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Microbiological Transformations. 23. A Surprising Regioselectivity of Microbiological Baeyer-Villiger Oxidations of Menthone and Dihydrocarvone

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Abstract *: Regioisomeric lactones (ee>98%) were obtained by microbiological Baeyer-Villiger oxidation of enantiomers of menthone and dihydrocarvone. The regioselectivity of the biotransformation is shown to be dependant upon the considered enantiomer. An improvement of our empirical model of the active site is suggested.*

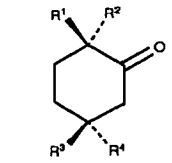
We have previously reported the enantioselective transformation of substituted cyclopentanones[1] and of bicyclic butanones[2] *via* microbiologically mediated Baeyer-Villiger (BV) type reactions. These reactions were carried out using a whole-cell procedure which is easier to use for synthetic purposes than purified enzymes[3]. Our work, as well as other recent examples[3,4], has shown that this type of reaction can be achieved on structurally different substrates and leads to quite different results depending upon these structures. Thus, whereas only one enantiomer of cyclopentanones was oxidized, the bicycloketones showed a surprisingly high regioselectivity[2a,c] and/or chemoselectivity[2b] depending upon the enantiomer. In this paper, we wish to describe the biooxidation of the enantiomers of two disubstituted cyclohexanones, menthone **1** and dihydrocarvone **2**, by *Acinetobacter* NCIB 9871 and *Acinetobacter* TD63 according to a whole-cell procedure[5].

These bacteria oxidized only the (+)-enantiomer of menthone **1** (cf. Table 1) and gave the lactone (+)-**5**[6], which was identical to the product obtained by chemical reaction (mCPBA, TFA, CH_2Cl_2, rt, 1h). Under the same conditions, (-)-menthone **1** was not metabolized and remained in the medium without undergoing further transformation. This fact is confirmed by the biotransformation of (+/-)-menthone which only led to (+)-**5**[7,8] .

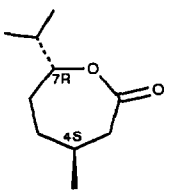
The results of the dihydrocarvone biotransformations were more surprising. As for (+)-**1**, (-)-dihydrocarvone **2** (obtained by hydrogenation of (+)-carvone with Zn / NaOH / EtOH / H_2O) only gave the expected lactone (+)-**6**[9]. On the other hand, (+)-**2** afforded a chemically unexpected compound, the lactone (-)-**7**[10,11],which shows unusual insertion of an oxygen atom between the carbonyl group and the least

substituted carbon atom. Also, it is interesting to note that, contrary to the case of chemical BV oxidation with the most common peracids, the double bond was not oxidized.

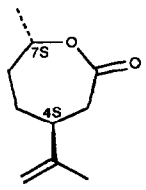
All our attempts to oxidize (+)-isomenthone **3** and (+)-isodihydrocarvone **4** failed. For example, a mixture of (+)-isomenthone **3** and (+)-menthone **1** gave only the lactone (+)-**5**, which shows that (+)-**3** is not a substrate for these bacteria.



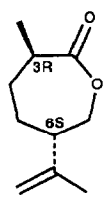
	R^1	R^2	R^3	R^4
(+)-1	H	i-Pr	Me	H
(-)-1	i-Pr	H	H	Me
(-)-2	H	Me	i-Propenyl	H
(+)-2	Me	H	H	i-Propenyl
(+)-3	H	i-Pr	H	Me
(+)-4	H	Me	H	i-Propenyl



(+)-5



(+)-6



(-)-7

Table 1 : Bioconversions by *Acinetobacter* NCIB 9871 (A) and *Acinetobacter* TD63 (B)

ketone	product	yield (%) A	B
(+)-1	(+)-5	90	61
(+/-)-1	(+)-5	45	43
(-)-2	(+)-6	80	95
(+)-2	(-)-7	73	66

Surprisingly, all these compounds, structurally very similar, exhibit a quite different behavior toward microbiological BV reactions. Menthone reacts as cyclopentanones did, i.e. one single enantiomer is transformed. On the other hand, both enantiomers of dihydrocarvone **2** undergo an oxidation, although each one with a different regioselectivity as it was the case for bicyclic butanones.Thus, it is particularly challenging to use these results in order to improve our previously proposed model of the active site of the enzyme[2c].

