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Studies on Reactions of the *N*-Phosphonium Salts of Pyridines. III. A New Method for the Activation of Amines *via* the *N*-Phosphonium Salts by Means of Oxidation of Phosphorous Acid and Its Esters in the Presence of Tertiary Amines

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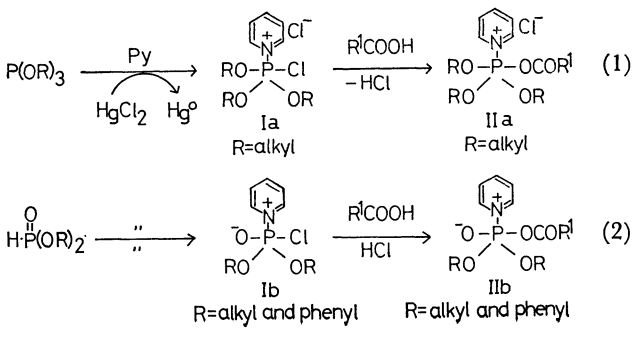
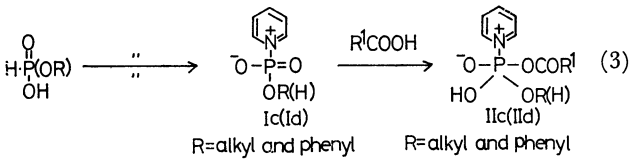
Amines were treated with the *N*-phosphonium salts of pyridine prepared by the oxidation of the phosphorous acid and its esters with mercuric chloride in pyridine; they yielded the corresponding amides in good yields upon acidolysis with carboxylic acids, together with metallic mercury in a nearly quantitative yield. The reaction was studied using phosphorous acid and its several esters, and was presumed to proceed *via* the activation of amines by the *N*-phosphonium salts of pyridine which were characterized by IR spectroscopy. This process for the activation of amino groups was successfully extended to peptide synthesis in pyridine at low temperature using diphenyl phosphite.

We obtained the *N*-phosphonium salts of pyridines by the oxidation of phosphorous acid and its esters in tertiary amines like pyridine.[1-3] The reactions of the salts with carboxylic acids were elucidated by the replacement of the chlorine atom on the salts by the acid (Eqs. 1 and 2), and the nucleophilic attack of the acid on the phosphorus atom (Eq. (3)).

A similar mechanism was proposed in the reaction of the salts (Ia-d) with amine.[3]



1) N. Yamazaki and F. Higashi, Part I, This Bulletin, **46**, 1235 (1973).
2) N. Yamazaki and F. Higashi, Part II, *ibid.*, **46**, 1239 (1973).
3) N. Yamazaki and F. Higashi, *Tetrahedron Lett.*, **1972**, 415.



The present paper deals with detailed studies on the reactions of the *N*-phosphonium salts with the amines, and the application to peptide synthesis.

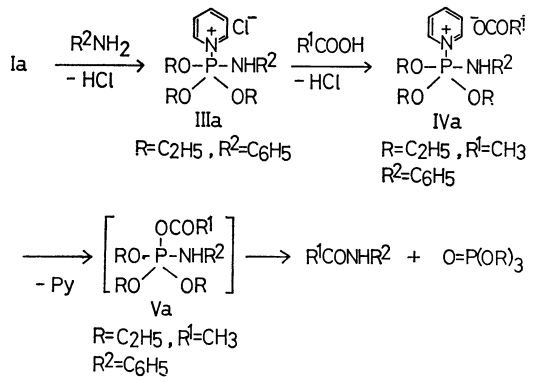
Results and Discussion

The *N*-phosphonium salt of pyridine (I) has been obtained by the oxidation of phosphorous acid and its esters with mercuric chloride in pyridine. When the salt in pyridine was refluxed with aniline for 1 hr and then with acetic acid for 1 hr, acetanilide was obtained in excellent yields. Similarly, several anilides were prepared by using phosphorous acid, its mono-, di- and tri-esters. The results are given in Table 1.

