taken up in the 3 *N* hydrochloric acid solution a considerable amount of acid-insoluble material appeared which proved to be identical with the symmetrical acid anhydride.

α,α'-Di-(12-oxo-*trans*-10-octadecenoyl)-glyceride.—The above esterification procedure was applied to 2.96 g. (0.01 mole) of 12-oxo-*trans*-10-octadecenoic acid using toluene as solvent and glycerol as the alcohol component. The solid reaction residue was taken up in 100 ml. of ether and the ether solution was washed with successive portions of 200 ml. of 10% potassium carbonate and water. The ether layer was dried over sodium sulfate and the ether was removed under reduced pressure. The residue was taken up in 50 ml. of hot ethanol. The solution was cooled to 0° and a precipitate was filtered off which after several recrystallizations from petroleum ether melted at 52–53°; it proved to be identical with 12-oxo-*trans*-10-octadecenoic anhydride; yield 1.2 g. When the filtrate was cooled to −20°, 1 g. of a material was obtained which was recrystallized from petroleum ether; m.p. 52–53°. This material gave a marked m.p. depression when admixed with the anhydride fraction.

Anal. Calcd. for $C_{39}H_{68}O_7$: C, 72.18; H, 10.56. Found: C, 72.03; H, 10.43.

β-Diethylmethylammoniumethyl 12-Oxo-*trans*-10-octadecenoate Iodide.—A solution containing 0.26 g. of β-diethylaminoethyl 12-oxo-*trans*-10-octadecenoate, 0.2 ml. of methyl iodide and 5 ml. of dry ether was allowed to stand overnight. The yellow precipitate was collected and recrystallized from acetone and ethyl acetate; yield, 0.3 g. (83%); m.p. 70–71°.

Anal. Calcd. for $C_{25}H_{46}INO_3$: C, 55.85; H, 9.00; N, 2.61. Found: C, 56.01; H, 9.07; N, 2.42.

Preparation of Anhydrides.—The anhydrides were prepared by the general method outlined for the preparation of the amides except that in place of an amine, one equivalent of the triethylamine salt of the carboxylic acid dissolved in 100 ml. of the reaction solvent was added to the mixed carbonic–carboxylic acid anhydride. The reaction product was worked up in a manner identical with that described above for the isolation of neutral amides.

SOMERVILLE, N. J.

[CONTRIBUTION FROM THE BIOCHEMISTRY AND CHEMISTRY DEPARTMENTS, UNIVERSITY OF PITTSBURGH]

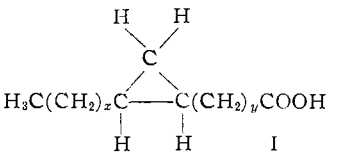
Studies on the Structure of Lactobacillic Acid. II. Position of the Cyclopropane Ring[1,2]

BY KLAUS HOFMANN, GINO J. MARCO AND GEORGE A. JEFFREY

RECEIVED MAY 3, 1958

A micromethod is described for the degradation of long-chain cyclopropane fatty acids. The procedure provides valid information regarding the position of the cyclopropane ring in this class of compounds. The cyclopropane ring of lactobacillic acid was shown to occupy the 11,12-position on the octadecanoic acid chain. X-Ray diffraction methods were used in the identification of the degradation products. Undecanedioic acid was found to be dimorphic, and X-ray diffraction data for both forms are presented. In addition to furnishing information regarding the ring position, the present degradation method provides a means to selectively remove the methylene bridge-carbon from the rest of the carbon chain of cyclopropane fatty acids. The biochemical implications of this finding are discussed.

Previous investigations from this Laboratory[3–5] have led to the conclusion that lactobacillic acid, a lipid constituent of various microörganisms, is a methyleneoctadecanoic acid of the general structure I



The results of chemical and physical studies and a comparison with synthetic methyleneoctadecanoic acids of known structure established the cyclopropane nature of lactobacillic acid, but the position of the cyclopropane ring and the stereochemistry of the molecule remained to be determined. In the present communication we wish to present results of degradative studies, on a micro scale, which locate the methylene bridge in lactobacillic acid between positions 11 and 12 on the octadecanoic acid chain.

