Enantioselectivity of the Microsomal Epoxide Hydrolase: Hydrolysis of (±)-*cis*-3-Bromo-1,2-epoxycyclohexane

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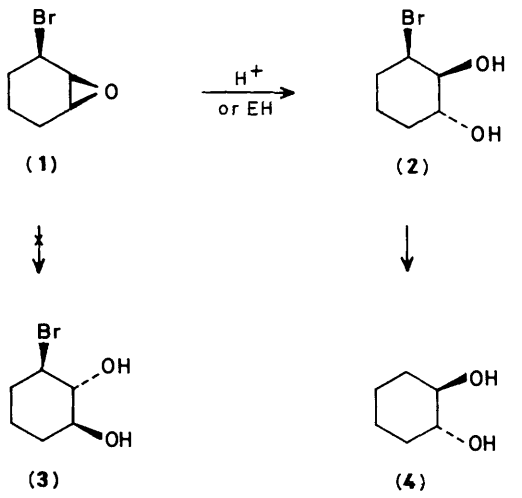
The acid-catalysed and the rabbit microsomal epoxide hydrolase-catalysed hydrolysis of (±)-*cis*-3-bromo-1,2-epoxycyclohexane (**1**) have been investigated. Both reactions were completely regio- and stereo-specific, giving *t*-3-bromocyclohexane-*r*-1,*t*-2-diol (**2**) as the only product. Epoxide (**1**) was found to be a much better substrate for the epoxide hydrolase than its *trans*-diastereoisomer. Under enzyme saturation conditions the hydrolysis was fairly enantioselective, as shown both by the biphasic shape of its kinetic profile and by the isolation of optically active (−)-(1*R*,2*R*,3*S*)-(**1**) and (−)-(1*R*,2*S*,3*R*)-(**2**) at incomplete reaction. The absolute configurations have been deduced by correlation with (−)-(*R*,*R*)-*trans*-cyclohexane-1,2-diol. At about 30% conversion, the enantiomeric excesses of unchanged (**1**) and formed (**2**) were 24—30% and 56—60%, respectively, and racemic (**2**) was obtained after complete hydrolysis. The results have been rationalized by a competitive inhibition of (+)-(1*S*,2*S*,3*R*)-(**1**) on the hydrolysis of (−)-(**1**) and fit the previously proposed picture describing the substrate enantioselection carried out by the microsomal epoxide hydrolase.

Epoxides are often found as products of the mono-oxygenase-catalysed biotransformations of xenobiotic compounds containing alkene or arene functions[1] and are thought to be the ultimate species responsible for the mutagenic and carcinogenic effects of several of these compounds.[2] It is also known that the biological activity of epoxy derivatives can be markedly affected by their relative and absolute stereochemistry.[3] These epoxides can be hydrolysed to the corresponding vicinal diols by the action of epoxide hydrolase (EH), an enzyme which plays an important role in the detoxification of epoxides[4] and, in certain cases, in their further metabolic activation.[5] Although EH is endowed with low substrate specificity, as required for an enzyme destined to carry out the biotransformations of a very broad class of xenobiotic compounds, it has been shown to be able to discriminate between different stereoisomers of substrates.[6-14] The stereochemical requirements of epoxide hydrolase appear therefore to be of crucial importance for a thorough comprehension of the structure-activity relationships of epoxides.

In previous investigations we have shown that both unsubstituted 1,2-epoxycyclohexane and its *trans*-3-bromo derivative are rather poor substrates for the rabbit microsomal EH[15] and exhibit some mutagenic activity towards V79 chinese hamster cells.[16] In contrast, *cis*-3-bromo-1,2-epoxycyclohexane was shown to be less toxic and less mutagenic, despite its similar electrophilic reactivity, as evaluated by the nicotinamide alkylation test.[16] This difference in biological activity was tentatively related to specific interactions with detoxifying enzymes. In this paper we report an investigation of the EH-promoted hydrolysis of (±)-*cis*-3-bromo-1,2-epoxycyclohexane.[17] The results have confirmed that this epoxide is a much better substrate for the rabbit EH than both its *trans*-diastereoisomer and the parent 1,2-epoxycyclohexane, and that the enzymatic hydrolysis is a fairly enantioselective process.

