amination of benzaldehyde with 2-aminoethanol according to the procedure of Cope and Hancock[20] for 2-alkylaminoethanols.

The cyclization of 2-benzylaminoethanol was carried out following the method described by Wenker[21] and by Leighton, Perkins and Renquist[22] for the preparation of ethylenimine from 2-aminoethanol. The intermediate 2-benzylaminoethylsulfuric acid could not be purified by trituration with ethanol as it was too soluble in this solvent; the crude product was used directly for the decomposition with the sodium hydroxide solution. The N-benzylethylenimine was obtained as a colorless liquid; b. p. 84–87° (8 mm.); $n^{20}D$ 1.5300; yield 24%.

Anal. Calcd. for $C_9H_{11}N$: C, 81.14; H, 8.34. Found: C, 80.82; H, 8.41.

(20) Cope and Hancock, THIS JOURNAL, **64**, 1503 (1942).
(21) Wenker, *ibid.*, **57**, 2328 (1935).
(22) Leighton, Perkins and Renquist, *ibid.*, **69**, 1540 (1947).

Acknowledgment.—The authors are indebted to Dr. James C. Vitucci for technical assistance in part of this work.

Summary

A series of substituted N-(2-chloroethyl)-dibenzylamine hydrochlorides has been prepared and tested as adrenergic blocking agents.

Substituents in the phenyl rings, other than methyl, greatly diminish or completely abolish the adrenergic blocking activity shown by N-(2-chloroethyl)-dibenzylamine (Dibenamine) hydrochloride.

DELAWANNA, NEW JERSEY RECEIVED JULY 11, 1949

[CONTRIBUTION FROM THE MASSACHUSETTS INSTITUTE OF TECHNOLOGY, DEPARTMENT OF CHEMISTRY]

Peptide Syntheses Using Energy-rich Phosphorylated Amino Acid Derivatives*

JOHN C. SHEEHAN AND VICTOR S. FRANK[1]

The synthesis of peptides and of peptide-like substances is known to take place with ease in living systems. For example, Borsook and Dubnoff[2] have shown that hippuric acid is formed rapidly in liver tissue supplied with glycine and benzoic acid in very dilute solution. However, it has been calculated from thermodynamic data that the change in free energy (ΔF) for this reaction is +2560 calories.[3] Expressed in terms of the equilibrium constant K, this corresponds to a value of approximately 10^{-2}. Consequently, hippuric acid would be more than 99% hydrolyzed at equilibrium under the conditions of the biosynthesis.

In a discussion of the free energy requirements for the synthesis of peptides *in vivo*, Borsook and Huffman[3] point out that equilibria involving peptides and their constituent amino acids would also lie well over on the side of hydrolysis. The synthesis of a typical peptide such as leucylglycine from the amino acids would be attended by a positive free energy change (ΔF = +2930 calories for leucylglycine). Since ΔF for the over-all process must be negative, the formation of a peptide bond in nature must be associated with another free energy-yielding reaction. In the currently accepted theory of the biogenesis of proteins, the driving force for the synthesis is attributed to the free energy supplied by intermediate phosphorylated compounds. The rupture of a "high-energy phosphate bond" is strongly "exergonic"[4] (ΔF about −11,000 calories for hydrolysis).[4,5,6] Compounds containing this high energy linkage are anhydrides or analogs of anhydrides. For example, the mixed anhydrides of phosphoric acid with acetic and glyceric acids are high energy compounds. Pyrophosphates are also high energy compounds. An example is adenosine triphosphate. Phosphorylated enols (*e. g.*, "phosphoenolpyruvic acid") and guanidino phosphates also contain high energy phosphate bonds. Examples of the latter type are phosphorylated creatine and arginine.

Phosphorylated compounds have been recognized as important intermediates in the synthesis of polysaccharides and in various fermentation processes.[5] Phosphoric acid derivatives are known to be responsible for the storage and transfer of energy in many cellular reactions.

The functions of phosphorylated compounds in metabolic processes have been reviewed by Lipmann.[6] This author has discussed the role of "energetic coupling reactions" in biological syntheses and has implied that the origin of the peptide bond in living cells may be ascribed to energy-rich acyl phosphates related to amino acids.

