dehydro-17-oxosparteine (lupanoline),[19] if $\Delta^{1(2)}$-dehydrosparteine were present, it should have been isolated by the procedures used here, but might not have been eluted during glc, because of the high polar (carbinolamine) character of the free base form of the compound. Clearly, additional studies on *L. arboreus* and other species known to produce both sparteine and lupanine are required.

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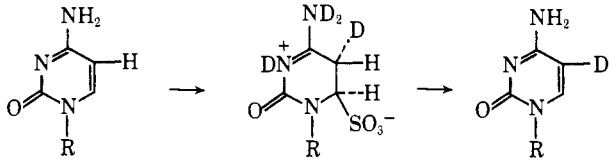
Bisulfite-Catalyzed Isotope Labeling of Cytidine 5′-Phosphate at Its 5 Position

Sir:

Bisulfite adds to the 5,6-double bond of pyrimidine nucleosides.[1-5] Cytidine undergoes a reversible addition of bisulfite to give 5,6-dihydrocytidine 6-sulfonate (**1**).[1,3] In a concentrated bisulfite solution the reaction rapidly reaches a point where cytidine and **1** are in an equilibrium. Regeneration of cytidine from **1** can be achieved either by removal of the bisulfite salt from the reaction mixture or by adjusting the pH of the mixture at a value higher than 8. A concomitant reaction that occurs in the equilibrium, cytidine ↔ **1**, is the deamination of **1** to give 5,6-dihydrouridine 6-sulfonate. We now wish to report that the bisulfite-catalyzed equilibrium between cytidine and **1** can be utilized for the isotope labeling of the 5 position of cytidine and cytidine 5′-phosphate. Conditions will be described with which practically no deamination of **1** takes place and yet a satisfactory incorporation of the isotope is obtained (see Scheme I).

Scheme I



1 in D_2O

Previous experiments have shown that more **1** is formed from cytidine in acidic bisulfite solutions than in a neutral bisulfite solution, and that the deamination of **1** is most pronounced at pH 4–6. Nmr studies have revealed that the preparation of **1** in 1 *M* $NaDSO_3$ at pD about 4, followed by the regeneration, in D_2O, of cytidine therefrom results in no incorporation of deuterium into the cytidine molecule.[3] It has now been found that when a D_2O solution of a mixture of cytidine 5′-phosphate and bisulfite is allowed to stand at pD 7.7, an exchange of deuterium with the hydrogen at C-5 of the cytosine ring takes place. The exchange was detected by directly measuring the nmr spectrum of the reaction mixture. Aside from signals at 3–6 ppm due to the adduct **1**,[3] signals of cytosine-ring protons were present at 6–8 ppm. As the exchange proceeded, the doublet signal centered at 8.01 ppm due to the 6-H of the cytosine ring changed into a triplet signal consisting of a singlet, due to the 6-H of the 5-D species, and a doublet, due to that of the 5-H species. This singlet was located at the middle of the doublet and the three peaks were well separated. Therefore the reaction extent can be accurately determined by measuring these signal strengths. As expected, the doublet at 6.13 ppm due to the 5-H decreased as a function of the time of treatment. However, this signal always remained a doublet, indicating no exchange of 6-H with deuterium. In order to minimize the hydrolysis of the 4-amino group during the reaction, we employed ammonium bisulfite as the source of the bisulfite salt, taking into account the recent report that an exchange amination of cytidine occurs in the presence of bisulfite.[6] The pH of the reaction mixture was fixed to the desired value by addition of sodium bisulfite. Extents of deuterium exchange and deamination were examined for cytidine 5′-phosphate using various reaction conditions. As Table I shows, both the incorporation of deuterium and the deamination proceeded slowly under the conditions employed. Both of these reactions were faster at a higher concentration of bisulfite and in more acidic solutions. However, it can clearly be seen that

Table I. Effect of Concentration and pH of Bisulfite Buffer on Deuterium Incorporation and Deamination of Cytidine 5′-Phosphate[a]

