filtration, washed with several small portions of ether, and dried in vacuo. The yellow olefin complexes could then be recrystallized from CH_2Cl_2–ether with ~10% acetone at −78 °C.

Demetalation of Olefin Complexes. An acetone solution of the olefin complex was treated with 2 equiv of NaI at room temperature, usually for ~1 h; with many of the complexes, the reaction was complete in much less time. For complexes **3a–h**, the reactions were carried out in a known volume of acetone in an ampule fitted with a serum cap. After 1 h, samples were withdrawn by syringe and injected into the gas chromatograph; the results were compared against known standards. The column was calibrated in order to determine relative yields. All reactions were found to be essentially quantitative (94–100% yield), and isomer purity as determined in the complexed olefins by ^1H and ^{31}C NMR spectroscopy was confirmed. For the remaining complexes, reactions in acetone-d_6 were examined by ^1H NMR spectroscopy and found to give quantitative conversion to the free olefin by comparison of relative integrated intensities of hydrocarbon peaks compared with the η^5-C_5H_5 (δ 5.03) signal for FpI formed.

For the $\Delta^{3,5}$-cholestadiene complex **3q**, treatment of the olefin complex with NaI–acetone followed by chromatography on silica gel (eluting with petroleum ether) gave a white solid (73%) which, when recrystallized from 95% EtOH, showed the following properties: mp 79.5–80.5 °C; ^1H NMR ($CDCl_3$) δ 6.05–5.2 (3 envelope m, 3 H, olefinic H), 2.5–0.6 (m, 41 H, ring H's and side chain H's and CH_3's as signals at δ 0.96, 0.90, 0.81, 0.70); UV (95% EtOH) λ (ϵ) 238 (17 600), 232 (15 800), and 248 nm (12 400); all are consistent with the structure for $\Delta^{3,5}$-cholestadiene.[75]

Preparation of the (*E*)-2-Pentene Complex (see Table III). The (*E*)-2-pentene complex was prepared by the method of Reger and Coleman.[76] Thus $Fp^+(THF)BF_4^-$ (2 equiv) (prepared from FpI and $Ag^+BF_4^-$ in THF) was stirred with (*E*)-2-pentene in CH_2Cl_2 for 10 h. Precipitation and recrystallization from CH_2Cl_2–ether at −78 °C furnished the (*E*)-2-pentene complex as a light yellow solid. Spectral properties are listed in Table III. The data were used to verify the isomeric purity of the (*Z*)-2-pentene complex **3d**.

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Registry No. **1a**, 69661-74-1; **1b**, 69661-73-0; **1c**, 69661-75-2; **1d**, 71988-05-1; **1e**, 69683-87-0; **1f**, 59980-61-9; **1g**, 71988-06-2; **1h**, 71988-07-3; **1i**, 71988-08-4; **1j**, 60003-79-4; **1k**, 59980-62-0; **1l**, 71988-09-5; **1m**, 42769-10-8; **1n**, 71988-10-8; **1o**, 72016-69-4; **1p**, 34807-88-0; **1q**, 71988-11-9; **1r**, 72028-88-7; **1s**, 72028-89-8; **1t**, 38960-10-0; **1u**, 71988-12-0; **1v**, 71988-13-1; **1w**, 71988-14-2; **2**, 34690-18-1; **3a**, 56336-61-9; **3b**, 56323-75-2; **3c**, 64942-00-3; (*Z*)-**3d**, 71988-16-4; (*E*)-**3d**, 72028-91-2; **3e**, 69661-77-4; **3f**, 59980-54-0; **3g**, 71988-18-6; **3h**, 60791-19-7; **3i**, 59744-57-9; **3j**, 59980-55-1; **3k**, 71988-20-0; **3l**, 71988-22-2; **3m**, 69661-80-9; **3n**, 71988-24-4; **3p**, 60349-23-7; **3q**, 72030-19-4; **3s**, 59744-57-9; **3t**, 37668-15-8; **4**, 12152-20-4; phenethyl tosylate, 4455-09-8; phenethyl bromide, 103-63-9; phenethyl chloride, 622-24-2; phenethyl mesylate, 20020-27-3; phenethyl triflate, 61795-01-5; 2-heptyl bromide, 1974-04-5; 2-hexyl mesylate, 4551-08-0; 2-hexyl tosylate, 4563-91-1; chloroethyl tosylate, 80-41-1; ethoxyethyl tosylate, 17178-11-9; allyl chloride, 107-05-1; allyl iodide, 556-56-9; ethyl 3-bromopropionate, 539-74-2; *p*-nitrophenethyl tosylate, 6948-72-7; (−)-menthyl tosylate, 7212-65-9; 2-methyl-3-pentyl tosylate, 1516-13-8; 2-methyl-3-pentyl triflate, 71988-87-9; 1-chloro-1-methylcyclohexane, 931-78-2; β-bromostyrene, 103-64-0; $[Fp]_2$, 12154-95-9.

