recently by Adelberg.[21] In isotopic competition experiments with an isoleucine-requiring mutant of *Neurospora*, he found that the presence of unlabeled threonine considerably reduced the incorporation of acetate-1,2-C^{14} into the dihydroxyisoleucine analog. Whereas isoleucine carbons 3 and 6 were undiluted in their activity, carbons 1, 2, 4 and 5 were diluted to a considerable degree. The conclusion that carbons 1, 2, 4 and 5 are derived from threonine carbons was supported by a subsequent experiment, in which he found that administration of threonine-1,2-C^{14} resulted in 1,2-C^{14} labeling in the dihydroxyisoleucine analog. Adelberg suggested a mechanism for isoleucine synthesis which also involves an intramolecular migration, differing in some detail from the one we have proposed. The mechanism proposed by us was chosen because of its analogy with known chemical and biological processes, as discussed previously with regard to its participation in valine biosynthesis.[4] At present, no data are available to choose between these, and final conclusions will have to await identification of intermediates in these biosynthetic processes.

(21) E. A. Adelberg, THIS JOURNAL, **76**, 4241 (1954).

PHILADELPHIA, PA.

[CONTRIBUTION FROM THE BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH, UNIVERSITY OF TORONTO]

Synthesis of Unsaturated α-Lecithins.[1] I. L-α-(Dioleyl)-lecithin

BY ERICH BAER, DMYTRO BUCHNEA AND ALAN G. NEWCOMBE

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A procedure permitting the synthesis of the optically pure enantiomeric forms of unsaturated α-lecithins has been developed. The synthesis of L-α-(dioleyl)-lecithin is described and its infrared spectrum, solubilities and other physical data are reported. The catalytic reduction of the L-α-(dioleyl)-lecithin offers a new route for the preparation of L-α-(distearoyl)-lecithin.

The isolation from natural sources of lecithins containing either two identical saturated[2-4] or unsaturated fatty acids[5] established the existence of these two types of phosphatides in nature and thus invalidated an earlier hypothesis that all natural lecithins contain one molecule each of a saturated and unsaturated fatty acid. In order to establish the structure and configuration of natural phosphatides, and to make pure representatives of these compounds readily accessible, methods for their synthesis were developed in this Laboratory. Our procedure for the synthesis of the enantiomeric forms of fully saturated α-lecithins has already been reported.[6-8] This procedure, employing phenylphosphoryl dichloride as the phosphorylating agent, unfortunately cannot be applied to the synthesis of unsaturated lecithins, since the phenyl group of the phenyl ester of the unsaturated lecithin, which would be an intermediate in this procedure, could not be removed by catalytic hydrogenolysis without the simultaneous reduction of the fatty acid double bond. Since in our experience phenylphosphoryl dichloride as a phosphorylating agent is superior in several respects to phosphorus oxychloride, it was deemed desirable to devise a procedure that would permit its use in the synthesis of unsaturated lecithins. Obviously, in this case, the removal of the protective phenyl group by catalytic hydrogenolysis would have to precede the introduction of the unsaturated fatty acid substituents into the glycerol moiety of the phosphatide. With this in mind a sequence of reactions was devised by means of which the synthesis of a fully unsaturated α-lecithin, namely, L-α-(dioleyl)-lecithin finally has been achieved. This compound was selected for synthesis not only because of the presence of oleic acid in many of the naturally occurring glycerolphosphatides,[9] but also in the hope that the unsaturated lecithin would prove to be a good substitute for beef heart lecithin in the serodiagnosis of syphilis.[10]

The synthesis was carried out as follows (see Reaction Scheme): D-acetone glycerol (I) was phosphorylated by means of phenylphosphoryl dichloride and quinoline, and the resulting acetone L-α-glycerylphenylphosphoryl chloride (II) was esterified with ethylene chlorohydrin in the presence of pyridine. The acetone L-α-glycerylphenylphosphoryl ethylene chlorohydrin (III) thus formed was freed of its phenyl group by catalytic hydrogenolysis and of its acetone group by mild acid hydrolysis, and the L-α-glycerylphosphoryl ethylene chlorohydrin (IV) was isolated in the form of its barium salt. Treating the barium salt with oleyl chloride and pyridine in anhydrous dimethylformamide gave the barium salt of L-α-dioleylglycerylphosphoryl ethylene chlorohydrin

(1) The feasibility of the procedure reported in this communication was tested by Dr. A. G. Newcombe by preparing a known saturated lecithin. The procedure was improved and applied to the synthesis of the unsaturated lecithin by Dr. D. Buchnea.

(2) A. Lesuk and R. J. Anderson, *J. Biol. Chem.*, **139**, 457 (1941).

(3) S. J. Thannhauser, J. Benotti, J. and N. F. Boncoddo, *ibid.*, **166**, 669 (1946).

(4) S. J. Thannhauser and N. F. Boncoddo, *ibid.*, **172**, 135 (1948).

(5) D. J. Hanahan and M. E. Jayko, THIS JOURNAL, **74**, 5070 (1952).

