Calculated Properties of Arene Oxides of Biological Interest. 1. Molecular Orbital Examination of Simple Models

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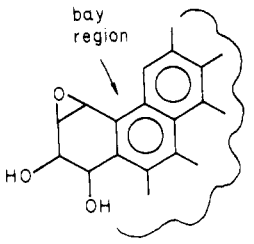
Received April 10, 1978

The INDO calculational formalism is shown to account for the relative ease of carbonium ion formation and for the variation in isomerization rates of some simple arene oxides. Transition-state energy differences provide a reasonable correlation with observed isomerization rates for benzene oxide, methyl-substituted benzene oxides, naphthalene 1,2-oxide, and phenanthrene 9,10-oxide.

Recent developments in the search for the molecular mechanism of carcinogenesis of aromatic hydrocarbons have led to a new model.[1] The essence of the model is the postulated formation of a relatively stable carbonium ion from an intermediate diol epoxide on a benzo ring of the aromatic hydrocarbon. The diol epoxide is produced in vivo by sequential action of hepatic monooxygenases and epoxide hydrases. Ring opening of the epoxide leads to a transient carbonium ion; the rate of formation of the ionic species is believed to be higher for the carcinogenic diol epoxides than for other possible diol epoxides. A crucial fate for the carbonium ion is its reaction with a critical nucleophilic component of the cell. The derivatized component is then involved in transformation of the cell.

A test of the validity of this concept would be to examine the relative ease of formation of carbonium ions generated from several aromatic hydrocarbons and derivatives thereof—particularly the diol epoxides. Because direct measurement of the rate of formation of such ions in the cell is not presently feasible and since few diol epoxides were available for study, a theoretical approach was taken. Jerina and Lehr,[1] in formulating the "bay region" (I)



I

hypothesis for correlating ease of carbonium ion formation with carcinogenic potential, used the perturbational Hückel molecular orbital (HMO) treatment to provide a straightforward indication of increased reactivity. The calculations indicated that those diol epoxides in which the epoxide group formed part of a bay region on a saturated, angular benzo ring would be the most reactive of the possible isomeric diol epoxides of a given hydrocarbon. Furthermore, comparisons of the predicted reactivity with the carcinogenicity of the parent hydrocarbons indicated a reasonable correlation.

Since this model does not incorporate more subtle features of the molecular species involved, a more detailed investigation is desirable. To begin to study this problem methodically, however, one should first establish the ability of the theoretical methods employed to correctly account for relevant properties of simpler epoxides.

Although there is little evidence at present which suggests that arene oxides themselves are ultimate carcinogens, a wide body of evidence has implicated substituted benzene oxides as toxic agents responsible for the hepatic centrolobular necrosis observed following administration of various benzene derivatives.[2-6] Chronic exposure to benzene itself is known to lead to bone-marrow damage.[7] Thus, the present calculations are intended to pave the way for more sophisticated computations on substituted benzene oxides as well as on diol epoxides of polycyclic aromatic hydrocarbons in the hope that insights will be gained into the mechanism of action of these highly biologically active molecules. The calculations reported here, then, are the initial results of examination of some simple arene oxides.

Few theoretical calculations of the properties of epoxides have been reported. The oxepin ⇌ epoxide equilibrium was examined by Stohrer and Hoffmann[8] using the extended Hückel theory (EHT); a model for the ring-opening polymerization of ethylene oxide was explored by Frenking et al. via the CNDO/2 method.[9] Comparative calculations using EHT, CNDO/2, and INDO were carried out by Mezey et al.;[10] these workers followed the reaction coordinate for formation (and decomposition) of ethylene oxide in detail. They found that only the INDO[11] method gave qualitatively correct potential-energy curves for the ring-opening process leading to carbonium ion formation. Thus, the approach chosen here involved the INDO formalism,[12] and the first effort was to determine whether or not an energy minimum for a logical conformation of benzene oxide could be obtained. Although X-ray diffraction data on crystalline benzene oxide are unavailable, Glusker et al. have reported crystal structures for phenanthrene 9,10-oxide, 7,12-dimethylbenzanthracene, 5,6-oxide, and benzo[*a*]pyrene 4,5-oxide.[15,16] The dimensions of the epoxide moiety are grossly similar among these structures. With the dimensions reported for phenanthrene 9,10-oxide of the epoxide and the ring to which it was fused, an initial geometry was generated. The potential energy well representing a relatively stable conformation of benzene oxide was probed by varying the location of the oxygen atom with respect to the benzene ring. One simplified test of conformational preference thus involved methodically displacing the oxygen atom on an *x*,*y*,*z* grid in 0.1-Å increments. The total energy of the molecule was recalculated after each displacement. The results in Table I indicate that an energy minimum is indeed found and that the location of the oxygen atom for the energy minimum is near that corresponding to the initial model taken from the X-ray data.

