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Application of CNDO/2 Theoretical Calculations to Interpretation of the Chemical Reactivity and Biological Activity of the *Syn* and *Anti* Diolepoxides of Benzo[*a*]pyrene

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CNDO/2 molecular orbital theoretical calculations performed on the *anti* and *syn* diolepoxides (**1** and **2**) of the potent carcinogen benzo[*a*]pyrene provide insight into the molecular structure and reactivity of these mutagenic and carcinogenic hydrocarbon metabolites. Hydrogen-bonded interaction between the 7-HO proton and the epoxide oxygen atom of **2** is shown to be absent in the normal semichair conformation of the tetrahydro ring, (H···O bond distance = 2.7 Å), but is energetically favored in a somewhat distorted puckered structure (H···O bond distance = 1.7 Å). Unexpectedly, internal H-bonding alters the relative electron density at C_9 and C_{10}, leading to prediction of the former as the more electrophilic center. Since all reactions of **2** take place exclusively at C_{10}, transannular H-bonding is concluded not to contribute significantly to the structure of **2**. Diolepoxide reactions with both weak and strong nucleophiles and with DNA are discussed and the mechanisms interpreted in terms of molecular structure as determined by the theoretical calculations.

INTRODUCTION

Carcinogenic polycyclic aromatic hydrocarbons undergo metabolic transformation catalyzed by the mixed-function oxidases and related enzymes to furnish complex mixtures of oxidized derivatives (*1*). Certain metabolites, specifically the arene oxides and diolepoxides, have recently been implicated as active forms of these carcinogens responsible for their mutagenic and carcinogenic effects (*2*). In particular, the diolepoxide derivative of benzo[*a*]pyrene (BP)[5] (+)-*trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene[6] (**1**) has been shown (*5–8*) to be the principal metabolite of this hydrocarbon which binds to nucleic acids *in vivo*, and the structure of resulting hydrocarbon-guanosine adduct has been elucidated (*4*, *9–13*). Syntheses of racemic **1** and the isomeric *syn*-BP-diolepoxide (**2**) have been reported (*2*, *3*, *14*, *15*);

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[5] Abbreviations used: BP, benzo[*a*]pyrene; THBP, 7,8,9,10-tetrahydrobenzo[*a*]pyrene.

[6] See page 498.

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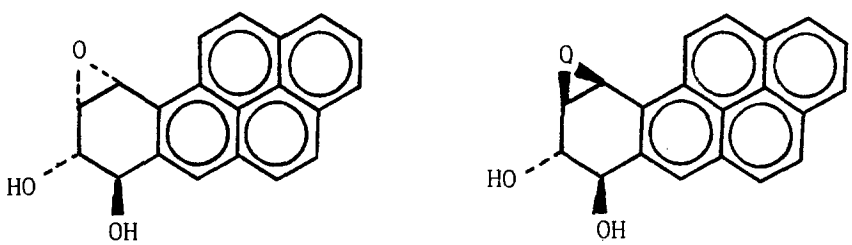
resolution of the metabolic precursor, the 7,8-dihydrodiol (*16–18*), and conversion of the resolved stereoisomers to the optically pure (+) and (–) enantiomers of the *anti*-diolepoxide **1** have also been achieved (*16*, *18*).

It was theorized by Hulbert (*19*) that the *syn* isomer should be more chemically reactive, and hence more biologically active, than the *anti*-diastereomer. This idea was based on the hypothesis that transannular hydrogen bonding between the oxide ring and the 7-hydroxyl group in **2** should provide anchimeric assistance to nucleophilic attack on the epoxide ring (Fig. 2). Supportive evidence was provided by Yagi *et al.*, who observed **2** to be more reactive than **1** with the thiolate anion (*14*). However, the available biological data have failed to support this concept. Thus, while both the *syn* and *anti* isomers exhibit exceptionally high mutagenic activity against Chinese hamster V79 cells, the *anti* isomer was found to be clearly more active in this respect (*20*, *21*). Also, the *anti* isomer has been found to be more potent than the *syn* in inhibiting the infectivity of the QB RNA phage virus (*22*). These discrepancies may be rationalized as



