perature of the reaction mixture dropped to 15°. The mixture was stirred for one-half hour and heated to the reflux temperature for three hours. It was filtered under nitrogen and the filtrate was fractionated through a 12-inch Vigreux column. The colorless product boiled at 157–158° at 0.55 mm. and weighed 86 g. (73%).

Anal. Calcd. for $C_{12}H_{10}ClOP$: Cl, 14.98; P, 13.08. Found: Cl, 14.88; P, 12.58.

(c) Diphenylphosphinic acid (109 g., 0.5 mole) was dissolved in 154 g. of phosphoric trichloride. Over a period of 1.5 hours 104 g. (0.5 mole) of phosphorus pentachloride was added. The temperature of the reaction mixture rose to 45°. The mixture was heated to 70–80° for three hours and fractionated through a 12-inch Vigreux column. The product boiled at 135–136° at 0.07 mm. and weighed 90 g. (76%).

Anal. Calcd. for $C_{12}H_{10}ClOP$: Cl, 14.98; P, 13.08. Found: Cl, 15.20; P, 12.72.

Diphenylphosphinic Acid.—(a) Both diphenylphosphinic chloride and diphenylphosphoranetrioic trichloride reacted very rapidly with water (or water vapor) to give quantitative yields of the acid. It was recrystallized from ethanol to form white needles, m.p. 190–192°.

(b) Diphenylphosphinodithioic acid, diphenylphosphinothioic chloride and diphenylphosphinothioic acid all were oxidized in benzene solution with 6 *N* nitric acid to form diphenylphosphinic acid which, after recrystallization from ethanol, melted at 190–192°.

(c) Steam was passed through 550 g. (2.2 moles) of diphenylphosphinodithioic acid at 125° for three hours. Analysis indicated that no reaction had occurred. Steam blowing was continued using preheated steam at 170° for 1.5 hours. Again there was practically no reaction. The temperature was raised to 210° and steam blowing was continued for 2.5 hours. As the reaction mass cooled, it solidified. The solid was broken up, filtered, washed with water and dried. This crude product weighed 400 g. (83%).

Anal. Calcd. for $C_{12}H_{11}O_2P$: P, 14.2; neut. equiv., 218. Found: P, 14.0; S, 0.54; neut. equiv., 207.

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CLEVELAND 17, OHIO

[CONTRIBUTION FROM THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH]

Synthesis of *N*-Phosphorylated Derivatives of Amino Acids[1]

BY SI-OH LI[2] AND ROBERT E. EAKIN

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The preparation of *N*-phosphorylated amino acids and their derivatives has been carried out in order to study their physical, chemical and biological properties and their possible involvement as intermediates in the biosynthesis of proteins. Such compounds were prepared by hydrogenolysis of appropriate *N*-dibenzylphosphoryl derivatives of amino acid esters and amino acid amides. The *N*-dibenzylphosphoryl amino acid esters and amides were prepared by the action of dibenzylphosphoryl chloride upon the corresponding compound in the presence of triethylamine. *N*-Phosphorylglycine and *N*-phosphoryl-DL-phenylalanine were obtained in crystalline form by hydrogenolysis of the corresponding *N*-dibenzylphosphoryl amino acid benzyl esters. The action of dibenzylphosphoryl chloride upon other functional groups of amino acids has been demonstrated.

When this investigation was undertaken phosphorylated amino acids had been suggested as the activated intermediates required for peptide syntheses.[3,4] These compounds would be analogous to the phosphorylated sugars and fatty acid intermediates in carbohydrate and lipid biogenesis, and a demonstration of their participation in peptide synthesis would indicate a mechanism for the utilization of the energy of adenosine triphosphate in the formation of amide bonds. The reactions of amino acids phosphorylated chemically at the carboxyl group had been investigated,[5] but little information was available on compounds in which the α-amino groups of amino acids are phosphorylated. Hence the preparation of such compounds was undertaken in order that a study could be made of their chemical, physical and biological properties.

The direct phosphorylation of α-amino acids with phosphorus oxychloride has been reported previously on two occasions,[6,7] but the yields obtained were low, and well characterized compounds were not isolated.

