lent of acid and 0.327 equivalent of carbonyl per 100 g. of sample. Calculated values for δ-formyl-α-hydroxyvaleric acid (VI) are 0.779 equivalent of both acid and carbonyl per 100 g.; 6-hydroxytetrahydropyran-2-carboxylic acid would be expected to give the same values under the conditions used.

A third portion of the ether solution equivalent to 0.09 mole of starting material was added to 30 ml. of water and oxidized with hydrogen peroxide after removal of ether. Evaporation of the resulting solution left a solid residue which after crystallization from benzene and ether gave 7 g. (60%) of glutaric acid which melted at 96–98° alone or mixed with an authentic sample.

6-Amino-2-hydroxyhexanamide (VIII).—The lactone VII (30 g., 0.23 mole) was dissolved in 80 g. of cold concentrated ammonium hydroxide with stirring and cooling, and the resulting solution, after addition of 75 g. of anhydrous ammonia, was hydrogenated over Raney nickel at 80 to 118° and 1200 p.s.i. hydrogen pressure in three hours. During suction filtration of the product, the solution was cooled by evaporation of ammonia with precipitation of 19 g. (55% yield) of 6-amino-2-hydroxyhexanamide, which melted at 111–113° after washing with absolute ethanol and then ether. Evaporation of the filtrate under vacuum at a maximum temperature of 40° left an additional 14 g. (40%) of less pure amide, m.p. 100–105°.

Anal. Calcd. for $C_6H_{14}O_2N_2$: C, 49.28; H, 9.64. Found: C, 49.0, 48.9; H, 9.5, 9.4.

The amide (2.3 g.) was warmed with 70 ml. of absolute ethanol and water (10 ml.) was added until the solid dissolved; on cooling 1.1 g. of 6-amino-2-hydroxyhexanoic acid, melting at 205–210° with decomposition, was deposited. Fischer and Zemplén[7] reported that the acid melted at 220–225° with decomposition.

Anal. Calcd. for $C_6H_{13}O_3N$: C, 48.28; H, 8.90; N, 9.5. Found: C, 48.39, 48.43; H, 8.91, 9.00; N, 9.74.

Acetic anhydride (0.05 mole) in 5 ml. of ether was slowly added to a stirred suspension of 7.3 g. (0.05 mole) of the amide in 50 ml. of ether at room temperature. After 18 hours the solid was removed by filtration and recrystallized from 40 ml. of ethanol to give 7 g. (74%) of 6-acetylamino-2-hydroxyhexanamide, m.p. 155–156°.

Anal. Calcd. for $C_8H_{16}O_3N_2$: C, 51.05; H, 8.57; N, 14.88. Found: C, 50.85, 51.01; H, 8.55, 8.57; N, 14.7, 14.8.

A second sample of 15 g. (0.12 mole) of lactone was dissolved in ammonium hydroxide, hydrogenated as before and the product evaporated under vacuum at a maximum temperature of 60°. The residual solid was dissolved in 25 ml. of water, 50 g. of 20% sodium hydroxide was added, the solution was cooled in ice and 20 g. (0.14 mole) of benzoyl chloride was added slowly with precipitation of 18 g. (60% yield) of 6-benzoylamino-2-hydroxyhexanamide, m.p. 161.5–162.0°, after two crystallizations from ethanol. Addition of more benzoyl chloride caused no further precipitation.

Anal. Calcd. for $C_{13}H_{18}O_3N_2$: C, 62.50; H, 7.27; N, 11.22. Found: C, 62.35, 62.52; H, 7.25, 7.18; N, 10.6, 10.9.

6-Benzoylamino-2-bromohexanoic Acid (X).—6-Benzoylamino-2-hydroxyhexanamide (4.03 g., 0.016 mole) and a solution of 10.6 g. of anhydrous hydrogen bromide in 16 g. of glacial acetic acid, sealed in a Carius tube, were heated at 110° for five hours. The product was stripped of acetic and hydrobromic acids at the water pump and the residue added to water. The oil (3.7 g., 61% yield) soon solidified and after crystallization from ethanol melted at 156–160° and contained 22.9% bromine compared with 25.4% calculated for $C_{13}H_{16}O_3NBr$. Eck and Marvel[8] reported a melting point of 166° for 6-benzoylamino-2-bromohexanoic acid.

Reaction of the crude bromoacid (3.7 g.) with aqueous ammonia following Eck and Marvel[8] gave 1.7 g. (58% yield) of *dl*-N^{ϵ}-benzoyllysine (XI). The melting point varied between 225° and 265° depending on the rate of heating; a reported value[8] is 265°.