1 **2**

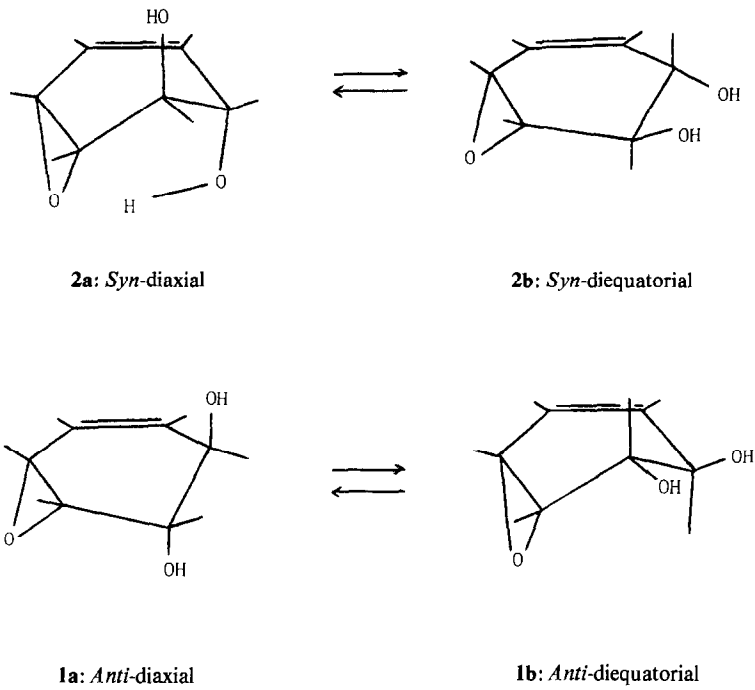
FIG. 1. Structures of the *anti*- and *syn*-BP-diolepoxides, **1** and **2** respectively.

due to selective rapid detoxification of the *syn* isomer by hydrolysis before it can react with DNA. However, kinetic studies (*23*) have shown the relative rates of aqueous hydrolysis to favor the *syn* isomer only moderately at pH 5. Under other conditions, a greater difference in stability in aqueous media has been reported (*24*, *25*).[7]

In the light of this experimental evidence, we felt a theoretical reevaluation of the reactivities of these two isomers warranted. To this end CNDO/2 molecular orbital

[6] The nomenclature employed for the diolepoxides of BP is essentially that proposed previously (*2*, *3*). *trans* refers to the relationship between the 7- and 8-hydroxy groups. *syn* and *anti* designate the relation of the benzylic hydroxyl group to the epoxide ring as being on the same or the opposite face of the molecule. Thus, there are two diastereomeric diolepoxides, *trans*-7,8-dihydroxy-*anti*-9,10-epoxy-THBP (**1**) and *trans*-7,8-dihydroxy-*syn*-9,10-epoxy-THBP (**2**). Each can exist as a pair of enantiomers, and the absolute configuration at each chiral center is specified by α and β, to designate whether the group is below or above the plane of the paper in the structural formula drawn in the conventional orientation according to the IUPAC rules. Thus 7β,8α-dihydroxy-9α,10α-epoxy-THBP is the absolute configuration represented in structure **1**. It is also the structure of the principal metabolite bound covalently to the RNA and DNA of mammalian (including human) cells *in vivo* (*4*). Its mirror image is the enantiomer 7α,8β-dihydroxy-9β,10β-epoxy-THBP, not depicted.

[7] Stability of the *syn* and *anti* diolepoxides in solution is found to be highly dependent upon the purity of the compound, the pH, and the presence in the medium of other components (e.g., proteins) which can catalyze their decomposition or react directly with them. It is important to note that slightly impure samples of **1** and **2** tend to decompose considerably more rapidly in solution than those of highest purity.



