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Amino Acids and Peptides. X.[1] Phosphorus in Organic Synthesis. V.[2] On the Mechanism for the Peptide Synthesis by Diphenyl Phosphorazidate[3]

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The peptide bond formation reaction by some analogs of diphenyl phosphorazidate (DPPA) was investigated, proving that DPPA is much superior in the Young test to diethyl phosphorazidate, di-*p*-nitrophenyl phosphorazidate, dimorpholyl phosphorazidate, and diphenyl phosphorochloridate. Some spectroscopic investigations on the DPPA method were also made. Possible mechanisms for the DPPA method are presented in Chart 1.

In our preceeding paper[1] we have reported diphenyl phosphorazidate (DPPA) may be used for the racemization-free peptide synthesis. We wish to describe here some considerations on the mechanism for the DPPA method.

First we investigated the coupling ability of some DPPA analogs using the Young test[5] which involves the coupling of benzoyl-L-leucine with glycine ethyl ester.

Diethyl phosphorazidate which was used for the preliminary experiments[1] on the reaction between phosphoryl azides and carboxylic acids caused the coupling of benzoyl-L-leucine with glycine ethyl ester hydrochloride to give benzoylleucylglycine ethyl ester with high optical rotation but in unfavorable yield. As the ethyl group is less electron-attractive than the phenyl group, the nucleophillic attack of the carboxylate anion to the phosphorus atom of diethyl phosphorazidate would be less favored.

This led us to investigate the use of di-*p*-nitrophenyl phosphorazidate which might be more easily attacked by the carboxylate anion. First we tried to prepare di-*p*-nitrophenyl phosphorazidate by the action of sodium azide on di-*p*-nitrophenyl phosphorochloridate[6] as in the preparation of DPPA.[1] However, no successful results were obtained. Thus, nitration of DPPA was examined to give the corresponding di-*p*-nitro derivative in good yield.

Indeed introduction of the nitro group at the *para* position of the phenyl group of DPPA increased the yield of benzoylleucylglycine ethyl ester, but racemization occurred to a great extent. Coexistence of 2 equivalents of N-hydroxysuccinimide known as a racemization-suppressing additive[7] in the N,N'-dicyclohexylcarbodiimide method neither improved the yield nor the extent of racemization.

Dimorpholyl phosphorazidate prepared from the corresponding chloride in the usual manner almost lacked the coupling ability to afford only a trace of benzoylleucylglycine ethyl

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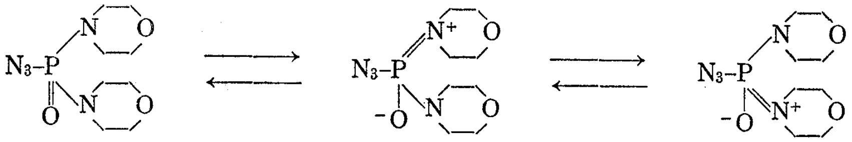
[1] Part IX: T. Shioiri and S. Yamada, *Chem. Pharm. Bull.* (Tokyo), **22**, 849 (1974).
[2] Part IV: Ref. 1).
[3] Preliminary communication: T. Shioiri, K. Ninomiya, and S. Yamada, *J. Am. Chem. Soc.*, **94**, 6203 (1972). Presented in part at the 9th Symposium on Peptide Chemistry, Shizuoka, November 24, 1971, Abstracts, p. 5; and at the Symposium on Organophosphorus Compounds, Tokyo, January 20, 1972, Abstracts, p. 17.
[4] Location: *Hongo, Bunkyo-ku, Tokyo, 113, Japan.*
[5] M.W. Williams and G.T. Young, *J. Chem. Soc.*, **1963**, 881.
[6] T. Ukita and H. Hayatsu, *J. Am. Chem. Soc.*, **84**, 1879 (1962); Z. Arnold and A. Holy, *Collection Czech. Chem. Commun.*, **27**, 2886 (1962).
[7] F. Weygand, P. Hoffmann, and E. Wünsch, *Z. Naturforsch.*, **21b**, 426 (1966).

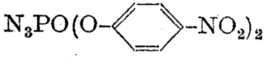
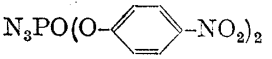
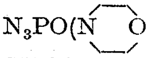
ester. When benzoyl-L-leucine was treated with an equimolecular mixture of dimorpholyl phosphorazidate and N-methylmorpholine in ethyl acetate, no spot of benzoylleucyl azide[5] which is easily formed by DPPA under similar reaction conditions could not be detected on a thin-layer chromatogram. This poor result is not unexpected, because the positive character of the phosphorus would be suppressed by the existence of $p\pi$—$d\pi$ orbital overlap as follows:



Diphenyl phosphorochloridate has already been known as a peptide coupling reagent,[8] but under the standard conditions described in Experimental the chloridate gave benzoylleucylglycine ethyl ester in miserable yield. Although a little improvement of the yield was observed when the Young test was carried out by the Zervas procedure[8] (see Experimental), extensive racemization occurred.

These results summarized in Table I showed that DPPA is the most suitable coupling reagent especially for the racemization-free peptide synthesis.

