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(N-PHOSPHORO)-α-AMINO ACIDS

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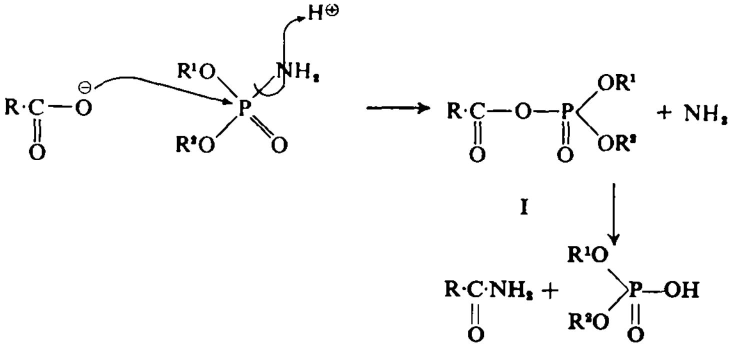
Abstract—α-(N-Dialkyl- and diaryl-phosphoroamido)-acids [II, R^3 = H] rearrange to give, initially, an α-amino acyl phosphate (e.g. IV) and a cyclic O-acyl phosphoroamidate (e.g. V), the relative proportions varying with starting material and solvent used. These initial products undergo further reactions leading to a variety of phosphates, the parent amino acid and oligopeptides.

Phosphoroamidates react with carboxylic acids to give carboxylic amides.[1,2] The initial product is presumably the acyl phosphate (I) which undergoes aminolysis by the amine liberated in the initial displacement. Such reactions are obviously intermolecular:[3] we now report on the related intramolecular reaction.



(i)

(N-Phosphoroamido)-acids are stable in alkali, but undergo very rapid hydrolysis in acid solution. Thus, N-phosphoroglycine (II; $R = R^1 = R^2 = R^3 = H$) is hydrolysed approximately sixty times faster than phosphoroamidic acid at pH 3·0,[4,5] the hydrolysis products being glycine and inorganic orthophosphate.[4] N-(O-Phenylphosphoro)-glycine (II; $R = C_6H_5$, $R^1 = R^2 = R^3 = H$) also undergoes rapid hydrolysis in acid solution giving glycine and monophenyl phosphate.[6] As neither peptides nor pyrophosphates can be detected[6] by paper chromatography, even in the presence of added glycine or orthophosphate, it seems likely that *hydrolysis*

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[1] Z. Skrowaczewska and P. Mastalerz, *Rocz. Chem.* **31**, 531 (1957); S. Goldschmidt and F. Obermeier, *Leibigs Ann.* **588**, 24 (1954).

[2] Z. A. Shabarova, L. G. Andronova, M. Bezdek and M. A. Prokof'ev, *Dokl. Akad. Nauk SSSR* **130**, 346 (1960).

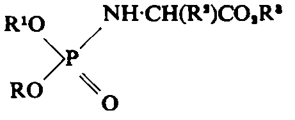
[3] N. K. Hamer, *J. Chem. Soc.* 46 (1965).

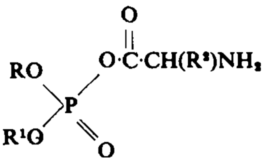
[4] T. Winnick and E. M. Scott, *Arch. Biochem.* **12**, 201 (1947).

[5] J. D. Chanley and E. Feageson, *J. Amer. Chem. Soc.* **85**, 1181 (1963).

