Anal. Calcd. for $C_{18}H_{21}O_4N$: C, 68.6; H, 6.7. Found: C, 68.4; H, 6.8.

B. By Oxidation of Dihydrocodeinone.—Dihydrocodeinone was oxidized with chromic anhydride under the general conditions described above. Purification was effected by chromatography on alumina, using benzene to elute the large amount of unchanged dihydrocodeinone and 60 to 80% chloroform in benzene to elute the 10-hydroxydihydrocodeinone. Recrystallization from benzene gave material of m.p. 200–202°.

The methiodide was prepared in and crystallized from methanol; m.p. 240–244° dec., $[\alpha]^{25}D$ −78° (*c* 0.98, water).

Anal. Calcd. for $C_{19}H_{24}O_4NI$: C, 49.9; H, 5.3. Found: C, 50.1; H, 5.4.

10-Hydroxydihydrocodeine Methine (10-Ketotetrahydro-α-methylmorphimethine) (V).—To 1.37 g. (3 mmoles) of 10-hydroxydihydrocodeine methiodide dissolved in 7 ml. of water was added 7 ml. of 60% aqueous potassium hydroxide and the mixture was heated under reflux (nitrogen atmosphere) for one hour. The mixture was extracted thoroughly with ether, the ether was evaporated, and the residue (0.92 g.) was crystallized several times from ether to give 0.75 g. (75% yield) of methine, m.p. 114–115°, $[\alpha]^{25}D$ −45° (*c* 0.81, ethanol).

Anal. Calcd. for $C_{19}H_{25}O_4N$: C, 68.9; H, 7.6. Found: C, 68.8; H, 7.7.

The **methiodide** was prepared in methanol and crystallized from ethanol; m.p. 259–260° after drying at 100° (1 mm.); $[\alpha]^{25}D$ −35.2° (*c* 0.93, water).

Anal. Calcd. for $C_{20}H_{28}O_4NI$: C, 50.7; H, 6.0; I, 26.8. Found: C, 50.4; H, 5.4; I, 27.2.

The **semicarbazone** was prepared in the usual fashion in aqueous solution. Basification with concd. ammonium hydroxide followed by extraction with chloroform and evaporation of the chloroform left a residue which was crystallized from butanone, m.p. 129–132°.

Anal. Calcd. for $C_{20}H_{28}O_4N_4$: N, 14.4. Found: N, 14.0.

Hydrogenolysis Experiments. A. Dihydrocodeine from 10-Hydroxydihydrocodeine.—A solution of 317 mg. (1 mmole) of 10-hydroxydihydrocodeine in 15 ml. of glacial acetic acid containing 0.5 ml. of 60% aqueous perchloric acid and 200 mg. of 5% palladium on carbon was hydrogenated at 40 to 50° and 30 lb. pressure. After several hours, hydrogen absorption ceased, the solution was filtered, and the filtrate was basified with sodium hydroxide and extracted with chloroform. Sublimation of the residue left on evaporation of the chloroform gave 230 mg. (76% yield) of dihydrocodeine, m.p. 105–107°. There was no depression in m.p. on admixture with an authentic sample of dihydrocodeine.

B. Tetrahydro-α-methylmorphimethine from 10-Ketotetrahydro-α-methylmorphimethine.—The keto methine (331 mg., 1 mmole), dissolved in 15 ml. of absolute ethanol to which 1 ml. of 60% aqueous perchloric acid had been added, was hydrogenated at room temperature and atmospheric pressure using 100 mg. of 5% palladium-on-carbon as catalyst. Hydrogen absorption ceased after exactly 2 moles of hydrogen had been consumed in two hours. Water (25 ml.) was added to dissolve the crystalline precipitate that had appeared in the hydrogenation mixture, the solution was filtered, and the filtrate was extracted with five equal volume portions of chloroform. Evaporation of the chloroform extracts left 340 mg. (81%) of crude tetrahydro-α-methylmorphimethine perchlorate. The aqueous alcohol was then basified and again extracted with chloroform from which 40 mg. (12%) of the oily free base was obtained.

Crystallization of the crude **perchlorate** above from absolute ethanol and drying at 100° (1 mm.) gave material of m.p. 224–225° (reported[20] m.p. 218–219°).

Anal. Calcd. for $C_{19}H_{28}O_7NCl$: C, 54.6; H, 6.8. Found: C, 54.3; H, 6.7.

The **methiodide** was prepared from the free base in methanol and recrystallized from absolute ethanol; m.p. 225–227°, mixed m.p. with an authentic sample of tetrahydro-α-methylmorphimethine methiodide (m.p. 226–227°), 225–227°.

The **hydrochloride** was prepared by adding absolute ethanolic hydrogen chloride to an isopropyl alcohol solution of the free base. After crystallization from isopropyl alcohol and drying at 100° (1 mm.), the hydrochloride had m.p. 226–227°, $[\alpha]^{25}D$ −31.2° (*c* 0.90, water) [reported[21] for tetrahydro-α-methylmorphimethine hydrochloride, m.p. 228°, $[\alpha]^{18}D$ −31.9 (*c* 0.97, water)].

(20) H. Wieland and M. Kotake, *Ann.*, **444**, 69 (1925).

(21) E. Speyer and K. Koulen, *ibid.*, **438**, 34 (1924).

BERKELEY, CALIFORNIA

[FROM THE BIOCHEMISTRY DEPARTMENT, UNIVERSITY OF PITTSBURGH SCHOOL OF MEDICINE]

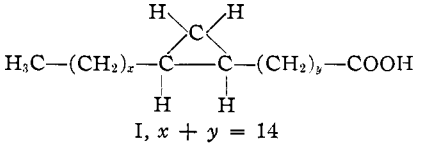
On the Structure of Lactobacillic Acid[1,2]

BY KLAUS HOFMANN, OTTO JUCKER, WILLIAM R. MILLER, ALFRED C. YOUNG, JR., AND FRED TAUSIG

RECEIVED SEPTEMBER 8, 1953

A method is presented for the isolation of dihydrosterculic acid from the kernel oil of *Sterculia foetida*, and synthetic procedures are described for the preparation of methyleneoctadecanoic acids possessing a *trans* configuration. A comparison of the infrared absorption spectra of lactobacillic and dihydrosterculic acid with those of synthetic *trans*-9,10- and 11,12-methyleneoctadecanoic acids offer strong support to the previously assigned cyclopropane structure for the naturally occurring acids.

