BISULFITE-CATALYZED TRANSAMINATION OF CYTOSINE AND CYTIDINE

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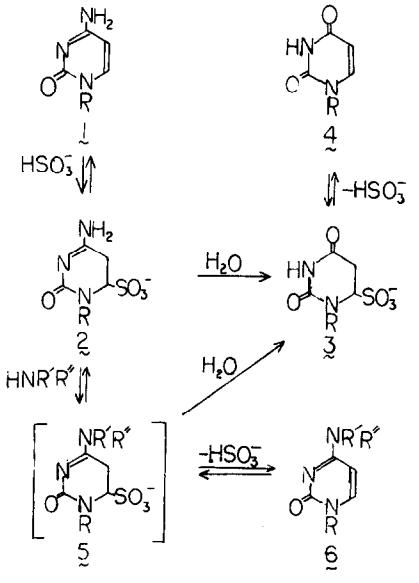
Summary: Sodium bisulfite catalyzes the reaction of cytosine derivatives with amines to give N^4-substituted cytosines. The reactions proceed in good yield, under mild conditions, with a number of aliphatic and aromatic amines. It is suggested that bisulfite may cause the cross-linking of nucleic acids and proteins by this mechanism.

It has recently been reported that $NaHSO_3$ reacts with cytosine derivatives under mild conditions by the path, 1 → 2 → 3 → 4.[1,2] This constitutes a useful method for the deamination of simple cytosine derivatives, and has been applied to the specific deamination of the cytosine residues of nucleic acids.[3] We now wish to report that certain amines can compete successfully with water as a nucleophile for the intermediate, 2 (see Scheme 1). The principal reaction product in such cases is the N^4 - alkyl - or arylcytosine,

Scheme 1



6, presumably formed via intermediate 5. This process is analogous to the known reactions of cytosine with hydroxylamine[4] and semicarbazide[5,6] derivatives. In these reactions, unlike the present case, the same nucleophile serves both to saturate the 5,6-double bond of cytosine, and to displace its amino group.

The successful reactions run, conditions used, and yields are summarized in Table I. The only product observed by thin layer chromatography (tlc), apart from the transamination products, 6, were uracil or uridine. The identities of 6a-g were established by comparison of their infrared and ultraviolet spectra and R_f [2-propanol - water (80:20)] with authentic samples synthesized by known procedures.[7-11] The new products, 6h and i were characterized by satisfactory elemental analyses and by the following properties: 6h, mp 290-293 (decomp); $\lambda_{max}^{pH\ 1}$ 283 mμ, 12,700; $\lambda_{max}^{pH\ 7}$ 276 mμ, ε 10,400; $\lambda_{max}^{pH\ 14}$ 290 mμ, ε 10,700; 6i, mp 205-206 (decomp); $\lambda_{max}^{pH\ 1}$ 288 mμ, ε 16,400; $\lambda_{max}^{pH\ 7}$ 280 mμ, ε 14,600; R_f, 2-propanol - water (80:20) (tlc), 6h 0.75, 6i, 0.80. The infrared and nuclear magnetic resonance spectra of 6h and 6i were in accord with their assigned structures. The variation of the yield (determined by tlc) with pH was investigated for the preparation of 6a. The results were: pH 3, 62%; pH 4, 64%, pH 5, 68%; pH 6, 76%; pH 7, 80%. The increased yield at pH 7, as compared to pH 5, can be ascribed to the decreased rate of the competing deamination reaction at the more basic pH.[1] The deamination rate also falls off at pH values more acidic than 5, but the beneficial effect of this on the yield is compensated for by the conversion of aniline to anilinium ion (pK_a 4.63). A more dramatic effect was seen in the reaction of cytosine with methylamine (pK_a 10.66). An excellent yield of 6d was obtained at pH 7.3, but only uracil was formed when the reaction was run at pH 5.0. The use of pH values above 7.5 for the reactions was avoided, as the rates became very slow. This was undoubtedly due to the adverse effect of increasing pH upon the equilibrium, 1 $\rightleftarrows$ 2.[1] Thus, the time required for the complete con-

TABLE I. PREPARATION OF SUBSTITUTED CYTOSINE DERIVATIVES

Product	R	R'	R''	mmoles[a] amine	% yield[b] (tlc)	% yield[c] (prep)	pH	solvent (ml)[d]	temp	time (hr)
6a[7]	H-	C_6H_5-	H-	30	80		7.0	40% C_2H_5OH (10)	37°	69
6b[7]	β-D-ribofuranosyl-	o-HOC_6H_4-	H-	10	54		4.1	57% C_2H_5OH (35)	50°	6
6c[7]	β-D-ribofuranosyl-	β-$C_{10}H_7$-	H-	10	30		5.0	60% C_2H_5OH (50)	40°	24
6d[8]	H-	CH_3-	H-	30	88	65	7.3	H_2O (12.5)	35°	66
6e[9]	β-D-ribofuranosyl-	CH_3-	H-	30	90	58	7.1	H_2O (10)	36°	288
6f[10]	H-	-CH_2CO_2H	H-	5	72	50	7.4	H_2O (7.5)	25°	96
6g[11]	H-	CH_3-	CH_3-	45	51		7.2	H_2O (10)	40°	144
6h	H-	-$CH_2CH_2CH_2CH_2$-		21	92	55	7.4	H_2O (10)	25°	108
6i	β-D-ribofuranosyl-	-$CH_2CH_2CH_2CH_2$-		36	80	58	6.7	H_2O (10)	48°	168