Results

Only one bromo diol was obtained when *cis*-3-bromo-1,2-epoxycyclohexane (**1**) was subjected to perchloric acid-catalysed hydrolysis in water—*t*-3-bromocyclohexane-*r*-1,*t*-2-diol (**2**). This was different from the known *c*-3-bromocyclohexane-*r*-1,*t*-2-diol (**3**), the only product of the hydrolysis of the diastereoisomeric *trans*-3-bromo epoxide, but was similarly transformed into *trans*-cyclohexane-1,2-diol by hydrogenolysis with Raney nickel. The acid-promoted hydrolysis of compound (**1**) was completely regio- and stereo-specific, water attack occurring with inversion only at C-1, furthest from the electron-withdrawing substituent, in agreement with the course of the HBr ring opening of (**1**).[19]



Enzymatic hydrolysis of the racemic *cis*-epoxide (**1**) was carried out at pH 7.4 with liver microsomes obtained from phenobarbital-pretreated rabbits. In contrast to its *trans*-3-bromo diastereoisomer, which required hours to be partially transformed by EH and yielded another epoxide, *trans*-2,3-epoxycyclohexanol, instead of a diol,[15] compound (**1**) was hydrolysed quickly to give the bromo diol (**2**) as the only product. Thus, both the acid- and enzyme-catalysed hydrolysis of (**1**) exhibited the same regio- and stereo-specificity.

The time course of the EH-promoted hydrolysis was examined at a 7 mg ml^{-1} microsomal protein concentration and a 2.3×10^{-2} M substrate concentration, *i.e.*, under conditions in

Table. Optical rotations, absolute configurations, and enantiomeric excesses of bromo diol (**2**) and bromo epoxide (**1**) recovered from incubations of (±)-(**1**) with rabbit liver microsomes

				Unchanged (**1**)			Formed (**2**)				
Run[a]	Incubated (±)-(**1**) (mg)	Incubation time (min)	Hydrolysis (%)[b]	mg	$[\alpha]_D^{25}$	Absolute config.	mg[c]	$[\alpha]_D^{25}$	Absolute config.	e.e.[d]	Total yield (%)
1	102	25	31.5	64.5	−48.6°[e]	1*R*,2*R*,3*S*	35.5	−25.3°	1*R*,2*S*,3*R*	78:22	95
2	100	25	30.5	62.5	−46.7°[f]	1*R*,2*R*,3*S*	33.5	−27.2°	1*R*,2*S*,3*R*	80:20	93
3	37	25	58	13.5			23.5		1*R*,2*S*,3*R*	67:33	94
4	47	180	100	0			51.7	0.0°		48:52	100

[a] The same amount of microsomal preparation (10 ml, 7 mg ml^{-1} protein) was used in each run. [b] Determined as the ratio of formed (**2**) to the incubated (±)-(**1**). [c] Corrected for an average 85% recovery of (**2**) (see Experimental section). [d] Determined by g.l.c. of bis(MTPA)esters of *trans*-cyclohexane-1,2-diol arising from hydrogenolytic debromination of (**2**). [e] $HClO_4$-catalysed hydrolysis of this sample of (**1**) gave (**2**), $[\alpha]_D^{25}$ +10.5°, 62:38 e.e. [f] $HClO_4$-catalysed hydrolysis of this sample of (**1**) gave (**2**), $[\alpha]_D^{25}$ +12.6°, 65:35 e.e.

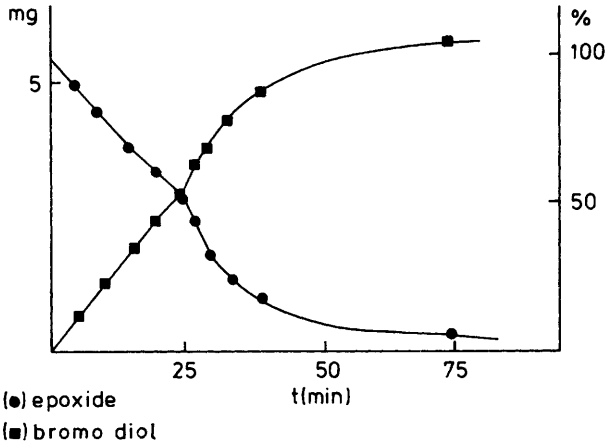


Figure. Kinetic profile of the enzymatic hydrolysis of (±)-*cis*-3-bromo-1,2-epoxycyclohexane (**1**) by microsomal epoxide hydrolase