Anal. Calcd. for $C_{13}H_{18}O_3N_2$: C, 62.4; H, 7.2; N, 11.2. Found: C, 62.7, 62.9; H, 7.3, 7.3; N, 11.1, 11.1.

The dibenzoate prepared by addition of benzoyl chloride to a solution of the above material in 10% sodium hydroxide melted at 141–143° alone and at 142–144° when mixed with *dl*-benzoyllysine (m.p. 144–145°) prepared from authentic *dl*-lysine hydrochloride.

Acknowledgment.—We are indebted to G. H. Magruder and to G. G. Ecke for portions of the experimental work.

EMERYVILLE, CALIFORNIA RECEIVED MARCH 12, 1951

[7] E. Fischer and G. Zemplén, *Ber.*, **42**, 4878 (1909).

[8] J. C. Eck and C. S. Marvel, *J. Biol. Chem.*, **106**, 387 (1934).

[CONTRIBUTION FROM THE SHELL DEVELOPMENT COMPANY]

Reactions of Acrolein and Related Compounds. V. Isomerization of 3,3-Diacyloxypropenes

BY CURTIS W. SMITH, DOUGLAS G. NORTON AND SEAVER A. BALLARD

Novel 1,3-diacyloxypropenes have been obtained by allylic rearrangement of 3,3-diacyloxypropenes. Addition of acetic acid to 3,3-diacetoxypropene has given 1,1,3-triacetoxypropane. Reactions of 1,3-diacetoxypropene and 3,3-diacetoxypropene with potassium cyanide have given a compound which may be α-acetoxyglutaronitrile.

Introduction

During an investigation of the properties of the known 3,3-diacetoxypropene (allylidene diacetate) from acrolein and acetic anhydride, it has been found that it can be isomerized in high yields to the previously unreported 1,3-diacetoxypropene. A study of equilibrium conditions for this isomerization was carried out and conversions to isomer as high as 31% were observed. This isomerization has also been carried out with 3,3-dibutyroxypropene (from acrolein and butyric anhydride), 3,3-diacetoxy-2-methylpropene[1] (from methacrolein and acetic anhydride) and 3,3-dipropionoxy-2-neopentylpropene (from α-neopentylacrolein and propionic anhydride).

As an extension of the above study it was found that acetic acid could be added to 3,3-diacetoxypropene to give the novel 1,1,3-triacetoxypropane. Other reactions of 1,3- and 3,3-diacetoxypropene have been carried out. During the course of this work certain improvements in the preparation of 3,3-diacetoxypropene were realized.

Discussion

Preparation of 3,3-Diacetoxypropene.—Previous preparations of 3,3-diacetoxypropene from acrolein and acetic anhydride with acidic catalysts have involved low reaction temperatures (<10°) and long reaction times[1,2] (5–10 hr.) or low yields (64%).[3] We have found that by adding

[1] J. H. Brant and F. R. Conklin (Eastman Kodak Co.), U. S. Patent 2,393,740 (1946).

[2] A. Kirrmann, *Bull. soc. chim.*, [5] **4**, 502 (1937).

[3] A. Wohl and R. Maag, *Ber.*, **43**, 3293 (1910).

acrolein dropwise to a 10% excess of acetic anhydride containing sulfuric acid as catalyst at 30–50° a 90% conversion to product and a 93% yield of 3,3-diacetoxypropene may be obtained in as little as 17 minutes reaction time. Although Kirrmann[2] was able to distil the 3,3-diacetoxypropene without neutralization of catalyst, we have found that in order to achieve careful fractionation of the product it is necessary to neutralize the catalyst, for the reaction is in equilibrium and reversion to acrolein and acetic anhydride proceeds readily.

Isomerization of 3,3-Diacyloxypropenes.—The isomer of 3,3-diacetoxypropene boiled about 16° higher (91–92° (10 mm.)) than the parent material. The infrared absorption spectrum with a strong broad band centering about 5.73 μ and a weaker, sharper band at 5.95 μ is that to be expected from 1,3-diacetoxypropene and is distinctly different from that of 3,3-diacetoxypropene with a strong sharp band at 5.68 μ and a weaker band at 6.06 μ. The bands at 5.95 and 6.06 μ are attributable to the ethylenic bonds. Generally speaking,[4] substitution on an olefin lowers the position of absorption attributed to the double bond and substitution at opposite ends of the double bond causes more lowering than two substituents on the same carbon. Examples in Table II bear this out. The broad band at 5.73 μ indicates two types of ester linkages (vinyl esters, 5.67 μ; saturated esters, 5.75 μ). It is interesting to note that the two acetoxy groups on the same carbon atom as in allylidene diacetate and propylidene diacetate lowers the position of absorption of the ester band from 5.75 to 5.68 μ.