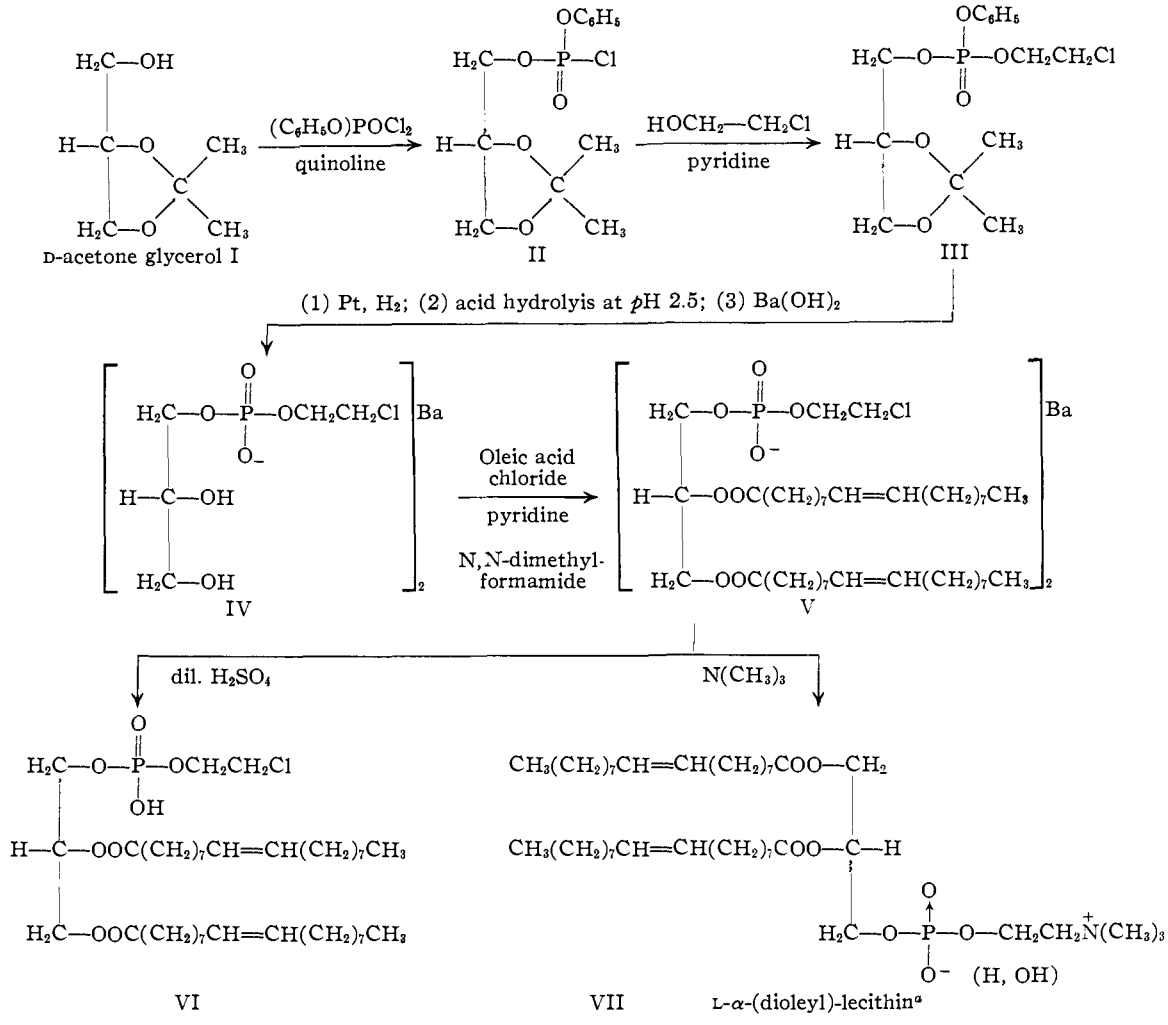
(6) E. Baer and M. Kates, *ibid.*, **72**, 942 (1950).

(7) E. Baer and J. Maurukas, *ibid.*, **74**, 158 (1952).

(8) E. Baer, *ibid.*, **75**, 621 (1953).

(9) The isolation of the dipalmitoleyllecithin from brewer's yeast had not then been reported by Hanahan and Jayko.

(10) The serological investigation of the unsaturated lecithin is being carried out by Drs. D. B. Tonks and R. H. Allen and Miss Evelyn Fowler, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa. Some of their findings were reported at the "Symposium on Recent Advances in the Study of Venereal Diseases," Washington, D. C., U. S. Public Health Service, 1955 (B. David Tonks, H. Rovelle Allen and Evelyn Fowler, The Use of a Synthetic Unsaturated (Dioleyl)-L-α-lecithin in Cardiolipin Antigens for the Serodiagnosis of Syphilis. A Comparison with Other Lecithins, Natural and Synthetic).



REACTION SCHEME

[a] A glycerolphosphatide is assigned the configuration of its glycerophosphoric acid moiety [see H. O. L. Fischer and E. Baer, *Chem. Revs.*, **29**, 287 (1941), E. Baer and M. Kates[8]]. Hence an α-lecithin that has been obtained from D-acetone glycerol is a derivative of L-α-glycerophosphoric acid, since its phosphoric acid is bound to the hydroxyl group formed by the reduction of the carbonyl group of acetone D-glyceraldehyde and its glycerol moiety thus possesses a steric arrangement that is opposite to that of D-glycerophosphoric acid, the reduction product of D-glyceraldehyde-3-phosphoric acid. The D- and DL-α-(dioleyl)-lecithins can be obtained by the same procedure, using L- or DL-acetone glycerol, respectively, as starting material.

