distillation gave 360.5 mg. (95% of theory) of pure oleic acid, m.p. 14–15°, $n^{22}D$ 1.4580.

Distearoyl-L-α-glycerylphosphorylethanolamine (IV).—In an all-glass hydrogenation vessel of 250-ml. capacity were placed 0.15 g. of platinic oxide[35] and 25 ml. of glacial acetic acid, and the oxide was reduced to platinum black. After replacing the hydrogen with nitrogen, the acetic acid was decanted and the catalyst was washed with three 25-ml. portions of glacial acetic acid. To the catalyst was then added a solution of 1.5 g. of L-α-(dioleoyl)-cephalin in 35 ml. of glacial acetic acid, the nitrogen was replaced by hydrogen, and the hydrogenation of the unsaturated cephalin was carried out at room temperature and at a pressure of approximately 50 cm. of water. The reduction was complete in 30 minutes, with the consumption of the theoretical amount of hydrogen. After replacing the hydrogen with nitrogen, and warming the mixture to 50°, the catalyst was removed by centrifugation and washed twice with small amounts of warm acetic acid. The combined solutions were brought to dryness under reduced pressure at a bath temperature of 40–50°. The L-α-(distearoyl)-cephalin, weighing 1.5 g. (theoretical yield), was stirred for 10 minutes with 25 ml. of 25% acetic acid, and the mixture was separated by centrifugation. The precipitate was treated in the same manner successively with three 15-ml. portions of anhydrous acetone. The cephalin was freed of acetone in an air current, and was dried *in vacuo* over sodium hydroxide. For recrystallization, the cephalin was dissolved in 95 ml. of chloroform, and to the solution now was added twice its volume of methanol. The solution, after clearing by centrifugation if necessary, was set aside at room temperature until spontaneous crystallization had set in, and then was placed in an ice-box at 5° for 12 hours. The cephalin was collected with suction on a buchner funnel, washed with pure ether, and dried *in vacuo*. The L-α-(distearoyl)-cephalin weighed 1.2 g. (80% recovery). It began to sinter slightly at 130°, turned amber at about 170° and coalesced suddenly with the formation of a meniscus at 180–181°,[36] $[\alpha]D$ +6.0° in a mixture of chloroform and acetic acid (9:1 v./v., *c* 5.5); authentic L-α-(distearoyl)-cephalin,[16,17] m.p. 180–182° with sintering at about 130–135°, $[\alpha]D$ +6.0°. *Anal.* Calcd. for $C_{41}H_{82}O_8NP$ (748.1): P, 4.14; N, 1.87. Found: P, 4.15; N, 1.83.

Preparation of a Silicic Acid Adsorption Column for the Chromatographic Purification of L-α-(Dioleoyl)-cephalin.—To 700 g. of silicic acid (Merck Reagent grade) was added with stirring 0.7 l. of methanol, the slurry was filtered with suction on a buchner funnel, and the silicic acid was washed on the filter with three 300-ml. portions of methanol. The silicic acid then was suspended in 700 ml. of chloroform, and the slurry was poured into a glass column (6 cm. width and 60 cm. length) equipped at its top with a 2 l. reservoir and at its base with a stopcock and a perforated procelain plate covered with a disk of filter paper. The silicic acid was washed with chloroform until it was translucent. To promote a uniform settling of the silicic acid, the tube was occasionally gently tapped.

Acknowledgment.—The work was assisted with funds allocated by the Province of Ontario under the National Health Grants Program of the Department of National Health and Welfare. The infrared spectrum was obtained through the courtesy of Dr. G. V. Marinetti of the Department of Biochemistry and of Dr. W. B. Mason of the Atomic Energy Project of the University of Rochester School of Medicine and Dentistry, and the determination was made possible in part by funds from the United States Atomic Energy Commission.

(35) Prepared as described in "Organic Syntheses," Coll. Vol. I, 2nd edition, John Wiley and Sons, Inc., New York, N. Y., 1948, p. 463, with the exception that the sodium nitrate was replaced by an equimolecular amount of potassium nitrate.

(36) The melting point determination was carried out in a capillary tube using an electrically heated bath of *n*-butyl phthalate and short-stem thermometers with a range of fifty degrees. The temperature of the bath was raised at a rate of 15–20° per minute up to 150°, and 4–5° thereafter.

