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

The Synthesis of O-(L-α-Glycerylphosphoryl)-ethanolamine and Some Comments on the Stereochemical Aspects of the Biosynthesis of Glycerolphosphatides from Carbohydrates

BY ERICH BAER AND HARVEY C. STANCER[1]

RECEIVED MAY 15, 1953

α-Glycerylphosphorylethanolamine (GPE), a moiety of cephalin and of plasmalogen, previously obtainable by the catalytic hydrolysis of plasmalogen only, is now readily accessible by synthesis. A procedure is described which permits the preparation of L-α-, D-α- and DL-α-GPE. The α-GPE of plasmalogen by comparison with synthetic material was identified as L-α-GPE. The plasmalogens thus join the naturally occurring α-lecithins and α-cephalins as members of the L-series. In view of the fact that the naturally occurring glycerolphosphatides which have been obtained in a pure state were found to be derivatives of L-α-glycerophosphoric acid (GPA), it is suggested that this compound is an intermediate in the biosynthesis of the glycerolphosphatides and most likely arises in the carbohydrate cycle where it is formed by the enzymatic and asymmetric reduction of dihydroxyacetone phosphate. Since this reduction yields only the L-α-GPA, all naturally occurring glycerolphosphatides arising from GPA should be α-isomers with the L-configuration.

In 1924, Feulgen and Rossenbeck[2] in their histochemical studies of the staining reactions of cells discovered a water-insoluble substance which gave a color reaction with fuchsin–sulfurous acid. Subsequent research by Feulgen and Voit[3a] and Feulgen, Imhäuser and Behrens[3b] revealed that the color reaction was given only after the tissue had been treated with acid or mercuric chloride. Several years later Feulgen and Bersin[4] reported the isolation of a crystalline phosphatide which gave the color reaction with fuchsin–sulfurous acid. The new substance was called plasmalogen.[5]

The plasmalogen on treatment with mercuric chloride broke down to form a fatty aldehyde and glycerylphosphorylethanolamine (GPE), and thus was identified as an acetal of GPE. The GPE was isolated in crystalline form and was believed to be a mixture of the α- and β-isomers. In 1951, Thannhauser, Boncoddo and Schmidt described the isolation of a crystalline phosphatide from beef brain.[6] This substance on treatment with mercuric chloride likewise yielded a crystalline GPE, which the authors found to possess a specific rotation of $-3.26°$[7] and was a pure α-isomer. The GPE was found to occur also in the free state. Its presence was demonstrated in the liver of pigs, rabbits, foetal sheep and rats,[8] and in the liver, spleen, kidney and brain of oxen.[9] Campbell and Work believe that the free GPE is a normal constituent of liver and other tissues. In spite of its widespread occurrence the isolation of GPE from biological material, even in small amounts, is a laborious and time-consuming task.

In earlier communications from this Laboratory it had been shown that the naturally occurring α-glycerophosphoric acid,[10] α-glycerylphosphorylcholine,[11] α-lecithins,[12] α-cephalins,[13] and by inference the α-GPE-moiety of the cephalins, possess the L-configuration. Plasmalogen and its GPE-moiety therefore could be expected to possess the same configuration.

To establish conclusively the configuration of these two substances, a pure enantiomer of α-GPE of known configuration was needed for comparison. In the Experimental Part a procedure is described by means of which optically pure L-α-GPE has been obtained. The same procedure can be used for the synthesis of D-α- and DL-α-GPE. The L-α-GPE was prepared as follows (see Reaction Scheme I): D-Acetone glycerol was phosphorylated with phenylphosphoryl dichloride in the presence of a slight excess of quinoline, giving rise to the formation of acetoneglycerylphenylphosphoryl chloride II, the main product, and bis-(acetoneglyceryl)-phenyl phosphate III. Without isolating compound II, the reaction mixture was treated immediately with N-carbobenzoxyethanolamine in the presence of a large excess of pyridine yielding acetoneglycerylphenylphosphoryl-N-carbobenzoxyethanolamine IV and perhaps a small amount of bis-(N-carbobenzoxyethanolamine)-phenyl phosphate. The acetoneglycerylphenylphosphoryl-N-carbobenzoxyethanolamine was separated from by-product III, and freed of its carbobenzoxy and phenyl groups, either consecutively or simultaneously, by catalytic hydrogenolysis using palladium and platinum as catalysts. Removal of the acetone group by a mild acid hydrolysis yielded O-(L-α-glycerylphosphoryl)-ethanolamine[14] which crystallized readily, forming long and narrow prisms measuring sometimes up to 7 mm. in length. The crystalline L-α-GPE was obtained in over-all yields ranging from 33 to 35%.

