T. WILLIAMS

Displacement chromatography has been applied to the separation of unsaturated fatty acids using charcoal as adsorbent and aqueous ethanol as solvent. Separations of saturated and various unsaturated acids of the same chain length have been made with acids varying from 4 to 18 carbons in chain length. Non-conjugated unsaturation decreases adsorption, carboxyl conjugated conjugation increases adsorption. The conjugated octadecadienoic acid (not carboxyl conjugated) is adsorbed less strongly than stearic acid, whereas conjugated octadecatrienoic acid is adsorbed more strongly than stearic acid. Linoleic and oleic acids are separable, but they both lie between myristic and palmitic acids in adsorbability. Impurities in fatty acid preparations which were not removed by repeated distillation were detected by chromatographic separation.

Introduction

Chromatography has proven to be a very valuable tool in lipid separations. It has been applied with success to sterols, phospholipids, triglycerides and to fatty acids and their derivatives. The usual techniques employ elution chromatography, but recently displacement separation has been found to be a promising technique for fractionation of saturated fatty acids,[2,3,4] fatty acid esters, and compounds of similar skeletal structure.[5]

Preliminary experiments reported by Claesson[6] indicated separation of saturated from unsaturated acids by displacement separation on silica gel, but no study was made of separations of various unsaturated acids from each other. The present investigation was made to study the effects of degree and kind of unsaturation upon adsorbability of fatty acids, and to apply the displacement technique to their separation.

Experimental

The apparatus used for the chromatographic separations was a modified Tiselius–Claesson interferometric adsorption analysis apparatus[7] provided with Hagdahl coupled filters.[8] The description of the apparatus and its use are found elsewhere.[9] Whenever possible, in addition to the refractive index observations, additional chemical or physical tests were made upon the effluent to identify zones. For example, titrations with alkali, titrations with bromine, measurements of ultraviolet absorption, and enzymatic determinations of linoleic acid[10] were made whenever appropriate.

The adsorbent used in these studies was a mixture of 1 part Darco G 60 charcoal and 2 parts Hyflo Super Cel and the column capacities varied according to need. Solvent mixtures of ethanol and water were chosen to dissolve slightly greater than the desired concentration of displacer substance.

When ultraviolet absorption of the effluent was measured, wave lengths were chosen such that using the 0.2 mm. flowing cuvette the maximum optical density during the experiment would not exceed the range of the Beckman spectrophotometer. Thus, in all cases, the measurements were not made at the wave length of maximum absorption. Even so, in some instances the optical densities observed were too high to be accurate.

The short chain acids used in this investigation were commercial preparations which were recrystallized or redistilled if single displacement tests for purity indicated that such purification was necessary. The final products as used had the following constants: crotonic acid, m.p. 73°; butyric acid, b.p. 162–163° (768 mm.); 2-pentenoic acid, b.p. 199° (760 mm.); *n*-valeric acid, b.p. 186° (760 mm.); 3-methyl-2-butenoic acid, m.p. 69°; isovaleric acid, b.p. 177° (760 mm.); sorbic acid, m.p. 134.5°; 2-hexanoic acid, m.p. 32.5°; *n*-caproic acid, b.p. 203° (760 mm.); 3-hexenoic acid, b.p. 208° (760 mm.); *n*-octanoic acid, b.p. 239° (760 mm.); and undecylenic acid, b.p. 275° (760

(1) (a) Supported in part by grants from the Research Corporation of New York, the National Dairy Council on behalf of the American Dairy Association and by a contract between the Office of Naval Research and the Texas A. and M. Research Foundation. Presented before the American Association of Biological Chemists, Cleveland, May 1, 1951. (b) Hormel Institute, University of Minnesota, Austin, Minn.

(2) Holman and Hagdahl, *Arch. Biochem.*, **17**, 301 (1948).

(3) Hagdahl and Holman, THIS JOURNAL, **72**, 701 (1950).

(4) Holman and Hagdahl, *J. Biol. Chem.*, **182**, 421 (1950).

(5) Holman, THIS JOURNAL, **73**, 3337 (1951).

(6) Claesson, *Rec. trav. chim.*, **T65**, 9 (1946).