Hydrogenation of the isomer gave a saturated derivative whose physical constants and infrared absorption spectrum were in agreement with those of the known 1,3-diacetoxypropane. The following formula shows the equilibrium between 3,3-diacetoxypropene and 1,3-diacetoxypropene.

$$\mathrm{CH_2{=}CHCHO + (CH_3CO)_2O} \xrightleftharpoons{\mathrm{H^+}} \mathrm{CH_2{=}CHCH(OCOCH_3)_2} \xrightleftharpoons{\mathrm{H^+}} \mathrm{CH_3COOCH{=}CHCH_2OCOCH_3}$$

Table I lists a series of experiments designed to study the above equilibrium. In several of the experiments a slight excess of acetic anhydride was added to minimize reversion to acrolein. The isomerization had apparently reached equilibrium after two hours at 110°. With longer reaction times polymerization becomes significant and there is a resultant decrease in yield. At higher temperatures the equilibrium is shifted in favor of acrolein, acetic anhydride and 1,3-diacetoxypropene at the expense of 3,3-diacetoxypropene. From these experiments it can be seen that in the preparation of 3,3-diacetoxypropene long reaction times and temperatures in excess of 50° are to be avoided in order to minimize isomer and polymer formation.

The conditions for the preparation of other diacyloxypropenes along with their properties and analyses are given in Table II. In general the 1,3-isomers have about 20° higher boiling points and higher refractive indices than the 3,3-isomers.

Reactions of 1,3-Diacetoxypropene.—The hydrogenation of 1,3-diacetoxypropene to give 1,3-diacetoxypropane proceeded rapidly at low pressure over nickel. By somewhat novel reactions either 1,3- or 3,3-diacetoxypropene with potassium cyanide gave a compound whose analysis and infrared absorption spectrum were in agreement with that to be expected of α-acetoxyglutaronitrile.

$$\begin{tikzcd}
\mathrm{CH_3COOCH_2CH{=}CHOCOCH_3} \arrow[dr, "\mathrm{KCN}"'] & & \mathrm{CH_2{=}CHCH(OCOCH_3)_2} \arrow[dl, "\mathrm{KCN}"] \\
& \mathrm{[NCCH_2CH{=}CHOCOCH_3]} \arrow[d, "\mathrm{HCN}"] & \\
& \mathrm{NCCH_2CH_2CH(CN)OCOCH_3} &
\end{tikzcd}$$

This reaction may proceed from 1,3-diacetoxypropene by replacement of the allylic acetoxy group by a nitrile radical followed by addition of HCN to the ethylenic bond. If the first reaction of the 3,3-isomer with potassium cyanide gave 1-acetoxy-3-cyanopropene by allylic rearrangement, subsequent addition of hydrogen cyanide would again give the α-acetoxyglutaronitrile.

An analogy for the above replacement of an acyloxy group by the nitrile group has been previously observed by Price and Kaplan[5] in the reaction of γ-anisyl-γ-butyrolactone with potassium cyanide to give the potassium salt of γ-anisyl-γ-cyanobutyric acid. As might be expected the 1,3-diacetoxypropene showed much less tendency to polymerize with peroxide catalyst than did the 3,3-isomer.

1,1,3-Triacetoxypropane.—We have found that acetic acid may be added to 3,3-diacetoxypropene with sulfuric acid as catalyst at 50° to give 1,1,3-triacetoxypropane. This triacetate gave a derivative with 2,4-dinitrophenylhydrazine which caused no depression of melting point in mixture with the 2,4-dinitrophenylhydrazone of acrolein. The loss of acetic acid in the preparation of this derivative is not too surprising in view of the fact that under the same conditions[6] β-ethoxypropionaldehyde loses ethanol to give the 2,4-dinitrophenylhydrazone of acrolein. Hydrogenolysis of the triacetate over Raney nickel at 150° gave the known 1,3-diacetoxypropane.