The lipides of *Lactobacillus arabinosus*[3,4] and *Lactobacillus casei*[5] contain significant amounts of a novel fatty acid of the composition $C_{19}H_{36}O_2$ for which we have chosen the name lactobacillic acid. The chemical behavior of lactobacillic acid (stability toward oxidation and lability on hydrogenation) and the presence in its infrared absorption spectrum of a maximum at 9.8 μ pointed to the presence of a cyclopropane ring. Based on these findings we have postulated the structure of a methyleneoctadecanoic acid (I) for lactobacillic acid.[3,4]



I, $x + y = 14$

The occurrence of fatty acids of the general structure I is not limited to the above mentioned lactobacilli. The kernel oil of the tropical tree

(1) Supported by grants from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council, the Rockefeller Foundation in New York, and Ciba Pharmaceutical Products, Inc., Summit, N. J.

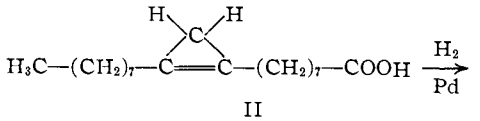
(2) A preliminary report of some of this work has appeared in the *Record of Chemical Progress*, **14**, 7 (1953).

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

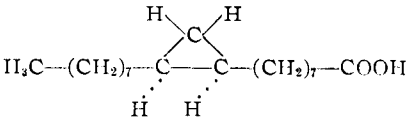
(4) K. Hofmann, R. A. Lucas and S. M. Sax, *J. Biol. Chem.*, **195**, 473 (1952).

(5) K. Hofmann and S. M. Sax, *ibid.*, **205**, 55 (1953).

Sterculia foetida contains as a major constituent a fatty acid (sterculic acid) of the composition $C_{19}H_{34}O_2$ possessing structure II.[6] This acid is converted into dihydrosterculic acid (III) when hydrogenated over palladium-in-ethanol. We regard dihydrosterculic acid as a *cis* isomer since it seems reasonable to assume that catalytic hydrogenation of II would lead to a *cis* addition of hydrogen. The close structural similarity between lactobacillic and dihydrosterculic acid made desirable a



II



III

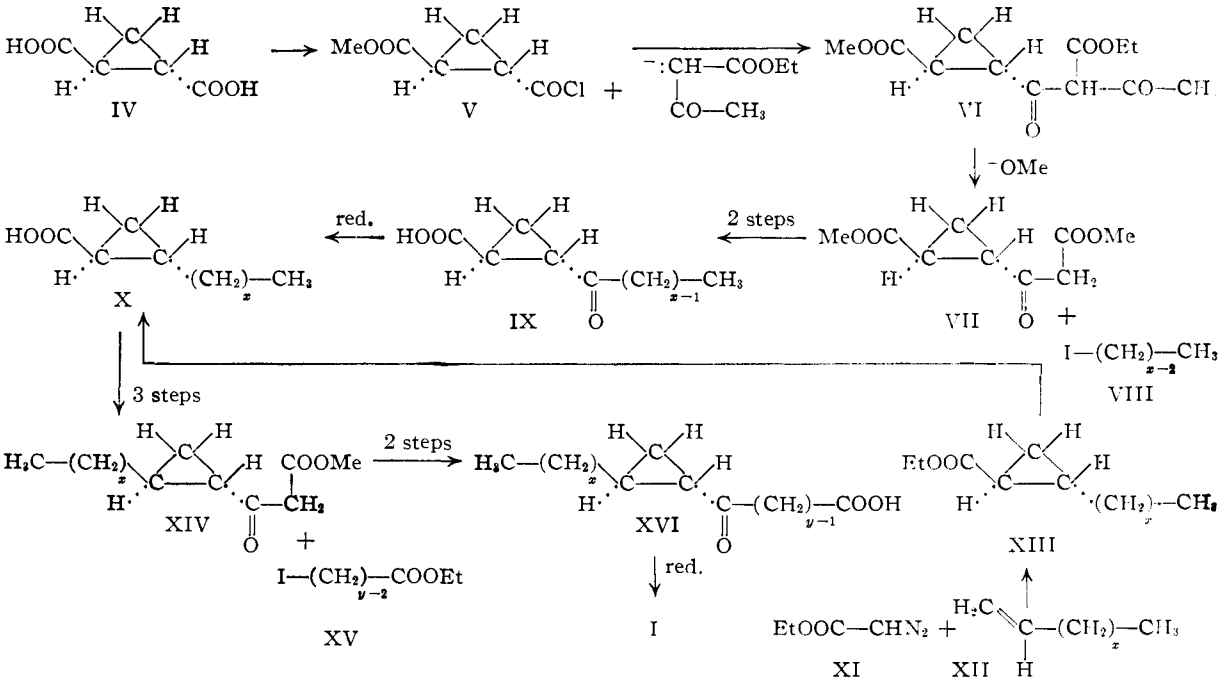
direct comparison between these two compounds. It was also of importance to compare these naturally occurring acids with synthetic models of established cyclopropane structure.

