neuronal activity responsible for actual behavior in response to light [(7); Y. Goh and D. L. Alkon, in preparation].
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13. The treatment condition of each animal was revealed after all voltage-clamp recordings had been analyzed and the quantitative measurements made.
14. A number of criteria for acceptability of impalement were used: (i) the resting potentials recorded by the voltage-recording and current passing electrodes should be approximately equal, (ii) the response to light recorded by each electrode should be approximately the same, (iii) the leak current at 0 mV should be ≤ 10 nA, (iv) the settling time of the voltage clamp should be ≤ 20 msec, and (v) the holding current at −60 mV should be ≤ 5 nA.
15. Corrections of I_A values for "leak" current were obtained by extrapolation from a linear portion of the current-voltage relation, which was generated with small positive and negative command pulses. I_A was also corrected for I_B, which was estimated from current values measured 1 second from the onset of the second of the twin pulses. This estimate agreed very closely with I_B values obtained with a test pulse (to 0 mV) occurring 30 μsec after a 5.0-second preliminary pulse to −10 mV. This correction for I_B was arrived at after numerous previous experiments (J. J. Shoukimas and D. L. Alkon, unpublished observations) that established I_B characteristics in the absence of I_A (after blocking with 4-aminopyridine).
16. The time constant for inactivation of I_A is much greater than the time constant of inactivation. Furthermore, there were no apparent between-group differences in the rising phase of I_A. Between-group differences in the decrement of I_A for the second of the twin pulses, therefore, were considered to largely reflect changes of inactivation of I_A rather than activation.
17. Other electrophysiological studies are also suggesting differences among the three type B cells with regard to changes during associative learning (T. Crow and D. L. Alkon, in preparation; J. Farley and D. L. Alkon, in preparation).
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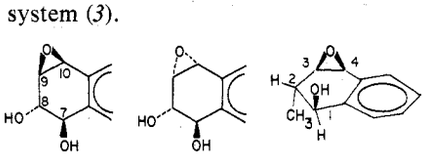
Internal Hydrogen Bond Formation in a *syn*-Hydroxyepoxide

Abstract. *The existence of an internal hydrogen bond in a compound representative of a* syn *diol epoxide (a possible intermediate in chemical carcinogenesis by certain polycyclic aromatic hydrocarbons) has been demonstrated by x-ray crystallographic and nuclear magnetic resonance studies. This internal hydrogen bond was found in 3,4-epoxy-2-methyl-1,2,3,4-tetrahydro-1-naphthol and was shown to persist in dioxane-water solutions containing up to 80 mole percent water. In this structure, the 1-hydroxy and 2-methyl groups are shown to occupy axial positions. In the* anti *diol epoxide, which has no internal hydrogen bond, analogous groups are equatorial. Crystals of the compound were unstable in the x-ray beam while the data were being collected (even at low temperatures), presumably as a result of decomposition.*

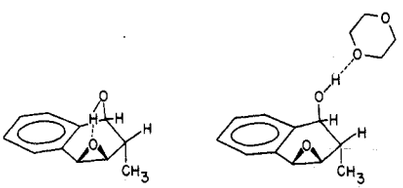
Initiation of carcinogenesis by benzo[*a*]pyrene (BaP) is considered by many to proceed by alkylation of DNA by an intermediate diol epoxide metabolite of BaP (*1*). Recent studies have shown that rat liver microsomes convert BaP, in part, to two diastereomeric diol epoxides [(−)-**1** and (+)-**2**] of high enantiomeric purity (*2*). The isomers are designated *syn* if the benzylic hydroxyl (the hydroxyl at position 7 of **1**) and epoxide groups project on the same side of the tetrahydrobenzene ring system (**1**), and *anti* if they lie on opposite sides (**2**). Isomer **2** has been identified as a potent carcinogen in newborn mice, and is thought to be the ultimate carcinogen of BaP in this system (*3*).



1 (*syn*) **2** (*anti*) **3** (*syn*)

Hulbert (*4*) pointed out that internal hydrogen bonding between the benzylic hydroxyl and epoxide groups is possible in *syn* diol epoxide **1**. Since this hydrogen bonding would weaken the C-O bonds of the epoxide, it was pointed out that nucleophilic attack at C(9) or C(10) of **1** would be facilitated. Indeed, the *syn* isomer (**1**) is about 160 times more reactive toward nucleophilic reaction at C(10) by the *p*-nitrothiophenolate ion in *t*-butyl alcohol than its *anti* isomer **2**, which appears not able to form such a stable intramolecular hydrogen bond (*5*). Presumably, then, the greater reactivity of the *syn* isomer causes it to interact with other macromolecules before it can reach the critical target for carcinogenesis. The structure of the *anti* BaP diol epoxide **2** has been studied by x-ray diffraction techniques (*6*). In addition, the probable three-dimensional structures of both isomers **1** and **2** have been constructed mathematically by merging known crystal structures (*7*).

