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STUDIES ON REACTIONS OF THE N-PHOSPHONIUM SALTS OF PYRIDINES—XII

PREPARATION OF PEPTIDES AND ACTIVE ESTERS BY A HYDROLYSIS-DEHYDRATION REACTION WITH PHOSPHONITES, AND THE APPLICATION OF THE REACTION TO POLYMER SYNTHESIS

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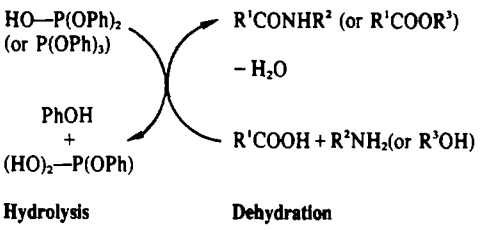
Abstract—Dehydration reactions accompanied by hydrolysis of the condensation reagent itself, generalized as "hydrolysis-dehydration," are seen in the formation of amides and esters using phosphites. The proposed concept was shown to be applicable also to the reactions with other phosphorus compounds, such as phosphinites, phosphonites and phosphonates. Aryl esters of these phosphorus compounds were effective for producing amides and esters, whereas alkyl esters were ineffective. Several peptides and active esters of amino acids were prepared using diphenyl phosphonites in the presence of tertiary amines and extended to the preparation of polypeptides and polyamides. Linear polypeptides and high molecular weight polyamides were obtained by means of diphenyl ethylphosphonite in N-methylpyrrolidone containing pyridine.

In our previous papers,[1,2] we showed that diphenyl and triphenyl phosphites reacted with carboxylic acids in the presence of pyridine to give acyloxy N-phosphonium salts of pyridine accompanied by dephenoxylation, which produced the corresponding amides and esters by aminolysis and alcoholysis. In these reactions, hydrolysis of diphenyl and triphenyl phosphites to monophenyl phosphite and phenol coupled to dehydration between carboxylic acids and amines, or alcohols to the corresponding amides and esters. Therefore, the reaction could be referred to as "hydrolysis-dehydration".



In the course of attempting to evolve the proposed concept of the hydrolysis-dehydration reaction using phosphites to other phosphorus compounds, we found that phosphinites, phosphonites and phosphonates also brought about coupling between carboxyl and amino or hydroxyl components via the hydrolysis-dehydration reaction, giving the corresponding amides and esters.

We describe here the reactions with these phosphorus compounds and its use for the preparation of peptides and active esters of amino acids, especially by means of diphenyl ethylphosphonite. This paper presents also the results of application of the reaction to the syntheses of polypeptides and polyamides.

RESULTS AND DISCUSSION

Diphenyl ethylphosphonite reacted with acetic acid in acetonitrile in the presence of imidazole, giving phenyl acetate and phenol in almost quantitative yields (Eq 1). When the reaction was carried out in the presence of aniline, the corresponding anilide was obtained together with almost the theoretical amount of phenol according to Eq 2; benzyloxycabonyl-glycinanilide(Z-glycinanilide) was produced in 93% yield by the reaction of diphenyl ethylphosphonite with Z-glycine and aniline at 40°C for 3 h in acetonitrile in the presence of imidazole. The anilide was also obtained in 94% yield when the reaction was conducted in the presence of pyridine instead of imidazole at 40°C for 12 h.

$$\underset{(R = Et)}{R—P(OPh)_2} + \underset{(R' = CH_3)}{R'COOH} \xrightarrow[\text{or Imidazole}]{\text{Pyridine}} R'COOPh + PhOH + R—P(OPh)(OH) \qquad \text{Eq 1.}$$

$$\underset{(R = Et)}{R—P(OPh)_2} + R'COOH + \underset{(R^2 = Phenyl)}{R^2NH_2} \xrightarrow[\text{or Imidazole}]{\text{Pyridine}} R'CONHR^2 + PhOH + R—P(OPh)(OH) \qquad \text{Eq 2.}$$

Triethylamine was employed as HCl-scavenger in peptide synthesis from a Z-amino acid and an amino acid ester hydrochloride. If pyridine is used as solvent, addition of triethylamine to reaction is avoided. It is therefore convenient to use pyridine for peptide synth-

esis, though the reaction was relatively slow and 12 h was neccessary to complete the reaction in pyridine at 40°C.

The phosphonite was also able to promote a coupling reaction between carboxyl and hydroxyl components, giving p-nitrophenyl Z-glycinate in 74% yield by reacting Z-glycine with p-nitrophenol in acetonitrile at 40°C for 12 h in the presence of imidazole.

