Formation of the (*R*)- and (*S*)-Enantiomers of Ethyl 3-Hydroxybutanoate and of 1-(1,3-Dithian-2-yl)-2-hydroxypropane by Microbial Reduction

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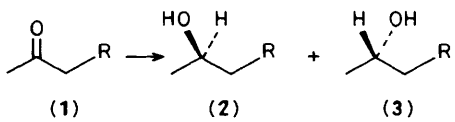
The (*R*) and (*S*) enantiomers of 3-hydroxybutanoate [(**2a**) or (**3a**)] and 1-(1,3-dithian-2-yl)-2-hydroxypropane [(**2b**) or (**3b**)] are obtained from ethyl 3-oxobutanoate (**1a**) and 1-(1,3-dithian-2-yl)-2-oxopropane (**1b**), respectively, using growing cultures from different strains of *Geotrichum candidum* and *Aspergillus niger*.

There is growing interest in small chiral molecules used as building blocks in the syntheses of natural products. Chiral building blocks are generally obtained from available natural products (sugars, amino-acids, hydroxyacids, *etc.*) or by microbial transformation of unconventional substrates. Both methods make available only one enantiomer, the antipode sometimes being reached only through tedious and expensive transformations, and this is a real limitation to the synthesis of natural products.

We now report the preferential reduction of ethyl 3-oxobutanoate (**1a**) to ethyl (*R*)-3-hydroxybutanoate (**2a**) or ethyl



(**1**) (**2**) (**3**)

a; R = CO_2Et

b; R = $\overline{SCH_2CH_2CH_2SCH}$

(*S*)-3-hydroxybutanoate (**3a**) using different strains of *Geotrichum candidum* and *Aspergillus niger*. Similarly when 1-(1,3-dithian-2-yl)-2-oxopropane (**1b**) was incubated with growing

Table 1. Results of the microbial reduction of (**1**).

Micro-organism	Substrate	Conversion[a] (%)	Enantiomers[b] (%)	
G.c.[c] CBS109.12	(**1a**)	96	(**2a**) 7	(**3a**)93
,,	(**1b**)	66	(**2b**)13	(**3b**)87
G.c. CBS233.76	(**1a**)	95	(**2a**)73	(**3a**)27
,,	(**1b**)	96	(**2b**)91	(**3b**) 9
A.n.[d] CBS102.12	(**1b**)	50	(**2b**)26	(**3b**)74
A.n. CBS108.47	(**1b**)	50	(**2b**)52	(**3b**)48
A.n. CBS134.54	(**1b**)	70	(**2b**)88	(**3b**)12
A.n. CBS626.66	(**1a**)	50	(**2a**)72	(**3a**)28
,,	(**1b**)	70	(**2b**)89	(**3b**)11
A.n. IPV283[e]	(**1a**)	98	(**2a**)75	(**3a**)25
,,	(**1b**)	95	(**2b**)92	(**3b**) 8

[a] Determined by g.l.c. analysis on a 2 m × 3 mm Pyrex column 5% SP100 on 100/120 Supelcoport: (**a**) 100—215 °C, 5 °C/min; (**b**) 210 °C. [b] Determined by g.l.c. analysis of their (+)-α-methoxy-α-trifluoromethylphenylacetates on a 50 m capillary column OV1: (**a**) 135—180 °C, 1 °C/min; (**b**) 170—210 °C, 1 °C/min. [c] G.c. = *Geotrichum candidum*. [d] A.n. = *Aspergillus niger*. [e] IPV = Istituto di Patologia Vegetale, Facoltà di Agraria, Università degli Studi di Milano.

cultures from different strains of *Geotrichum candidum* and *Aspergillus niger*, excess of (*R*)-1-(1,3-dithian-2-yl)-2-hydroxypropane (**2b**) or (*S*)-1-(1,3-dithian-2-yl)-2-hydroxypropane (**3b**)[1] was obtained.

A general procedure for microbial reduction is as follows. An alcoholic solution of the substrate (**1**) (50 mg) was added to a culture of the micro-organism (50 ml), which had been shaken for 3 days, and the mixture shaken for one further day. The resulting mixture was extracted twice with diethyl ether and the ether extract dried over sodium sulphate and the ether evaporated off. The composition of the crude extract was determined by g.l.c. analysis. The enantiomeric composition of the secondary alcohol produced was determined by the following procedure. The dried extract (*ca.* 1 mg) was added to a clear solution of (+)-α-methoxy-α-trifluoromelthylphenylacetyl chloride (5 mg)[2] in pyridine (0.1 ml) and tetrachloromethane (0.1 ml); after standing at room temperature overnight the samples were analysed by g.l.c. The results are summarized in Table 1.

To the best of our knowledge this is the first report of a microbial reduction of a ketone to an excess of the (*R*)- or (*S*)-enantiomer of a secondary alcohol, using growing cultures from different strains of the same micro-organism. There are a few reported examples of the microbial generation of secondary alcohols of opposite configuration but these use different micro-organisms on the same substrate,[3—5] or the same micro-organism on a modified substrate.[6]

Our results may be explained by the presence, in *Aspergillus niger* and *Geotrichum candidum*, of more than one oxidoreductase, which generate secondary alcohols of opposite configuration, but at different concentrations depending on the strain from which the culture is obtained.

The possibility of regulating the stereochemical course of microbial reduction by choosing the appropriate strain of a micro-organism could provide a method for the preparation of secondary alcohols of either (*R*) or (*S*) configuration.

It is noteworthy that the enantioselectivity is higher when (**2b**) is the preferred enantiomer from (**1b**), while baker's yeast,[1] *Hansenula anomala* (CBS110) and *Kloekera saturnus* (CBS5761), produce (**3b**) in more than 99% enantiomeric excess.

Received, 9th January 1984; Com. 033

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