(1) This paper forms part of a thesis to be submitted by H. C. Stancer to the Department of Pathological Chemistry of the University of Toronto in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) R. Feulgen and H. Rossenbeck, *Z. physiol. Chem.*, **135**, 230 (1924).

(3) (a) R. Feulgen and K. Voit, *Arch. ges. Physiol.*, **206**, 389 (1924); (b) R. Feulgen, K. Imhäuser and M. Behrens, *Z. physiol. Chem.*, **180**, 161 (1929).

(4) R. Feulgen and Th. Bersin, *ibid.*, **260**, 217 (1939).

(5) See appendix.

(6) S. J. Thannhauser, N. F. Boncoddo and G. Schmidt, *J. Biol. Chem.*, **188**, 417 (1951).

(7) S. J. Thannhauser, N. F. Boncoddo and G. Schmidt, *ibid.*, **188**, 423 (1951).

(8) P. N. Campbell and T. S. Work, *Biochem. J.*, **50**, 449 (1952).

(9) D. M. Walker as quoted by Campbell and Work.[8]

(10) E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **128**, 491 (1939).

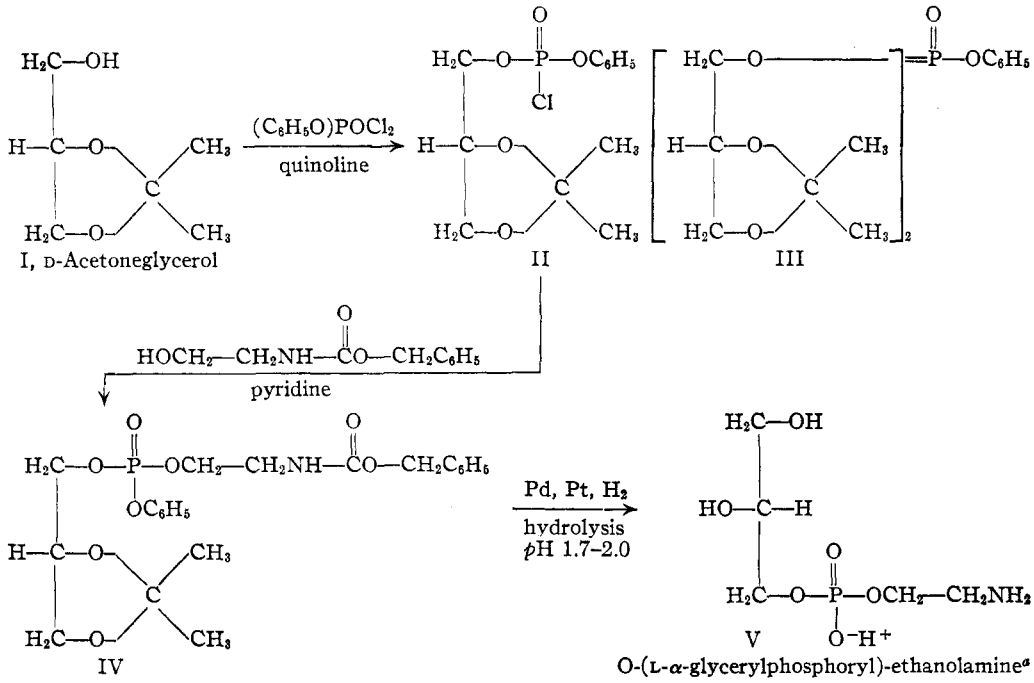
(11) E. Baer and M. Kates, THIS JOURNAL, **70**, 1394 (1948).

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

(13) E. Baer, J. Maurukas and M. Russell, *ibid.*, **74**, 152 (1952).

(14) The guiding principles in establishing the steric classification of enantiomeric glycerides and related compounds have been outlined by H. O. L. Fischer and E. Baer in *Chem. Revs.*, **29**, 287 (1941). The α-GPE obtained from D-acetoneglycerol has the L-configuration since the position of the phosphoric acid is opposite to that in D-α-glycerophosphoric acid, the reduction product of D-glyceraldehyde-3-phosphoric acid.

REACTION SCHEME I



[a] The D-α- and DL-α-glycerylphosphorylethanolamines are prepared by using as starting material L-acetoneglycerol or DL-acetoneglycerol, respectively.

On degradation by acid or alkali, it forms mixtures of L-α-, DL-α- and β-GPA.[15]

The melting point (86–87°) and the specific rotation (−2.9°) of the synthetic product agreed well with those reported by Feulgen and Bersin (86–87°) and by Thannhauser, Boncoddo and Schmidt (−3.2°) for the natural α-GPE,[16] thus establishing the latter and the plasmalogen as members of the L-series.[17]

The fact that the acetal phosphatides, the lecithins,[12,18,19] cephalins[13] and, as we found recently, the phosphatidyl serines[20] are derivatives of L-α-GPA, which is known to be formed by the asymmetric and enzymatic reduction of dihydroxyacetone phosphate,[10] an intermediate in carbohydrate metabolism, is strong evidence in support of a concept stated by us on other occasions,[14,21] namely, that most likely all of the GPA for the biosynthesis of the glycerolphosphatides is supplied by way of the carbohydrate cycle and that the naturally occurring glycerolphosphatides formed from GPA should be α-isomers and possess the L-configuration.