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[CONTRIBUTION FROM THE PROCTOR & GAMBLE CO., MIAMI VALLEY LABORATORIES]

Preparation and Properties of Various Fatty Compounds Containing Lactic Acid

BY J. C. WOOTTON AND E. S. LUTTON

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The following new fatty compounds containing lactic acid have been prepared and the phase behavior of these compounds is described: O-palmitoyllactic acid, monomorphic; 1-mono-O-palmitoyllactin, dimorphic (metastable form, fleeting); 1-O-palmitoyllactyl-2,3-dilactin, monomorphic (α, stable); 1-palmitoyl-2,3-dilactyllactin, monomorphic (α, stable); tri-O-palmitoyllactin, dimorphic (α, fairly stable); 1-O-palmitoyllactyl-2,3-dipalmitin, dimorphic (unusually stable super-α). 1-Palmitoyl-2,3-dilactin, monomorphic (α, stable), has been reported previously. Of particular interest were 1-O-palmitoyllactyl-2,3-dilactin, 1-palmitoyl-2,3-dilactyllactin and 1-palmitoyl-2,3-dilactin. These triglycerides, each with one long and two short chains, showed stable α-forms; likewise, having two unesterified hydroxyl groups in the molecule, they possessed surface activity of the same order of magnitude as monoglycerides such as 1-monoölein.

Introduction

A series of lactic acid glycerides of known structure was made as part of a continuing program for the preparation of new compounds of potential value as components of edible products. In general, the synthetic approach was the same as that described by Goldblatt and co-workers.[1] Lactic acid was introduced into the glyceride molecule as the O-benzyllactic acid derivative. The benzyl group was subsequently removed by hydrogenolysis to yield the final product or to render the hydroxyl group of the lactic acid radical available for further reaction. The phase behavior of these compounds was compared with that of related but more familiar fatty compounds. Because of the hydrophilic groups, these compounds were also tested for interfacial activity in fat–water systems.

Experimental

Synthesis.—The following are typical preparations of the lactic acid glycerides and related compounds not previously described in the literature.

O-Palmitoyllactic Acid.—A solution of 27 g. (0.15 mole) of benzyl lactate, prepared as described by Fein and Fisher,[2] and 18 g. of pyridine in 150 ml. of dry chloroform was chilled in ice, and 41.2 g. (0.15 mole) of palmitoyl chloride was added dropwise with swirling. After standing for 4 days at room temperature, the solution was diluted with ether, water-washed three times, dried over sodium sulfate,

(1) L. A. Goldblatt, D. A. Yeadon and M. Brown, THIS JOURNAL, 77, 2477 (1955).

(2) M. L. Fein and C. H. Fisher, *J. Org. Chem.*, 15, 530 (1950).

and finally evaporated on the steam-bath. A yield of 61.8 g. (98%) of crude product was obtained, $n^{30}D$ of 1.4711. A small amount of palmitic acid was removed from this material by dissolving it in 5 volumes of benzene, cooling to 0° and filtering off the precipitate.

To a 150 ml. solution of 70 g. of benzyl O-palmitoyllactate in ethyl acetate was added 3 g. of powdered Pd catalyst.[3] The material was hydrogenolyzed overnight under a hydrogen pressure of 50 p.s.i. on a Parr hydrogenation apparatus. The hydrogen uptake was slightly greater than quantitative. The catalyst was filtered off and the solvent evaporated on a Rinco evaporator to obtain 51 g. (93% yield) of product. Repeated recrystallization of this material from 5 volumes of petroleum ether at 0° gave a solid melting at 55–58°. *Anal.* Calcd. for $C_{19}H_{36}O_4$: C, 69.47; H, 11.05; acid value, 171; sapn. value, 342. Found: C, 69.72; H, 11.22; acid value, 170; sapn. value, 341.

1-Mono-O-palmitoyllactin.—To a solution of 8 g. (0.039 mole) of 1-lactylacetoneglycerol, prepared as described by Feldmann and Fischer,[4] in 10 ml. of dry chloroform, 3.5 g. (0.044 mole) of dry pyridine was added dropwise with swirling followed by 10.8 g. (0.039 mole) of palmitoyl chloride. After standing 3 days at room temperature, the solution was diluted with ether (to lower its density) and washed six times with water. The ether solution was dried over sodium sulfate and evaporated on the steam-bath to yield 17.3 g. (100% yield) of 1-O-palmitoyllactylacetoneglycerol, a liquid with a $n^{30}D$ of 1.4460 and a sapn. value of 253. Distillation of a sample of this material gave a liquid, $n^{30}D$ 1.4457, which was analyzed. *Anal.* Calcd. for $C_{28}H_{46}O_6$: C, 67.85; H, 10.03; sapn. value, 254. Found: C, 67.93; H, 10.28; sapn. value, 256.