Several peptides and active esters of amino acids were prepared using diphenyl ethylphosphonite under several conditions in yields upto 94% with high optical purity (Tables 1 and 2). No difficulties were observed with the side chains of glutamine and methionine in the carboxly component and of tyrosine in the amino component.

Presence of tertiary amines facilitated the reaction, improving the yield in the reaction of Z-glycine and aniline (Table 3). The yield of Z-glycinanilide increases with an increase of basicity of tertiary amine having small steric hindrance around nitrogen atom. Lower yields by α-picoline, 2,6-lutidine and triethylamine may be due to steric hindrance in spite of adequate basicity.

Diphenyl ethylphosphonite and tertiary amines are involved stoichiometrically in the reaction. The effect of the employed amounts of imidazole and diphenyl ethylphosphonite was examined in the reaction of Z-glycine and aniline in acetonitrile. The results, shown in Fig. 1, indicate an increase of the yield of Z-glycinanilide until the ratios of imidazole and phosphonite over the carboxyl component reach limiting values of 2 and 1, respectively. Above these values, no substantial effect of further addition of imidazole or phosphonite could be found. More than 50% yield of the anilide was obtained by use of a quarter or a half equivalent of diphenyl ethylphosphonite, indicating that some of the resulting monophenyl ethylphosphonite from diphenyl ethylphosphonite participates further in coupling as shown in Scheme 1.

These results suggest that the reaction proceeds through a similar mechanism (hydrolysis-dehydration reaction) to those with phosphites.[1] Acyloxy N-phosphonium salt of pyridine or imidazole 1 is formed by reaction of dipenyl ethylphosphonite with a carboxylic adid, involving release of a phenolate anion from the phosphonite. 1 is converted into 2 and an amide or an

Table 2. Preparation of active esters of amino acids by means of diphenyl ethylphosphonite in the presence of imidazole[a]

Active Esters	Yield(%)	mp, °C[c]
Z-Gly-O-C$_6$H$_4$-NO$_2$	74	128
Z-Gly-O-C$_6$H$_4$-COOCH$_3$	75	121
Z-Gly-O-C$_{10}$H$_7$	72	109–10
Z-Gly-S-C$_6$H$_5$	74	71–2
Z-Phe-O-C$_6$H$_4$-NO$_2$(L)[b]	71	125–6

[a] The reaction was carried out in acetonitrile at 40° for 12 h using an equivalent of the phosphonite.
[b] $[\alpha]_D$ − 25° (c = 2, DMF) [lit.[6] − 24·5° (c = 2, DMF)].
[c] After recrystallization.

Table 3. Preparation of Z-glycinanilide using dipheny ethylphosphonite in the presence of various tertiary amines[a]

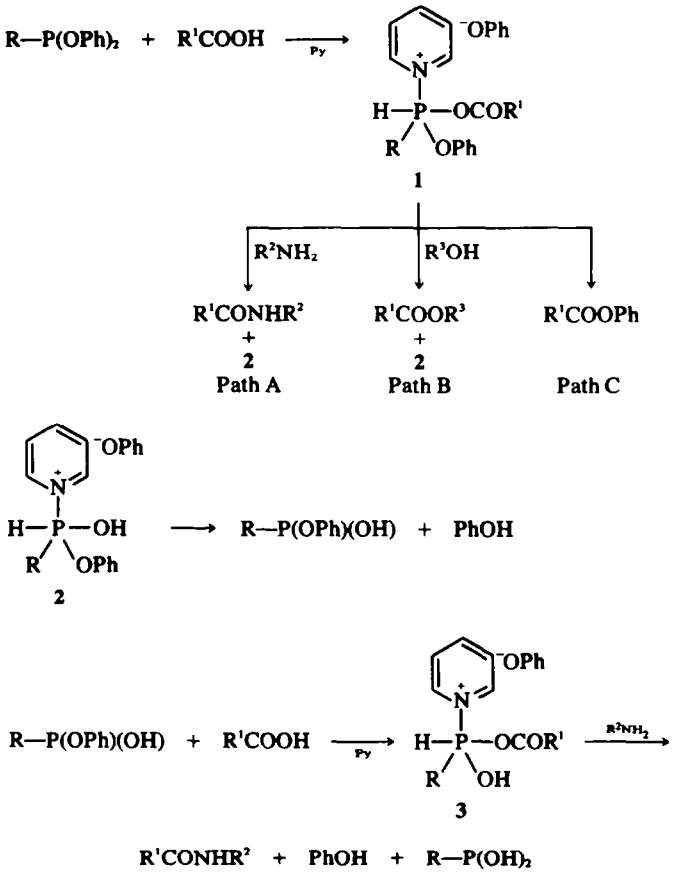
Tertairy amines	pKa	Z-glycinanilide Yield(%)
Imidazole	7·12	93
γ-Picoline	6·02	56
β-Picoline	5·52	42
Pyridine	5·23	34(84)[b]
α-Picoline	5·97	31
2,6-Lutidine	6·99	25
Triethylamine	10·87	8
None	—	8