(1) Supported by Grants from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council; Ciba Pharmaceutical Products, Inc., Summit, N. J., and the U. S. Public Health Service.

(2) A preliminary communication reporting some of the results of this investigation has appeared: G. J. Marco and K. Hofmann, *Federation Proc.*, **15**, 308 (1956).

(3) K. Hofmann and R. A. Lucas, THIS JOURNAL, **72**, 4328 (1950).

(4) K. Hofmann, R. A. Lucas and S. M. Sax, *J. Biol. Chem.*, **195**, 473 (1952).

(5) K. Hofmann, O. Jucker, W. R. Miller, A. C. Young, Jr., and F. Tausig, THIS JOURNAL, **76**, 1799 (1954).

In 1952,[4] it was observed that treatment of lactobacillic acid with hydrogen bromide in glacial acetic acid resulted in formation of an oily acidic product arising from addition of the elements of hydrogen bromide to the cyclopropane ring. This method of opening the ring now has been repeated with larger samples of lactobacillic acid, and also was applied to three other acids of the cyclopropane series, namely, *trans*-DL-9,10- and *trans*-DL-11,12-methyleneoctadecanoic acids and dihydrosterculic acid (*cis*-DL-9,10-methyleneoctadecanoic acid). The ring position and stereochemistry of the latter three acids is known with certainty.[5,6] Bromine analyses of the crude reaction products demonstrated a practically quantitative reaction with hydrogen bromide of all the acids studied. Dehydrobromination in boiling *s*-collidine under nitrogen converted the hydrobromination products from each acid into a mixture of monoethenoid fatty acids. Iodine number determinations performed with the dehydrobrominated materials indicated the following olefinic acid percentage content: lactobacillic acid, 59.0; dihydrosterculic acid, 70.6; *trans*-9,10-methyleneoctadecanoic acid, 67.3; *trans*-11,12-methyleneoctadecanoic acid, 89.7. (These figures are based on a theoretical iodine number of 85.7 for a monoethenoid nonadecanoic acid.) The crude dehydrohalogenated materials derived from each acid then were subjected to hydroxylation with performic acid, and the ensuing

(6) H. Hofmann, S. F. Orochena and C. W. Yoho, *ibid.*, **79**, 3608 (1957).

reaction products were oxidized further, first with periodate and then with silver oxide. The oxidation products were divided into a neutral and an acidic fraction, and the latter was separated into various components by chromatography on buffered silicic acid columns. Klenk and Bongard[7] described a buffered silica gel column for the separation of odd and even mono- and dibasic acids up to twelve carbon atoms. Our experience with this column was not encouraging, possibly because the "aged" silica gel prepared in our laboratory possessed properties differing from those of the gel employed by the German investigators. In the Experimental section we describe a column, prepared with commercially available silicic acid, which has given consistently good results. For example, a synthetic mixture of the composition *n*-heptanoic acid (10.94 mg.), undecanedioic acid (4.26 mg.), sebacic acid (3.96 mg.), azelaic acid (3.66 mg.) and suberic acid (3.84 mg.) readily was resolved into its constituents with recoveries of the various acids of 104.4, 97.3, 91.5, 93.5 and 96.7%, respectively (Fig. 1). The individual acids were

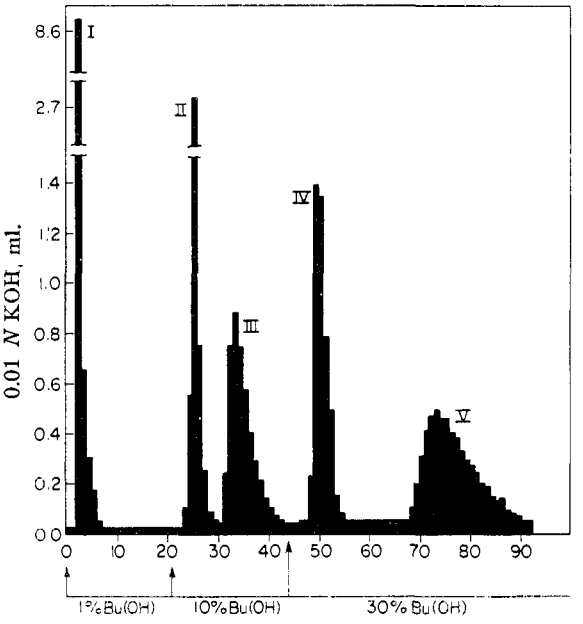


Fig. 1.—Chromatogram of synthetic mixture of mono-, and dibasic acids; see text for details.

