nate. The product was twice washed by suspension in cold acetone and finally oven dried; yield, 247 g. (64%), m.p. 128–134°. After two crystallizations from ethanol the m.p. was 135–136°.

Anal. Calcd. for $C_{17}H_{16}N_2O_4S$: N, 8.13; S, 9.31. Found: N, 7.82; S, 9.28.

The reaction mixture from which the aforementioned salt had been filtered, together with the acetone washings, was diluted with ten to fifteen volumes of water to precipitate 40 g. of 2-acetylaminonaphthalene. After crystallization from ethanol, this amide melted at 133–134° and did not depress the melting point when mixed with authentic 2-acetylaminonaphthalene.

(*b*) *2-Acetylamino-1-naphthalenesulfonic acid* (II). When pyridine (87 ml.) was added to a suspension of 2-amino-1-naphthalenesulfonic acid (158 g.) in acetic anhydride (190 ml.), considerable heat was generated and all of the solid dissolved. The mixture was heated to reflux for 1 hr. and then 36% hydrochloric acid (480 ml.) was added with efficient cooling of the reaction mixture in order to minimize elimination of the sulfonic acid group. Upon standing in the cold the solution deposited 118 g. (63%) of II as a white powder of suitable purity to be directly halogenated. Dilution of the filtrate with water caused precipitation of varying quantities of 2-acetylaminonaphthalene.

Although the 2-acetylamino-1-naphthalenesulfonic acid was not successfully recrystallized without decomposition, a sample was prepared for analysis by solution in the minimum quantity of cold water and precipitation with 36% hydrochloric acid. The sample was then dried *in vacuo* over solid potassium hydroxide.

Anal. Calcd. for $C_{12}H_{11}NO_4S$: S, 12.08. Found: S, 11.62.

2-Acetylamino-1-chloronaphthalene (III) was instantaneously precipitated when 250 ml. of water saturated with chlorine at room temperature was poured slowly, with stirring, into a solution of 5 g. of 2-acetylamino-1-naphthalenesulfonic acid (II) in 200 ml. of water. After 10 min. the precipitate was collected, washed with water and with potassium bisulfite solution, and again with water. The dried material (3.5 g., 85%) melted at 143–147°. Crystallization from absolute ethanol raised the m.p. to 146–147°, which was not depressed when authentic 2-acetylamino-1-chloronaphthalene, prepared by the method of Franzen and Stäuble[4] was admixed.

When the pyridinium salt of II was chlorinated in exactly the same manner as for the free acid, 2-acetylamino-1-chloronaphthalene was again obtained in 85% yield.

2-Acetylamino-1-bromonaphthalene (IV) was precipitated in quantitative yield when a solution of bromine (5–10% excess) in acetic acid (15 ml.) and water (1 ml.) was added slowly at room temperature, with stirring, to a solution of 2-acetylamino-1-naphthalenesulfonic acid II (10 g.), or an equivalent weight of the pyridinium salt of II, in water (200 ml.). Recrystallization from absolute ethanol readily gave IV in colorless needles, m.p. 140–141°, which did not depress the melting point of authentic IV prepared by the method of Langenbeck and Hölscher.[5]

2-Acetylamino-1-iodonaphthalene (V) was obtained in exactly the same manner (80% yield) by addition of iodine monochloride (7 g.), dissolved in a mixture of acetic acid (20 ml.) and water (3 ml.), to a solution of II (10 g.), or an equivalent weight of the pyridinium salt in water (200 ml.); m.p. 155–162°. Recrystallization from absolute ethanol raised the melting point to 163°, which was not depressed by admixture of authentic V prepared by the method of Willstaedt and Scheiber.[3]

1-Bromo-2-(2-thenoyl)naphthalene (VII). 2-Acetylamino-1-bromonaphthalene (IV) was hydrolyzed[9] and the resulting amine was converted to 1-bromo-2-naphthoic acid by the method of Boyes, Grieve, and Rule.[10] The acid chloride (VI) was formed when a mixture of 145 g. of 1-bromo-2-naphthoic acid and 290 g. of thionyl chloride was allowed to stand for 24 hr. at room temperature. Evaporation under reduced pressure and crystallization of the residue once from heptane gave 147 g. (95%) of material of sufficient purity for the subsequent Friedel-Crafts reaction.