(15) E. Baer, H. C. Stancer and I. A. Korman, *J. Biol. Chem.*, **200**, 251 (1953).

(16) We wish to express our appreciation to Professor S. J. Thannhauser and Dr. G. Schmidt for a generous gift of their precious natural α-GPE.

(17) The optical purity of the α-GPE from plasmalogen is proof that no racemization has occurred during the liberation of plasmalogen and thus precludes any migration of phosphoric acid during the alkaline treatment of the native lipid material. Hence the native acetal phosphatide should have the same configuration as plasmalogen.

(18) E. Baer and M. Kates, *Science*, **109**, 31 (1949).

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

(20) The synthesis of L-α-distearoyl phosphatidyl L-serine has been accomplished by J. Maurukas of this Laboratory and will be reported soon. A comparison of the specific rotation of the synthetic L-α-distearoyl phosphatidyl L-serine with that of the reduction product of the phosphatidyl L-serine of ox brain revealed that the phosphatidyl moiety of the natural phosphatidyl serine is the α-isomer and possesses the L-configuration. The phosphatidyl serines thus form the fourth class of glycerolphosphatides to which we were able to assign a configuration.

(21) E. Baer, *J. Biol. Chem.*, **189**, 235 (1951).

Experimental Part

Monophenylphosphoryl Dichloride (MPD).—The MPD was prepared either according to Jacobsen[22] using, however, the slightly modified procedure reported by Brigl and Müller[23] or, more recently, according to the procedure of Zenftman and McGillivray,[24] which has the advantage of yielding almost exclusively MPD. In both cases the MPD was carefully fractionated *in vacuo* and the fraction boiling from 103–106° at 9 mm. (calcd. P, 14.7; found P, 14.6) was used.

D-Acetoneglycerol.—The acetoneglycerol was prepared by following in general the simplified procedure of Baer and Fischer[25] but carrying out the catalytic reduction of the acetone-D-glyceraldehyde at atmospheric pressure.[26]

Acetone-L-α-glycerylphenylphosphoryl-N-carbobenzoxyethanolamine. Phosphorylation (Step 1).—In a dry[27] one-liter, three-necked, thick-walled round flask with ground glass joints and equipped with an oil-sealed, motor-driven and efficient stirrer, calcium chloride tube and a dropping funnel, were placed 63.3 g. (0.30 mole) of monophenylphosphoryl dichloride and 100 ml. of glass beads (6–7 mm. diameter). The flask was immersed in a cold-bath (−10°) and a mixture of 39.6 g. (0.30 mole) of freshly prepared D-acetoneglycerol[28] and 39.0 ml. (0.33 mole) of dry quino-

(22) G. Jacobsen, *Ber.*, **8**, 1519 (1875).

(23) P. Brigl and H. Müller, *ibid.*, **72**, 2121 (1939).

(24) H. Zenftman and R. McGillivray, *C.A.*, **45**, 9081 (1951) British Patent 651,656.

(25) E. Baer and H. O. L. Fischer, *J. Biol. Chem.*, **128**, 463 (1939).

(26) E. G. Ball, "Biochemical Preparations," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1952, p. 31 (submitted by E. Baer).

(27) During both steps of the phosphorylation anhydrous conditions were maintained at all times.

(28) It is important that the specific rotation of the acetoneglycerol be not lower than +13.5°. Lower values indicate the presence of moisture which causes a considerable reduction in the formation of the desired phosphorylation product. If the specific rotation is below the indicated value, the acetoneglycerol is dissolved in ether once more, dried over anhydrous potassium carbonate, and, after the removal of the ether, is fractionally distilled *in vacuo*.

line[29,30] was added dropwise in the course of ten minutes to the vigorously stirred mixture of MPD and glass beads. The addition of the mixture of acetoneglycerol and quinoline was begun at a fairly rapid rate and decreased toward the end. Five minutes after the last of this mixture had been added the cold-bath was removed and the contents of the flask were allowed to attain room temperature. The main reaction product, the acetone-L-α-glycerylphenylphosphoryl chloride, without isolation was immediately esterified with N-carbobenzoxyethanolamine.