A mechanism of enzymatic BV oxidations was proposed by Walsh[12] and Schwab[3c] in the case of cyclohexanone oxygenase and implied an nucleophilic attack of ketone by a hydroperoxiflavine leading to the formation of a hydroxyperoxiflavine intermediate. We previously suggested that the positioning of this intermediate in the active site and also some stereoelectronic effects[13] would direct the regioselectivity and therefore the enantioselectivity of the reaction[14]. In our model, the active site is represented as a cube (cf. Figure 1). The peroxidic bond is immobile at the bottom of this cube by hypothesis. The rest of the molecule is allowed to rotate around the O-C(1) bond in order to authorize the migrating bond to be antiperiplanar to the peroxidic bond. To explain the observed regioselectivity of bicyclic butanones, we supposed the existence of steric interactions inside the active side, which prevented the hydroxyperoxide intermediate from adopting some configurations. Such a "forbidden zone" had been localized at the upper left corner of the cube. The extension of this model to the above cyclohexanones **1** and **2** now leads us to suppose an equatorial introduction of the hydroperoxide, since an axial orientation would imply occupation of the "forbidden zone". Thus, the

hydroxyperoxides from (+)-**1**, (-)-**2** and (+)-**2** should adopt the positions described in Figure 2 in order to lead to lactones **5**, **6** and **7**. Obviously, some more steric interactions must be considered in order to explain the absence of the BV oxidation of (-)-**1**, (+)-**3** and (+)-**4**. Thus, a comparison of the configurations of the different hydroxyperoxides drawn in Figure 2 shows that :

- an equatorial or an axial methyl group at C(2) is allowed but not an equatorial isopropyl group
- an equatorial methyl or isopropenyl group at C(3) is allowed but not an axial methyl group

Presumably, another forbidden zone must be situated in the lower right part of the cube model.[15]

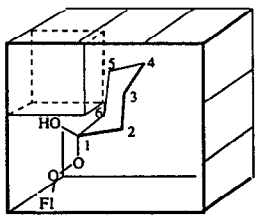


Figure 1 : Positioning of the hydroxyperoxide intermediate in the active site

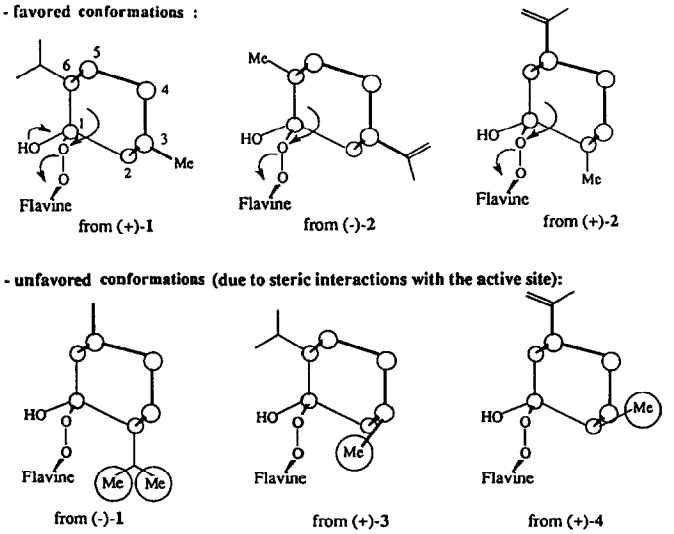


Figure 2 : Top view of different hydroxyperoxide intermediates.

Currently, work is in progress in order to further develop this model as well as to continue the study of the synthetic applications of these reactions.

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References

(1) (a) Alphand, V.; Archelas, A.; Furstoss, R. *J. Org. Chem.* **1990**, *55*, 347. (b) Alphand, V.; Archelas, A.; Furstoss, R. *Biocatalysis* **1990**, *3*, 73.

(2) (a) Alphand, V.; Archelas, A.; Furstoss, R. *Tetrahedron Lett.* **1989**, *30*, 3663. (b) Königsberger, K.; Alphand, V.; Furstoss, R.; Griengl, H. *Tetrahedron Lett.* **1991**, *32*, 499. (c) Alphand, V.; Furstoss, R. *J. Org. Chem.* in press

(3) (a) Taschner, M. J.; Black, D. J. *J. Am. Chem. Soc.* **1988**, *110*, 6892. (b) Abril, O.; Ryerson, C. C.; Walsh, C.; Whitesides, G. M. *Bioorg. Chem.* **1989**, *17*, 41. (c) Schwab, J., M.; Li, W., B.; Thomas, L., P. *J. Am. Chem. Soc.* **1983**, *105*, 4800.

(4) (a) Ouazzani-Chahdi, J.; Buisson, D.; Azerad, R. *Tetrahedron Lett.* **1987**, *28*, 1109. (b) Levitt, M. S.; Newton, R. F.; Roberts, S. M.; Willetts, A. J. *J. Chem. Soc., Chem. Commun.* **1990**, 619. (c) Carnell, A. J.; Roberts, S. M.; Sik, V.; Willetts, A. J. *J. Chem. Soc., Chem. Commun.* **1990**, 1438.