The present communication deals with the preparation of dihydrosterculic acid from the kernel oil of *Sterculia foetida*, with the synthesis of two fatty acids of the general structure I (*trans*-DL-9,10-methyleneoctadecanoic acid (x and y = 7), and *trans*-DL-11,12-methyleneoctadecanoic acid (x = 5, y = 9), and with a comparison of some of the properties of these compounds with similar properties of lactobacillic acid.

conversion into dihydrosterculic acid he subjected the purified sterculic acid to hydrogenation over palladium. Since sterculic acid exhibits a pronounced tendency toward polymerization we developed a simple method for the direct isolation of dihydrosterculic acid from the kernel oil. The oil was hydrogenated over palladium-in-ethyl acetate and the hydrogenated material saponified. The ensuing fatty acids were converted into the methyl esters and these were subjected to fractional distillation in a spinning band-type fractionating column.[4] Saponification of the highest boiling fraction afforded an acid exhibiting properties akin to those reported for dihydrosterculic acid.[6]

trans-Cyclopropane-1,2-dicarboxylic acid (IV) was selected as a logical starting material for the synthetic work, since it was important to have available models of established stereo-structure for comparison with the natural materials. The monomethyl ester chloride V of IV was condensed with sodio ethyl acetoacetate and the resulting crude diketo ester VI exposed to the action of sodium methoxide in methanol to give methyl *trans*-γ-keto-α,β-methyleneadipate (VII).[7] Alkylation of VII with *n*-butyl iodide (VIII, x = 5) followed by saponification and decarboxylation gave *trans*-4-keto-2,3-methylenenonanoic acid (IX, x = 5) which was converted into *trans*-2,3-methylenenonanoic acid (X, x = 5) by the Huang-Minlon modification of the Wolff–Kishner reduction.[8] Alkylation of VII with *n*-hexyl iodide (VIII, x = 7) followed by saponification and reduction gave *trans*-2,3-methyleneundecanoic acid (X, x = 7).

Poor yields were realized in this method of synthesis and other routes to acids of structure X were explored. A superior procedure involves condensa-



Nunn's scheme[6] for the isolation of sterculic acid involves saponification of the kernel oil followed by low temperature fractional crystallization of the urea complexes of the resulting fatty acids. For

(6) J. R. Nunn, *J. Chem. Soc.*, 313 (1952).

(7) S. Stallberg-Stenhagen, *Archiv Kemi Mineral. Geol.*, A22, No. 19, 1 (1946).

(8) Huang-Minlon, THIS JOURNAL, 68, 2487 (1946).

tion of ethyl diazoacetate XI with olefins of the general structure XII followed by saponification of the resulting esters XIII. Starting with octene-1 (XII, $x = 5$), decene-1 (XII, $x = 7$), and dodecene-1 (XII, $x = 9$), 2,3-methylenenonanoic acid (X, $x = 5$), 2,3-methyleneundecanoic acid (X, $x = 7$), and 2,3-methylenetridecanoic acid (X, $x = 9$), respectively, were obtained in satisfactory yields. The 2,3-methylenenonanoic and undecanoic acids were identical with the respective acids prepared from *trans*-cyclopropane-1,2-dicarboxylic acid and thus must possess a *trans* configuration. An extensive search in the mother liquors from the crystallization of the *trans*-acids failed to reveal the presence of *cis* isomers indicating that the addition of ethyl diazoacetate to olefins of structure XII results in the predominant formation of *trans*-cyclopropanecarboxylic acids.

For conversion into *trans*-9,10-methyleneoctadecanoic acid the acid chloride of X ($x = 7$) was transformed into methyl *trans*-3-keto-4,5-methylenetridecanoate (XIV, $x = 7$) in the manner described for the conversion of V into VII. Alkylation of the keto ester (XIV, $x = 7$) with methyl ϵ-iodocaproate (XV, $y = 7$) followed by saponification and decarboxylation yielded *trans*-8-keto-9,10-methyleneoctadecanoic acid (XVI, x and $y = 7$) which was reduced[8] to give *trans*-9,10-methyleneoctadecanoic acid (I, x and $y = 7$).

trans-11,12-Methyleneoctadecanoic acid (I, $x = 5$, $y = 9$) was obtained from *trans*-2,3-methylenenonanoic acid (X, $x = 5$) by the same sequence of reactions, with methyl η-iodocaprylate (XV, $y = 9$) serving as the alkylating reagent.

The two methyleneoctadecanoic acids were carefully purified by distillation of their methyl esters in a spinning band-type fractionating column,[4] and the acids resulting from the saponification of center cuts were subjected to low temperature recrystallization to constant melting point from acetone and petroleum ether.

The infrared absorption spectra of the synthetic acids are compared with those of lactobacillic acid and dihydrosterculic acid in Fig. 1.[9] It will be noted that the positions and extinctions of the major absorption bands are identical for all the compounds. Especially noteworthy is the presence in all of the spectra of a sharp absorption band at 9.8 μ which had been assigned to the cyclopropane ring.[3,10] These findings offer strong support to the previously postulated cyclopropane structure for lactobacillic and dihydrosterculic acid. An inspection of the 9.8 to 15 μ region of the spectra reveals small differences between the curves of the two synthetics on the one hand and the natural acids on the other. The chemical behavior of the models paralleled that of lactobacillic acid. They were stable to potassium permanganate in acetone solution and underwent hydrogenolysis in the presence of Adams catalyst. Nonadecanoic acid was isolated from the hydrogenation products of *trans*-11,12-methyleneoctadecanoic acid and identified by mixed melting point determination and X-ray analysis with an authentic sample. Nonadecanoic acid was previously identified as one of the hydrogenation products of lactobacillic acid.[3,4]

