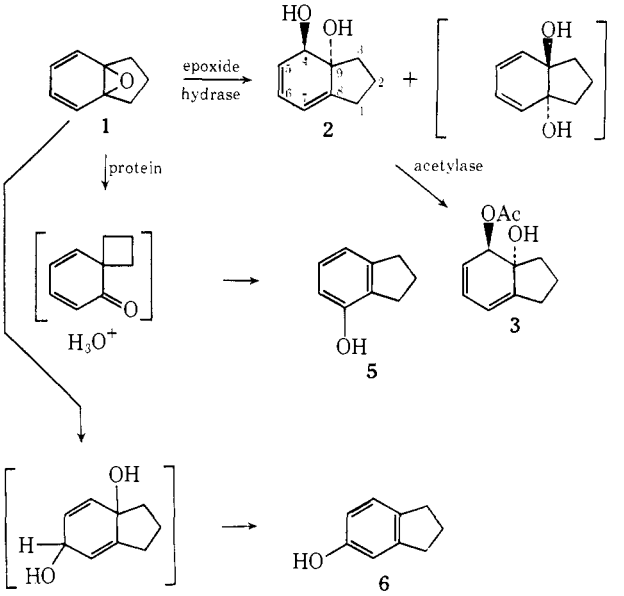
Enzymatic Hydration of 8,9-Indan Oxide. Homoallylic Addition of Water

Sir:

The importance of arene oxides as key intermediates in the metabolism of aromatic substrates to phenols, dihydrodiols, catechols, and mercapturic acid precursors has led to a detailed study of the enzymatic hydration of arene oxides to dihydrodiols with a hepatic epoxide hydrase.[1-4] Both arene and alicyclic oxides are converted to *trans*-diols[1,4,5] whose absolute stereochemistry depends on the substrate.[3] In order to assess the parameters which affect the course of enzymatic hydration of arene oxides, 8,9-indan oxide,[6] 9,10-tetralin oxide,[6] and 9,10-naphthalene oxide (1,6-oxido[10]annulene)[7] were investigated.[8]

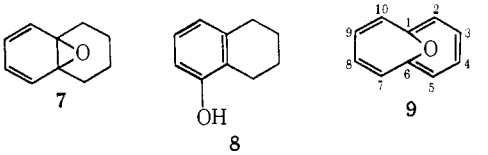
Three nonphenolic products were obtained after incubation of 8,9-indan oxide (**1**) with intact but not with boiled microsomes. *The major metabolites, trans-4,9-dihydro-4,9-dihydroxyindan* (**2**, *2–4% conversion, R_f 0.22) and its 4-O-acetate* (**3**, *0.1–1% conversion, R_f 0.55*), *result not from the expected 1,2 but from an unusual 1,6 addition of water to* **1**. The molecular ion of **2** corresponds to $C_9H_{12}O_2$ (found 152.084, calcd 152.083) with a base peak at 134 (M − 18). The uv spectrum (λ_{max}^{MeOH} 266 mμ) is in good agreement with that expected of a 4,9-dihydro- rather than an 8,9-dihydroindan.[9] The nmr spectrum[10] of **2** with a J of 5.7 Hz for the coupling between the 4 and 5 protons is compatible only with the *trans*-diol as revealed by an inspection of Dreiding models. The absolute stereochemistry of the hydroxyl function at C-9 was deduced from the CD curve of **2**, since the sign of the Cotton effect of such conjugated dienes is related to their skew sense.[11] Although changes in the conformations of **2** affect the magnitude of the skew angle, the skew sense is unequivocal, so that the observed negative CD curve for **2** requires that the diene be skewed in the sense of a left-handed helix and that the absolute configuration about the carbinol atoms be 4*R*,9*R* as shown. The concentration of the diol **2** was estimated by an assumed extinction coefficient for the diene of 4700.[9] The $[\theta]^{25}_{266}$ value calculated in this manner was −65,900° (*c* 0.022, methanol). This value is, as expected from a less flexible diene system, much higher than the value for the corresponding (−)-*trans*-(1*R*,2*R*)-1,2-dihydro-1,2-dihydroxybenzene obtained from enzymatic hydration of benzene oxide.[3] In addition to **2**, varying amounts of its 4-O-acetate (**3**) were formed, presumably by the action of hepatic acetylases on **2**. That **3** was the 4-O-acetate of **2** was conclusively demonstrated by its mass spectrum, uv spectrum, CD curve, and the presence in the nmr of a singlet due to a $-OC(=O)CH_3$ group at δ 2.01 and a doublet due to C*H*OR at 5.28 (*vs.* 4.02 in **2**). Both **2** and **3** are converted by mineral acid (30 min, 60°, 1 *N* HCl) to 4-hydroxyindan and a trace of 5-hydroxyindan.