(75) Crabbé, P.; Léon, C. *J. Org. Chem.* **1970**, *35*, 2594.

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Stereoselectivity in the Epoxide Hydrase Catalyzed Hydrolysis of the Stereoisomeric 4-*tert*-Butyl-1,2-epoxycyclohexanes

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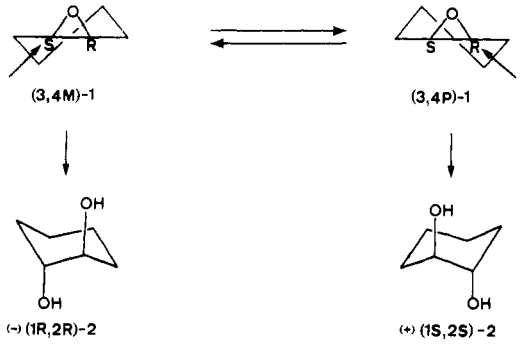
The steric course of the rabbit liver microsome promoted hydrolysis of the racemic cis and trans forms of the title epoxide was investigated. The (+) and (−) forms of *c*-4-*tert*-butylcyclohexane-*r*-1,*t*-2-diol were the only hydrolysis products, indicating that the enzyme reaction takes place exclusively by diaxial opening of the oxirane ring. The absolute configuration and maximum rotation of this diol were determined by correlation with (1*S*,3*R*)-*cis*-3-*tert*-butylcyclohexanol. Of the four stereoisomers of the epoxide, the (1*S*,2*R*,4*S*) form was by far the best substrate and the (1*R*,2*S*,4*S*) form the worst. At low conversion, high enantiomeric excesses of the (−)-diol and the (+)-diol were obtained, respectively, from the (±)-trans and the (±)-cis epoxide; optical purities decreased with increasing conversion. The results are discussed in terms of the helicity of the cyclohexane ring and of the orientation of the *tert*-butyl group with respect to the oxirane ring.

The microsomal epoxide hydrase is an important enzyme involved in the metabolism of xenobiotic compounds, playing a fundamental role in the detoxification of the often highly carcinogenic and mutagenic epoxides that are formed by the action of monooxygenases on alkenes and arenes.[1] Although the sensitivity of the hydrase to substrate structure is rather low, as can be expected from its role in attacking compounds that are foreign to the biological system within which it acts, several cases have been reported of a high stereoselectivity that enables it to discriminate between diastereoisomers and enantiomers of some compounds. Practically nothing being known so far on the detailed structure of the hydrase and little on that of its active site and reaction mechanism, the investigation of the structural and steric requirements for substrate reactivity can provide helpful, though indirect, hints on the geometry of the active site. Whereas a wide range of different epoxides has been investigated with regard to the influence of structural variations on their ability to act as substrates for the hydrase, often with rather unpredictable

(1) F. Oesch, *Xenobiotica*, **3**, 305 (1973); F. Oesch, "Mises au Point de Biochimie Pharmacologique", G. Siest and C. Heusghem, Eds., Masson, Paris, 1977, p 128.

0022-3263/80/1945-0299$01.00/0

results,[1,2] data on the importance of their relative and absolute configurations are much less abundant. In all known cases, hydrase-catalyzed opening of the oxirane ring takes place exclusively in the anti mode, in agreement with an A2 type mechanism of nucleophilic attack by water, for which there are several lines of experimental evidence.[3] In the case of the stilbene oxides, the cis isomers are attacked in a highly selective manner on the (*S*) epoxide carbon, independently from the presence of electron donating or attracting groups on one of the phenyl rings.[4] *trans*-Stilbene oxide reacts much slower, and its (*S,S*) enantiomer is hydrolyzed only 2.3 times faster than the (*R,R*) one.[4a] In the case of styrene oxide, attack by water takes place exclusively on the primary carbon, and there is very little, if any, discrimination between the enantiomers.[3a,5] The (+)-diol is formed from 9,10-epoxystearic acid with low stereo- and regioselectivity, since the enantiomeric excess does not change with the percent of conversion.[4a] More data are available on benzene and polycyclic arene oxides, but steric requirements are not easily rationalized since one observes cases of preferential attack on (*S*) carbons (benzene oxide, naphthalene 1,2-oxide)[5] or on (*R*) carbon (phenanthrene 9,10-oxide)[5] and high regio- and low stereoselectivity (benzo[*a*]pyrene 7,8-oxide) or vice versa (benzo[*a*]pyrene 4,5-oxide).[6] For the saturated analogue of benzene oxide, cyclohexene oxide (**1**), a preference for attack on the (*S*) carbon, with formation of the (*R,R*)-(−)-diol **2** of 70% optical purity, was observed at 10% conversion.[5] This being about the only information available on the stereoselectivity of the hydrase-promoted hydrolysis of an epoxycyclohexane, it was the purpose of the present work to investigate the hydrolysis of simple conformationally rigid cyclohexene oxides in order to ascertain how the reactivity changes with ring helicity and relative and absolute configuration. For this purpose, we chose the trans and cis isomers of 4-*tert*-butyl-1,2-epoxycyclohexanes **6** and **8**, in which the anchoring group is sufficiently far from the reaction site to minimize direct steric interaction with the nucleophile.