[6] D. W. Hutchinson, Ph.D. Thesis, Cambridge (1960).

occurs without rearrangement to the amino-acyl phosphate (III) for it is well known that such mixed anhydrides decompose to oligopeptides in aqueous solution, even in the absence of added amino acids.[7]


II


III

α-(N-Phosphoroamido)-acids were originally prepared,[8] as magnesium salts, by phosphorylation of the parent amino acid using phosphorus oxychloride and magnesium oxide. A more convenient general method, involving phosphorylation of an α-amino acid ester with a dialkyl or diaryl phosphorochloridate in pyridine, was developed by Zervas and Katsoyannis.[9] However, in our hands, attempts to prepare N-diphenylphosphoroglycine benzyl ester by the Zervas method resulted in an impure product which smelled strongly of phenol and which could not be purified readily. When the reaction was performed in acetonitrile using N,N-dimethyl formamide as catalyst,[10] N-diphenylphosphoroglycine benzyl ester (II; $R = R^1 = C_6H_5$, $R^2 = H$, $R^3 = C_7H_7$) was obtained in high yield. It seems likely that under the Zervas conditions, pyridine caused partial debenzylation of the product and further decomposition ensued.

Our initial experiments were carried out with N-diphenylphosphoroglycine (II; $R = R^1 = C_6H_5$, $R^2 = R^3 = H$), prepared by catalytic hydrogenolysis of the corresponding benzyl ester in dioxan solution. Lyophilization immediately following hydrogenation gave a colourless gum whose IR spectrum contained a band ($\nu = 1725$ cm^{-1}) confirming the presence of the carboxyl group. Attempts to crystallize the acid or to isolate it as a salt failed. In the presence of a neutral solution of S-benzyl thiuronium chloride, a precipitate of S-benzyl thiuronium monophenyl phosphate was formed. Glycine and oligoglycines, together with small quantities of diphenyl phosphate, P^1,P^2-diphenyl pyrophosphate, inorganic phosphate and polyphosphates were detected in the filtrate.

In trifluoroacetic acid solution, N-diphenylphosphoroglycine gave diphenyl phosphoric acid, in accordance with (i), but small quantities of oligoglycines were again detected. Under reflux in inert solvents, e.g. benzene, t-butanol, precipitates were produced, examination of which by paper chromatography and paper electrophoresis showed the presence of glycine, oligoglycines, 2:5-diketopiperazine and several phosphates including monophenyl phosphate, diphenyl phosphate and P^1,P^2-diphenyl pyrophosphate. Although peptide formation had occurred it was apparent that the course of the reaction was complex. Since the known reactions between diphenyl esters of various N-substituted phosphoroamidic acids and carboxylic acids[1] are considerably slower than the rearrangements here observed the initial reaction(s) of N-diphenylphosphoroglycine seem likely to be intramolecular.

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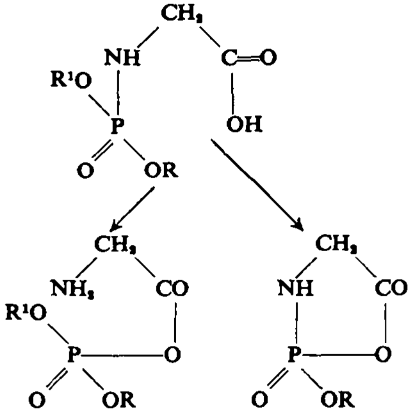
[7] W. Grassmann and E. Wünsch, *Fortschr. Chem. Org. Naturstoffe*, **13**, 444 (1956).

[8] C. Neuberg and W. Oertel, *Biochem. Z.* **60**, 491 (1914).

[9] L. Zervas and P. G. Katsoyannis, *J. Amer. Chem. Soc.* **77**, 5351 (1955).

[10] F. Cramer and M. Winter, *Chem. Ber.* **94**, 989 (1961).

Intramolecular nucleophilic attack by carboxylate at phosphorus could lead to cleavage of the P—N bond, giving IV, or to cleavage of a P—O bond to give V.