isolated from the respective chromatographic bands and identified by melting point, by mixed melting point with an authentic sample and by the X-ray powder diagram. Repeatedly during the course of this investigation the observation was made that samples of undecanedioic acid which had been obtained from the chromatographic column and recrystallized from ethanol–water at −10° gave an X-ray powder diffraction pattern which differed from that of preparations of this acid prior to chromatographic treatment. Upon recrystallization from the melt, crystals of the original form were obtained. X-Ray data for undecanedioic acid, which have not been reported previously, are given in Table I.

(7) E. Klenk and W. Bongard, *Z. physiol. Chem.*, **290**, 181 (1952).

TABLE I

X-RAY POWDER DATA FOR α- AND β- UNDECANEDIOIC ACID,[a] λ CuK_a = 1.542 Å.

β-Form			α-Form		
2θ	*d*, Å.	*I*	2θ	*d*, Å.	*I*
8.0	*11.02*	s	7.0	*12.60*	s
19.0	*4.64*	vs	13.9	6.35	vw
20.5	4.33	vw	17.3	5.13	vw
22.8	*3.90*	vs	19.1	*4.66*	vs
24.9	3.57	w	22.1	*4.02*	s
27.6	*3.23*	s	23.5	*3.79*	vs
33.6	2.66	vw	26.6	3.35	vw
35.4	2.54	vw	28.5	*3.13*	s
38.0	2.37	vw	29.6	3.02	vw
40.2	*2.24*	s	39.1	2.30	w
			41.4	2.18	w

[a] The *d* spacings of the strongest lines are italicized.

The β-form was obtained by recrystallization from ethanol–water at −10° or by crystallization from the melt. The α-form ensued whenever a sample of the β-form was subjected to chromatography and the isolated material was recrystallized from ethanol–water at −10°. It is well known that in the long-chain fatty acids, the formation of different polymorphic forms is dependent not only upon temperature and mode of crystallization but also, very sensitively, upon purity, and this is the most likely explanation of this observation. The assignment of α and β to these dimorphic forms of undecanedioic acid conforms with single crystal data recorded by Caspari[8,9] for the C_9-and C_{13}-dicarboxylic acids. The principal spacings which correspond to the strong lines in the powder patterns of the two forms of the C_9, C_{11}, and C_{13}-dicarboxylic acids are shown in Table II. Our data for the undecanedioic acid fits in with that of the homologous series. In the α-series, the average increment in *c* spacing is 1.1 Å. per methylene group and in the β series it is about 1.0 Å., since there are two molecules, head to head, along the length of the *c*-axis. The indexing of the spacings was deduced from Caspari's[8,9] single crystal data

TABLE II

PRINCIPAL SPACINGS FOR THE α- AND β-FORMS OF AZELAIC, UNDECANEDIOIC AND BRASSYLIC ACIDS IN Å.

	α-Series					β-Series			
	d_{002}	d_{011}	d_{110}	d_{200}	d_{211}	d_{002}	d_{020}	d_{100}	d_{101}
C_9	10.40	4.70	4.06	3.74	3.16[a]	9.30	4.79	3.87	3.30[a]
						9.56	4.67	3.87	3.28[b]
C_{11}	12.60	4.67	4.02	3.80	3.14	11.02	4.64	3.90	3.24
C_{13}	14.90	4.75	4.06	3.77	3.11[a]	13.30	4.70	3.93	3.21[b]

[a] Calculated from the single crystal data of Caspari.[8,9] [b] Reported from powder photographs by A. R. Normand, T. D. M. Ross and E. Henderson, *J. Chem. Soc.*, 2632 (1926).

	a	*b*	*c*	β
α-Azelaic	9.72	4.83	27.14	129°30′
α-Brassylic	9.63	4.82	37.95	128°28′
β-Azelaic	5.61	9.58	27.20	136°31′

and information which he gives about the intensities of the reflections.