[a] The reaction was carried out at 40° for 3 h in acetonitrile in the presence of two equivalents tertiary amines.
[b] The yield was obtained by prolonged reaction time of 12 h.

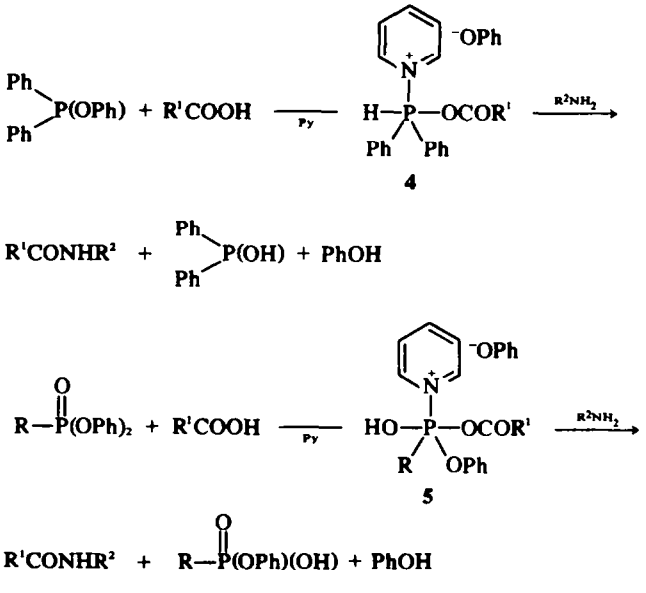
Table 1. Preparation of peptides by means of diphenyl ethylphosphonite[a]

Peptides	Tertiary Amines	Solvents	Yield (%)	mp (°C)
Z-Gly-Gly·OEt	Py	Py	92	80
	Im	CH_3CN	92	—
Z-Phe-Gly·OEt(L)	Py	Py	90	108–9
	Im	CH_3CN	90	—
Z-Gly-Tyr·OEt(L)[b]	Im	CH_3CN	86	125–6
Z-Met-Gly·OEt(DL)	Im	CH_3CN	93	72–3
Z-Gln-Gly·OEt(L)[c]	Im	CH_2Cl_2	78	168–9

[a] The reaction was carried out at 40° for 12 h using an equivalent amount of diphenyl ethylphosphonite.
[b] $[\alpha]_D$ + 19·5° (c = 5, EtOH) [lit.[5] + 19·8° (c = 5, EtOH)].
[c] $[\alpha]_D$ − 7·0° (c = 1, DMF) [lit.[6] − 7·2° (c = 1, DMF)].



Scheme 1



Scheme 2.

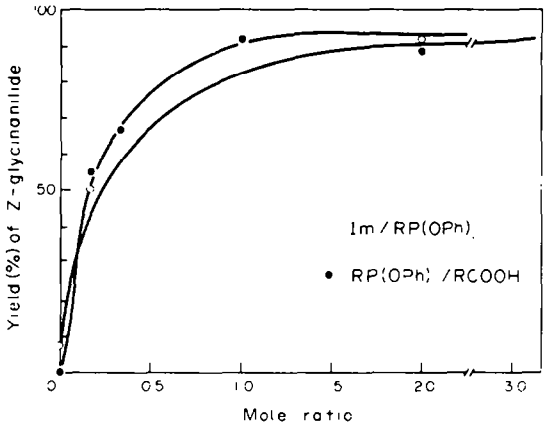


Fig. 1.

ester by aminolysis or alcoholysis with an amino or a hydroxyl component (Path A or B). 2 then changes to monophenyl ethylphosphonite. Different from monophenyl phosphite resulted from phosphites, the monophenyl ethylphosphonite is hydrolyzed further to the free acid, ethylphosphonous acid, promoting another coupling reaction via 3 to give an amide or an ester. A side reaction, intramolecular reaction of a phenolate anion on 1 with the acyloxy group, produces phenyl ester (Path C), which is dominant in the absence of an amino component as mentioned before in the text.