A solution of 8.8 g. (0.2 mole) of 1-O-palmitoyllactylacetoneglycerol in 50 ml. of ether was chilled to 0° and 50 ml. of concentrated hydrochloric acid, also chilled to 0°, was added. The mixture was stored in ice for 25 minutes with occasional swirling; 300 ml. of ice-water was added, the mixture shaken, and the layers separated. The aqueous layer was extracted twice more with ether. The ether layers were combined, water-washed three times, dried over sodium sulfate, and evaporated on the steam-bath to obtain 6.1 g. (91% yield) of a white solid melting at 55–60°. After two recrystallizations from ethyl acetate at 0°, 2 g. (30% yield) of solid was obtained melting at 68.5–70°. *Anal.* Calcd. for $C_{22}H_{42}O_6$: C, 65.63; H, 10.52; sapn. value, 279; hydroxyl value, 279. Found: C, 66.02; H, 10.50; sapn. value, 279; hydroxyl value, 271.

1-O-Palmitoyllactyl-2,3-dilactin.—Fifteen ml. of dry pyridine and 18.5 g. (0.046 mole) of 1-mono-O-palmitoyllactin were dissolved in 75 ml. of dry chloroform and chilled in ice. To the chilled and swirled solution—22.2 g. (0.112 mole) of freshly distilled O-benzyllactic acid chloride was added dropwise. After standing for three days at room temperature, the solution was diluted with ether (to reduce its density) and washed successively once with water, twice with 1% hydrochloric acid, and three times with water. After drying over sodium sulfate, the solution was evaporated with a Rinco evaporator at a bath temperature of 45° to obtain 35 g. (100% yield) of a light yellow liquid, $n^{30}D$ 1.4870. *Anal.* Calcd. for $C_{42}H_{62}O_{10}$: C, 69.39; H, 8.60; sapn. value, 309. Found: C, 69.47; H, 8.20; sapn. value, 312.

The 1-O-palmitoyllactyl-2,3-dibenzyllactin thus obtained was dissolved in 130 ml. of purified glacial acetic acid, 3.0 g. of palladium (10%) on powdered carbon catalyst added, and the material hydrogenolyzed overnight under 50 p.s.i. hydrogen pressure on a Parr hydrogenation apparatus. The catalyst was filtered off and the solvent evaporated on a Rinco evaporator to obtain 23.8 g. (95% yield) of a product melting at 26°, $n^{30}D$ of 1.4566. *Anal.* Calcd. for $C_{28}H_{50}O_{10}$: C, 61.50; H, 9.15; sapn. value, 410. Found: C, 61.48; H, 9.30; sapn. value, 421.

1-Palmitoyl-2,3-dilactyllactin.—A mixture of 10 g. (0.021 mole) of 1-palmitoyl-2,3-dilactin (prepared in the manner described by Goldblatt[5]) and 6 ml. of dry pyridine was dissolved in 50 ml. of dry chloroform. The solution was chilled in ice and 10 g. (0.050 mole) of O-benzyllactic acid chloride added dropwise with swirling. After standing for three days, the solution was diluted with ether, washed three times with water, dried over sodium sulfate, and evaporated on a Rinco evaporator without heating. The 15.2 g. of yellowish oil thus obtained was dissolved in 75 ml. of acetone, cooled to −30°, and filtered. The filtrate was again cooled to −30° and filtered. Evaporation of the acetone left 12.8 g. (76% yield) of a liquid with a $n^{30}D$ of 1.4841 and a sapn. value of 348 (theory, 351). This material was dissolved in 75 ml. of ethyl acetate, 3 g. of palladium-on-carbon catalyst freshly prepared according to the procedure of Verkade[6] was added, and the mixture shaken overnight on a Parr hydrogenation apparatus under a hydrogen pressure of 50 p.s.i. The catalyst was filtered off and the filtrate evaporated to obtain 7.5 g. of a liquid melting at 10° and with a $n^{25}D$ of 1.4572. *Anal.* Calcd. for $C_{31}H_{54}O_{12}$: C, 60.17; H, 8.80; sapn. value, 453. Found: C, 60.12; H, 8.75; sapn. value, 454.