(V), which on heating with trimethylamine[11] in benzene at 60° for a period of 4 days yielded a mixture of L-α-(dioleyl)-lecithin and oleyllysolecithin that was separated by chromatography on silicic acid.[12]

The L-α-(dioleyl)-lecithin thus obtained was a wax-like material whose analytical values for carbon, hydrogen, nitrogen and phosphorus, and molecular ratios of oleic acid:phosphoric acid:choline, agreed closely with those required by theory for the lecithin structure shown by formula VII. The synthesis of L-α-(dioleyl)-lecithin recently has been accomplished also *via* the bromo analogs of compounds III, IV, V and VI, and will be reported later.

The L-α-(dioleyl)-lecithin was found to be readily soluble at room temperature in methanol, ethanol or chloroform, and in contrast to the fully saturated lecithins,[6] also in ether and in 90% acetone. The considerable solubility of dioleyllecithin (and presumably of other unsaturated phosphatides) in moist acetone indicates that the conventional procedures for the isolation of phosphatides from tissues, egg yolk and other natural sources permit loss of considerable amounts of the more unsaturated phosphatides. This has been noted by members of our department who have been interested in the determination of tissue lipids. In the case of liver

(11) An attempt to obtain ester-bound choline *in situ* by the action of trimethylamine on phosphatidyl ethylene chlorohydrin has been reported by A. Grün and F. Kade (*Ber.*, **45**, 3367 (1912), German Patent, 240,075).

(12) The separation of phosphatides from fats and free fatty acids on saccharose, magnesium oxide or silicic acid columns was studied by B. Borgström (*Acta Physiol. Scand.*, **25**, 101 (1952)). His observation that the adsorptive capacity of silicic acid for phosphatides is far superior to either saccharose or magnesium oxide, not only was confirmed in our laboratory but we found that it affords also an excellent means for separating lecithins from lysolecithins. While our manuscript was in the hands of the editors an interesting paper by C. H. Lea, D. N. Rhodes and R. D. Stoll (*Biochem. J.*, **60**, 353 (1955)) appeared describing in detail the separation of lecithins and lysolecithins on a silicic acid column.

for example the loss can amount to as much as 40% of the organic phosphorus soluble in fat solvents.[13] The specific ($[\alpha]_D$ +6.2°) and molecular (M_D +49.0°) rotations of L-α-(dioleyl)-lecithin are identical with those of L-α-(distearoyl)-lecithin[6] and L-α-(dipalmitoleyl)-lecithin.[5] Furthermore, the catalytic reduction of the L-α-(dioleyl)-lecithin in a mixture of ethanol and chloroform with platinum as catalyst gave in good yield optically pure L-α-(distearoyl)-lecithin. The synthesis of unsaturated lecithins by our method hence proceeds without racemization.

The L-α-(dioleyl)-lecithin like L-α-(dipalmitoleyl)-lecithin is quite stable toward atmospheric oxidation. A sample of the synthetic lecithin exposed in a thin layer to air for a period of 5 days had neither become colored nor had it changed its iodine number. Thus it appears that the rapid deterioration in air, so frequently reported for the unsaturated lecithins obtained from natural sources, is not a property of the pure unsaturated lecithins but is caused by compounds that accompany the natural lecithins throughout the various stages of the isolation and purification and which are still unidentified. In view of the unexpected stability of pure unsaturated, synthetic as well as natural,[5] lecithins toward oxygen it was thought worthwhile to emphasize this fact.

The infrared spectrum of the L-α-(dioleyl)-lecithin (Fig. 1) in general resembles closely those of the saturated lecithins,[8] except that it also exhibits absorption bands at 13.2, 13.8 and 15.08 μ. The presence of a fairly strong band in a position where *trans*-double bonds are known to absorb (10.33 μ) suggested the possibility that elaidinization of the oleic acid had occurred during the synthesis. This possibility was eliminated by the finding that the fatty acid obtained after saponifying the synthetic product was pure oleic acid. It would appear that the absorption band at 10.33 μ is due mainly to the covalent phosphate group since all phosphatides possess a strong band in this region.

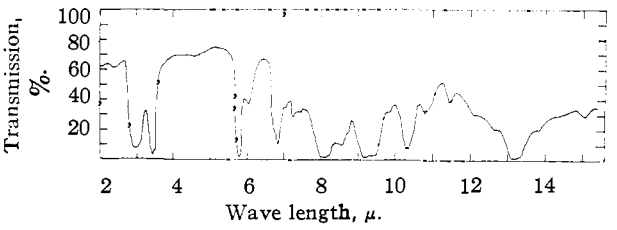


Fig. 1.—Infrared spectrum of L-α-(dioleyl)-lecithin.

In alcohol, dioleyllecithin and cadmium chloride form in almost theoretical yield an ethanol-insoluble addition compound that consists of two moles of lecithin and three moles of cadmium chloride, and thus has the same composition as the corresponding compounds of the saturated lecithins[6] and glycollecithins.[14] Its specific rotation ($[\alpha]_D$ +3.7°) is similar to those reported by Levene and West[15] for cadmium chloride addition compounds of egg yolk lecithin ($[\alpha]_D$ +2.0 to +4.2°).