IV V

Compound IV is both a mixed anhydride, and an amine, and intermolecular acylation would be expected to give 2:5 diketopiperazine or an oligopeptide, with elimination of a di-ester of orthophosphoric acid. Compound V is a mixed anhydride and also a cyclic phosphoroamidate[11] related to ethylene phosphate. The instability of such five-membered cyclic phosphates is wellknown,[12] and under appropriate conditions V might be expected to undergo further reaction following one of two routes. Phosphorus-oxygen bond cleavage[13] would give the phosphoroamidic monoester (II: $R^1 = R^3 = H$), whereas P—N cleavage would produce the aminoacyl phosphate ester, (III: $R^1 = H$). Mono-esters of phosphoroamidic acid are well known[14,15] to give pyrophosphates under a wide variety of conditions, and a reaction of this type, following P—O cleavage in V, would account for the formation of P^1,P^2-diphenyl pyrophosphate. In support of this contention, P^1,P^2-diphenyl pyrophosphate was found to be the major phosphorus-containing product when N-diphenylphosphoroglycine was heated under reflux in pyridine, a solvent known[3] to catalyse acylation by phosphoroamidic mono-esters. In contrast, using t-butanol as solvent, monophenyl phosphate was produced in 80% yield. In all cases, glycine and oligoglycines were formed.

Since nucleophilic attack at the phosphorus atom in phosphoroamidates is normally bimolecular,[16] the relative amounts of IV and V formed from II($R^2 = R^3 = H$) will depend on the ease of displacement of a dialkyl or diaryl phosphate ion as opposed to an alkoxide or phenoxide ion. Attempts to prepare simple alkyl esters of II resulted in the formation of oils, difficult to purify, but N-di-*p*-nitrobenzylphosphoroglycine was crystalline. Its solution in pyridine slowly gave rise to

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[11] A. Katchalsky and M. Paecht, *Bull. Res. Council Israel*, **2**, 312 (1952); H. Keller, H. Netter and B. Niemann, *Z. physiol. Chem.* **313**, 244 (1958).

[12] J. Kumamoto, J. R. Cox and F. H. Westheimer, *J. Amer. Chem. Soc.* **78**, 4858 (1956).

[13] *Cf.* A. Cosmatos, I. Photaki and L. Zervas, *Chem. Ber.* **94**, 2644 (1961).

[14] V. M. Clark, G. W. Kirby and Sir Alexander Todd, *J. Chem. Soc.* 1497 (1957).

[15] V. M. Clark and S. G. Warren, *J. Chem. Soc.* 5509 (1965).

[16] N. K. Hamer, *J. Chem. Soc.* 2731 (1965).

P^1,P^2-di-*p*-nitrobenzyl pyrophosphate, isolated in 65% yield. In t-butanol and trifluoroacetic acid solutions, the major phosphorus-containing product was *di-p-nitrobenzyl phosphoric acid.*

To provide an example in which the route II($R^2 = R^3 = H$) → V was favoured, N-di-*p*-nitrophenylphosphoroglycine was prepared. Since catalytic hydrogenolysis of the corresponding benzyl ester was accompanied by reduction of the nitro-groups, it was made from the t-butyl ester, the protecting group being removed by treatment with trifluoroacetic acid.[17] After 1 hr at room temperature, the solvent was removed and the resultant oil dissolved in anhydrous ethanolic hydrochloric acid. Paper chromatographic examination of the reaction mixture showed the presence of glycine, oligoglycines, inorganic orthophosphate and mono-*p*-nitrophenyl phosphate—no di-*p*-nitrophenyl phosphate could be detected. Thus, the route II → V seems to be the exclusive pathway for the decomposition of N-di-*p*-nitrophenylphosphoroglycine under these conditions.

Intramolecular rearrangement of N-diphenylphosphoroglycine to the mixed anhydride (III: $R = R^1 = C_6H_5$, $R^2 = H$) is only possible if the entering and leaving groups at the phosphorus atom are at less than 180° to each other—unless there is a considerable amount of bond breaking prior to attaining the transition state. That the rearrangement of II to III is intramolecular is supported by the failure of N-diphenylphosphoroglycine methyl ester to react with acetic acid. Moreover, the stability of N-diphenylphosphoro-β-alanine under conditions causing rearrangement of the glycine analogue seems to indicate that attack to give the five-membered cyclic system (V) is much easier than that to form the corresponding six-membered ring.

Haake and Westheimer[18] studied the hydrolysis and ^{18}O exchange of ethylene hydrogen phosphate and found that the entering and leaving groups at phosphorus must be at either 90° or 120° to each other. It seems likely therefore that this is true also in the rearrangement of II to III.