The phosphorylation of α-amino acids by diaryl- and dialkylphosphoryl halides has been attempted in two laboratories. In the first case[8] diphenylphosphoryl chloride was used, but the products obtained were the diphenyl phosphoric acid salts of the amino acids rather than the desired diphenyl phosphoramides. In the second instance[9] diisopropylphosphoryl fluoride was tried, but without success.

The phosphorylation of α-amino acid esters by dialkyl- and diarylphosphoryl halides has been accomplished and these products can serve as intermediates for the preparation of *N*-phosphoryl amino acids providing methods can be devised for the cleavage of both the phosphoryl and carboxyl esters. Amino acid esters have been phosphorylated with diphenylphosphoryl chloride[10] and with dialkylphosphoryl chlorides.[9,11] In both cases the reactions took place without difficulty and well characterized products were obtained. By hydrogenolysis of *N*-diethyl- and *N*-diisopropylphosphorylglycine benzyl esters, Wagner-Jauregg, *et al.*,[11] obtained the corresponding *N*-dialkylphosphoryl-

(1) This communication is from part of a dissertation submitted to the Graduate School of the University of Texas in partial fulfillment of requirements for the Ph.D. degree, May, 1954.

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(3) F. Lipmann, *Advances in Enzymol.*, **1**, 99 (1941); *Harvey Lecture*, **44**, 119 (1948); *Federation Proc.*, **8**, 597 (1949).

(4) P. P. Cohen and R. W. McGilvery, *J. Biol. Chem.*, **166**, 261 (1946); **169**, 119 (1947); **171**, 121 (1947).

(5) H. Chantrenne, *Nature*, **160**, 603 (1947); **164**, 576 (1949); *Biochem. Biophys. Acta*, **2**, 286 (1948); **4**, 484 (1950).

(6) C. Neuberg and W. Oertel, *Biochem. Z.*, **60**, 491 (1914).

(7) T. Winnick and E. M. Scott, *Arch. Biochem.*, **12**, 201 (1947).

(8) A. Bernton, *Ber.*, **55**, 3361 (1922).

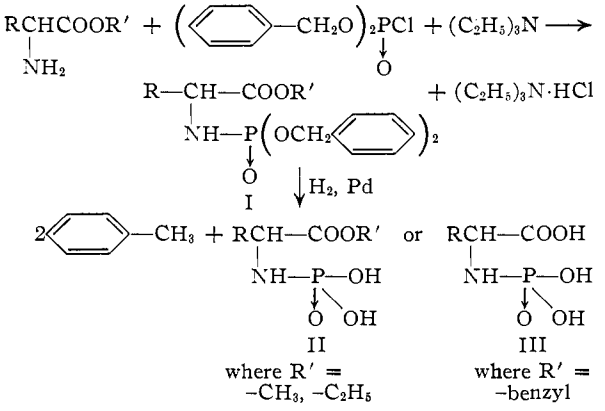
(9) T. Wagner-Jauregg, J. J. O'Neill and W. H. Summerson, THIS JOURNAL, **73**, 5202 (1951).

(10) L. T. Sciarini and J. S. Fruton, *ibid.*, **71**, 2940 (1949).

(11) T. Lies, R. E. Plapinger and T. Wagner-Jauregg, *ibid.*, **75**, 5755 (1953).

glycine, but they did not remove the alkyl groups from the phosphoryl group. Sciarini and Fruton[10] cleaved the phenyl groups by hydrogenolysis from the phosphoryl group of *N*-diphenylphosphoryl-L-glutamic acid diethyl ester and obtained a product whose elementary composition approximated the theory for the di-sodium salt of *N*-phosphoryl-L-glutamic acid diethyl ester, but the product was not further characterized. These investigators reported undertaking use of dibenzylphosphoryl chloride upon benzyl esters of amino acids but at the time of reporting had not obtained any crystalline products.