(7) Tiselius and Claesson, *Arkiv for Kemi Mineral Geol.*, **15B**, 18 (1942).

(8) Hagdahl, *Acta Chem. Scand.*, **2**, 574 (1948).

(9) Holman and Hagdahl, *Anal. Chem.*, **23**, 794 (1951).

(10) Holman, unpublished data.

mm.). The saturated acids, lauric, myristic, palmitic and stearic, were all commercial products which had been shown by previous experiments to be sufficiently pure for the present purposes. Undecanoic acid, m.p. 21°, was prepared from undecanoic acid by hydrogenation. Palmitoleic acid prepared from the C_{16}-fraction of the methyl esters of cod liver oil had an iodine value of 93.3. Although this sample was only approximately 93% pure, because its chief impurity was palmitic acid, it was suitable for demonstration of separation of palmitic and palmitoleic acids.

Oleic acid (I.V. 85, equiv. wt. 281, m.p. 13.3°) was prepared from olive oil by the low temperature crystallization method of Brown and Shinowara.[11] Elaidic acid (I.V. 87.5, equiv. wt. 286, m.p. 42–43°) was prepared from oleic acid by selenium elaidinization.[12] Petroselinic acid, equiv. wt. 285, m.p. 29°, was prepared from parsley seed oil.[13] Linoleic acid (I.V. 175) was prepared by the usual bromination-debromination procedure.[14] 10,12-Octadecadienoic acid, m.p. 55–56°, was prepared from dehydrated castor oil by the method of von Mikusch.[15] Linolenic acid was prepared by bromination-debromination[14] and β-eleostearic acid, m.p. 68–69°, was isolated from tung butter. Arachidic acid, m.p. 73°, was prepared by malonic acid synthesis from octadecyl bromide.[16]

Experimental Results

The separation of butyric and crotonic acids is shown in Fig. 1. The column used had a capacity of 15.4 ml., the solvent was water and the displacer 0.8% *n*-caproic acid. The sample consisted of 46 mg. of butyric and 79 mg. of crotonic acid. The interferometric measurements indicated that the chromatogram had 3 major zones and one minor one just in advance of the displacer. Bromine titrations of 1.0-ml. fractions indicated that zone A was a saturated compound (butyric acid) and that zone B was unsaturated (crotonic acid). The minor zone between B and the displacer was also found to absorb bromine. This zone was found in all chromatograms in which *n*-caproic acid was used, and represents an unsaturated impurity that is inseparable from *n*-caproic acid by repeated fractional distillation.

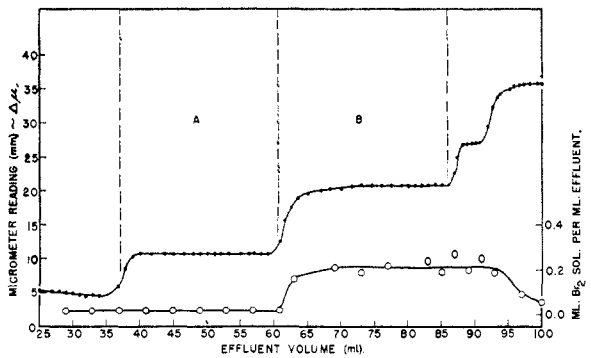

Fig. 1.—Displacement separation of 46 mg. of butyric and 79 mg. of crotonic acids in water on a 15.4-ml. coupled filter column using 0.8% caproic acid as displacer: ·····, interferometer measurements; O-O, bromine titrations.

Separation of 86 mg. of *n*-valeric acid and 102 mg. of α,β-unsaturated valeric acid is shown in Fig. 2. The column was 15.4-ml. capacity, water the solvent, and the displacer 0.8% *n*-caproic acid. The interferometric measurements indicated 4 zones in the chromatogram. Bromine uptake of 1.0-ml. fractions indicated that the substance in zone A was saturated (*n*-valeric acid) and that substances B and C were unsaturated. Measurement of light absorption at 2330 Å. in a 0.2-mm. cuvette showed that zones B and C contained conjugated unsaturation. Zone B represents α,β-unsaturated valeric acid and zone C the impurity present in the caproic acid displacer.