The rearrangement we report has been extended to N-phosphoro-derivatives of DL-phenylalanine, L-phenylalanine, DL-valine and DL-leucine. Qualitatively similar reactions were observed in all cases. Hydrolysis of the oligopeptide derived from L-phenylalanine gave the parent amino acid with undiminished optical rotatory power indicating that oligomerization was not accompanied by racemization.

EXPERIMENTAL

Paper chromatographic and electrophoretic data

Electrophoretic buffer system. A. Pyridine–AcOH–water (1:10:89), pH 3·5. Electrophoretic runs were carried out on Whatman No. 1 paper at 20 v/cm.

Solvent systems for paper chromatography. B. Butan-1-ol–AcOH–water (4:1:1); *C.* EtOH–0·1M K_2CO_3 (65:35). Ascending chromatograms were run on Whatman No. 1 paper throughout. Phosphorus containing compounds were detected by the method of Hanes and Isherwood.[19] Amides were detected by the chlorine–potassium iodide-starch treatment of Rydon and Smith.[20] Amino acids were detected by ninhydrin spray (0·1%, w/v in butan-1-ol).

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[17] H. C. Beyerman and J. S. Bontekoe, *Rec. Trav. Chim.* **81**, 699 (1962).

[18] P. C. Haake and F. H. Westheimer, *J. Amer. Chem. Soc.* **83**, 1102 (1961).

[19] C. S. Hanes and F. A. Isherwood, *Nature, Lond.* **164**, 1107 (1949).

[20] H. N. Rydon and P. W. G. Smith, *Nature, Lond.* **169**, 922 (1952).

N-*Diphenylphosphoro-α-amino acid benzyl esters*

The amino acid benzyl ester toluene-*p*-sulphonate[21] (0·033 mole) in dry acetonitrile (67 ml) containing N,N-dimethylformamide (3·3 ml) was stirred at 0° and dry Et_3N (10 ml) added. After a short while, dropwise addition, with stirring, of diphenyl phosphorochloridate (10·0 g; 0·037 mole) was commenced, and completed within 15 min. Stirring was continued, at room temp, for 1½ hr. The reaction mixture was then poured into ice-cold water (700 ml). The crude N-diphenylphosphoro-α-amino acid benzyl ester rapidly separated as a colourless oil.

(a) N-*Diphenylphosphoroglycine benzyl ester.*[13] The oil crystallized readily on standing. The solid was collected, washed well with water and dried *in vacuo* (11·6 g, 89%). A sample, recrystallized from ether 4x, formed colourless needles of N-diphenylphosphoroglycine benzyl ester m.p. 60–61°. (Found: C, 63·2; H, 5·2; N, 3·7. Calc. for $C_{21}H_{20}NO_5P$: C, 63·5; H, 5·0; N, 3·5%.)

(b) N-*Diphenylphosphoro*-DL-*phenylalanine benzyl ester.*[13] The oil crystallized very readily. The solid was collected, washed well with water and dried (12·8 g, 80%). A sample recrystallized from ether (4 x) gave white crystals of N-diphenylphosphoro-DL-phenylalanine benzyl ester m.p. 90–91°. (Found: C, 69·1; H, 6·0; N, 3·1. Calc. for $C_{28}H_{26}NO_5P$: C, 69·0; H, 5·3; N, 2·9%.)

(c) N-*Diphenylphosphoro*-L-*phenylalanine benzyl ester.* The oil crystallized very readily. The solid was collected, washed well with water and dried *in vacuo* (87%). A sample was recrystallized from ether 3x to give colourless crystals, m.p. 86°. $[\alpha]_D^{21}$ −5·2° (CCl_4); M_D^{21} −24·8° (CCl_4). (Found: C, 68.2; H, 5.3; N, 3.1. $C_{38}H_{26}NO_5P$ requires: C. 69.0; H, 5.4; N, 2.9%.)