2a: *Syn*-diaxial **2b**: *Syn*-diequatorial

1a: *Anti*-diaxial **1b**: *Anti*-diequatorial

FIG. 2. The conformational equilibrium between the diaxial and diequatorial forms of the diastereomeric *syn*- and *anti*-diolepoxides of benzo[*a*]pyrene (partial structures). The diaxial conformer of the *syn* isomer (**2a**) is more favorably disposed to hydrogen-bonded interaction between the 7-HO and the oxiranyl oxygen than the *syn*-diequatorial conformer (**2b**) or either of the *anti* diastereomer conformers (**1a, b**).

calculations were performed on both diolepoxides and on their protonated forms. The results presented herein are discussed in terms of the observed reactivities, mutagenicities, and DNA binding.

EXPERIMENTAL

Method of calculation. All calculations were done by the CNDO/2 method of Pople and Beveridge (*26*). The original program (*27*) was modified to accept larger molecules. The electron density at each atom was calculated according to the formula:

$$Q_a = \sum_{a}^{A} \sum_{i}^{\text{occ}} 2c_{ai}^2,$$

where the first summation is over all the atomic orbitals belonging to atom A, the second summation is over the occupied molecular orbitals, and c_{ai} is the coefficient of the ath component of the ith orbital.

Description of chemical structure. Atomic coordinates from X-ray determinations have not been used in order to avoid anomalies due to crystal packing or refinement

procedures. Rather, average bond parameters (*28*) were used; specifically (all distances in Angstroms) C–C 1.54, C–C (aromatic) 1.394, C–C (benzylic) 1.51, C_9–C_{10} (epoxide) 1.516, C–O (hydroxyl) 1.426, C–O (epoxide) 1.47, C–H 1.09, and O–H 0.97. The numbering system corresponds to the accepted IUPAC nomenclature (Fig. 3). The pyrene portion was considered to be planar and consist of regular hexagons. C_7 and C_8 were assumed to be tetrahedral. The angle between the triangular epoxide plane and an average plane described by C_8–C_9–C_{10}–C_{10a} is taken as 98.1°.

As pointed out by Hulbert (*19*), both isomers can exist in two conformations (Fig. 2). The *anti*-diequatorial (**1b**) and *syn*-diaxial (**2a**) conformers are favored due to eclipsing between the C_8–C_9 bonds in the less favored conformers (**1a**, **2b**). The CNDO/2 calculations were performed on the preferred conformations (**1b**, **2a**). In the initial series of calculations the tetrahydro ring was treated as a semi-chair with the dihedral angle between C_9 and C_{10} and the plane of the pyrene ring system equal to 25°. With this structural input the O_{epox} to 7-HO proton distance in the *syn* isomer was 2.68 Å, and the related O–H–O angle was 131°. In the second series the C_8–C_9 bond was twisted so that C_9–C_{10} was coplanar with the pyrene ring, thus changing the above distance and angle to 1.72 Å and 147°, respectively.

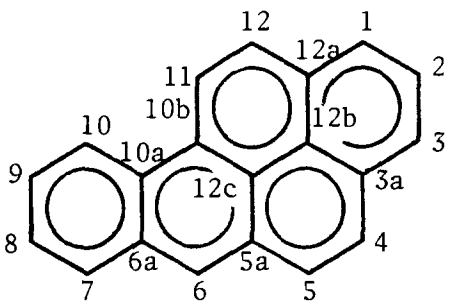


FIG. 3. Numbering system of benzo[*a*]pyrene according to IUPAC nomenclature.