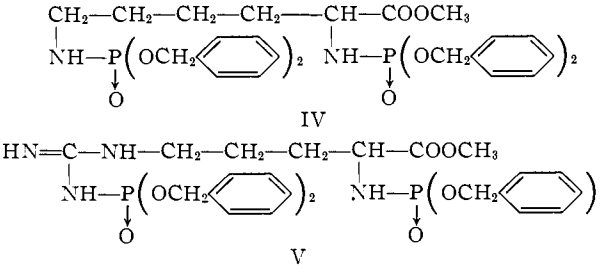
In the investigations reported here, a general method was developed which is based upon the reaction in organic solvents of dibenzylphosphoryl chloride (DBPCl) with an amine and the subsequent removal of the benzyl groups on the phosphoryl group by hydrogenolysis[12,13] to give the compounds shown in the equations



Synthesis of *N*-Phosphoryl Amino Acid Esters.—Two general methods have been used for the phosphorylation of amino acid esters: (a) Liberation of an amino acid ester from its hydrochloride salt with ammonia and phosphorylation of the free ester with DBPCl in the presence of triethylamine.[14] However, this method cannot be applied to amino acid benzyl esters since no liberation of the free base occurs upon the addition of ammonia to the suspension of the salts in chloroform. (b) Phosphorylation of the hydrochloride salt of the amino acid esters by the direct reaction with DBPCl in the presence of *two* moles of triethylamine.[14] The yields by this method are slightly lower than those obtained in method a, but it is much more convenient and can be used for the phosphorylation of amino acid benzyl esters.

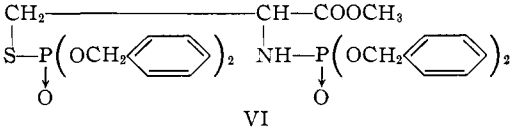
By these methods the phosphorylations of about sixteen amino acid esters have been carried out. The compounds formed are soluble in organic solvents but insoluble in water.

In the course of study of the phosphorylation of amino acid esters, it was found that DBPCl not only reacts with the α-amino group but also with other functional groups present in the amino acid molecules. On the basis of elementary analysis the product isolated from the reaction of DBPCl with lysine methyl ester presumably has the structure IV, and the product formed from arginine methyl



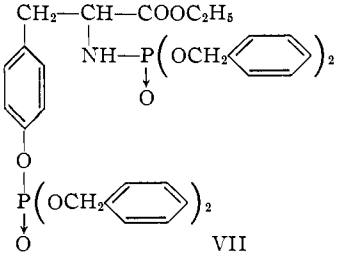
ester gives a negative Sakaguchi test[15] for arginine's guanido group indicating structure V, in which both the guanido group and the α-amino group have reacted.

The α-amino group of cysteine methyl ester can be phosphorylated with diisopropylphosphoryl chloride without the reagent's attacking the thiol group.[16] With phosphorus oxychloride and cysteine, conditions can be used whereby the thiol group is more reactive than the amino group and from the phosphorylated mixture S-phosphorylcysteine can be obtained.[17] However, the reaction product of cysteine methyl ester with DBPCl gave a negative sulfhydryl group test with alkaline sodium nitroprusside and an analysis of nitrogen and phosphorus corresponding to approximately that of structure VI, in which both the sulfhydryl group and the α-amino group have been phosphorylated.



When two equivalents of DBPCl react with cystine methyl ester both α-amino groups are phosphorylated.

When tyrosine ethyl ester was allowed to react with a single mole of DBPCl, the isolated product was phosphorylated only on the amino group and gave a positive Millon test for phenol; with 2 moles of DBPCl, however, the reaction product gives a negative phenol test and an approximate analysis of nitrogen and phosphorus corresponding to that for the structure VII in which both the phenolic group and the α-amino group have been phosphorylated.



The behavior of DBPCl with hydroxy amino acid esters is similar to that reported for diisopropyl-

(12) F. A. Atherton, H. T. Openshaw and A. R. Todd, *J. Chem. Soc.*, 382 (1945).

(13) S. O. Li, *Acta Chem. Scand.*, **4**, 610 (1950).

(14) S. O. Li, THIS JOURNAL, **74**, 5959 (1952).

(15) F. C. Koch, "Practical Methods in Biochemistry," William Wood and Co., Baltimore, Md., 1934, pp. 46–47.

(16) R. E. Plapinger and T. Wagner-Jauregg, THIS JOURNAL, **75**, 5757 (1953).