(d) N-*Diphenylphosphoro*-DL-*valine benzyl ester.* The oil did not crystallize. It was extracted with ether (100 ml) and the extract dried (Na_2SO_4). Evaporation *in vacuo* gave a colourless oil (8·6 g, 59%) which crystallized after several weeks at 0°. Recrystallized from ether it gave white crystals of N-*diphenylphosphoro*-DL-*valine benzyl ester* m.p. 44–48°. (Found: C, 65·6; H, 6·4; N, 3·2. $C_{24}H_{26}NO_5P$ requires: C, 65·6; H, 5·9; N, 3·2%.)

(e) N-*Diphenylphosphoro*-DL-*leucine benzyl ester.* The oil crystallized on standing at 0°. The solid was collected, washed with water and dried *in vacuo* (9·0 g, 61%). A sample recrystallized from ether gave colourless crystals of N-*diphenylphosphoro*-DL-*leucine benzyl ester* m.p. 52–53°. (Found: C, 65·7; H, 6·0; N, 3·2. $C_{25}H_{28}NO_5P$ requires: C, 66·1; H, 6·2; N, 3·1%.)

Hydrogenation of N-*diphenylphosphoro-α-amino acid benzyl esters*

A solution of the N-diphenylphosphoro-α-amino acid benzyl ester (1 mmole) in dry dioxan (30 ml) was hydrogenated at room temp and atm. press. using 10% Pd—C as catalyst. Uptake of H_2 was complete after about 5 hr. The catalyst was filtered off. A white precipitate slowly formed in the resultant dioxan solution. Precipitation was complete after 2 days. The precipitate was filtered off, washed with dioxan and dried *in vacuo* (ca. 100 mg).

The filtrate was examined by paper chromatography and found to contain diphenyl phosphate, P^1P^2-diphenyl pyrophosphate and the parent amino acid. The precipitate was shown by paper chromatography and paper electrophoresis to contain inorganic orthophosphate, inorganic polyphosphates, monophenyl phosphate, P^1P^2-diphenyl pyrophosphate, diphenyl phosphate, the parent amino acid and oligopeptides.

N-*Diphenylphosphoroglycine.* A solution of N-diphenylphosphoroglycine benzyl ester (397 mg, 1 mmole) in dry dioxan (30 ml) was hydrogenated as above. When uptake of H_2 was complete, the catalyst was at once filtered off and the solvent immediately evaporated from the filtrate by freeze drying. N-diphenylphosphoroglycine was left as a gummy residue (307 mg, 100%). (IR max at ν 1725 cm^{-1}.)

Reactions of N-*diphenylphosphoroglycine in various solvents*

(a) *Benzene.* N-Diphenylphosphoroglycine (307 mg) was dissolved in dry benzene (25 ml) and heated under reflux for 1½ hr. After a few min precipitation commenced and the reaction was complete after 1½ hr. The precipitate was collected, washed with ether and dried *in vacuo* (65 mg). Paper chromatography of the filtrate indicated diphenyl phosphate and P^1P^2-diphenylpyrophosphate. For the precipitates, chromatographic data are given below.

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[21] P. C. Crofts, J. H. H. Markes and H. N. Rydon, *J. Chem. Soc.* 3610 (1959).

Compound	R_f values Solvent *B*	 Solvent *C*	Migration (cm) in buffer *A*
Glycine	0·14	0·46	6·4
Glycylglycine	0·18	0·52	14·7
Triglycine	0·12	0·50	14·4
2:5-Diketopiperazine	0·32	—	2·8
Precipitate	0·00 → 0·10, 0·14, 0·18, 0·32	0·13 → 0·45, 0·52	2·8, 6·4, 14·7, 9·8 → 13·5

(b) t-*Butanol.* N-Diphenylphosphoroglycine (307 mg) was dissolved in t-butanol (40 ml) and heated under reflux for 2 days. The solvent was removed by lyophilization and the residue dissolved in EtOH. Addition of cyclohexylamine (0·5 ml) gave a white precipitate which on recrystallization from aqueous EtOH gave pure di-(cyclohexylammonium) monophenyl phosphate[22] (301 mg, 81%) m.p. 216°. (Found: C, 58·5; H, 8·2; N, 7·0; P, 8·3; Calc. for $C_{18}H_{33}N_2O_4P$: C, 58·2; H, 8·9; N, 7·5; P, 8·3%.) Paper chromatographic examination of the mother liquor showed the presence of glycine and oligoglycines.