Results

All of the experiments were performed with the same lot of rather active rabbit liver microsomes that were stored at −40 °C, in order to ensure the comparability of data of different runs, since it was found that the enzymic activity changed considerably from one preparation of microsomes to another. However, even with less active preparations similar optical purities were obtained at a given percent of conversion. Substrate concentrations (0.03–0.05 M) were certainly far in excess of the K_m of the enzyme (reported values with styrene oxide as the substrate range between 0.25 mM for the crude microsomes and 0.67 mM for the purified enzyme[7]). Conditions were therefore adequate for ensuring a meaningful comparison of rate data for different substrates.

Table I. Conversion of (±)-*trans*- and (±)-*cis*-4-*tert*-Butyl-1,2-epoxycyclohexane into *c*-4-*tert*-Butylcyclohexane-*r*-1,*t*-2-diol

substrate	incubation time, min	% hydrolysis	diol 7 $[\alpha]^{25}_D$, deg	diol 7 ee, %
(±)-**6**	5	28	−26.1	95
	10	47	−24.1	88
	20	58	−16.8	61
	30	68	−9.8	36
	60[a]	100	0.0	0
(±)-**8**	20	30	+20.0	73
	45	51	+18.8	69
	60	56	+17.2	63
	120	67	+13.0	47

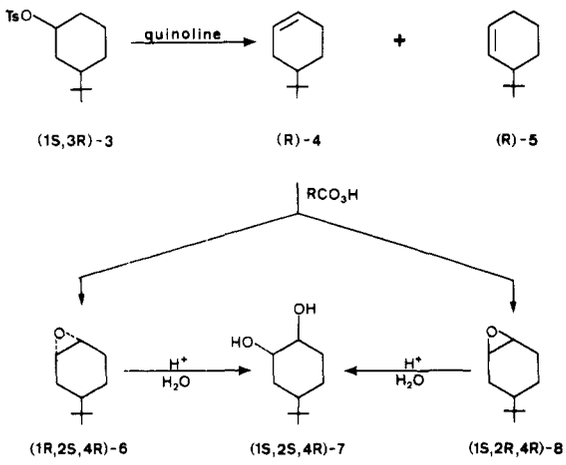
[a] With a less active lot of microsomes after 60 min the conversion was only 60% and the diol 7 had $[\alpha]^{25}_D$ −16.0°, with an ee of 58%.

In order to interpret the data, we needed to know the absolute configuration and maximum rotation of the diaxial diol **7** that was expected, and found, to be the only hydrolysis product of the epoxides **6** and **8**. This was achieved by converting optically pure (1*S*,3*R*)-(−)-*cis*-3-*tert*-butylcyclohexyl tosylate (**3**) into a 38:62 mixture of 3- and 4-*tert*-butylcyclohexene (**5** and **4**) that was transformed into the corresponding epoxides without separating the two olefins. The unseparated mixture of cis and trans epoxides was hydrolyzed with dilute aqueous H_2SO_4 to the corresponding glycols. Although three glycols were present in this mixture, the diol **7** was easily obtained pure because of its presence as the main component and of its much lower solubility compared to that of the other two diols deriving from **5**. (1*S*,2*S*,4*R*)-(+)-**7**, $[\alpha]^{25}_D$ +27.4°, was thus correlated with (1*S*,3*R*)-(−)-**3**, the absolute configuration and maximum rotation of which had previously been determined.[8]

The enzymic hydrolysis of cyclohexene oxide (**1**) was repeated, and the previous results[5] indicating an 85:15 preference for attack at the (*S*) carbon to give an excess of (*R,R*)-(−)-cyclohexane-1,2-diol (**2**) were confirmed. It was also found, as expected for an achiral substrate, that the stereoselectivity was independent of the extent of conversion, remaining the same after 100% as it was after 10% of hydrolysis.