For purposes of subsequent modification of the parent benzene oxide structure, e.g., attachment of methyl groups, it was desirable to use a geometry generated from standard (for INDO)[11] bond angles and distances. The total energy of such a structure (dimensions in Figure 1) was found to be only slightly higher (2.1 kcal) than the minimum-energy

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Table I. Variation in Oxygen Atom Position in Benzene Oxide

position, 0.1-Å increments	total E, kcal/mol	ΔE, kcal/mol
initial[a]	−39669.48	0
x + 0.1	−39669.33	+0.1
x − 0.1	−39664.65	+4.8
y + 0.1	−39670.78	−0.3
y − 0.1	−39642.97	+26.5
z + 0.1	−39664.36	+5.1
z − 0.1	−39664.19	+5.3
y + 0.2	−39633.49	+36.0
y + 0.1, x + 0.1	−39663.05	+6.4
y + 0.1, x − 0.1	−39674.26	−4.8[b]
y + 0.1, z + 0.1	−39663.13	+6.3
y + 0.1, z − 0.1	−39662.95	+6.5
y + 0.1, z − 0.2	−39671.64	−2.2
y + 0.2, x − 0.2	−39662.95	+6.3
y + 0.1, x − 0.1, z + 0.1	−39667.58	+1.9
y + 0.1, x − 0.1, z − 0.1	−39667.31	+2.2

[a] Initial Cartesian coordinates (numbering as in Figure 1):

	x	y	z
O(1)	−0.3114	−1.2217	0.7381
C(2)	0.0	0.0	0.0
C(3)	0.0	0.0	1.4722
C(4)	1.2942	0.0	2.1874
C(5)	2.5000	−0.0723	1.4749
C(6)	2.4998	−0.0847	−0.0057
C(7)	1.2887	−0.0003	−0.7148
H(8)	−0.7801	0.4521	−0.5374
H(9)	−0.7765	0.4503	2.1136
H(10)	1.3030	0.0655	−1.8027
H(11)	3.3442	−0.1595	−0.5481
H(12)	3.4452	−0.1197	2.0156
H(13)	1.3112	0.0566	3.2757

[b] Calculated minimum-energy position.

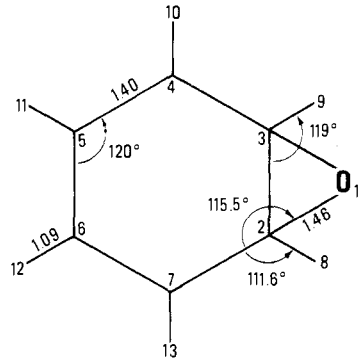


Figure 1. Dimensions of "standard" benzene oxide: dihedral angle 6–7–2–8 = 156.8°; dihedral angle 6–7–2–1 = 290°.

structure of Table I; the "standard" structure was then used as the comparative basis for INDO calculations on subsequently modified models.