We report here the crystal structure of a tetrahydronaphthalene epoxide **3** containing a benzylhydroxyl group located *syn* to the epoxide group (**3**). This compound is structurally related to the *syn* naphthalenediol epoxide (**1**), with the C(8) hydroxyl group of **1** replaced in **3** by a methyl group on C(2) (equivalent to C(8) of **1**). Compound **3** was prepared from *trans*-2-methyl-1-acetoxy-1,2,3,4-tetrahydronaphthalene by the following series of reactions: *N*-bromosuccinimide (NBS) bromination at C(4), dehydrobromination, and epoxidation to yield a mixture of the acetate of **3** and a diastereomeric acetate with the epoxide and acetate groups in a *trans* relation. The isomeric acetates were separated by alumina chromatography, and the *syn* acetate was hydrolyzed to **3**. Purification of **3** was effected by crystallization from an ether-pentane solution (*8*).



4 **5**

The ^1H nuclear magnetic resonance (NMR) spectrum of **3** in either CCl_4 or [$^2H_8$]dioxane revealed a small coupling of 2 Hz between H(1) and H(2), which suggests that the C(1) hydroxyl and C(2) methyl groups occupy axial positions. The ^1H NMR spectrum of **3** in CCl_4 showed an H(1)-OH coupling of 12 Hz

Table 1. Atomic parameters as fractions of cell edges with estimated standard deviation values in parentheses.

Atom	*x*	*y*	*z*	Atom	*x*	*y*	*z*
O(1)	0.4476(3)	0.7269(3)	−0.1283(4)	H(O1)	0.417(5)	0.647(5)	−0.051(7)
O(2)	0.4245(3)	0.6013(3)	0.2567(4)	H(C1)	0.679(3)	0.930(3)	−0.014(5)
C(1)	0.6138(4)	0.8298(4)	0.0350(6)	H(C2)	0.488(4)	0.888(3)	0.201(5)
C(2)	0.5727(4)	0.8758(4)	0.2448(6)	H(C3)	0.500(3)	0.771(3)	0.536(5)
C(3)	0.5247(4)	0.7542(4)	0.3805(6)	H(C4)	0.639(4)	0.607(4)	0.525(6)
C(4)	0.6229(4)	0.6575(4)	0.3922(6)	H(C5)	0.868(5)	0.558(5)	0.438(7)
C(5)	0.8891(4)	0.6194(4)	0.3145(6)	H(C6)	1.101(5)	0.592(4)	0.226(6)
C(6)	1.0161(5)	0.6420(4)	0.1948(8)	H(C7)	1.117(4)	0.756(4)	−0.073(6)
C(7)	1.0182(5)	0.7257(5)	0.0265(7)	H(C8)	0.892(4)	0.850(3)	−0.149(5)
C(8)	0.8902(5)	0.7887(4)	−0.0239(6)	H1(C11)	0.861(5)	1.006(5)	0.450(7)
C(9)	0.7608(4)	0.7669(4)	0.0936(6)	H2(C11)	0.765(4)	1.101(4)	0.314(5)
C(10)	0.7585(4)	0.6810(4)	0.2623(6)	H3(C11)	0.707(4)	1.029(3)	0.540(5)
C(11)	0.7295(5)	1.0175(4)	0.3933(7)				

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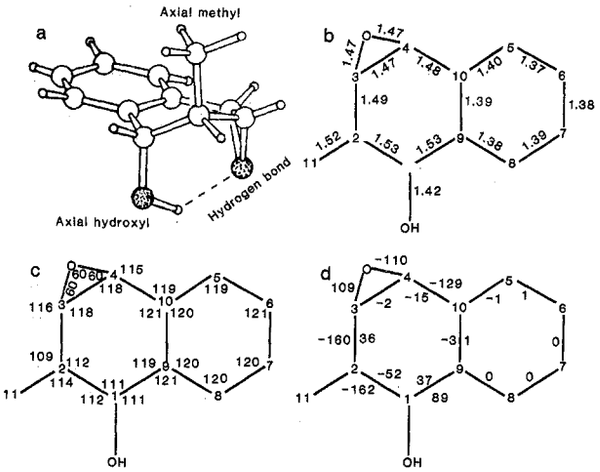

Fig. 1. (a) General view of the molecule drawn by the computer program VIEW (*13*). Oxygen atoms are stippled and hydrogen atoms are smaller than oxygen atoms. The internal hydrogen bond is indicated by broken lines. (b) Bond lengths (estimated standard deviations) 0.002 to 0.006 Å. (c) Interbond angles (estimated standard deviation values 0.2° to 0.3°). (d) Torsion angles (estimated standard deviation values 0.3° to 0.6°); the positive sense is clockwise.