Experimental

Preparation of 3,3-Diacetoxypropene.—To a stirred solution of 5.5 moles (561 g.) of acetic anhydride containing 2.1 g. (0.021 mole) of sulfuric acid (0.25% of total weight), 5 moles (280 g.) of acrolein was added over a period of seven minutes. The temperature was maintained at 50° throughout the addition and for ten minutes thereafter. At this time, 7 g. (0.085 mole) of powdered anhydrous sodium acetate was added and the product was distilled. After removal of 8.5 g. of acrolein and 90 g. of acetic anhydride, a total of 713 g., b.p. 90–100° (40 mm.), (90% conversion) of 3,3-diacetoxypropene and 34 g., b.p. 90–97° (10 mm.), (4.3% conversion) of 1,3-diacetoxypropene were obtained. This corresponds to a 93% yield of 3,3-diacetoxypropene taking credit for recovered acrolein and acetic anhydride. The physical constants of the product are given in Table II.

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[4] R. S. Rasmussen, *Fortschr. Chem. org. Naturstoffe*, **5**, 348 (1948).

[5] C. C. Price and W. Kaplan, THIS JOURNAL, **66**, 477 (1944).

[6] R. L. Shriner and R. C. Fuson, "Identification of Organic Compounds," 2nd Edition, John Wiley and Sons, Inc., New York, N. Y., 1940, p. 143.

TABLE I

ISOMERIZATION OF 3,3-DIACETOXYPROPENE[a]

Acetic anhydride added, %wt.	Temp., °C.	Time, hr.	3,3-Diacetoxypropene consumed, %	Conversion to 1,3-diacetoxypropene, %	Yield[b] of 1,3-diacetoxypropene, %	Recovery of acrolein and acetic anhydride,[c] %	Residue, %w of 3,3-diacetoxypropene
None	22	28	11	Little if any	..	6	5
5	50	1	15	8	89	6	1
5	50	8	17	10	80	5	2
None	50	24	26	11	76	11	3
5	80	1	28	15	88	11	2
5	110	0.16	23	11	89	11	1
None	110	1	40	18	82	18	4
5	110	2	58	31	78	18	9
5	110	4	52	24[d]	72	19	4
None	150	0.16	34	13	69	14	6
None	150	1	66	21	63	32	12
None	150	2	76	17	41	35	23

[a] In these experiments 3,3-diacetoxypropene with 0.25%w sulfuric acid and with or without added acetic anhydride was heated in sealed glass tubes for the specified time, neutralized with twice the theoretical equivalents of sodium acetate and distilled. The sulfuric acid was added as a 10%v solution in ether. [b] Takes credit for acrolein and acetic anhydride as equivalent weight of 3,3-diacetoxypropene. [c] These figures do not include the acetic anhydride added. [d] In this experiment a 5% conversion to 1,1,3-triacetoxypropane was observed.

TABLE II

PREPARATION AND PROPERTIES OF DIACYLOXYPROPENES

		Preparation[b]				Properties						Analyses, %			
						Boiling point			Infrared bands, μ			Carbon		Hydrogen	
Expt.	Compound, propene	Temp., °C.	Time, min.	Conv., %	Yield,[a] %	°C.	Mm.	$n^{20}D$	Ester	Olefin	Formula	Theory	Found	Theory	Found
1	3,3-Diacetoxy-[c]	50	17	90	93	184 75	760 10	1.4203	5.68	6.06	$C_7H_{10}O_4$	...	...	..	..
2	1,3-Diacetoxy-	110	120	31	78	91–92	10	1.4384	5.73	5.95	$C_7H_{10}O_4$	53.16	53.04 52.95	6.37	6.39 6.40
3	3,3-Dibutyroxy-	35	90	79	88	65–66	1	1.4280	5.69	6.03	$C_{11}H_{18}O_4$	61.66	61.74	8.47	8.46
4	1,3-Dibutyroxy-	110	240	12	62	81	1	1.4448	5.73	5.96	$C_{11}H_{18}O_4$	61.66	61.66	8.47	8.47
5	3,3-Diacetoxy-2-methyl-	70	240	70	92	87	10[d]	1.4250[d]	5.67	6.04	$C_8H_{12}O_4$	...	...	..	..
6	1,3-Diacetoxy-2-methyl-	150	120	>8	>50	74–79	2	1.4450	5.72	5.91	$C_8H_{12}O_4$	55.8	56.0	7.0	7.2
7	3,3-Dipropionoxy-2-neopentyl-[e]	40–45	60	44	81	71.2	0.5	1.4370	5.75	6.08	$C_{14}H_{24}O_4$	65.59	65.22	9.44	9.40
8	1,3-Dipropionoxy-2-neopentyl-	110	240	16	95	90	0.5	1.4509	5.78	6.01	$C_{14}H_{24}O_4$	65.59	65.75	9.44	9.47