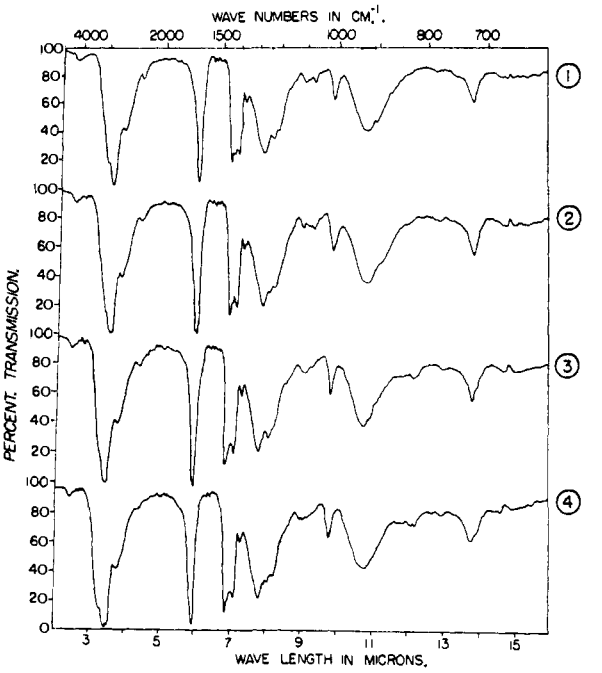


Fig. 1.—Infrared absorption spectra of cyclopropane fatty acids: 1, *trans*-DL-11,12-methyleneoctadecanoic acid; 2, *trans*-DL-9,10-methyleneoctadecanoic acid; 3, lactobacillic acid from *L. casei;* 4, dihydrosterculic acid.

Comparison of a number of physical properties of the synthetic acids and their amides with similar properties of lactobacillic acid and lactobacillamide (Table I) demonstrates the similarity, but nonidentity of these compounds. All three acids exhibit the same long spacing value, but lactobacillamide differs in its long spacing from the two synthetic amides. The long spacing intensity distribution of lactobacillic acid resembles closely that of *trans*-11,12-methyleneoctadecanoic acid but differs significantly from that of the 9,10-isomer.[11]

TABLE I

COMPARISON OF SOME PHYSICAL PROPERTIES OF SYNTHETIC AND NATURALLY-OCCURRING CYCLOPROPANE FATTY ACIDS AND THEIR AMIDES

	Acid		Amide	
Compound, acid	M.p., °C.	Long spacing value, Å.	M.p., °C.	Long spacing value, Å.
Lactobacillic	28–29	41.0	80–82	36.3
trans-DL-9,10-Methyleneoctadecanoic	33.6–35	41.0	86.2–87.2	39.6
trans-DL-11,12-Methyleneoctadecanoic	36.5–37.2	41.0	84–85	39.7

Experimental[12]

Preparation of Dihydrosterculic Acid (III).—A sample of

(9) The infrared absorption spectra of the cyclopropane intermediates described in this paper have been recorded and are on file in our laboratory.

(10) J. M. Derfer, E. E. Pickett and C. E. Boord, THIS JOURNAL, **71**, 2482 (1949).

(11) We wish to express our apreciation to Dr. E. S. Lutton of the Procter and Gamble Company, for the X-ray work.

(12) The melting points were determined with short-stem Anschütz thermometers and are uncorrected. The microanalyses were performed in our microanalytical laboratory by Mr. George L. Stragand.

Sterculia foetida kernel oil[13] (10.35 g.) dissolved in ethyl acetate (100 ml.) was hydrogenated in the presence of a palladium-on-charcoal catalyst (2 g.) until the hydrogenation came to an end (uptake 870 ml. at 28° and 737 mm.). The catalyst was then removed by filtration and the filtrate evaporated to dryness *in vacuo*. The oily residue was refluxed for five hours with methanol (110 ml.) and 5 *N* potassium hydroxide (110 ml.) and the fatty acids isolated in the usual manner (yield 8.98 g.). The acids were converted into the methyl esters by short exposure to diazomethane. Distillation of the methyl esters gave essentially three fractions corresponding in boiling point to esters of fatty acids possessing 16, 18 and 19 carbon atoms. The amounts of these various fractions were C_{16}-esters, 12%; C_{18}-esters, 15%; C_{19}-esters, 52%; and higher boiling residues 20%. The center cut of the C_{19}-ester fraction (3.95 g.), b.p. 188.7–189° (3 ± 0.05 mm.) was saponified and the resulting dihydrosterculic acid purified by low temperature recrystallization from acetone at −20°, m.p. 39.7–40.5° (lit. m.p. 38.8–39.8°[6]).

Anal. Calcd. for $C_{19}H_{36}O_2$: C, 77.0; H, 12.2; neut. equiv., 296. Found: C, 77.0; H, 12.0; neut. equiv., 296.

Monomethyl Ester of *trans*-Cyclopropane-1,2-dicarboxylic Acid (V).—A mixture of *trans*-cyclopropane-1,2-dicarboxylic acid (13 g.), methyl *trans*-cyclopropane-1,2-dicarboxylate (9.2 g.) and concentrated hydrochloric acid (3 g.) was heated in a modified claisen flask at 140–145° until the mixture became homogeneous. The temperature was then lowered to 100°, methanol (4 ml.) was added and the mixture refluxed at 100° for two hours. An additional amount of methanol (1.5 ml.) was then added and the mixture heated for two additional hours. Distillation at 10 mm. gave methyl *trans*-cyclopropane-1,2-dicarboxylate, b.p. 90–93° (10.1 g.), and monomethyl ester, b.p. 145–148° (9.5 g.). The monomethyl ester solidified and was recrystallized from petroleum ether, m.p. 40.5–43.0°.

Anal. Calcd. for $C_6H_8O_4$: C, 50.0; H, 5.6; neut. equiv., 144. Found: C, 50.1; H, 5.6; neut. equiv., 145.

Methyl *trans*-γ-Keto-α,β-methyleneadipate (VII).—The above monomethyl ester (10 g.), oxalyl chloride (10.8 g.), and benzene (20 ml.) were mixed under anhydrous conditions and the mixture kept at room temperature until the gas evolution subsided. The solution was then heated at 40–45° for one hour and the excess of oxalyl chloride and benzene removed by distillation. The acid chloride was freed from oxalyl chloride by one evaporation with benzene (10 ml.), and was added to an ice-cold suspension of sodio ethyl acetoacetate, prepared in the usual manner from powdered sodium (1.8 g.), ethyl acetoacetate (11 g.) and benzene (150 ml.). Following the addition of the acid chloride the reaction mixture was refluxed for 10 minutes, poured into ice-water, and acidified with 10% sulfuric acid. The mixture was extracted with several portions of ether, and the combined ether extracts were washed with water, dried over sodium sulfate and evaporated. The resulting crude diketo ester (VI) was dissolved in a solution of sodium (1.9 g.) in methanol (50 ml.) and the mixture kept at room temperature for 4 hours. The solution was concentrated to a small volume *in vacuo*, the residue acidified to congo red with 10% sulfuric acid and the keto ester VII isolated in the usual manner; b.p. 163–168° (18 mm.), yield 8 g. (57%), $n^{20}D$ 1.4680.