(11) Brown and Shinowara, THIS JOURNAL, **59**, 6 (1937).
(12) Bertman, *Chem. Weekblad*, **33**, 3 (1936).
(13) Hilditch and Jones, *J. Soc. Chem. Ind.*, **46**, 174T (1927).
(14) McCutcheon, *Org. Syntheses*, **22**, 75, 82 (1942).
(15) von Mikusch, THIS JOURNAL, **64**, 1580 (1942).
(16) Bleyberg and Ulrich, *Ber.*, **64**, 2504 (1931).

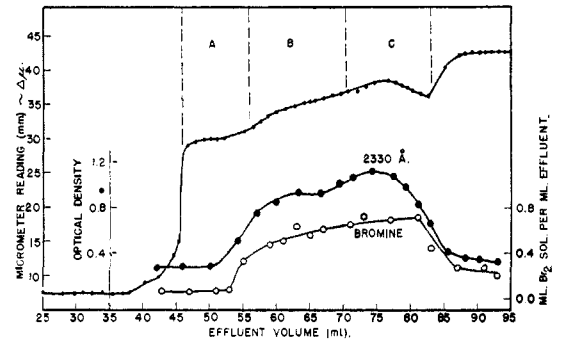

Fig. 2.—Displacement separation of 86 mg. of *n*-valeric acid and 102 mg. of α,β-unsaturated valeric acid in water on a 15.4-ml. coupled filter column using 0.8% caproic acid as displacer.

Separation of 70 mg. of isovaleric acid and 80 mg. of α,β-unsaturated isovaleric acid is shown in Fig. 3. The column had a capacity of 13.5 ml., the solvent was water and the displacer 0.8% *n*-caproic acid. The interferometric measurements indicated 4 zones in the chromatogram but only 3 were discernible by titration of the 1.0-ml. fractions with alkali. Bromine titration indicated that zones B and C were unsaturated substances. Thus, zone A was found to be isovaleric acid, zone B, unsaturated isovaleric acid, and zone C the impurity associated with the caproic acid displacer.

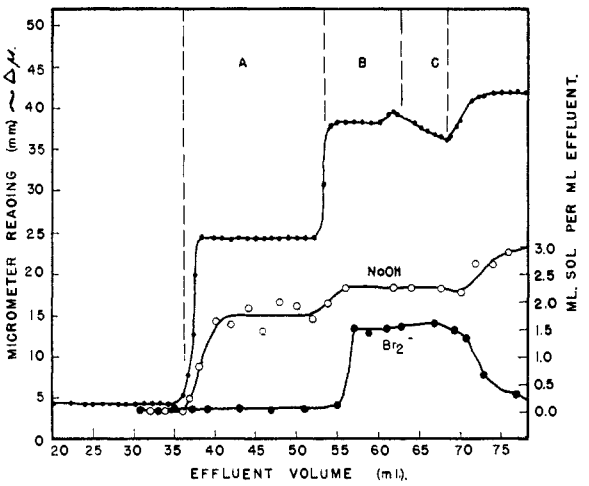

Fig. 3.—Displacement separation of 70 mg. of isovaleric acid and 80 mg. of α,β-unsaturated isovaleric acid in water on a 13.5-ml. coupled filter column using 0.8% caproic acid as displacer.

The separation of 74 mg. of caproic acid and 144 mg. of sorbic acid shown in Fig. 4 was performed on a 20.5-ml. column in 40% ethanol using 1.0% caprylic acid as displacer. The adsorption diagram indicates that a zone having a very high refractive index (B) was between two zones of lower refractive index, the latter being the displacer. Titrations of the 1.0-ml. fractions indicated that this zone also had a higher molar concentration of acid than did the displacer, a rather unusual occurrence. Measurement of the light absorption at 2960 Å. (on the side of the sorbic acid absorption maximum) verified that zone B was sorbic acid.

