STEREOSELECTIVE HYDROLYSIS OF CIS- AND TRANS-STILBENE OXIDES BY HEPATIC MICROSOMAL EPOXIDE HYDROLASE

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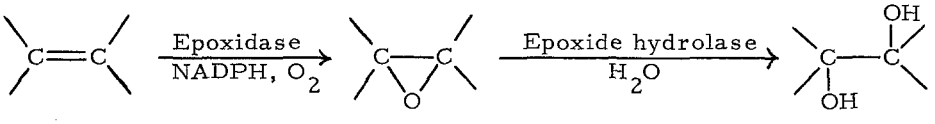
SUMMARY

Stereochemistry in the hydrolysis of isomeric cis- and trans-olefin oxides by hepatic microsomal epoxide hydrolase has been described. cis- and trans-Stilbene oxides used as model substrates for this purpose were hydrolyzed with a strict stereoselectivity by rabbit liver microsomes to d-threo- and meso-1, 2-diphenyl-1, 2-ethanediols, respectively. The previously established absolute configuration of the d-threo-glycol (1R, 2R) indicates it to be formed, in the enzymatic reaction process, as a result of the selective attack of a hydroxyl anion on only one oxiran carbon of the symmetric cis-olefin oxide. The rate of the enzymatic glycol formation reaction is much higher in cis-stilbene oxide than in the trans-isomer.

Hepatic microsomal epoxide hydrolase (1, 2) or epoxide hydrase (3) has recently been demonstrated to play an important role in the metabolism of foreign olefins (2, 4) and arenes (3) to vicinal glycols and of oleic acid (5) to threo-9, 10-dihydroxystearic acid *via* the corresponding intermediate epoxides. The role of this non-specific enzyme *in vivo* is not only hydrolysis of epoxides formed from



olefins and arenes by the catalytic action of microsomal epoxidase, a mixed function oxidase requiring NADPH as a cofactor and molecular oxygen, but also considered to be detoxication by reason that the conversion of olefin oxides, many of which are

well known to be toxic (6), to polar glycols facilitates their excretion (7, 8). In this connection, an interesting suggestion has recently been made that proximal active forms of some potent carcinogens such as aflatoxins, pyrrolizidine alkaloids, and polycyclic aromatic hydrocarbons would be respective epoxides formed in the animal body (9). Based on this assumption, epoxide hydrolase must be considered to play a key role in the carcinogenesis due to the above mentioned substances.

Very recently valuable information has been accumulated on the stereochemical course of the enzymatic glycol formation from acyclic (1, 2, 4), monocyclic (10), and polycyclic (7, 11-13) olefin oxides. However, it remains to solve an important problem about the mode of action of the hydrolase on isomeric cis- and trans-olefin oxides. The present paper deals with optically stereoselective hydrolysis of cis- and trans-stilbene oxides used in this investigation as model substrates for a promising approach to this problem.

Stilbene oxides were uniformly emulsified in 0.1 M phosphate buffer, pH 7.4, containing Tween 80 by the method of Dean et al. (14) and incubated at 37° in nitrogen-filled flasks with washed rabbit liver microsomes prepared by the previously reported method (15). The reaction was terminated at various intervals by the addition of an aqueous sodium hydroxide solution. TLC of ethereal extracts of the mixtures indicated that only the corresponding glycols were microsomal reaction products. For identifying the glycols formed, the areas of the adsorbent layer where they appeared as quenching bands under a UV-lamp were collected and extracted thoroughly with hot methanol. The methanolic extracts were trimethylsilylated in the standard manner (16) and analyzed by GLC on a succinate polyester column. The gas chromatograms obtained indicated that the glycol formed from cis-stilbene oxide was only threo-1,2-diphenyl-1,2-ethanediol and the one from the trans-oxide only meso-1,2-diphenyl-1,2-ethanediol (TABLE I). After isolation as crystals from the ethereal extracts by silica gel column chromatography, the glycols were further identified

by superimposability of their IR spectra with those of the corresponding authentic specimens. Since by the GLC method used at least 0.1% of the isomeric glycol to the other was found to be detected each other as far as examined with standard mixtures of the authentic specimens, the above mentioned results show that the