The results obtained in the hydrolysis of the epoxides (±)-**6** and (±)-**8** in the presence of rabbit liver microsomes after different incubation times are given in Table I and graphically presented in Figure 1. In all cases, only the diaxial diol **7** was formed, none of the corresponding diequatorial diol being detectable by the gas-chromatographic method that could reveal the presence of less than

(2) F. Oesch, N. Kaubisch, D. M. Jerina, and J. W. Daly, *Biochemistry*, **10**, 4858 (1971); F. Oesch, *Biochem. J.*, **139**, 77 (1974).
(3) (a) R. P. Hanzlik, M. Edelman, W. J. Michaely, and G. Scott, *J. Am. Chem. Soc.*, **98**, 1952 (1976); (b) R. P. Hanzlik, S. Heideman, and D. Smith, *Biochem. Biophys. Res. Commun.*, **82**, 310 (1978); (c) G. C. DuBois, E. Appella, W. Levin, A. Y. H. Lu, and D. M. Jerina, *J. Biol. Chem.*, **253**, 2932 (1978).
(4) (a) T. Watabe and K. Akamatsu, *Biochim. Biophys. Acta*, **279**, 297 (1972); (b) P. M. Dansette, V. B. Makedonska, and D. M. Jerina, *Arch. Biochem. Biophys.*, **187**, 290 (1978).
(5) D. M. Jerina, H. Ziffer, and J. W. Daly, *J. Am. Chem. Soc.*, **92**, 1056 (1970).
(6) D. R. Thakker, H. Yagi, W. Levin, A. Y. H. Lu, A. H. Conney, and D. M. Jerina, *J. Biol. Chem.*, **252**, 6328 (1977).
(7) G. Belvedere, J. Pachecka, L. Cantoni, E. Mussini, and M. Salmona, *J. Chromatogr.*, **118**, 387 (1976); P. Bentley, F. Oesch, and A. Tsugita, *FEBS Lett.*, **59**, 296 (1975).
(8) G. Bellucci, G. Ingrosso, A. Marsili, E. Mastrorilli, and I. Morelli, *J. Org. Chem.*, **42**, 1079 (1977).



0.5% of the latter isomer. Both enantiomers of the trans epoxide **6** were found to be much better substrates than cyclohexene oxide (Scheme I). Starting from the racemic mixture, the (1*S*,2*R*,4*S*) enantiomer reacted much more rapidly, with attack on the (1*S*) carbon, than the (1*R*,2*S*,4*R*) one, so that the (1*R*,2*R*,4*S*)-(−)-diol **7** was obtained with a high enantiomeric excess in the early stage of the hydrolysis, and only after complete hydrolysis was the diol racemic. A rough estimate of the ratio of hydrolysis rates of the two enantiomers on the basis of the optical yield at low conversion indicates that (1*S*,2*R*,4*S*)-**6** reacts at least 40 times faster than its antipode in the racemic mixture; however, a more accurate value would require measurements on the presently unavailable separated enantiomeric epoxides, since it is likely that they may act as competitive inhibitors for each other.

The racemic cis epoxide **8** reacted more slowly than the trans isomer (±)-**6**, and the isolated diol **7** was, somewhat surprisingly, dextrorotatory; the (1*S*,2*R*,4*R*) enantiomer is more easly hydrolyzed by attack on the (2*R*) carbon to give the (1*S*,2*S*,4*R*)-(+)-diol **7** than the (1*R*,2*S*,4*S*) is by attack on the (2*S*) carbon. The stereoselectivity is lower, with an about 6:1 ratio between the rates of the faster and of the slower reaction.

Discussion

Cyclohexene oxide, a meso compound, can actually be visualized as a 50/50 mixture of two chiral rapidly equilibrating enantiomeric monoplanar conformers that can be indicated as the (3,4 M) and the (3,4 P) forms according to their helicities.[9] In accordance with the demonstrated A2 mechanism of the enzyme-catalyzed hydrolysis, it should involve a typical diaxial (antiparallel) opening of the oxirane ring according to the Fürst–Plattner rule.[10] The epoxide should therefore react in the enzyme active site preferentially in the (3,4 M) conformation, in order to explain the formation of an excess of the (1*R*,2*R*) diol **2**. The preference is however not very high, since the 85:15 ratio of (1*R*,2*R*)-**2** to (1*S*,2*S*)-**2** indicates a difference of about 1 kcal/mol in the $\Delta G^{\ddagger}$ of the two diastereoisomeric transition states. The fact that 2α,3α- and 2β,3β-epoxycholestanes, which have the oxirane ring fused to a cyclohexane ring held rigidly in the (3,4 P) monoplanar conformation, are both attacked exclusively on the (*R*) carbon to give the same diaxial diol[11] confirms that the wrong helicity does not prevent, even if it may slow down, the enzyme-promoted hydrolysis; of course, the nonavailability of the enantiomers of the two epoxycholestanes that would have the right helicity did not allow us to evaluate the importance of helicity in the case of such bulky epoxides. The 4-*tert*-butyl-1,2-epoxycyclohexanes **6** and **8** provide simpler substrates on which to answer this question. Being conformationally rigid and chiral they exhibit a stereochemical behavior that is different from