The displacement separation of 4 six-carbon acids is shown in Fig. 5. The column capacity was 40.0 cc., the solvent 40% ethanol and the displacer 0.8% sorbic acid. The sample consisted of 84 mg. of β,γ-unsaturated caproic acid, 104 mg. of caproic acid and 65 mg. of α,β-unsaturated caproic acid. The interferometric curve indicated 4 major zones in the chromatogram. At 118 ml. of eluate, the refractive index became so high as to exceed the range of the

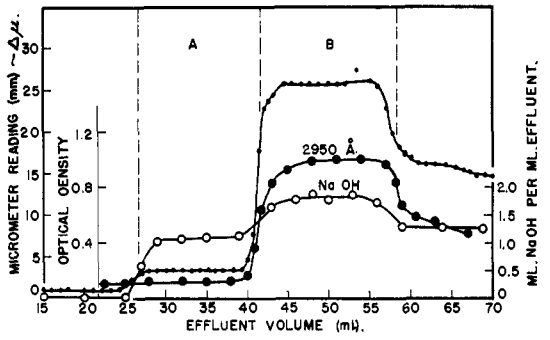

Fig. 4.—Displacement separation of 74 mg. of caproic acid and 144 mg. of sorbic acid in 40% aqueous ethanol using 1.0% caprylic acid as displacer.

micrometers, and so the channels of the interferometer cuvette were crossed over[17] to permit further observations. Bromine uptake of 1.0-ml. fractions indicated that zone A and the displacer readily absorbed bromine. Light absorption measurements at 2960 Å. in the 0.2-mm. cell indicated that none of the components prior to the displacer (sorbic acid) had carboxyl conjugated double unsaturation. Light absorption at 2310 Å. indicated that zone C had carboxyl conjugated unsaturation (α,β-unsaturated caproic acid). Thus zone A was found to be β,γ-unsaturated caproic acid, zone B was caproic acid and zone C, α,β-unsaturated caproic acid.

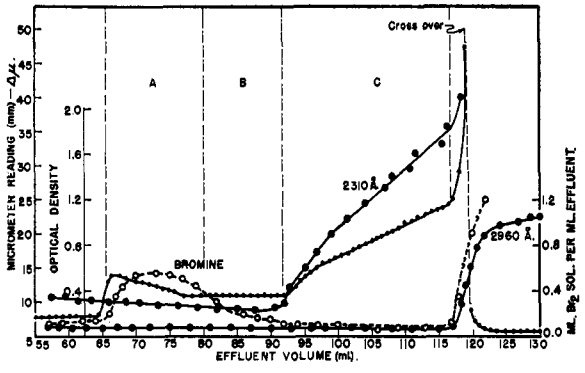

Fig. 5.—Displacement separation of 84 mg. of β,γ-unsaturated caproic, 104 mg. of caproic and 65 mg. of α,β-unsaturated caproic acids in 40% aqueous ethanol on a 40.0-cc. coupled filter column using 0.8% sorbic acid as displacer.

Figure 6 illustrates the separation of 94 mg. of undecenoic acid and 152 mg. of undecanoic acid on a 28.0-cc. column in 50% ethanol using 1.0% lauric acid as displacer. Zone A was identified as the unsaturated component of the sample by bromine titrations of the 1.0-ml. fractions. Separation of 97 mg. of palmitoleic acid and 100 mg. of palmitic acid is shown in Fig. 7. The column was 28-cc. capacity, the solvent 95% ethanol and the displacer 1.0% stearic acid. Interferometric measurements indicate two distinct zones before the displacer, and bromine titrations again indicate that zone A is the unsaturated constituent.

In the experiment shown in Fig. 8 the separation of two saturated acids and one unsaturated acid was accomplished. The column had a capacity of 40.0 cc., the solvent was 95% ethanol, the displacer was 1.0% stearic acid and the sample contained 80 mg. of myristic, 55 mg. of oleic and 100 mg. of palmitic acids. This experiment illustrates a result sometimes observed in our experience, that separations are accomplished which cannot be discerned with the interferometer alone. In this case, bromine titrations indicated that the oleic acid was present in the first half of the second step observed interferometrically. The step heights for oleic and palmitic acids were so nearly alike that

(17) Holman, *Anal. Chem.*, **22**, 832 (1950).