TABLE I. Rf and Retention Time Values of Derivatives of Stilbene

Compound	Rf[a]	Retention times (min)[b]	
		15% Succinate polyester	0.75% SE-30
cis-Stilbene oxide[c]	0.85	Not eluted	6.7
trans-Stilbene oxide[d]	0.88	Not eluted	11.1
d,l-threo-1,2-Diphenyl-1,2-ethanediol[e]	0.32	Not eluted	Not eluted
meso-1,2-Diphenyl-1,2-ethanediol[f]	0.32	Not eluted	Not eluted
TMS d,l-threo-1,2-Diphenyl-1,2-ethanediol[g]	——	13.4	19.6
TMS meso-1,2-Diphenyl-1,2-ethanediol[g]	——	11.9	19.3

a The chromatogram was obtained in benzene and acetone (6:1) on a plate coated with silica gel containing an inorganic phosphor agent and visualized under a UV-lamp. The ethereal extracts of the incubation mixtures showed no quenching spot other than the unchanged substrates and the glycols formed. b A Shimadzu gas chromatograph model GC-1C equipped with a flame ionization detector was used. Succinate polyester was coated on Shimalite (60-80 mesh) and SE-30 on Chromosorb-W (60-80 mesh), and they were packed in glass columns (180 cm x 4 mm). GLC conditions used were column temperatures: 160° (succinate polyester) and 130° (SE-30) and nitrogen as the carrier gas: 35 ml/min. c and d The cis- and trans-oxides were prepared from cis- and trans-stilbenes, respectively, by the method of Reif et al. (19). e Prepared from cis-stilbene by the method of Swern et al. (20) f Prepared by the previously reported method (21). g TMS means trimethylsilylated.

oxiran ring of both oxides are hydrolyzed trans with a strict stereoselectivity by microsomal epoxide hydrolase. Our previous demonstrations of mechanistic similarity in the enzymatic hydrolysis of epoxysteroids to their acid-catalyzed

one and of inhibition of the reaction with a steroid aziridine have led us to the conclusion that the active center of the hydrolase has a dissociating hydrogen interactive with the oxygen atom of oxirans and the reaction is completed by a concerted back-side attack of a hydroxyl anion from water to form the corresponding trans-diaxial glycols (13). The present results obtained with stilbene oxides are also in good accordance with this conclusion (Fig. 1).