Phosphorylation (Step 2).—To the hard, white reaction mixture was added immediately 100 ml. of anhydrous pyridine[31] and the mixture was stirred until a fine suspension had formed. The flask was then immersed in a water-bath at 15–20° and a solution of 58.5 g. (0.30 mole) of carbobenzoxyethanolamine[32,33] in 120 ml. of pyridine,[31] prepared under anhydrous conditions and brought to room temperature, was added to the vigorously stirred mixture. The stirring was continued for 2 hours.

Isolation of the Phosphorylation Product.—The reaction flask was attached to a receiver and the pyridine was removed as completely as possible *in vacuo* (8–10 mm.) at a bath temperature of 35–40°. To the viscous residue was added 200 ml. of petroleum ether (b.p. 35–60°), the mixture was stirred for a few minutes and then was permitted to separate. The petroleum ether extract was decanted and the extraction was repeated once more with 200 ml. of petroleum ether.[34]

The petroleum ether-insoluble residue then was treated in the same manner with four 200-ml. portions of diethyl ether. The diethyl ether extracts were filtered and washed in succession as rapidly as possible, with two 150-ml. portions of ice-cold 5 *N* sulfuric acid, 150 ml. of distilled water, 150 ml. of a half-saturated solution of sodium bicarbonate and finally with 150 ml. of distilled water. The ether solution after drying with anhydrous sodium sulfate was concentrated under reduced pressure (bath 35–40°) and the last traces of the solvent were removed by keeping the residue in a vacuum of 0.2 mm. at a bath temperature of 40° for a period of five hours. The almost colorless oil weighed 114.2 g. (corresponding to a yield of 81.8% of theory calculated for D-acetoneglycerol). The oil was found to be readily soluble in ether, acetone, benzene, chloroform, dioxane, ethanol or methanol and insoluble in petroleum ether or water; $n^{23}D$ 1.5227 ± 0.0013. In substance it exhibited a rotation of −0.6° in a 1 dm. tube. No significant rotations were observed for either chloroform (*c* 13) or benzene (*c* 12) solutions of the oil.

Anal. Calcd. for $C_{22}H_{28}O_8NP$ (465.4): C, 56.77; H, 6.06; N, 3.00; P, 6.65; acetone, 12.48. Found: C, 56.82; H, 6.10; N, 2.99; P, 6.72; acetone, 12.40.[35]

O-(L-α-Glycerylphosphoryl)-ethanolamine.—Catalytic hydrogenolysis: 18.6 g. (0.040 mole) of acetone-L-α-glycerylphenylphosphoryl-N-carbobenzoxyethanolamine was dissolved in 140 ml. of 99% ethanol and the clear solution together with 4.65 g. (0.0435 mole) of freshly prepared palladium black[36] was shaken vigorously in an all-glass reduction vessel of 800–1000 ml. capacity in an atmosphere of pure hydrogen at room temperature and at a pressure of 40–50 cm. of water until the absorption of hydrogen ceased. This usually occurred at the end of one hour and the absorption of approx. 1050 ml. of hydrogen. The hydrogen was then replaced with nitrogen, the mixture was filtered, the catalyst washed with 40 ml. of 99% ethanol and the combined filtrates were transferred to another hydrogenation vessel of the same capacity. After adding 5.4 g. (0.022 mole) of freshly prepared platinic oxide[37] and 4.0 ml. of 5.0 *N* sulfuric acid, the mixture was again shaken in an atmosphere of hydrogen until the absorption of hydrogen ceased. The volume of hydrogen consumed and the time required for the completion of the reduction varied from 5000–6000 ml. and from 1 to 2.5 hours, respectively. After replacing the hydrogen with nitrogen the catalyst was filtered off, washed with 99% ethanol and the combined filtrates were concentrated *in vacuo* at a bath temperature of 30–35° to a volume of approx. 50 ml.

Deacetonation.—To the concentrate was added 200 ml. of distilled water and the mixture, usually possessing a *p*H of 1.7–1.9, was left to stand at room temperature (25°) until 6–7 hours had elapsed from the time at which the sulfuric acid had been added. To remove the sulfate ions and a small amount of glycerophosphoric acid, an aqueous solution of neutral lead acetate was added cautiously until no further precipitation occurred.[38] The mixture was centrifuged sharply and into the decanted and still slightly opalescent supernatant solution was passed hydrogen sulfide. The lead sulfide was removed and the clear solution, to which capryl alcohol was added periodically to prevent foaming, was concentrated *in vacuo* at 35–40° to a small volume. If on testing a small sample of the concentrate with neutral lead acetate it was found that glycerophosphoric acid was still present, the concentrate was treated once more with neutral lead acetate and hydrogen sulfide. The glycerophosphoric acid-free solution was concentrated *in vacuo* (bath 35–40°) to a viscous oil.