Chromatography of the acidic oxidation products derived from the four cyclopropane fatty acids gave

(8) W. A. Caspari, *J. Chem. Soc.*, 3235 (1928).
(9) W. A. Caspari, *ibid.*, 2709 (1929).

TABLE III

MELTING POINTS OF DIBASIC ACIDS[a]

Source of acids	Band II C_{11}	Band III C_{10}	Band IV C_9	Band V C_8
Ref. compounds	108.5–109.5	127.5–128.5	104.5–105.5	135.0–136.0
Lactobacillic acid	109.0–110.0	126.5–128.0	.....	.....
	(108.5–109.5)	(126.5–128.0)	.....	.....
11,12-Methyleneoctadecanoic acid	108.5–109.5	127.0–128.0	.....	.....
	(108.5–109.5)	(126.5–128.0)	.....	.....
Dihydrosterculic acid	.....	.....	104.0–105.0	134.5–135.5
	.....	.....	(103.5–104.5)	(135.0–136.0)
9,10-Methyleneoctadecanoic acid	.....	.....	104.0–105.0	135.0–136.0
	.....	.....	(103.5–105.0)	(134.5–135.5)

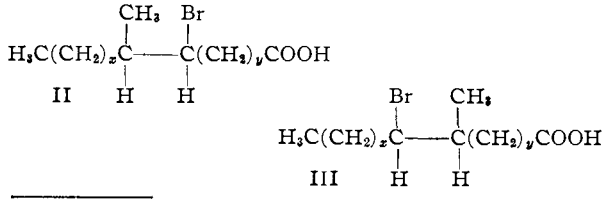
[a] Melting points of 1:1 mixtures with the respective reference compound are given in parentheses.

the patterns illustrated in Fig. 2. The monocarboxylic acid fraction (band I) was not investigated. The acids from the other major bands (bands II to V) were isolated and identified by the methods employed with the synthetic mixture. The acid isolated from band II was identified as undecanedioic acid; the acids corresponding to bands III, IV and V proved to be sebacic, azelaic and suberic acids, respectively (see Table III).

From inspection of Fig. 2 it is apparent that the two methyleneoctadecanoic acids with established 9,10-position of the cyclopropane ring afforded approximately equal proportions of azelaic and suberic acids as the major dibasic acid fragments, whereas 11,12-methyleneoctadecanoic and lactobacillic acids gave approximately equal proportions of undecanedioic and sebacic acids under identical degradative conditions. These findings suggest that our degradation procedure provides valid information regarding the ring position in this series of compounds. The results leave little doubt regarding the 11,12-position of the cyclopropane ring in lactobacillic acid, a conclusion which is supported by recent X-ray studies.[10]

While this study was in progress we became acquainted with the very elegant oxidation method of Lemieux and von Rudloff.[11] In order to ascertain that this more direct method affords results comparable to those which we had obtained with the standard oxidation method, a sample of the dehydrobromination products derived from dihydrosterculic acid was oxidized by the Lemieux procedure and the acidic fragments were analyzed chromatographically. The ensuing pattern was indistinguishable from the one illustrated in Fig. 2, pattern D.

The complex sequence of events leading to formation of the characterized degradation products may receive brief consideration at this point. It seems plausible to assume that the attack of hydrogen bromide on the cyclopropane ring proceeds



predominantly according to Markownikoff's rule. and that the main components of the bromo acid mixture possess structures II and III. However, the presence of acids of structures IV and V cannot be excluded. Elimination of the elements of hydrogen bromide from these four bromo acids may result in formation of eight olefinic acids, but not in equal proportions necessarily. However, two of these possibilities, namely, compounds VI and VII, are likely to be present in significant and approximately equivalent proportions since they are the most plausible precursors of the identified dibasic acids. These materials may arise from compound II by dehydrobromination in accordance with, or in violation of, Saytzeff's rule. Two other compounds, namely, VIII and IX, also may give rise to the identified dicarboxylic acids; both materials could arise from compound IV.

