The Reaction of 2,4-Dinitrofluorobenzene with Nucleotides

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Summary A nucleotide 2,4-dinitrophenyl ester is the major product of reaction of nucleotides with 2,4-dinitrofluorobenzene in the absence of trialkylamine whereas trialkylamine catalyses the reaction of fluoride ion with the 2,4-dinitrophenyl ester resulting in the formation of nucleoside phosphorofluoridate.

NUCLEOSIDE phosphorofluoridates, *e.g.*, thymidine-5′ phosphorofluoridate, are of increasing interest as intermediates in the chemical synthesis of the internucleotide bond[1] and as inhibitors of nucleolytic enzymes.[2] They are prepared by the reaction of 2,4-dinitrofluorobenzene with a nucleotide[3] where a 2,4-dinitrophenyl nucleoside phosphate is a transitory intermediate.[4] We have defined conditions under which the 2,4-dinitrophenyl ester is the major product and shown that, under other conditions, it is possible to convert a nucleotide 2,4-dinitrophenyl ester into the nucleoside phosphorofluoridate by reaction with fluoride ion.

Thymidine-5′ phosphate as its tri-n-butylammonium salt (0·9 m.mole) in dry dimethylformamide (4 ml) was allowed to react with 2,4-dinitrofluorobenzene (3·0 m.mole) at 25 °C for 48 h. After isolation on DEAE-cellulose,[5] the ammonium salt of thymidine-5′ 2,4-dinitrophenyl phosphate (0·41 mmol) was obtained, the other significant products being thymidine-5′ phosphorofluoridate (0·15 m.mole) and thymidine-5′ phosphate (0·17 m.mole). Under essentially the same conditions, in the presence of two additional equivalents of trialkylamine, the predominant product is thymidine-5′ phosphorofluoridate.[1,4]

The ammonium salt of thymidine-5′ 2,4-dinitrophenyl phosphate (0·078 m.mole)[5] in water (0·3 ml) containing triethylamine (0·7 m.mole) was allowed to react with sodium fluoride (0·5 m.mole) at 25 °C for 48 h. The ammonium salt of thymidine-5′ phosphorofluoridate (0·06 m.mole) was isolated after chromatography on DEAE-cellulose.[1,4] Some thymidine-5′ 2,4-dinitrophenyl phosphate (0·012 m.mole) was recovered.

Thus, the reaction of 2,4-dinitrofluorobenzene with thymidine-5′ phosphate without *excess* of trialkylamine yields thymidine-5′ 2,4-dinitrophenyl phosphate as the major product. This reaction, which is to be expected because of the susceptibility of 2,4-dinitrofluorobenzene to nucleophilic attack, provides a convenient alternative route to nucleotide 2,4-dinitrophenyl esters.[5] These esters are useful enzyme substrates[5,6] and the 2,4-dinitrophenyl derivative is potentially useful as a chemical protecting group in view of its ready lability with hydroxide ion[4,7] and ammonia and primary amines.[4,8] This route to 2,4-dinitrophenyl esters is also of interest because aromatic derivatives of nucleic acids show a specific affinity for benzoyl DEAE-cellulose[9,10] and naphthoyl cellulose[1] which is useful in nucleic acid chemistry. The reaction of fluoride with thymidine-5′ 2,4-dinitrophenyl phosphate must involve specific catalysis by trialkylamine, analogous to the reaction with simple 2,4-dinitrophenyl phosphates.[8] It provides a convenient route to thymidine-5′ phosphorofluoridate. It also suggests that nucleotide derivatives other than 2,4-dinitrophenyl esters could be useful intermediates in this type of synthesis.[8,11]

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