(5) Culture conditions were : 1,2-cyclohexanediol as carbon source, 30°C and biotransformation conditions were : 1,2-cyclohexanediol as cosubstrate, pH 7.1, 25°C (for more detailed procedure cf. ref.2c).

(6) (4S,7R)-(+)-4-Methyl-7-iso-Propyl-2-oxo-oxepanone **5** : IR (neat) 1720 cm^{-1}; ^1H NMR δ (ppm) 4.05 (dd,1H,J=9.0Hz,J=4.4Hz), 2.50 (m,2H), 1.85 (m,4H), 1.60 (m,1H), 1.30 (m,1H), 1.04 (d,3H,J=6.6Hz), 0.97 (dd,6H,J=6.8Hz,J=2.7Hz); ^{13}C NMR δ (ppm) 174.46 (C), 84.74 (CH), 42.69 (CH_2), 37.57 (CH_2), 33.42 (CH); 31.06 (CH_2), 30.50 (CH), 23.83 (CH_3), 18.38 (CH_3), 17.18 (CH_3); $[\alpha]_D^{25}$=+20.5 (c=1.4 $CHCl_3$).

(7) (-)-menthone **1** is recovered with low yield (20%) due to its volatility under bioconversion conditions (0.2 v/v/m aeration) and is partially epimerized during work-up.

(8) As far as we know, this is the first example of biological oxidation of (+)-**1**. Only (-)-**1** was studied and, in spite of the use of hydrolase inhibitors, only the hydroxyacid of **5** had been isolated (cf. (a) Croteau, R.; Sood, V. K.; Renstrom, B.; Bhushan R. *Plant. Physio.* **1984**, *76*, 647. (b) Nakajima, O.; Iriye, R.; Hayashi, T. *Nippon Nogeikagaku Kaishi* **1976**, *50*, 403. (c) Schukla, O. P.; Bartholomus R. C.; Gunsalus, I. C. *Can. J. Microbiol.* **1987**, *33*, 489.)

(9) (4S,7S)-(+)-7-Methyl-4-iso-Propenyl-2-oxo-oxepanone **6** : IR (neat) 1710 cm^{-1}; ^1H NMR δ (ppm) 4.74 (m,2H), 4.46 (qd,1H,J=6.5Hz,J=6.4Hz), 2.70 (m,2H), 2.30 (m,1H), 2.0-1.6 (m+s,7H), 1.37 (d,3H,J=6.4Hz); ^{13}C NMR δ (ppm) 177.60 (C), 148.49 (C), 110.12 (CH_2), 76.60 (CH), 41.88 (CH), 40.29 (CH), 35.96 (CH_2), 34.39 (CH_2), 22.62 (CH_3), 20.19 (CH_3), $[\alpha]_D^{25}$=+46.2 (c=1.1 $CHCl_3$).

(10) (3R,6S)-(-)-3-Methyl-6-iso-Propenyl-2-oxo-oxepanone **7** : IR (neat) 1725 cm^{-1}; ^1H NMR δ (ppm) 4.82 (m,1H), 4.72 (m,1H), 4.17 (d,2H,J=6.3Hz), 2.75 (m,1H), 2.28 (m,1H), 2.00 (m,1H), 1.8-1.6 (m+s,6H), 1.22 (d,3H,J=6.7Hz); ^{13}C NMR δ (ppm) 177.60 (C), 145.74 (C), 111.14 (CH_2), 71.57 (CH_2), 46.55 (CH), 37.29 (CH), 34.23 (CH_2), 31.88 (CH_2), 21.76 (CH_3), 18.44 (CH_3), $[\alpha]_D^{25}$=-35.8 (c=1.6 $CHCl_3$).

(11) These results are quite different from these ones previously reported[3b] using a purified cyclohexanone oxygenase (EC.1.14.13-), extracted from *Acinetobacter* NCIB 9871 and immobilized on a polyacrylamide gel. In this case, the product of the biotransformation of (+)-**2** was described as lactone **6**.

(12) Walsh, C. T.; Chen, Y. C. J. *Angew. Chem. Int., Ed. Engl.* **1988**, *27*, 333.

(13) Deslonchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon Press: Oxford, 1983; pp 313-314.

(14) A similar model, based on different hypotheses, has been recently proposed by Taschner et al. (personal communication).

(15) At the present time, we only consider this model as being a working hypothesis and not necessarily an accurate representation of the real enzymatic active site. Besides, it has not yet been proven that these reactions are really achieved by one single enzyme nor that only one single active site is involved. Nevertheless, it is interesting to notice that the results described by Taschner[3a] et al (BV oxidation of dimethylcyclohexanones by a purified monooxygenase) agree with our model.