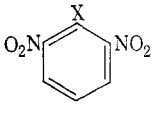
THE REACTION OF 1-FLUORO-2,6-DINITROBENZENE WITH SOME AMINO ACIDS*

By J. F. K. WILSHIRE†

One of the earliest and still one of the most successful methods for the determination of the *N*-terminal amino acids of peptides and proteins is the Sanger method[1] which employs the reagent 1-fluoro-2,4-dinitrobenzene.‡ From time to time, other nitroaryl reagents have been used, for example, 2,4,6-trinitrobenzenesulphonic acid[2] and 1-fluoro-4-methoxycarbonyl-2-nitrobenzene,[3] but none has yet found wide acceptance among protein chemists. Potentially the most useful of these reagents appears to be 2,4,6-trinitrobenzenesulphonic acid which has recently been used as a colorimetric reagent in place of ninhydrin in the automatic amino acid analyser[4] and has the advantage that the amino acid being analysed can be recovered as its *N*-2,4,6-trinitrophenyl derivative after colorimetry.



(I) X = F
(II) X = Cl
(III) X = NHCH(*R*)CO_2H

In view of a recent report[5] that 1-fluoro-2,6-dinitrobenzene (I)‡ reacts one-half as fast as 2,4-FDNB with aniline in ethanol, it seemed likely that 2,6-FDNB would have a similar order of reactivity *vis-à-vis* 2,4-FDNB towards amino acids and might also be a useful reagent in protein chemistry. 2,6-FDNB (I) was therefore prepared (in 90–96% yield) by the reaction of potassium fluoride with 1-chloro-2,6-dinitrobenzene (II) in dimethyl sulphoxide and made to react with a number of representative amino acids§ under the conditions normally used for the preparation of 2,4-DNP-amino acids (aqueous ethanolic solution containing sodium bicarbonate at room temperature). In the event, high yields of the corresponding 2,6-DNP-amino acids (III) were obtained (see Tables 1 and 2).

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† Division of Protein Chemistry, CSIRO Wool Research Laboratories, Parkville, Vic. 3052.

‡ Abbreviations: 2,4-FDNB for 1-fluoro-2,4-dinitrobenzene; 2,6-FDNB for 1-fluoro-2,6-dinitrobenzene; DNP for *N*-dinitrophenyl.

§ The reaction of 2,6-FDNB with alcohols, phenols, thiophenols, and anilines has already been reported.[6]

[1] Sanger, F., *Biochem. J.*, 1945, **39**, 507.

[2] Okuyama, T., and Satake, K., *J. Biochem., Tokyo*, 1960, **47**, 454.

[3] Holley, R. W., and Holley, A. D., *J. Am. chem. Soc.*, 1952, **74**, 5445.

[4] Satake, K., Take, T., Matsuo, A., Tazaki, K., and Hiraga, Y., *J. Biochem., Tokyo*, 1966, **60**, 12.

[5] Parker, R. E., and Read, T. O., *J. chem. Soc.*, 1962, 3149.

[6] Vorozhtsov, N. H., Sokolenko, V. A., and Yakobson, G. G., *Izv. sib. Otdel. Acad. Nauk SSSR.*, 1962, 87 (*Chem. Abstr.*, 1963, **59**, 1507*a*).

Both L-tyrosine and L-histidine gave bis-2,6-DNP-derivatives, which shows that the imidazole and phenolic side-chains of these amino acids also reacted readily with the reagent. L-Lysine appeared to give the bis-2,6-DNP-amino acid but a suitable crystallization solvent for the product could not be found.

TABLE 1

N-2,6-DINITROPHENYL DERIVATIVES OF SOME AMINO ACIDS

Compound	Parent Compound	M.P. of 2,6-DNP-Derivative	Yield (%)	Solvent[a]
(1)	Glycine	182–184°	86	A
(2)	L-Alanine	145–147	78	A
(3)	L-Phenylalanine	138–140	75	A
(4)	L-Proline	136–138	84	A
(5)	L-Serine	154–156[b]	79	B
(6)	L-Threonine	123–125[b]	76	A
(7)	L-Valine	97–99	85	C
(8)	L-Tyrosine	142–145	93	A
(9)	L-Histidine	225 (dec.)	79	D
(10)	L-Lysine	105–110[b]	69	—[c]

[a] Recrystallization solvent: A, aqueous ethanol; B, water; C, hexane–methylene chloride; D, aqueous acetone.

[b] Product isolated by ether extraction of acidified solution (see Experimental).

[c] No suitable crystallization solvent found. Analysis performed on crude product.