which the enzyme active site was saturated* and the reaction proceeded at a rate around its $V_{max.}$. The hydrolysis was followed to 90% conversion by determining by g.l.c. the amounts of unchanged epoxide and formed diol at time intervals after extraction of samples of the incubation mixture with ethyl acetate and addition of the diastereoisomeric bromo diol (**3**), which was not formed from the hydrolysis of (±)-(**1**), as a standard. The results are reported in the curves of the Figure, showing the formation of (**2**) and the simultaneous disappearance of (**1**) during 75 min. Both curves exhibited biphasic shapes, the rates being roughly constant up to about 50% reaction, but showing afterwards an evident increase. This course is typical for enantioselective enzyme reactions in which the two enantiomers of a racemic substrate are consumed at different rates.[13,20]

This enantioselection was checked by isolation of products from experiments carried out on a larger scale. Different amounts of (±)-(**1**) were incubated for the same time with the same amount of microsomal preparation. The unchanged epoxide was then isolated by selective extraction with light petroleum and the produced diol was subsequently extracted with ethyl acetate. The yields of both products were individually determined by g.l.c. Duplicate runs (1 and 2 in the Table) gave reproducible results. Both the recovered epoxide (**1**) and the produced bromo diol (**2**) were found to be laevorotatory in runs stopped at about 30% conversion. Perchloric acid-catalysed hydrolysis of this recovered laevorotatory epoxide gave dextrorotatory (**2**), thus confirming that the excess (−)-enantiomer of (**3**) arose by a preferential hydrolysis of (+)-(**1**). In an experiment in which complete hydrolysis of (±)-(**1**) was achieved by increasing the incubation time, racemic (**2**) was isolated.

The absolute configuration of epoxide (**1**) and of bromo diol (**2**) were established by correlation with *trans*-cyclohexane-1,2-diol (**4**), of known configuration.[11] A sample of (**2**), $[\alpha]_D$ − 34.9°, was debrominated with Raney nickel in the presence of calcium carbonate to give (−)-(*R*,*R*)-(**4**), $[\alpha]_D$ − 37.3°. Thus, the (1*R*,2*S*,3*R*)-configuration could be assigned to (−)-(**2**), and, since this bromo diol is formed from the parent *cis*-bromo epoxide with inversion at C-1, the (1*S*,2*S*,3*R*)-configuration was attributed to (+)-(**1**).

Bis[α-methoxy-α-(trifluoromethyl)phenylacetic (MTPA)] esters of (**2**) were chosen as suitable diastereoisomeric derivatives for analysis by chromatographic techniques in order to obtain an accurate evaluation of the enantiomeric excess of this bromo diol. Attempts at esterification of (±)-(**2**) with MTPA chloride gave, however, unsatisfactory results since incomplete esterification was often obtained on heating and using a large excess of the reagent, and the two bis(esters) were detected in ratios different from the expected 50:50 ratio, pointing to some kinetic resolution. Better results were obtained in the reaction of MTPA chloride with *trans*-cyclohexane-1,2-diol, which yielded the two diastereoisomeric bis(esters) in exactly equal amounts, as shown by g.l.c. analysis. The samples of optically active (**2**) obtained from the enzymatic hydrolyses of (±)-(**1**) were then subjected to hydrogenolytic debromination followed by conversion into MTPA derivatives, which were analysed by g.l.c. The results, reported in the Table, show that a fair excess (56—60% e.e.) of the (−)-(1*R*,2*S*,3*R*)-diol (**2**) and a moderate excess (24—30% e.e.) of the (−)-(1*R*,2*R*,3*S*)-epoxide (**1**) were obtained at about 30% conversion (runs 1 and 2 of the Table). The e.e. of compound (**2**) decreased with increasing conversion and became zero after complete hydrolysis. Thus, both enantiomers of epoxide (**1**) are substrates for EH, but the (+)-(1*S*,2*S*,3*R*)-isomer is hydrolysed first when the racemic mixture is used as the substrate.