Anal. Calcd. for $C_9H_{12}O_5$: C, 54.0; H, 6.0. Found: C, 53.9; H, 6.4.

***trans*-4-Keto-2,3-methylenenonanoic Acid (IX, *x* = 5).**—A mixture of VII (3.55 g.), *n*-butyl iodide (3.60 g.), anhydrous potassium carbonate (12.0 g.) and methyl *n*-propyl ketone (40 ml.) was stirred and refluxed for 16 hours under anhydrous conditions at a bath temperature of 120°. The mixture was poured on cracked ice, acidified with 10% sulfuric acid and the organic layer separated. The aqueous layer was extracted with ether and the combined organic layers washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue was dissolved in a solution of potassium hydroxide (13 g.) in water (13 ml.) and methanol (200 ml.), and the mixture kept at 45° for 24 hours. The keto acid was isolated in the usual manner and recrystallized from petroleum ether at −20°; m.p. 48–49°, yield 0.58 g. (17%).

Anal. Calcd. for $C_{10}H_{16}O_3$: C, 65.1; H, 8.7. Found: C, 64.8; H, 8.6.

***trans*-4-Keto-2,3-methyleneundecanoic Acid (IX, *x* = 7).**—A mixture of VII (3.75 g.), *n*-hexyl iodide (3.98 g.), anhydrous potassium carbonate (12.0 g.) and methyl *n*-propyl ketone (40 ml.) was stirred and refluxed for 16 hours under anhydrous conditions at a bath temperature of 120°. The resulting keto ester was isolated and saponified in the manner described above. The keto acid was recrystallized from petroleum ether at −20°; m.p. 67–68°, yield 1.21 g. (31%).

Anal. Calcd. for $C_{12}H_{20}O_3$: C, 67.9; H, 9.5. Found: C, 67.6; H, 9.7.

***trans*-2,3-Methylenenonanoic Acid (X, *x* = 5). A. From *trans*-4-Keto-2,3-methylenenonanoic Acid (IX, *x* = 5).**—The keto acid (0.56 g.) was reduced[8] with potassium hydroxide (0.58 g.), hydrazine hydrate (0.78 g.) in diethylene glycol (3 ml.), and the reduced acid isolated in the usual manner; b.p. 114–118° (0.01 mm.), yield 0.17 g. (33%).

***p*-Bromophenacyl ester:** from petroleum ether, m.p. 89.2–89.6°. *Anal.* Calcd. for $C_{18}H_{23}O_3Br$: C, 58.8; H, 6.5; Br, 21.7. Found: C, 58.9; H, 6.3; Br, 21.8.

***p*-Phenylphenacyl ester:** from petroleum ether, m.p. 103.2–104.4°. *Anal.* Calcd. for $C_{24}H_{28}O_3$: C, 79.1; H, 7.7. Found: C, 79.3; H, 8.0.

B. From Octene-1 (XII, *x* = 5).—A three-necked flask fitted with a reflux condenser, a mechanical stirrer and a dropping funnel was charged with a mixture of octene-1 (45.0 g.), di-*n*-butyl ether (30.0 g.), and copper powder (1 g.) and the mixture heated at 110° while ethyl diazoacetate (100.0 g.)[14] was added slowly with stirring over a period of three hours.

Following the addition, the temperature was maintained at 120–130° for an additional hour when the mixture was cooled to room temperature and filtered through a sintered glass disc. The filtrate was diluted with ether (300 ml.) and the solution washed with 6 *N* hydrochloric acid, 10% sodium bicarbonate and saturated sodium chloride and dried over sodium sulfate. The solvents were removed *in vacuo* and the residue distilled through a 10-inch Vigreux column to give a main fraction boiling at 75–80° (0.05 mm.) representing crude ethyl *trans*-2,3-methylenenonanoate; yield 43.5 g. (57%), $n^{26}D$ 1.4342.

The above ester was refluxed under nitrogen for six hours with a mixture of 6 *N* potassium hydroxide (300 ml.) and methanol (120 ml.) and the saponifiable material isolated in the usual manner.

This resulting crude acid (41.0 g.) was converted into the S-benzylthiuronium salt[15] which was recrystallized twice from 95% ethanol; yield 60 g., m.p. 143–144°.

Anal. Calcd. for $C_{18}H_{28}O_2N_2S$: N, 8.3. Found: N, 8.2.

For conversion to the free acid, the S-benzylthiuronium salt was suspended in 9 *N* hydrochloric acid (400 ml.) and the suspension extracted with five 200-ml. portions of ether. The combined ether extracts were washed with water, dried over sodium sulfate and the ether removed *in vacuo*. The acid (30 g.) was converted into the methyl ester with diazomethane and the ester distilled in a spinning-band column.[4] The fraction boiling at 78–79° (3 ± 0.05 mm.) was collected; yield 23.0 g., $n^{22}D$ 1.4382. Saponification of this fraction gave *trans*-2,3-methylenenonanoic acid, b.p. 115–117° (0.05 mm.), yield 20 g., $n^{22}D$ 1.4512.

Anal. Calcd. for $C_{10}H_{18}O_2$: C, 70.6; H, 10.6; neut. equiv., 170. Found: C, 70.4; H, 10.4; neut. equiv., 171.