It was later definitely proposed by Lipmann[7] and by Cohen and McGilvery[8] that the potential energy required for peptide bond formation may be supplied by acyl phosphates. A recent publication of Chantrenne[9] suggests that the high-energy

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(1) Swift Amino Acid Fellow, 1947–1949. Present address: Research Department, Merck and Co., Inc., Rahway, N. J.

(2) Borsook and Dubnoff, *J. Biol. Chem.*, **132**, 307 (1940).

(3) Schmidt, "The Chemistry of the Amino Acids and Proteins," C. C. Thomas, Springfield, Ill., 1945. Chapter XV by Borsook and Huffman includes some thermodynamical considerations of peptide synthesis.

(4) Kalckar, *Chem. Revs.*, **28**, 71 (1941).

(5) Green and Colowick, *Ann. Rev. Biochem.*, **13**, 155 (1944). This review discusses many of the processes which involve phosphorus compounds, in addition to methods for their chemical and biological preparation.

(6) Lipmann, *Adv. in Enzymology*, **1**, 99 (1941).

(7) Lipmann, *ibid.*, **6**, 231 (1946).

(8) Cohen and McGilvery, *J. Biol. Chem.*, **166**, 261 (1946); **169**, 199 (1947).

(9) Chantrenne, *Nature*, **160**, 603 (1947).

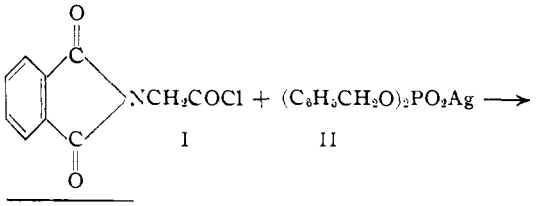
intermediates may be diacyl phosphates or substituted phosphoric acids in which the carboxylic groups of amino acids are "activated" by phosphate groups bound to nucleic acids or to other cell constituents. Chantrenne inferred that simple acyl phosphates do not act as acylating agents and therefore are not likely intermediates in peptide bond synthesis.

Evidence that phosphorylated amino acids are involved in the biogenesis of peptides would be strengthened by the isolation and characterization of the proposed intermediate derivatives, the simplest of which may take the form of a substituted glycyl phosphate. Substances with this type of structure have not been found in nature, although biochemical evidence favors their existence.[10,11,12] No synthesis of a substance of this type has been reported.

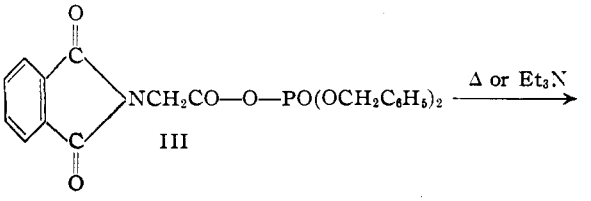
The syntheses of two model phosphorylated glycine derivatives are described in the present work. It has been demonstrated that phthalylglycyl dibenzyl phosphate is an acylating agent, and a method is described by means of which peptide derivatives have been synthesized using this acyl phosphate under simulated physiological conditions.

In this investigation, phthalimidoacetyl (phthalylglycyl) dibenzyl phosphate was synthesized in high yield from phthalylglycyl chloride and silver dibenzyl phosphate. The silver salt[13] was prepared from pure, crystalline dibenzyl phosphate. The latter compound has been prepared by Lossen and Kohler[14] by partial saponification of tribenzyl phosphate, and by Todd and coworkers[15] from dibenzyl phosphite. In order to obtain material of high purity it was found preferable to synthesize barium dibenzyl phosphate[16] by Lynen's procedure followed by interaction with sodium sulfate and subsequent acidification.

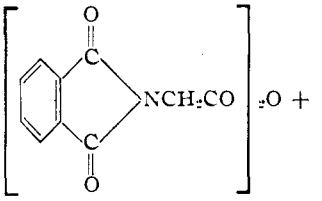
Phthalylglycyl dibenzyl phosphate (III) was synthesized by agitating a suspension of the dry silver salt in a benzene solution of phthalylglycyl chloride at room temperature. The product is a low-melting crystalline substance which readily disproportionated on attempted recrystallization from benzene or in the presence of a small amount of triethylamine to the corresponding anhydrides (IV) and (V).