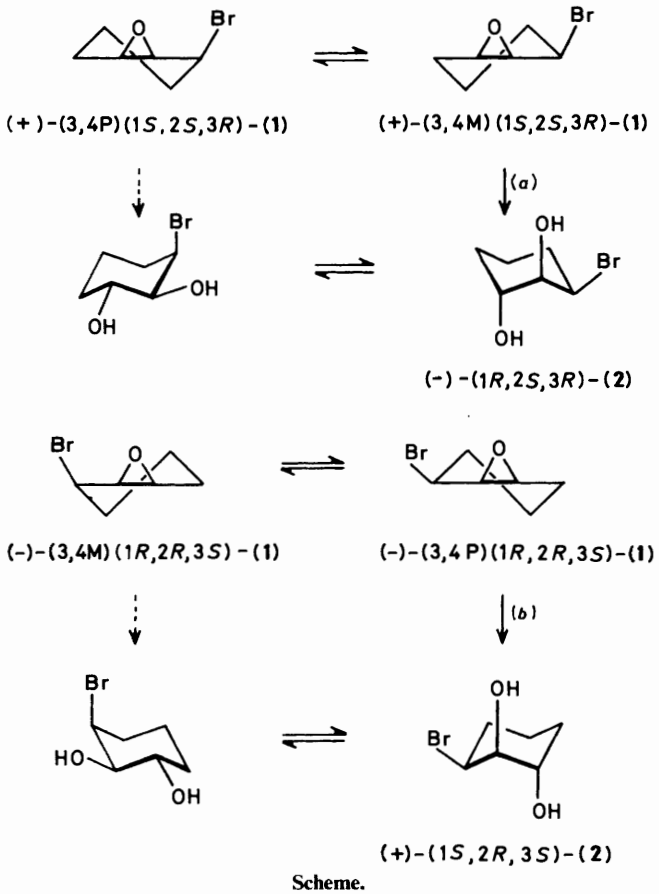
Discussion

The results of the present investigation show that the EH-promoted hydrolysis of (±)-*cis*-3-bromo-1,2-epoxycyclohexane is completely regio- and stereo-specific and is a fairly enantioselective process. The formation of the sole bromo diol (±)-(**2**)

* Values of K_M ranging between 2μM and 0.7mM have been reported for EH with different substrates: P. Bentley, F. Oesch, and A. Tsugita, *FEBS Lett.*, 1975, **59**, 296; see also ref. 3.

at complete conversion indicates that both enantiomers of the epoxide undergo anti-attack by water at C-1. An anti-opening at C-2 would have produced diastereoisomer (**3**), no trace of which was detected in the product. A conformationally favourable antiparallel attack on the more stable half-chair form of the epoxide with pseudoequatorial bromine, leading to a chair-like transition state, can be used to explain this steric course in the case of the acid-catalysed reaction and is also likely to occur in the enzymatic reaction. In the latter case, the same product (**2**) could also have been formed by parallel attack at C-1 on a half-chair conformer with pseudoaxial bromine, resulting in a boat-like transition state, if this normally unfavourable transition state could be stabilized by the enzyme. However, previous results obtained with epoxycyclohexane substrates [11-13] have shown that antiparallel opening through the usual chair-like transition state is highly preferred in enzymatic reactions. One exception to this rule is provided by the exclusive parallel opening at C-1 of *trans*-3-t-butyl-1,2-epoxycyclohexane.[12] In this case, however, antiparallel attack at C-2 is severely hindered by the steric effect of the bulky t-butyl group.

The course of the enzymatic hydrolysis of (±)-(**1**) can thus be depicted as shown in the Scheme, where the dashed arrows refer



Scheme.

to the less probable boat-like transition state pathways involving pseudoaxial conformers of the substrate, and (3,4M) and (3,4P) denote the helicities of the alternative monoplanar conformations of the six-membered ring of the two enantiomers of the epoxide.