Experimentally, the acid-catalyzed ring-opening preferences of the three monomethylbenzene oxides to produce phenols are known to occur with high specificity.[17] Thus both 1- and 3-methylbenzene oxide isomerize predominantly to 2-methylphenol while 4-methylbenzene oxide isomerizes mainly to 4-methylphenol. A test of those preferences by calculation would be to determine the energy difference between the ground state or protonated epoxide state and the carbonium ion transition states required for the dominant and minor ring-opened species. The results of such INDO calculations are seen in Figure 2. It is evident that the positive energy change is less in each case for the known preferred pathway. That is, the preferred pathway has a lower activation energy in the model reactions. Additional calculations on naphthalene 1,2-oxide and its two ring-opened hydroxycarbonium ions

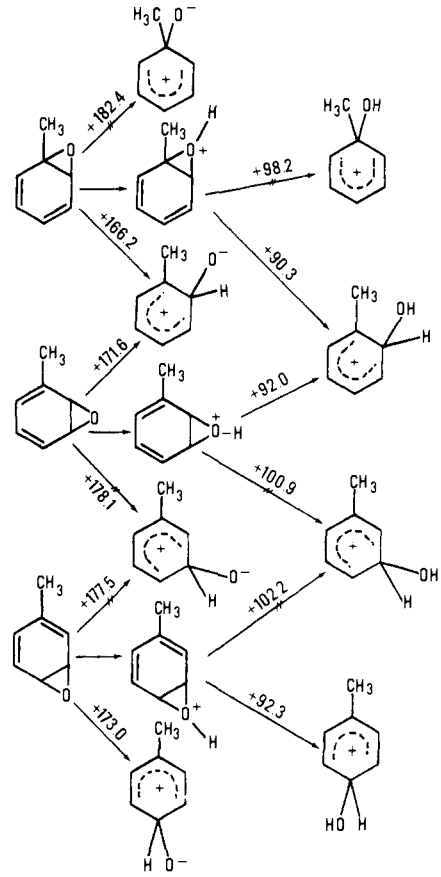
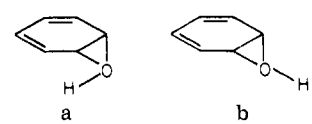


Figure 2. Energy differences in kilocalories for reaction pathways.

are also consistent with the predominant isomerization to 1-naphthol;[18] thus, the energy difference for formation of the 1-hydroxy ion from the protonated epoxide (+100.4 kcal/mol) is lower than that for the formation of the 2-hydroxy species (+113.5 kcal/mol). Similarly, the calculated energy difference for formation of the 1-hydroxy ion from the protonated phenanthrene 1,2-oxide[18] (+93.9 kcal/mol) is less than the energy difference for the 2-hydroxy ion (+104.2 kcal/mol). Experimentally, the epoxide ring-opening preference for benzanthracene 5,6-oxide is not so clear-cut, the reported yields of 5- and 6-hydroxybenzanthracene (from dehydration of the 5,6-diol) being 43 and 33%, respectively.[19] The calculated energy differences are very nearly the same for the two possible pathways (+113.1 kcal/mol for the 5-hydroxy ion and +113.9 kcal/mol for the 6-hydroxy ion). The many approximations used in the semiempirical calculations preclude attaching great quantitative significance to these energy differences; however, within the context of parallel changes in structure among the various arene oxides, they appear to account very well for observed qualitative results and trends.

In the course of examination of the proposed reaction pathway (Figure 2), it was necessary to decide on a preferred conformation of the protonated epoxide; comparative INDO calculations indicated that the extended "exo" conformer, b, was favored by 1.7 kcal/mol. The exo-



a b

geometry was adopted for the calculations on the methyl-substituted benzene oxides indicated in Figure 2. These dimensions are the same as those in Figure 1 for benzene

Table II. Molecular Orbital Indices for Arene Oxides

compd	HOMO, au	ΔE^a (protonated epoxide), kcal/mol	ΔE^a (zwitterion), kcal/mol	oxygen $2p_y$[b] population
benzene oxide	−0.4079	−298.0	+175.9	1.897
phenanthrene 9,10-oxide	−0.3954	−313.4	+199.6	NC[c]
benzanthracene 5,6-oxide	−0.3603	−316.6	NC[c]	NC[c]
naphthalene 1,2-oxide	−0.3951	−308.9	+188.0	1.904
3-methylbenzene oxide	−0.3922	−301.6	+171.6	1.897
4-methylbenzene oxide	−0.4005	−303.4	+173.0	1.897
4,5-dimethylbenzene oxide	−0.3941	−306.9	+173.2	1.898
1-methylbenzene oxide	−0.4038	−305.4	+166.2	1.899
1,4-dimethylbenzene oxide	−0.3959	−308.9	+167.7	1.900

[a] Relative to calculated ground-state energy. These represent anticipated intermediate structures along the isomerization reaction coordinate. [b] Electron population of the 2p orbital on oxygen oriented along the y axis in a coordinate system common to all the compounds; it is presumably the principal bonding orbital for an approaching proton. [c] NC = not calculated.