RESULTS

The results of the CNDO/2 calculations are summarized in Table 1. Computer-generated graphical representations of the isomeric diolepoxide structures are depicted in Fig. 4. The calculated charge densities in the *anti* and *syn* isomers (I and III) were found in the first approximation to be essentially identical to one another at every atom, excepting C_8 and O_8. Internal hydrogen bonding, if present in the *syn* isomer, should be reflected in altered charge density at the epoxide C_9 and C_{10} atoms with respect to the same atoms of the *anti* isomer. Since this effect is not evident, hydrogen bonding of this type does not contribute significantly to this structure. Lack of hydrogen-bonded interaction is not surprising, since the bond distance (2.7 Å) between the 7-HO proton and the epoxide oxygen atom exceeds the normal range for hydrogen-bonding which is typically on the the order of 1.7 Å. It is interesting to note, however, that the calculations indicate that the positive charge density at C_{10} exceeds that at C_9 for both isomers. This would predict C_{10} as the preferred site of nucleophilic attack on the epoxide ring, in agreement with the experimental findings (*3*, *4*, *9–14*).

TABLE 1

CALCULATED CHARGE DENSITY

	C_1	C_2	C_3	C_{3a}	C_4	C_5	C_{5a}	C_6	C_{6a}	C_7	C_8	C_9	C_{10}	C_{10a}	C_{10b}	C_{11}	C_{12}	C_{12a}	C_{12b}	C_{12c}
I *Anti*	−0.013	0.005	−0.013	0.034	−0.009	−0.009	0.029	−0.017	0.002	0.140	0.190	−0.019	0.082	−0.002	0.032	−0.008	−0.009	0.034	0.014	0.014
II *Anti*-H^+	0.019	0.004	0.021	0.024	0.026	−0.026	0.066	−0.033	0.029	0.132	0.207	0.027	0.139	−0.059	0.069	−0.041	0.034	0.018	0.026	0.004
III Syn 2.7 Å	−0.014	0.005	−0.014	0.035	−0.010	−0.008	0.028	−0.016	0.000	0.139	0.179	−0.023	0.084	0.004	0.029	0.007	−0.010	0.035	0.014	0.016
IV Syn-H^+ 2.7 Å	0.018	0.004	0.020	0.025	0.025	−0.025	0.065	−0.032	0.029	0.128	0.192	0.022	0.139	−0.051	0.066	−0.040	0.033	0.020	0.026	0.006
V Syn 1.7 Å	−0.013	−0.004	−0.013	0.034	−0.009	−0.009	0.031	−0.019	0.005	0.153	0.118	0.083	0.065	0.001	0.029	−0.009	−0.008	0.034	0.015	0.014
VI Syn-H^+ 1.7 Å	0.022	0.004	0.023	0.026	0.029	−0.024	0.073	−0.027	0.028	0.140	0.132	0.142	0.098	−0.061	0.058	−0.048	0.041	0.020	0.025	0.001

	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8	H_9	H_{10}	H_{11}	H_{12}
I Anti	−0.008	−0.009	−0.008	−0.008	−0.008	−0.005	−0.010	−0.031	−0.033	0.043	−0.009	−0.008
II Anti-H^+	0.008	0.013	0.011	0.012	0.010	0.015	0.008	−0.004	0.008	0.110	−0.026	0.010
III Syn 2.7 Å	−0.008	−0.010	−0.008	−0.009	−0.009	−0.006	−0.017	−0.015	−0.042	0.066	−0.010	−0.009
IV Syn-H^+ 2.7 Å	0.008	0.013	0.011	0.012	0.010	0.016	0.023	0.031	−0.001	0.133	−0.027	0.009
V Syn 1.7 Å	−0.009	−0.010	−0.008	−0.009	−0.009	−0.007	−0.029	−0.005	−0.037	0.039	−0.011	−0.009
VI Syn-H^+ 1.7 Å	0.007	0.012	0.010	0.011	0.009	0.014	0.014	0.033	0.009	0.107	−0.022	0.010

