1-Cyano-4-dimethylamino-pyridinium Salts: New Water-soluble Reagents for the Cyanylation of Protein Sulphydryl Groups

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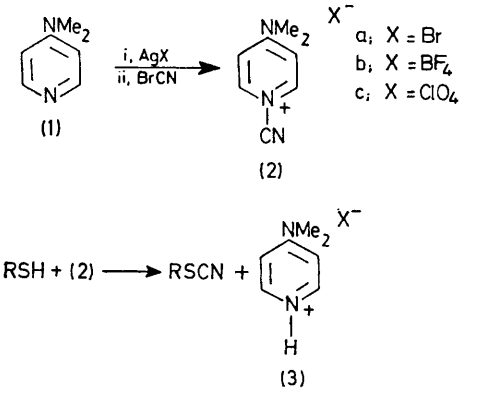
Summary 1-Cyano-4-dimethylaminopyridinium perchlorate or fluoroborate rapidly react with thiols in neutral or acidic medium: in 11 min at pH 3·6, 98% of the catalytic activity of papain is inhibited; cysteine residues nos. 7 and 19 of the reduced B-chain of bovine insulin are quantitatively cyanylated at pH 3·5, then the *N*-peptide bonds are selectively cleaved at pH 9·5.

1-ACYLATED DERIVATIVES of 4-dimethylaminopyridine can be considered as stabilised *N*-acylpyridinium ions.[1,2] 1-Cyano-4-dimethylaminopyridinium perchlorate and fluoroborate[3] have been prepared and their reactions with protein sulphydryl groups in aqueous media investigated.

The cyanylation of cysteine residues of proteins[4] inhibits the catalytic activity of cysteine-active enzymes and allows the selective alkaline cleavage of the protein chain *via* *N*-acyl-2-iminothiazolidine intermediates.[5,6]

At 20 °C 4-dimethylaminopyridine (**1**) reacts with cyanogen bromide in ether to give the hygroscopic 1-cyano-4-dimethylaminopyridinium bromide (**2a**). The non-hygroscopic stable fluoroborate (**2b**), m.p. (decomp.) 120 °C, and perchlorate (**2c**), m.p. (decomp.) 190 °C can be obtained in 40% yield from (**2a**) by anion exchange, or more easily by the reaction of BrCN with AgX–dimethylaminopyridine complexes[7] at 20 °C (60% yield). *E.g.*, addition of a solution of BrCN (0·022 mol) in MeCN (10 ml) to a mixture of (**1**) and $AgClO_4$ (0·020 mol each) in MeCN (20 ml), followed by filtration, precipitation with EtOAc, and recrystallisation (MeCN–EtOAc) gives (**2c**): ν_{max} (Nujol) 2260 (CN), 1665, and 1595 cm^{-1}; λ_{max} (H_2O): 301 nm



ϵ 25,500; δ(MeCN): 3·42 (6H, s, Me), 7·12 (2H, d, β–H), and 8·20 (2H, d, α–H). Hydrolysis of (**2**) yields (**3**) (λ_{max} 282 nm) without ring opening;[1] at 25 °C, pH 3·6, $t_{\frac{1}{2}}$ = 60 min†; at pH 5·2, $t_{\frac{1}{2}}$ = 7·5 min (0·1N acetate buffer).

† Under these conditions cystine and methionine do not accelerate the rate of hydrolysis but histidine and tyrosine do.

Cysteine quantitatively reacts with (**2c**) in water to give 2-iminothiazolidine-4-carboxylic acid.[8] After 11 min of incubation with a 20-fold molar excess of (**2c**) at pH 3·6 and 25 °C, papain loses more than 98% of its catalytic activity (tested towards a specific substrate: *N*-benzoxycarbonyl-glycyl-*p*-nitro-phenyl ester). Cysteine residues nos. 7 and 19 of the reduced B-chain of bovine insulin are quantitatively cyanylated at pH 3·5‡ and the modified protein can be isolated by gel chromatography on Sephadex G 25 (eluent: 10% AcOH).§ At pH 9·5 (24 h; 39 °C) the protein chain is cleaved to give three fragments corresponding to the B^{1}—B^{6}, B^{7}—B^{18} and B^{19}—B^{30} sequences, which are separated on a Biogel P6 column (1·2 × 200 cm; eluent: 0·02N-HCl).

The reaction of the water-soluble reagent (**2**) with protein sulphydryl groups at pH 2—7 can therefore be easily followed by u.v. spectrophotometry and leads to isolable thiocyanates without oxidative side reactions. Then the protein chain may be selectively cleaved in alkali.[5]

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‡ The protein is dissolved in a solution of 7M urea–0·1M acetate buffer and incubated for 15 min at 25 °C with a 3-fold excess of (**2**). The reaction may occur from pH 2 to 7.

§ Minor peaks may be due to the partial cyanylation of histidine and tyrosine residues. Hydrolysis at pH 9·5 gives back the starting residues because unpurified cyanylated protein leads to the same fragmentation as the purified one.

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[2] E. Guibé-Jampel and M. Wakselman, *Chem. Comm.*, 1971, 267; *Bull. Soc. chim. France*, 1971, 2555.
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[7] For an analogous reaction see: E. Guibé-Jampel, G. Bram, M. Wakselman, and M. Vilkas, *Synthetic Comm.*, 1973, **3**, 111. The complexes (**1**)–0·5 $AgClO_4$ and (**1**)–0·5 $AgBF_4$ may be isolated.
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