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REGIOSELECTIVITY IN ENZYMATIC HYDRATION OF CIS-1,2-DISUBSTITUTED [^{18}O]-EPOXIDES

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SUMMARY

The hydration of cis-β-methylstyrene oxide, cis-2,3-octene oxide, and their ^{18}O-enriched forms by epoxide hydrase of rat liver microsomes has been investigated. Both cis epoxides underwent quantitative enzymatic hydration yielding exclusively the corresponding threo diols, indicating that complete stereochemical inversion at a single oxirane carbon had occurred. Mass spectral analysis of diols formed enzymatically from the ^{18}O enriched epoxides indicated they were formed with great regioselectivity, 89% and 85% of the ^{18}O being located at the benzylic carbon of the styrene diol and at C-3 of the octane diol, respectively.

Liver microsomal epoxide hydrases (E.C. 4.2.1.63) are thought to serve a protective function by converting chemically reactive epoxide formed *in situ* by cytochrome P-450 oxygenases to less toxic 1,2-diols (1). The mechanism by which the hydration is effected is not known, but the overall course of the reaction has been characterized reasonably well. Both (+) and (-) enantiomers of mono-, 1,1-, and cis-1,2-disubstituted oxiranes (epoxides) bearing at least one lipophilic substituent are excellent substrates, while trans-1,2-, tri-, and tetra-substituted oxiranes do not serve as substrates and fail to inhibit the hydration of styrene oxide. In contrast to this relative lack of optical and geometrical selectivity, certain other features of

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the reaction are quite specific. For example, epoxides of cyclic olefins and arene oxides give only trans-1,2-diols, and cis-1,2-disubstituted oxiranes give only threo-1,2-diols.

Studies with $^{18}OH_2$ and with a number of ^{18}O-labeled mono- and 1,1-disubstituted oxiranes have revealed another remarkably specific feature of the reaction, namely, that the incoming hydroxyl always (> 95%) attacks the least hindered oxirane carbon (2). With such substrates there is a clear distinction in the steric and electronic characteristics of the two potential reaction centers, and the observed pattern of enzymatic hydration is clearly consistent with a mechanism that emphasizes the role of the hydroxide nucleophile. However, with cis-1,2-disubstituted oxiranes the steric and electronic differences at the two potential reaction centers are very slight by comparison, and one could easily expect that the enzymatic hydration of these substrates would show little, if any, regiospecificity. We have now studied the enzymatic hydration of two ^{18}O-labeled cis-1,2-disubstituted oxiranes and find, surprisingly, that their hydration is only slightly less regiospecific than with the other types of substrates.

MATERIALS AND METHODS

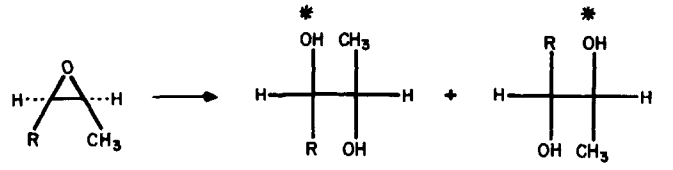
Cis-2-octene and cis-β-methyl styrene, as well as their corresponding epoxides, diols (both erythro and threo) and diol-acetonides were obtained as described previously (3). [^{18}O]-epoxides were prepared as described previously (2) by N-bromosuccinimide oxidation in $^{18}OH_2$/THF, except that in this case diazabicyclononane (DBN) in THF was used in place of K_2CO_3/methanol for conversion of the bromohydrins to the epoxides. Despite an unexpected decrease in the cis/trans ratio, we were able to obtain β-methylstyrene oxide, containing 21.4 atom-

% excess ^{18}O and having a 56:44 cis/trans ratio, in ca. 25% overall yield. 2,3-Epoxyoctane containing 27.4 atom % excess ^{18}O and having a 40:60 cis/trans ratio was obtained similarly. Pure cis-[^{18}O]-2,3-epoxyoctane was isolated by preparative glc (6' x 1/8", 10% Carbowax 20 M on 100-120 mesh AWDCMS Chromosorb W; oven, injector, and detector at 110° C). Other aspects of this study, including microsomal incubations, diol isolations, and mass spectral analyses for ^{18}O label were performed as described previously (2,4). Male Holtzman rats were used as the source of the liver microsomes.