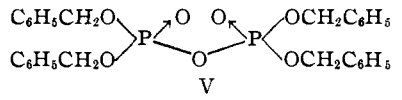
I II



III

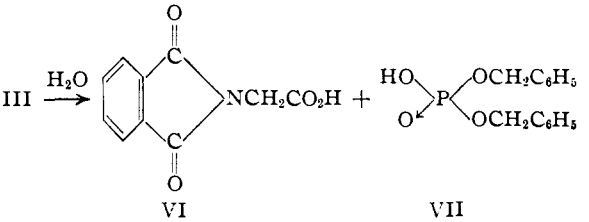


IV

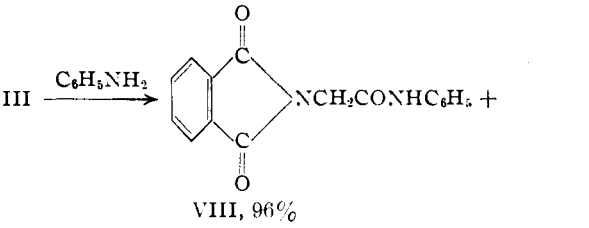


V

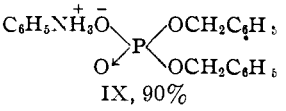
Disproportionation also took place on long standing at room temperature. The mixed anhydride was easily decomposed by aqueous dioxane to the corresponding acids (VI and VII).



VI VII



VIII, 96%



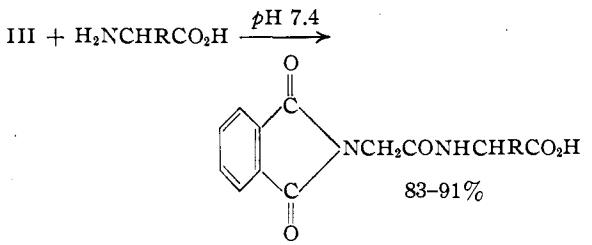
IX, 90%

Reaction of III with an excess of aniline was very rapid and exothermic. Phthalylglycyl anilide (VIII) was obtained in almost the theoretical yield. The anilinium salt of dibenzyl phosphate (IX) was also obtained in high yield, and was converted to the free acid. No evidence for the presence of N-(dibenzylphosphoryl)-aniline (the corresponding phosphorylated product) could be found. A similar reaction was observed with an excess of benzylamine. The N-benzylamide (X) was obtained in excellent yield, and the only by-product isolated was the benzylammonium salt of dibenzyl phosphate (XI). These results demonstrate that the compound was in fact the desired acyl phosphate and not an equimolar mixture of the symmetrical anhydrides. The amides (VIII) and (X) could not be formed in high yields from such a mix-

(10) Steward and Street, *Ann. Rev. Biochem.*, **16**, 495 (1947).
(11) Elliott, *Nature*, **161**, 128 (1948).
(12) Speck, *J. Biol. Chem.*, **179**, 1405 (1949).
(13) Lynen's procedure for this salt may lead to a product contaminated with disilver monobenzyl phosphate.
(14) Lossen and Kohler, *Ann.*, **262**, 211 (1891).
(15) Atherton, Howard and Todd, *J. Chem. Soc.*, 1111 (1948).
(16) Lynen, *Ber.*, **73**, 373 (1940).

ture which would have just half the acylating power of the anhydride (III).

Phthalylglycyl dibenzyl phosphate has also been shown to be capable of acylating glycine and DL-phenylalanine. Reactions were carried out at room temperature by adding a solution of the mixed anhydride in dioxane to a buffered (*p*H 7.4) aqueous solution of the amino acid. Phthalyl dipeptides were obtained in high yield by this procedure. Thus it has been demonstrated that a model acyl phosphate derived from an amino acid does react with amino acids to form true peptide bonds under simulated physiological conditions. Since these same phthalyl dipeptides have been transformed[17] to glycylglycine and glycyl-DL-phenylalanine, the procedure constitutes a synthesis of peptides.