Anhydrous stannic chloride (26 g.) was added dropwise, during a period of 30 min., to a stirred mixture of 1-bromo-2-naphthoyl chloride (VI, 27 g.), thiophene (8.4 g.) and benzene (100 ml.) maintained at a temperature of 10°. After being stirred for 2 hr. the mixture was hydrolyzed with dilute hydrochloric acid. Evaporation of the organic layer, which had been washed with aqueous sodium carbonate, left a residue which crystallized from ethanol to give 26.5 g. (83%) of 1-bromo-2-(2-thenoyl)naphthalene (VII), m.p. 95–96°.

Anal. Calcd. for $C_{15}H_9BrOS$: Br, 25.20; S, 10.11. Found: Br, 25.42; S, 10.51.

An *oxime of* VII crystallized from ethanol, m.p. 191–192°.

Anal. Calcd. for $C_{15}H_{10}BrNOS$: N, 4.22. Found: N, 4.25.

2-(2-Thenoyl)-1-naphthonitrile (VIII) was prepared by heating a mixture of VII (5 g.), cuprous cyanide (1.6 g.), and pyridine (15 ml.) to reflux for 16 hr. in an atmosphere of nitrogen. The nitrile (3.3 g., m.p. 128–132°) obtained by diluting the reaction mixture with water and extracting with ether was crystallized from aqueous ethanol or from a mixture of benzene and petroleum ether; yield, 2.6 g., m.p. 131.5–132.5°.

Anal. Calcd. for $C_{16}H_9NOS$: C, 72.98; H, 3.44; N, 5.32. Found: C, 73.14; H, 3.31; N, 5.31.

The nitrile (VIII, 9.5 g.) hydrolyzed slowly when heated to reflux with sulfuric acid (20 g.), water (260 ml.), and ethanol (280 ml.). After a reflux period of 6 days a 62% yield of *2-(2-thenoyl)-1-naphthoic acid* (IX) was obtained by distilling the ethanol and extracting the residual mixture with benzene. The benzene solution was extracted with 10% aqueous sodium carbonate and acid IX was precipitated by acidification of the alkaline solution; m.p. 155–157°. Recrystallization from acetic acid raised the melting point to that reported by Sandin and Fieser.[1]

1-(2-Thenoyl)-2-naphthoic acid (X). A mixture of 1,2-naphthalic anhydride (10 g.), thiophene (4.2 g.), and carbon disulfide (100 ml.) was heated on a steam bath and stirred vigorously while anhydrous aluminum chloride (11.4 g.) was added in small portions over a period of 2 hr. The mixture was heated for 2.5 hr., then cooled, and the carbon disulfide layer was decanted. Hydrolysis of the residue with ice and hydrochloric acid yielded a crude organic acid that was purified by crystallization of the sodium salt as described by Sandin and Fieser.[1] The only isomer obtained was 1-(2-thenoyl)-2-naphthoic acid (X), m.p. 220–222°; yield, 3.8 g. (27%).

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An Improved Synthesis of Glycerylphosphorylcholine