The reaction can be assumed to proceed *via* *N*-phosphonium salt(III) carrying aniline obtained by the reaction of I with aniline. III is converted into IV on treatment with carboxylic acids. IV is unchanged in pyridine at ordinary temperature, but decomposes to the anilide *via* V at higher temperatures (see Scheme 1).

In order to confirm the mechanism, III and IV were separated as syrups containing pyridine hydrochloride.[1] They do not seem to have been purified because of contamination of pyridine hydrochloride and of difficulty in crystallization. Though V could not be isolated, it was considered to take part in the reaction from the result of steric effect of carboxylic acids upon the yield of the anilides.

The structure of Ia ($R=C_2H_5$-) has been discussed previously.[1] The IR spectrum of IIIa ($R=C_2H_5$-), like that of Ia ($R=C_2H_5$-), showed characteristic bands at 1630, 1580 and 1485 cm^{-1} due to $\nu_{C=N^+}$ of the *N*-phosphonium salt of pyridine, and a strong band at 1600 cm^{-1} due to $\nu_{C=C}$ of anilino moiety. From the results and the appearance of the bands due to pyridine hydrochloride after the reaction of Ia ($R=C_2H_5$–) with aniline, IIIa ($R=C_2H_5$–) was assumed to be produced by replacement of a chlorine atom (or ion) on

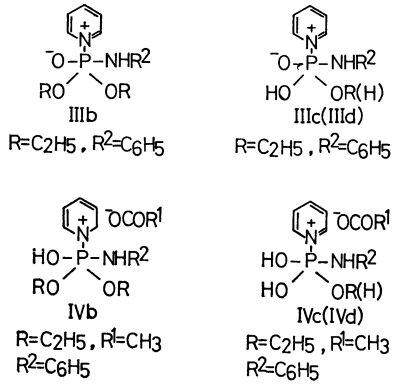


Scheme 1.

Ia ($R=C_2H_5$–) with anilino moiety. The IR spectrum of IVa ($R=C_2H_5$–) also showed bands due to $\nu_{C=N^+}$ of the *N*-phosphonium salt, νC=C of anilino moiety and $\nu_{C=O}$ of acetoxy group.

The reaction with the triester is summarized in Scheme 1.

The reactions with phosphorous acid (Id) and its other esters (Ib and Ic) might proceed in a similar way. The corresponding *N*-phosphonium salts of pyridine were prepared from phosphorous acid (IIId and IVd), its monoester (IIIc and IVc) and its diester (IIIb and IVb), and characterized by IR spectroscopy. These can be envisaged as follows.



The reaction of III with carboxylic acids took place easily and gave better results than that of II with amines. For example, IIId from phosphorous acid gave acetanilide in a nearly quantitative yield (97%) by the reaction with acetic acid for only 1 hr of reflux, whereas in the case of IId more than 2 hr of reflux with aniline was required to obtain the anilide in the same yield.[2]

This would be attributed to the difference in reactivity between IId and IIId toward nucleophiles, since the formation of IId was almost completed under these conditions, the ether extract from the reaction mixture of Id and acetic acid containing no acetic acid.[1]

The facility of acidolysis of III can be explained in terms of the electromeric effect of the anilino group on III. The effect may cause a decrease of electron-density on the phosphorus atom through the migration of the electron of phosphorus-nitrogen (aniline) bond toward the nitrogen atom, resulting in an increase in electrophilicity on the phsophorus atom. Consequently, II can be readily attacked by the carboxylate anions to give the corresponding anilides by way of IV and V.

In the reactions with the monoalkyl esters, the yield of the anilide was unexpectedly low, but that of monophenyl ester was excellent (Table 1). The increase in pK_a of carboxylic acids did not improve the yield (see trifluoroacetanilide), suggesting that the rate-determining step in the reaction with monoalkyl esters is the nucleophilic attack of aniline in the phosphorus atom on Ic. Therefore, the presence of electron-attracting groups such as the phenyl group, increased the electrophilicity on the phosphorus atom, facilitating the attack of aniline on Ic. On the other

hand, the electron-donating nature of alkyl groups retarded the reaction.