R = H, $C_6H_5CH_2$

Alternate methods for the preparation of phthalylglycyl dibenzyl phosphate were attempted. The reaction of silver phthalylglycinate with dibenzyl chlorophosphonate[15] led to the formation of tarry products from which the desired compound could not be isolated. A quantitative yield of triethylamine hydrochloride was obtained from the interaction of phthalylglycyl chloride with triethylammonium dibenzyl phosphate, but only the symmetrical anhydrides could be separated from the reaction product.

Carbobenzoxyglycyl dibenzyl phosphate has also been prepared from the corresponding acid chloride and silver dibenzyl phosphate in 50% yield. This mixed anhydride gave N-carbobenzoxyglycine-N-benzyl amide when treated with an ethereal solution of benzylamine. The benzyl amide was prepared independently from the acid chloride.

We wish to express our appreciation to Swift and Company for the support of a fellowship for one of us (V.S.F.).

Experimental[18]

Silver Dibenzyl Phosphate (II).—The procedure is a modification of that of Lynen.[16] A solution of barium dibenzyl phosphate (6.92 g., 0.01 mole) in the minimum amount of hot water was added to 50 ml. of a saturated sodium sulfate solution. Barium sulfate was removed by filtration (Celite), and the filtrate was acidified with sulfuric acid. Dibenzyl phosphate crystallized in colorless needles (5.2 g.). Recrystallization from chloroform–petroleum ether (30–60°) afforded 5.08 g. (91%) of the product as clusters of fine needles, m. p. 78–79° (reported[14] m. p. 78–79°). Calcd. for $C_{14}H_{15}O_4P$: neut. equiv., 278. Found: neut. equiv., 277.

A solution of 10.0 g. (0.036 mole) of dibenzyl phosphate in approximately 100 ml. of 50% alcohol containing 1.44 g. (0.036 mole) of sodium hydroxide was added in the dark to a hot, concentrated aqueous solution of 6.1 g. (0.036 mole) of silver nitrate. After storage in the dark for two days, the solution was filtered. The filtrate was concentrated to a volume of about 20 ml., filtered, and the combined residues were washed with water and with alcohol. The colorless crystalline product was pulverized and dried at 75° for twelve hours, and at 110° (0.5 mm.) for six hours. The silver salt (II) was obtained in 90.5% yield (12.5 g.), m. p. 212–216° (dec.) (Lynen[16] reported m. p. 216° (dec.)).

Dibenzyl Phosphite.—The procedure employed is a modification of that of Todd.[19] A solution of 108 g. (1 mole) of benzyl alcohol in 121 g. (1 mole) of dimethylaniline was added dropwise in two and one-half hours to a stirred solution of 68.7 g. (0.5 mole) of phosphorus trichloride in 375 ml. of dry benzene at 0–5°. Stirring was continued for thirty minutes more at 0°, and the mixture was allowed to stand at room temperature overnight. Water (250 ml.) was added to the stirred mixture at 25–28° (ice-bath cooling) and the layers were separated. The benzene layer was washed with 250 ml. of a saturated sodium chloride solution, two 250-ml. portions of dilute ammonium hydroxide (*p*H 8) and two 250-ml. portions of water. The last aqueous layer was chloride-free. The benzene solution was dried over sodium sulfate. Concentration under reduced pressure gave 81.0 g. of a yellow, viscous liquid. The crude product was evaporatively distilled in two portions in a short-path alembic type still. Seventy-five grams (57%) of colorless dibenzyl phosphite was collected at 110–120° (0.5 μ); $n^{25}D$ 1.5520 (Todd, *et al.*,[19] reported $n^{18}D$ 1.5521).

Dibenzyl Benzylaminophosphonate.[19]—Dibenzyl phosphite (1.705 g., 6.5 millimoles) was converted to dibenzyl chlorophosphonate by treatment with gaseous chlorine in carbon tetrachloride solution (10 ml.) at −5 to −10°. Excess chlorine and hydrogen chloride were removed under reduced pressure while the solution was concentrated to a volume of approximately 5 ml. A solution of 0.695 g. (6.5 millimoles) of benzylamine and 0.660 g. (6.5 millimoles) of triethylamine in 10 ml. of carbon tetrachloride was added at −10° with stirring. After standing overnight the mixture was filtered and the residue extracted with 50 ml. of carbon tetrachloride. By concentration of the combined filtrate and extract there was obtained 1.53 g. (64%) of crude material, m. p. 77–79°. Recrystallization from benzene–*n*-hexane gave 1.11 g. (46%) of colorless needles, m. p. 81–83°. A sample was recrystallized to a constant melting point of 83.0–84.2° from ethylene dichloride and hexane (reported m. p. 84–85°).