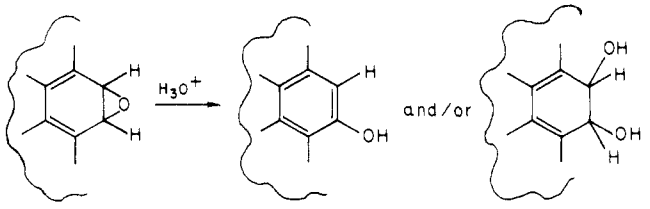
Table III. Correlation Parameters for Arene Oxides

compd	ref	TSE,[a] kcal/mol	ΔTSE[b]	log k_{H^+}, M^{-1} s^{-1} calcd	log k_{H^+}, M^{-1} s^{-1} obsd
benzene oxide	18	200.8	0	0.40	0.30[c]
phenanthrene 9,10-oxide	18	200.4	−0.4	0.33	0.36
naphthalene 1,2-oxide	18	208.5	7.7	1.73	1.40
benzanthracene 5,6-oxide	18	203.2[d]	2.4	0.86	1.00
3-methylbenzene oxide	26	209.6	8.8	1.92	1.98
4-methylbenzene oxide	26	211.1	10.3	2.18	2.67
4,5-dimethylbenzene oxide	26	213.8	13.0	2.65	3.31[c]
1-methylbenzene oxide	26	215.1	14.3	2.87	2.02
1,4-dimethylbenzene oxide	27	213.5[d]	12.7	2.98	3.01

[a] Transition-state energy (energy difference between ground-state reactant and ring-opened carbonium ion; net charge is negative because of addition of the energy of a proton to the ground-state structure). [b] TSE difference relative to benzene oxide as the "parent" compound. [c] Corrected for symmetry, i.e., ring opening either way yields the same product; thus, the single-position rate is half the observed rate. [d] Weighted average based on the ratio of products formed.

oxide with the addition of a proton with a bond length of 0.96 Å and bond angle of 110° with respect to each C–O epoxide bond.

These computations were subsequently extended to the xylene oxides and to phenanthrene 9,10-oxide. Table II lists a number of calculated properties of some of these compounds. These may be compared with an experimentally determined property, log k_{H^+}, in Table III; this value is the logarithm of the acid-catalyzed rate for the reactions



There is not an obvious correlation with the energy difference for zwitterionic formation or protonation of the epoxide. Although the zwitterionic energies were included for possible correlation consideration, those intermediate structures are anticipated in the nonacid-catalyzed k_0 pathway. Furthermore, the energies of the highest occupied molecular orbital (HOMO) and the electron populations of the reacting p orbital on oxygen (O_{2p_y}) are not consistently related to the reaction rate; in fact, the electron populations seem rather unaffected by substituent changes on the parent structure, benzene oxide.

Table III also lists some additional properties of these molecules; in particular, a transition-state energy (TSE) and a ΔTSE value was calculated for each—based on benzene oxide as a parent molecule. Linear-regression analysis indicated a highly significant correlation with ΔTSE, as shown in eq 1. The values in parentheses are the standard errors of the coefficients.

$$\log k_{H^+}(\pm 0.492) = 0.176(\pm 0.031)\Delta\text{TSE} + 0.436(\pm 0.286) \quad (1)$$

$$n = 9;\ r^2 = 0.82;\ P(\text{F}) = 0.01$$

These initial studies indicate that the INDO formalism provides calculated properties consistent with the known direction and rate of isomerization of several arene oxides. It should be noted, of course, that the calculations do not include solvent effect or counterion contributions. The reported properties are those of bare molecules and ions; nevertheless, comparative treatment of all congeners in a series by this approach has been shown repeatedly to provide valid and informative results (for example, see ref 20 and 21).

