4 and **6** as well as of **3** and **7** show superimposable fragmentation patterns, with base peaks at m/e 135 (*cf.* **13**) in each case. Differences in peak intensities are negligible except for a stronger peak at m/e 302 in **6** and m/e 260 in **7**, both corresponding to $M^+ - 28$.

The expected stereochemistry of the photoproducts of **2** and **5**, based on mechanistic analogy with the photolysis products of α,β-epoxy ketones,[2b,8] is shown in structures **4**, **6**, and **8**. Work designed for detailed proofs of these structures is currently in progress.[14]

Acknowledgments. The authors from E. T. H. (D. B., T. I., K. S., and O. J.) gratefully acknowledge the financial support of the Schweiz. Nationalfonds zur Förderung der wissenschaftlichen Forschung, CIBA AG., Basel, and J. R. Geigy AG., Basel.

(14) Satisfactory elemental analyses and spectral data were obtained for all new crystalline compounds. The products **6**, **8**, **9**, and **11**, which could not be obtained in crystalline form, were carefully purified by column and preparative thin layer chromatography (2 mm, precoated Merck silica gel plates; one spot for each compound). The resulting samples gave satisfactory empirical formulas (high-resolution mass spectra) and spectral data.

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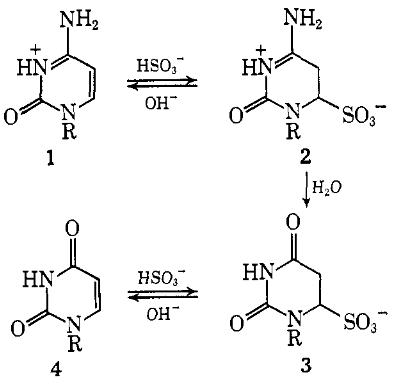
Received September 3, 1969

Reactions of Uracil and Cytosine Derivatives with Sodium Bisulfite. A Specific Deamination Method

Sir:

An important feature of the chemistry of uracil and cytosine is the susceptibility of their ring systems to nucleophilic attack at the 5,6 double bond. A number of synthetic transformations of uracil nucleosides have recently been shown to proceed through the intramolecular addition of a sugar substituent to that double bond.[1-4] It is believed that the transient addition of an external nucleophile is involved in deuterium exchange processes of uracil,[5] in the mechanism of cleavage of uracil by hydroxylamine,[6] and in the mode of action of thymidine synthetase.[7] We now wish to report that sodium bisulfite adds reversibly to the 5,6-double bond of uracil and its nucleosides, and that the favorable equilibrium with this reagent permits their complete conversion, under physiological conditions, to the dihydrouracil derivatives **3**. This reaction is reversed in basic solution. Cytosine derivatives react similarly and are converted by sodium bisulfite to saturated adducts of structure **2**. This conversion is analogous to the initial step of the reaction of the potent mutagen, hydroxylamine, with cytosine derivatives.[6] Unlike hydroxylamine, however, bisulfite does not react further to displace the amino group of the adduct. Rather, **2** deaminates upon standing in aqueous solution to give **3**. Adjustment of the pH of the solution to 10 converts **3** to the uracil derivative **4**. This sequence constitutes a method for the specific deamination of cytosine derivatives, under mild conditions, in the presence of the other common nucleic acid components. Adenosine and guanosine do not react with sodium bisulfite. The above reaction sequence is comparable to that involved in the hydrolytic deamination of cytidine by hot, aqueous, acidic buffer.[8-11] In that case, however, the equilibrium to the adduct corresponding to **2** is unfavorable, this adduct is present in very low concentration, and the conditions required by the reaction are too vigorous for it to be useful for preparative or mutagenic purposes.[12]