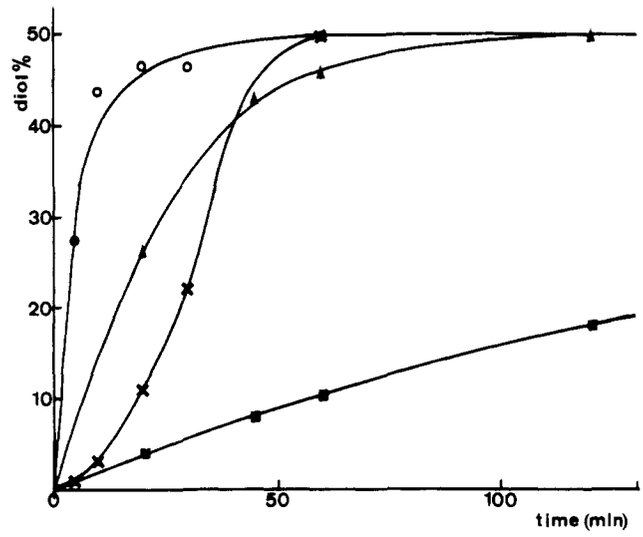
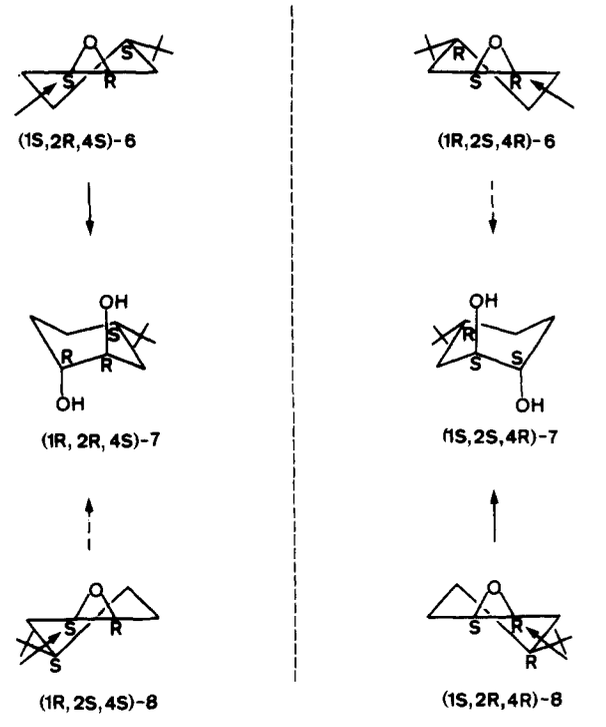


Figure 1. Formation of (+)- and (−)-*c*-4-*tert*-butylcyclohexane-*r*-1,*t*-2-diol from (±)-*trans*- and (±)-*cis*-4-*tert*-butyl-1,2-epoxycyclohexane: (○) (−)-**7** from (±)-**6**; (×) (+)-**7** from (±)-**6**; (▲) (+)-**7** from (±)-**8**; (■) (−)-**7** from (±)-**8**.

Scheme I



(9) R. S. Cahn, C. Ingold, and V. Prelog, *Angew. Chem., Int. Ed. Engl.*, **5**, 385 (1966); the absolute configurations of the enantiomeric half-chair (monoplanar) conformers of cyclohexene oxide cannot be identified by the *R*,*S* convention but are designated best by the helicity around one of the CH_2–CH_2 bonds. Although this is not explicitly said in the third paper by Cahn, Ingold, and Prelog, it is implicit in the discussion (p 406 and 409). (3,4 M) means an (M) helicity around the 3,4 bond, independently from the fact that numbering is started from the (*S*) or (*R*) carbon.

(10) A. Fürst and P. A. Plattner, "Abstracts of Papers of the 12th International Congress of Pure and Applied Chemistry", New York, 1951, p 409.

(11) T. Watabe, K. Kiyonaga, K. Akamatsu, and S. Hara, *Biochem. Biophys. Res. Commun.*, **43**, 1252 (1971).

