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RELATIVE AND ABSOLUTE STEREOCHEMISTRY OF DIOLS OBTAINED FROM MICROBIAL OXIDATION OF 3-METHYLCYCLOHEXENE

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Although a considerable literature exists on the microbial oxidation of aliphatic and aromatic compounds,(1) comparatively few studies have examined microbial oxidations of simple alicyclic hydrocarbons.(2) In order to determine the stereochemistry of the transformation of a carbon-carbon double bond into a vicinal glycol, we examined the products formed by the action of a mutant strain of *Pseudomonas putida* (*P. putida* 39/D) on (±) 3-methylcyclohexene. A portion of the crude oxidation products obtained with *P. putida* 39/D grown in the presence of (±) 3-methylcyclohexene was converted to bis-trimethylsilyl ethers, with hexamethyl disilazane in pyridine, and examined by v.p.c. under conditions (see Table 1) where authentic samples(3) of the four isomeric 1,2-dihydroxy-3-methylcyclohexanes could be separated. The only volatile compounds observed under these conditions were those of the bis-trimethylsilyl ethers of the *cis* diols 1a and 2a.

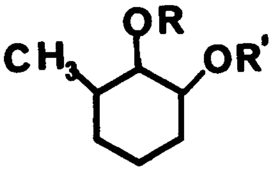
Table 1

C.G. Analysis of Diols as Trimethyl Silyl Ethers T=75°; 1% QF-1

Compound	Relative retention time (R_f)	Compound	R_f
trans, *cis*	1.00	*cis*, *trans* (2a)	1.34
cis, *cis* (1a)	1.25	*trans*, *trans*	1.77

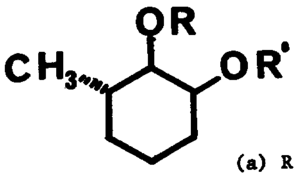
In order to ascertain whether one or both enantiomers of 3-methylcyclohexene were oxidized, the crude diol fraction was reacted with one equivalent of 3,5-dinitrobenzoyl chloride and the mixture (1b, 2b, 2c and 2d) separated. The stereochemistry assigned to the diols was confirmed by isolation of 1b and 2d and analysis of their nmr spectra. Each compound was optically active, establishing that *P. putida* was stereoselective in oxidizing both enantiomers. In order to determine whether one or both enantiomers were oxidized the absolute stereochemistry of each diol was determined using a combination of chemical and physical methods.

The diol 1a (α_D3.7°; $CHCl_3$, c=1.2) prepared by hydrolysis of 1b was converted to the dibenzoate 1c, using benzoyl chloride in pyridine and the c.d. curve of the purified dibenzoate determined. The c.d. curve exhibited a negative band at 237 nm and a positive band at 222nm. We have shown(4) earlier that this c.d. pattern of 1c requires the absolute stereo-



1

(a) R = R' = H
(b) R = H,
R' = 3,5-dinitrobenzoyl
(c) R = R' = benzoyl



2

(a) R = R' = H
(b) R = H,
R' = 3,5-dinitrobenzoyl
(c) R = 3,5-dinitrobenzoyl
R' = H
(d) R = R' = 3,5-dinitrobenzoyl

chemistry be to 3(R)-methyl-1(S),2(R)-dihydroxycyclohexane.

The diol 2a (α_D-35.2°, $CHCl_3$, c=1.2) was prepared by hydrolysis of 2d and the absolute stereochemistry of the methyl group established by oxidation to (+)-2(S)-methyl adipic acid of known absolute stereochemistry.(5) This result, in conjunction with the known relative stereochemistry of 2a, establishes 2a as 3(S)-methyl-1(S),2(R)-dihydroxycyclohexane.

The observation that the absolute stereochemistry at the carbons bearing hydroxyl groups in each case is 1(S),2(R) also suggests that either the same or a very similar enzyme is involved. These results represent one of the few authenticated cases where microbial oxidation of an olefin results exclusively in the formation of a *cis* diol.

REFERENCES

(1) D. T. Gibson, *Crit. Revs. Microbiol.* *1*, 199 (1971).

(2) B. B. Mukherjer, G. Kraidman and I. D. Hill, *Applied Microb.* *25*, 447 (1973).

(3) H. Ziffer, J. I. Seeman, R. J. Highet and E. A. Sokoloski, *J. Org. Chem.* in press.

(4) H. Ziffer, D. M. Jerina, D. T. Gibson and V. M. Kobal, *J. Am. Chem. Soc.* *95*, 4048 (1973).

(5) C. F. Wong, E. Auer and R. T. LaLonde, *J. Org. Chem.* *35*, 517 (1970).