The obvious premise for such correlations is the probability that the molecular environment at the receptor for the molecules being compared is reasonably constant; a further assumption is made that the model interaction for the series is the dominant one. While the INDO treatment of these molecules and carbonium ions is consistent in the series, there are well-known limitations in any detailed analysis of the computational results.[11] For these reasons, the calculated absolute energies of the reactant molecules are not meaningful, and the energy differences have significance only in the relative sense; it is this relative comparison, however, which enables us to draw some useful conclusions about the biological effect of structural changes among the group of compounds studied.

Not included in this work are more sophisticated results involving geometry optimization of the ground- and transition-state models and the extension of the models to include the diol epoxides. Jerina and Lehr[1] have used

a comparison of delocalization energy differences among polycyclic hydrocarbon diol epoxides to account for relative carcinogenicities; however, their calculations cannot take into account the presence or relative configuration of the hydroxyl groups in the diol epoxides. In light of the success of the present calculations and recent experimental evidence for marked differences in the relative mutagenicity[22,23] and carcinogenicity[24,25] of such isomeric diol epoxides, a more elaborate calculational study—both of the monocyclic and polycyclic derivatives—is in progress.

Acknowledgment. Many helpful discussions have been held with colleagues of the Lilly Research Laboratories, especially D. B. Boyd, R. B. Hermann, R. E. McMahon, and P. J. Murphy; the advice and help of N. L. Allinger, Roald Hoffmann, and R. E. Lehr are also gratefully acknowledged.