that of cyclohexene oxide, particularly because, starting from one of the racemic mixtures, one gets a diol whose optical purity decreases with increasing percent of conversion. The fact that of the four stereoisomers of these epoxides three are hydrolyzed enzymatically faster than the unsubstituted cyclohexene oxide is in accordance with what is known about alkyloxiranes, in which the presence of a lipophilic chain on the epoxide ring improves their properties as substrates for the hydrase.[1,2,3b] This has been attributed to a favorable hydrophobic interaction in the active site. In the case of the epoxides 6 and 8, the rather different rates of reaction of the four stereoisomers show that the orientation of the lipophilic side chain in the enzyme–substrate complex plays an important role in determining the ease of the hydrolytic attack. By far the fastest reacting stereoisomer is the (1*S*,2*R*,4*S*) trans epoxide 6, in good agreement with the results mentioned above for cyclohexene oxide, since it has the six-membered ring held rigidly in the favorable conformation of (3,4 M) helicity, and attack takes place on the (*S*) carbon. However, in the case of the cis epoxide 8, nucleophilic attack prefers the (*R*) carbon of the enantiomer having the (3,4 P) conformation, and the ca. 6:1 ratio in rates corresponds to a difference in $\Delta G^{\ddagger}$ of about 1 kcal/mol in favor of the transition state involving the (1*S*,2*R*,4*R*) epoxide. By taking into account the fact that the wrong helicity of this enantiomer should increase the $\Delta G^{\ddagger}$ by about 1 kcal/mol, one can deduce that the right disposition of the substituent with respect to the oxirane ring more than compensates the unfavorable conformational aspect, thus becoming the predominant factor in determining the relative reactivities of the two enantiomers; the decrease in transition state energy connected with such a disposition can be roughly estimated at 2 kcal/mol. Apparently, the better substrates are those in which the *tert*-butyl group is to the right of the plane perpendicular to the epoxide ring and passing through the oxygen atom and the midpoint of the oxirane C–C bond, if one views the molecules with the epoxide ring oriented as depicted in Scheme I. This should imply a corresponding geometry of the active site in which a sufficiently large hydrophobic cavity is asymmetrically situated with respect to the catalytic site in such a way as to accommodate the *tert*-butyl groups of (1*S*,2*R*,4*S*)-6 and (1*S*,2*R*,4*R*)-8 at the moment of nucleophilic attack better than those of their enantiomers. Work is being continued with other substrates, having substituents of different sizes and polarities in positions 3 and 4 of epoxycyclohexane, in order to refine this model for the reaction site.

Experimental Section

Melting points were determined on a Kofler block and are uncorrected. GLC analyses were performed with a C. Erba Fractovap Model GV and a Perkin-Elmer Model F11 instrument. Optical rotations were measured in water (*c* 0.7 to 1.4) for *trans*-1,2-cyclohexanediol and in ethyl acetate (*c* 0.7 to 2.5) for *c*-4-*tert*-butylcyclohexane-*r*-1,*t*-2-diol with a Perkin-Elmer Model 141 photoelectric polarimeter. $MgSO_4$ was always used as the drying agent. Petroleum ether used for the extractions was the fraction of boiling range 40–60 °C and that for the crystallizations was the fraction of boiling range 60–80 °C.

Starting Materials. Commercial cyclohexene oxide was purified by fractionation. 4-*tert*-Butylcyclohexene was prepared by dehydration of the commercial mixture of *cis*- and *trans*-4-*tert*-butylcyclohexanols.[12] Pure *trans*-4-*tert*-butylcyclohexene oxide (6) was obtained by the reported method.[13] The pure cis epoxide 8 was prepared from 6 with the standard three-step sequence,[14] consisting of the acetolysis of 6 to the glycol monoacetate, followed by conversion to tosylate, and final treatment with methanolic potassium hydroxide. However, performing the third step as described[14] gave a very low yield of 8, the main products being probably a glycol monomethyl ether and a chlorohydrin, obtained at the expenses of the first formed epoxide by addition of methanol and of the hydrochloric acid used for diluting the reaction mixture. Much better yields of 8 were obtained with a shorter refluxing time (30 min) followed by dilution with water.

(+)-*c*-4-*tert*-Butylcyclohexane-*r*-1,*t*-2-diol (7). The (1*S*,3*R*)-(−)-tosylate of *cis*-3-*tert*-butylcyclohexanol (3) ($[\alpha]^{25}_{D}$ −23.1°) was heated in quinoline as described[10] and the crude product filtered through a silica gel column to give a 38:62 mixture of (*R*)-(−)-3-*tert*-butylcyclohexene (5) and (*R*)-(+)-4-*tert*-butylcyclohexene (4) (GLC: 3-m glass column, 2.5-mm i.d., packed with 10% Carbowax 20M on silanized Chromosorb W 80–100 mesh; column 75 °C, evaporator and detector 180 °C, nitrogen flow 25 mL/min; relative retention times of 5 and 4, 1:1.15). A solution of this mixture (1.27 g) in $CHCl_3$ (12 mL) was treated with *m*-chloroperoxybenzoic acid (2.40 g), left at 5 °C for 24 h, filtered, washed with 10% aqueous sodium carbonate and water, dried, and evaporated. The residue (1.30 g) was shown by GLC to consist of 64% of *trans*- and *cis*-4-*tert*-butylcyclohexene oxides (unseparated under the conditions employed), 32% of *trans*-3-*tert*-butylcyclohexene oxide, and 4% of *cis*-3-*tert*-butylcyclohexene oxide (GLC: same column as above; column 130 °C, evaporator and detector 180 °C, nitrogen flow 45 mL/min; relative retention times for *cis*-3-*tert*-butylcyclohexene oxide, 1, *trans*-3-*tert*-butylcyclohexene oxide, 1.17, and *trans*- and *cis*-4-*tert*-butylcyclohexene oxides (6 and 8, unseparated), 1.41).