a, R = β-D-2-deoxyribofuranosyl
b, R = β-D-ribofuranosyl
c, R = H

The ultraviolet absorbtion of uracil was rapidly lost after a sample was dissolved in a 1 M solution of $NaHSO_3$ at pH 7 ($t_{1/2}$ = 9.7 min at 24°). No ultraviolet-absorbing spots were observed when the reaction mixture was examined by thin layer chromatography on cellulose. After exposure of the plate to ammonia, however, a single ultraviolet-absorbing spot [R_f 0.33 in 2-propanol–water (70:30)] was detected. (When a portion of the reaction mixture was brought to pH 10 with ammonia, uracil was regenerated.) The product was isolated by crystallization from the reaction mixture and analyzed as a monohydrate of $C_4H_5N_2O_5SNa$. Its infrared spectrum (KBr) contained peaks at 8.20, 8.44, and 9.44 μ, which are attributed to a sulfonate group.[13] The nmr spectrum (D_2O) lacked the uracil low-field H-5 and H-6 absorbances, exhibiting instead multiplets at τ 5.55 (one proton) and 6.93 (two protons). This resembled the spectra of known 6-substituted dihydrouracils[14] and was consistent with structure **3** (R = H) for the product of the reaction. Cytosine derivatives showed a similar reactivity to sodium bisulfite. The unstable adducts **2** have not yet been isolated in pure

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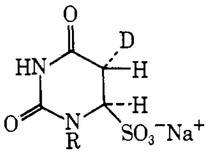
form, and their structural assignment is based upon the information below. Dilution (1:10) of a solution of **2a** in 2.26 *M* $NaHSO_3$, pH 5.5, caused it to revert to deoxycytidine. Adjustment of the pH of the original solution to 10 had the same effect. Data on the nmr spectra of the adducts **5a**, **5b**, **6a**, and **6b**, prepared in D_2O, are given in Table I. The H-5 and H-6 protons, in each case, appeared as moderately sharp singlets ($J_{5,6} < 3$ cps). The small coupling constants were consistent with diequatorial positions for the 5 and 6 protons.[14] This indicated that the addition of the sulfite group and the deuteron was stereoselective and *trans.*

Table I

Compd	Chemical shifts, τ, in D_2O H-5	H-6	H-1′
5a	6.96	5.05	4.10 (t), 4.30 (t)
5b	6.98	5.05	4.35 (d), 4.65 (d)
6a	6.78	5.06	4.00 (t), 4.40 (t)
6b	6.76	5.06	4.40 (d), 4.68 (d)



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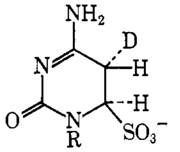

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a, R = β-D-2′-deoxyribofuranosyl
b, R = β-D-ribofuranosyl

Upon addition of alkali to the reaction mixtures containing **5a**, **5b**, **6a**, and **6b**, the spectra of the parent uracil and cytosine nucleosides reappeared. No deuterium incorporation had taken place, demonstrating that the elimination also proceeded with *trans* stereochemistry. The H-1′ protons of the adducts **5a**, **5b**, **6a**, and **6b** appeared as pairs of doublets or triplets. This was attributed to the existence of the adducts as mixtures of two diastereomers. Similar observations have been made in the cases of the photohydrates of uridine and uridine 5′-phosphate.[15,16]