Several phosphorus compounds were tried in the reaction of Z-glycine and aniline in the presence of imidazole (Table 4). Of these compounds, diphenyl alkylphosphonite and phenylphosphonite, and phenyl diphenylphosphinite were markedly effective. On the other hand, diethyl and monoethyl ethylphosphonites failed to give the anilide, which may be attributed to the fact that these phosphonites are unable to form the salts (I and II) because of the low nucleophilicity of the ethoxy groups. Similarly, diphenyl ester of phosphonic acid was effective, but diethyl ester was not. Both triphenyl and triethyl phosphoates did not promote the reaction.

As with phosphonites, the reactions with phosphinite and phosphonate may be represented as follows (Scheme 2).

The reaction of producing an amide as described above was extended to the polycondensation reaction of α-amino acids, and of dicarboxylic acids and diamines using diphenyl ethylphosphonite which has been extremely effective for peptide synthesis.

Polycondensation of several amino acids were carried out using diphenyl ethylphosphonite in pyridine or imidazole, and the results are given in Table 5, which shows that polypeptides are produced in pyridine in high yields from free amino acids which are known to easily cyclize to dimers by ordinary methods.[4] Imidazole did not give favourable results.

The polymer from glycine shows an infrared spectrum with three amide bands at 1630, 1550 and 1240 cm^{-1} characteristic of polyglycine. All other polymers were

Table 4. Preparation of Z-glycinanilide using various phosphorus compounds in the presence of imidazole[a]

Phosphorus compounds	Z-glycinanilide Yield(%)
$Et\text{-}P(OPh)_2$	93
$n\text{-}Bu\text{-}P(OPh)_2$	93
$Ph\text{-}P(OPh)_2$	84
$Et\text{-}P(OEt)_2$	0
Et-P(OH)(OEt)	0
	70
	28(11)[b]
	0
$O{=}P(OPh)_3$	0
$O{=}P(OEt)_3$	0

[a] The reaction was carried out at 40° for 3 h in acetonitrile

[b] Pyridine was used instead of imidazole

Table 5. Polycondensation of several amino acids using diphenyl ethylphosphonite in pyridine at 40° for 18 h

Amino acids	ηinh[a]	Yield(%)
Glycine	0·17	70
L-Alanine	0·18	73
L-Leucine	0·25	60
L-Phenylalanine	0·11	46

[a] Measured in dichloroacetic acid at 30°

identified as polypeptides from their infrared spectra and elementary analyses. These results indicated that linear polycondensation took place in this reaction mixture with diphenyl ethylphosphonite.

The polycondensation of L-leucine in various solvents was carried out in the presence of two equivalent amounts of pyridine (Table 6). It is seen that polymer with a relatively higher viscosity was produced in pyridine

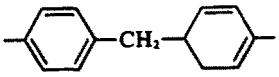
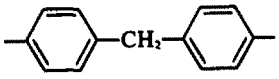
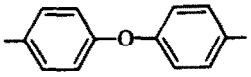
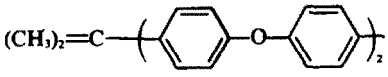
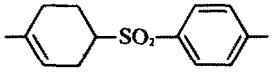
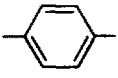
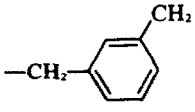
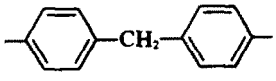
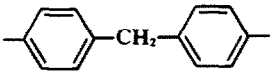
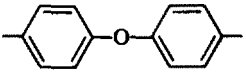
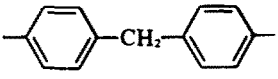
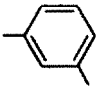
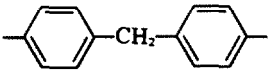
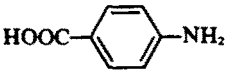
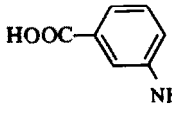
Table 6. Polycondensation of L-leucine in various solvents[a]

Solvents	Yield(%)	ηinh[b]
Pyridine	60	0·25
Benzene	96	0·12
n-Hexane	82	0·12
Acetonitrile	82	0·12
Dichloromethane	82	0·12
Dioxane	64	0·14
DMF	35	0·15
NMP	36	0·15

[a] The reaction was carried out at 40° for 18 h in the presence of two equivalents of pyridine

[b] Measured in dichloroacetic acid at 30°

Table 7. Polycondensation of various diamines with dicarboxylic acids in NMP containing pyridine[a]