Crystalline GPE.—The colorless oil,[39] weighing 8.2 g., was dissolved in 140 ml. of distilled water and the solution after adding 14 g. of Amberlite IR 120 (in the hydrogen phase) was stirred for 1.5 hours. The Amberlite was removed, a few drops of capryl alcohol added, the water distilled off *in vacuo* (bath 30–35°), and the oily residue was dissolved in 12 ml. of distilled water. To this solution was added 18 ml. of 99% ethanol, and the solution cleared by centrifugation. To the decanted supernatant was added 100 ml. of 99% ethanol, and the crystallization of the oily precipitate was induced mechanically with a glass rod. Usually the first crystals began to appear within the first half-hour forming clusters of narrow prisms which when permitted to form slowly and undisturbed often attained considerable length (6–7 mm.). The form of the crystals was identical with that reported for the natural compound.[7] The mixture was allowed to stand at room temperature until the crystallization was complete. The crystals were collected with suction on a buchner funnel, washed with 20 ml. of 95% ethanol and dried *in vacuo* over calcium chloride. The crystalline O-(L-α-glycerylphosphoryl)-ethanolamine (monohydrate) weighed 4.06 g. (43.5% of theoretical calculated for acetone-L-α-glycerylphenylphosphoryl-N-carbobenzoxyethanolamine) and was free of potassium. For analytical purposes the L-α-GPE (4.06 g.) was reprecipitated from water (10 ml.) with 99% ethanol (60 ml.) and the oil was allowed to crystallize; recovery 81% (3.29 g.);

(29) Synthetic quinoline was refluxed over barium oxide, and fractionally distilled within narrow limits of the boiling point.

(30) In one phosphorylation the quinoline was replaced by anhydrous dimethylaniline. In this experiment the acetoneglycerol was added dropwise and under anhydrous conditions to the vigorously stirred and cooled (−10°) mixture of dimethylaniline and phenylphosphoryl dichloride. The primary phosphorylation product II was treated with N-carbobenzoxyethanolamine in the presence of pyridine, and the isolation of the final phosphorylation product IV was carried out as described above. The acetone-L-α-glycerylphenylphosphoryl-N-carbobenzoxyethanolamine was obtained in a yield of 56%, which is considerably lower than that obtained by using quinoline in the first phosphorylation step. *Anal.* Calcd. for $C_{22}H_{28}O_8NP$ (465): N, 3.00; P, 6.65. Found: N, 2.97; P, 6.70.

(31) Pyridine of a good commercial grade was refluxed over barium oxide and distilled with the exclusion of moisture.

(32) E. Chargaff, *J. Biol. Chem.*, **118**, 417 (1937).

(33) W. G. Rose, THIS JOURNAL, **69**, 1384 (1947).

(34) The bis-(acetoneglyceryl)-phenyl phosphate is soluble in petroleum ether and is removed.

(35) The acetone determinations were carried out according to the method of Messinger, *Ber.*, **21**, 3366 (1888). Before distilling, however, the acid hydrolysate was made alkaline to phenolphthalein to prevent the distillation of phenol.

(36) J. Tausz and N. von Putnoky, *ibid.*, **52**, 1573 (1919).

(37) The catalyst was 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 (A. H. Cook and R. P. Linstead, *J. Chem. Soc.*, 952 (1934).

(38) The reductive cleavage, the deacetonation and the subsequent removal of the sulfate, glycerophosphoric acid and excess lead ions should be carried out the same day. The solution of the GPE then can be stored in the cold overnight without too great a loss of GPE.

(39) In several preparations this oil, on precipitation from water with ethanol, either failed to form crystalline glycerylphosphorylethanolamine or, if crystals did form, they were coated with a sticky material which was difficult to remove. The ease of crystallization, as well as the yield and the purity of the GPE, was greatly improved by treating its aqueous solution with a cation exchange resin (Amberlite IR 120).

m.p. 86–87°[40]; over-all yield 35.6%, $[\alpha]^{26}D$ −2.9° (water, *c* 7.6); reported for GPE obtained from plasmalogen, m.p. 86–87°[4,41] and $[\alpha]D$ −3.26° (water, *c* 5).[7]

Anal. Calcd. for $C_5H_{14}O_6NP \cdot H_2O$ (233.2): C, 25.75; H, 6.92; N, 6.00; P, 13.29. Found: C, 25.48; H, 7.07; N, 5.84; P, 13.5.

At room temperature (23–25°) the L-α-GPE (monohydrate) is insoluble in acetone, ether, chloroform, ethyl acetate, benzene, petroleum ether, dioxane, anhydrous pyridine or dimethyl formamide, slightly soluble in 95% ethanol, readily soluble in methanol or glacial acetic acid, and very soluble in water. The water of crystallization can be removed by keeping the substance *in vacuo* (0.2 mm.) over phosphorus pentoxide at 100° for a period of six hours. The residue, at room temperature, is a colorless, hard glass.