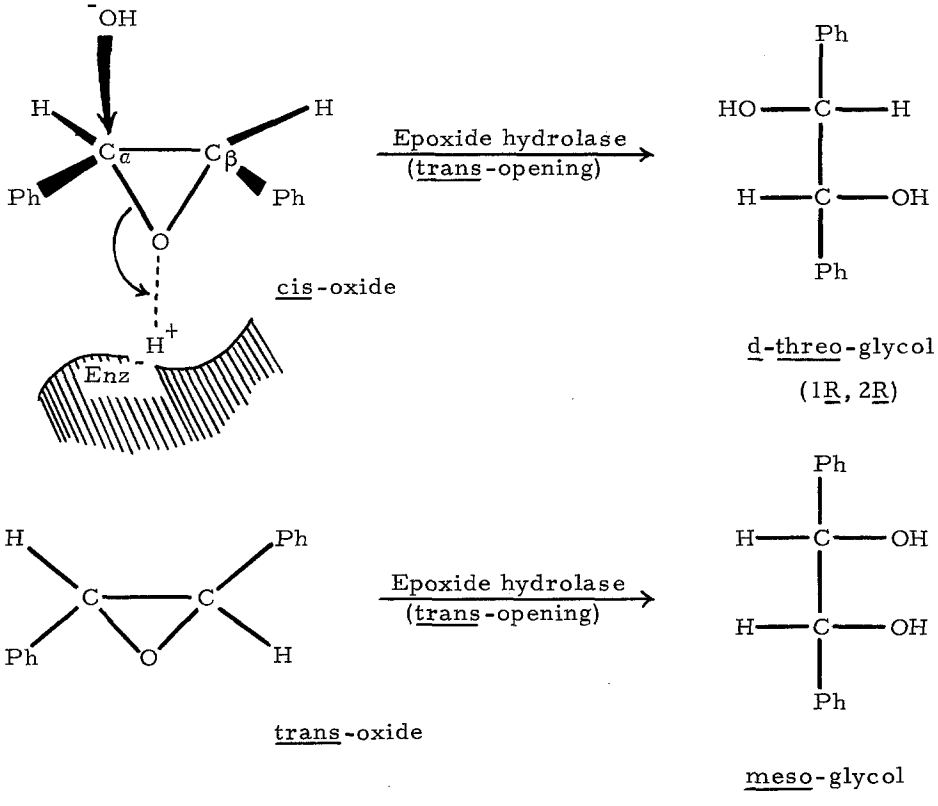


Fig. 1. Optically stereoselective glycol formation from cis- and trans-stilbene oxides by microsomal epoxide hydrolase. $Enz^- H^+$ represents the electronic character of the active center of the enzyme.

The threo-glycol formed from cis-stilbene oxide had an optical activity of $[\alpha]_D$ + 91° (c= 0.66 in absolute alcohol, recorded on a JASCO's ORD/UV-5 spectropolarimeter) and melted at 147°. Based on the earlier demonstration that the d-

threo-glycol ($[\alpha]_D$ + 92° in absolute alcohol; mp 146°) has an (R, R)-configuration with its asymmetric carbon atoms (17), it is evident that upon hydrolytic opening of the cis-oxide the hydroxyl anion attacks selectively on only one oxiran carbon atom (marked α in Fig. 1) in spite of the equal possibility of the bond cleavage between $C\alpha$—O and $C\beta$—O. The hindered attack of the hydroxyl anion on C_β might be attributable to a steric repulsion involving the three dimensional enzyme protein structure around its active center. Similar evidence for the selective formation of only one optically active trans-glycol by the action of microsomal epoxide hydrolase on an epoxide has been obtained with cyclic olefin oxides (10-13) and arenes (10).

TABLE II. Hydrolysis of cis- and trans-Stilbene Oxides by Rabbit Liver Microsomal Epoxide Hydrolase [a]

Substrate	Glycol formed	Rate of the reaction [b] ($\times 10^{-9}$ M/min/mg microsomal protein)	Relative rat of the rate
cis-Stilbene oxide	d-(1R, 2R)-1, 2-Diphenyl-1, 2-ethanediol	1728	785
trans-Stilbene oxide	meso-1, 2-Diphenyl-1, 2-ethanediol	2.2	1

a Final concentrations of the substrates and Tween 80, used for emulsifying the water-insoluble substrates, in the mixtures were adjusted to 10^{-3} M and 0.04%, respectively, and of the microsomal protein 0.41 mg and 8.2 mg (equivalent to 0.01 g and 0.2 g of the liver, respectively)/ml for the cis- and trans-olefin oxides, respectively. The reaction was terminated at various intervals by the addition of 10N NaOH so that the alkali made a final concentration of 1N. And the reaction mixtures were mechanically extracted with three time volumes of ether following saturation of them with sodium chloride. b The reaction rates were estimated by determining the unchanged substrates by GLC under the same conditions as shown in TABLE I. The data are arithmetic mean values of at least three experiments.

A remarkable difference was observed in the rates of the enzymatic hydrolysis of cis- and trans-stilbene oxides (TABLE II), indicating a steric hindrance effect of the trans-arranged phenyl groups on an approach of the oxiran oxygen to the active center of the enzyme since, chemically, trans-stilbene oxide is known to be more susceptible to an acid-catalyzed hydrolysis than the cis-oxide (18).

Both stilbene oxides are completely stable in water at pH higher than 5.0, so that they were recovered quantitatively when incubated for a long time with boiled microsomes under the same conditions as mentioned above.

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