A similar effect caused by the electronic nature of alkyl and phenyl groups was also expected in the reactions with the diesters, but no effect was observed. It seems reasonable to assume that the reaction of Ib with aniline is unaffected by the electrophilicity on the phosphorus atom because of an easy replacement reaction of the chlorine atom (or ion) in Ib by the anilino group in a similar manner to Eq. (2).

The yield of the anilides was affected by the steric effect of amines, carboxylic acids and tertiary amines, similarly to those in the reactions of I with carboxylic acids.[1,2]

The steric effect of carboxylic acids upon the yield was reflected in the lower yields of pivalanilide than those of acetanilide (see Table 1). Pivalanilide was obtained in nearly the same amount by acidolysis of III as by aminolysis of II. The results led us to consider that the nucleophilic attack of carboxylate anions on the phosphorus atom is involved in the reaction, by possible destruction of the phosphorus-nitrogen (pyridine) bond to give V.

Another possible route of reaction *via* anilinophosphoramides was excluded by the fact that diethyl anilinophosphoramidate yielded no acetanilide when refluxed with acetic acid in pyridine.

Carboxylic amides were proved to be produced through a similar activation of amines to that of carboxylic acids. They were also obtained in the presence of both amines and carboxylic acids.[3] Consequently, three procedures were available for the preparation of carboxylic amides by means of the *N*-phosphonium salts of pyridines, *viz.*, aminolysis after activation of carboxyl component (method A), acidolysis after that of the amino component (method B) and the activation instantly followed by the coupling reaction in the presence of both components (method C).

The three methods were tested in the reaction of benzyloxycarbonyl-glycine and ethyl glycinate in order to find the most effective one for peptide synthesis. Both activation and coupling reactions were carried out at low temperature (45 °C) to prevent racemization. The activation procedures were achieved by adding the amino and/or carboxyl components to the mixture prepared by the oxidation of diphenyl phosphite with mercuric chloride in pyridine.[3] Methods B and C were preferable to A, indicating that even in the presence of both components the amino component was preferably activated to yield the peptide in a good yield (Table 2).

A variety of phosphorus compounds were examined

Table 1. Preparation of anilides through activation of aniline using various phosphorus compounds and mercuric chloride in tertiary amines

Phosphorus compounds	Tertiary amines	Anilides	Yield, %
Phosphorous Acid	Py	Acetanilide	97
Phosphorous Acid	Py	*N*-Methylacetanilide	53
Phosphorous Acid	Py	Pivalanilide	27
Phosphorous Acid	2-Me-Py	Acetanilide	69
Phosphorous Acid	4-Me-Py	Acetanilide	77
Monoethyl Ester	Py	Acetanilide	33
Monoethyl Ester	Py	Trifluoroacetanilide	28
Monoisopropyl Ester	Py	Acetanilide	45
Monophenyl Ester	Py	Acetanilide	96
Diethyl Ester	Py	Acetanilide	93
Diisopropyl Ester	Py	Acetanilide	95
Diisopropyl Ester	Py	Pivalanilide	65
Diphenyl Ester	Py	Acetanilide	95
Triethyl Ester	Py	Acetanilide	62
Triisopropyl Ester	Py	Acetanilide	86

Table 2. Peptide synthesis using diphenyl phosphite in pyridine by several methods

Peptides	Yield, %[a]	Method[b]	Mp, °C	$[\alpha]_D$
Z-Gly-Gly-OEt	84	A	80	
Z-Gly-Gly-OEt	92	B	80	—
Z-Gly-Gly-OEt	95	C	80	
Z-Phe-Gly-OEt (L)	90	B	108—109	−17.7° (*c* 2, EtOH)
Z-Gly-Tyr-OEt (L)	90	B	125—126	+19.8° (*c* 5, EtOH)
Z-α-Glu-Gly-OEt (L)	70	B	122—123	—
Z-Glu(NH_2)-Gly-OEt (L)	79	C	166—168	−6.5° (*c* 1, DMF)
Z-Met-Gly-OEt (DL)	93	C	72—73	—

a) The coupling reactions were carried out at 45 °C for 12 hr.
b) Method A; Through activation of carboxyl components.
Method B; Through activation of amino components.
Method C; Activation and coupling in the presence of both components.