	O_7	O_8	O_{EPOX}	$H_{7\text{-HYDROX}}$	$H_{8\text{-HYDROX}}$	H_{EPOX}
I Anti	−0.270	−0.259	−0.133	0.136	0.143	
II Anti-H^+	−0.251	−0.234	−0.013	0.164	0.181	0.292
III Syn 2.7 Å	−0.265	−0.274	−0.139	0.149	0.139	
IV Syn-H^+ 2.7 Å	−0.237	−0.268	−0.017	0.143	0.180	0.290
V Syn 1.7 Å	−0.294	−0.255	−0.175	0.177	0.144	
VI Syn-H^+ 1.7 Å	−0.249	−0.241	−0.046	0.141	0.184	0.294

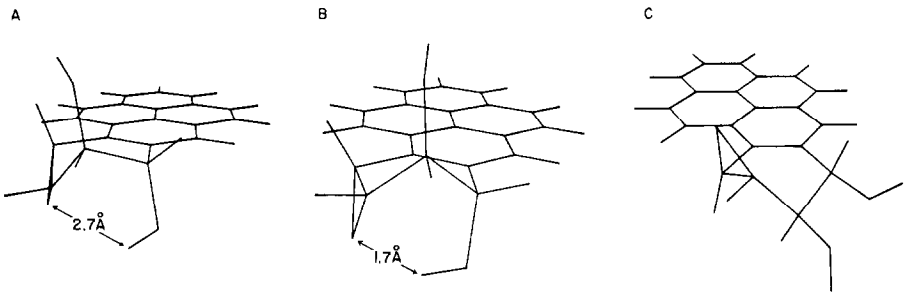


FIG. 4. Computer-generated graphical representations of the (A and B) *syn*-diaxial (**2a**) and (C) *anti*-diequatorial (**1b**) structures of the BP-diolepoxides.

In the second approximation, the optimum bond distance (*29*) of 1.7 Å between the 7-HO proton and the epoxide oxygen atom was assumed. To achieve this, the C_8–C_9 bond had to be twisted to make the C_9–C_{10} bond coplanar with the pyrene ring. This altered the conformation of the tetrahydro ring from a semi chair to a puckered structure. The energy released from H-bond formation more than compensated for the consequent ring strain, since the total energy of V is found to be lower than that of III (Table 2). The net energy difference of 0.0659 a.u. between structures III and V is considerable (41 kcal/mol, since 1 a.u. = 623 kcal/mol); the steric strain introduced is taken into account in these calculations. Internal H-bonding causes a rather dramatic decrease in the electron density at C_9 and, unexpectedly, an increase in the negative charge density at C_{10}. On this basis nucleophilic attack would be predicted to occur preferentially at C_9 rather than C_{10}. Although metabolic products arising from covalent binding at C_9 have not yet been detected; conceivably they may be found among the lesser products still unidentified.

Since acid catalysis of the reactions of epoxides (*30*) and diolepoxides (*23*, *25*) is well known, it was of interest to determine the effect of proton addition to the O_{epox} atom of **1** and **2**. As expected, protonation caused a large decrease in the electron density of this oxygen atom (Table 1: II, IV, and VI) and decreased the extent of H-bonding as shown by the increased electron density on $H_{7\text{-}HO}$ of VI in comparison with V. More significantly, protonation decreased the electron density on the carbon atoms of the tetrahydro ring, except for C_7 which showed a slight increase. The effect was in the order $C_8 < C_9 < C_{10}$ for II and IV, but $C_{10} < C_8 < C_9$ in the case of VI. However, VI proved lower in total energy than IV (Table 2), indicating it to be the more favorable protonated structure.

TABLE 2

CALCULATED TOTAL ENERGY

	Energy (a.u.)
I Anti	−108.3459
II Anti-H^+	−108.8025
III Syn 2.7 Å	−108.3499
IV Syn-H^+ 2.7 Å	−108.8089
V Syn 1.7 Å	−108.4158
VI Syn-H^+ 1.7 Å	−108.8735

DISCUSSION

The foregoing results provide some insight into the relative chemical reactivity and the extraordinary biological properties of these diolepoxide derivatives of BP.