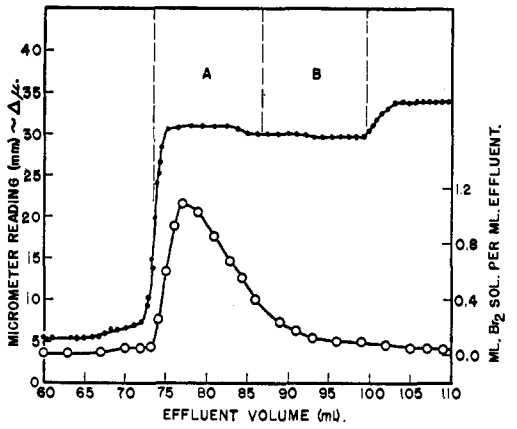

Fig. 6.—Displacement separation of 94 mg. of undecenoic acid and 152 mg. of undecanoic acid on a 28.0-ml. coupled filter column in 50% ethanol using 1.0% lauric acid as displacer: O-O, bromine titrations.

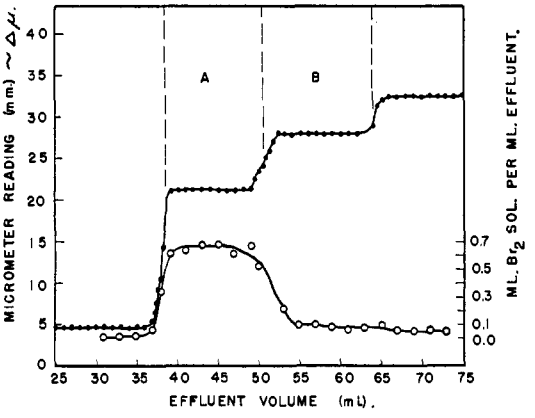

Fig. 7.—Displacement separation of 97 mg. of palmitoleic acid and 100 mg. of palmitic acid in 95% ethanol on a 28.0-ml. coupled filter using 1.0% stearic acid as displacer: O-O-O, bromine titrations.

they were not distinguishable. In other words, the products of concentration and refractive index increment for oleic and palmitoleic acids were almost equal. In a similar experiment in which petroselinic acid was substituted for oleic acid, the same results were found. This clearly points out the need for more than one means of observation on the

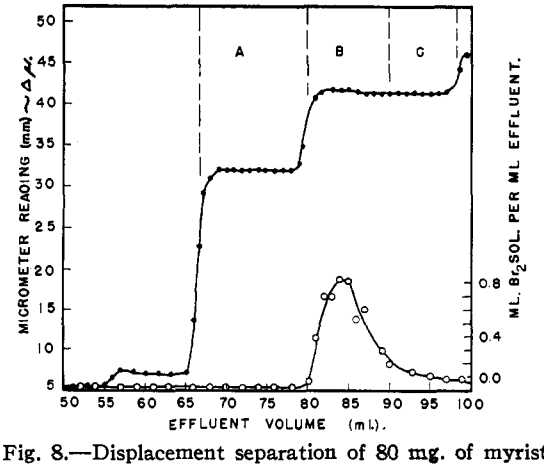

Fig. 8.—Displacement separation of 80 mg. of myristic, 55 mg. of oleic and 100 mg. of palmitic acids in 95% ethanol on a 40.0-ml. coupled filter using 1.0% stearic acid as displacer.

effluent. These experiments also indicate that the presence of one double bond in an 18-carbon acid lessens the adsorbability greatly, causing it to be between the 14- and 16-carbon saturated acids in its adsorptive property in this system. When linoleic acid was substituted for oleic acid in a similar experiment it also appeared between myristic and palmitic acids in the chromatogram, but in this case the linoleic acid zone was discernible in the interferometer curve because it had a step height sufficiently higher than the palmitic acid which followed it.