(17) F. Binkley, *J. Biol. Chem.*, **195**, 283 (1952).

phosphoryl chloride in that it too is inactive toward the hydroxyl groups of the methyl esters of serine, threonine, and phenylserine, even though the reagent (DBPCl) has been reported to react with alcohols.[18]

To convert these dibenzylphosphoryl compounds to the corresponding derivatives in which the phosphoramide group is not esterified, catalytic hydrogenolysis was carried out by conventional procedures. However, only *N*-phosphoryl amino acid methyl esters of DL-phenylalanine and DL-tryptophan have been isolated in crystalline form.[14] The hydrogenolysis products from all the other dibenzylphosphoryl amino acid methyl esters came out as glassy, transparent, viscous liquids. All attempts to isolate products in crystalline form either as the free phosphoramides or as sodium or barium salts have been unsuccessful.

Synthesis of *N*-Phosphoryl Amino Acids.—The use of benzyl esters of amino acids in the preparation of *N*-phosphoryl amino acids seemed the preferred method of synthesis in that it eliminates the necessity of saponifying the carboxyl ester because the conditions for the hydrogenolysis of the dibenzylphosphoryl ester groups should also cause the simultaneous cleavage of the carboxybenzyl ester. By phosphorylation of amino acid benzyl esters the *N*-dibenzylphosphoryl derivatives of DL-phenylalanine benzyl ester, L-tyrosine benzyl ester and L-glutamic acid dibenzyl ester were obtained in crystalline form (Table I); the same derivatives of DL-alanine and DL-serine were viscous oils, and the glycine derivative formed a soft waxy solid after standing at room temperature for several months. These compounds have properties very similar to those of the corresponding (carboxy) methyl and ethyl esters.

The catalytic hydrogenolysis of these *N*-dibenzylphosphoryl amino acid benzyl esters was carried out in the usual manner; however, except in the case of the phenylalanine and glycine derivatives the products isolated do not have compositions consistent with those required for the *N*-phosphoryl amino acids.

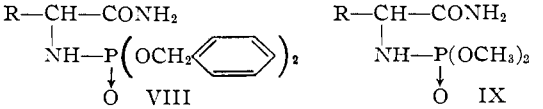
The products obtained from the hydrogenolysis of *N*-dibenzylphosphoryl-L-glutamic acid dibenzyl ester was only slightly soluble in water. Titration with alkali gave curves showing only two breaks: one at *p*H 3.3, the other at *p*H 5.5. The compound has no glutamic acid activity when tested with a glutamic acid requiring microörganism, *Lactobacillus arabinosus*, although *N*-phosphorylphenylalanine can serve as a source of phenylalanine for this same organism. These findings taken with the carbon and hydrogen analyses would favor a structure corresponding to *N*-dibenzylphosphoryl-L-glutamic acid rather than the expected *N*-phosphoryl-L-glutamic acid. However, an acid hydrolysate of the isolated compound upon chromatographic analysis gives a ninhydrin spot whose R_f value is different from that of glutamic acid. Further work to characterize the product has not yet been completed.

In the case of *N*-dibenzylphosphoryl-L-tyrosine benzyl ester, there was no hydrogen up-take during the catalytic hydrogenolysis, hence reduction with sodium in liquid ammonia was tried. In the course of this reaction the theoretical amount of sodium reacted, yet no *N*-phosphoryltyrosine could be obtained. The only isolatable compound had a very low percentage of carbon and hydrogen and gave no reaction when tested with ninhydrin.

From these results it seems that the debenzylation by hydrogenolysis of *N*-dibenzylphosphoryl amino acid benzyl esters may not offer a satisfactory general method for the preparation of *N*-phosphoryl amino acids. Probably the hydrogenolysis procedure causes decomposition of the P–N bond of the desired reaction product and results in the formation of other compounds due to secondary reactions. The difficulties and unsatisfactory results encountered also have been experienced by other investigators. For example Friedman, *et al.*,[19] reported that they have not been able to isolate the desired product from hydrogenolysis of dibenzylphosphoryl derivatives of bis-β-chloroethylamine, and Kircherov[20] reported that attempted removal of the ester groups of the ester amides of the type $(PhO)_2PONHR$ by hydrogenation led to cleavage of the P–N bond and the desired phosphoramides could not be obtained.

Attempted Synthesis of *N*-Phosphoryl Amino Acid Amides.—The preparation of *N*-(amino)-phosphoryl amino acid (carbox) amides, if accomplished, would be advantageous for several reasons: (1) they likely would be more easily crystallized and purified than the corresponding compounds having a free carboxyl group; and (2) the *N*-phosphoryl amino acids might be prepared enzymatically from the amides by the use of purified peptidases which would cleave the carboxamide but leave intact the phosphoramide linkage.