and a high field resonance at δ 2.57 for the hydroxyl hydrogen. These data suggest that the hydroxyl hydrogen is located in the shielding cone of the epoxide group (as indicated by hydrogen bonded structure **4**), and the *anti* relationship between H(1) and the O-H gives rise to a large coupling (*9*). Since intramolecular hydrogen bonding would most likely be favored in nonpolar solvents, we then obtained the spectrum of **3** in the more basic solvent, dioxane, in order to determine if hydrogen bonding to the solvent (as depicted by **5**) would compete with the intramolecular hydrogen bonding shown in **4**. External hydrogen bonding would destroy the *anti* relationship between H(1) and the OH hydrogens, and should cause a reduction in the H(1)-OH coupling. However, the H(1)-OH coupling of **3** in [$^2H_8$]dioxane is 11.0 Hz, an indication that the intramolecular hydrogen bond is still favored. Spectra of **3** were then recorded in solutions of water and [$^2H_8$]dioxane solutions. The magnitude of the H(1)-OH coupling remained at 11.0 Hz in solutions containing up to 80 mole percent water (20 mole percent dioxane) (*10*).

Crystals of **3** were grown and the structure was determined by x-ray diffraction techniques (*11*). Three-dimensional data were collected on an automated four-circle diffractometer with the use of the variable θ-2θ scan mode and graphite-crystal monochromatized MoKα radiation. Since crystal decomposition occurred rapidly on irradiation with x-rays, data were collected on a crystal cooled to −80°C. The minimum scan rate was 6 deg min^{-1}. Of 1828 independent experimental data, 1276 had intensity, I, greater than 1.0 $\sigma(I)$ (σ is an estimated standard deviation based on counting statistics). Values of $\sigma(F)$ were calculated as $\sigma(F) = (F/2)\{(\sigma^2(I)/I^2) + \delta^2)\}^{1/2}$ where δ is an instrumental uncertainty constant (0.013) determined experimentally and F is the structure amplitude of each reflection. The data were corrected for loss of intensity as a function of time. The structure was solved by the Patterson superposition technique and refined by a full-matrix least-squares procedure. All hydrogen atoms were found from Fourier difference maps and included in the final refinement cycles. The refinement terminated with $R = 0.091$, $wR = 0.062$ [where $w = 1/\sigma^2(F_o)$ for reflections above the observational threshold of $\sigma(I)$ and $R = \Sigma \| F_o | - | F_c \| / \Sigma | F_o |$]. Presumably the decomposition of the crystal induced by x-rays during data collection affects these R values. Atomic parameters are given in Table 1. A list of observed and calculated structure factors is available.

The structure determination shows that the hydroxyl-epoxide hydrogen bond (**3**) is indeed intramolecular (Fig. 1a). The bond lengths, interbond angles, and torsion angles, except those involving hydrogen atoms, are listed in Fig. 1, b to d, respectively. The internal hydrogen bond, O(1)-H(O1)···O(2) is 2.792(3) Å in length with an O-H···O angle of 142(3)°. (The numbers in parentheses refer to the estimated standard deviations with respect to the last digit quoted.) This hydrogen bond has been shown to be sufficiently strong to exist in concentrated aqueous dioxane solutions. The ring pucker is such that C(2), which bears the methyl group, deviates more from the general plane of the ring system than does C(3), which is part of the epoxide ring system. The epoxide ring is approximately symmetrical with C-O distances of 1.466(4) and 1.473(3) Å, with no distortion due to steric effects (*12*). The two ring substituents—the hydroxyl group on C(1) and the methyl group on C(2)—occupy axial positions in this structure. The hydroxyl groups in *syn* diol epoxides (*1*) are known from ^{1}H NMR data also to occupy axial positions [whereas they are known to be equatorial in the *anti* diol epoxide of BaP (*6*)]. By analogy with **3**, a similar strong internal hydrogen bond may exist in **1**, even in highly aqueous solutions. This axial conformation may affect possible interactions with DNA prior to alkylation since the internal hydrogen bond is strong.

The disorder in the crystal structure as a result of radiation damage is demonstrated by additional peaks in electron density maps. A detailed analysis was not possible but the peaks were consistent with cleavage of a C-O bond of the epoxide ring. As a result, the formation of a ketone might be expected. In acid solution triols are formed, presumably by ring opening at the benzyl position [C(4)].

Thus the existence of an internal hydrogen bond, rather than intermolecular hydrogen bond formation, is demonstrated in the crystalline state and is shown to persist in aqueous dioxane solutions, up to at least 80 mole percent water.

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9. Similar ^{1}H NMR data for the *syn*-naphthalene-diol epoxide in $^2H_6$-labeled dimethylsulfoxide was observed (*5*).
10. Whether internal hydrogen bonding in **3** persists in aqueous solutions with more than 80 mole percent water remains to be determined.
11. Crystals are triclinic, space group $P\bar{1}$. Data collected at −80°C, MoKα radiation. Unit cell dimensions: $a = 8.098(7)$, $b = 9.938(6)$, $c = 6.279(6)$, $\alpha = 93.03(6)$, $\beta = 104.35(7)$, $\gamma = 113.60(5)°$, crystal size: 0.30 by 0.18 by 0.10 mm.
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