(13) Dr. C. C. Lucas informs us that data from a recent reinvestigation of these losses are being prepared for publication elsewhere.

(14) E. Baer, THIS JOURNAL, **75**, 5533 (1953).

(15) P. A. Levene and C. J. West, *J. Biol. Chem.*, **34**, 175 (1918).

Ether or chloroform extracts of biological material in addition to the desired phosphatides often contain appreciable amounts of electrolytes, carbohydrates, amino acids and other substances, that normally are insoluble in these solvents. The solubility of these contaminants in ether or chloroform doubtless is due to a solubilizing effect of the phosphatides. A qualitative study of this effect was made by us several years ago[16] using the saturated lecithins and cephalins that we had obtained by synthesis. Although it was found that these phosphatides made our test substances (sodium chloride, sodium sulfate, glucose and glycogen) slightly soluble in chloroform, they seemed to have no effect on the solubility of these substances in ether. This was not surprising since the saturated lecithins and cephalins are practically insoluble in ether. Our studies have now been continued but on a more quantitative basis and using the much more ether-soluble L-α-(dioleyl)-lecithin. In order to study its effect on the solubility of sodium chloride, glucose, saccharose or serine, a weighed amount of the finely powdered substance was added to a 2-ml. portion of a 5% solution of the unsaturated lecithin in either chloroform or ether, and the mixture was shaken in a closed vessel at 25° for one hour. To determine the amount of material that had dissolved, the remaining solid was recovered by centrifugation and, after washing with small amounts of the pure solvent and drying *in vacuo*, was weighed. In experiments that were carried out in the same manner, but omitting lecithin, all four substances were recovered quantitatively. Of the four test substances, glucose and saccharose were found to be soluble to an appreciable amount in ether as well as in chloroform (Table I). For example, 5% solutions of the unsaturated lecithin in moist ether were found to contain glucose or saccharose in amounts that were equal to 6 or 3%, respectively, of the weight of the lecithin. The solubilizing effect of the lecithins thus established provides at least a partial explanation for the presence of certain otherwise insoluble materials in ether or chloroform extracts of natural phosphatides.

TABLE I

APPROXIMATE SOLUBILITY VALUES IN 100 ML. OF A 5% SOLUTION OF L-α-(DIOLEYL)-LECITHIN AT 25° IN MG.

	Anhydrous ether	Moist ether	Chloroform
Sodium chloride	0	25	0
L-Serine	0	15	0
Glucose	125	315	175
Saccharose	15	150	115

The synthesis of unsaturated α-lecithins containing fatty acids other than oleic acid, and of unsaturated α-cephalins is in progress in this Laboratory.

Experimental Part

Acetone L-α-Glycerylphenylphosphoryl Ethylene Chlorohydrin (III). Phosphorylation.—In a flask equipped with a calcium chloride tube, an oil-sealed stirrer and a dropping funnel was placed 71.4 g. (0.34 mole) of monophenylphosphoryl dichloride[17] and 100 ml. of glass beads (approx. 6

(16) E. Baer, J. Maurukas and M. Russell, THIS JOURNAL, **74**, 152 (1952).

(17) Prepared by the method of H. Zenftman and R. M. McGillivray, *C. A.*, **45**, 9081 (1951), British Patent 651,656.

mm. in diameter). The flask was immersed in a cold-bath at −12 to −15° and 55.5 g. (0.43 mole) of pure distilled anhydrous quinoline (synthetic) was added to the rapidly stirred mixture in a thin stream, followed by the dropwise addition of 44.9 g. (0.34 mole) of freshly prepared D-acetone glycerol[18] over a period of 20 minutes. About 20 minutes after the acetone glycerol had been added, 200 ml. of pure distilled anhydrous pyridine was added in one portion, followed, after the formation of a fine suspension, by the addition of 28.0 g. (0.35 mole) of freshly distilled ethylene chlorohydrin over a period of 10 minutes. The stirring was continued for 30 minutes at −10° and for 1 hour at room temperature. At the end of this period the reaction mixture was freed from glass beads, the beads were washed with ether, and the ether was added to the reaction mixture. After diluting the reaction mixture with 1 liter of ether, the precipitate, consisting mainly of quinoline and pyridine hydrochlorides, was removed by filtration with suction and the filtrate was concentrated *in vacuo* as far as possible at a bath temperature of 40–45° to remove most of the pyridine. The concentrate, a thick oil, was dissolved in 1.5 l. of ether, and the ether solution was washed rapidly in succession with two 500-ml. portions of ice-cold 5 *N* sulfuric acid, 500 ml. of water, two 500-ml. portions of saturated sodium bicarbonate solution and finally three 500-ml. portions of water. The ether solution, after drying overnight with 200 g. of anhydrous sodium sulfate, was concentrated under diminished pressure[19] and the residual thick oil was freed of the rest of the solvent by keeping it in a good vacuum (0.1 mm.) at 40–50° for several hours. The acetone L-α-glycerylphenylphosphoryl ethylene chlorohydrin (III) weighed 101.0 g., that is, 84.7% of the theoretical amount calculated for D-acetone glycerol. The substance is readily soluble in ether, methanol, ethanol, acetone or chloroform, and insoluble in water; $[\alpha]^{25}D$ −0.24° in substance; $[\alpha]^{25}D$ +3.5° in chloroform (*c* 10).