Amide: from aqueous methanol, m.p. 107–107.5°. *Anal.* Calcd. for $C_{10}H_{19}ON$: C, 70.9; H, 11.3; N, 8.3. Found: C, 70.9; H, 11.4; N, 8.5.

***p*-Bromophenacyl ester:** from ether–petroleum ether, m.p. 90.5–91°. No depression with the same ester prepared according to method A. *Anal.* Calcd. for $C_{18}H_{23}O_3Br$: C, 58.8; H, 6.5; Br, 21.7. Found: C, 58.9; H, 6.3; Br, 21.8.

***p*-Phenylphenacyl ester:** from ether–petroleum ether, m.p. 104.5–105.5°. No depression with the same ester prepared according to method A. *Anal.* Calcd. for $C_{24}H_{28}O_3$: C, 79.1; H, 7.7. Found: C, 79.3; H, 8.1.

***trans*-2,3-Methyleneundecanoic Acid (X, *x* = 7). A. From *trans*-4-Keto-2,3-methyleneundecanoic Acid (IX, *x* = 7).**—The keto acid (1.21 g.) was reduced[8] with potassium hydroxide (1.06 g.), hydrazine hydrate (1.43 g.) in diethylene

(13) We wish to express our sincere appreciation to Dr. J. R. Nunn for supplying us with this material.

(14) E. B. Womack and W. B. Nelson, *Org. Syntheses*, **24**, 56 (1944).

(15) J. J. Donleavy, THIS JOURNAL, **58**, 1004 (1936).

glycol (7.2 ml.) to give the desired acid, b.p. 120–122° (0.03 mm.), yield 1.01 g. (89%).

S-Benzylthiuronium salt: from ethanol, m.p. 139–140°. *Anal.* Calcd. for $C_{20}H_{32}O_2N_2S$: C, 65.9; H, 8.8. Found: C, 65.1; H, 8.5.

Amide: from methanol, m.p. 103–104°. *Anal.* Calcd. for $C_{12}H_{23}ON$: C, 73.0; H, 11.7; N, 7.1. Found: C, 73.2; H, 11.7; N, 7.3.

p-**Bromophenacyl ester**: from petroleum ether, m.p. 92–93°. *Anal.* Calcd. for $C_{20}H_{27}O_3Br$: C, 60.8; H, 6.9; Br, 20.2. Found: C, 61.0; H, 6.7; Br, 20.2.

p-**Phenylphenacyl ester**: from petroleum ether, m.p. 105–106°. *Anal.* Calcd. for $C_{26}H_{32}O_3$: C, 79.6; H, 8.2. Found: C, 79.6; H, 8.4.

B. **From Decene-1 (XII, x = 7)**.—The condensation of decene-1 (59.0 g.), ethyl diazoacetate (96.0 g.) in di-*n*-butyl ether (40 g.) in the presence of copper powder (2 g.), as described for the preparation of ethyl *trans*-2,3-methylenenonanoate, gave ethyl *trans*-2,3-methyleneundecanoate (51.6 g.), b.p. 88–91° (0.04 mm.), $n^{20}D$ 1.4418. Saponification gave *trans*-2,3-methyleneundecanoic acid which was purified by recrystallization from petroleum ether at −20°; yield 19 g., m.p. 33.8–34.0°.

Anal. Calcd. for $C_{12}H_{22}O_2$: C, 72.7; H, 11.2; neut. equiv., 198. Found: C, 72.7; H, 11.1; neut. equiv., 198.

S-Benzylthiuronium salt: from ethanol, m.p. 152–153°. *Anal.* Calcd. for $C_{20}H_{32}O_2N_2S$: C, 65.9; H, 8.8; N, 7.7. Found: C, 66.0; H, 8.6; N, 7.7.

Amide: from methanol, m.p. 108–109°; no depression with the same derivative prepared according to method A. *Anal.* Calcd. for $C_{12}H_{23}ON$: C, 73.0; H, 11.7; N, 7.1. Found: C, 73.0; H, 11.5; N, 6.9.

p-**Bromophenacyl ester**: from ether–petroleum ether, m.p. 94.0–95.0°; no depression with the same ester prepared according to method A. *Anal.* Calcd. for $C_{20}H_{27}O_3Br$: C, 60.8; H, 6.9; Br, 20.2. Found: C, 61.0; H, 6.7; Br, 19.9.

p-**Phenylphenacyl ester**: from ether–petroleum ether, m.p. 106–107°; no depression with the same ester prepared according to method A. *Anal.* Calcd. for $C_{26}H_{32}O_3$: C, 79.6; H, 8.2. Found: C, 79.6; H, 8.4.

trans-**2,3-Methylenetridecanoic Acid (X, x = 9)**.[16]—The condensation of dodecene-1 (71.0 g.) with ethyl diazoacetate (96.0 g.) in di-*n*-butyl ether (48 g.) in the presence of copper powder (2 g.) gave ethyl *trans*-2,3-methylenetridecanoate (67.0 g.), b.p. 114–142° (0.05 mm.). This material was saponified to give the acid (55.6 g.) which was recrystallized from petroleum ether; m.p. 49.5–50.5°.

Anal. Calcd. for $C_{14}H_{26}O_2$: C, 74.3; H, 11.6; neut. equiv., 226. Found: C, 74.4; H, 11.9; neut. equiv., 231.

Methyl *trans*-3-Keto-4,5-methyleneundecanoate (XIV, x = 5).—To an ice-cold suspension of sodio ethyl acetoacetate in benzene, prepared in the usual manner from powdered sodium (3.15 g.) and ethyl acetoacetate (19.40 g.) in benzene (170 ml.), was added *trans*-2,3-methylenenonanoyl chloride (23.5 g.); b.p. 75–78° at 0.1 mm. (prepared from the acid (22.0 g.) and thionyl chloride (20.0 g.)). The mixture was refluxed for 15 minutes, poured on cracked ice and acidified to congo red with 5% sulfuric acid. The water layer was separated, extracted with ether and the combined organic layers washed with water, dried over sodium sulfate and the solvents removed *in vacuo*. The residue was dissolved in a solution of sodium (3.5 g.) in methanol (190 ml.) and the mixture kept at room temperature for 12 hours. The solution was then concentrated to a small volume *in vacuo* at 25° bath temperature and the residue diluted with ice-water and acidified to congo red with 10% sulfuric acid. The keto ester was isolated in the usual manner and distilled; b.p. 110–114° (0.03 mm.), yield 19.2 g., $n^{22}D$ 1.4539. The compound forms a dark red color with ferric chloride in aqueous ethanol.