[a] Takes credit for recovered aldehyde and anhydride and recovered 3,3-isomer in the isomerization experiments. [b] In these experiments 0.25%w of sulfuric acid was added as catalyst. In the reaction of the acid anhydrides with the aldehydes (1,5,7) a 10% excess of anhydride was used. In the isomerization experiments (2,6,8) a 10% excess anhydride was added to minimize reversion to aldehyde and anhydride. Equimolar amounts of acrolein and butyric anhydride were used in expt. 3. No extra butyric anhydride was added in expt. 4. [c] For earlier references to the preparation of 3,3-diacetoxypropene see references 1 and 2. [d] Reference 2 gives the b.p. 100° (21 mm.) and refractive index, $n^{20}D$ 1.4256 for 3,3-diacetoxy-2-methylpropene. [e] Neopentylacrolein (b.p. 149–50°, $n^{20}D$ 1.4363, d^{20}_4 0.840) was prepared by G. Hearne and M. Adams (Shell Development Co.), U. S. Patent 2,486,842 (1949).

Phosphoric and *p*-toluenesulfonic acids were also found to catalyze the reaction.

The presence of water in the reaction mixture is deleterious in that it reacts with an equivalent of acetic anhydride thus displacing the equilibrium (shown earlier in this paper) to the left and producing acetic acid a part of which adds to the diacetoxypropenes to further complicate the reaction.

Isomerization of 3,3-Diacetoxypropene.—A solution containing 100 g. of 3,3-diacetoxypropene, 5 g. of acetic anhydride and 0.14 ml. (0.0025 mole) of sulfuric acid was heated at 110° for two hours in a sealed glass vessel. The acid was then neutralized with 0.5 g. (0.0058 mole) of powdered anhydrous sodium acetate. Distillation of the product gave 23 g. of a mixture of acrolein and acetic anhydride, 42 g. of unreacted 3,3-diacetoxypropene and 31 g. of 1,3-diacetoxypropene, b.p. 85–95° (10 mm.). Analyses and physical constants are given in Table II.

Hydrogenation of 1,3-Diacetoxypropene.—1,3-Diacetoxypropene (80 g.) was hydrogenated over Harshaw nickel at room temperature and 30 p.s.i. for two hours. Distillation gave 59 g. of product which boiled at 88–90° (10 mm.), $n^{20}D$ 1.4184. A sample of 1,3-diacetoxypropane prepared from 1,3-propanediol with acetic anhydride boiled at 88–90° (10 mm.), $n^{20}D$ 1.4192. The infrared absorption of the two samples of 1,3-diacetoxypropane were essentially identical with an ester band at 5.75 μ which is at distinct variance with that of 1,1-diacetoxypropane whose ester band is at 5.68 μ. The low yield, 74%, in the hydrogenation may have been due to hydrogenolysis since 20 g. of low boiling material was obtained which had an odor suggestive of acetic acid and propyl acetate.

Addition of Acetic Acid to 3,3-Diacetoxypropene.—A solution of 100 g. (0.63 mole) of 3,3-diacetoxypropene, 38 g. (0.63 mole) of acetic acid and 0.14 ml. (0.0025 mole) of sulfuric acid was heated in a sealed glass vessel at 50° for 18 hours. The mixture was cooled and 0.5 g. (0.008 mole) of sodium acetate was added. Distillation gave, besides 38 g. of a mixture of acrolein, acetic acid and acetic anhydride, 37.5 g. of unreacted 3,3-diacetoxypropene, 22 g. of 1,3-diacetoxypropene, and 31 g. of 1,1,3-triacetoxypropane, b.p. 100–101° (0.8 mm.), $n^{20}D$ 1.4270.

Anal. Calcd. for $C_9H_{14}O_6$: C, 49.54; H, 6.47; sapn. equiv., 72.6. Found: C, 49.48, 49.52; H, 6.55, 6.44; sapn. equiv., 69.5.

The infrared absorption spectrum had a broad band at 5.68 μ.

Treatment of 2 g. of the triacetate with 1.5 g. of 2,4-dinitrophenylhydrazine in 100 ml. of ethanol containing 2 ml. of hydrochloric acid gave a derivative shown by melting point and mixed melting point, 165°, to be identical with the 2,4-dinitrophenylhydrazone of acrolein.

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.

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[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

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[10] Holman, unpublished data.