The expected dihydrodiol (**4**) was obtained only in trace amounts (<0.2% conversion, R_f 0.29). The mass spectrum of **4** exhibits a parent ion at *m/e* 152 and a base peak at 134 (M − 18). A uv spectrum with a λ_{max}^{MeOH} at 262 mμ and a levorotatory CD curve suggest that **4** is *trans*-8,9-dihydro-8,9-dihydroxyindan (**4**). Acid readily aromatizes **4** to a mixture of 4-hydroxy- (two parts) and 5-hydroxyindan (three parts).

During incubation, 8,9-indan oxide (**1**) isomerizes spontaneously to 4-hydroxyindan (**5**), probably *via* a cyclohexadiene intermediate, and to trace amounts of 5-hydroxyindan. This is surprising, since acid-catalyzed isomerization of **1** leads exclusively to 5-hydroxyindane (**6**).[6] The isomerization of **1** to **4** is probably protein catalyzed, as reported previously for the isomerization of benzene oxide.[1] Aqueous acetamide[1] also catalyzes isomerization of **1** to yield nine parts of **5** and one part of **6**.

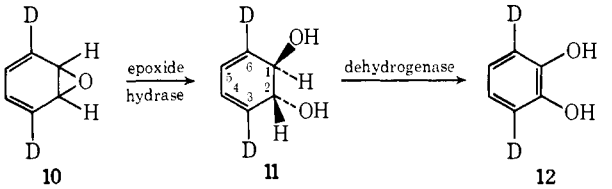
Incubation of the homologous but more stable 9,10-tetralin oxide (**7**) with microsomes led only to small amounts of 5-hydroxytetralin (**8**). In general stable oxides, such as cyclohexene oxide, are poor substrates for epoxide hydrase which prefers acid-labile oxides, such as styrene, naphthalene, indene, and benzene oxides. Another stable oxide, 9,10-naphthalene oxide,



which exists solely in the oxepin form (1,6-oxido[10]-annulene) (**9**)[6,7] was not a substrate for epoxide hydrase.

