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MICROBIAL HYDROXYLATION OF CYCLOHEXYLCYCLOHEXANE: SYNTHESIS OF AN ANALOGUE OF LEUKOTRIENE-B_3

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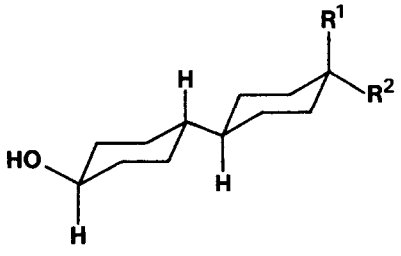
Summary: Cyclohexylcyclohexane was transformed into cyclohexylcyclohexanediols using selected fungi: 4,4'-cyclohexylcyclohexanediol was converted into an analogue of leukotriene-B_3.

Oxygenases are capable of functionalising formally non-activated carbon centres[1]. Except in the area of steroid research, little work has been done in the way of using these enzymes to provide useful synthons for the preparation of medicinally important compounds[2]. For some time we have been interested in the utilisation of whole-cell systems for the controlled hydroxylation of cyclic and polycyclic substrates; we report a quantitative investigation into the biohydroxylation of the readily available hydrocarbon cyclohexylcyclohexane[3].

Sixty-one cultures (including fungi, plant callus, streptomycetes and bacteria) were screened for the hydroxylation of cyclohexylcyclohexane. Four fungi, Beauvaria bassiana, Cunninghamella blakesleeana, Absidia cylindrospora and Gongronella lacrispora gave the most interesting results (Table). B. bassiana, C. blakesleeana and G. lacrispora gave a major polar product identical in retention time on g.c. to authentic 4,4'-e,e-cyclohexylcyclo-

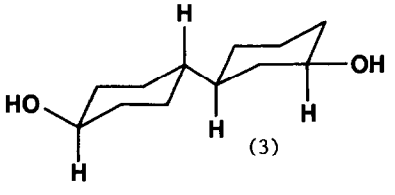


(1) R^1 = H, R^2 = OH

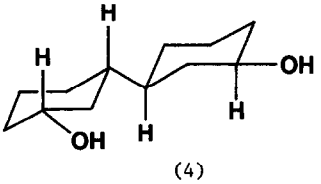
(2) R^1 = OH, R^2 = H

(5) R^1, R^2 = O

(6) R^1 = R^2 = H



(3)



(4)

hexanediol (1)[4]. Two or three other polar products (2)-(4) were observed for all three fungi. A. cylindrospora gave a relatively small amount of the 4,4'-e,e-diol and larger quantities of two other polar compounds.

Before scale up and full identification of the diols various parameters of the bioconversions were examined and the following observations were made:-

a) Apart from the differences in specificity mentioned above, the conversion time courses for B. bassiana, A. cylindrospora and C. blakesleeana were broadly similar. Diol products were not observed in the reaction mixture until approximately 24 h. After this period the diol concentration rose steadily to reach a maximum after 6 days, then after 9 days the concentration began to fall. In the later stages of the conversion, a product identical in retention time (g.c.) to authentic 4'-hydroxycyclohexylcyclohexan-4-one (5) was formed along with other similar materials which were probably positional isomers of the hydroxyketone.

b) The diol products were presumably formed via monohydroxy intermediates. Cyclohexylcyclohexan-4-ol (6) was readily converted into the 4,4'-e,e-diol (1) by C. blakesleeana with high efficiency (better than 20% conversion). Likewise conversion of the 4,4'-e,e-diol (1) into the 4,4'-hydroxyketone (5) could be demonstrated.

c) Optimum conversions to diol products were obtained using high biomass levels (with B. bassiana and C. blakesleeana diol production was proportional to biomass concentration up to ca. 40 g dry wt/l), glucose-free media (e.g. distilled water or spent broth), high pH (approx. 8) and moderate aeration.

d) Cyclohexylcyclohexane could be conveniently added to reaction mixtures using dimethylformamide (DMF) as co-solvent, provided the final DMF concentration did not exceed 2.7% v/v.

e) High substrate concentrations were found to inhibit diol formation by B. bassiana (optimum substrate concentration 1-2 g/l) but not by C. blakesleeana.

The conversion reactions were scaled up successfully to 50 litres or 500 litres giving yields similar to those shown in the Table at small scale. Scale up of the hydroxylation of cyclohexylcyclohexane (12 g; 40 litres) using B. bassiana gave, after a three-day fermentation, extraction and chromatography, 4,4'-e,e-cyclohexylcyclohexanediol (380 mg) identical with authentic material. A 500 litre (500 g) scale fermentation under the same conditions gave, after work-up and separation, 4,4'-e,e-cyclohexylcyclohexanediol (2.4 g) and 3,4'-e,e-cyclohexylcyclohexanediol (3) (1.8 g) identified by n.m.r. and mass spectroscopy. Similarly a 500 litre (500 g) scale conversion catalysed by A. cylindrospora gave the 3,4'-e,e-cyclohexylcyclohexanediol (3.6 g) and 3,3'-e,e-cyclohexylcyclohexanediol (4.7 g). The latter compound was identified by n.m.r. and mass spectroscopy and by the observation of optical activity $[\alpha]_D^{21}$ = +11.5, (c=2, methanol). Finally a 50 litre scale conversion catalysed by C. blakesleeana using 50 g of cyclohexylcyclohexane gave, after a three day fermentation process, 4,4'-e,e-diol (8g), and 3,4'-e,e-diol (3) (3.4 g) in the broth

representing a 19% conversion to these two diols.