Anal. Calcd. for $C_5H_{14}O_6NP$ (215.1): C, 27.89; H, 6.55. Found: C, 27.63; H, 6.38.

Vicinal Glycol Titration with Periodic Acid.—The titration was carried out according to Voris, Ellis and Maynard[42] on 5.0-ml. aliquots of a solution of 41.4 mg. (0.178 mM.) of crystalline GPE in 15 ml. of distilled water. After 90 minutes, 0.0592 mM. of the diester had consumed 0.0592 mM. of periodic acid or 100% of the theoretical amount calculated for the α-glycerylphosphorylethanolamine monohydrate.

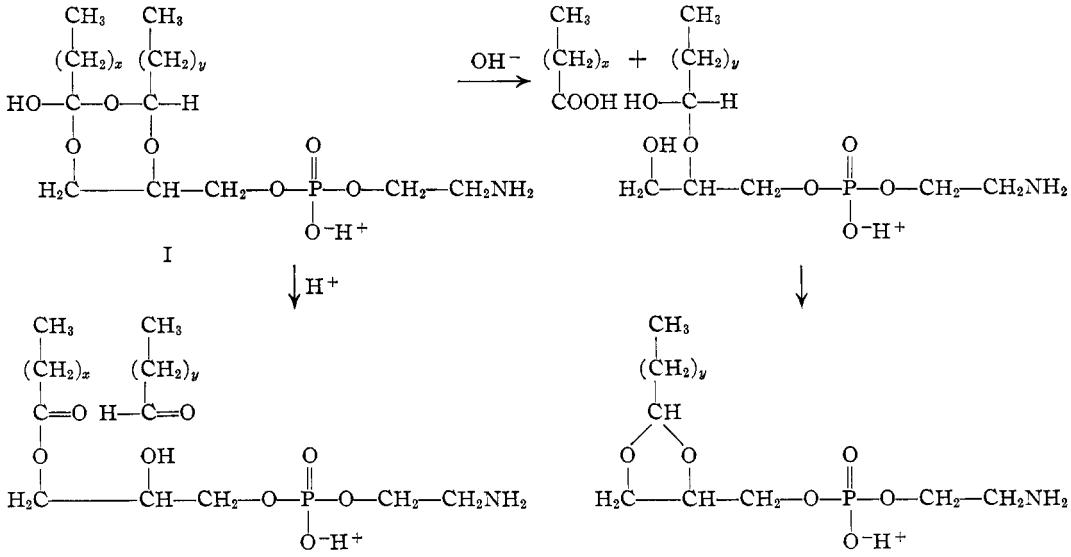
X-Ray Diffraction Patterns.—The synthetic L-α-glycerylphosphorylethanolamine monohydrate and that obtained from plasmalogen gave distinct and identical X-ray powder diffraction patterns with Ni-filtered CuKα radiation (λ 1.5418). The intensities of the diffraction rings of the patterns were estimated visually on an arbitary scale and are quoted in parentheses after the crystal spacings: 7.16 (3), 5.75 (3), 5.05 (7), 4.59 (1), 4.17 (3), 3.89 (10), 3.68 (8), 3.41 (2), 3.28 (2), 3.14 (3), 3.06 (1/2), 2.89 (1/2), 2.82 (2), 2.60 (3), 2.48 (1), 2.40 (1), 2.33 (5), 2.28 (1/2), 2.20 (2), 2.15 (2), 2.09 (1/2), 2.03 (1/2), 1.991 (2), 1.939 (1/2), 1.910 (1), 1.868 (1/2), 1.832 (1), 1.776 (1), 1.685 (1/2), 1.660 (1), 1.635 (1).

Alternative Procedure for Removal of the Carbobenzoxy and Phenyl Groups.—The simultaneous removal of the carbobenzoxy and phenyl groups was accomplished by carrying out the catalytic hydrogenolysis of the acetone-L-α-glycerylphenylphosphoryl-N-carbobenzoxyethanolamine (5.1 g.) in glacial acetic acid (50 ml.) using as catalyst a 1:1 (w./w.) mixture of platinic oxide and palladium (2.1 g.). It was found, however, that to achieve a satisfactory cleavage, the mixed catalyst had to be added in two portions (0.5 g. and 1.6 g.). The glacial acetic acid solution, freed of catalysts, on concentrating *in vacuo* yielded a viscous oil. This oil, on trituration with three 50-ml. portions of anhydrous ether, turned into a solid which weighed 2.39 g. and in general contained 25–30% less acetone than required by theory for acetone-L-α-glycerylphosphorylethanolamine. The substance, obviously a mixture of acetone-L-α-GPE and L-α-GPE, was freed of the rest of the acetone by hydrolysis with dilute acetic acid (50 ml. of 25% acetic acid, 6 hours). The acetone and solvents were removed *in vacuo* and the residue was marked up as described above. The crystalline L-α-GPE (monohydrate) was obtained in a yield of 40.4% (1.03 g.); over-all yield 33.0%.