(1) D. M. Jerina, J. Daly, B. Witkop, P. Zaltzman-Nirenberg, and S. Udenfriend, *Arch. Biochem. Biophys.*, **128**, 176 (1968).
(2) D. M. Jerina, J. Daly, B. Witkop, P. Zaltzman-Nirenberg, and S. Udenfriend, *J. Amer. Chem. Soc.*, **90**, 6525 (1968).
(3) D. M. Jerina, H. Ziffer, and J. W. Daly, *ibid.*, in press.
(4) D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nirenberg, and S. Udenfriend, *Biochemistry*, **9**, 147 (1970).
(5) L. C. Leibman and E. Ortiz, *Mol. Pharmacol.*, 4, 201 (1968).
(6) E. Vogel and H. Günther, *Angew. Chem. Intern. Ed. Engl.*, **6**, 385 (1967).
(7) E. Vogel, M. Biskup, W. Pretzer, and W. A. Böll, *ibid.*, **3**, 642 (1964) and A. Shani and F. Sondheimer, *J. Amer. Chem. Soc.*, **89**, 6310 (1967).
(8) After incubation under nitrogen with rabbit liver microsomes (20,000 × g supernatant) at pH 9 for 25 min at 37°, substrate and products were extracted into ethyl acetate and isolated by preparative tlc (SiO_2–GF, chloroform–benzene–ethyl acetate, 1:1:1). Phenolic products ($R_f \sim 0.8$) were further analyzed on a 0.025% HiEff 1A and 0.02% phosphoric acid on 100–140 mesh glass bead column at 110–125°.
(9) S. W. Staley and T. J. Henry, *J. Amer. Chem. Soc.*, **91**, 1239 (1969).
(10) $\delta^{ppm}_{CDCl_3}$, 100 MHz; 0.7–1.0 (m, 2 protons, CH_2), 0.14–2.2 (m, 4 protons, CH_2's), 4.02 (d, J = 5.7 Hz, 1 proton, 4-C*H*–OH), 5.85 (t, 1 proton, 6-H), 5.98 (d, J = 5.6 Hz, 1 proton, 7-H), and 6.06 (q, J = 5.6, 5.7 Hz, 1 proton, 5-H).
(11) U. Weiss, H. Ziffer, and E. Charney, *Tetrahedron*, **21**, 3105 (1968).

The unexpected 1,6 opening of indan oxide (**1**) necessitated a reexamination of the enzymatic hydration of benzene oxide, in which 1,6 opening would have gone undetected in previous studies.[1,3] Incubation of 1,2-benzene-3,6-^{2}H oxide (**10**, 1.06 atoms of deuterium)[12] with epoxide hydrase produced (−)-*trans*-1,2-dihydro-1,2-dihydroxybenzene-3,6-^{2}H (**11**, 1.08 atoms of deuterium) as proved by nmr spectroscopy. Enzymatic dehydrogenation[1] of **11** afforded catechol-3,6-^{2}H (**12**, 1.12 atoms of deuterium) without loss of deuterium, a clear proof that enzymatic hydration of benzene oxide does not proceed by homoallylic but *only* by 1,2-*trans* addition of water. *Isomerization of 1,2-benzene oxide-3,6-^{2}H to phenol-^{2}H (1.06 atoms of deuterium) during incubation with microsomes occurs without loss of deuterium.*



10 **11** **12**

The enzymatic homoallylic hydration of an arene oxide so far is unique for 8,9-indan oxide (**1**) where steric factors may well prevent normal 1,2 hydration and cause stereospecific entry of water at the homoallylic position. Enzymatic hydrations of 16α,17α- and 16β,-17β-epoxysteroids[13] and of 2,3-oxidosqualenes[14] provide further examples of control by steric factors. The significance of hepatic epoxide hydrase to drug metabolism prompts us to continue our studies on the effect of electronic and steric factors on enzymatic hydration of epoxides, and the purification and properties of the enzyme.[15]

(12) 1,4-Cyclohexadiene-3,6-^{2}H (1.10 atoms of deuterium) was purchased from Isomet Corp. and converted to **10**.[1] Deuterium was analyzed by mass spectrometry and localized by nmr spectroscopy.

(13) H. Breuer and R. Knuppen, *Biochim. Biophys. Acta*, **49**, 620 (1961).

(14) E. J. Corey, K. Lin, and M. Jautelat, *J. Amer. Chem. Soc.*, **90**, 2724 (1968).

(15) F. Oesch, D. M. Jerina, C. R. Creveling, and J. Daly, *Fed. Proc.*, in press.