TABLE 2

ANALYTICAL DATA FOR *N*-2,6-DINITROPHENYL DERIVATIVES

Compound	Molecular Formula	Found (%)			Calc. (%)		
		C	H	N	C	H	N
(1)	$C_8H_7N_3O_6$	39·9	3·2	17·3	39·8	2·9	17·4
(2)	$C_9H_9N_3O_6$	42·2	3·5	16·5	42·4	3·6	16·5
(3)	$C_{15}H_{13}N_3O_6$	54·6	4·1	12·5	54·4	4·0	12·7
(4)	$C_{11}H_{11}N_3O_6$	47·0	4·0	14·7	47·0	3·9	14·9
(5)	$C_9H_9N_3O_7$	40·1	3·5	15·4	39·9	3·3	15·5
(6)	$C_{10}H_{11}N_3O_7$	41·8	3·9	14·5	42·1	3·9	14·7
(7)	$C_{11}H_{13}N_3O_6$	46·2	4·6	14·9	46·6	4·6	14·8
(8)	$C_{21}H_{15}N_5O_{11},H_2O$	47·7	3·2	13·3	47·5	3·2	13·2
(9)	$C_{18}H_{13}N_7O_{10}$	44·9	2·9	20·0	44·4	2·7	20·1
(10)	$C_{18}H_{18}N_6O_{10}$	45·6	4·0	17·5	45·2	3·8	17·6

2,6-FDNB can therefore be regarded as a useful reagent for the characterization of α-amino acids. Work which is now in progress to assess its usefulness as a reagent for the determination of the *N*-terminal amino acids of peptides and proteins will be reported elsewhere.

Experimental

Reagent grade potassium fluoride was heated in an oven to 120°, powdered, and kept at 120° for at least 24 hr before use. All melting points are uncorrected. The elementary analyses were carried out by the Australian Microanalytical Service, Melbourne. All analytical samples were dried under vacuum over P_2O_5 at 60° for 4 hr.

Preparation of 1-Fluoro-2,6-dinitrobenzene

1-Chloro-2,6-dinitrobenzene (Aldrich Chem. Co.) (40 g) was stirred in dimethyl sulphoxide (Fluka grade used without further purification) (48 g) with powdered anhydrous potassium fluoride (24 g) for 8 hr on the steam-bath. The reaction mixture was poured onto ice and the semi-solid mixture extracted with benzene–ether (1 : 1; 300 ml). The organic layer was washed successively with water, with 1M sodium bicarbonate (2 × 100 ml), and finally with water (3 × 100 ml) before being dried over anhydrous Na_2SO_4. Removal of the solvent left a solid which was crystallized from light petroleum to give 1-fluoro-2,6-dinitrobenzene as colourless rhombs (25·0 g), m.p. 60–62° (lit.[6] m.p. 60–61°). The filtrate was evaporated to dryness and the oily residue rapidly crystallized. This product was covered with a little cold pentane and filtered to give a further quantity of product (10·1 g, m.p. 60–62° with slight softening at 57°). The absence of starting material in both products was shown by thin-layer chromatography (silica gel G: benzene–light petroleum (2 : 1)). Total yield was 35·1 g (96%). Although this product was pure enough for the reaction with amino acids, a further purification was obtained by distilling a portion (29·9 g) under vacuum. The distillate (28·9 g) had b.p. 130°/0·4 mm and m.p. 62–63°.

When the fluoride exchange reaction was carried out for a longer period (24 hr), a slightly lower yield (90%) of product, m.p. 57–60°, was obtained.

This procedure gives a somewhat higher yield than that described by Vorozhtsov *et al.*[6] who carried out the fluoride exchange reaction in the absence of solvent.

Reaction of 1-Fluoro-2,6-dinitrobenzene with Amino Acids

The reaction with L-proline is typical. Sodium bicarbonate (504 mg; 6 mmole) and L-proline (345 mg; 3 mmole) were dissolved in water (17 ml). 2,6-FDNB (558 mg; 3 mmole) in ethanol (20 ml) was added and the mixture stirred (magnetically) for 2 hr. The resultant orange solution was diluted with water (20 ml) and extracted with ether. The lower alkaline layer (occasionally the addition of sodium chloride was required to separate the layers) was removed and acidified with 1N hydrochloric acid to give a yellow solid (707 mg; 84% yield), m.p. 135–137°. The analytical sample (needles from aqueous ethanol) had m.p. 136–138°.

Occasionally (see Table 1), when no precipitate was obtained on acidification, or the yield of 2,6-DNP-amino acid was low, or the oily product could not be induced to solidify, the acidified solution was extracted with ether. The ether extract was washed with saturated salt solution (3 × 25 ml) and dried over anhydrous sodium sulphate. Removal of the solvent gave the 2,6-DNP-amino acid either directly as a solid or as an oil which solidified readily on trituration with pentane. In the case of the bis-2,6-DNP-derivative of L-lysine, solidification was very slow and was only complete after 2 weeks.

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