Dicarboxylic acids HOOC-R-COOH	Diamines NH_2-R-NH_2	Yield(%)	ηinh[b]
$-(CH_2)_2-$		97	0·32
$-(CH_2)_4-$		97	0·97(0·91)[c]
		97	1·65
		100	1·95[d]
		62	0·22
		100	0·39
		85	0·26
$-(CH_2)_6-$		100	1·05
$-(CH_2)_8-$		96	1·12
		98	1·57
		98	0·30
		98	0·43
		97	0·21
		97	0·29

[a] The reaction was carried out at 100° for 6 h using diphenyl ethylphosphonite
[b] Measured in H_2SO_4 at 30°
[c] Diphenyl phenylphosphonite was used
[d] Measured in NMP at 30°

solution, and that hydrocarbons such as n-hexane and benzene, haloalkane such as dichloromethane and ether solvent such as dioxane, gave polymer in higher yields than high polar amide solvents such as DMF and N-methylpyrrolidone (NMP), despite the heterogeneity of the reaction system in these nonpolar solvents.

Similarly, the direct polycondensation reaction of diamines with dicarboxylic acids was carried out at 100° for 6 h in NMP containing pyridine (Table 7). Combination of aromatic diamines with dicarboxylic acids gave polymers of higher viscosity than aliphatic diamine. On the other hand, 4,4'-diaminodiphenylsulfone and p-phenylenediamine yielded polymer solutions of low viscosity, probably because of the lower basicity of the former diamine and of the lower solubility of the resulting polymer from the latter diamine in the reaction mixture. Among aliphatic dicarboxylic acids, succinic acid produced polyamide with extremely low molecular weight. This unfavorable result might be due to the cyclization involving succinimide structure which might terminate the polycondensation reaction. Aromatic dicarboxylic acids even with an aromatic diamine as well as aromatic amino acids did not form highly viscous polymer solutions.

EXPERIMENTAL

Materials. Triphenyl and triethyl phosphates were obtained from a commercial source. Diphenyl alkylphosphonites,[8] diethyl ethylphosphonite,[9] monoethyl ethylphosphonite,[10] monophenyl diphenylphosphinite,[9] diphenyl n-butylphosphonate[11] and diethyl ethylphosphonate[12] were prepared by literature procedures.

Preparation of Peptides and Active Esters of Amino Acids by Means of Diphenyl Ethylphosphonite. A mixture of equimolar amounts of an Z-amino acid (12·5 mmole), an amino acid ester hydrochloride and diphenyl ethylphosphonite in 40 ml of pyridine was stirred at 40° for 12 h. The reaction was carried out in 40 ml of acetonitrile in the presence of triethylamine (12·5 mmole), when imidazole (25 mmole) was used in place of pyridine. The reaction mixture was worked up by the previously reported procedure[1] to give peptide. In the case of preparing ethyl Z-L-glutaminylglycinate, methylene chloride was used as solvent and the precipitated peptide was filtered off, washed with 2N-hydrochloric acid, sodium bicarbonate solution, water, methylene chloride and petroleum ether. Similarly, the active ester was obtained by the reaction of equivalents of diphenyl ethylphosphonite (12·5 mmole), Z-amino acid, a hydroxyl component and two equivalents of imidazole (25 mmole) at 40° for 12 h in the absence of triethylamine. Preparation of Z-glycinanilide was carried out at 40° for 3 h in acetonitrile in the presence of two equivalents of several tertiary amines, and by varying the amounts of imidazole and diphenyl ethylphosphonite. The reaction was conducted also using several phosphorus compounds under identical conditions.

Polycondensation of Amino Acids in Pyridine. Diphenyl ethylphosphonite (25 mmole) was added to a mixture of amino acids (25 mmole) in 20 ml of pyridine and then the reaction mixture was stirred at 40° for 18 h. Polycondensation in various solvents was carried out as above in 20 ml of solvents containing two equivalents of pyridine. The polymer was obtained by work-up procedure described in the previous papers.[3]

Polycondensation of Diamines with Dicarboxylic Acids. A mixture of diphenyl ethylphosphonite (20 mmole) and equivalents of diamines (10 mmole) and dicarboxylic acids was heated at 100° for 6 h in 40 ml of NMP containing 10 ml of pyridine. For the polycondensation reaction of p- and m-aminobenzoic acids (20 mmole), an equivalent amount of the phosphonite (20 mmole) was used. Polymers were obtained by pouring the reaction mixtures into methanol followed by filtration and drying.

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