In the attempted synthesis of these phosphorylated amino acid amides, procedures similar to those used in preparing the corresponding esters were employed. So far the *N*-dibenzylphosphoryl amino acid amides (VIII) of glycine and DL-alanine have been isolated (Table II). These compounds are moderately soluble in water, soluble in methanol, ethanol and chloroform, slightly soluble in carbon tetrachloride and ether, and insoluble in petroleum ether. Hydrogenolysis of these compounds gives water-soluble and hygroscopic residues which have as yet neither been crystallized nor converted into well characterized barium or morpholine salts.



In an attempt to convert *N*-dibenzylphosphoryl amino acid esters into their corresponding amides by treating the compounds with methanol saturated with ammonia, three new compounds have been obtained, which, on the basis of elementary analyses, very likely have the structures represented by IX. Probably the original carboxylic acid esters have been transformed by ammonolysis to the cor-

(18) A. R. Todd, *Bull. soc. chim. France*, 933 (1948).

(19) O. M. Friedman, D. L. Klass and A. M. Seligman, THIS JOURNAL, **76**, 916 (1954).

(20) V. F. Kircherov, *Zhur. Obshchei Khim.* (*J. Gen. Chem.*), **19**, 126 (1949) [*C. A.*, **43**, 6178 (1949)].

TABLE I

N-DIBENZYLPHOSPHORYL AMINO ACID ESTERS

Products, *N*-Dibenzylphosphoryl	R and R′ in I R	R′	Yield, %	M.p., °C.	Formula	Nitrogen, % Calcd.	Found	Phosphorus, % Calcd.	Found	Carbon, % Calcd.	Found	Hydrogen, % Calcd.	Found
DL-Serine methyl ester[a]	$-CH_3O$	$-CH_3$	54		$C_{18}H_{22}O_6NP$	3.69	3.48			56.9	56.4	5.82	6.14
DL-Threonine methyl ester	$-C_2H_4O$	$-CH_3$	51.7	52–54[b] (waxy)	$C_{19}H_{24}O_6NP$	3.56	3.42	7.89	7.91				
DL-Phenylserine methyl ester	$-C_7H_7O$	$-CH_3$	51	115–116 dec.	$C_{24}H_{26}O_6NP$	3.07	2.76	6.82	6.83				
DL-Valine methyl ester	$-C_4H_7$	$-CH_3$	78	39–41[b] (waxy)	$C_{20}H_{26}O_4NP$	3.58	3.47	7.93	7.84				
DL-Leucine methyl ester	$-C_4H_9$	$-CH_3$	75	45–46	$C_{21}H_{28}O_4NP$	3.45	3.92	7.64	7.62				
L-Glutamic acid dimethyl ester[c]	$-C_4H_7O_2$	$-CH_3$	87[d]		$C_{21}H_{26}O_7NP$	3.22	3.05						
DL-Phenylalanine benzyl ester	$-C_7H_7$	$-C_7H_7$	70.7	67–69	$C_{30}H_{30}O_4NP$	2.72	2.79	6.02	6.01	69.8	69.11 69.09	5.83	5.93 5.89
L-Tyrosine benzyl ester	$-C_7H_7O$	$-C_7H_7$	77.5	54–55	$C_{30}H_{30}O_6NP$	2.63	2.62	5.83	5.82	67.7	66.19	5.64	5.80
Glycine benzyl ester[e]	$-H$	$-C_7H_7$	72[d]		$C_{23}H_{24}O_4NP$	3.29	3.28	7.30	7.47				
DL-Alanine benzyl ester[f]	$-CH_3$	$-C_7H_7$	75.5[d]		$C_{24}H_{26}O_4NP$	3.18	3.02	7.03	7.05				
L-Glutamic acid dibenzyl ester	$-C_{10}H_{11}O_2$	$-C_7H_7$	71	45–47[b] (waxy)	$C_{33}H_{34}O_7NP$	2.38	2.27	5.29	5.14				

[a] Obtained as a viscous sirup. [b] Taken on a Fisher–Johns block and uncorrected. [c] Obtained as a viscous oil. [d] Calculated from the up-take of hydrogen in hydrogenolysis. [e] Obtained as a sirup which crystallized out in waxy form when standing at room temperature for several months, m.p. 143–144° (Fisher–Johns block and uncorrected). [f] Obtained as a viscous oil, which could not be crystallized.

