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THE CYTOCHROME P-450 CATALYZED OXIDATION OF 1-METHYLCYCLOHEXENE. COMPETITION BETWEEN HYDROXYLATION AND EPOXIDATION AND ABSOLUTE STEREOCHEMISTRY OF THE EPOXIDATION

Giuseppe Bellucci,* Cinzia Chiappe, Franco Marioni
Dipartimento di Chimica Bioorganica, Università di Pisa, Via Bonanno 33, 56126 Pisa, Italy
Pier Giovanni Gervasi, Annalisa Lippi
Istituto di Mutagenesi e Differenziamento del CNR, Via Svezia 10, 56100 Pisa, Italy

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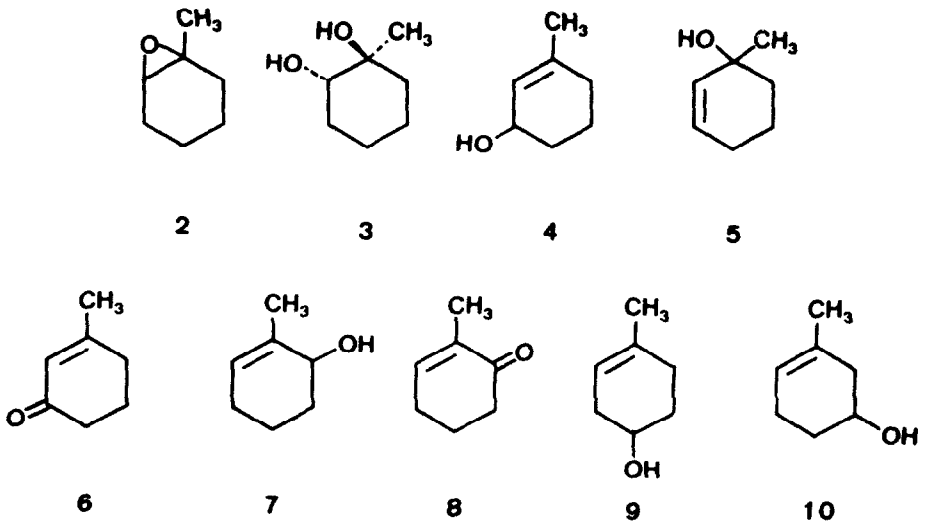
Abstract: The title reaction gives allylic hydroxylation and epoxidation products in about 2:1 ratio, 1-methyl-1,2-epoxycyclohexane being formed with a 46% e.e. of the (1R,2S) enantiomer.

Xenobiotic compounds containing an olefinic double bond are oxidized in mammals by Cytochrome P-450 dependent monooxygenases,[1] to give mainly epoxides and allylic alcohols. Whereas the latter metabolites usually are devoid of toxic effects, epoxides, unless rapidly detoxified by hydrolysis to diols by the action of epoxide hydrolases[2] or by glutathione conjugation,[3] can covalently bind to cellular macromolecules. These changes can lead to toxic, mutagenic and carcinogenic effects,[4] the severity of which can depend upon the absolute stereochemistry of the epoxide.[5] A 1-methylcyclohexene (**1**) partial structure is present in a number of terpenoid compounds and its epoxide has been shown to be a poor substrate for the epoxide hydrolases.[6] The competition between hydroxylation and epoxidation and the steric course of the latter biotransformation is therefore of concern, and is the subject of the present communication.

1-Methylcyclohexene (20 mM), free from autoxidation products, was incubated for 30 min at 37°C with liver microsomal preparations[7] (5 mL, 2 mg protein mL^{-1} in 100 mM phosphate buffer, pH 7.4) obtained from rats untreated and pretreated with phenobarbital (PB), a powerful Cytochrome P-450 inducer, in the presence of an NADPH regenerating system (0.5 mM $NADP^+$, 10 mM glucose 6-phosphate, 5 mM $MgCl_2$ and 2 units of glucose 6-phosphate dehydrogenase). The reactions were stopped by saturating with NaCl the incubation mixtures followed by extraction with ethyl acetate. The known products **2-10** were identified and quantified by GC-MS, by comparison with authentic samples.

Table I reports the product amounts and also includes, for comparison purposes, the selectivity recently reported for the autoxidation of **1**.[8] Diol **3** arose by partial hydrolysis of epoxide **2** by the microsomal epoxide hydrolase.[6] Ketones **6** and **8** were formed by microsomal oxidation of



the initially formed allylic alcohols **4** and **7**. No product of methyl oxidation was found, in agreement with the very low amount of 1-hydroxymethylcyclohexene found in the autoxidation.[8] Considering that the tertiary alcohol **5** is formed in the autoxidation of **1** by rearrangement of a first-formed allylic radical at C(3), it can be concluded that the microsomal oxidation essentially occurs at the same sites involved in the autoxidative reaction, although with different selectivity. PB induction produced a large increase in all biotransformation routes and also resulted in a significant amount of hydroxylation at the non-allylic positions C(5) and, to a lesser degree, C(4).