The results of the Table show that process (*a*) is predominant over (*b*) in the early stages of the hydrolysis of the racemic substrate, despite the fact that, as shown by the reaction-time profiles of the Figure, process (*b*), prevailing during the second half of the reaction, is faster. Similar observations have been previously reported for the EH-promoted hydrolysis of both acyclic epoxides,[20] and epoxides fused to a six-membered ring [13,21] performed under enzyme saturation conditions, and can be rationalized by a competitive inhibition of one enantiomer of the substrate on the hydrolysis of its antipode. In the case of epoxide (**1**), the (+)-(1*S*,2*S*,3*R*)-enantiomer appears to fit better into the EH active site, giving a more stable ES complex, which releases the diol product, (−)-(1*R*,2*S*,3*R*)-(**2**), with a lower $V_{max.}$ relative to the less stable ES complex of (−)-(1*R*,2*R*,3*S*)-(**1**), which produces (+)-(1*S*,2*R*,3*S*)-(**2**). A rough value of 1.5—2 for the ratio of the respective $V_{max.}$ for the two processes (*b*) and (*a*) is obtained from the slopes of the reaction-time profiles of the Figure before and after 50% hydrolysis. Owing to its higher affinity for the enzyme, as long as (+)-(**1**) is present it acts as a competitive inhibitor of the hydrolysis of (−)-(**1**), so that only when most of the former enantiomer has been consumed can the hydrolysis of the latter occur at its $V_{max.}$, thus resulting in a faster diol production.

The above explanation for the microsomal EH-catalysed hydrolysis of (±)-(**1**) fits the general picture describing the substrate enantioselection carried out by this enzyme towards epoxycyclohexanes and epoxytetrahydropyrans,[11-14] as well as epoxides of polycyclic aromatic hydrocarbons.[22] According to this picture, the enantioselection is determined mainly by the helicity of the monoplanar conformation of the six-membered ring and by the presence of lipophilic substituents appropriately disposed with respect to the oxirane ring. The most stable ES complexes are obtained from substrate stereoisomers having a (3,4M) helicity and bearing substituents placed to the right of the oxirane ring, when the molecule is viewed with oxygen on the front topside as in the Scheme. The (+)-(1*S*,2*S*,3*R*)-enantiomer of (**1**) in its more stable conformation with pseudoequatorial bromine has the favourable (3,4M) helicity, in addition to the correct orientation of the substituent to give the more stable ES complex. This form is therefore expected to react first. In contrast, the (−)-antipode of (**1**) in the same conformation has the unfavourable (3,4P) helicity and a wrong orientation of the bromine substituent and is not expected to bind as well to the enzyme active site. On the other hand, its alternative conformation with pseudoaxial bromine has the required (3,4M) helicity, but water attack at C-1 in this conformation would involve an unfavourable boat-like transition state, which should presumably be reflected in a slower rate of diol release. This is the opposite of the observed result, indicating a faster release of the (+)-(1*S*,2*R*,3*S*)-diol (**2**) from the ES complex of (−)-(**1**) relative to that of (−)-(1*R*,2*S*,3*R*)-(**2**) from the complex of (+)-(**1**). The occurrence of enzymatic hydrolysis through the less stable pseudoaxial conformation can therefore be reasonably excluded, at least as a dominant pathway, for both enantiomers of (**1**).

The steric course of the hydrolysis of (±)-(**1**) is therefore comparable to that of the conformationally rigid (±)-*cis*-3-t-butyl-1,2-epoxycyclohexane [12] and (±)-*trans*-4-t-butyl-1,2-epoxycyclohexane.[11] The extent of enantioselection observed for (±)-(**1**) (around 60% e.e.) at about 30% hydrolysis is, however, lower than that found at comparable conversion for these t-butyl derivatives (>95% e.e.), where the orientation of the bulky substituent appears to play an important role in determining a selective binding to a hydrophobic pocket present in the enzyme active site. On the other hand, the enantioselection observed for (±)-(**1**) is only moderately higher than that reported for (±)-*trans*-4,5-dimethyl-1,2-epoxycyclohexane (30% e.e. at 27% hydrolysis),[13] where the effects of the two substituents cancel each other because of their symmetrical disposition and the enantioselection is mainly due to ring helicity effects. The fact that both enantiomers of (**1**) are by far better substrates for EH than the unsubstituted 1,2-epoxycyclo-

hexane and its *trans*-3-bromo derivative suggests a favourable interaction of the *cis*-3-bromine atom with the active site of the enzyme. This may contribute to the observed much easier detoxification of (**1**).[16] However, the above mentioned comparisons of enantioselection as a function of substituents indicate that this *cis*-bromine atom, perhaps owing to its relatively small size, makes only a modest contribution to the selective binding of the two enantiomers at the hydrophobic pocket, but affects the reaction course basically by maintaining the two enantiomers of (**1**) preferentially in pseudoequatorial conformations, whose helicity differences are responsible for the observed substrate enantioselection.