Anal. Calcd. for $C_{13}H_{22}O_3$: C, 69.0; H, 9.8. Found: C, 68.8; H, 9.7.

Methyl *trans*-3-Keto-4,5-methylenetridecanoate (XIV, x = 7).—The preparation of this keto ester was carried out as described above. From *trans*-2,3-methyleneundecanoyl chloride (22.3 g., b.p. 103–107° (0.03 mm.)), was obtained ethyl *trans*-3-keto-4,5-methylenetridecanoate, 19 g., b.p. 129–132° (0.06 mm.), $n^{22}D$ 1.4550. The substance exhibited a positive ferric chloride test.

(16) This compound was prepared by Mr. Walter E. Behnke.

Anal. Calcd. for $C_{15}H_{26}O_3$: C, 70.8; H, 10.3. Found: C, 70.6; H, 10.1.

trans-**8-Keto-9,10-methyleneoctadecanoic Acid (XVI, x and y = 7)**.—A mixture of methyl *trans*-3-keto-4,5-methylenetridecanoate (16.8 g.), methyl ϵ-iodocaproate (16.9 g.), dried potassium carbonate (30 g.), and methyl *n*-propyl ketone (100 ml.) was refluxed for 16 hours under anhydrous conditions at a bath temperature of 115°. The mixture was cooled, filtered, the filter residue washed with ether and the organic layer poured on cracked ice and acidified to congo red with 10% sulfuric acid. The combined organic extracts were washed with water, dried and the solvents removed *in vacuo*. The resulting yellow oil was saponified with a solution of potassium hydroxide (52 g.) in water (52 ml.) and methanol (780 ml.) for 24 hours at 45°. The keto acid was isolated in the usual manner and recrystallized from acetone; yield 13.7 g. (67%), m.p. 60.4–61.4°.

Anal. Calcd. for $C_{19}H_{34}O_3$: C, 73.5; H, 11.0; neut. equiv., 310. Found: C, 73.5; H, 10.9; neut. equiv., 311.

trans-**10-Keto-11,12-methyleneoctadecanoic Acid (XVI, x = 5, y = 9)**.—Alkylation of methyl 3-keto-4,5-methyleneundecanoate (17.3 g.) with methyl η-iodocaprylate (21.7 g.) in the presence of potassium carbonate (40 g.) in methyl *n*-propyl ketone (110 ml.) followed by saponification of the initial condensation product gave the keto acid which was recrystallized from acetone; yield 13.3 g. (56%), m.p. 61–62°.

Anal. Calcd. for $C_{19}H_{34}O_3$: C, 73.5; H, 11.0; neut. equiv., 310. Found: C, 73.5; H, 10.9; neut. equiv., 311.

trans-**9,10-Methyleneoctadecanoic Acid (I, x and y = 7)**.—The *trans*-8-keto-9,10-methyleneoctadecanoic acid (2.5 g.) was reduced[8] with hydrazine hydrate (1.35 ml.), potassium hydroxide (1.53 g.) and diethylene glycol (12 ml.). The resulting crude acid (2.3 g.) was recrystallized five times from petroleum ether at −20°; m.p. 32.4–32.6°. The acid derived from two reductions (4.2 g.) was converted into the methyl ester with diazomethane and the ester distilled in a spinning band column.[4] The fractions boiling at 185.8–186.1° (3 ± 0.05 mm.) (2.65 g.) were combined and saponified and the resulting acid recrystallized three times from petroleum ether at −20°; m.p. 33.6–35°.

Anal. Calcd. for $C_{19}H_{36}O_2$: C, 77.0; H, 12.2; neut. equiv., 296. Found: C, 76.8; H, 12.0; neut. equiv., 298.

S-Benzylthiuronium salt: from ethanol, m.p. 137–138°. *Anal.* Calcd. for $C_{27}H_{46}O_2N_2S$: C, 70.1; H, 10.0. Found: C, 69.9; H, 10.2.

Amide: from methanol and water, m.p. 86.2–87.2°. *Anal.* Calcd. for $C_{19}H_{37}ON$: C, 77.2; H, 12.6; N, 4.7. Found: C, 77.3; H, 12.6; N, 4.9.

trans-**11,12-Methyleneoctadecanoic Acid (I, x = 5, y = 9)**.—The reduction of *trans*-10-keto-11,12-methyleneoctadecanoic acid (7.2 g.) with hydrazine hydrate (3.9 ml.) and potassium hydroxide (4.4 g.) in diethylene glycol (35 ml.) was carried out as described.[8] The acid derived from two reductions (10 g.) was converted into the methyl ester with diazomethane and the ester distilled in a spinning band column.[4] The fractions boiling at 190.4–190.6° (3 ± 0.05 mm.) (6.8 g.) were combined, saponified, and the resulting acid recrystallized from petroleum ether at −20°; yield 4.9 g. m.p. 36.5–37.2°.

Anal. Calcd. for $C_{19}H_{36}O_2$: C, 77.0; H, 12.2; neut. equiv., 296. Found: C, 77.1; H, 12.2; neut. equiv., 298.

S-Benzylthiuronium salt: from ethanol, m.p. 137–138°. *Anal.* Calcd. for $C_{27}H_{46}O_2N_2S$: C, 70.1; H, 10.0. Found: C, 70.1; H, 10.0.