A separation of natural and conjugated isomers of linoleic acid from stearic acid is shown in Fig. 9. The separation was achieved using a 38.3-ml. column, absolute ethanol as solvent and 0.9% arachidic acid as displacer. The sample contained 90 mg. of linoleic acid, 140 mg. of stearic acid and 120 mg. of 10,12-octadecadienoic acid. Observations with the interferometer clearly indicated 4 zones in the chromatogram. Measurements of ultraviolet absorption indicated that zone B was the conjugated diene component. It was also observed that the initial portions of zone A contained some chromophoric substance, probably an oxidation product of one of the unsaturated acids. When the 1-ml. fractions were cooled to 5°, the earliest fraction which yielded crystals was found to be the 82nd ml. shown by the arrow. Thus, zone A was the linoleic acid component. This was verified by bromine titrations which showed qualitatively that zones A and B absorbed bromine whereas zones C and D did not. Thus it was shown that the non-conjugated and conjugated dienoic acids were adsorbed less strongly than their saturated analog stearic acid.

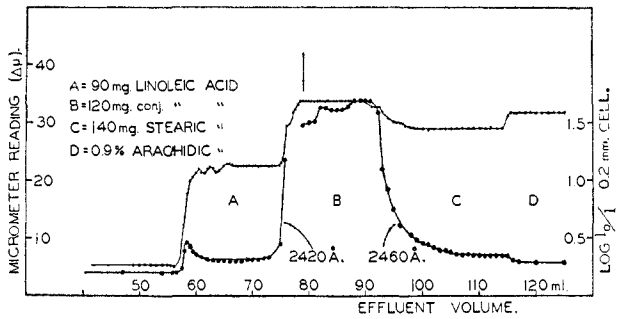

Fig. 9.—Displacement separation of 90 mg. of linoleic acid, 140 mg. of stearic acid and 120 mg. of 10,12-octadecadienoic acid on a 38.3-ml. coupled filter in absolute ethanol, using 0.9% arachidic acid as displacer. Arrow shows first 1.0-ml. fraction depositing crystals at 5°.

The separation of linolenic, stearic, β-eleostearic and arachidic acids is shown in Fig. 10. The column used had a capacity of 38.3 ml., the solvent was absolute ethanol, the sample contained 80 mg. of linolenic acid, 120 mg. of stearic acid and 120 mg. of β-eleostearic acid, and the displacer was 0.9% arachidic acid. From interferometric measurements it was clear that the chromatogram contained 4 major zones. Zones A and B were separated by a small zone having a higher refractive index than either A or B. As a means of identifying the β-eleostearic acid zone, light absorption at 2820 Å was measured in a flowing cuvette having 0.2 mm. light path with the Beckman spectrophotometer. These measurements also indicated the presence of the small zone between zones A and B by its increased light absorption. The very strong light absorption of zone C as well as its high refractive index identified this zone as β-eleostearic acid. Bromine titrations of 1-ml. fractions of the entire chromatogram indicated that zones A and C absorbed bromine whereas zone B and the displacer (D) did not. Thus, zone A was linolenic acid, zone B was stearic acid and zone C, β-eleostearic acid. When the 1-ml. fractions were cooled to 5° all fractions above the point indicated by the arrow deposited crystals. This confirmed the identity of zone A as linolenic acid. The small zone lying between linolenic and stearic acids was probably some chromophoric oxidation product of one of the unsaturated acids. It may be concluded from this experiment that non-conjugated trienoic acid (linolenic) is adsorbed less strongly than its saturated analog, whereas conjugated trienoic acid (β-eleostearic) is more strongly adsorbed than its saturated analog.

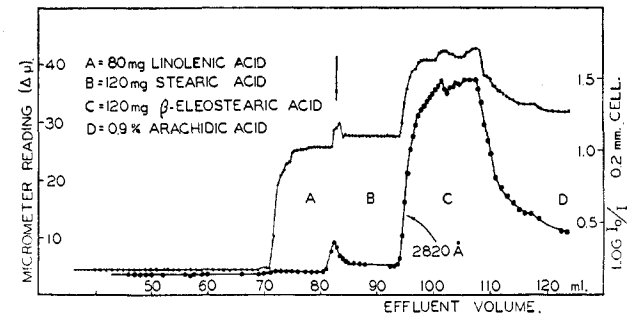

Fig. 10.—Displacement separation of 80 mg. of linolenic acid, 120 mg. of stearic acid and 120 mg. of β-eleostearic acid in absolute ethanol on an 83.3-ml. coupled filter using 0.9% arachidic acid as displacer. Arrow shows first 1.0-ml. fraction depositing crystals at 5°.

