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SOME N-6 ACYLATED CYTOSINES

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In an attempt to obtain a blocking group for the 6-amino group of cytosine which is more easily removed under mild acid conditions than the acetyl group, the preparation and properties of some β-haloacetyl-N-6-aminocytosines were investigated.

When anhydrous cytosine was dissolved in trifluoroacetic anhydride, the solution allowed to stand for some hours at room temperature and the anhydride removed in vacuo, a quantitative yield of N-6-trifluoroacetylcytosine, as shown by its analysis and u.v. spectra, was obtained. In aqueous solution however, and even in dry ethanol with sodium bicarbonate in suspension, the compound rapidly decomposed to give cytosine. The value for the λ_{max} in ethanol was 330 mμ, but due to the rapid decomposition of the compound, no value for the molar extinction coefficient could be obtained.

N-6-Trichloroacetyl- and N-6-dichloroacetyl-cytosine were prepared in a similar way, but due to the high boiling point of the anhydride, the products were precipitated from solution with a 50% v/v mixture of ether/petroleum ether (60-80°). The products were found to decompose to cytosine when dried, probably due to the difficulty in removing all traces of the chloroacid or anhydride, and they were kept under ether. No elemental analysis could be obtained for the products, however the u.v. spectra were those expected for N-6-trichloroacetyl- and N-6-dichloroacetyl-cytosine with λ_{max} 245 and 315 mμ for the former and λ_{max} 250 and 305 mμ for the latter compound. An attempt to make N-6-monochloroacetylcytosine in a similar way in the presence of an excess of the anhydride at room temperature was not successful, and when carried out at 60° it was found to be impossible to remove all the unreacted anhydride by precipitation of the product with ether/petroleum ether. The following two methods were used for the preparation of N-6-monochloroacetylcytosine:- i) Anhydrous cytosine (0.5 g.) was suspended in N,N-dimethylformamide (60 ml.). Anhydrous sodium bicarbonate (1.0 g.) and monochloroacetic anhydride (0.8 g.) were added, and the mixture stirred vigorously at room temperature. When all the cytosine had reacted (20 min.), the mixture was filtered and poured into ether. The resulting solid was filtered off, washed with ether and dried. The product was stirred with water (200 ml.) at room temperature for

1 hr. and filtered to give a pale yellow product (0.4 g.) ii) Anhydrous cytosine (0.5 g.) was suspended in chloroform (80 ml.). Tributylamine (1.25 ml.) and mono-chloroacetic anhydride (1.75 g.) were added. The suspension was boiled under reflux for 24 hr., filtered, the precipitate washed with chloroform and dried (0.77 g.). The product was stirred with water (100 ml.) for 1 hr. at room temperature, the suspension filtered, and the product dried (0.65 g.). The compound can be recrystallised by dissolving in DMSO at room temperature, adding water and cooling.

The products obtained from both reactions gave elemental analyses consistent with the formula for N-6-monochloroacetylcytosine. The products had λ_{max} 246, ε 8,000, λ_{max} 300 mμ, ε 4,800 in ethanol. The compound decomposes at elevated temperatures.

Comparison of the Stabilities of N-6 Acylated Cytosines

Cytosine derivative	Time taken for 50% production of cytosine			
(N-6-acylated)	Dry ethanol	0.1NHCl	0.01NKOH	MeOH/NH_3(15%)
Trifluoroacetyl-	⁎	⁎	⁎	⁎
Trichloroacetyl-	1 hr.	⁎	⁎	⁎
Dichloroacetyl-	$4\frac{1}{2}$ hr.	⁎	⁎	⁎
Monochloroacetyl-	*	$13\frac{1}{2}$ min.	≠	70 sec.
Acetyl-	*	130 min.	340 min.	57 min.

*	Stable for more than 24 hr.
⁎	Decomposition was too fast to measure the time taken for 50% decomposition.
≠	See below

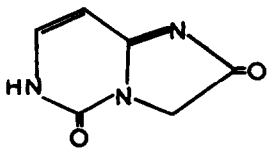
All the compounds appeared to be stable in pyridine solution at room temperature for 24 hr.

From these figures, it can be seen that, as would be expected, increasing the electron attracting power of the acyl group decreases its stability towards both acid and alkali. However, most of the acyl substituents were too labile to be of any use in synthetic work, although they might be of some use for reactions of short duration in non-aqueous solvents. The monochloroacetylcytosine appears to be reasonably stable even in acid solutions and could thus provide a useful blocking group when working with cytosine derivatives.

The action of alkali on monochloroacetylcytosine

Chromatography and electrophoresis of an alkaline hydrolysate of monochloroacetylcytosine (0.1NKOH for 20 min. at room temperature)showed the presence of two main

ultraviolet absorbing compounds. One of these was cytosine and the other was a compound which was uncharged at pH 3.5. Comparison with previous work on similar compounds[1] suggested that the compound was probably I,



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formed by nucleophilic attack by the N-3 of cytosine with displacement of the chloride ion from the chloroacetyl group. Compound I was made on a preparative scale by heating N-6-monochloroacetylcytosine (0.6 g.) in water (80 ml.) on a boiling water bath for 30 min., keeping the pH of the solution at 6 by the addition of 0.4 N KOH. The solution was concentrated to 30 ml. and the crystals which formed were filtered off. The elemental analysis was that to be expected for compound I and its u.v. spectrum showed λ_{max} 304 mμ, ε 10,600, at pH 7. The compound decomposes at elevated temperatures.

1. G.B. Chheda and R.H. Hall, Biochemistry, 5, 2082 (1966).
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