Appendix

According to Feulgen and Bersin[4] there is a possibility that plasmalogen is a secondary product and is formed by the action of alkali on a native acetal phosphatide. The results of a quantitative study by Schmidt, Ottenstein and Bessman[43] of the hydrolysis of the phosphatide extracts of brain, containing native acetal phosphatide, and of plasmalogen, seems to confirm Feulgen and Bersin's suspicion.

SCHEME II



Schmidt, *et al.*, on the basis of their experiments assume that the native acetal phosphatides "are alkali-labile compounds of glycerylphosphorylethanolamine lipide acetals with other lipide groups." Formula I (Scheme II), which we propose tentatively, might describe the structure of the native acetal phosphatides. In this formula it is assumed that the "other lipide group" is a fatty acid which, in its ortho form, is esterified with both glycerol and the fatty aldehyde hemiacetal of GPE. That the suggested hemiacetal ester structure, which would stabilize the aldehyde, is feasible is shown by the work of Montgomery, Hann and Hudson who prepared the acetylated hemiacetal of arabinose tetraacetate.[44,45] Fatty acid bound in such a manner should be alkali-labile.[46]

(40) Determined in a capillary tube using an electrically heated bath of *n*-butyl phthalate and a short-stem thermometer with a range of 50 degrees.

(41) Thannhauser, Boncoddo and Schmidt (7) report that α-GPE obtained by catalytic hydrolysis of beef brain plasmalogen sinters at 88° and melts at 155°. A gift of the natural α-GPE from Professor Thannhauser and Dr. Schmidt permitted us to make simultaneous determinations of the melting points (40) of the natural and synthetic materials. It was found that both the natural material (after two recrystallizations from water and ethanol) and the synthetic material sintered slightly at 84° and formed clear melts at 86–87°.

(42) L. Voris, G. Ellis and L. A. Maynard, *J. Biol. Chem.*, **133**, 491 (1940). The publication seems to contain a typographical error. If the buffer is made up as reported by these authors its *p*H is outside the stated limits of 4.4 to 7. A suitable buffer, however, is obtained by increasing the amount of disodium phosphate (12 H_2O) from 12 g. to 17 g.

(43) G. Schmidt, B. Ottenstein and M. J. Bessman, *Fed. Proc.*, **12**, 265 (1953).

(44) E. Montgomery, R. M. Hann and C. S. Hudson, THIS JOURNAL, **59**, 1124 (1937).

(45) See also H. W. Post, "The Chemistry of the Aliphatic Ortho-Esters," Reinhold Pub. Corp., New York, N. Y., 1943, p. 106.

(46) The instability of an ortho-acid structure, similar to that shown in formula I (Reaction Scheme II), toward alkali has been reported by

On removal of the fatty acid by alkali, ring closure could take place with the formation of plasmalogen. From the fact that no migration of phosphoric acid occurs during the treatment of the native acetal phosphatide with alkali[17] it can be deduced that the aldehyde is attached to the β-position. An acetal phosphatide possessing the proposed structure I would not be expected to give rise to the formation of an acid-soluble phosphate ester in the plasmal reaction, unless it has been pretreated with alkali. This is precisely the behavior observed by Schmidt, *et al.*, for the native acetal phosphatide.

Pigman and Isbell (W. W. Pigman and H. S. Isbell, *J. Research Natl. Bur. Standards*, **19**, 189 (1937)). See also W. W. Pigman and R. M. Goepp, "Chemistry of the Carbohydrates," Academic Press, Inc., New York, N. Y., 1948, pp. 158–159.

Acknowledgment.—This work has been supported by grants from the National Research Council, Canada, and the Nutrition Foundation, Inc., New York, to both of which the authors wish to express their sincerest thanks. We wish also to thank Professor E. W. Nuffield of the Department of Geological Sciences, University of Toronto, for the X-ray diffraction patterns.