An inspection of Fig. 2 shows the presence of two minor components in the dicarboxylic acid region of the chromatograms derived from *trans*-9,10-methyl-

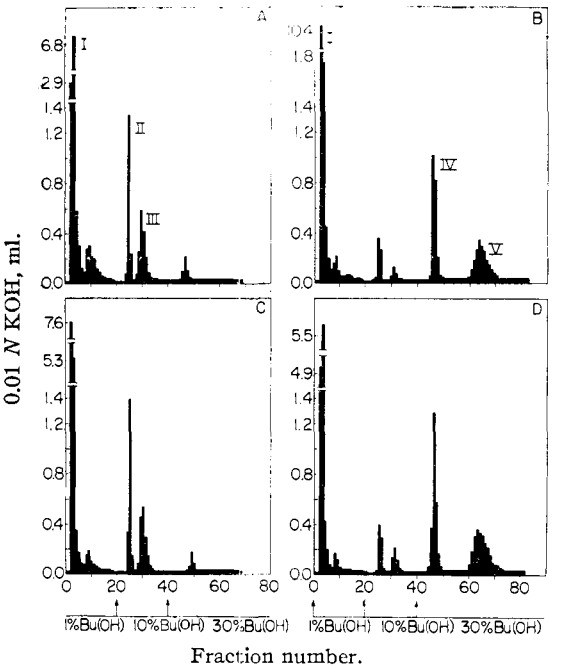
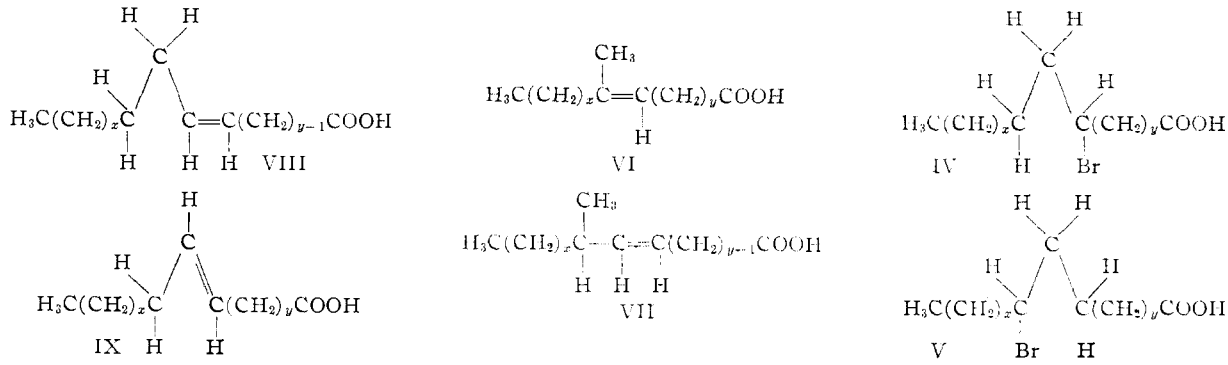


Fig. 2.—Chromatograms of acidic degradation products from: A, *trans*-DL-11,12-methyleneoctadecanoic acid; B, *trans*-DL-9,10-methyleneoctadecanoic acid; C, lactobacillic acid; and D, dihydrosterculic acid; sample size, 36.19, 44.10, 47.52 and 38.63 mg., respectively.

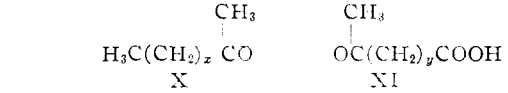
(10) T. Brotherton and G. A. Jeffrey, THIS JOURNAL, 79, 5132 (1957).

(11) R. U. Lemieux and E. von Rudloff, *Can. J. Chem.*, 33, 1701 (1955).



eneoctadecanoic and dihydrosterculic acids. The same components also were present among the oxidation products derived from the Lemieux oxidation. The very limited supplies of these compounds precluded exact identification, but the location on the chromatogram points to undecanedioic and sebacic acids as likely possibilities. The formation of small amounts of these acids is readily explicable since dehydrobromination products of V could produce the necessary olefinic precursors. The nature and origin of the small band in the azelaic acid region on the chromatograms derived from *trans*-11,12-methyleneoctadecanoic and lactobacillic acids was not investigated.