Anal.[20] Calcd. for $C_{21}H_{22}O_3NP$: C, 68.70; H, 6.03; P, 8.45. Found: C, 67.65; H, 6.06; P, 8.20.

Phthalylglycyl Dibenzyl Phosphate (III).—A solution of 2.73 g. (12 millimoles) of phthalylglycyl chloride (I) in 100 ml. of dry benzene was shaken with 4.65 g. (12 millimoles) of silver dibenzyl phosphate (II) in a 250 ml. glass-stoppered Erlenmeyer flask for four hours at room temperature. A second portion of the silver salt (II) (1.16 g., 25% molar excess) was added and the suspension

(17) Sheehan and Frank, THIS JOURNAL, **71**, 1856 (1949).

(18) All melting points are corrected. We are indebted to Mr. S. M. Nagy and his associates for the microanalyses.

(19) Atherton, Openshaw and Todd, *J. Chem. Soc.*, 382 (1945).

(20) It is well known that the presence of phosphorus in organic compounds interferes with the determination of carbon.[21,22,23] Low carbon values are usually obtained. The difficulty may be overcome by addition of lead oxide, lead chromate, potassium dichromate or copper oxide to the combustion tube and to the sample. In certain instances "these and other subterfuges fail."[23] The values obtained for carbon in this work frequently were low by 1–2% using copper oxide.

(21) Evans and Tilt, *Am. Chem. J.*, **44**, 364 (1910).

(22) Hilpert, *Ber.*, **46**, 951 (1913).

(23) Silbert and Kirner, *Ind. Eng. Chem., Anal. Ed.*, **8**, 353 (1936).

was shaken for two additional hours. Silver chloride and excess silver dibenzyl phosphate were removed by filtration (2.88 g.). This residue was digested with concentrated nitric acid, washed with water and dried. The yield of silver chloride was quantitative (1.72 g.).

The benzene solution was filtered with Celite in order to remove traces of silver salts. The chloride-free filtrate was concentrated to a colorless oil by freeze-drying. Crystallization was induced by seeding with crystalline material (obtained in a previous experiment from a similar colorless oil after keeping it for four days at room temperature). The product, after standing overnight at room temperature, was triturated with 20 ml. of dry ether, with a mixture of 20 ml. of dry ether and 5 ml. of dry benzene, and finally with 20 ml. of ether. Phthalylglycyl dibenzyl phosphate (III) was obtained in the form of small needles in 91% yield (5.11 g.), m. p. 63–65°. A sample of this product was digested with concentrated nitric acid until a clear solution was obtained. Upon addition of hydrochloric acid no silver chloride was precipitated.

Anal. Calcd. for $C_{24}H_{20}O_7NP$: C, 62.15; H, 4.33; N, 3.01; P, 6.69. Found: C, 60.70, 60.40, 61.13; H, 4.38, 4.45, 4.33; N, 3.27; P, 6.53.

Reaction of Silver Phthalylglycinate with Dibenzyl Chlorophosphonate.—Silver phthalylglycinate was prepared in 96.5% yield according to the method of Reese.[24] A suspension of 1.56 g. (5 millimoles) of this salt in 50 ml. of dioxane was shaken with a dioxane solution of dibenzyl chlorophosphonate prepared from 1.31 g. (5 millimoles) of dibenzyl phosphite as described above. After six hours a dark brown mixture was obtained, and a silver mirror had deposited on the sides of the flask. Filtration with Celite gave a brown solution which could not be clarified with Darco. Concentration under reduced pressure afforded a viscous, brown tar from which the desired compound (III) could not be obtained.