The displacement separation of oleic and linoleic acids has been difficult to demonstrate. Numerous experiments under various conditions failed to differentiate the two zones of these acids as judged from refractive index data obtained by the interferometer, or by bromine titrations of small fractions. In the experiment shown in Fig. 11, separation of oleic and linoleic acids was demonstrated by the use of an enzymatic reaction specific for linoleic acid. Lipoxidase has been shown to oxidize only those unsaturated fatty acids with methylene interrupted polyunsaturated systems.[18] Under suitable conditions this enzyme can be used as a specific reagent for the quantitative determination of microgram quantities of linoleic and related acids.[10,18]

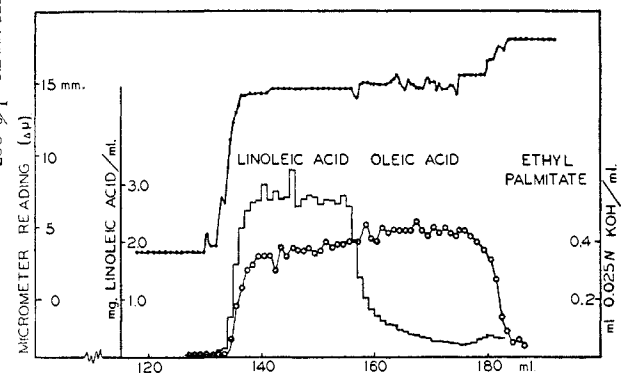

Fig. 11.—Displacement separation of 100 mg. of oleic acid and 100 mg. of linoleic acid in 75% ethanol on a 38.3-ml. coupled filter using 0.8% ethyl palmitate as displacer: O-O-O, titrations with alkali.

The system to demonstrate separation of oleic and linoleic acids used a coupled filter column of 38.3-ml. capacity, 75% ethanol as solvent and 0.8% ethyl palmitate as displacer. The sample consisted of 100 mg. of linoleic acid and 100 mg. of oleic acid. Observations were made with the interferometer and 1.0-ml. fractions were cut throughout the course of the experiment. From each fraction 0.15 ml. was taken for linoleic acid determination. The remainder was titrated with standard alkali. The results allow the conclusions that (1) oleic and linoleic acids are separable, and (2) that the linoleic acid separated is still in the natural form which can be attacked by the enzyme. This experiment again emphasizes that separations can be achieved which are not distinguishable with the interferometer or by titrations. The products of concentration and refractive index increment for the oleic and linoleic acid zones were so nearly alike as to be indistinguishable. Thus the need is apparent for more than one means of observation, and for very specific means of identification wherever possible.

Discussion

Chromatographic techniques have been shown

(18) Bergström and Holman, *Adv. Enzymol.*, **8**, 425 (1948).

to be of great value in separations of saturated and unsaturated acids. The customary elution separation has been used by numerous investigators with success for the separation and isolation of both saturated and unsaturated fatty acids. The present investigation has indicated the usefulness of Tiselius' displacement technique[19] in separating unsaturated acids from 4 to 18 carbon atoms in length. Thus displacement chromatography can be used for separation of saturated and unsaturated acids and for separation of acids differing in degree and kind of unsaturation whenever the advantages of this type of chromatography make it desirable.

From the experiments shown and from other experience gained in this type of adsorption work, it can be concluded that within a family of acids of equal chain length, increasing unsaturation decreases the adsorbability on Darco G 60 charcoal if the unsaturation is non-conjugated. On the other hand, if the double bonds are conjugated either with the carboxyl group or with themselves, the adsorbability is increased. Increasing conjugated unsaturation increases adsorption.

The usefulness of displacement chromatography in separation of fatty acids on the basis of kind and degree of unsaturation has been demonstrated. The experiments presented here also indicate the usefulness of the technique in the detection of impurities. In many cases, preparations thought to be pure were found to contain impurities detectable by displacement separation. This is most striking in the case of the *n*-caproic acid which had been subjected to three fractional distillations prior to use, yet still contained an unsaturated impurity. The detection of small amounts of unexpected chromophoric substances in the chromatograms involving the long chain polyunsaturated acids suggests the possible usefulness of this method in their purification and in studies of their oxidation products.