RESULTS AND DISCUSSION

Preliminary microsomal incubations with cis/trans mixtures of unlabeled β-methylstyrene oxide and 2,3-epoxyoctane consistently showed that only the cis isomers were detectably utilized by the hydrase enzyme, and that only the threo diols were produced. The same was observed when pure cis-[^{18}O]-2,3-epoxyoctane was incubated on a preparative (10 mg) scale with rat liver microsomes. Thus, the stereochemical course of these hydrations involved the trans addition of water, with inversion of configuration at one of the oxirane carbons. This result is consistent with other observations of trans diol formation from epoxides of cyclic olefins and arenes catalyzed by microsomes from several species of mammals (5-7) and with the reported conversions of cis-9,10-epoxystearic acid and cis-stilbene oxide to threo-9,10-dihydroxystearic acid and threo-stilbene diol, respectively, by rabbit liver microsomes (5,6).

Results obtained with the ^{18}O-labeled epoxides are given in Figure 1; again, only threo diols were formed. Furthermore, the distribution of ^{18}O observed in the product diols reflects a high degree of regiospecificity in the hydration process. This



Substrate	Product (±5%)	
1, R = C_6H_5-	89%	11%
2, R = C_5H_{11}-	85%	15%

Figure 1. Regioselectivity of Enzymatic Hydration of cis-1,2-Disubstituted Oxiranes.

is somewhat surprising in view of the rather small differences in steric and electronic effects imposed by the substituents on the two oxirane carbons. However, the pattern of the regiospecificity is consistent with that observed with other oxiranes, and reinforces our earlier suggestion that epoxide hydrase action involves specific activation of water, probably by means of general base catalysis, followed by nucleophilic attack of hydroxide at the less hindered oxirane carbon (2).

An intriguing contrast to the pronounced regiospecificity observed with epoxide hydrase is the relative lack of optical specificity reported for this enzyme. Although diol products from meso oxiranes show some asymmetric induction, discrimination between enantiomers of substrate oxiranes is generally slight, and with styrene oxide it is nil (6,8). A simple model for epoxide hydrase which could account for its generally high regioselectivity but generally low enantioselectivity would consist of a small polar catalytic site region, flanked by two (or more) hydrophobic regions for side chain binding, such that both enantiomers of a substrate can be bound with nearly equal efficiency. The catalytic site would have to be a small pocket or depression which could be occupied only by a water molecule and an oxirane

ring having at least one unhindered aspect, such as a CH_2 end group or a cis-H-C-C-H face, in order to explain the exclusion of dissolved nucleophiles, as well as the lack of either substrate or inhibitor activity on the part of trans-1,2- and more highly substituted oxiranes. The regiospecificity of the enzymatic reaction, then, appears to be mainly that which is generally characteristic of nucleophilic attack on oxirane rings, and is governed largely by steric factors. The fact that the enzymatic hydration process actually shows somewhat greater regiospecificity than analogous solution reactions of epoxides with base may be due either to small substrate orienting effects associated with binding, or to differences in solvation of the reactants. In solution, hydrogen bonding to the oxirane oxygen (i.e. weak or incipient acid catalysis) would tend to weaken the bond from oxygen to the most highly substituted carbon. This in turn would tend to diminish the regioselectivity of the hydroxide nucleophile for the least hindered carbon in proportion to the degree of proton transfer to the oxirane oxygen. Since neither this effect, nor any significant specificity for binding substrate enantiomers is observed with epoxide hydrase, one might conclude that general acid catalysis may not be a significant feature of the catalytic mechanism. This tentative conclusion is supported by the failure of aziridines and oxazirenes to inhibit significantly the hydration of styrene oxide (9). Steroidal aziridines have in fact been shown to be hydrated in parallel fashion to structurally analogous oxiranes by rabbit liver epoxide hydrase (10). In contrast, 2,3-iminosqualene is a potent inhibitor of 2,3-oxidosqualene lanosterol cyclase, an enzyme which depends upon carbonium ion generation via epoxide protonation as an essential part of the mechanism (11).

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