In addition to providing useful information regarding the position of the cyclopropane ring, the present degradation procedure makes possible the selective removal of the methylene bridge carbon from the rest of the carbon chain of lactobacillic acid and related compounds. Treatment with hypoiodite of both the neutral and the acidic oxidation fragments from all four acids studied resulted in formation of iodoform, which was identified by melting and mixed melting point determinations. Two oxidation products, namely, compounds **X** and **XI**, are the only plausible precursors for iodoform formation. It follows logically that,



since the methyl ketone methyl group of these compounds must have its origin in the methylene bridge carbon of the original acids, the iodoform carbon must be derived from this source. The application of this scheme of degradation to radioactive biosynthetic lactobacillic acid samples may provide a clue regarding the biosynthesis of this compound. Work along these lines is in progress.

Experimental

General Comments.—Details are presented for the degradation of lactobacillic acid; the degradation of other acids followed exactly the same procedures. Melting points were determined with a Kofler-type micro hot-stage, and are uncorrected. Solvents were freshly distilled. Chloroform was freed of alcohol by distillation over calcium oxide. Extractions were performed in counter-current fashion by the use of separatory funnels with Teflon stopcocks requiring no lubrication. The iodine numbers were determined according to the method of Hoffman and Green.[12]

Degradation of Lactobacillic Acid. Hydrobromination.—Lactobacillic acid (222 mg.), isolated from *Agrobacterium tumefaciens*,[13] was dissolved in 5 ml. of a 30% solution of hydrogen bromide in glacial acetic acid, and the mixture was heated at 100° for three hours in a sealed tube. The solvent was removed *in vacuo*, the residue was dissolved in ether and the ethereal solution was washed successively with two 10-ml. portions of water, one 10-ml. portion of 5% sodium bicarbonate and two 10-ml. portions of water and dried over sodium sulfate. The ensuing oil was dried to constant weight *in vacuo* at room temperature; yield 264 mg. (93.7%).

Anal. Calcd. for $C_{19}H_{37}O_2Br$: Br, 21.2. Found: Br, 21.0.

Dehydrobromination.—The hydrobromination products (250 mg.) were dissolved in *s*-collidine (5 ml.) and the solution was refluxed under nitrogen for one hour at a bath of 190–195°. The ensuing suspension was cooled and the collidine hydrobromide removed by filtration through a pad of glass wool. Water (20 ml.) was added to the clear filtrate, and the solution was cooled with ice-water. Sulfuric acid (50%) was added slowly until the solution was acid to congo red and the mixture was extracted with ether. The ethereal extracts were washed successively with two 10-ml. portions of water, one 10-ml. portion of 5% sodium bicarbonate and two 10-ml. portions of water, and dried over sodium sulfate. The resulting oil was dried to constant weight at room temperature *in vacuo;* yield 210 mg., iodine number 50.6.

Oxidation. a. Standard Procedure.—The dehydrohalogenation products (220 mg.) were dissolved in 88% formic acid (2 ml.) and 30% hydrogen peroxide (0.5 ml.) was added. The mixture was shaken for 10 minutes with cooling under the tap, and then was kept at 40° for 2.5 hours with occasional shaking. The solvents were removed *in vacuo*, 3 *N* potassium hydroxide (15 ml.) was added, and the solution was heated at 100–105° for 3 hours under nitrogen. Water (15 ml.) was added and the mixture was acidified to congo red with 50% sulfuric acid. The hydroxylated acids were isolated in the usual manner; yield 182 mg. This material was dissolved in 95% ethanol (10 ml.), the solution was heated at 40° and a solution of periodic acid (496 mg.) in water (10 ml.) was added. The mixture was kept at room temperature for three hours with occasional shaking, and then was poured into 20 ml. of water. The split products were isolated in the usual manner, and dissolved in methanol (6 ml.). Silver oxide (prepared from 1.5 g. of silver nitrate) was added and the slurry was shaken at room temperature while *N* sodium hydroxide was added slowly to the phenolphthalein end-point. Then more sodium hydroxide (2 ml.) was added and shaking was continued for 10 minutes. The slurry was filtered through a thin layer of Hyflo Filtercel and the clear filtrate was diluted with water (15 ml.) and ether extracted. The neutral split products were isolated from the ether extract in the usual manner; yield 35 mg. From the alkaline solutions and washings the acidic split fragments were isolated, and dried *in vacuo* at room temperature; yield 119 mg.