Anal. Calcd. for $C_{14}H_{20}O_6PCl$ (350.7): C, 47.95; H, 5.79; P, 8.83; Cl, 10.10; acetone, 16.56. Found: C, 47.88; H, 5.70; P, 8.80, 8.85; Cl, 10.25; acetone, 16.25.

Barium Salt of L-α-Glycerylphosphoryl Ethylene Chlorohydrin (IV). Catalytic Hydrogenolysis.—Acetone L-α-glycerylphenylphosphoryl ethylene chlorohydrin (78.0 g., 0.222 mole) was dissolved in 750 ml. of 99% ethanol and the clear solution to which 12 g. of platinum oxide (Adams catalyst)[20] had been added was shaken vigorously in an all-glass reduction vessel in an atmosphere of pure hydrogen at room temperature (approx. 22°) and a pressure of 40–50 cm. of water until the absorption of hydrogen ceased. The hydrogenolysis seemed to be complete at the end of 4 hr. with an uptake of hydrogen of approx. 90% of theory. After replacing the hydrogen with nitrogen, the mixture was filtered, the catalyst was washed with a small amount of ethanol, and the combined filtrates were concentrated *in vacuo* to a thick sirup.

Deacetonation.—To the concentrated solution of the acetone L-α-glycerylphosphoryl ethylene chlorohydrin in water, which was strongly acid, was added gradually a saturated aqueous solution of barium hydroxide until the *p*H of the solution was approx. 2.5. After standing for 12 hours at room temperature, the *p*H was adjusted with barium hydroxide solution to a value between 8 and 9, and the excess of the barium ions was removed with carbon dioxide. The aqueous solution, freed from barium carbonate, was brought to dryness *in vacuo* at a bath temperature of 40 to 45°. The solid material was dissolved in 200 ml. of methanol, the solution was cleared by centrifugation and the methanol was removed *in vacuo* at low temperature. The amorphous and strongly hygroscopic residue was quickly pulverized and was dried over phosphorus pentoxide in a high vacuum until constant weight was reached. The barium salt of L-α-glycerylphosphoryl ethylene chlorohydrin (IV) was obtained in a yield of 97% of theory (65.0 g.). It was found to be readily soluble in water, methanol, ethanol or dimethylformamide but insoluble in ether; $[\alpha]D$ −0.83° in 2 *N* HCl (*c* 9). No rotation could be observed for 10% solutions of the barium salt in either water or ethanol (1-dm. tube).

Anal. Calcd. for $(C_5H_{11}O_6PCl)_2Ba$ (604.5): C, 19.87; H, 3.67; P, 10.25; Cl, 11.73; Ba, 22.72. Found: C, 19.90; H, 3.64; P, 10.00; Cl, 11.69; Ba, 22.70.

Dioleyl-L-α-glycerylphosphoryl Ethylene Chlorohydrin (VI).—Into a dry flask was placed 19.0 g. (0.031 mole) of the barium salt of L-α-glycerylphosphoryl ethylene chlorohydrin, 24 g. (0.30 mole) of anhydrous pyridine and 100 ml. of pure distilled anhydrous dimethylformamide, and to the solution was added in one portion 45.1 g. (0.15 mole) of freshly distilled oleyl chloride.[21] The closed flask was placed in an oven at 70° for a period of 40 hours. At the end of this time, flask and contents were brought to room temperature, 20 ml. of a mixture of crushed ice and water was added, and the flask was cooled occasionally with water to prevent a rise in temperature. After two hours the mixture, that had separated into two layers, was poured with stirring into 500 ml. of distilled water and kept in a cold bath at −10° for 30 minutes. The heavy pasty material was collected by centrifugation and was triturated successively with two 150-ml. portions of ice-cold water, and two 150-ml. portions of cold (−10°) acetone, each time separating the mixture by centrifugation. The viscous oil thus obtained, after thorough removal of acetone *in vacuo* (0.1 mm.), was dissolved in 50 ml. of anhydrous and peroxide-free ether, the solution was cleared by centrifugation, and the barium salt was precipitated by the addition of 250 ml. of 99% ethanol. After keeping the mixture at −10° for one hour, it was centrifuged and the precipitate was dried *in vacuo* over phosphorus pentoxide to constant weight. The barium salt of dioleyl-L-α-glycerylphosphoryl ethylene chlorohydrin (V), a spongy material that could be compressed to a light-colored solid of wax-like appearance and consistency, weighed 31.0 g. (59.5% of theory). This material was used for the condensation with trimethylamine.

Anal. Calcd. for $C_{82}H_{150}O_{16}Cl_2P_2Ba$ (1662.3): C, 59.24; H, 9.10; Cl, 4.27; P, 3.73; Ba, 8.26; oleic acid, 67.96. Found: C, 59.16; H, 9.21; Cl, 4.51; P, 3.85; Ba, 7.95; oleic acid, 68.23.