Expt no.	Concn of bisulfite (*M*)	pH[b]	pD[c]	Incorp of deuterium, % 24 hr	72 hr	Deamination, % 24 hr	72 hr
1	1.06	7.1		33.0		4.3	
2	0.96	7.5	7.7	14.2	24.6	0.6	2.1
3	1.88	7.2		53.6		6.4	
4	1.75	7.5	7.6	29.6	54.3		3.8
5	2.62	7.2		68.3		12.7	
6	2.48	7.5	7.6	46.2	68.7	2.3	8.5
7	2.40	7.7	7.9	29.4	54.6	1.5	3.1

[a] Disodium cytidine 5′-phosphate, 100 mg, was dissolved in 1 ml of D_2O. To this was added ammonium sulfite and sodium bisulfite in the amounts as listed below, and the resulting solution was allowed to stand at 37° in a tightly stoppered tube. Experiment number/$(NH_4)_2SO_3 \cdot H_2O$/$NaHSO_3$: 1/134 mg/20 mg; 2/134 mg/7.5 mg; 3/268 mg/30 mg; 4/268 mg/14 mg; 5/402 mg/40 mg; 6/402 mg/20 mg; 7/402 mg/10 mg. The extent of deamination was determined as follows. An aliquot (10 μl) was withdrawn and mixed with concentrated ammonia (10 μl) and the whole solution was subjected to two-dimensional cellulose thin-layer chromatography. The chromatographic solvents used were: first dimension, isobutyric acid–0.5 *N* NH_4OH (10:6, v/v); second dimension, isopropyl alcohol–concentrated HCl–water (75:17:8, by vol). Only two spots corresponding to cytidine 5′-phosphate and uridine 5′-phosphate were detected. Each of these compounds was eluted from the chromatogram with 0.01 *N* HCl and its quantity was determined by the uv spectrum. As a reference, a blank solution was prepared by eluting a non-uv absorbing zone of the same size on the thin-layer plate. [b] The pH values are those determined for corresponding H_2O solutions. [c] Reading on a pH meter.

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(4) H. Hayatsu, *J. Amer. Chem. Soc.*, **91**, 5693 (1969).
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(6) R. Shapiro and J. M. Weisgras, *Biochem. Biophys. Res. Commun.*, **40**, 839 (1970).

under certain conditions an incorporation of deuterium more than 10% of the theoretical amount can be obtained without appreciable deamination. The effect of temperature on the reaction at pD 7 in 0.9 *M* bisulfite was also studied. As the temperature was raised from 0 to 60°, both the incorporation of deuterium and the deamination increased in a parallel fashion.

This reaction was carried out in T_2O. Cytidine or cytidine 5′-phosphate was treated with 1 *M* bisulfite in T_2O (0.5 Ci/ml) at pH 7.5 and at 37° for 25 hr. After removal of bisulfite as an insoluble barium salt, the nucleoside or nucleotide was recovered from the reaction mixture by paper chromatography and counted for its radioactivity. The recovery was generally higher than 90% as based on the amount of the starting material used. The paper chromatography confirmed that no appreciable deamination occurred during the reaction. As Table II shows, incorporation of tritium

Table II. Bisulfite-Catalyzed Incorporation of T into Cytosine Derivatives

Compd	T incorporated, cpm/0.1 μmol (% of 1-atom exchange per molecule)[c]		
	With bisulfite buffer	With phosphate buffer[d]	Without salt
Cytidine	28,200[a] (13.2)	314 (0.15)	80 (0.04)
Cytidine 5′-phosphate	18,900[b] (10.3)		
Thymidine	195 (0.09)		