The kinetics and equilibrium of the uracil (0.02 *M*)–sodium bisulfite (1 *M*) reaction were studied at 24° by ultraviolet spectroscopy. The equilibrium was completely in favor of **3c** at pH <8, then fell off sharply to 50% **3c** at pH 9.25 and 0% **3c** at pH >10.2. The following expression was derived (neglecting activity coefficients and the slight consumption of bisulfite in the formation of **3c**): $K_e = [1]([H^+] + K_s)([H^+] + K_u)/([uracil][H^+]^2)$. The values of K_s and K_u (the dissociation constants for bisulfite and uracil) were determined by titration to be 3.7×10^{-7} and 5.2×10^{-10}, respectively, at the ionic strength of the reaction mixture. By substituting these values and the experimental equilibrium data into the above equation, a consistent value of 1.3×10^3 was calculated for K_e, the equilibrium constant. The pseudo-first-order rate constant, *K*, for the forward reaction rose in inverse proportion to $[H^+]$ in the pH range 2–6. It reached a maximum at pH 7–8 and fell off with a further rise in pH. This could be explained by the assumption that the reaction involved a rate-determining attack by sulfite ion upon neutral uracil. The following expression was derived: $K = K^*K_s[H^+]/\{([H^+] + K_s)([H^+] + K_u)\}$, where K^* is a pH-independent rate constant. Using a value of 1.7×10^{-3} sec^{-1} for K^*, excellent agreement was obtained between the observed rates and those calculated from this equation. A comparison of the reaction rates of uracil and several of its derivatives was made at pH 7.2 (24°, 1 *M* $NaHSO_3$). The observed values (sec^{-1}) of *K* were: uracil 1.4×10^{-3}; uridine 9.5×10^{-4}; deoxyuridine 6.8×10^{-4}; uridine 5′-phosphate 3.4×10^{-4}. In the case of thymine, the overall equilibrium for its reversible reaction with bisulfite was found to be less favorable. At 24°, in 1 *M* $NaHSO_3$, a maximum loss of 40% of its ultraviolet absorbtion was observed in the pH range 4–6. The equilibrium constant was estimated at 0.8.

The variation of the equilibrium of the reaction, deoxycytidine + $NaHSO_3 \rightleftharpoons$ **2a**, with pD was studied by nmr in 2.26 *M* $NaHSO_3$ in D_2O solution, 37°, using 0.22 *M* (initial) deoxycytidine. The following values were obtained: pD <6, 100% **2a**; pD 6.6, 71% **2a**; pD 7.0, 55% **2a**; pD 7.4, 33% **2a**; pD 7.7, 26% **2a**; pD >8.2, 0% **2a**. At a given pD, it was found that lower temperature favored the formation of **2a**. Thus, at pD 7.4, 6°, 67% of **2b** was present. The rate of the forward reaction was too rapid for convenient measurement at 37°, pD 4 or higher, under the above conditions. A half-life of 8 min was observed at pD 3.0, and one of 12 min was obtained at pD 2.7. This decline of the rate with decreasing pD resembled the situation observed with the uracil–bisulfite reaction and suggested that the rate-determining step may involve addition by sulfite ion.

Upon standing in aqueous solution, the cytosine adducts **2** deaminated to the corresponding uracil adducts **3**. This was demonstrable by thin layer chromatography, nmr spectroscopy, and the isolation of **3c** from a cytosine–bisulfite reaction mixture.

The deamination reaction (**2a** → **3a**) was studied. This was done in unbuffered bisulfite solutions (pD adjusted with DCl) in D_2O at 37°, over the pD range 3.0 to 6.6. A maximum at pD 5.8 was observed, $k = 3.0 \times 10^{-4}$ sec^{-1}, which fell off to $k = 8.6 \times 10^{-5}$ at pD 6.6 and 7.8×10^{-5} sec^{-1} at pD 4.9. At pD 3.0 no appreciable deamination was observed, unless citrate buffer (of the same pD) was added. These results suggest the involvement of the protonated species **2a**, and of catalysis by buffer anions.

To demonstrate the preparative utility of the reaction, 50 mg of deoxycytidine was allowed to react with 238 mg of sodium bisulfite in 1 ml of H_2O at 25°. After 14 hr of reaction, the excess bisulfite was removed by precipitation with $Ba(OH)_2$ and the solution was neutralized with a cation exchange resin. Crystallization of the reaction mixture afforded a 94% yield of deoxyuridine. In a similar fashion, 1 mg of cytidylyl-(3′–5′)-adenosine was converted to a single product, identical in R_f and ultraviolet spectrum with uridylyl-(3′–5′)-adenosine.