The dioleyl-L-α-glycerylphosphoryl ethylene chlorohydrin (VI) was obtained in the following manner: 5 g. of the barium salt was dissolved in 500 ml. of peroxide-free ether, and the solution was washed in succession with 500 ml. of ice-cold 0.5 *N* sulfuric acid and three 500-ml. portions of distilled water. The ether solution was evaporated under diminished pressure at a bath temperature of 30–35°, and the residue was dried *in vacuo* (0.1 mm.) over phosphorus pentoxide and paraffin for 24 hr. The amorphous dioleyl-L-α-glycerylphosphoryl ethylene chlorohydrin (VI) is readily soluble in ether, chloroform, tetrachloromethane or petroleum ether, but is insoluble in water, acetone, ethanol, methanol or dioxane; $[\alpha]^{25}D$ +2.1° in dry and ethanol-free chloroform (*c* 10).

Anal. Calcd. for $C_{41}H_{76}O_8PCl$ (763.5): C, 64.50; H, 10.03; P, 4.06; Cl, 4.65; iodine no., 66.4. Found: C, 64.53; H, 9.95; P, 4.10; Cl, 4.76; iodine no., 65.9.

Dioleyl-L-α-glycerylphosphorylcholine (VII). Condensation.—Into a heavy-walled Carius combustion tube (60–70 cm. length, 19 mm. inside diameter) that for most of its length was immersed in an acetone–solid carbon dioxide cold-bath (−70°) and contained a solution of 25 g. of the barium salt of dioleyl-L-glycerylphosphoryl ethylene chlorohydrin in 60 ml. of anhydrous benzene (Analar), was distilled under anhydrous conditions 40 ml. of trimethylamine. The trimethylamine was passed through a column of soda lime.[22] The tube was sealed, removed from the cold bath

(18) Prepared in "Biochemical Preparations," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1952, p. 31. It is important that the specific rotation of the D-acetone glycerol be not lower than +13.5°. See E. Baer and H. C. Stancer, THIS JOURNAL, **75**, 5410 (1953), footnote 28.

(19) This and all following vacuum distillations were carried out in a nitrogen atmosphere.

(20) 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.

(21) The oleyl chloride had been prepared with thionyl chloride that was purified by successive distillations from quinoline and boiled linseed oil as described in A. I. Vogel's "Text-Book of Practical Organic Chemistry," Longmans, Green and Co., London, New York, Toronto, 1948, p. 185.

(22) It was found that a considerably better yield of the condensation product is obtained if the trimethylamine is distilled over soda lime that has been treated before use with gaseous ammonia. A suitable soda lime preparation is obtained by exposing 100 g. of soda lime to 50 ml. of ammonia gas for a period of 12 hours.

and brought to room temperature. After mixing its contents thoroughly, it was placed in an oven at 60° for 4 days. At the end of this period the tube, after cooling in a mixture of acetone and solid carbon dioxide, was opened and allowed to attain gradually room temperature. The escaping trimethylamine was absorbed in hydrochloric acid. After most of the trimethylamine had distilled off, the contents of the tube were transferred to a distilling flask, using peroxide-free ether for rinsing, and the rest of the trimethylamine and solvents were removed under diminished pressure at a bath temperature of 30–35°. The residue was dissolved in 400 ml. of anhydrous and peroxide-free ether and the solution, after clearing by centrifugation, was brought to dryness under reduced pressure at low temperature. The last traces of solvent were removed by keeping the substance in a vacuum of 0.1 mm. or better for several hours. The remaining material was triturated with five 100-ml. portions of 99% ethanol, separating the mixture each time by centrifugation. The combined extracts were evaporated under reduced pressure and the residue was freed of alcohol *in vacuo* (0.1 mm.) at a bath temperature of 30–40°.

Separation of the Condensation Products by Chromatography.—A clear solution of the mixture of condensation products (20.0 g.) in 500 ml. of chloroform was passed through an adsorption column of 600 g. of silicic acid (approx. 50 cm. in height).[23] The column was washed with chloroform until a test of the effluent showed that it was free of solute. This required approx. 1 l. of the solvent. The adsorbate was divided by two light-colored, narrow bands into three zones, of which the upper one contained most of the lysolecithin, the middle one the lecithin, and the lower one a mixture of substances low in nitrogen. Immediately below the colored band at the top there was a narrow translucent zone that contained also lysolecithin. To obtain the lecithin-containing zone as pure as possible, the column was extruded by a gentle pressure with nitrogen, and care was taken to collect for elution only that section of the column that was framed by the translucent zone and the lower colored band. This material was made into a slurry with methanol, transferred to a tube that contained a small layer of pure charcoal (Norit GSX), and the lecithin was recovered by elution with methanol. Approx. 700 ml. of methanol was required. The combined eluates were concentrated under reduced pressure at 30–35° to one third of the original volume. To the concentrate was added 5% (by volume) of distilled water and 25 g. each of Amberlites IR-4B (XE-59) and IRC-50 (XE-97), and the mixture was shaken for 90 minutes. The Amberlites were removed by filtration, washed with methanol, and the combined filtrates were concentrated as far as possible under reduced pressure at 30–35°. The residue was taken up in 150 ml. of dry ether, the solution was cleared by centrifugation and concentrated under reduced pressure. The last traces of solvent were removed by keeping the lecithin in a high vacuum (0.05–0.1 mm.) at room temperature until the weight was constant. The dioleyl-L-α-glycerylphosphorylcholine (VII), a wax-like material, weighed 13.0 g. It was found to be readily soluble in ether, methanol, ethanol, chloroform or 90% acetone, and moderately soluble in warm petroleum ether (b.p. 30–60°); $[\alpha]^{25}D$ +6.2° in a mixture of equal volumes of chloroform and methanol (*c* 5). The lecithin thus obtained usually was free of lysolecithin as shown by a negative test for hemolytic activity. If lysolecithin was found to be present the chromatographic separation on silicic acid was repeated.

