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Asymmetric Reduction of Karahanaenone with Various Microorganisms

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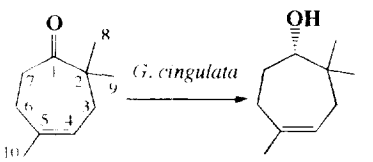
Abstract-Both enantiomers of karahanaenol have been prepared by microbial asymmetric reduction of karahanaenone. The (*S*)-enantiomer with high enantiomeric excess was obtained by *Glomerella cingulata*, *Aspergillus niger*, *Streptomyces aureofaciens* and *Bacillus subtilis* respectively. In contrast, the (*R*)-enantiomer of karahanaenol was obtained with *Fusarium solani*.

Karahanaenone is a monoterpene present in hop oil[1]. This unique and peculiar compound with its fragrance and flavour is one of the most important compounds for the flavouring industry[2]. Our main objective is to make a compound with a unique fragrance and flavour and which possesses a fruity and a woody odor. The reduction of karahanaenone by $NaBH_4$ has been reported[3]. Chemical and biological methods have been applied to the asymmetric reduction of karahanaenone into karahanaenol however the enantioselectivity was poor. In this paper, we report a novel method for reducing karahanaenone with various microorganisms into karahanaenol with excellent enantioselectivity.

We selected the following fungi *Aspergillus niger* IFO 4414, *Glomerella cingulata*[4], *Fusarium solani*[4] and *Rhizoctonia solani*[4], and the bacterium *Bacillus subtilis* IFO 3936, and actinomyces *Streptomyces aureofaciens* IFO 3187. All the microorganisms reduced karahanaenone during a period of 24hrs. The results are summarized as following in the table below

Table : Microbiological Reduction of karahanaenone

	$[\alpha]_D^{20}$	ee	Conf.	Yield
Glomerella cingulata	-33	100%	*S*	98%
Aspergillus niger	-33	100%	*S*	98%
Rhizoctonia solani	-28	70%	*S*	97%
Streptomyces aureofaciens	-33	100%	*S*	96%
Bacillus subtilis	-33	100%	*S*	98%
Fusarium solani	+28	70%	*R*	80%



karahanaenone (*S*)-karahanaenol

100% e.e.

The absolute configuration of the secondary alcohol was determined from esters obtained by the reaction of each karahanaenol (*S*)-(-) and (*R*)-(+)-(α)-MTPA chlorides according to Mosher's methods[5,6]. Enantiomerically pure karahanaenol has not been described in the literature before.

Both enantiomers of karahanaenol have a mint like odor, The (*S*)-enantiomer had a notably fresher odor than the (*R*).

Experimental

Reduction on analytical scale. Fungi case: Mycelia were respectively transplanted to the culture medium (15ml in a 50ml Petri dish) and furthermore incubated at 27°C under a static situation for 3days. After the growth of the fungus, the substrate was added directly to each medium (7.5mg/15ml) and further incubated at the same condition for 24h. Bacteria and Actinomyces case: Mycelia were then transplanted to the culture medium (100ml in a 200ml Erlenmeyer flask) and stirred for 3days at 28°C. After the growth of the fungus, the substrate was added directly to each medium (50mg/100ml) and were further incubated at the same condition for 24h. Nutrient broth for microbial transformation: *A. niger*, *F. solani*, *G. cingulata*: polypeptone 5g, KCl 0.5g, $MgSO_4 \cdot 7H_2O$ 0.01g, succharose 15g, glucose 15g, H_2O 1l. *R. solani*: glucose 50g, KNO_3 10g, K_2HPO_4 5g, $MgSO_4 \cdot 7H_2O$ 2.5g, $FeCl_3 \cdot 6H_2O$ 0.02g, H_2O 1l. *B. subtilis*: polypeptone 5g, Yeast extract 0.2g, $MgSO_4 \cdot 7H_2O$ 0.1g, H_2O 1l. *S. aureofaciens*: oatmeal 5g, H_2O 1l. Aliquots were withdrawing periodicaly and monitored by GLC on a chiral column : CHROMPACK WCOAT Fused silica Cp-Cyclodextrin-B-236-M-19(50m × 0.25mm , N_2 flow rate 1ml/min).

Reduction on preparative scale. Karahanenone was added to the resulting mycelia of the grown cell by a process similar to the analytical scale. After 24hrs, the reaction mixture was extracted with dichloromethane with a continuous liquid-liquid extractor and concentrated under reduced pressure. The products were purified by column chromatography (silica gel, hexane/ethyl acetate ratio) to give the enantiomerically pure alcohol. Optical rotations were measured on a Japan Spectroscopic CO. LTD DIP-140 in $CHCl_3$. The enantiomeric excesses are determined by GLC on chiral column [retention time (min) of alchohols : (1*S*)-13.7, (1*R*)-13.4].

Determination of absolute configuration of ( )-karahanaenol. [$\Delta\delta = \delta(-)$MTPA-$\delta(+)$MTPA ;(C1) proton; 4.88-4.91=-0.03, (C2) gem Methyl; 0.93-0.86=+0.07, 0.83-0.81=+0.02 (C4) proton; 5.35-5.34=+0.01, (C5) Methyl; 1.71-1.74=-0.03, (C6) proton; 2.08-2.13=-0.05, 1.95-2.01=-0.06].

References and Notes

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