responding carboxamides (as was desired), but at the same time a transesterification of the two phosphoryl ester groups has occurred, the dimethylphosphoryl esters being formed from the original dibenzylphosphoryl esters. Compounds presumed to be *N*-dimethylphosphoryl amino acid amides of DL-alanine, DL-phenylalanine and L-glutamic acid have been isolated (Table II). These compounds are water-soluble and hygroscopic. Titration curves (sodium hydroxide) show no free phosphoryl or carboxyl groups present in the molecules.

Experimental[21]

Preparation of Dibenzyl Hydrogen Phosphite.—Of the two procedures tried (Atherton, *et al.*,[12] Sheehan and Frank[23]) the former gave better yields and was followed in its essential features. A mixture of 121 g. of dimethylaniline and 108 g. of benzyl alcohol was added slowly with stirring and cooling to 74 g. of phosphorus trichloride dissolved in approximately 300 ml. of benzene. After the addition (taking approximately 40 minutes), 54 g. of benzyl alcohol was added over a 10-minute period. The following day, 200 ml. of water was added to dissolve the precipitate and the benzene layer was separated and washed successively with 200 ml. of water, twice with 200 ml. of 5 *N* ammonium hydroxide and finally with 200 ml. of water. The benzene solution was dried with anhydrous sodium sulfate and the solvent and benzyl chloride removed *in vacuo;* and the crude product (96 g.), which crystallized in white needles when kept in the refrigerator, distilled at 10^{-4} mm. 76.6 g. was collected as colorless viscous oil in the range 120–130°. The crude product was, however, found to be pure enough for use directly in the synthesis of dibenzylphosphoryl chloride, so the preparation usually was not vacuum distilled.

Preparation of Dibenzylphosphoryl Chloride (DBPCl).—DBPCl was always prepared at the time it was used from dibenzyl hydrogen phosphite and sulfuryl chloride according to the published procedure.[24]

***N*-Dibenzylphosphoryl Amino Acid Methyl Esters.**—The compounds were prepared from DBPCl and the appropriate amino acid ester by a procedure described previously.[14] Their analyses are reported in Table I.

***N*-Dibenzylphosphoryl Derivatives of L-Lysine and L-Arginine Methyl Ester.**—By procedure a, a lysine derivative was obtained from the reaction of equal molar amounts of DBPCl and the amino acid methyl ester hydrochloride. It is a yellow viscous sirup, which solidifies when kept in the refrigerator. The analysis of this compound indicates that even though only one equivalent of DBPCl was used the product isolated was the one in which both the ϵ-amino group and the α-amino group have been phosphorylated.

Anal. Calcd. for $C_{35}H_{44}O_8N_2P_2$ (IV): N, 4.03; P, 8.89. Found: N, 4.18; P, 8.98.

The reaction product of equal molar amounts of DBPCl and arginine methyl ester hydrochloride was obtained as a white crystalline compound, m.p. 91–93°, that gave a negative response to Sakaguchi reagent for guanido compounds.

Anal. Calcd. for $C_{35}H_{42}O_8N_4P_2$ (V): N, 8.22; P, 9.1; C, 61.6; H, 6.20. Found: N, 8.78 (Dumas); P, 9.0; C, 60.06; H, 6.32.

***N*-Dibenzylphosphoryl Derivatives of L-Cysteine and L-Cystine Methyl Esters.**—The product from the reaction of equimolar amounts of DBPCl and cysteine methyl ester hydrochloride was obtained as a yellow sirup which could not be induced to crystallize. The product was soluble in ether, benzene, chloroform and carbon tetrachloride, insoluble in petroleum ether and water. It did not solidify when refrigerated for several days. This product gave a negative sulfhydryl test with alkaline sodium nitroprusside.

Anal. Calcd. for $C_{18}H_{22}O_5NPS$ (I): N, 3.54; P, 7.86. Calcd. for $C_{32}H_{36}O_8NP_2S$ (VI): N, 2.14; P, 9.47. Found: N, 2.59; P, 9.93

The cystine derivative was obtained as a white crystalline substance from the reaction of 3 g. of the amino acid methyl ester hydrochloride and 2 equivalents of DBPCl; yield 5.1 g. (73%). In a capillary tube the compound began to melt at 96–97° and was completely melted at 100° forming a clear liquid.