[a] Cytidine, 10 μmol, was dissolved in 50 μl of 2 *M* $(NH_4)_2SO_3$–2*M* $NaHSO_3$ (20:1, v/v, pH 7.4). To this was added 50 μl of T_2O (1 Ci/ml), the final pH being 7.5, and the solution was incubated at 37° for 24 hr; 1 *M* barium chloride (100 μl) was then added to the solution and the resulting precipitate was removed by centrifugation. The precipitate was washed three times with water and the washings were combined with the supernatant. The solution was evaporated to dryness and the residue was chromatographed on paper by the solvent system of isopropyl alcohol–concentrated HCl–water (75:17:8, by vol). Cytidine was eluted from the chromatogram with 0.01 *N* HCl. The absorbance at 280 mμ and the radioactivity were determined for this solution. Repeated evaporation with H_2O before the paper chromatographic step did not affect the results. This fact indicated that the easily exchangeable tritium atoms on the cytidine molecule had been completely removed by the procedure described above. [b] Cytidine 5′-phosphate Na_2, 1 μmol, was treated in a 100-μl reaction solution as described in footnote *a*. In order to avoid coprecipitation of barium cytidylate, the reaction solution was diluted with 5 ml of H_2O before the addition of 100 μl of 1 *M* barium chloride. Subsequent work-up was the same as in footnote *a*. [c] The counting efficiency differed from sample to sample, ranging between 19 and 22%. [d] A 1:1 mixture (by vol) of 1 *M* sodium phosphate buffer, pH 7.5, and 1 *M* NaCl was used instead of 1 *M* bisulfite.

by catalysis of bisulfite into cytidine and cytidine 5′-phosphate proceeded to an extent of about 10% of the theoretical value. Absence of incorporation into thymidine confirmed that the exchange did occur specifically at the 5 position of the cytosine ring. Little incorporation of tritium into cytidine observed in phosphate buffer demonstrated the catalytic effectiveness of bisulfite.

Normally, both the addition and the elimination, cytidine $\rightleftarrows$ **1**, proceed in a trans configuration, resulting in the regeneration of 5-H cytidine. A partial participation of the cis mechanism in this process could lead to a gradual accumulation of 5-D cytidine. Another possible mechanism is a slow exchange of the C_5–H by deuterium at the level of compound **1**.

The labeling of the 5 position of cytidine by deuterium has been previously carried out in a weakly acidic carboxylate buffer at 95°, accompanying deamination.[7,8] In contrast to this, the bisulfite-catalyzed labeling reported here proceeds in a neutral solution at 37° or below, causing little deamination. It should be noted that bisulfite, at pH 7, does not appear to cleave the phospho diester bond of a tRNA[9] or diribonucleoside monophosphates.[3] It is of interest to investigate the applicability of this new method of specific labeling to other cytosine-containing compounds of biochemical importance.

Acknowledgment. Professor T. Ukita of our Faculty is gratefully acknowledged for his encouragement throughout this research.

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Electronic Structure of the Open Forms of Three-Membered Rings

Sir:

The purpose of this communication is to report nonempirical self-consistent-field and configuration-interaction studies[1] of the open forms of several three-membered ring systems of the type

where A, B, and C are CH_2, CH^-, NH, or O. The main interest is in the electronic structure of the ring-open forms and in particular in the features of the electronic structure which determine the presence or absence of stereoselectivity in electrocyclic and cycloaddition reactions involving these species.

The systems which have been studied included cyclopropane, cyclopropyl anion, aziridine, ethylene oxide, oxazirane, and ozone. The electronic structure of open forms of these species is essentially a linear combination of a resonating π system, $B{=}A^+{-}C^- \leftrightarrow {}^-B{-}A^+{=}C$, and a π diradical, $\cdot B{-}A{-}C\cdot$. For the systems considered here the dipolar contributions ^+B–A–C^- or ^-B–A–C^+ are relatively small. For ring opening and subsequent cycloaddition to multiple bonds there is a competition between concerted and nonconcerted modes of reaction. Here the concerted mode is used to designate the idealized situation in which there are two concerted steps, the ring opening and subsequent cycloaddition. It is well established that the electronic structure of the resonating π system is such that the concerted steps are favored.[2] In the case of a

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(2) R. B. Woodward and R. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, 8, 1475 (1969).