Experimental

M.p.s were taken on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 photoelectric polarimeter. G.l.c. analyses were carried out with a Dani 3800 instrument. Amberlite IRA-400-OH was obtained by washing commercial Amberlite IRA-400-Cl with 20% aqueous NaOH. Epoxide (±)-(**1**) was obtained by chromatography over a silica gel column of a 3:1 mixture of *cis*- and *trans*-3-bromo-1,2-epoxycyclohexane prepared by addition of acetyl hypobromite to 3-bromocyclohexene, followed by deacetylation and epoxide ring closure.[19] Elution with light petroleum–diethyl ether (98:2) gave, in succession, the pure *trans*- and *cis*-epoxides. (+)-α-Methoxy-α-trifluoromethylphenylacetic acid chloride was prepared from the (−)-acid and thionyl chloride.[23]

Acid-catalysed Hydrolysis of (±)-(**1**).—A sample of (±)-(**1**) (0.2 g) was stirred for 24 h with 1.75M aqueous $HClO_4$ (5 ml) at room temperature. After addition of NaCl, the resulting solution was repeatedly extracted with ether. Evaporation of the washed and dried ($MgSO_4$) extracts gave a single (g.l.c.) solid product consisting of (±)-(**2**), m.p. 130—132 °C [after crystallization from light petroleum–diethyl ether (lit.,[15] m.p. 130—132 °C)].

Microsomal Preparations.—Liver microsomes were obtained[11] from male New Zealand white rabbits (about 3 kg) pretreated with sodium phenobarbital [35 mg/(kg day) for 3 days by intraperitoneal injection]. Livers were removed and homogenized in two volumes of 5×10^{-2}M Tris-HCl buffer (pH 7.4) containing KCl (1.15% w/v), and the resulting suspensions were centrifuged at 9 000 g for 20 min. The supernatant was further centrifuged at 105 000 g for 2 h to give microsomal pellets which were resuspended in two volumes of the same buffer to a final protein concentration of 7 mg ml^{-1} and used directly or stored at −40 °C.

Enzymatic Hydrolyses.—A. Kinetic profile. The microsomal preparation (1.3 ml) was pre-heated at 37 °C and added to a series of flasks containing samples of (±)—(**1**) (5.5 mg, 0.03 mmol) dissolved in ethanol (0.1 ml) and the mixtures were incubated with shaking at 37 °C. At given times, ranging between 5 and 75 min, the reactions were stopped by addition of NaCl and immediate cooling at −40 °C, and the incubation mixtures were extracted with 3 × 3 ml of ethyl acetate. The extracts were concentrated under reduced pressure and subjected to g.l.c. analysis, which always indicated the presence of the epoxide (**1**) and the bromo diol (**2**), but not the diastereoisomeric *c*-3-bromocyclohexane-*r*-1,*t*-2-diol (**3**). A stock solution of (**3**) (0.1 ml, 28.6 mg ml^{-1}) in ethyl acetate was then added to the extracts as a standard, and the amounts of epoxide (**1**) and bromo diol (**2**) were determined by g.l.c. [20 m capillary column, 0.25 mm i.d., filled with SE52, column 150 °C, evaporator and detector 210 °C, nitrogen flow 2 ml min^{-1}; relative retention times: (**1**), 1; (**3**), 1.3; (**2**), 1.5] using a calibration curve obtained with the pure reference compounds. The average recovery of bromo diol (**2**) from fresh microsomes and of epoxide (**1**) from boiled microsomes was evaluated by blank experiments to be 85% and >95% respectively. In particular, no bromo diol was produced from the epoxide and boiled microsomes, showing that no spontaneous hydrolysis of (**1**) had occurred. The total yields of compounds (**1**) and (**2**) determined from each incubation of (±)-(**1**), after correction for the recovery of products, were around 100%. The amounts of (**1**) and (**2**) found at different times are shown in the Figure.