According to the hypothesis of Hulbert (*19*), the susceptibility of the *syn*-BP-diolepoxide isomer to nucleophilic attack is enhanced by intramolecular H-bonding. While the stabilizing effect of such bonding is confirmed by the CNDO/2 calculations, a surprising decrease in electron density at C_9 and increase in negative charge density at C_{10} are also observed. While this would predict preferential reaction at C_9, the only available experimental evidence demonstrates that both **1** and **2** react with water (*23*, *25*), *tert*-butylthiolate (*3*), and nucleic acids (*4*, *9–12*) preferentially at C_{10}. However, these reactions were conducted in aqueous media, conditions under which it is probable that intermolecular H-bonded association with the solvent or cations present may be of greater importance than intramolecular association. Under these conditions, the intermediate is more likely to exist in a structure intermediate between the protonated and unprotonated forms, IV and III, both of which predict C_{10} as the preferred reaction site, in agreement with the findings.[8] An alternative possibility is an SN_1 mechanism involving proton assisted ring-opening prior to nucleophilic attack at C_{10}.

With weaker nucleophiles, such as methanol or water, the evidence supports the SN_1 mechanism. Thus, substantial amounts of *cis* ring-opened products are formed during methanolysis (*31*) and hydrolysis (*23*, *25*, *31*) of both **1** and **2**. Kinetic studies of aqueous solvolysis conducted by Keller *et al.* (*23*) showed the rates of hydrolysis of the *syn* and *anti* isomers in 1 : 1 dioxane-water to be pH dependent and relatively similar. However, the *syn* isomer was found to undergo exclusive *cis* addition of water (within the limits of experimental detection) at pH 5, whereas the *anti* form furnished the isomeric tetraols arising from both *trans* and *cis* addition. These studies and the subsequent more thorough kinetic studies by Yang *et al.* (*25*) over a wider pH range support the largely SN_1 character of these reactions. This conclusion is also consistent with previous findings concerning hydrolysis of arene oxides which show that at acidic pH the rate-limiting step is carbon–oxygen cleavage preceded by protonation of oxygen (*32*, *33*). The observed strong *cis* stereoselectivity of hydration of the *syn* isomer has been explained by Yang *et al.*, as a consequence of H-bonding between the hydroxyl groups at C_7 and C_9 of the carbonium ion intermediate and steric interference between the remaining hydroxyl group at C_8 and the incoming water molecule, directing attack to the opposite ring face (Fig. 5) (*25*). While this explanation appears reasonable and compatible with the carbonium ion structure, it would be premature to accept as valid this or any other mechanism at this time in the absence of direct experimental evidence.

With stronger nucleophiles [*tert*-butylthiolate (*3*), methoxide (*31*), *p*-nitrothiophenolate (*31*), aniline (*31*)] ring-opening is *trans*-stereospecific (or highly *trans*-stereoselective), and an SN_2 mechanism is clearly implicated. In all cases nucleophilic attack occurs at C_{10} of both **1** and **2**. Intermediates between the proton- or cation-associated structures, II and IV, and the free forms, I and III, are most probable, the extent of proton or cation association being dependent upon pH, cation concentration, ionic strength, and other factors. Kinetic measurements have been carried out only for

[8] In aqueous media, facile solvolysis to afford the tetraols also occurs, complicating attempts to obtain accurate kinetic rate data for reactions with nucleophiles.