(19) Tiselius, *Arkiv Kemi Mineral. Geol.*, **16A**, 18 (1943).

COLLEGE STATION, TEXAS RECEIVED MAY 3, 1951

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY AND NUTRITION, TEXAS AGRICULTURAL AND MECHANICAL COLLEGE SYSTEM, COLLEGE STATION]

Displacement Analysis of Lipids. VII. Carrier Separation of Unsaturated Fatty Acids[1a]

BY RALPH T. HOLMAN[1b]

The positions of a series of single unsaturated fatty acids in a carrier displacement chromatogram have been determined using methyl esters of even saturated acids in one series and methyl esters of odd saturated acids in another using Darco G 60 as adsorbent and 95% ethanol as solvent. It was found that increasing the number of isolated double bonds decreases the adsorption, changing a saturated acid to an unsaturated acid to an acetylenic acid decreases adsorption, conjugation of double bonds increases adsorption. Differences in adsorbability of *cis*- and *trans*-isomers are slight. One isolated double bond in the molecule decreases adsorption roughly equivalent to 2 less carbon atoms. Separation of stearic and linoleic acids and of linoleic acid and its conjugated isomer by carrier displacement are demonstrated.

Carrier displacement chromatography introduced by Tiselius and Hagdahl[2] makes use of relatively large quantities of substances whose adsorption isotherms lie between those of the components of a sample. When the sample plus these additional substances are displaced, the constituents arrange themselves in order of adsorbability, and the components of the sample are sandwiched between much larger zones of the added substances. Thus, small quantities of sample constituents travel at the fronts of the zones of the more abundant "carriers," and are, under ideal conditions, completely separated from each other. Such a technique has been found applicable to separations of amino acids and peptides[2] and separations of saturated fatty acids.[3] The study of fatty acids has now been extended to a series of unsaturated fatty acids to obtain qualitative information on the influence of kind and degree of unsaturation upon the position occupied by an acid within a carrier system of methyl esters of saturated fatty acids.

Experimental

The apparatus used in this investigation was a modification of the Tiselius–Claesson adsorption–analysis apparatus[4] equipped with Hagdahl coupled filters.[5] The apparatus and its use is described elsewhere.[6] The interferometric observations were made with a 76-mm. cuvette at 37°. The adsorbent was 1 part Darco G 60 and 2 parts Hyflo Supercel. The carrier systems were similar to those used in the study of carrier separation of saturated acids.[3] The acids were chromatographed in two carrier systems. One system consisted of approximately 120 mg. of methyl palmitate, 95 mg. of methyl myristate and 60 mg. of methyl laurate displaced by 1.0% methyl stearate. The other system consisted of approximately 150 mg. of methyl heptadecanoate, 130 mg. of methyl pentadecanoate, and 75 mg. of methyl tridecanoate displaced by 1.0% methyl stearate. The solvent, unless otherwise specified, was 95% ethanol. The esters used were prepared from commercially available acids and last traces of acid were removed from the ester by treatment with aluminum oxide. All esters were sufficiently pure for the present use as determined by single displacement experiments. The unsaturated fatty acids were prepared by standard procedures.[7]

In the simple carrier experiments, the acid component was located by titration of 1.0-ml. fractions with approximately 0.02 *N* KOH. In the experiments involving linoleic acid, its concentration was determined in the 1.0-ml. frac-

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(2) Tiselius and Hagdahl, *Acta Chemica Scand.*, **4**, 394 (1950).

(3) Holman, THIS JOURNAL, **73**, 1261 (1951).

(4) Tiselius and Claesson, *Arkiv for Kemi Mineral. Geol.*, **15B**, 18 (1942).

(5) Hagdahl, *Acta Chemica Scand.*, **2**, 574 (1948).

(6) Holman and Hagdahl, *Anal. Chem.*, **23**, 794 (1951).

(7) Ralston, "Fatty Acids and Their Derivatives," John Wiley and Sons, Inc., New York, N. Y., 1948.