B. Preparative incubations. Incubations were carried out as described under *A*, but using 10 ml of microsomal preparation and the quantities of (±)-(**1**) reported in the Table. At the required time each reaction was stopped by cooling at −40 °C, the mixture was extracted with 3 × 10 ml of light petroleum, and the organic layer was separated by centrifugation. G.l.c. of these extracts revealed the presence of the sole epoxide (**1**). The combined extracts from each experiment were then reduced to an exactly known volume by evaporation at room temperature, a proper amount of a stock solution of (**3**) (2.0 mg ml^{-1}) in ethyl acetate was added as a standard to 1 ml of the extract, and the amount of epoxide (**1**) was determined by g.l.c. The remaining part of the extract was evaporated under reduced pressure and the optical rotation of the liquid residue, consisting of the unchanged epoxide (**1**), was measured in light petroleum.

The aqueous phase containing bromo diol (**2**) was then saturated with NaCl and extracted with 5 × 10 ml of ethyl acetate. G.l.c. analysis of these combined extracts showed the presence of the sole diol (**2**). After concentration under reduced pressure to an exactly known volume, the proper amount of the same stock solution of (**3**) was added to 1 ml of each extract and the amount of (**2**) was determined by g.l.c. and corrected for an 85% recovery. The remaining part of the extract was evaporated under reduced pressure and the crude solid residue, consisting of (**2**), was subjected to the measurement of the optical rotation in ethyl acetate. The results obtained in four experiments are reported in the Table.

Determination of Enantiomeric Excesses.—The crude residue consisting of (−)-(**2**), obtained by evaporation of the ethyl acetate extracts of the incubations of (±)-(**1**), was dissolved in ethanol (3 ml) and treated with 0.5 ml of a suspension of Raney nickel previously washed with ethanol, and with Amberlite IRA-400-OH (0.25 g). After being stirred at room temperature for 24 h, the solution was filtered and evaporated under reduced pressure to give a solid product consisting of *trans*-cyclohexane-1,2-diol (**4**), (by g.l.c.). This product was dissolved in dry pyridine (2 ml) and treated with a triple molar excess of (+)-MTPA chloride. After 24 h at room temperature, the reaction mixture was diluted with water and extracted with ether, and the extract was washed with 2M HCl and aqueous Na_2CO_3 solution, dried ($MgSO_4$), and evaporated. G.l.c. analysis of the residue (20 m capillary column, 0.25 mm i.d., filled with SE52, column 240 °C, evaporator and detector 260 °C, nitrogen flow 3 ml min^{-1}) showed two peaks, due to the diastereoisomeric bis(MTPA) esters of (**4**), with relative retention times of 1 and 1.05, in the ratios reported in the Table.

Preliminary experiments carried out starting either from (±)-(**4**) or from (±)-(**2**) had shown that the above sequence always led to complete esterification, giving 50(2):50(2) mixtures of the two bis(MTPA) esters.

The crude epoxide (−)-(**1**), recovered by evaporation of the light petroleum extract of the incubations of (±)-(**1**), was stirred with 1.75M $HClO_4$ (1 ml) for 24 h. After addition of NaCl, the resulting solution was extracted with ether. The extracts were dried ($MgSO_4$) and evaporated to give bromo diol (**2**), whose optical rotation was measured in ethyl acetate. This product

was then subjected to the reaction sequence described above for the determination of enantiomeric excess.

The results are reported in the Table.