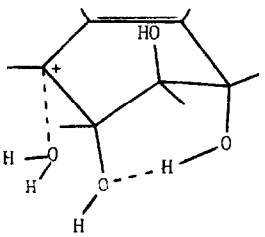


FIG. 5. Stabilization of the carbonium ion intermediate obtained from acid-catalyzed hydrolysis of **2a** by H-bonding.

reaction of *p*-nitrothiophenolate with **1** and **2** (*14*). These reactions were conducted in *tert*-butanol plus dimethyl sulfoxide in the absence of water. Under these conditions reaction of the *syn* isomer was found to be faster by a factor of 160. This finding was taken as evidence by Yagi *et al.* (*14*) for the internally H-bonded intermediate V. However, attack of the nucleophile has been shown subsequently to occur at C_{10} (*31*), rather than at C_9 as predicted by this structure. Moreover, the observed greater reactivity of the *syn* isomer is not consistent with the calculated charge densities which are quite similar for the most electrophilic carbon atom of the epoxide rings of I, III, and V (Table 1). The results are explicable, however, on the assumption that internal H-bonding promotes reaction by stabilizing the transition complex (Fig. 6). The critical points is that H-bonding does not aid thiolate attack on the epoxide ring, but once the addition is initiated intramolecular association facilitates reaction by delocalization of negative charge in the transition state. Behavior of this type has been documented previously. Thus, Houminer observed a similar rate enhancement by β-hydroxyl groups in the reaction of azide ion with steroidal epoxides (*34*). His results indicated that H-bonding became important only after azide addition. Although the charge density in the ground state of the H-bonded form of **2** favors attack at C_9, reaction is more probable at C_{10} because the developing positive charge at this site can be delocalized through the pyrene ring. It should be emphasized that the reactions with *p*-nitrothiophenolate were conducted in the absence of water, conditions favoring intermolecular H-bonding. Similar rate enhancement is improbable for analogous reactions of this or other nucleophiles in aqueous media or *in vivo*.

Reactions of the BP diolepoxides with nucleic acids are presumed to be of greatest significance with respect to their biological activities. Both isomeric diolepoxides have been shown to be highly efficient alkylating agents of DNA, RNA, and purine and

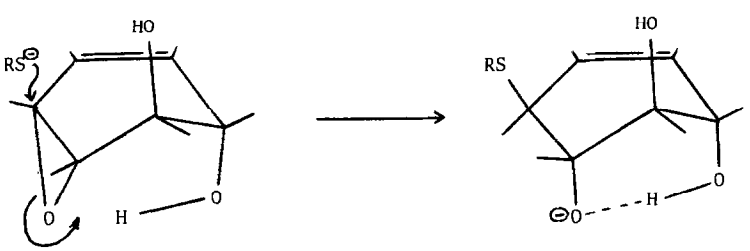


FIG. 6. Facilitation of reaction of **2a** with nucleophiles through stabilization of the transition complex by intramolecular H-bonding.

pyrimidine homopolymers (*9*, *10*, *22*). The structures of the major bound products following degradation to the nucleotide or nucleoside level have been determined and shown to involve covalent bonding between the C_{10} benzylic positions of **1** and **2** and the 2-NH_2 group of guanosine (*4*, *9*, *12*). Ring-opening is predominantly *trans*-stereospecific. These results are consistent with an SN_2 mechanism involving attack by the nucleophilic center on C_{10} of the hydrated forms of I and III at neutral pH, i.e., intermediates between the extremes of I and II and III and IV, respectively.

Can differences in chemical reactivity and molecular structure account for the observed differences in biological activities of the *syn* and *anti* isomers? The CNDO/2 calculations indicate these stereoisomers to be structurally quite similar and predict no dramatic differences in their reactivity in the ground state. The kinetic studies of hydrolysis tend to support this conclusion. One factor not considered in the theoretical treatment which may contribute significantly to differences in reactivity with cellular macromolecules, if such do indeed exist, is the fact that the diolepoxides formed metabolically are optically active and the nucleic acids and protein reactants are optically active and possess secondary structure which may favor preferential reaction of only one enantiomer. There is now evidence that the enantiomers of **1** and **2** do indeed exhibit differences in mutagenic activity (*35*). A definitive answer to these questions is dependent upon greater information concerning other factors including relative rates of enzymatic detoxification, DNA repair, etc., which enter this complex problem.

ACKNOWLEDGMENTS

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