(c) *Pyridine.* N-Diphenylphosphoroglycine (307 mg) was dissolved in pyridine (10 ml) and left at room temp for 1 week. The solvent was evaporated and the residual gum dissolved in EtOH. Addition of cyclohexylamine (0·5 ml) gave a white precipitate which, on recrystallization from water, gave pure di-(cyclohexylammonium)-P^1P^2-diphenyl pyrophosphate[23] (158 mg, 60%) m.p. 256–258°. (Found: C, 54·4; H, 7·3; N, 11·8. Calc. for $C_{24}H_{38}N_2O_7P_2$: C, 54·6; H, 7·3; N, 11·7%.) Paper chromatographic examination of the crude reaction mixture indicated the formation of inorganic orthophosphate, monophenyl phosphate, diphenyl phosphate, glycine and oligoglycines.

(d) *Trifluoroacetic acid.* N-Diphenylphosphoroglycine (307 mg) was dissolved in trifluoroacetic acid (10 ml) and the solution allowed to stand at room temp for 1 hr. The solvent was removed by lyophilization and the residual gum dissolved in EtOH. Addition of cyclohexylamine (0·5 ml) gave a white precipitate which on recrystallization from EtOH gave pure cyclohexylammonium diphenyl phosphate[24] (176 mg, 50%) m.p. 199–200° undepressed on admixture with an authentic sample). Paper chromatographic examination of the filtrate indicated the formation also of inorganic orthophosphate, monophenyl phosphate, P^1P^2-diphenyl pyrophosphate, glycine and oligoglycines.

Hydrolysis of the oligopeptide formed from N-*diphenylphosphoro*-L-*phenylalanine*

N-Diphenylphosphoro-L-phenylalanine benzyl ester (0·970 g, 2 mmoles) was hydrogenated in dioxan in the usual way, and the white precipitate which formed was filtered and dried (185 mg). After refluxing the precipitate overnight with 20% HCl (3 ml) the resulting solution contained only one substance giving a positive ninhydrin test, and on electrophoresis it moved as phenylalanine. The solution was made slightly alkaline with $NaHCO_3$ and shaken for 2 hr with a solution of 1-fluoro-2, 4-dinitrobenzene (0·28 g) in EtOH (5 ml). The EtOH was evaporated and the residue dissolved in water. Excess 1-fluoro-2,4-dinitrobenzene was removed by ether extraction. Acidification of the aqueous layer with HCl precipitated the crude dinitrophenyl derivative of L-phenylalanine. This was recrystallized from aqueous MeOH, m.p. 184–185°; $[\alpha]_D^{21}$ −107° (glacial AcOH); M_D^{21} −356° (glacial AcOH).

An authentic sample of the dinitrophenyl derivative of L-phenylalanine was recrystallized from aqueous MeOH, m.p. 185–186°; $[\alpha]_D^{21}$ −110° (glacial AcOH); M_D^{21} −364° (glacial AcOH).

N-*Di*-p-*nitrobenzylphosphoroglycine benzyl ester.*[26] Glycine benzyl ester toluene-*p*-sulphonate (3·2 g, 0·0105 mole) in dry acetonitrile (20 ml) containing N,N-dimethylformamide (1·0 ml) was stirred at 0° while dry Et_3N (3·0 ml) was added. After 15 min, di-*p*-nitrobenzyl phosphorochloridate (4·05 g, 0·0105 mole) was added slowly. Stirring was continued at room temp for 1½ hr. The reaction

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[22] M. Miyano, *J. Amer. Chem. Soc.* **77**, 3524 (1955).