This mixture of epoxides was stirred with 13 mL of 1 N aqueous H_2SO_4 at room temperature for 24 h and then extracted with ethyl ether. The extracts were washed with water and saturated aqueous $NaHCO_3$, dried, and evaporated to give a residue (1.0 g) containing some unreacted *trans*-3-*tert*-butylcyclohexene oxide, *c*-4-*tert*-butylcyclohexane-*r*-1,*t*-2-diol (7, 75%), *t*-3-*tert*-butylcyclohexane-*r*-1,*t*-2-diol (17%), and *c*-3-*tert*-butylcyclohexane-*r*-1,*t*-2-diol (8%) (GLC: 1-m glass column, 2.5-mm i.d., packed with 10% Carbowax 20M on silanized Chromosorb W 80–100 mesh; column 170 °C, evaporator and detector 200 °C, nitrogen flow 25 mL/min; relative retention times for *c*-3-*tert*-butylcyclohexane-*r*-1,*t*-2-diol, 1, *t*-3-*tert*-butylcyclohexane-*r*-1,*t*-2-diol, 1.58, and *c*-4-*tert*-butylcyclohexane-*r*-1,*t*-2-diol (7), 2.19). Two crystallizations of this mixture from petroleum ether gave the pure (GLC) diol (1*S*,2*S*,4*R*)-(+)-7: mp 118–119 °C; $[\alpha]^{25}_{589}$ +27.4°, $[\alpha]^{25}_{546}$ +30.9°, $[\alpha]^{25}_{436}$ +49.8°, $[\alpha]^{25}_{365}$ +71.9° (*c* 1.93, AcOEt) (lit.[14] (±)-7, mp 141–142 °C).

Microsomal Preparations. Liver microsomes were obtained from male New Zealand white rabbits (2.5–3 kg) pretreated with sodium phenobarbital for 3 days (35 mg/(kg day) by intraperitoneal injection). Livers were removed and homogenized in four volumes of 0.01 M phosphate buffer (pH 7.4) containing KCl (1.15% w/v) with a Teflon tissue grinder, and the resulting suspensions were centrifuged at 10 000*g* for 30 min. The supernatant was further centrifuged at 105 000*g* for 2 h to give microsomal pellets which were resuspended in the same buffer to a final protein concentration of ca. 16 mg/mL and used directly or stored at −40 °C. All of the incubations reported in Table I were performed with a single lot of microsomes prepared by pooling the livers of two rabbits.

Enzymatic Incubations. A. Cyclohexene Oxide. Cyclohexene oxide (100 mg) in 1.5 mL of ethanol was added to 12 mL of microsomal preparation and 8 mL of 0.01 M phosphate buffer (pH 7.4), and the mixture was incubated with shaking at 37 °C. After 30 min, the incubation was terminated by cooling it below 0 °C. In order to remove the unreacted epoxide, the mixture was extracted five times with 10 mL of petroleum ether by vigorous shaking followed by brief centrifugation to separate the emulsion and freezing. Bromocyclohexane was added as a standard to the combined extracts, and the amount of cyclohexene oxide was determined by GLC by comparison with a blank experiment carried out with boiled microsomes. It was found that 10% of the oxide had reacted (GLC conditions: 2-m glass column, 2.5-mm

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i.d., packed with 3% ethylene glycol succinate on silanized Chromosorb W 80–100 mesh; low isotherm 70 °C, temperature increment 6 °C/min, high isotherm 200 °C, evaporator and detector 200 °C, nitrogen flow 30 mL/min).

The incubation mixture was then diluted with methanol (20 mL), the protein fraction removed by centrifugation, the solution evaporated under reduced pressure, and the residue extracted five times with 3 mL of ethyl ether. Evaporation of the combined extracts followed by one crystallization of the residue from petroleum ether gave 8 mg of slightly impure *trans*-1,2-cyclohexanediol, $[\alpha]^{25}_D$ −26.4°. After a second crystallization, the optical rotation rose to $[\alpha]^{25}_D$ −36.5° (ee 78.7%).

In another experiment carried out under identical conditions but with an incubation time of 8.5 h, no unreacted epoxide was recovered by extraction with petroleum ether. The extraction with ethyl ether of the residue obtained by evaporation of the incubation mixture, after removal of the protein fraction, gave crude *trans*-1,2-cyclohexanediol which was purified by sublimation at 1 mm to give the pure diol, $[\alpha]^{25}_D$ −33.5° (ee 72%). Further extraction with ethyl acetate and sublimation yielded a second crop of diol with $[\alpha]^{25}_D$ −31.4° (ee 67.5%). Crystallization from petroleum ether of the combined products from the two extractions (65 mg) gave a sample of diol with $[\alpha]^{25}_D$ −32.8° (ee 70.5%), and a further crystallization changed the optical rotation to $[\alpha]^{25}_D$ −34.6° (ee 74.5%). These experiments indicate that the optical purity of the diol changes little with percent conversion. They also show that crystallization of the diol is accompanied by a slight increase in optical purity. We believe that the most reliable value is obtained by sublimation and therefore that the actual optical purity of the enzymatically formed diol is around 70 ± 2%.

