THE CHEMICAL BASIS OF HYDRAZINE MUTAGENESIS

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Hydrazine acts as a mutagen towards bacteria (Lingens, 1961, 1964) and bacteriophages (Orgel, 1960; Freese et al., 1961). No satisfactory explanation for its action has been given but it is observed that, despite high lethality, a considerable proportion of the mutational events appear to be of the transition type; the pH optimum is in the neighbourhood of 8.3 (Orgel, 1960).

It is well known that the purine bases are essentially unaffected by hydrazine hydrate or the anhydrous reagent. However, uracil is converted by ring-cleavage to pyrazolone, thymine (I) to 4-methylpyrazolone (II) and cytosine to 3-aminopyrazole. These products are also formed from the corresponding bases in nucleotide linkage, the order of reaction rates being U>C>T* (inter al., Baron and Brown, 1955; Temperli et al., 1964).

In more dilute solution at about pH 8.0 we have found, following initial experiments by Dr. H. Paulus, a reactivity

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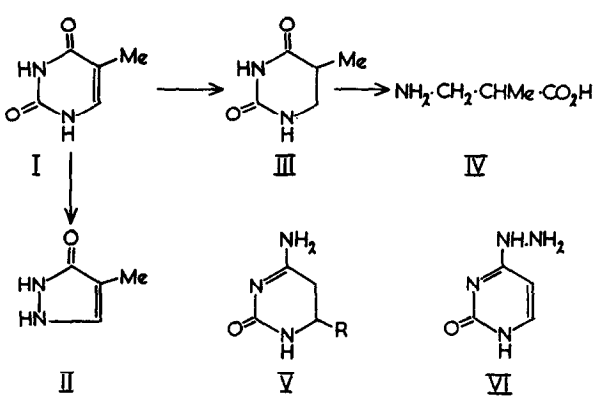
* Abbreviations: Uracil, Thymine, Cytosine and 5-methylcytosine are U, T, C and 5-MeC respectively.

order T>U>5-MeC>C, confirming an earlier observation (Freese et al., 1961; but compare Temperli et al., 1961; Budovskii et al., 1964). Reaction rates were followed by optical density decrease at the λ_{max} of the pyrimidine. Initial experiments gave erratic results, not unusual with hydrazine solutions (Andrieth and Ogg, 1951), but it was then observed that oxygen was required and that copper metal exerted a catalytic effect, leading to better reproducibility. Thus, after prior stirring with a clean copper wire for 2 min. and with continuous aeration, reaction proceeded at 21°C in 5M-hydrazine at pH 8.0 to give the following half-lifes: T, 4 min.; U, 10 min.; 5-MeC, 4.75 hr.; C, 24 hr. Under these conditions no pyrazolone formation was observed.

Under preparative conditions the product, in the case of T, was characterised as 5,6-dihydroT (III), m.p. and mixed m.p. 262°, R_F, 0.6 (in n-butanol-acetic acid-water, 5/2/3). In other smaller scale experiments a more basic product, R_F 0.38, was obtained which was also a reduction product since under the same conditions it was also formed from dihydroT. Both



it and dihydroT, on strong acid hydrolysis gave rise to α-methyl-β-alanine (IV). Although reaction with 5-MeC was much slower, the product, too, gave (IV) on acid hydrolysis.

The reduction of the 5,6-double bond in these pyrimidines is not surprising since the conditions used are those which give rise to the powerful reducing entity, di-imide (Hünig et al., 1965). However, sodium azodicarboxylate, another di-imide generator, did not appear to reduce thymine. Alternative mechanisms for the reduction are not ruled out.

There is accumulating evidence that dihydroC but not dihydroU residues, either in polynucleotide templates or in nucleoside triphosphate substrates, are recognised in enzymatic transcription and translation processes in vitro (Grossman, Kato and Orce, 1966; Rottman and Cerutti, 1966; Cerutti, Miles and Frazier, 1966; but see Roy-Burman, Roy-Burman and Visser, 1966). We suggest that reduction of the 5,6-double bond of cytosine and its derivatives may account for the production of transition mutants by hydrazine. A close analogy clearly exists between the dihydro-derivative (V; R=H) and the water (V; R=OH) and hydroxylamine (V; R=NH.OH) adducts, both of which have been implicated in C → T transition mutagenesis (Drake, 1963; Howard and Tessman, 1964; Ono et al., 1965; Phillips et al., 1965). The lethality of hydrazine, however, may be due to its high reactivity towards T residues as suggested by Freese et al. (1961).

Recently Lingens and Schneider-Bernlöhr (1965) have noted the conversion of C to N^4-aminoC (4-hydrazino-2-pyrimidone) by hydrazine at pH 6.0 and we find that the reaction takes place, but more slowly, at pH 8. It is possible that this process is mutagenic, but further work is

necessary to show whether or not N^4-aminoC is a primary reaction product (c.f. Brown and Phillips, 1965). The oxygen requirement for the lethal and mutagenic effects of hydrazine should be revealing as it has been in a corresponding study with hydroxylamine (Freese et al., 1965). A study of dihydroC and of N^4-aminoC derivatives shows that the predominant tautomeric state in aqueous solution is as shown in V (R=H) and VI respectively (Brown and Hewlins, unpublished work).

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