While the organisms employed in the above large scale conversions usefully provided two major products for subsequent chemical modification, a more selective conversion of cyclohexylcyclohexane into the 4,4'-e,e-diol (1) was achieved using other Cunninghamella sp. strains recently isolated in our laboratories. Some of the latter strains were shown to give a 50% conversion of cyclohexylcyclohexane (1 g/l) into (1) in shake flask experiments. The diol (1) was used to prepare the carboxylic acid (7) [an interesting structural analogue of the highly chemotactic agent leukotriene B_3 (8)[5]] by the method outlined in the Scheme.

We have shown that a simple fermentation process can be used to prepare selected derivatives of a lipophilic hydrocarbon. The earlier observations of Fonken et al[3] have been extended considerably so that multigram quantities of three different diols were obtained. We are exploring the controlled hydroxylation of other polycyclic compounds.

Table: Transformation of Cyclohexylcyclohexane using Four Fungi[a]

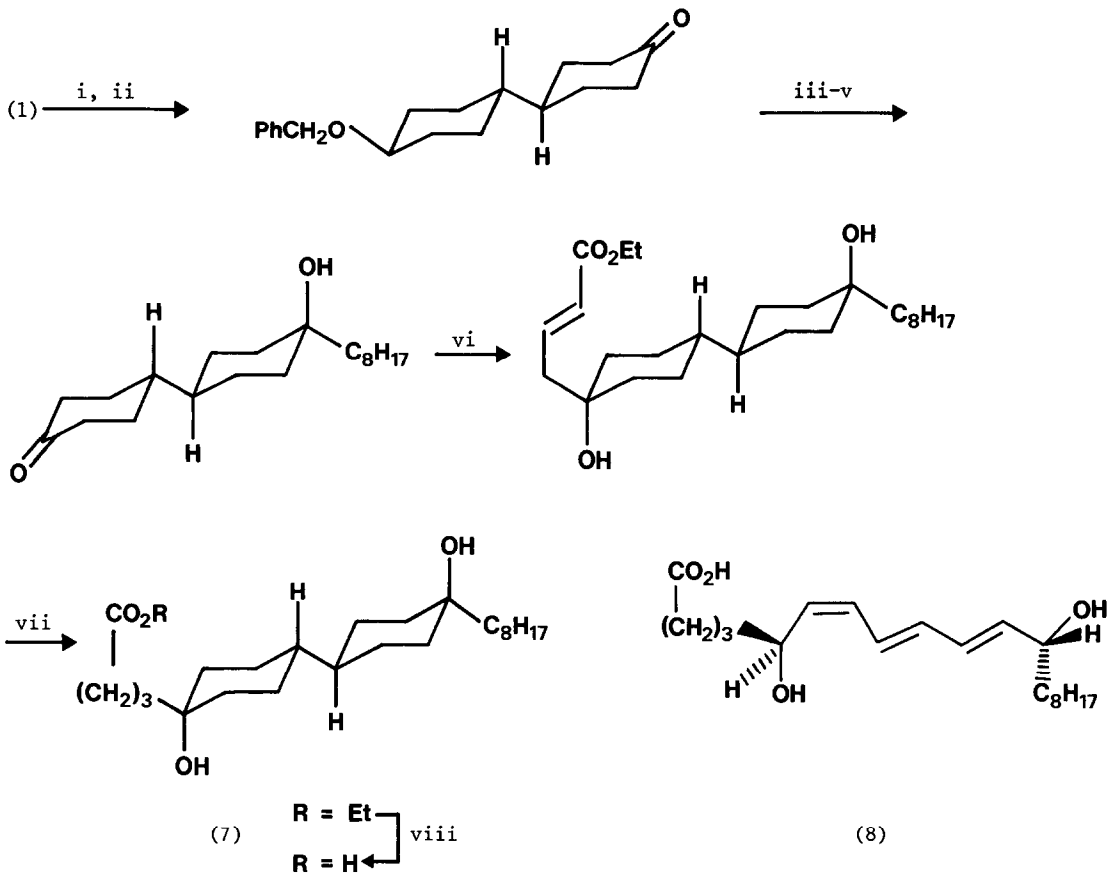
Organism	Product (cyclohexylcyclohexane-diol)	Concentration of product in broth (mg/l)[b]
A. cylindrospora C2589	4,4'-e,e-diol (1)	12
	4,4'-a,e-diol (2)[c]	3
	3,4'-e,e-diol (3)	43
	3,3'-e,e-diol (4)	ca 25[d]
B. bassiana C2592	4,4'-e,e-diol (1)	85
	4,4'-a,e-diol (2)	1
	3,4'-e,e-diol (3)	31
	3,3'-e,e-diol (4)	ca 5[d]
C. blakesleeana C1809	4,4'-e,e-diol (1)	151
	4,4'-a,e-diol (2)	15
	3,4'-e,e-diol (3)	60
	3,3'-e,e-diol (4)	ca 10[d]
G. lacrispora C2586	4,4'-e,e-diol (1)	132
	4,4'-a,e-diol (2)	68
	3,4'-e,e-diol (3)	92
	3,3'-e,e-diol (4)	not detected

[a] Conversions were carried out using growing cells and the growth medium was corn steep liquor/dextrose. Cyclohexylcyclohexane in DMF was added to a final concentration of 1 g/litre after two days growth of fungus.

[b] By g.c. analysis.

[c] Authentic compound available by route given in reference 4.

[d] Estimated from t.l.c.



Reagents: i, $PhCH_2Br$, NaH, toluene then chromatography; ii, Jones oxidation; iii, $C_8H_{17}Br$, Mg, Et_2O then chromatography; iv, H_2, Pd/C, EtOAc; v, pyridinium chlorochromate, NaOAc, CH_2Cl_2; vi, $BrCH_2CH{=}CHCO_2Et$, Zn/Cu, benzene; vii, H_2, Pd/C, EtOH; viii, NaOH, H_2O, CH_3OH.

SCHEME

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