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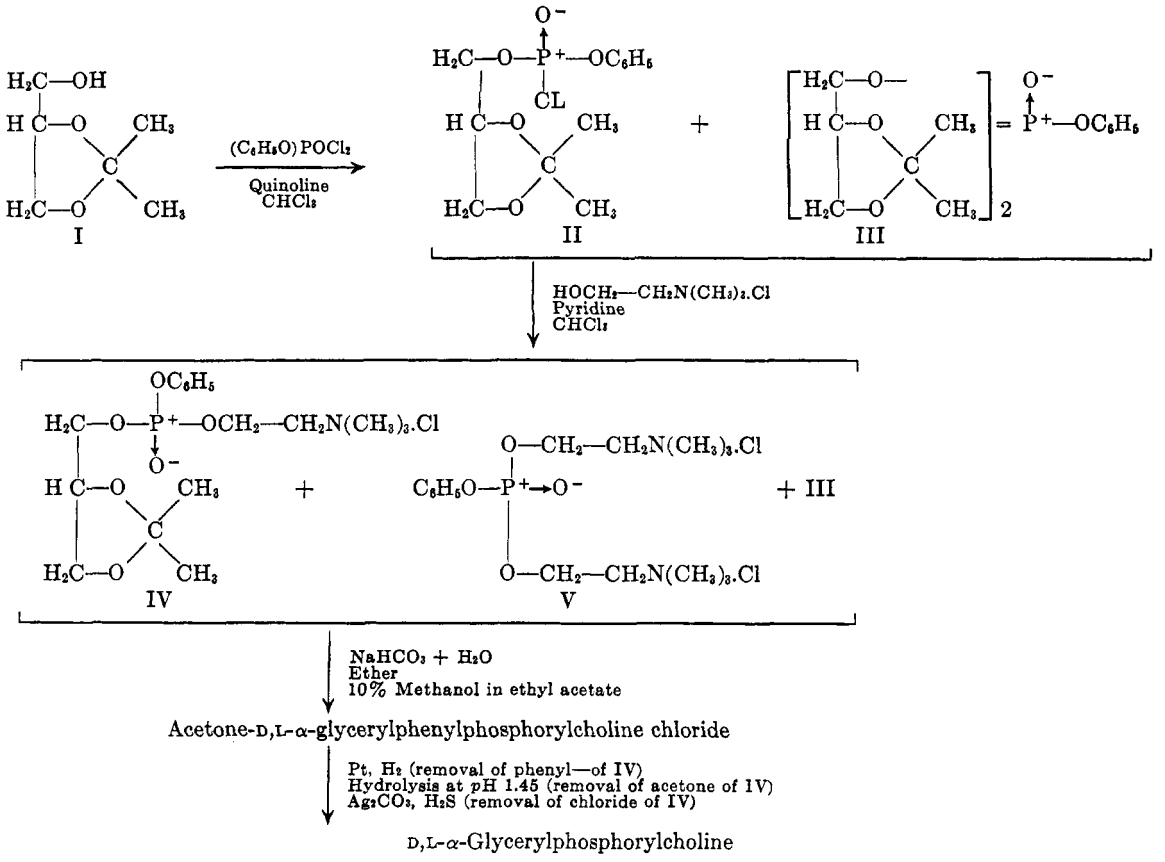
Recent investigation in this laboratory has demonstrated a pronounced effect of synthetic lecithin on blood cholesterol levels. This lecithin also has shown definite ability to dissolve experimentally produced atheromatous plaques in rabbits' aortas. Consequently, it became desirable to extend the investigations to related substances such as glycerylphosphorylcholine.

From several methods available for the preparation of this substance[1-3] we first chose the Baer and Kates procedure.[4] This procedure, although yielding the pure glycerylphosphorylcholine, was found to be cumbersome, costly, and time consuming for the preparation of the relatively large quantity required. The development of a new and more practical procedure for the synthesis of racemic α-glycerylphosphorylcholine was, therefore, undertaken. The procedure reported in this paper utilizes products of metabolism.[4,10] The procedure illustrated in the reaction scheme is as follows: The D, L-α-acetone glycerol (I) was phosphorylated with monophenylphosphoryl dichloride in the presence of dry quinoline and anhydrous and ethanol-free chloroform in a cold bath at the temperature of −10°. The reaction mixture consisting of acetone-D,L-α-glycerylphenylphosphoryl chloride (II), bis(acetone-D,L-α-glyceryl) phenylphosphate (III), and monophenylphosphoryl dichloride was