Anal. Calcd. for $C_{36}H_{42}O_{10}N_2P_2S_2$: N, 3.58; P, 7.87. Found: N, 3.56; P, 7.62.

Reaction of DBPCl with L-Tyrosine Ethyl Ester.—The product obtained by procedure a from 5 g. of L-tyrosine ethyl ester (commercial product) and one equivalent of DBPCl was a white crystalline compound melting at 104–105°; yield 8.2 g. (73%).

Anal. Calcd. for $C_{25}H_{28}O_6NP$ (I): N, 2.98; P. 6.61. Found: N, 3.06; P, 6.55.

With twice the equivalent amount of DBPCl, the product was a yellow waxy solid, m.p. 95–97° (Fisher–Johns block, uncorrected). Elementary analysis and the negative phenol test indicate that both the phenolic group and the α-amino group have been phosphorylated.

Anal. Calcd. for $C_{39}H_{41}O_9N_2P_2$ (VII): N, 1.88; P, 8.35. Found: N, 2.25 (Kjeldahl), 2.57 (Dumas); P, 8.08.

(21) Melting points corrected. Nitrogen detemined by micro-Kjeldahl method. Phosphorus determined by the method of Lowry and Lopez[22] after preliminary digestion with sulfuric acid and nitric acid. Carbon and hydrogen analyzed by the Clark Microanalytical Laboratory, Urbana, Ill.

(22) O. H. Lowry and J. A. Lopez, *J. Biol. Chem.*, **162**, 421 (1946).

(23) J. C. Sheehan and V. S. Frank, THIS JOURNAL, **72**, 1312 (1950).

(24) F. R. Atherton, H. T. Howard and A. R. Todd, *J. Chem. Soc.*, 1106 (1948).

TABLE II

N-DIALKYLPHOSPHORYL AMINO ACID AMIDES

	R in VIII	Yield, %	M.p., °C.	Formula	Nitrogen, % Calcd.	Found	Phosphorus, % Calcd.	Found	Carbon, % Calcd.	Found	Hydrogen, % Calcd.	Found
N-Dibenzylphosphoryl												
glycine amide	-H	63.3	103–104.5	$C_{16}H_{19}O_4N_2P$	8.75	8.63	9.70	10.1				
DL-alanine amide	$-CH_3$	64	97–99	$C_{17}H_{21}O_4N_2P$	8.05	7.64 (Dumas)	8.91	8.63				
	R in IX											
N-Dimethylphosphoryl												
DL-alanine amide	$-CH_3$	72.2	111–112	$C_5H_{13}O_4N_2P$	14.3	14.9	15.8	16.2	30.6	30.79	6.64	6.73
DL-phenylalanine amide	$-C_7H_7$	83.6	148–149	$C_{11}H_{17}O_4N_2P$	10.6	10.3	11.7	11.8	48.5	48.89	6.25	6 45
L-glutamic acid diamide	$-C_3H_6O_2N$	94	117–120 dec.	$C_7H_{16}O_5N_3P$	16.6	16.4	12.3	11.6	33.2	35.22	6.32	6.41

Preparation of Amino Acid Benzyl Ester Hydrochlorides.—The DL-alanine derivative was prepared by the method of Miller and Waelsch[25] in 72% yield, m.p. 94–95°.

Anal. Calcd. for $C_{10}H_{14}O_2NCl$: N, 6.49. Found: N, 6.40.

The DL-serine derivative was prepared by the same method; yield 62%, m.p. 141–142°.

Anal. Calcd. for $C_{10}H_{14}O_3NCl$: N, 6.05. Found: N, 6.13.

L-Tyrosine benzyl ester hydrochloride was prepared by the method of Sachs and Brand[26]; yield 76%, m.p. 189–191° dec.

Anal. Calcd. for $C_{16}H_{18}O_3NCl$: N, 4.55. Found: N, 4.38.