TABLE 3. PREPARATION OF Z-Gly-Gly-OEt USING VARIOUS PHOSPHORUS COMPOUNDS

Phosphorous compounds	Yield, %[a)]
Phosphorous Acid	4
Monophenyl Ester	20
Diisopropyl Ester	18
Diphenyl Ester	95
Triisopropyl Ester	23

a) The reactions were carried out at 45 °C for 12 hr by method C.

in the reaction between benzyloxycarbonyl-glycine and ethyl glycinate by method C (Table 3). Diphenyl phosphite was found to be most effective. This may be due to the increase in electrophilicity of the phosphorous atom caused by the electron-attracting nature of the phenyl groups in the phosphite.

Several other peptides were also synthesized using diphenyl phosphite by methods B and C. The results are given in Table 2. Good yields without detectable racemization were obtained within experimental errors.

It is surprising that the presence of the free side chains of glutamic acid, glutamine and methionine in the carboxyl component, and of tyrosine in the amino component did not cause any difficulty in the reactions. For example, the α-carboxyl rather than the γ-carboxyl group in glutamic acid referred to be activated, producing the α-peptide in a high yield. Similarly, a peptide of glutamine was obtained accompanied by no side reactions such as nitrile formation.

The oxidation mixture involving the *N*-phosphonium salts of pyridine could activate not only carboxyl and amino groups but also hydroxyl groups.[3)] Thus, a compeptitive activation of the amino and hydroxyl groups was expected in the reaction between ethyl tyrosinate and benzyloxycarbonyl-glycine, giving the corresponding amide and ester. The result shows that the amino group in ethyl tyrosinate was preferably activated to give the amide without yielding any detectable amount of the ester.

Experimental

Preparation of I, III and IV. Preparation of I was described in previous papers.[1,2)] A mixture of equimolar amounts of triethyl phosphite (2.1 g, 12.5 mmol) and mercuric chloride (3.5 g) was refluxed for 1 hr in 20 ml of pyridine, and for 1 hr after addition of aniline (1.2 g, 12.5 mmol). From the mixture, IIIa ($R=C_2H_5-$) was obtained as an ether-insoluble syrup by a similar procedure to that for Ia($R=C_2H_5-$). When IIIa ($R=C_2H_5-$) was treated with acetic acid at room temperature, IVa ($R=C_2H_5-$) was also obtained as a syrup. Similarly, IIIb~IIId and IVb~IVd were obtained.

Preparation of Anilides through the Activation of Aniline. A mixture of equimolar amounts of phosphorous acid and its esters (12.5 mmol) and mercuric chloride was heated at 115 °C for 1 hr in 20 ml of tertiary amines. The resulting mixture was treated with aniline (12.5 mmol) for 1 hr under reflux, and then with equimolar amounts of carboxylic acids for 1 hr. The resulting anilides were isolated and purified according to the previously reported procedures.[3)] The anilides thus obtained had melting points and infrared spectra identical with those of authentic samples.

Preparation of Peptides using Phosphorous Acid and Its Esters in Pyridine. A mixture of equimolar amounts of phosphorous acid and its esters (12.5 mmol) and mercuric chloride was refluxed for 1 hr in 20 ml of pyridine.

Method A: The oxidation mixture was treated with a benzyloxycarbonyl amino acid (12.5 mmol) in 10 ml of pyridine. After 1 hr, an amino acid ester hydrochloride (12.5 mmol) was added to the mixture, and then the solution was kept at 45 °C for 12 hr. The peptide formed was isolated and purified as described previously.[3)]

Method B: The oxidation mixture was treated as in method A with an amino acid ester hydrochloride, a benzyloxycarbonyl amino acid being added after 1 hr, and the mixture kept under the same conditions.

Method C: A mixture of a benzyloxycarbonyl amino acid and an amino acid ester hydrochloride in 20 ml of pyridine was added to the oxidation mixture, and then the solution was kept under the same conditions as for method A.