TORONTO 5, CANADA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, BUCKNELL UNIVERSITY, AND SCHOOL OF CHEMISTRY, RUTGERS UNIVERSITY]

On Cyclic Intermediates in Substitution Reactions. III. The Alkaline Hydrolysis of ε-Bromocaproic and ζ-Bromoenanthic Acids

BY HAROLD W. HEINE, EDWARD BECKER AND JOHN F. LANE[1]

RECEIVED MARCH 5, 1953

The rates of aqueous decomposition have been determined for the sodium salts of ε-bromocaproic acid and ζ-bromoenanthic acid. It has been established that (1) the rates of decomposition, as measured by the release of bromide ion, are of the first order with respect to the ion of the haloacid; (2) the entropies of activation are negative and decrease markedly in the order $\epsilon > \zeta$; (3) the heats of activation decrease in the order $\epsilon > \zeta$. These results are discussed in terms of mechanisms involving simple hydrolysis and a cyclic intermediate.

In part I[2] of this series it was shown that in the alkaline hydrolysis of some aliphatic α-, β- and γ-bromoacids, the reactivities increased in the order $\alpha < \beta <<< \gamma$ and that the entropies of activation were positive (about 10 e.u.). The results suggested that in nucleophilic displacement involving intermediate formation of 3-, 4- and 5-membered rings the rate of the displacement process is governed largely by the heat of activation which increases markedly with the strain attending ring formation.

All the bromoacids studied in this investigation involved bromine substitution on the α-, β- or γ-carbons. For the sake of completeness it seemed desirable to determine the behavior of some bromoacids where the carboxylate ion was further removed from the seat of substitution. The present paper reports a study of the hydrolysis of sodium ε-bromocaproate and sodium ζ-bromoenanthate in solutions containing sodium bicarbonate.

Experimental

ε-Bromocaproic Acid.—This acid was prepared according to the procedure of Heine and Jones.[3] The acid was recrystallized from petroleum ether, m.p. 32.5°.

Anal. Calcd. for $C_6H_{11}O_2Br$: Br, 40.9. Found: Br, 40.3.

ζ-Bromoenthanic Acid.—A solution of 78 g. (1.2 moles) of potassium cyanide in 160 ml. of water is added slowly and with stirring to a solution of 244 g. (1.0 mole) of hexamethylene dibromide in 650 ml. of 95% ethanol and the mixture is refluxed for 15 hours. The alcohol (500 ml.) is removed by distillation and the residue is extracted with three 100-ml. portions of benzene. The benzene solutions are combined and washed with 50 ml. of 1 *N* NaOH and then with 50 ml. of water. The benzene is removed by distillation. The residue is vacuum distilled to yield 152 g. of unreacted hexamethylene dibromide boiling at 113–128° (10 mm.), 66 g. of crude ω-bromoenanthonitrile boiling at 129–140° (10 mm.), and 26 g. of crude hexamethylene dicyanide boiling at 140–168° (10 mm.).

Hydrolysis of the crude bromonitrile is effected by refluxing with 48% hydrobromic acid using a twofold molar excess

(1) Deceased, October 17, 1952.

(2) J. F. Lane and H. W. Heine, THIS JOURNAL, **73**, 1348 (1951).

(3) H. W. Heine and H. Jones, *ibid.*, **73**, 1361 (1951).

TABLE I

RATES OF AQUEOUS DECOMPOSITION OF ε-BROMOCAPROATE AND ζ-BROMOENANTHIC ACIDS IN 0.25 *M* SODIUM BICARBONATE

Time, min.	Vol. of 0.05 *N* $AgNO_3$, ml.	10^3k_1 (min.$^{-1}$)	Time, min.	Vol. of 0.05 *N* $AgNO_3$, ml.	10^3k_1 (min.$^{-1}$)
Sodium ε-bromocaproate, $t = 50.00°$			Sodium ζ-bromoenanthate, $t = 70.10°$		
61.75	1.71	1.38	51.5	1.09	4.89
106.3	2.54	1.38	102.5	1.91	4.83
173.1	3.71	1.40	164.3	2.70	4.86
269.6	5.20	1.39	218.6	3.21	4.85
309.6	5.73	1.38	269.5	3.59	4.87
357.8	6.40	1.39	347.6	4.00	4.86
∞	15.61	Mean 1.39	∞	4.90	Mean 4.86

TABLE II

RATE CONSTANTS FOR THE FIRST-ORDER DECOMPOSITION OF ε- AND ζ-BROMOACIDS IN 0.25 *M* SODIUM BICARBONATE

Temp., °C.	10^3k_1 (min.$^{-1}$)	Mean	Temp., °C.	10^3k_1 (min.$^{-1}$)	Mean
ε-Bromocaproic acid			ζ-Bromoenanthic acid		
50.00	1.41	1.40	59.80	1.79	1.78
	1.39			1.79	
	1.44			1.75	
	1.38			1.79	
	1.39		70.10	4.86	4.85
62.72	6.41	6.39		4.86	
	6.38			4.84	
	6.38			4.84	
70.00	13.2	13.2	79.90	12.0	12.1
	13.3			11.9	
	13.2			12.2	
				12.2	