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Letter

Preparation of substituted (*R*)-2-alkanols by microbial hydroxylation

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Abstract

Hydroxylation of alkanenitriles (C_6 to C_{10}) and of 2-octanone, 2-nonanone and 2-decanone by *Helminthosporium* species NRRL 4671 occurs predominantly at the (ω-1) position to give the corresponding (*R*)-2-alkanols in > 95% enantiomeric purity.

Keywords: Carbinol; Hydroxylation; *Helminthosporium*; NRRL 4671

Microbial hydroxylation of a prochiral methylene group to generate a chiral alcohol is a potentially powerful tool in synthetic organic chemistry, but its practical application has been largely limited to the modification of larger molecules such as steroids, terpenes and antibiotics [1]. The application of biocatalytic methods to the production of simple, low molecular weight chiral alcohols in the C_5 to C_{10} range has focused on the enantioselective hydrolysis of esters (e.g. [2]) or the reduction of carbonyl groups (e.g. [3–5]), the latter process normally generating carbinol according to Prelog's rule [6] as shown in Eq. (1). Most reductions operating in this manner afford the (*S*) configuration of product, although application of the C–I–P sequence rules can also result in assignment of the (*R*) descriptor to the product of a Prelog reduction [7].

(1)

S = small substituent, L = large substituent

Some isolated oxidoreductase enzymes are known to operate with anti-Prelog selectivity [8], and anti-Prelog microbial reduction by *Yarrowia lipolytica* of several methyl ketones to the corresponding (*R*) methyl carbinols occurs in good yield to give products of 48–100% e.e. [9]. In this communication we report the formation in high enantiomeric purity of cyano- and ketosubstituted (*R*)-methyl carbinols in the C_6 to C_{10} range by the direct hydroxylation of a methylene group using the fungus *Helminthosporium* species NRRL 4671. These products are those that would arise from anti-

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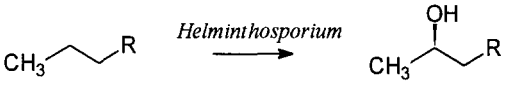
Prelog reduction of the corresponding ketones. The reaction is described in detail below for the preparation of (*R*)-9-hydroxy-2-decanone.

Helminthosporium species NRRL 4671, obtained from the USDepartment of Agriculture, Northern Regional Research Laboratories, Peoria, Ill, was maintained on 4% malt agar slopes, grown at 27°C and stored at 4°C. Two slopes of *Helminthosporium* species NRRL 4671 were used to inoculate 15 1-l Erlenmeyer flasks each containing 200 ml of an autoclaved medium composed of V-8 vegetable juice (200 ml) and calcium carbonate (3 g) per litre of distilled water, adjusted to pH 7.2 by the addition of 1 M sodium hydroxide prior to sterilization. The flasks were allowed to stand overnight at 27°C, then placed on a rotary shaker at 180 rpm, and growth continued for a further 72 h at 27°C. The fungus was then harvested by vacuum filtration (Büchner funnel), and resuspended in 15 1-l Erlenmeyer flasks each containing 200 ml of distilled water, resulting in ca. 90 g (wet weight) of mycelial growth per flask. 2-Decanone (1 g in 30 ml of 95% ethanol) was then distributed among the flasks, which were replaced on the rotary shaker at 180 rpm, 27°C for a further 72 h. The fungus and aqueous medium were then separated by filtration as before, the aqueous medium extracted with dichloromethane (continuous extraction, 72 h), and the fungus discarded. Concentration of the medium extract gave the crude product, which was submitted to flash chromatography using a benzene ether 10% stepwise gradient to give (*R*)-9-hydroxy-2-decanone (12%); oil; ^{1}H NMR δ 1.1 (2H, t), 1.2–1.7 (12H, m, including 3H, d), 2.1 (3H, s), 2.4 (2H, m) and 4.2 (1H, m) ppm; ^{13}C δ 23.9, 24.1, 25.8, 29.5, 29.6, 29.8, 39.6, 44.1, 68.4 and 109.0 ppm; MS *m/z* (%) 172(2), 96(31), 71(100); $[\alpha]_D$ −9.7 (c 2, $CHCl_3$). The yields and ee values quoted in Table 1 refer to isolated, purified, homogeneous material and arise from the combination of (only) homogeneous column fractions without further purification that could lead to changes in stereochemical enrichment values. All products were identified by a combination of NMR and mass spectral analysis, and enantiomeric purities determined by ^{1}H

Table 1
Hydroxylations by *Helminthosporium* species NRRL 4671



Substrate	Product	Yield (%)	$[\alpha]_D$ ($CHCl_3$)	Config.	E.E.(%)
Hexanenitrile	5-Hydroxyhexanenitrile [a]	10	−28.2	*R*	> 95
Heptanenitrile	6-Hydroxyheptanenitrile [b]	14	−10.8	*R*	90
Octanenitrile	7-Hydroxyoctanenitrile [c]	8	−8.6	*R*	> 95
Nonanenitrile	8-Hydroxynonanenitrile d	13	−9.4	*R*	> 95
Decanenitrile	9-Hydroxydecanenitrile [e]	19	−9.6	*R*	> 95
2-Octanone	7-Hydroxy-2-octanone [f]	14	−9.0	*R*	> 95
2-Nonanone	8-Hydroxy-2-nonanone [g]	12	−7.9	*R*	> 95
2-Decanone	9-Hydroxy-2-decanone [h]	12	−9.7	*R*	> 95

[a] Accompanied by 4-hydroxyhexanenitrile (0.4%).
[b] Accompanied by 5-hydroxyheptanenitrile (2%) and7-hydroxyheptanenitrile (3%).
[c] Accompanied by 6-hydroxyoctanenitrile (1%).
[d] Accompanied by 9-hydroxynonanenitrile (1%).
[e] Accompanied by 10-hydroxydecanenitrile (1%) and9-ketodecanenitrile (4%).
[f] Accompanied by 6-hydroxy-2-octanone (2%) and octane-2,7-dione (17%).
[g] Accompanied by 9-hydroxy-2-nonanone (1%), 7-hydroxy-2-nonanone (2%), and nonane-2,8-dione (1%).
[h] Accompanied by 10-hydroxy-2-decanone (1%) and decane-2,9-dione (1%).

NMR analysis of the appropriate C*H*OH resonance at 200 MHz in the presence of tris[3-(heptafluoropropyl hydroxymethylene)-D-camphorato] europium(III). Absolute configurations were assigned where possible by comparison of optical rotation values with those reported for samples of known configuration [10–12], and for other products by correlation of rotation and NMR shift parameters (in the presence of tris[3-(heptafluoropropyl hydroxymethylene)-D-camphorato] europium(III)) within the series. The NMR method was calibrated by the use of racemic standards generated by sodium borohydride reduction of the corresponding ketones. The results of other biotransformations performed in an identical manner are summarized in Table 1.

In most of the examples studied, the major product of the biotransformation is the (*R*)-configuration (ω-1) hydroxylation product. Although the isolated yields are not high, they are not optimized and the ready availability of the substrate, combined with the simplicity of the biotransformation procedure, makes this an attractive method for the preparation of those (*R*) methyl carbinols listed in Table 1. A further investigation of the hydroxylation of other terminally substituted alkanes by this procedure is currently under way.

Acknowledgements

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