Table I. Products of the rat liver microsomal oxidation and selectivity of the autoxidation of 1-methylcyclohexene.

	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Microsomal Oxidation[a]									
Untreated	21	13	46	1	13	10	1	-	-
PB pretreated	174	119	347	12	34	43	4	5	54
Autoxidation Selectivity[b]	15	-	24	29	-	28	-	-	-

[a] Products are given as nmol (mg protein)$^{-1}$. [b] Given in mol %. 2-Hydroxy-1-methylene-cyclohexane (2%) and 1-hydroxymethylcyclohexene (1%) are also reported (see ref. 8).

The Cytochrome P-450 catalyzed oxidation of cyclohexene in rat had been shown to lead to cyclohexene oxide and 2-cyclohexen-1-ol in approximatively equivalent amounts.[9] The present data show that with 1-methylcyclohexene the ratio of epoxidation (evaluated as the sum of **2** and **3**) to hydroxylation is about 1:2 both with untreated and with PB treated microsomes. The ratio between the hydroxylation at C(3) and that at C(6) in the Cytochrome P-450 catalyzed oxidation is much higher than that found in the autoxidation of **1**. Furthermore, very little or no allylic rearrangement to give the tertiary alcohol **5** is found in the microsomal hydroxylation, in contrast to the autoxidation. Substantial scrambling of the allylic positions has been reported for the microsomal hydroxylation of 3,3,6,6-tetra-deuteriocyclohexene, methylenecyclohexane and β-pinene.[10] This supports a non concerted mechanism consisting of an allylic hydrogen abstraction by an oxo-iron species of P-450 to give a radical pair intermediate that collapses very rapidly to the alcohol,[11] the degree of allylic scrambling being related to the stability and steric accessibility of the carbon radical center and to the rate of recombination of the carbon radical with the metal-bound oxygen. From this point of view, the absence or the very scarce formation of the tertiary alcohol **5** is probably due, rather than to a concerted oxygen insertion mechanism, either to a recombination that is faster than the reorganization of the first-formed free radical at C(3) or, more probably, to a steric effect by the methyl substituent to the transfer of the metal-bound oxygen to the C(1), end of the delocalized allylic radical.

The absolute stereochemistry of the epoxidation of **1** by PB induced microsomes was investigated by HPLC analysis of the two diastereoisomeric 2-[α-methoxy-α-(trifluoromethyl)-phenyl]acetates of diol **3** arising by hydrolysis of **2**.[6] As the microsomal epoxide hydrolase catalyzed hydrolysis of **2** was shown to occur with considerable substrate enantioselection,[6] the incubations were continued for a time long enough to ensure a complete enzymatic hydrolysis of the epoxide, thus avoiding kinetic resolution effects. The diol, separated by column chromatography, was reacted with (+)-(S)-MTPA chloride. The two monoesters were in a 73:27 ratio in favour of the one corresponding to the 1R,2R diol. Since the microsomal epoxide hydrolase catalyzes opening of **2** with inversion of configuration at C(2),[6] the epoxide formed in the microsomal oxidation of **1** contained a 46% e.e. of the 1R,2S enantiomer.

Only few data are available on the enantioselectivity of the microsomal epoxidation of simple prochiral alkenes. A very low product enantioselection has been found with rat liver microsomes in the case of styrene,[12] whereas higher values of the e.e. (40-50% in favour of the S enantiomer) have been reported for propene, 1-butene and 1,3-butadiene.[13] However, the e.e. decreased with increasing substitution on the double bond, trisubstitution leading to a racemic epoxide.[13] A discussion of these results in terms of enantioselection is complicated by the fact that microsomes

contain a number of different P-450 isozymes, which may contribute to product formation with different enantioselectivity. However, the remarkable e.e. found with the trisubstituted olefin **1** indicates that for this compound there is at least one isozyme leading essentially to the (1R,2S) epoxide, which is not completely counterbalanced by other isozymes endowed with opposite product enantioselectivity. This type of enantioselection could be related to the presence of two rapidly equilibrating dissimmetrical half-chair conformations of the cyclohexene ring, which may favour prochiral recognition by the Cytochrome P-450 isozymes. Work is in progress in order to check this hypothesis.

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