B. *trans*- and *cis*-4-*tert*-Butylcyclohexene Oxides. The epoxide **6** or **8** (100 mg) was incubated as described above for cyclohexene oxide. The incubations were terminated by cooling after the times reported in Table I, and the mixtures were extracted three times with 30 mL of ethyl ether. The combined extracts were analyzed by GLC under the following conditions: 3-m glass column, 2-mm i.d., packed with 2% ethylene glycol succinate on silanized Chromosorb W 80–100 mesh; low isotherm 80 °C, high isotherm 200 °C, temperature increment 2.5 °C/min, evaporator and detector 200 °C, nitrogen flow 30 mL/min; relative retention times for **6** or **8**, 1, and **7**, 2.21. The percentage of hydrolysis, determined from the ratios of glycol produced to unreacted epoxide, is reported in Table I. Blank experiments carried out with artificial mixtures of epoxide and diol showed that the recovery was identical for both compounds, and no chemical hydrolysis occurred. The extracts were then evaporated, and the residues were crystallized from petroleum ether to give the pure (GLC) diol. No variation in optical purity occurred on crystallization, since two or more consecutive crystallizations gave products with unchanged optical rotations. Alternatively, the crude products of the incubations were taken up in cold petroleum ether in order to remove the unreacted epoxide, and the insoluble diol was sublimed at 1 mm. The optical purity of the same sample of diol was identical after crystallization or sublimation. The optical rotations and optical purities of the diols obtained by stopping the incubations at different times are reported in Table I.

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Registry No. **3**, 31062-01-8; **4**, 61062-50-8; **5**, 61062-49-5; (1*R*,2*S*,4*R*)-**6**, 71961-99-4; (1*S*,2*R*,4*S*)-**6**, 71962-00-0; (±)-**6**, 71962-01-1; (+)-**7**, 71962-02-2; (−)-**7**, 71962-03-3; (1*S*,2*R*,4*R*)-**8**, 35650-41-0; (1*R*,2*S*,4*S*)-**8**, 72274-34-1; (±)-**8**, 71962-04-4; *trans*-3-*tert*-butylcyclohexene oxide, 20887-61-0; *cis*-3-*tert*-butylcyclohexene oxide, 20887-60-9; *t*-3-*tert*-butylcyclohexane-*r*-1,*t*-2-diol, 25348-58-7; *c*-3-*tert*-butylcyclohexane-*r*-1,*t*-2-diol, 25348-59-8; cyclohexene oxide, 286-20-4; *trans*-1,2-cyclohexanediol, 1460-57-7.

Energy Sufficient α-Amino Peroxides as Potential Sources of Excited-State Carbonyls

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Products from the thermolysis of α-amino peroxides **2** and **3** reveal a normal di-*sec*-alkyl peroxide decomposition route rather than an intramolecular base-catalyzed elimination. With peroxide **2**, *N*-phenyl-*N*-methylformamide and benzophenone were produced in 92 and 97% yield, respectively. Peroxide **3** gave *N*-phenyl-*N*-methylformamide and 1-mesityl-3,3-diphenylpropane-1,2-dione in yields of 82 and 89%, respectively. From thermochemical calculations, it was deduced that peroxides **2** and **3** are energy sufficient to generate excited-state products upon thermolysis. Extremely low chemiluminescence quantum yields (Φ_{CL}) were observed for **3**, in both the absence and the presence of acceptors (9,10-dibromoanthracene, 9,10-diphenylanthracene, and rubrene). Luminescence from the thermolysis of **2** was too weak to obtain Φ_{CL} values. The lack of efficient excited-state product formation in these energy sufficient reactions is discussed. A proposed mechanism for bioluminescence in the flavin system is also considered in relationship to the thermolyses of **2** and **3**.

A necessary condition for the production of excited-state products in a chemical reaction is that there be sufficient energy. The energy requirement is such that the enthalpy of reaction plus some fraction of the activation energy be equal to or greater than the difference in energy between the ground-state and excited-state product.[1] This can be seen in Figure 1, as well as the need for a nuclear configuration of the two surfaces so that crossing occurs. In addition, the probability of crossing from one energy surface to the other is dependent on the difference between the slopes of the two surfaces near the crossing point.[2] A smaller difference between the slopes will favor crossing. Furthermore, the crossing will be facilitated by an increase in density of vibrational states of the two surfaces at the crossing point. These latter two factors will also be dependent on the relative placement of the two surfaces. Lastly, the probability of crossing to P* will depend on the total electronic spin states of the species associated with the two surfaces.

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