***N*-Dibenzylphosphoryl Amino Acid Benzyl Esters.**—These were prepared and isolated analogous to the corresponding methyl and ethyl carboxyl esters.[14] Recrystallization was effected from benzene and petroleum ether. Data from the compounds which have not been reported previously are summarized in Table I. When ammonia was passed through chloroform suspensions of the benzyl ester hydrochloride of DL-phenylalanine and DL-serine and the resulting solutions treated with DBPCl in the presence of an equivalent amount of triethylamine as described previously (*i.e.*, procedure a[14]), a compound, melting at 107–108°, was isolated in 20–30% yield in both cases. This product was identified on the basis of its melting point and its elementary analysis as dibenzylamidophosphonate, a known compound.[12]

Anal. Calcd. for $C_{14}H_{16}O_3NP$: C, 60.6; H, 5.77; N, 5.05. Found: C, 61.11; H, 5.75; N, 5.09.

Preparation of *N*-Phosphoryl-DL-phenylalanine.—3.4 grams of twice-recrystallized *N*-dibenzylphosphoryl DL-phenylalanine benzyl ester was subjected to hydrogenolysis using 0.3 g. of palladium black in 170 ml. of dry methanol under conventional conditions. After hydrogenolysis (requiring about 90 minutes) and removal of the solvent an oily product was obtained from which 1.5 g. of material solidified at room temperature. Treatment of the solid with methanol extracted a soluble product which crystallized out upon addition of ether (yield 1.0 g.) melting at 163–164° dec. after additional recrystallization from methanol and ether.

Anal. Calcd. for $C_9H_{12}O_5NP$ (III)·H_2O (263.1): C, 41.1; H, 5.33; P, 11.8; N, 5.32. Found: C, 41.28; H, 5.80; P, 12.1; N, 5.50.

(25) H. K. Miller and H. Waelsch, THIS JOURNAL, **74**, 1092 (1952).

(26) H. Sachs and E. Brand, *ibid.*, **75**, 4610 (1953).

The compound is freely soluble in water. The methanol-insoluble residue was soluble in water, decomposed at about 280°, and the analysis of this material approximates that of an N-phosphorylated phenylalanyl phenylalanine monohydrate (Calcd. N, 6.82; P, 7.57. Found: N, 6.05; P, 7.96, 7.32).

Preparation of *N*-Phosphoryl Glycine.—One gram of dibenzylphosphorylglycine benzyl ester (oily product) was refluxed with Ranev nickel in methanol to destroy impurities that might subsequently poison the palladium catalyst. After hydrogenolysis (approximately 70 minutes) with palladium black, the catalyst was removed by filtration, and the product precipitated from the solution by the addition of two volumes of acetone. After standing in the refrigerator for several hours the crystalline material was filtered, washed with acetone, and dried in a vacuum desiccator over sulfuric acid. It is very hygroscopic. In a capillary tube the compound softened at about 70° and completely melted at about 115° dec., yield 0.1 g.

Anal. Calcd. for $C_2H_6O_5NP$ (III): N, 9.03; P, 20.0. Found: N, 9.31 (Dumas), 9.30 (Kjeldahl); P, 19.4.

Additional information concerning the structure and properties of *N*-phosphoryl-DL-phenylalanine and *N*-phosphorylglycine will be reported in a later paper.

Reaction of DBPCl with Amino Acid Amides.—The amide hydrochloride of the appropriate amino acid was dissolved in methanol, the free amide liberated with cooling by the calculated amount of freshly prepared sodium methoxide in methanol, the solution concentrated under reduced pressure, the precipitate of sodium chloride filtered off and washed with a small amount of methanol, the filtrate and washings evaporated, and the residue then suspended in chloroform. To the chloroform solutions were added equal molar amounts of triethylamine and DBPCl, slowly with stirring and cooling. The compounds were isolated in the usual manner described previously and their analyses are recorded in Table II. Recrystallization was effected from chloroform and petroleum ether.

Reaction of *N*-Dibenzylphosphoryl Amino Acid Esters with Ammonia.—Three to 5 grams of pure *N*-dibenzylphosphoryl amino acid ester was dissolved in 100 ml. of dry methanol which had been saturated with ammonia at 0° and kept in a glass-stoppered erlenmeyer flask at room temperature. After one week, the solvent was removed under reduced pressure on a steam-bath. The residue was taken up in methanol and a crystalline product thrown out by the addition of ether. The compounds so prepared were recrystallized from methanol and ether. Their analyses are reported in Table II.

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