References and Notes

(1) D. M. Jerina, R. E. Lehr, H. Yagi, O. Hernandez, P. M. Dansette, P. G. Wislocki, A. Wood, R. L. Chang, W. Levin, and A. H. Conney in "In Vitro Metabolic Activation in Mutagenesis Testing", F. J. de Serres, J. R. Fouts, J. R. Bend, and R. M. Philpot, Eds., Elsevier/North-Holland Biomedical Press, Amsterdam, 1976, pp 159–177.
(2) E. G. Toranzo, T. Gillesse, M. Mendenhall, G. J. Traiger, P. G. Riley, R. P. Hanzlik, and R. A. Wiley, *Toxicol. Appl. Pharmacol.*, **40**, 415 (1977).
(3) B. B. Brodie, D. Watson, A. K. Cho, G. Sipes, G. Krishna, and J. R. Gillette, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 160 (1971).
(4) J. R. Mitchell, W. D. Reid, B. Christie, J. Moscowitz, G. Krishna, and B. B. Brodie, *Res. Commun. Chem. Pathol. Pharmacol.*, **2**, 877 (1971).
(5) J. R. Gillette, *Biochem. Pharmacol.*, **23**, 2785 (1974).
(6) D. J. Jollow, J. R. Mitchell, N. Zampaglione, and J. R. Gillette, *Pharmacology*, 11, 151 (1974).
(7) R. Snyder and J. J. Kocsis, *CRC Crit. Rev. Toxicol.*, **3**, 265 (1975).
(8) W.-D. Stohrer and R. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, 11, 285 (1972).
(9) G. Frenking, H. Kato, and K. Fukui, *Bull. Chem. Soc. Jpn.*, **48**, 6 (1975).
(10) P. Mezey, R. E. Kari, A. S. Denes, I. G. Csizmadia, R. T. Gosavi, and O. P. Strausz, *Theor. Chim. Acta*, **36**, 329 (1975).
(11) J. A. Pople and D. L. Beveridge, "Approximate Molecular Orbital Theory", McGraw-Hill, New York, N.Y., 1970, pp 80–83 and Appendix A.
(12) The computer program used for the calculations reported here is BNDO, an extensively modified version of QCPE No. 141,[13] developed by Dr. Donald B. Boyd of the Lilly Research Laboratories.[14]
(13) P. Dobosh, *QCPE*, **10**, 141 (1969).
(14) D. B. Boyd, *J. Phys. Chem.*, **78**, 2604 (1974).
(15) J. P. Glusker, H. L. Carrell, D. E. Zacharias, and R. G. Harvey, *Cancer Biochem. Biophys.*, 1, 43 (1974).
(16) J. P. Glusker, D. E. Zacharias, H. L. Carrell, P. P. Fu, and R. G. Harvey, *Cancer Res.*, **36**, 3951 (1976).
(17) D. M. Jerina, N. Kaubisch, and J. W. Daly, *Proc. Natl. Acad. Sci. U.S.A.*, **68**, 2545 (1971).
(18) P. Y. Bruice, T. C. Bruice, P. M. Dansette, H. G. Selander, H. Yagi, and D. M. Jerina, *J. Am. Chem. Soc.*, **98**, 2965 (1976).
(19) J. C. Wiley, Jr., C. S. Menon, D. L. Fischer, and J. F. Engel, *Tetrahedron Lett.*, 2811 (1975).
(20) R. B. Hermann, H. W. Culp, R. E. McMahon, and M. M. Marsh, *J. Med. Chem.*, **12**, 749 (1969).
(21) D. B. Boyd, R. B. Hermann, D. E. Presti, and M. M. Marsh, *J. Med. Chem.*, **18**, 408 (1975).
(22) A. W. Wood, P. G. Wislocki, R. L. Chang, W. Levin, A. Y. H. Lu, H. Yagi, O. Hernandez, D. M. Jerina, and A. H. Conney, *Cancer Res.*, **36**, 3358 (1976), and references cited therein.
(23) A. W. Wood, R. L. Chang, W. Levin, H. Yagi, D. M. Jerina, and A. H. Conney, *Biochem. Biophys. Res. Commun.*, **77**, 1389 (1977), and references cited therein.
(24) J. Kapitulnik, P. G. Wislocki, W. Levin, H. Yagi, D. M. Jerina, and A. H. Conney, *Cancer Res.*, **38**, 354 (1978).
(25) T. J. Slaga, A. Viaje, D. L. Berry, W. Bracken, S. G. Buty, and J. D. Scribner, *Cancer Lett. (Amsterdam)*, **2**, 115 (1976).
(26) G. J. Kasperek, T. C. Bruice, H. Yagi, and D. M. Jerina, *J. Chem. Soc., Chem. Commun.*, 784 (1972).
(27) G. J. Kasperek, T. C. Bruice, H. Yagi, N. Kaubisch, and D. M. Jerina, *J. Am. Chem. Soc.*, **94**, 7876 (1972).

Synthesis and Quantitative Structure–Activity Relationships of Some Antibacterial 3-Formylrifamycin SV *N*-(4-Substituted phenyl)piperazinoacethydrazones

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Received February 21, 1978

A series of 14 3-formylrifamycin SV *N*-(4-substituted phenyl)piperazinoacethydrazones has been synthesized and evaluated for their antimicrobial activity. The compounds were found active against *Bacillus subtilis*, *Staphylococcus aureus*, *Mycobacterium phlei*, and *Mycobacterium tuberculosis* but not as active as rifampin. The compounds also exhibited significant activity against *Clostridium perfringens* and in this bacterial system some were more active than rifampin. The QSAR showed that the activity against *B. subtilis* depended only on lipophilicity, and the regression equation was linear. A parabolic relationship between the antibacterial activity and lipophilicity of the compounds was found in *Staph. aureus*. Additionally, the activity was dependent upon the electronic and steric effects of the phenyl substituents. The sensitivity of *M. phlei* to the compounds was found to correlate well with a linear combination of hydrophobic, electronic, and steric parameters. No statistically significant correlation was possible between the physicochemical parameters studied and the activity of the compounds against *C. perfringens* and *M. tuberculosis*.

5-Nitro-2-furaldehyde *N*-(4-nitrophenyl)piperazinoacethydrazone (1) was reported in 1971 to be active against *Mycobacterium tuberculosis*.[1] Since nitrofurans, in general, do not have antitubercular properties, the activity

0022-2623/78/1821-1301$01.00/0