Reaction of Phthalylglycyl Chloride with Triethylammonium Dibenzyl Phosphate.—A solution of 2.24 g. (0.01 mole) of the acid chloride (I) in 25 ml. of benzene was added dropwise to an agitated solution of 2.78 g. (0.01 mole) of dibenzyl phosphate and 1.01 g. (0.01 mole) of triethylamine in 25 ml. of benzene. A colorless precipitate formed instantly. The mixture was warmed at 50° for five minutes and then allowed to stand at room temperature for two hours. The precipitate was removed by filtration and dried. This material (2.18 g.) was digested with ice-water in order to dissolve triethylamine hydrochloride, and excess aqueous silver nitrate was added. The silver chloride was obtained in quantitative yield (1.42 g.).

Concentration of the benzene solution by freeze-drying gave a light yellow oil. The oil was converted to a pasty solid by trituration with dry petroleum ether. This product was extracted with chloroform at room temperature. The colorless residue (m. p. 229–236°) was recrystallized from nitrobenzene, forming platelets, m. p. 240–241°, identified as phthalylglycine anhydride (IV) by mixed melting point determination. The yield was 1.09 g. (58%).

Tetrabenzyl pyrophosphate (V) was obtained as an oil from the chloroform extract by addition of petroleum ether. Upon recrystallization from ether-cyclohexane 1.91 g. (71%) of colorless needles was obtained, m. p. 59–61°. The melting point was not depressed when mixed with an authentic sample.[25]

Reaction of III with Triethylamine.—Two drops of triethylamine were added to a solution of 0.930 g. (0.002 mole) of III in 25 ml. of benzene. A precipitate formed immediately. This was filtered and washed with ether, yielding 0.333 g., m. p. 235–238°. Recrystallization from nitrobenzene afforded 0.321 g. (85%) of phthalylglycine anhydride, m. p. 240–241°. Evaporation of the combined filtrate and ether wash gave tetrabenzyl pyrophosphate as a light yellow oil. Crystallization from ether-petroleum ether yielded 0.420 g. (78%) in the form of colorless needles, m. p. 59.5–61.0°.

The attempted preparation of III from silver dibenzyl phosphate and phthalylglycyl chloride in refluxing dioxane also led to the formation of the symmetrical anhydrides. Disproportionation also took place when the acyl phosphate (III) was heated in benzene and when a benzene solution of III was allowed to stand at room temperature for ten days.

Reaction of III with Aniline.—A solution of 0.930 g. (0.002 mole) of III in 10 ml. of dry dioxane was added to a solution of 0.372 g. (0.004 mole) of aniline in 15 ml. of benzene. The reaction was exothermic. Phthalylglycine anilide (VIII) crystallized as long needles (0.54 g.). Recrystallization from alcohol afforded 0.51 g. (91%), m. p. 230.5–231.5°. When mixed with an authentic sample of phthalylglycyl anilide there was no depression in melting point.

Upon addition of petroleum ether to the mother liquor, 0.713 g. of crude anilinium dibenzyl phosphate (IX) was obtained. This material was recrystallized from chloroform–petroleum ether, giving 0.695 g. (90.3%) of colorless needles, m. p. 115–116°. Treatment with 10% sodium hydroxide followed by acidification with hydrochloric acid yielded 0.49 g. (88%) of dibenzyl phosphate, m. p. 76–78°. Purification was effected by recrystallization from chloroform–petroleum ether (m. p. 78–79°) and the product was identified by a mixed melting point determination.

Reaction of III with Benzylamine.—A solution of 0.930 g. (0.002 mole) of III in 10 ml. of dioxane was added to 0.428 g. (0.004 mole) of benzylamine in 15 ml. of benzene. A vigorous reaction ensued and the solution was allowed to cool to room temperature. The benzyl amide (X) crystallized as long, colorless needles. Recrystallization from alcohol gave 0.51 g. (87%), m. p. 216.0–217.5°. The same amide was also synthesized from phthalylglycyl chloride (2.96 g., 13.3 millimoles) and excess benzylamine (3.0 g.). The crude product (3.67 g., m. p. 215–217°) was recrystallized from alcohol, yielding 3.58 g. (91.7%) of colorless needles, m. p. 216–218°. The melting point was unchanged on recrystallization from alcohol.

Anal. Calcd. for $C_{17}H_{14}O_3N_2$: C, 69.39; H, 4.79; N, 9.54. Found: C, 69.17; H, 4.60; N, 9.67.