[a] Mmoles of cytosine (or cytidine) and $NaHSO_3$ were kept at 1 and 12.5, respectively. [b] The solution was brought to pH 12, with NaOH. The substances (1, 4, and 6) were separated by tlc on cellulose in 2-propanol-water (80:20) for a, b, and f; in 2-propanol-HCl-water (65:16.7:18.3) for c and d; in 1-butanol-water-NH_3 (86:14:1) followed by 2-propanol-water (80:20) for e, g, h, and i. The amounts of 1, 4, and 6 were determined spectrophotometrically. Yields are based upon consumed 1. [c] The solution was brought to pH 8 and the product isolated by chromatography on Amberlite CG 120 resin. In the case of 6f, a subsequent crystallization from aqueous HCO_2H was needed. The yields are based upon the starting amounts of 1, and upon 6 isolated. [d] Where the workup was conducted both by tlc and preparatively, the conditions given are those of the preparative reaction.

sumption of cytosine in its reaction with aniline and bisulfite was 6.5 hr, 25°, at pH 6, and 40 hr, 37°, at pH 7. Prolongation of the reaction times after consumption of the starting materials, cytosine or cytidine, generally led to decreased yields of 6. This was undoubtedly due to the sequence: 6 →5 →3 →4. In one reaction, the conversion of an alkylcytosine to cytosine (6→ 5 →2 →1) was demonstrated. This was achieved by allowing 6d to react with a solution of 1.3 g $NaHSO_3$, 2.5 ml conc. NH_3 and 5 ml of H_2O at pH 7.4, 25°, for 120 hr. A 74% yield of cytosine (by tlc) was obtained.

The transamination reaction failed entirely with certain amines. Thus, when cytosine was allowed to react with imidazole or morpholine under a variety of conditions, only uracil was produced. Similarly, the reactions of cytidine with α-naphthylamine and cytosine with p-phenylazoaniline gave, apart from deamination, only traces of unidentified fluorescent substances. Steric effects may have been responsible for the failure of the α-naphthylamine and morpholine reactions, and also for the slowness of the reactions conducted with dimethylamine (Table I) as compared to those with methylamine.

Apart from the obvious synthetic value of this reaction at the nucleoside level, it opens up a number of important possibilities for the chemical modification of nucleic acids. Polynucleotides containing N^4 - alkylcytosines have been of interest for physical and enzymatic studies,[12] but have hitherto been preparable only the polymerization of suitable monomers. The reaction may also be of use for the introduction of "reporter groups"[13,14] into nucleic acids.

In our previous paper it was suggested that the catalysis of the deamination of cytosine derivatives by sodium bisulfite made the latter a genetic hazard to living organisms.[1] Bisulfite has subsequently been shown to be a mutagen for phage λ and for E. Coli.[15,16] The results here raise new possibilities for the manner in which bisulfite may inflict biological damage. The formation of 6f is a model for a bisulfite - catalyzed cross-linking of protein and nucleic acid molecules. Ultraviolet light has been

shown to link cysteine and uracil residues[17], and the resultant protein - nucleic acid cross links are believed to be a significant factor in the killing of microorganisms by ultraviolet irradiation.[18] Similarly, the formation of 6c suggests that bisulfite can catalyze the binding of carcinogenic aromatic amines to nucleic acids.[7]

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References

1. Shapiro, R., Servis, R. E., and Welcher, M., J. Amer. Chem. Soc. 92, 422 (1970).
2. Hayatsu, H., Watayu, Y., and Kai, K., J. Amer. Chem. Soc. 92, 724 (1970).
3. Shapiro, R., Cohen, B. I., and Servis, R. E., Nature, in press (1970).
4. Phillips, J. H., and Brown, D. M., Progr. Nucleic Acid Res. Mol. Biol. 7 349 (1967).
5. Hayatsu, H., Takeishi, K.-I., and Ukita, T., Biochim. Biophys. Acta. 123, 445 (1966).
6. Kikugawa, K., Hayatsu, H., and Ukita, T., Biochim. Biophys. Acta 134, 221 (1967).
7. Shapiro, R., and Klein, R. S., Biochemistry 6, 3576 (1967).
8. Szer, W., and Shugar, D., Acta Biochim. Pol. 13, 177 (1966).
9. Fox, J. J., Van Praag, D., Wempen, I., Doerr, I. L., Cheong, L., Knoll, J. E., Eidinoff, M. L., Bendich, A., and Brown, G. B., J. Amer. Chem. Soc. 81, 178 (1959).
10. Janion, C., and Shugar, D., Acta Biochim. Pol. 15, 261 (1968).
11. Wempen, I., Duschinsky, R., Kaplan, L., and Fox, J. J., J. Amer. Chem. Soc. 83, 4755 (1961).
12. Brimacombe, R. L. C., and Reese, C. B., J. Mol. Biol. 18, 529 (1966).
13. Burr, M., and Koshland, Jr., D. E., Proc. Nat. Acad. Sci. U. S. 52, 1017 (1964).
14. Hille, M. B., and Koshland, Jr., D. E., J. Amer. Chem. Soc. 89, 5745 (1967)
15. Hayatsu, H., and Miura, A., Biochem. Biophys. Res. Commun. 39, 156 (1970).
16. Mukai, F., Hawryluk, I., and Shapiro, R., Biochem, Biophys. Res. Commun. 39, 983 (1970).
17. Smith, K. C., and Aplin, R. T., Biochemistry 5, 2125 (1966).
18. Smith, K. C., Hodgkins, B., and O'Leary, M. E., Biochim. Biophys. Acta 114, 1 (1966).