Tri-O-palmitoyllactin.—A solution of 5.8 g. (0.019 mole) of trilactin, prepared as described by Feldmann and Fischer,[4] 10 ml. of dry pyridine and 125 ml. of dry chloroform was chilled in ice. To the chilled solution, 18.6 g. (0.068 mole) of palmitoyl chloride was added dropwise with swirling. After standing two days at room temperature, the solution was diluted with ether, washed three times with water, dried over sodium sulfate, and finally evaporated on the steam-bath to obtain 25 g. of liquid, 12.5 g. of which was chromatographed on a column 1.5″ in diameter and packed with 24″ of silica gel. Elution with 3 liters of hexane removed only traces of material from the column. Elution with 2300 ml. of benzene removed 2.0 g. of material which was discarded. Elution with 1500 ml. of a 10% solution of ether in benzene removed 7.7 g. of material, m.p. 31°. On standing for several days, this m.p. rose due to polymorphic change and the material was eventually found to have a m.p. of 50°. *Anal.* Calcd. for $C_{60}H_{110}O_{12}$: C, 70.44; H, 10.84; sapn. value, 329. Found: C, 70.64; H, 10.83; sapn. value, 329.

1-O-Palmitoyllactyl-2,3-dipalmitin.—A solution of 4 g. (0.01 mole) of 1-mono-O-palmitoyllactin, 3 g. of dry pyridine and 25 ml. of chloroform was chilled in ice. Palmitoyl chloride (6.1 g., 0.022 mole) was added dropwise with swirling to the chilled solution. After standing for two days at room temperature, the solution was diluted with ether to lower its density and washed successively once with water, once with 1% hydrochloric acid and three times with water. The ether layer was dried over sodium sulfate and evaporated on the steam-bath to obtain 9.0 g. (100% yield) of a solid melting at 38–40°. One recrystallization from hexane raised the melting point range to 48–51° and a second recrystallization did not change it further. A final recrystallization from 5 volumes of ethyl acetate raised the melting point to 63–64°. The final yield was 40%. *Anal.* Calcd. for $C_{54}H_{102}O_8$: C, 73.74; H, 11.69. Found: C, 73.78; H, 11.81.

Thermal and X-Ray Diffraction Technique.—Melting point and diffraction data were obtained by a previously described procedure.[7] (Rapid complete m.p.'s and regular complete m.p.'s were determined for each compound, the former for metastable phases, especially alpha; the latter for stable form only.) Except for solvent-crystallized stable forms, which were put into capillaries as powders, all studies were on melted samples drawn into capillaries.

Flat film X-ray diffraction patterns were made on all polymorphic forms either as "rod pellets" or in Pyrex glass capillaries with a General Electric XRD-1 unit employing CuKα nickel-filtered radiation and a 0.025″ pinhole system. Sample-to-film distance was normally 5 cm., but was 10 cm. for determining long spacings.

Thermal and X-ray diffraction data are listed in the following order: compound, polymorphic form: m.p., °C.; long spacing, L.S. (in Å.); short spacings, S.S. (in Å.). Unless indicated otherwise, m.p.'s of metastable forms are rapid, complete m.p.'s; m.p.'s of stable forms are complete m.p.'s. Relative intensities of diffraction lines are indicated by: very strong, vs; strong, s; medium, m; and weak, w.

O-Palmitoyllactic acid, stable form: m.p. 56.2°; L.S., 38.7; S.S., 5.20w+, 4.78s+, 4.43m, 4.07s, 3.81w, 3.68s, 3.55w.

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TABLE I

THE SURFACE ACTIVITY OF VARIOUS LACTIC ACID GLYCERIDES AT AN OIL/WATER INTERFACE AT 37°

Additive (1% by weight dissolved in cottonseed oil)	Interfacial tension, dynes cm.$^{-1}$
None	23.6
1-Palmitoyl-2,3-dilactyllactin	13.7
1-Palmitoyl-2,3-dilactin	11.3
1-Monoölein	10.3
1-Mono-O-palmitoyllactin	8.9
1-O-Palmitoyllactyl-2,3-dilactin	7.3

1-Mono-O-palmitoyllactin, metastable form: m.p. 34.2; stable form (from ethyl acetate and melt): m.p. 70°; L.S., 45.8; S.S., 5.20m, 4.50m+, 4.19vs, 3.99w, 3.86w, 3.67m−, 3.46m−.

1-Palmitoyl-2,3-dilactin, stable α-form: m.p. 31.5°; L.S., 31.5; S.S., 4.20s.

1-O-Palmitoyllactyl-2,3-dilactin, stable α-form: m.p. 26.1°; L.S., 42.7; S.S., 4.13s+.

1-Palmitoyl-2,3-dilactyllactin, stable α-form: m.p. 12.1°; L.S., 42.0; S.S., 4.14s.

Tri-O-palmitoyllactin, α: m.p. 30.5°; L.S., 28.9; S.S., 4.15s; stable form (from hexane): m.p. approximately 50°; L.S., 30.7; S.S., 5.91m, 4.71m, 4.51m, 4.28s−, 4.05s.