Sodium bisulfite should be a reagent of considerable utility in nucleic acid chemistry both for its ability to convert uracil and cytosine derivatives to the saturated adducts **2** and **3** and for its catalysis of the deamination of cytosine to uracil. There has been considerable interest in methods for converting uracil to saturated

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derivatives for analytical reasons and because of the occurrence of dihydrouracil in tRNA.[17–19] A similar transformation is involved in the well-known photohydration reaction of uracil,[20,21] which has important consequences for the functioning of nucleic acids.[22,23]

In addition to its synthetic use in nucleotide chemistry, we believe that sodium bisulfite should be of value as a reference mutagen. It is the first reagent to be described that can *specifically* convert one common, naturally occurring nucleic acid heterocycle to another. It should finally be noted that sodium bisulfite is a food additive and sulfur dioxide is an atmospheric pollutant. It now seems probable that these substances may constitute a genetic hazard to living organisms.

Acknowledgments. We wish to thank the National Institutes of Health (Grant GM-11437) and the Damon Runyon Memorial Fund for Cancer Research (Grant DRG-976) for their support, and Mr. Josef Weisgras for his aid with the experiments.

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Carbon–Carbon and Carbon–Hydrogen Bond Length–Bond Overlap Correlations

Sir:

The well-known environmental changes in single carbon–carbon bond lengths have been attributed to differences in hybridization of carbon atoms.[1] The bonds have been classified into sp^n–sp^m types (n, m being 1, 2, 3) and a rule is found[2] that carbon–carbon single bond distance decreases by 0.04 Å when one of the participating carbon atoms changes the hybridization type from sp^3 to sp^2, or sp^2 to sp. However, there is sufficient evidence in the literature to indicate that useful correlations may be obtained when the hybridization in molecules is not restricted to the special cases sp, sp^2, and sp^3. For example, it has been found that J(^{13}C–H), assumed to depend mainly on the percentage of s character in the bonds,[3] of the bridgehead carbon atom of norbornane is much larger than that of the usual sp^3–sp^3 bond.[4] Similarly, the enhanced acidity and reduced reactivity of aryl positions adjacent to fused strained rings toward electrophilic substitution indicate that the atomic orbitals of the fused aryl carbon used to construct the strained ring have higher p character than the simple sp^2 hybrids suggest.[5] The theoretical approaches also suggest models which do not restrict the hybridization parameters (exponent n in sp^n) to integers. Thus the hybrids calculated by the maximum overlap approximation,[6] which are of the general sp^n (n = a noninteger) form, lead to correlations of bond energy,[7] chemical shift,[8] or spin–spin coupling (^{13}C–H)[9] with hybridization. Other theoretical approaches, like local orbital prediction of the hybridization based on the CNDO/2 MO wave functions, also lead to noninteger hybrid parameters.[10] On the other hand, an estimate of the s:p ratio from experimental bond lengths, following the idea that hybrid radius is a function of hybridization,[11] was made, and noninteger hybrid parameters were suggested.[12] All this indicates that the restriction of hybridization parameters to integers is neither justified nor useful in approximate descriptions of the bonding in molecules.

Here we considered various single C–C and C–H bonds and established a correlation between the bond lengths and corresponding bond overlaps based on hybrids with noninteger values of the exponent n, m. It is found that hybridization characterized by noninteger exponents obtained by the maximum overlap approximation can account for C–C and C–H bond length variations and be useful in predictions of the environmental changes of single C–C bonds in molecules for which the experimental data are not complete or available. Bond overlaps depend on the amount of electron density in the region between the atoms forming the bond and are expected, therefore, to be a useful bond index, especially for similar bonds, when bonded electrons are approximately equally bound to their own atoms, and there is no high polarity of bonds.[13,14] This is, for example, a justification for Coulson's well-known definition of bond orders,[15] and is implied in various semiempirical methods in which overlap or integrals proportional to overlap are allowed to depend on the bond length.[16,17]

Bond overlap for some 20 hydrocarbons for which experimental bond lengths are known have been evaluated. Hybrid orbitals characterizing the bonds have been obtained by the method of maximum overlap. The method of calculation has been previously described in detail.[6] The computations were not directly based on the variational theorem in which an energy expression is minimized with respect to a set of parameters. Instead, a basis set consisting of local hybrid orbitals was assumed, and parameters specifying the hybridization were varied to maximize the total overlap. Briefly, in the method we search for optimal parameters a_i, b_i, of individual hybrids ψ_i =

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