Amide: from methanol–water, m.p. 84–85°. *Anal.* Calcd. for $C_{19}H_{37}ON$: C, 77.2; H, 12.6; N, 4.7. Found: C, 77.4; H, 12.5; N, 4.9.

Catalytic Hydrogenation.—A sample of the acid (1.0 g.) when hydrogenated in glacial acetic acid (100 ml.) in the presence of prehydrogenated Adams catalyst (7 g.) absorbed 0.91 mole equivalent of hydrogen in 12 hours. The catalyst was removed by filtration, the filtrate concentrated to a small volume *in vacuo* and the residue dissolved in ether. The ether solution was washed with water and dried over sodium sulfate and the ether evaporated. The residue (1.0 g.) was subjected to fractional crystallization at −20° first from petroleum ether and then from acetone to give a solid fraction, 42 mg., m.p. 66.8–67.6°. No depression of the melting point was observed when this material was admixed with

an authentical sample of nonadecanoic acid. The X-ray diffraction pattern of the acid was identical with that of nonadecanoic acid; long spacing value 45.0 Å. A sample of the acid (17 mg.) was converted into the tribromoanilide, m.p. 126.5–127.5°, which did not depress the melting point of an authentic sample of the tribromoanilide of nonadecanoic acid.

Anal. Calcd. for $C_{25}H_{40}ONBr_3$: C, 49.2; H, 6.6. Found: C, 49.3; H, 6.1.

PITTSBURGH, PENNSYLVANIA

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WASHINGTON]

The Action of Lecithinase D on Lecithin. The Enzymatic Preparation of D-1,2-Dipalmitolein and D-1,2-Dipalmitin[1]

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The action of lecithinase D of *Cl. perfringens* type A toxin on pure lecithins in 98% ether–2% alcohol has been studied. When (dipalmitoleyl)-L-α-lecithin was used as substrate, D-1,2-dipalmitolein and phosphorylcholine were obtained as products. When the substrate was (dipalmitoyl)-L-α-lecithin, D-1,2-dipalmitin and phosphorylcholine were found. The yield of products was 90% or greater. The reaction proceeded smoothly and reproducibly in this solvent system and was followed by a direct titration of the phosphorylcholine in the reaction medium.

In 1941, MacFarlane and Knight[2] reported that the toxic filtrate of *Clostridium welchii* cultures could attack lecithin with the production of a diglyceride and phosphorylcholine. Since then this enzyme system, called lecithinase D,[3] has been found in *Cl. oedemantiens*,[4] *Cl. bifermentans*,[4] *the* venom of *Bothrops alternatus*,[5] and in the brain tissue of the rabbit, dog and bull.[6] It has been found to be active primarily on lecithin[2] and to a lesser degree on sphingomyelin,[7] and inactive toward cephalin,[7] phosphatidylserine,[7] lysolecithin,[8] cerebrosides[8] and glycerylphosphorylcholine.[8]

Previously it had been shown in this Laboratory[9,10] that the lecithinase A of snake venoms and pancreatin can attack lecithin in solvent systems such as diethyl ether or 95% ether–5% ethyl alcohol. In a continuation of the study of the mode and specificity of action of the phospholipide-hydrolyzing enzymes, we have found that the lecithinase D of the toxic filtrate of *Clostridium perfringens* type A cultures can attack lecithin in solvent systems such as diethyl ether or 98% ether–2% ethyl alcohol. When a pure, unsaturated lecithin, (dipalmitoleyl)-L-α-lecithin is used as a substrate, the only products of the enzymatic action are an asymmetrical, unsaturated diglyceride, D-1,2-dipalmitolein, and phosphorylcholine. Similarly, the saturated lecithin, (dipalmitoyl)-L-α-lecithin yields the asymmetrical, saturated diglyceride, D-1,2-dipalmitin and phosphorylcholine. In both cases, yields of 90% or greater are obtained.

The reaction mixture consisted of a solution of the lecithin in the ether–alcohol solvent to which was added the enzyme solution in water. A homogeneous mixture was obtained and remained so throughout the course of the reaction. When this system was used for the assay of the enzyme activity of a toxic filtrate or a kinetic study on the enzyme action, additional alcohol was added at the end of the incubation period to stop the reaction and to allow for the direct titration of the liberated phosphorylcholine in the reaction mixture with methanolic NaOH to the cresol red end-point. Thus the progress of the hydrolysis could be followed conveniently and reproducibly by this procedure. Previous methods for following this reaction included the determination of the water-soluble phosphate which was formed[2] and a manometric assay of the amount of CO_2 liberated from a bicarbonate buffer by the phosphorylcholine.[8] It is felt that the presently described titrimetric procedure represents a less laborious and more accurate means for measurement of the progress of the reaction.

The isolation of the products could be accomplished by the addition of excess water to the reaction mixture. The ether fraction, which contained the diglyceride, was removed and washed with water. When an excess of enzyme was used, it has been our experience that the ether fraction consisted solely of the diglyceride. It is interesting to note that the partition ratio of the intact lecithin between ether and water is apparently in favor of the water, for this compound can be extracted almost quantitatively from the ether by the addition of water. Consequently, any contamination by unreacted substrate is minimal. Phosphorylcholine can be isolated as the calcium salt from the original aqueous fraction and washings.

As had been pointed out by MacFarlane and Knight[2] and confirmed and extended by Zamecnik, *et al.*,[8] calcium ions are apparently the primary activators for this enzyme system. In agreement with these observations, we have found that calcium was necessary for the enzymatic action to

(1) Presented in part before the Division of Biological Chemistry at the 123rd Meeting of the American Chemical Society, Los Angeles, Calif., March 15-20, 1953.

(2) M. G. MacFarlane and B. C. J. G. Knight, *Biochem. J.*, **35**, 884 (1941).

(3) Inasmuch as there is considerable confusion as to the proper nomenclature of these enzymes, we prefer to use the term lecithinase D to designate that enzyme capable of removing phosphorylcholine from lecithin.

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