REACTION SCHEME



monophenylphosphoryl dichloride first described by Jacobsen[5] and later widely adopted by Baer and his co-workers to the syntheses of optically active phospholipids[6-9] and their biological end further esterified with choline chloride in the presence of a large excess of dry pyridine and anhydrous, ethanol-free chloroform. The isolation of acetone -D,L-α-glycerylphenylphosphorylcholine chloride (IV) from the reaction mixture was effected by virtue of its insolubility in ethyl ether and solubility in water and alcohol. The protective phenyl-, acetone, and chloride was removed by hydrogenolysis in the presence of platinum catalyst, acid hydrolysis, and silver carbonate treatment, respectively. Drying the residue to a constant weight over phosphorus pentoxide yielded D,L-α-glycerylphosphorylcholine which crystallized readily from absolute ethanol with periodic addition of anhydrous ethyl ether. The crystalline D,L-α-glycerylphosphorylcholine melted at 159–

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160°. This procedure is equally suited for the preparation of L-α-glycerylphosphorylcholine and D-α-glycerylphosphorylcholine. The racemic α-glycerylphosphorylcholine has been obtained in a crystalline form and in an overall yield of 53.5%.

EXPERIMENTAL

Acetone-D,L-α-glycerylphenylphosphorylcholine chloride (IV) (a). *Phosphorylation.* Into a dry two-necked 200-ml. round bottomed flask equipped with ground glass joints, magnetic stirrer, calcium chloride tube, and a dropping funnel were placed 0.063 mole (13.29 g.) of double-distilled monophenylphosphoryl dichloride[5] and 10 ml. of anhydrous, ethanol-free chloroform. The flask was immersed in a water-bath (−5°) and 0.063 mole (8.5 g.) of freshly prepared D,L-α-acetone glycerol[11] in 10 ml. of anhydrous, ethanol-free chloroform and 0.063 mole (8.1 g.) of anhydrous synthetic quinoline[12] was added dropwise in the course of 30 min. to the vigorously stirred phosphorylating mixture. After the last drop of acetone glycerol was added, the cold bath was removed and the stirring was continued for 15 min. at room temperature 25° and for 20 min. in the water bath at 35°. At the end of this period the water bath was removed and a mixture consisting of 0.126 mole (10.0 g.) of anhydrous pyridine[13] and 35 ml. of anhydrous, ethanol-free chloroform was added gradually in the course of 30 min. to the vigorously stirred reaction mixture. This was followed immediately by the addition of 0.063 mole (8.8 g.) of choline chloride (freshly recrystallized from 99% ethanol).[14] The reaction mixture, protected from moisture, was allowed to stir for a period of 16 hr. at room temperature (24–26°).

(b). *Isolation of acetone-D,L-α-glycerylphenylphosphorylcholine chloride.* The reaction mixture was brought to dryness under reduced pressure at a water bath temperature of 40°. The oily residue was then placed into 100 ml. of distilled water and suspended quinoline was removed in a separatory funnel. The aqueous solution then was neutralized to litmus paper by the addition of solid sodium bicarbonate and the liberated quinoline and pyridine removed by ether extraction. Two 50-ml. portions of anhydrous ethyl ether was used. The clear aqueous solution was concentrated under reduced pressure and at a water bath temperature not exceeding 40°. The last traces of water were removed by keeping the residue over phosphorus pentoxide *in vacuo* desiccator of 0.25 mm. for a period of 18 hr. The residue then was extracted at room temperature (27°) with three 60-ml. portions of methanol and anhydrous ethyl acetate[15] mixture (1:9) and the combined extracts were brought to dryness under reduced pressure at a water-bath temperature not exceeding 40°. If at this time the oil still contained solid material, the treatment with methanol and ethyl acetate was repeated. The acetone-D,L-α-glycerylphenylphosphorylcholine chloride, after drying *in vacuo* (0.25 mm.), weighed 23.63 g. (92.0%). The nearly colorless oil was found to be readily soluble in water, ethanol, methanol, acetic acid, and chloroform; moderately soluble in benzene, carbon tetrachloride, and ethyl acetate; and insoluble in ethyl ether, acetone, and petroleum ether.