b. Lemieux Procedure.—A mixture of the dehydrobromination products from dihydrosterculic acid (202 mg.), anhydrous potassium carbonate (214 mg.), sodium metaperiodate (663 mg.), water (200 ml.) and potassium permanganate (12 mg.) was shaken until all ingredients were dissolved. The solution was kept at room temperature for 20 hours, and then was acidified to congo red with 50%

(12) H. D. Hoffman and C. E. Green, *Oil and Soap*, **16**, 236 (1939).

(13) K. Hofmann and F. Tausig, *J. Biol. Chem.*, **213**, 125 (1955).

sulfuric acid. Isolation of the acidic and neutral products in the usual manner afforded 37 mg. of neutral and 174 mg. of acidic split products.

c. **Hypoiodite Oxidation.**—Samples (5 to 10 mg.) of both the neutral and the acidic degradation products from lactobacillic acid were dissolved in dioxane (0.2–0.4 ml.) in a centrifuge tube and 10% sodium hydroxide (0.5 ml.) was added. Iodine–potassium iodide reagent (6.35 g. of potassium iodide plus 3.13 g. of resublimed iodine in 25 ml. of water) was added dropwise until the iodine color remained, and the tube was heated at 60° for 10 minutes. More reagent was added, if necessary, to maintain the iodine color. The mixture was cooled, the excess of iodine was decolorized by addition of 10% sodium hydroxide, and water (1 ml.) was added. The iodoform was collected, washed with water, and dried; m.p. 117–118°. No melting point depression was observed when this material was admixed with an authentic sample of iodoform.

Degradation of Dihydrosterculic Acid.—Treatment with hydrogen bromide in glacial acetic acid of 229 mg. of dihydrosterculic acid (prepared as described previously[5]) afforded 277 mg. of bromo acids; found Br, 20.4%. This material on dehydrobromination gave 205 mg. of a material with an iodine number of 60.5. On oxidation 30 mg. of neutral and 125 mg. of acidic products were obtained.

Degradation of *trans*-DL-9,10-Methyleneoctadecanoic Acid.—Treatment with hydrogen bromide in glacial acetic acid of 184 mg. of the synthetic acid[5] afforded 213 mg. of bromo acids; found Br, 20.9%. This material on dehydrobromination gave 165 mg. of a material with an iodine number of 57.7. On oxidation 28 mg. of neutral and 92 mg. of acidic products were obtained.

Degradation of *trans*-DL-11,12-Methyleneoctadecanoic Acid.—Treatment with hydrogen bromide in glacial acetic acid of 231 mg. of the synthetic acid[5] afforded 285 mg. of bromo acids; found Br, 20.6%. This material on dehydrobromination gave 210 mg. of a material with an iodine number of 76.9. On oxidation 38 mg. of neutral and 120 mg. of acidic products were obtained.