1-O-Palmitoyllactyl-2,3-dipalmitin, α: m.p. 41.0°; L.S., 50.0; S.S., 4.15s+; super-α (from hexane or ethyl acetate or from α after one week at 38°): m.p. 63.0°; L.S., 42.0; S.S., 5.26w, 4.42vw+, 4.07vs, 3.68vw (a third form of a less pure specimen, from hexane: m.p. 58.2°; L.S., 44.9; S.S., 4.40w, 4.26s+, 4.02s−, 3.85s, 3.47vw).

Surface Activity.—One of the interesting features of the lactic acid glycerides is their surface activity. The compounds 1-palmitoyl-2,3-dilactin and 1-O-palmitoyllactyl-2,3-dilactin, although triglycerides, both contain two unesterified hydroxyl groups in the molecule and have surface activities similar to those of monoglycerides. The interfacial tensions of several lactic acid glycerides dissolved in cottonseed oil at an oil–water interface are listed in Table I. The interfacial tension was determined by a drop-weight method[8] at a temperature of 37°. Distilled water was used and the oil was a refined, bleached, and deodorized cottonseed oil.

Discussion

The chief feature of interest in the phase behavior of these compounds is the stability in an α-phase of the three glycerides 1-palmitoyl-2,3-dilactin, 1-O-palmitoyllactyl-2,3-dilactin and 1-palmitoyl-2,3-dilactyllactin. This behavior is reminiscent of the high, but not complete, alpha stability of 1-stearoyldiacetin, etc.[9] The alpha stability of the dimorphic tri-O-palmitoyllactin is also quite high. The 1-O-palmitoyllactyl-2,3-dipalmitin shows unusual behavior with a normal α-form and a form from melt called super-alpha because of its single strong, short spacing and its resemblance to forms of that name observed for 2-butyroyldipalmitin and 2-butyroyldistearin.[10] (A third form was obtained from hexane with a specimen of lower purity.)

The acid compound, O-palmitoyllactic acid, appeared to be monomorphic.

The monoglyceride, 1-mono-O-palmitoyllactin, was dimorphic but with a lower melting form so fleeting as to defy X-ray characterization.

The influence of the lactic moiety on surface activity is not easily assessed. As might be expected, the possession of two hydroxyl groups by triglyceride compounds gives them a surface activity comparable to that of monoölein. It is of interest that for the first time synthetic triglycerides have been observed which show both surface activity and stability in the α-phase.

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CINCINNATI 31, OHIO

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

The Sulfonation of Chitosan[1,2]

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Sulfation of chitosan with pyridine–chlorosulfonic acid yielded an amorphous sodium salt which had an anticoagulant activity of 56 I. U./mg. It had a molecular weight (by light scattering) of 456,000 or a D. P. of 1280. It was approximately twice as toxic as heparin. A homogeneous sulfation method was established, using the sulfur trioxide–N,N-dimethylformamide complex in an excess of N,N-dimethylformamide. Sulfated chitosan thus obtained had an anticoagulant activity of 50 I. U./mg. and a D. P. (by light scattering) of 530. Its acute LD_{50} (mouse, intravenous) was about equal to that of heparin.

In a preliminary communication[2] from this Laboratory, we reported the preparation of a sulfated chitosan with a high degree of anticoagulant activity by treatment of chitosan with chlorosulfonic acid in pyridine. Essentially simultaneously with the publication of our results, Doczi and co-workers[3] reported the preparation of a sulfated chitosan, although no experimental details were given or have appeared to date. Coleman and associates[4] reported the preparation of sulfated chitosan of low anticoagulant activity by treatment of chitosan with sulfur dioxide and sulfur trioxide. Ricketts[5] also treated dried chitosan with chlorosulfonic acid in pyridine and isolated a product which exhibited very little anticoagulant activity.

We report herein the details of our sulfation[2] of essentially (90%) N-deacetylated chitosan with chlorosulfonic acid and pyridine. The success of

(1) Supported by the Bristol Laboratories, Inc., Syracuse, N. Y., under contract with The Ohio State University Research Foundation, Project 432(1951–1954).

(2) Reported in part in THIS JOURNAL, **75**, 1519 (1953), and in U. S. Patent 2,832,766 (1958).

(3) J. Doczi, A. Fischman and J. A. King, *ibid.*, **75**, 1512 (1953).

(4) L. L. Coleman, L. P. McCarty, D. T. Warner, R. F. Willy and J. H. Flokstra, *Abstracts Papers Am. Chem. Soc.*, **128**, 19L (1953); British Patent 746,870 (1956); *C. A.*, **51**, 1258 (1957).

(5) C. R. Ricketts, *Research*, **6**, 17S (1953).