Anal. Calcd. for $C_{17}H_{29}O_6NPCl$ (409.89): C, 49.82; H, 7.13; N, 3.41; P, 7.56. Found: C, 49.13; H, 7.15; N, 3.26; P, 7.41.

D,L-α-*Glycerylphosphorylcholine. Removal of protective phenyl—, acetone and chloride groups* (IV). The acetone compound of D,L-α-glycerylphenylphosphorylcholine chloride (22.8 g.) was dissolved in 200 ml. of 99% ethanol and placed in a polyethylene bottle together with 4.0 g. of platinic oxide (Adams catalyst). The mixture was shaken vigorously in an atmosphere of pure hydrogen at room temperature and at an initial pressure of 42 cm. of water until the absorption of hydrogen had ceased. In about 4 hr., 5000 ml. of hydrogen were consumed. The hydrogen was replaced by nitrogen and the hydrogenation mixture freed from the platinum catalyst by gravity filtration.[16] After washing the catalyst with 20 ml. of 99% ethanol, the combined ethanol filtrate and washing was concentrated to dryness under the reduced pressure and at a water bath temperature not exceeding 40°. The residue (approximately 19 g.), dissolved in 400 ml. of distilled water and diluted to *p*H 1.45 by the addition of distilled water (200 ml.), was permitted to stand at room temperature (25°) for a period of 2 hr. To remove the protective chloride ions, 7.6 g. of silver carbonate was added and vigorously stirred until no precipitation of silver chloride occurred on the addition of silver nitrate to a small specimen of the reaction mixture. The silver salts were removed by filtration; the soluble silver ions were removed by passing hydrogen sulfide gas, followed by filtration. The combined aqueous filtrate and washing were brought to dryness at a reduced pressure in a 35–40° water bath. In order to remove the last traces of water the colorless oil was placed *in vacuo* (0.25 mm.) desiccator over fresh phosphorus pentoxide and kept there until a constant weight was reached. The glass-like substance, weighing 14.2 g., was dissolved in 20 ml. of warm (40 to 50°) 99% ethanol and precipitated by the gradual addition of 250 ml. of ethyl ether. The mother-liquor was decanted and the oily residue dried *in vacuo* over phosphorus pentoxide to a constant weight (13.8 g.). The D,L-α-glycerylphosphorylcholine was obtained in colorless crystalline form by recrystallizing it from 95 ml. of hot (70 to 80°) 99% ethanol and the periodic addition of ethyl ether to speed up crystallization; yield 9.2 g. (66.5% yield from acetone-D,L-α-glycerylphenylphosphorylcholine chloride or 53.5% overall yield). The synthetic diester is a crystalline substance which is readily soluble in methanol, ethanol, and water, and insoluble in benzene, ethyl ether, petroleum ether, acetone, or carbon disulfide, m.p. 159–160°.[17]

Anal. Calcd. for $C_8H_{22}O_7NP$ (275.2): C, 34.90; H, 8.00; P, 11.27; N, 5.09. Found: C, 34.72; H, 8.09; P, 11.32; N, 5.04.

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(12) Synthetic quinoline and barium oxide were shaken for eight hours and the quinoline distilled *in vacuo*.

(13) An ACS grade pyridine was refluxed over barium oxide and distilled with the exclusion of moisture.

(14) The powdered choline chloride was dried in the vacuum drying pestle over phosphorus pentoxide (0.20 mm.).

(15) Commercial ethyl acetate tends to dissolve inorganic sodium salts. It was dried over anhydrous potassium carbonate for forty-eight hours.

(16) Special precautions must be exercised at this stage to prevent ignition of the alcohol by the platinum catalyst. At no time should the platinum catalyst be allowed to dry in the air.

(17) Determined on the glass slide using Fisher's melting point apparatus and long-stem thermometer with a range of 300°.