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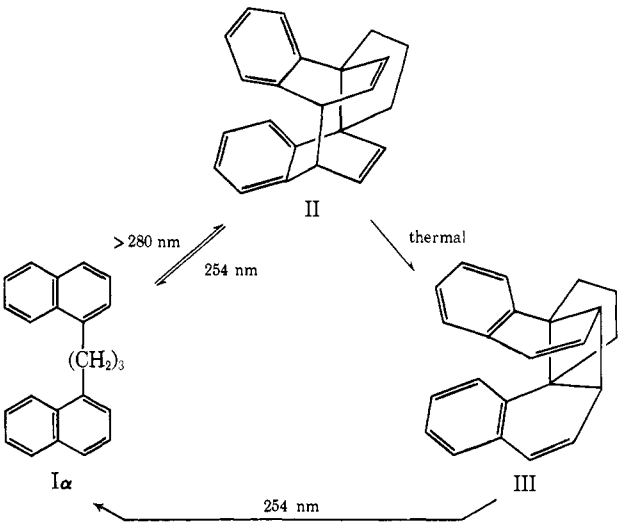
Reversible Intramolecular Photodimerization of 1,3-Bis(α-naphthyl)propane

Sir:

We have found that irradiation of 1,3-bis(α-naphthyl)-propane (Iα) results in intramolecular photodimerization of the naphthalene nuclei to give the anthracene-like dimer II. Such photodimerization has not previously been observed for naphthalene or any of its simple derivatives, although it does occur in the naphthalene paracyclophane.[1] It has been postulated[2,3] as the mode of photodimerization of various β-alkoxynaphthalenes but the structure assigned to these dimers is still in dispute.[4]



The photoisomerization of Iα was discovered during a study of the fluorescence of various dinaphthylalkanes, designed to yield information about the geometric requirements for intramolecular excimer formation. Only the symmetrical 1,3-dinaphthylpropanes Iα and the corresponding β-naphthyl isomer Iβ, which can form perfectly overlapping sandwich pairs, exhibit strong excimer interaction.[5] The small (*ca.* 4 kcal) activation energy involved in excimer formation has been attributed to the rotation barrier in the propane chain. At temperatures above −30°, where the excimer is the main fluorescent species, the fluorescence of Iα is much more subject to thermal quenching than is that of Iβ.

The absorption spectra of solutions of Iα as irradiation[6] proceeds at 25° clearly indicate that a photochemical reaction is occurring. The absorption spectrum of Iβ is not changed under these conditions. The spectra of a degassed 10^{-3} *M* solution of Iα in methylcyclohexane are shown in Figure 1. The absorption decreases to 20% of its original value within 15 min and reaches a steady state (18%) within an hour. It appears that a small amount of Iα remains.

If this solution is heated briefly or allowed to stand overnight, the spectrum changes to curve 3. Irradiation of either this solution or the original unheated solution with a low-pressure Hg lamp (primarily 254 nm, through the quartz portion of the apparatus) regenerates the initial spectrum of Iα (curve 4). The lower absorbance of the solution thus obtained relative to that of the original is suggestive of a photostationary state. These data indicate that upon irradiation Iα forms a compound, II, having a weaker absorbance. Further, II is thermally unstable and gives III (λ_{max} 265

(1) H. H. Wasserman and P. M. Keehn, *J. Amer. Chem. Soc.*, **91**, 2374 (1969); **89**, 2270 (1967).

(2) J. S. Bradshaw and G. S. Hammond, *ibid.*, **85**, 3953 (1963).

(3) J. S. Bradshaw, N. B. Nelsen, and D. P. Rees, *J. Org. Chem.*, **33**, 259 (1968).

(4) M. Sterns and B. K. Selinger, *Aust. J. Chem.*, **21**, 2131 (1968).

(5) C. J. Dempster and E. A. Chandross, Abstracts, 156th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1968, No. PHYS 25.

(6) A Rayonet reactor (N. E. Ultraviolet Co.) was used; the lamps were low-pressure Hg (400 W total) with a phosphor whose emission peaks at 300 nm. Irradiation was through Pyrex in a sealed degassed apparatus which had 1- and 10-mm quartz absorption cells attached.