[23] H. G. Khorana and A. R. Todd, *J. Chem. Soc.* 2257 (1953).

[24] N. S. Corby, G. W. Kenner and A. R. Todd, *J. Chem. Soc.* 1234 (1952).

[25] A. Vollmar and M. S. Dunn, *J. Org. Chem.* **25**, 387 (1960).

[26] D. M. Theodoropoulos, J. Gazopoulos and I. Soucherlis, *Nature, Lond.* **185**, 606 (1960).

mixture was then poured into ice-cold water (200 ml). A pale yellow oil rapidly separated out. The oil crystallized on standing to give N-di-*p*-nitrobenzylphosphoroglycine benzyl ester (4·1 g, 76%). A sample recrystallized from $CHCl_3$–pet. ether (60–80°) afforded colourless crystals m.p. 110–111°. (Found: C, 54·1; H, 4·3; N, 8·7. Calc. for $C_{23}H_{22}N_3O_9P$: C, 53·6; H, 4·3; N, 8·2%.)

N-*Di*-p-*nitrobenzylphosphoroglycine*.[9] N-Di-*p*-nitrobenzylphosphoroglycine benzyl ester (670 mg) was shaken with a solution of NaOH (120 mg) in 50% aqueous EtOH (50 ml) for 1 hr. Acidification of the resultant solution with conc. HCl gave a precipitate which on recrystallization from EtOH gave N-di-*p*-nitrobenzylphosphoroglycine (400 mg, 81%) m.p. 145–147° (dec). (Found: N, 9·6; P, 7·6. Calc. for $C_{16}H_{16}N_3O_9P$: N, 9·9; P, 7·3%.)

Reaction of N-*Di*-p-*nitrobenzylphosphoroglycine in various solvents*

(a) t-*Butanol*. N-Di-*p*-nitrobenzylphosphoroglycine (100 mg) was heated under reflux in t-butanol (20 ml) for 2 days. A white precipitate formed and was filtered off (22 mg) and the filtrate evaporated to dryness. Paper chromatographic examination of the precipitate showed the presence of glycine, oligoglycines and small quantities of phosphates. The residue obtained from the filtrate was shaken with $CHCl_3$ and the white crystalline material produced was collected. Recrystallized from EtOH, it gave pure di-*p*-nitrobenzyl phosphoric acid (57 mg, 82%) m.p. 174–176° (unchanged on admixture with an authentic sample).

(b) *Pyridine*. N-Di-*p*-nitrobenzylphosphoroglycine (80 mg) was dissolved in pyridine (3 ml). After 1 day a precipitate began to form: it was collected after 1 week. Recrystallization from $CHCl_3$ gave the crystalline diglycine salt of P^1P^2-di-*p*-nitrobenzyl pyrophosphoric acid (23 mg, 41%) m.p. 152–153°. (Found: C, 35·8; H, 4·3; P, 10·9. Calc. for $C_{18}H_{24}N_4O_{15}P_2$: C, 36·1; H, 4·1; P, 10·35%.) The filtrate was shown by paper chromatography to contain glycine, P^1P^2-di-*p*-nitrobenzyl pyrophosphate and di-*p*-nitrobenzyl phosphate. Evaporation of the filtrate gave an oily residue which formed a precipitate on treatment with $CHCl_3$ (2 ml) and excess cyclohexylamine. Recrystallization of the precipitate from water gave di-(cyclohexylammonium)-P^1P^2-di-*p*-nitrobenzyl pyrophosphate (15 mg, 24%).

(c) *Trifluoroacetic acid*. N-Di-*p*-nitrobenzylphosphoroglycine (100 mg) was dissolved in trifluoroacetic acid (3 ml). After 1 day at room temp the solvent was evaporated to give a pale yellow gum which solidified on standing. Paper chromatographic examination of the solid showed the presence of glycine, oligoglycines and one P-containing product: di-*p*-nitrobenzyl phosphate. Crystallization of the solid from EtOH gave di-*p*-nitrobenzyl phosphoric acid (36 mg, 46%) m.p. 172–174°.