Anal. Calcd. for $C_{44}H_{86}O_9PN$ (804.2): C, 65.71; H, 10.78; P, 3.85; N, 1.74; iodine no., 63.1. Found[24]: C, 65.73, 65.78, 65.75; H, 10.74, 10.79, 10.72; P, 3.88, 3.86, 3.87; N, 1.76, 1.73, 1.79; iodine no., 62.3, 61.4, 63.0.

The unsaturated lecithin on hydrolysis gave in good yield pure oleic acid: m.p. 14–15°, $n^{23}D$ 1.4582; authentic oleic acid, m.p. 14°, $n^{20}D$ 1.4582.

Recovery of Oleic Acid and Choline.—A solution of 503.0 mg. of dioleyl-L-α-lecithin in 50 ml. of a mixture of equal volumes of ethanol and 2 *N* aqueous potassium hydroxide solution was refluxed for 8 hr. The solution was concentrated to a volume of approx. 25 ml. in a stream of nitrogen and transferred quantitatively to a separatory funnel. After acidifying the mixture with 7 ml. of 10 *N* sulfuric acid, it was extracted with three 25-ml. portions of low boiling petroleum ether. The combined petroleum ether extracts were washed with two 10-ml. portions of water.

(1) **Oleic Acid.**—The petroleum ether was removed in a stream of nitrogen at 50–60°, and the residue was titrated according to the method of Stetten,[25] using, however, thymol blue as indicator. Calcd. oleic acid: 353.3 mg. Found: oleic acid, 342.5 mg. (96.9% recovery).

(2) **Choline.**—The *p*H of the combined aqueous solutions was adjusted approx. to a value of 4 with concentrated ammonium hydroxide, and the choline was determined gravimetrically in form of its reineckate. Calcd. choline reineckate: 262.9 mg. Found: choline reineckate 261.5 mg. (99.3% recovery).

Cadmium Chloride Compound of L-α-(Dioleyl)-lecithin.—A solution of 0.7 g. of cadmium chloride (2.5 H_2O) in 0.5 ml. of water, and 18 ml. of 99% ethanol, was added gradually and with stirring to a solution of 1.0 g. of L-α-(dioleyl)-lecithin in 30 ml. of 99% ethanol. After standing for a short time in the ice-box, the mixture was centrifuged and the precipitate was transferred to a small buchner funnel with 5 ml. of ethanol. The precipitate was washed with ether, dried *in vacuo* at room temperature, and reprecipitated from chloroform (10 ml.) with 99% ethanol (50 ml.). The L-α-(dioleyl)-lecithin cadmium chloride addition compound was obtained in a yield of 97% (1.3 g.); $[\alpha]D$ +3.7° in chloroform (*c* 10).

Anal. Calcd. for $[C_{44}H_{86}O_9NP]_2[CdCl_2]_3$ (2158.2): C, 48.97; H, 8.03; N, 1.30: P, 2.87; Cd, 15.63; Cl, 9.86; choline, 11.22; oleic acid, 52.34. Found: C, 48.87; H, 8.08; N, 1.31; P, 2.86; Cd, 15.40; Cl, 10.37; choline, 10.86; oleic acid, 53.23.

Distearoyl-L-α-glycerylphosphorylcholine.—0.7 G. of platinum oxide (Adams catalyst) suspended in 100 ml. of glacial acetic acid was reduced with hydrogen. After replacing the hydrogen with nitrogen, the acetic acid was decanted, and the platinum catalyst was washed in succession with a 100-ml. portion of glacial acetic acid and three 100-ml. portions of 99% ethanol. After placing in the reduction vessel a solution of 5.4 g. of L-α-(dioleyl)-lecithin in 200 ml. of a mixture of 99% ethanol and chloroform (3/1, v./v.), the reduction was carried out in the usual manner. The reduction was complete in approx. 40 min. with the consumption of 375 ml. of hydrogen (calcd. volume of hydrogen for 25° and 744 mm., 335 ml.). The catalyst was removed by centrifugation and washed twice with small amounts of a chloroform–ethanol mixture (1/2), and the combined solutions were brought to dryness under reduced pressure at 40°. The residue was dissolved in 25 ml. of a warm mixture of petroleum ether (b.p. 35–60°) and chloroform (9/1, v./v.), the solution was freed of traces of catalyst by centrifugation, and the solvents were evaporated under reduced pressure. The L-α-(distearoyl)-lecithin, after drying *in vacuo* (0.1 mm.) over phosphorus pentoxide and paraffin shavings, weighed 5.3 g. (98.3% of theory). Reprecipitation of the lecithin from chloroform with ether[8] yielded 4.3 g. (81.2% of theory) of pure and crystalline L-α-(distearoyl)-lecithin $[\alpha]^{25}D$ +6.2° in chloroform–methanol (1/1, *c* 4). The lecithin started to sinter at about 90° forming translucent droplets. On further heating (20°/min. up to 210° and from there on 10°/min.) the droplets gradually darkened to coalesce suddenly with the formation of a meniscus at 230–231°. Reported for L-α-(distearoyl)-lecithin[8]: $[\alpha]^{25}D$ +6.1°; m.p. with formation of meniscus 230.5–231.5°.