Chromatographic Procedures.—A mixture of silicic acid[14] (12 g.) and a 2 *M* phosphate buffer of *p*H 7.05 (6 ml.) was ground in a glass mortar until uniform. The resulting wet powder was suspended in chloroform containing 1% of 1-butanol v./v. (35 ml.) and the slurry was poured into a chromatogram tube (15 × 350 mm.). Entrapped air was removed by stirring, and the column was subjected to one pound of nitrogen pressure to ensure firm packing The column was then layered with 5 mm. of sand, followed by a filter disk and another 5 mm. layer of sand. The sample to be analyzed (25–50 mg.) was dissolved in chloroform containing 30% of 1-butanol v./v. (1 ml.) and the solution was delivered to the top of the column. The meniscus was allowed to reach the level of the filter paper, and two washings of 1 ml. each of chloroform containing 1% 1-butanol v./v. were used to effect a quantitative transfer of the sample to the column. The liquid level was allowed to reach the filter paper level after each washing. The column was mounted on a Technicon automatic fraction collector set at a drop rate of 50 drops per minute, and the impulse counter was adjusted to collect fractions of 280 drops each (approximately 5 ml.). The column was then eluted with mixtures of chloroform and 1-butanol having a butanol content v./v. of 1, 10 and 30%; the volume of eluent required was 100–130 ml., 100–130 ml. and 200–250 ml., respectively. Each eluting solvent was equilibrated with 2 *M* dipotassium phosphate prior to use. In order to maintain approximately equal drop rates with these various solvents it was necessary to apply positive nitrogen pressure to the top of the column; the pressure was increased with the various eluents from 0.2–0.3 to 0.5–0.7 to 1 pound, respectively. Individual fractions were titrated with 0.01 *N* methanolic potassium hydroxide to the phenolphthalein end-point, mixing being effected by bubbling a stream of nitrogen through the solutions. The meniscus of each eluent was allowed to fall to the top of the column prior to introducing the next solvent. The columns were operated at a temperature of 27 ± 2°. Typical chromatograms are illustrated in Figs. 1 and 2. The material recovery was calculated by comparing the milliequivalents of alkali required to titrate the combined effluents from the column to the milliequivalents of alkali necessary to titrate the total sample introduced; in three experiments recoveries of 98.5, 95.6 and 99.5% were realized.

Isolation and Identification of Acids.—The contents of the titrated tubes from each band were pooled, and the solvents evaporated. The dry residue was dissolved in water (5 ml.) and the solution was acidified to congo red with 50% sulfuric acid. The acids were extracted with ether, and the extracts washed with two 5-ml. portions of water. For separation from phenolphthalein the acids were re-extracted into 5% sodium bicarbonate, and were isolated in the usual manner. Normally 1–2 mg. of the dibasic acids were obtained, although a second recrystallization was often necessary. Identical fractions from several chromatograms were combined and the acids recrystallized from dilute ethanol at −10°. The melting and mixed melting point data are summarized in Table III. Samples of the dibasic acids were powdered in a micro-mortar and placed into glass capillaries of 0.3 mm. diameter and 0.01 mm. wall thickness for the X-ray photographs which were taken on a 57.3 mm. radius camera with nickel filtered CuK radiation. The powder patterns of the unknowns were compared with those obtained under identical conditions with authentic samples.

PITTSBURGH, PA.

(14) $SiO_2 \cdot xH_2O$, 100 mesh, analytical grade, prepared according to L. L. Ramsey and W. I. Patterson, *J. Assoc. Offic. Agric. Chem.*, **31**, 441 (1948), obtained from Mallinckrodt Chemical Works, New York, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF CLARK UNIVERSITY]

Ultracentrifugation of Chemically Reacting Systems

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The validity of the application to chemically reacting systems of Archibald's suggestion for direct ultracentrifuge molecular weight determinations has been examined. It has been shown that the equations for the calculation of the total solute concentrations at either meniscus in the ultracentrifuge cell, applicable both to solutions of monodisperse ideal solutes and to solutions of polydisperse non-ideal solutes are also valid for chemically reacting systems. The possible effects of the centrifugal field on the chemical equilibrium in reacting systems have been discussed. The interpretation of such direct molecular weight ultracentrifuge experiments appears to be much more straightforward than that of the corresponding sedimentation velocity experiments.

Introduction

Recently the study of chemically reacting systems by sedimentation velocity methods has been subjected to a theoretical reinterpretation.[1,2] It has been shown that, neglecting diffusion, even for very fast reactions one should expect serious errors in both the sedimentation rates and the relative concentrations of reacting components as obtained by classical interpretation. In fact, the chemical reaction, even though infinitely rapid, can be ex-

(1) G. A. Gilbert, *Disc. Faraday Soc.*, **20**, 68 (1955).

(2) G. A. Gilbert and R. C. H. Jenkins, *Nature*, **177**, 853 (1956).