Microbial Epoxidation of Long-chain Terminal Olefins

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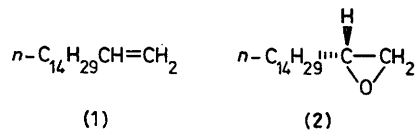
Summary Terminal olefins were oxidized biochemically by using *Corynebacterium equi* (IFO 3730) to give the corresponding optically pure (*R*)-(+)-epoxides.

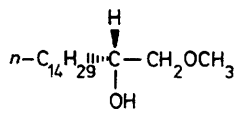
ASYMMETRIC reactions are important in synthetic chemistry; however, although a number of important natural compounds contain chiral carbon atoms bearing oxygen atoms,[1] formation of chiral carbon–oxygen bonds by asymmetric oxidation has achieved limited success. There have been several studies on the asymmetric epoxidation of olefins using chiral phase transfer catalysts,[2] chiral epoxidizing reagents,[3] or organometallic complexes with chiral ligands,[4] but the optical yields were generally low, and we thought that enzymatic oxidation of olefins would be a more useful method for obtaining optically active epoxides.

A number of micro-organisms were examined and *Corynebacterium equi* (IFO 3730) showed the best growth in an inorganic medium† containing hexadec-1-ene (**1**) as the sole carbon source. The following preparative experiments were carried out using this bacterium.



(1)

(2)



(3)

In a 500 ml Erlenmeyer flask 50 ml of the sterilized inorganic medium, 0·05 ml of polyoxyethylene sorbitan mono-oleate, 1 ml of n-octane, and 0·25 ml of (**1**), was inoculated with 1 ml of a suspension of *C. equi* and the whole was incubated for 48 h at 30 °C on a rotary shaker. The broth was extracted three times with 50 ml portions of ether, and the combined extracts were concentrated under reduced pressure. G.l.c. analysis showed that 1,2-epoxyhexadecane (**2**) was formed in a yield of 41% based on consumed substrate. The product was isolated in a larger scale experiment, in which 5% (v/v) of (**1**) was used, and identified as (**2**) by comparing its ^{1}H n.m.r., i.r., and mass spectra with those of an authentic specimen prepared by the epoxidation of (**1**) with *m*-chloroperbenzoic acid. Elemental analyses also supported this structure.

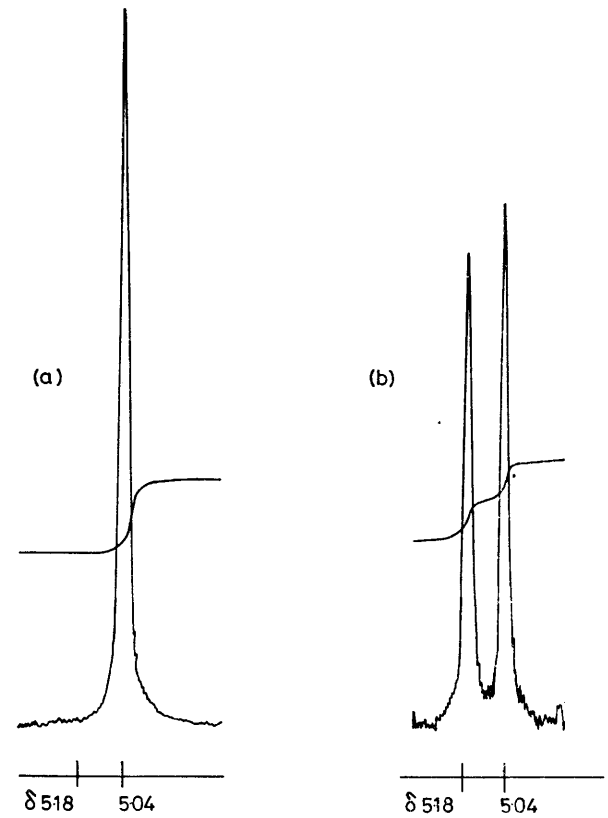


FIGURE. ^{1}H N.m.r. signal due to methoxy groups of (a) chiral and (b) racemic 1-methoxyhexadecan-2-ol (**3**) with Eu(tfc)$_3$, recorded on a Varian HA-100 instrument at 100 MHz in $CDCl_3$.

The epoxide obtained by microbial oxidation showed $[\alpha]_D^{25}$ +9·64 (*c* 3·71, n-hexane). Its optical purity was determined by conversion into 1-methoxyhexadecan-2-ol (**3**) by refluxing (**2**) with methanol containing a small amount of sodium methoxide. The ^{1}H n.m.r. peak due to

† The medium consisted of: $(NH_4)_2HPO_4$ (10 g), K_2HPO_4 (2 g), $MgSO_4 \cdot 7H_2O$ (0·3 g), $FeSO_4 \cdot 7H_2O$ (10 mg), $ZnSO_4 \cdot 7H_2O$ (8 mg), $MnSO_4 \cdot 7H_2O$ (8 mg), yeast extract (0·2 g), and H_2O to make 1000 ml (pH 7·2).

the methoxy group of (**3**) prepared from racemic (**2**) was split into two peaks by adding 0·3 mol. equiv. of the chiral shift reagent, tris[3-(**2**,2,2-trifluorohydroxyethylidene)-(+)-camphorato]europium [Eu(tfc)]. However, under the same conditions, (**3**) derived from the product of microbial oxidation showed a methoxy signal due to only one enantiomer as shown in the Figure. Thus, the optical purity of the epoxide (**2**) obtained by microbial oxidation was 100%, within the experimental error of the n.m.r. measurements. Coke and Golding and their co-workers[5] have synthesized optically active 1,2-epoxy-butane and -propane whose optical rotations and absolute configurations have been correlated. Comparison of the signs of the optical rotations shows that enzymatic epoxidation of (**1**) proceeds stereospecifically to give (*R*)-(+)-**1**,2-epoxyhexadecane.[5] May and Schwartz have also obtained (*R*)-(+)-7,8-epoxyoct-**1**-ene by enzymatic epoxidation using *Pseudomonas oleovorans*, but the stereospecificity was somewhat lower than in the present experiment.[6]

C. equi has been shown to be able to catalyse the epoxidation of other terminal olefins with carbon chains longer than fourteen. Although chemical yields are not yet high, the present enzymatic epoxidation, because of its highly stereospecific manner, may have some applicability in synthetic organic chemistry.

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