TABLE I. The Young Test by DPPA Analogs

Coupling reagent	Bz-Leu-Gly-OEt			
	Yield (%)	mp (°C)	$[\alpha]_D$	L-Isomer[a] (%)
$N_3PO(OCH_2CH_3)_2$	28	156—159	−31.0	91
	92	145—146	−14.0	41
	72	144—145	−15.3	45[b]
	trace	—	—	—
$ClPO(OPh)_2$	10	146—147	<− 0.3[c]	<1
$ClPO(OPh)_2$	36	146—148	− 1.4	4[d]

a) excluding L-isomer present as racemate[1,5]
b) with 2 equiv. of N-hydroxysuccinimide
c) The observed rotation was too small to measure accurately.
d) by Zervas procedure[8]

Next we made some spectroscopic investigations about racemization during the Young test using DPPA. When an equimolecular mixture of benzoyl-L-leucine and triethylamine in chloroform was treated with an equimolecular DPPA at 0°, no oxazolone formation mainly responsible for the racemization[9] could be detected on the infrared absorption at 1830 cm^{-1} even after 1 hr. It should be noted, however, that the week but appreciable oxazolone absorption appeared within 0.5 hr on a similar treatment of a mixture of benzoyl-L-leucine and triethylamine by DPPA in methylene chloride at 10—15°. This is in accord with the result of the Young test by DPPA in methylene chloride which was proved to be not so advantageous.[1] Nevertheless it seems that the DPPA method operated under the standard conditions is remarkably resistant to racemization, especially in a highly polar solvent dimethylformamide.[1]

In consequence, the processes shown in Chart 1 warrant consideration for the coupling by DPPA. The carboxylate anion may attack the phosphorus atom of DPPA to give the

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[8] A. Cosmatos, I. Photaki, and L. Zervas, *Chem. Ber.*, 94, 2644 (1961).
[9] M.W. Williams and G.T. Young, *J. Chem. Soc.*, 1964, 3701.

pentacovalent phosphorus compound (I) and the mixed anhydride (II). Equilibrium will exist between I and II. The amino component will attack I and II to give the amide. However, when the Young test was carried out after the formation of II by the action of diphenyl phosphorochloridate with benzoyl-L-leucine in the presence of triethylamine according to the Zervas procedure, both the yield and the optical purity were inferior to those of the DPPA method. This shows that direct attack of the amino component to II will occur to a lesser extent. The intramolecular migration of the azido group of I from the phosphorus to the carboxyl carbon by an *Sni* type rearrangement will give the carboxylic acid azide (III), which may be also formed from II by an *S*$_N$2 type reaction of the azide anion with II. The intermediacy of the acyl azide (III) was well demonstrated by the detection of benzazide[1] on a thin-layer chromatogram when benzoic acid was condensed with *n*-butylamine by a mixture of DPPA and triethylamine. The acyl azide (III) is an intermediate of the well-known azide method which is essentially free of racemization. Thus III will give the amide without racemization. When the amino component reacts with I, the coupling reaction may proceed in a concerted manner as shown in Chart 1, because the Young test by DPPA gave a successful result in the optical purity as well as the yield.

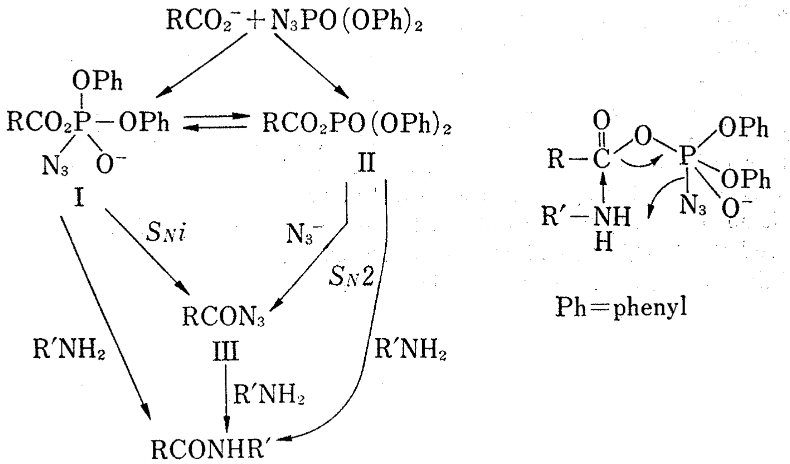

Chart 1

Experimental

Melting points were determined on a hot stage apparatus and uncorrected. Infrared (IR) spectra were determined in nujol, and proton magnetic resonance spectra in deuteriochloroform.

