

observed optical absorptions are the result of intermolecular charge-transfer complex formation.

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Reaction of Fluoro-2,4-dinitrobenzene with Amino-Acids and Peptides

THE reaction of fluoro-2,4-dinitrobenzene with the terminal amino-group of peptides and proteins under mild alkaline conditions has been widely used for the quantitative assay of *N*-terminal amino-acids¹. In the corresponding reaction with amino-acids, it has been shown² that some 2,4-dinitrophenyl-peptide is formed, the proportion of the DNP-peptide increasing with the separation of the amino- and carboxyl-functions.

A peptide linkage is readily formed between an *N*-substituted amino-acid DNP-ester and an amino-acid³ and this mechanism could be responsible for DNP-polymer formation through the intermediate DNP-amino-acid DNP-ester.

The bimolecular nucleophilic substitution of fluorine by a carboxylate ion should be related to the basicity of the anion present in the alkaline solution⁴. The nucleophilic power of the attacking reagent would then increase with increasing value of pK_1 for the substituted amino-acid. The reaction-rate is also influenced by the ion-pairing tendency of carboxylate ion in the substrate, following the same order of accelerating power as in the above sequence.

O-dinitrophenylation would be more likely to occur in the presence of an excess of fluoro-2,4-dinitrobenzene than when an excess of amino-acid is present. Increasing yields of DNP-amino-acid have been obtained by raising the concentration of amino-acid in the mixture⁵, or raising the reaction temperature and evaporating the mixture to dryness⁶. Once the DNP-amino-acid DNP-ester is formed, the reaction of this 'mixed anhydride' with a free amino-group would occur in competition with the reaction of fluoro-2,4-dinitrobenzene at the same amino-group.

The intermediate DNP-amino-acid DNP-ester has now been isolated by paper chromatography of the products of reaction between fluoro-2,4-dinitrobenzene and β -alanine. The products from a typical reaction are (R_F values in brackets), ethyl 2,4-dinitrophenyl ether (0.95), *bis*-2,4-dinitrophenyl ether (0.73), 2,4-dinitrophenyl- β -alanine 2,4 dinitrophenyl ester (0.63), 2,4-dinitrophenol (0.53), 2,4-dinitroaniline (0.46), 2,4-dinitrophenyl- β -alanine (0.26), 2,4-dinitrophenyl- β -alanyl- β -alanine (0.06), and 2,4-dinitrophenyl-di- β -alanyl- β -alanine (0.01). The solvent system was 95 per cent propanol, 95 per cent acetic acid and petroleum ether (b.p. 100–120°), (20 : 6 : 100 v/v), moving on Whatman No. 1 paper buffered with 0.1 *M* citric acid. Elution of the 'active ester' and subsequent reaction with β -alanine gave β -alanyl- β -alanine and 2,4-dinitrophenol. A similar intermediate phosphate DNP-ester has been isolated, in high yield, from the

reaction between a monophosphate ester and fluoro-2,4-dinitrobenzene in the presence of pyridine⁶. This base must be excluded from reactions involving amino-acids, due to the concurrent ring opening of the intermediate pyridinium fluoride in the presence of a primary amino-group.

The reaction of fluoro-2,4-dinitrobenzene with a series of ω -amino-acids, including aspartic acid and glutamic acid, also produced considerable amounts of DNP-polymers. In the case of glutamic acid the identity of the polymers as γ -glutamyl peptides has been shown by comparison with authentic material prepared by the phthaloylglutamic anhydride procedure⁷. The corresponding α -glutamyl compounds were made from the acid chloride of phthaloylglutamic acid γ -methyl ester.

In related reactions with glycine, glycyglycine, and diglycyglycine, the proportion of dimer was only significant with the latter compound, although the formation of DNP-glycyglycine from DNP-glycine has been reported². Observations on higher molecular weight starting material are rendered difficult by the low mobility of the DNP-polymers on chromatograms. The results of these experiments are shown in Table 1.

Table 1. DNP-POLYMERS FORMED BY REACTION OF MONOMER WITH FLUORO-2,4-DINITROBENZENE

Monomer	pK_1	No. of monomer units in polymer				
		2	3	4	5	
Glycine	2.34	trace	—	—	—	—
β -Alanine	3.60	+	+	+	—	—
ϵ -Aminocaproic acid	4.43	+	+	+	+	+
Glutamic acid	2.19	+	+	+	—	—
	4.25	+	+	trace	—	—
Aspartic acid	1.88	+	+	trace	—	—
	3.65	+	+	trace	—	—
Glycyglycine	3.12	+	trace	?	?	?
Diglycyglycine	3.26	+	?	?	?	?

Reaction of an excess of fluoro-2,4-dinitrobenzene with glutamic acid at pH 8.5 and 40°, washing with hexane followed by further reaction with glutamic acid, gave substantially increased yields of the DNP- γ -dimer with little of the higher polymers. The free dipeptide was obtained by fast hydrogenolysis using a large excess of platinum catalyst, followed by extraction from the *m*-phenylenediamine produced⁸. This reaction is now being applied to the synthesis of peptides containing aminoalkylphosphonic acid residues.

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Solid-State Polymerization of Phosphonitrilic Chloride

SINCE the work of Mesrobian *et al.*¹ on the polymerization of acrylamide, many monomers have been polymerized in the solid state by γ -rays or electron beam^{2–4}. Hayashi and Okamura⁵ have recently irradiated cyclic monomers and found that the rate of polymerization increases up to the melting point of