Conversion of (−)-(1R,2S,3R)-(**2**) *into* (−)-(R,R)-*Cyclohexane*-1,2-*diol*.—A sample of (**2**) (78 mg), $[\alpha]_D$ −34.9°, in ethanol (5 ml) was treated with 2 ml of a suspension of Raney nickel previously washed with ethanol, and $CaCO_3$ (50 mg), and the mixture was refluxed for 3 h. After being cooled the solution was filtered and evaporated under reduced pressure and the residue was subjected to sublimation (80—100 °C/1 mmHg) to give a pure sample of *trans*-cyclohexane-1,2-diol, $[\alpha]_D$ −37.3° (*c* 1.06, water), e.e. 80%.[11]

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References

1 E. W. Maynert, R. L. Foreman, and T. Watabe, *J. Biol. Chem.*, 1970, **245**, 5234; D. M. Jerina and J. W. Daly, *Science*, 1974, **185**, 573.
2 L. Ehrenberg and S. Hussain, *Mutat. Res.*, 1981, **86**, 1.
3 W. Levin, M. K. Buening, A. W. Wood, R. L. Chang, B. Kedzierski, D. R. Thakker, D. R. Boyd, G. S. Gadaginameth, R. N. Armstrong, H. Yagi, J. M. Karle, T. J. Slaga, D. M. Jerina, and A. H. Conney, *J. Biol. Chem.*, 1980, **255**, 9067.
4 F. Oesch, 'Enzymatic Basis of Detoxication,' ed. W. B. Jakoby, Academic Press, New York, 1980, vol II, pp. 277—290.
5 A. H. Conney, W. Levin, A. W. Wood, H. Yagi, R. E. Lehr, and D. M. Jerina, 'Advances in Pharmacology and Therapeutics,' ed. Y. Cohen, Pergamon Press, Oxford, 1978, vol **9**, pp. 53—62.
6 D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltmann-Niremberg, and S. Underfriend, *J. Am. Chem. Soc.*, 1968, **90**, 6525.
7 D. M. Jerina, M. Ziffer, and J. W. Daly, *J. Am. Chem. Soc.*, 1970, **92**, 1056.
8 D. R. Thakker, H. Yagi, W. Levin, A. Y. H. Lu, A. H. Conney, and D. M. Jerina, *J. Biol. Chem.*, 1977, **252**, 6328.
9 P. M. Dansette, V. B. Makedonska, and D. M. Jerina, *Arch. Biochem. Biophys.*, 1978, **187**, 290.
10 R. N. Armstrong, W. Levin, and D. M. Jerina, *J. Biol. Chem.*, 1980, **255**, 4698.
11 G. Bellucci, G. Berti, G. Ingrosso, and E. Mastrorilli, *J. Org. Chem.*, 1980, **45**, 299.
12 G. Bellucci, G. Berti, R. Bianchini, P. Cetera, and E. Mastrorilli, *J. Org. Chem.*, 1982, **47**, 3105.
13 G. Bellucci, G. Berti, M. Ferretti, E. Mastrorilli, and L. Silvestri, *J. Org. Chem.*, 1985, **50**, 1471.
14 G. Catelani and E. Mastrorilli, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2717.
15 G. Bellucci, G. Berti, M. Ferretti, F. Marioni, and F. Re, *Biochem. Biophys. Res. Commun.*, 1981, **102**, 838.
16 G. Bellucci, A. Lippi, F. Marioni, L. Citti, P. G. Gervasi, and G. Turchi, *Chem.-Biol. Interactions*, 1984, **51**, 77.
17 A preliminary communication on this was presented at IV Convegno Nazionale della Divisione di Chimica Farmaceutica della S.C.I., Palermo, October 1983, Abstracts p. 88.
18 R. A. B. Bannard and L. R. Hawkins, *Can. J. Chem.*, 1961, **39**, 1539.
19 P. L. Barili, G. Bellucci, F. Marioni, and V. Scartoni, *J. Org. Chem.*, 1975, **40**, 3331.
20 T. Watabe, N. Oshikawa, and Y. Yoshikawa, *Biochem. Pharmacol.*, 1981, **30**, 1695; T. Watabe, N. Ozawa, and A. Hiratsuka, *ibid.*, 1983, **32**, 777.
21 P. L. Barili, G. Berti, G. Catelani, F. Colonna, and E. Mastrorilli, *J. Chem. Soc., Chem. Commun.*, 1986, 7.
22 R. N. Armstrong, B. Kedzierski, W. Levin, and D. M. Jerina, *J. Biol. Chem.*, 1981, **256**, 4726.
23 J. A. Dale, D. L. Dull, and H. S. Mosher, *J. Org. Chem.*, 1969, **34**, 2543.

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