Di-*p*-nitrophenyl Phosphorazidate——i) In Carbon Tetrachloride: To DPPA (2.81 g, 0.01 mole) in carbon tetrachloride (15 ml) was added fuming nitric acid ($d=1.5$, 5 ml) during 10 min at −5——10°. The mixture was stirred at 25° for 43 hr, and poured onto ice, followed by the addition of chloroform. The white precipitates were filtered, and from the filtrate was separated the organic layer. The water layer was extracted with chloroform. The combined organic extracts were dried over sodium sulfate, and evaporated to give yellow crystals, which were recrystallized from carbon tetrachloride to afford slightly yellow prisms (1.40 g, 38%), mp 105—107°.

ii) To DPPA (8.27 g, 0.03 mole) was added fuming nitric acid ($d=1.5$, 26 ml) at 0—5° during 1 hr 15 min with stirring. After the addition, the mixture was stirred at 0—5° for 0.5 hr. The yellow reaction mixture was poured onto ice-water (100 ml) to give slightly yellow precipitates, which were filtered, washed with water (200 ml), and dried over potassium hydroxide and phosphorus pentoxide *in vacuo* overnight. The precipitates were dissolved in methylene chloride (50 ml), and the solution was washed three times with water. Drying over sodium sulfate followed by evaporation gave a yellow solid (9.3 g, 85%), mp 104—108°. Recrystallization from methylene chloride followed by washing the crystals with diisopropyl ether gave colorless prisms, mp 108—110°. *Anal.* Calcd. for $C_{12}H_8O_7N_5P$: C, 39.46; H, 2.21; N, 19.18. Found: C, 39.37; H, 2.30; N, 18.99. IR ν_{max}: 2200 cm^{-1} (N_3). NMR δ: 7.4, 8.3 (8H, doublet-doublet, AB type, $J=8$ Hz).

Dimorpholyl Phosphorazidate——A mixture of dimorpholyl phosphorochloridate[10] (12.7 g, 0.05 moles) and sodium azide (4.9 g, 0.075 moles) in dry acetone (150 ml) was refluxed for 18 hr. To the cooled sus-

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[10] H.A.C. Montgomery and J.H. Turnbull, *J. Chem. Soc.*, **1958**, 1963.

pension was added dry diethyl ether (100 ml), and the mixture was filtered. The filtrate was evaporated *in vacuo* to leave a slightly yellow oil (12.38 g), which was crystallized by scratching. Recrystallization from a mixture of acetone and *n*-hexane gave 1.11 g of colorless prisms, mp 167—187°, whose structure is undetermined. The mother liquor was evaporated to give a slightly yellow oil which crystallized upon standing at room temperature. Washing the crystals with a mixture of dry diethyl ether and *n*-hexane gave 9.05 g (69%) of colorless hygroscopic needles, mp 44—46°. *Anal.* Calcd. for $C_8H_{16}O_3N_5P$: C, 36.78; H, 6.17; mol. wt. 261.22. Found: C, 37.19; H, 6.28. Mass Spectrum *m/e*: 261 (M^+). IR ν_{max}: 2200 cm^{-1} (N_3). NMR δ: 3.1 (8H, multiplet), 3.6 (8H, multiplet).

The Young Test; General Procedure——To a stirred mixture of benzoyl-L-leucine[5] (0.47 g, 2 mmoles) and glycine ethyl ester hydrochloride (0.31 g, 2.2 mmoles) in dimethylformamide (5 ml) was added a coupling reagent (2.2 mmoles) in dimethylformamide (5 ml) at 0°, followed by the addition of triethylamine (0.42 g, 4.2 mmoles) in dimethylformamide (5 ml) at 0° during 5—10 min. The mixture was stirred at 0° for 0.5 hr, and then at 20° for 4 hr.

The reaction mixture was diluted with a mixture of benzene (50 ml) and ethyl acetate (100 ml), and successively washed with 5% aqueous hydrochloric acid (2 × 10 ml), water (10 ml), saturated aqueous sodium chloride (2 × 10 ml), saturated aqueous sodium bicarbonate (2 × 10 ml), water (10 ml), and saturated aqueous sodium chloride (2 × 10 ml). Drying over sodium sulfate followed by evaporation gave a mixture of crystals and an oil, which subjected to a silica gel column chromatography (Wakogel C-200, 70 g). Elution with a mixture of chloroform and ethyl acetate (10: 1) gave colorless crystals of benzoylleucylglycine ethyl ester, whose structure was confirmed by thin-layer chromatographic and infrared spectral comparisons with the authentic ones.[5] These crystals were weighed, and the mp (lit. values:[5] L-isomer, 156.5—157°; racemate, 146°) was determined. Optical rotations were measured in ethanol at 21—23° (c=2—3). The results are summarized in Table I.

The Young Test Using Diphenyl Phosphorochloridate by the Zervas Procedure[8]——To a stirred mixture of benzoyl-L-leucine (0.47 g, 2 mmoles) and triethylamine (0.20 g, 2 mmoles) in dimethylformamide (8 ml) was added diphenyl phosphorochloridate (0.595 g, 2.2 mmoles) in dimethylformamide (3 ml) at 0°. After 10 min stirring, glycine ethyl ester hydrochloride (0.31 g, 2.2 mmoles) was added to the reaction mixture, followed by the addition of triethylamine (0.22 g, 2.2 mmoles) in dimethylformamide (4 ml) during 5 min. The mixture was stirred at 0° for 1 hr, and at 20—25° for 22 hr. The reaction mixture was treated as in the general procedure of the Young test. The result is shown in Table I.