N-*Di*-p-*nitrophenylphosphoroglycine* t-*butyl ester*. Glycine t-butyl ester hydrochloride (176 mg, 0·0115 mole) in dry acetonitrile (20 ml) containing N,N-dimethylformamide (0·05 ml) was stirred at 0° while dry Et_3N (0·2 ml) was added. After 15 min di-*p*-nitrophenyl phosphorochloridate[27] (411 mg, 0·0115 mole) was added slowly. Stirring was continued at room temp for 1½ hr and the reaction mixture was then poured into water. The yellow oil which separated was extracted with $CHCl_3$. The $CHCl_3$ solution was dried and evaporated to give a pale yellow oil which crystallized on standing. Recrystallization from benzene gave the pure N-*di*-p-*nitrophenylphosphoroglycine* t-*butyl ester* (469 mg, 90%) m.p. 113°. (Found: C, 47·6; H, 4·4; N, 8·9. $C_{18}H_{20}N_3O_9P$ requires: C, 47·7; H, 4·4; N, 9·3%.)

Action of trifluoroacetic acid on N-*di*-p-*nitrophenylphosphoroglycine* t-*butyl ester*

N-Di-*p*-nitrophenylphosphoroglycine t-butyl ester (100 mg) was dissolved in trifluoroacetic acid and the solution stood at room temp (1 hr). The solvent was then evaporated to give a pale yellow oil which showed peaks in the IR at 1865 cm^{-1} and 1790 cm^{-1}. The oil was dissolved in EtOH and dry HCl was passed into the solution. On addition of ether a white precipitate (17 mg) formed. Paper chromatographic examination of this precipitate showed it to contain glycine and various oligoglycines. The filtrate was similarly shown to contain inorganic orthophosphate and mono-*p*-nitrophenyl phosphate; no di-*p*-nitrophenyl phosphate could be detected.

N-*Diphenylphosphoro*-β-*alanine benzyl ester*. β-Alanine benzyl ester toluene-*p*-sulphonate (11·6 g, 0·033 mole) in dry acetonitrile (67 ml) containing N,N-dimethylformamide (3·3 ml) was stirred at 0° while dry Et_3N (10 ml) was added. After 15 min diphenyl phosphorochloridate (10·0 g, 0·051 mole)

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[27] T. Ukita and H. Hayatsu, *J. Amer. Chem. Soc.* **84**, 1879 (1962).

was added dropwise with stirring over 15 min. The reaction mixture was then poured into ice-cold water (700 ml) and the crude product separated out as an oil. The oil was extracted with ether and the ethereal solution dried over Na_2SO_4. Evaporation of the solution gave a colourless oil which crystallized on standing at 0°. Recrystallization from ether gave pure N-*diphenylphosphoro-β-alanine benzyl ester* (12·3 g, 91%) m.p. 50–52°. (Found: C, 63·9; H, 5·6; N, 3·4. $C_{22}H_{22}NO_5P$ requires: C, 64·2; H, 5·4; N, 3·4%.)

Action of heat on N-*diphenylphosphoro-β-alanine*

N-Diphenylphosphoro-β-alanine benzyl ester (0·42 g) in dioxan (20 ml) was submitted to catalytic hydrogenation at room temp and atm. press. using 10% Pd–C as catalyst. When the uptake of H_2 was complete, the catalyst was filtered off and the filtrate evaporated to give a gummy residue of N-diphenylphosphoro-β-alanine (0·339 g, 97%). This residue was heated under reflux in benzene for 12 hr. Paper chromatographic examination of the reaction mixture showed that no decomposition had occurred; starting material was recovered (0·319 g, 94%).

Investigation of the effect of heating N-*diphenylphosphoroglycine methyl ester*[9] *with acetic acid*

A solution containing N-diphenylphosphoroglycine methyl ester (321 mg, 0·001 mole) and AcOH (0·06 ml, 0·001 mole) in dry benzene (25 ml) was heated under reflux for 6 hr. Paper chromatographic examination of the solution showed that no reaction had occurred.

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