Benzylammonium dibenzyl phosphate was obtained from the mother liquor and was converted to dibenzyl phosphate in the manner described above. The yield was 0.465 g. (93.8%), m. p. 77–79°.

Reaction of III with Glycine.—The mixed anhydride (III) (1.09 g., 2.34 millimoles) in 10 ml. of dioxane was added to a solution of 0.353 g. (4.7 millimoles) of glycine in 10 ml. of a boric acid–borax buffer (prepared by adding 0.05 *M* borax to 0.2 *M* boric acid to *p*H 7.4). About 5 ml. of dioxane was added to make a clear solution. After standing overnight at room temperature the mixture was concentrated under reduced pressure. The residue was recrystallized from alcohol giving 0.51 g. (83%) of crude phthalylglycylglycine, m. p. 180–190°. Recrystallization from alcohol yielded 0.478 g. (78%), m. p. 229–231°. The melting point did not depress when mixed with an authentic sample. An additional crop obtained from the mother liquor was recrystallized from alcohol, yielding 0.08 g. (13%), m. p. 227–230°.

Reaction of III with DL-Phenylalanine.—The acylation was carried out as described above, using 0.930 g. (0.002 mole) of III and 0.66 g. (0.004 mole) of DL-phenylalanine. The crude product (0.585 g., 83%) was recrystallized from isoamyl alcohol, giving colorless needles, m. p. 197–198°. This material was identical with phthalylglycyl-DL-phenylalanine (mixed melting point determination).

N-Carbobenzoxyglycyl Dibenzyl Phosphate.—A solution of 0.622 g. (0.0027 mole) of N-carbobenzoxyglycyl chloride in 35 ml. of dry ether was shaken with 1.05 g. (0.0027 mole) of pulverized silver dibenzyl phosphate at 0° for thirty minutes. The mixture, after standing overnight at 4°, was filtered to remove silver salts (0.605 g.) and the filtrate was evaporated under reduced pressure at

(24) Reese, *Ann.*, **242**, 1 (1881).

(25) Prepared by the method of Todd, *et al.*, *J. Chem. Soc.*, 674 (1947).

−10 to 0°. The oily residue was washed several times by centrifugation with the cold (0°), dry *n*-pentane. The halogen-free product (0.640 g., 50%) was obtained in clusters of colorless needles, m. p. 76.5–77.5°.

Anal.[26] Calcd. for $C_{24}H_{24}O_7NP$: C, 61.40; H, 5.15; P, 6.62. Found: C, 59.43; H, 5.37; P, 6.79.

N-Carbobenzoxyglycine-N-benzyl amide was prepared by adding a few drops of benzylamine to a solution of approximately 10 mg. of N-carbobenzoxyglycyl dibenzyl phosphate in 10 ml. of ether. Colorless rosettes were obtained, m. p. 115.5–116.8°. Recrystallization from ether gave the pure amide, m. p. 115.8–116.8°.

Anal. Calcd. for $C_{17}H_{18}O_3N_2$: C, 68.43; H, 6.08; N, 9.39. Found: C, 68.70; H, 6.37; N, 9.17.

The same amide was prepared from an ethereal solution of the acid chloride and an excess of benzylamine. The product crystallized from ether in fine needles, m. p. 115.8–116.6°. A mixture with the amide obtained from N-carbobenzoxyglycyl dibenzyl phosphate showed no depression in melting point.

(26) The sample was mixed with cupric oxide before combustion. In the absence of cupric oxide the values found were: C, 46.99; H, 5.10.

Summary

Syntheses of two model high-energy phosphorus compounds derived from glycine are described. Both phthalylglycyl dibenzyl phosphate and N-carbobenzoxyglycyl dibenzyl phosphate have the properties of acylating agents. In addition, it has been demonstrated that phthalylglycyl dibenzyl phosphate reacts with glycine and with DL-phenylalanine to form phthalyl peptides under simulated physiological conditions. These reactions represent the first *in vitro* synthesis of peptide bonds employing amino acyl phosphate derivatives.