Anal. Calcd. for $C_{44}H_{90}O_9NP$ (808.2): P, 3.83; N, 1.73. Found: P, 3.86; N, 1.75.

On hydrolysis, the L-α-(distearoyl)-lecithin[8] gave in practically quantitative yields stearic acid (98.8%) and choline (99.5%).

Discussion

Configuration and Structure of Naturally Occurring Glycerophosphatides.—With the exception of the plasmalogens, members of all the principal types of α-glycerolphosphatides have been synthesized in our laboratory in the configuration in which they occur in nature. It may be therefore an appropriate time to draw attention to the fre-

(23) Silicic acid (Merck, Reagent Grade).

(24) The reported analytical values are of three independent preparations of L-α-(dioleyl)-lecithin.

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TABLE II

CONFIGURATION AND STRUCTURE OF GLYCEROLPHOSPHATIDES ISOLATED FROM NATURAL SOURCES

Glycerophosphoric acid of phosphatides (38): L-α-

Glycerylphosphorylcholine	Glycerylphosphorylethanolamine		Glycerylphosphorylserine
Beef pancreas autolysates[37]: L-α-	Beef brain[18]: L-α-		Ox-brain[36]: L-α-
Lecithins	**Cephalins**	**Plasmalogen**	**Phosphatidyl serine**
Distearoyllecithin	Distearoylcephalin		
From a hydrogenated mixture of egg yolk or brain phosphatides[8]: L-α-	From a hydrogenated mixture of egg yolk phosphatide[16]: L-α-	Beef brain[18]: L-α-	Ox-brain[38]: L-α-
Partially unsatd. lecithin of egg yolk or brain	Partially unsatd. cephalin of egg yolk		
Parent compd. of distearoyllecithin above[6,39]: L-α-	Parent compd. of distearoylcephalin above[16]: L-α-		
Dipalmitoyllecithin *Cisticercus fasciolaris*[6]: L-α-			
Dipalmitoyllecithin Yeast[40]: L-α-			

quency with which we have observed the α-structure and L-configuration in natural glycerophosphatides. Indeed, our observations suggest that this may be the only form in which these phosphatides occur. The isolation of phosphatides and biologically related substances displaying optical activity from a variety of natural sources (2–5, 26–29) left no doubt as to the occurrence of the α-isomers. The common assumption of the natural occurrence of β-isomers was based solely on the presence of β-glycerophosphoric acid in the hydrolysis products of natural phosphatides. Baer and his associates[30–32] established beyond doubt that the hydrolysis of glycerolphosphatides is accompanied by a reversible migration of phosphoric acid that will account for most, if not all, of the β-glycerophosphoric acid to be found in the hydrolysis products of natural glycerolphosphatides. The possibility that the β-forms of glycerolphosphatides occur in nature indeed seems remote at the present time. Although the optical activity of some of the natural phosphatides indicated that they possess an α-structure, the available optical data were neither sufficient to establish their structural and optical purity, nor to determine their configuration. This unsatisfactory state of affairs was brought to an end when Fischer and Baer[33–35] succeeded in synthesizing the dextro- and levorotatory α-glycerophosphoric acids by a method that clearly revealed their stereochemical relationship to the D- and L-glyceraldehydes. This work not only established the configuration of these key-substances but provided the foundation for the elucidation of the configuration of natural glycerolphosphatides either by chemical or enzymatic means.

In the ensuing years procedures were developed in this Laboratory that for the first time not only made it possible to synthesize pure α-glycerophosphatides, but to obtain these phosphatides in both of their enantiomeric forms. By comparing the optical properties of the corresponding synthetic and natural phosphatides we were able to establish unambiguously both the configuration and structure of various naturally occurring lecithins,[6] cephalins,[16] plasmalogen,[18] phosphatidylserine[36] and their biological intermediates, namely, glycerylphosphorylcholine,[37] glycerylphosphorylethanolamine,[18] glycerylphosphorylserine[36] and phosphatidic acids.[38] In order to show more clearly the uniform structural and configurational pattern of the naturally occurring glycerolphosphatides that has emerged from our investigation, our scattered data have been collected and with certain pertinent data from the literature are presented in Table II.

It is noteworthy that the natural glycerolphosphatides and their phosphorus-containing intermediates (GPC, GPE, GPS) thus far investigated were found without exception to be derivatives of L-α-glycerophosphoric acid. The occurrence of the glycerolphosphatides in nature apparently in only one of the two possible enantiomeric forms would suggest a common principle or pathway at some point in their synthesis. Our findings seem to justify the prediction made 16 years ago by Professor Hermann O. L. Fischer and one of the authors (E.B.)[34] that the glycerolphosphatides of higher plants and animals would be found to possess the α-structure and L-configuration.

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