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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Kazushige Kai,* Yusuke Wataya, Hikoya Hayatsu
Faculty of Pharmaceutical Sciences, University of Tokyo
Bunkyo-ku, Tokyo 113, Japan
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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

(1) For general information about these methods, see, for instance, R. G. Parr, "Quantum Theory of Molecular Electronic Structure," W. A. Benjamin, New York, N. Y., 1964.
(2) R. B. Woodward and R. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, 8, 1475 (1969).

π diradical, the electronic structure involves degenerate molecular orbitals with opposite stereochemical preference. Thus the concerted and nonconcerted modes of reaction are thought to be equally favorable.[3]

In order to determine the amount of π-diradical character for the open form of these three-membered rings, the lowest energy molecular orbital wave function has been determined for the quasi-planar open forms[4] of each system using the self-consistent-field method with the orbitals approximated as linear combinations of Gaussian orbitals.[5] This wave function may be written, in abbreviated form, as

$$\varphi_1 = \pi_-^2$$

For these systems there is a second electronic configuration

$$\varphi_2 = \pi_+^2$$

which is nearly degenerate with the lowest electronic configuration, φ_1. Here, π_+ signifies that the $p\pi$ atomic orbitals on the terminal atoms both enter the molecular orbital with a positive coefficient and π_- indicates that these atomic orbitals enter the molecular orbital with coefficients of opposite sign.

The coefficients of φ_1 and φ_2 have been determined for each of the molecules mentioned above using the configuration-interaction method.[1] Once these coefficients are known it is straightforward to determine the amount of π-diradical character, since the wave function for the π diradical

$$\varphi_D = (1/\sqrt{2})(\pi_+^2 - \pi_-^2)$$

to a first approximation is just a linear combination of these two electronic configurations with equal weight and opposite sign.[6] Table I contains the predicted amount of π-diradical character for the open form of several three-membered rings. The main point to be derived from this table is that among these species there are large differences in the amount of π-diradical character. (Since more diradical character means a smaller fraction of the electronic wave function has a stereochemical preference, one should expect appreciable variations in the degree of stereoselectivity in the reaction of these species.) At present the experimental evidence to test this prediction is rather limited; however, it has been reported that the 2 + 3 addition of tetracyanoethylene oxide to olefins[7] and the similar cycloadditions of the aziridines[8] are highly stereoselective (approximately 100%), whereas the pyrolysis of pyrazolines is only moderately stereoselective (30–40%).[9] Assuming each of these reactions proceeds *via* a 0,0 intermediate, this difference can be interpreted conveniently by considering the predicted amount of π-diradical character for each of the ring-open forms.

Table I. π-Diradical Character of the Open Forms of Several Three-Membered Rings[a,b]

Molecule	Calcd weight, % π_+^2	Calcd weight, % π_-^2	Diradical character,[c] %
Cyclopropane	40	60	80
Cyclopropyl anion	4	96	8
Aziridine	15	85	30
Ethylene oxide	19	81	38
Oxazirane	10	90	20
Ozone	15	85	30

[a] The open forms of these ring systems are CH_2–CH_2–CH_2, CH_2–CH–CH_2, CH_2–NH–CH_2, CH_2–O–CH_2, CH_2–NH–O, and O–O–O. [b] Calculations were carried out for each molecule in the quasi-planar open forms of each ring system for a bond angle of 120°, except for oxazirane, where the bond angle was 126°. The details of these calculations will be reported in a subsequent paper. [c] Note that the diradical wave function $\varphi_D = (1/\sqrt{2})[\pi_+^2 - \pi_-^2]$ is not orthogonal to π_-^2. This presents a problem in interpreting just what one means by the per cent of diradical character. Here we have taken it to be the per cent of the electron density which can be represented by the square of φ_D.

In comparing reaction paths for these species, one needs to consider the stability of the 0,0 open form compared to other geometrical forms. In this connection it should be noted that there seems to be a relationship between the per cent of diradical character and the stability of the open form; *i.e.*, the smaller the diradical character the greater the stability of the 0,0 open form. This may be particularly important in the case of the open forms of cyclopropane, since the amount of diradical character is about 80%. In fact, the recent experiments of Condit and Bergman suggest that the pyrolysis of 2-methyl-3,4-diazabicyclo[3.3.0]oct-3-ene may not involve the 0,0 open form of cyclopropane.[10]

It is hoped that these predictions of the per cent of diradical character of the open forms of three-membered ring systems will stimulate further experimental and theoretical work into the nature of the stereoselectivity of reactions involving the open forms of these systems.

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(3) L. Salem, *Chem. Commun.*, 981 (1970).
(4) The term "quasi-planar open forms" refers to the orientation of the terminal methylene groups, so that the hydrogens lie in the plane of the ring atoms; see ref 5 for further details.
(5) A. K. Q. Siu, W. M. St. John, III, and E. F. Hayes, *J. Amer. Chem. Soc.*, **92**, 7249 (1970), contains details of the calculations for trimethylene. Similar calculations were carried out for each species mentioned in Table I.
(6) Here it has been assumed that there is no significant amount of charge transfer from one terminal atom to the other.
(7) W. J. Linn, and R. E. Benson, *J. Amer. Chem. Soc.*, **87**, 3657 (1965).
(8) See, for instance, R. Huisgen, W. Scheer, and H. Huber, *ibid.*, **89**, 1753 (1967), and references therein.
(9) R. J. Crawford and A. Mishra, *ibid.*, **87**, 3768 (1965).
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Edward F. Hayes,* Albert K. Q. Siu
Department of Chemistry, Rice University
Houston, Texas 77001
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An Experimental Method for Estimating Substituent Effects on Transition-State Structure

Sir:

The success of the transition-state theory of reaction rates has led to frequent discussions of the *structure* of transition states. This has in turn led to a number of speculations and calculations concerning ways of estimating changes in transition-state structure in a series of related reactions, of which Hammond's postulate is perhaps the best known.[1] The problem