CAMBRIDGE 39, MASSACHUSETTS RECEIVED AUGUST 31, 1949

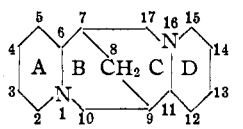
[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

The Total Synthesis of Sparteine and an Isosparteine by Reductive Cyclization[1]

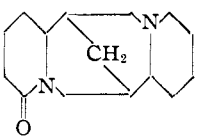
BY NELSON J. LEONARD AND ROGER E. BEYLER[2]

The total synthesis of *dl*-sparteine (I)[3] and the resolution of racemic sparteine[4] have been announced from this Laboratory. It is the purpose of this paper to disclose the details of the synthesis and resolution of sparteine, to report the synthesis of an isosparteine, and to discuss the stereochemistry of these $C_{15}H_{26}N_2$ compounds.

The reduction of *dl*-lupanine (II) (an alkaloid found in the racemic form in *Lupinus albus*,[5a] *Lupinus termis*[5b] *Podalyria buxifolia*,[5c] *Podalyria sericea*[5c] and *Virgilia capensis*[5d]) to "deoxylupanine" (later shown to be *dl*-sparteine (I), an alkaloid occurring in the racemic form in *Cytisus proliferus*,[5e]) was reported in 1928 by Clemo and Leitch.[5b] At that time the structure of both alkaloids was unknown, and because *l*-sparteine,[6] the form of I most abundantly available in nature,[7] could not be racemized and "deoxylupanine" was not resolved, the relation between "deoxylupanine" and *l*-sparteine was unclear. Clemo, Raper and Tenniswood[8] later succeeded in resolving *dl*-lupanine and in reducing *d*- and *l*-lupanine to *l*- and *d*-sparteine, respectively. Their work established the identity of "deoxylupanine" with *dl*-sparteine and also constituted the synthesis of sparteine from an alkaloid source.

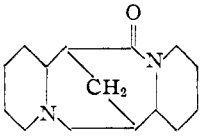


I

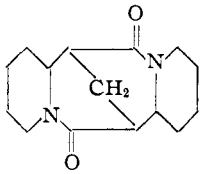


II

In an approach toward the total synthesis of sparteine, Clemo, Morgan and Raper[9] prepared *dl*-oxosparteine (III)[10] by a multi-step procedure starting with ethyl 2-pyridylacetate. Although the carbonyl group in *dl*-oxosparteine (III) is structurally similar to that in *dl*-lupanine (II), reduction of III to I could not be accomplished with reagents available at that time. However, the synthesis of *dl*-oxosparteine served to establish the identity of III with the alkaline ferricyanide oxidation product of I. Since the appearance of our first communication,[3] Clemo, Raper and Short have reported a successful reduction of



III



IV

(1) This investigation was supported in part by a grant from the Research Board of the University of Illinois.

(2) Present address: Merck and Co., Inc., Rahway, New Jersey.

(3) Leonard and Beyler, THIS JOURNAL, **70**, 2298 (1948).

(4) Leonard and Beyler, *ibid.*, **71**, 737 (1949).

(5) (a) Schmidt, *Arch. Pharm.*, **235**, 192 (1897); (b) Clemo and Leitch, *J. Chem. Soc.*, 1811 (1928); (c) White, *New Zealand J. Sci. Tech.*, **25B**, 137 (1944); (d) White, *ibid.*, **27B**, 478 (1946); (e) White, *ibid.*, **25B**, 103 (1943).

(6) The use in this paper of *l* to indicate negative rotation and *d* to indicate positive rotation is consistent with the usage of previous workers in this field.

(7) *l*-Sparteine has been found in *Cytisus scoparius*, *C. ratisbonensis*, *C. proliferus*, *Genista aetnensis*, *Lupinus barbiger*, *L. laxus*, *L. luteus*, *L. niger*, *Retama sphaerocarpa*, *Spartium junceum* and *Chelidonium majus* (Henry, "The Plant Alkaloids," 4th edition J. and A. Churchill, Ltd., London, England, 1949, pp. 116–119).

(8) Clemo, Raper and Tenniswood, *J. Chem. Soc.*, 429 (1931).

(9) Clemo, Morgan and Raper, *ibid.*, 1025 (1936).

(10) The nomenclature used by Clemo and others is "oxysparteine," which the authors consider misleading.