Production of (*R*)-1-(1,3-Dithian-2-yl)propan-2-ol by Microbial Reduction

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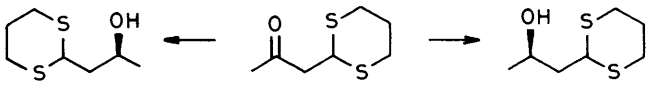
The reduction of several selected carbonyl compounds with growing cultures of *Streptomyces* sp., *Aspergillus niger*, and *Geotrichum candidum* has been studied. The production of (*R*)-1-(1,3-dithian-2-yl)propan-2-ol of high enantiomeric purity has been achieved by reduction of dithianylacetone with a *Streptomyces* sp.

The peculiarity of the dithianyl group makes the enantiomerically pure forms of 1-(1,3-dithian-2-yl)propan-2-ol (DHP) (**1**) and (**3**) the ideal substitutes for ethyl 3-hydroxybutanoates as four-carbon chiral building blocks in the syntheses of enantiomerically pure natural products. (*S*)-DHP (**1**) is readily obtained, from (1,3-dithian-2-yl)acetone (**2**) in high chemical yield, and >99% enantiomeric excess (e.e.), by reduction with fermenting Baker's yeast.[1] Ethyl (*S*)-3-hydroxybutanoate is obtained by Baker's yeast reduction of ethyl 3-oxobutanoate in *ca.* 50% chemical yield, and an e.e. never exceeding 95—97%.[2] Ethyl (*R*)-3-hydroxybutanoate is available by depolymerization of poly(hydroxybutanoate)[3] in high enantiomeric purity. Since the importance and utility of a chiral building block is greatly increased by the availability of both antipodes of high enantiomeric purity we studied the microbial reduction of dithianylacetone (**2**) in order to find micro-organisms able to produce (*R*)-DHP. We found[4] that (*R*)-DHP (**3**) of *ca.* 80% e.e. was produced by reduction with growing cultures of *Aspergillus niger* and *Geotrichum candidum*.



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

The search for better conditions allowed us to raise the e.e. of (*R*)-DHP, obtained by *A. niger*-mediated reduction, from 80 to 90% by increasing the substrate concentration, in the culture medium, from 1 to 4 mg ml^{-1} (4 mg ml^{-1} is the highest concentration possible without apparent formation of insoluble crystals of dithianylacetone). The (*R*)-DHP produced by reduction with *G. candidum* reaches its highest e.e. (90%) with a substrate concentration of 2 mg ml^{-1}. While working at increasing the enantiomeric purity of (*R*)-DHP produced by reduction performed by *A. niger* and *G. candidum*, we found a more promising strain of a *Streptomyces* species.[5] In fact (*R*)-DHP of 99% e.e. is obtained in satisfactory yield when dithianylacetone (**2**) is subjected to reduction with cultures of *Streptomyces* sp. (IPV 2645)† grown in the right conditions. Particularly important is the shaking rate: if growth and reduction are performed at 150 rev min^{-1} <50% of dithianylacetone is reduced in 24 h, and the e.e. of (*R*)-DHP is only 75%; if reduction is operated at 90 rev min^{-1} a 70% conversion and 99% e.e. are reached. The enantiomeric purity depends on dithianylacetone concentration as well; increasing the concentration from 1 to 2.5 mg ml^{-1} lowers the e.e. of (*R*)-DHP from 99 to 95%. Therefore we are able to obtain both enantiomers of DHP in enantiomeric pure form.

† IPV stands for Istituto di Patologia Vegetale, Facoltà di Agraria, Università degli Studi di Milano, from where the cultures are available.

Table. Absolute configuration and e.e. (%) of alcohols obtained by microbial reduction

Substrate	*Streptomyces* sp.[a]	*A. niger*[b]	*G. candidum*[c]	Baker's yeast
Dithianylacetone	*R*(99)	*R*(90)	*R*(90)	*S*(>99)[d]
Ethyl 3-oxobutanoate	*R*(85)	*R*(80)	*R*(89)[e]	*S*(96)[e]
t-Butyl 3-oxobutanoate	*R*(92)	*S*(48)	*S*(85)	*S*(77)[f]
2,2,2-Trifluoroacetophenone	*S*(84)	*S*(88)	*S*(92)	*R*(44)[g]
Ethyl 4-chloro-3-oxobutanoate	*S*(30)	*S*(8)	*R*(42)	*S*(55)[h]
(Phenylthio)acetone	(00)	*R*(16)	*R*(84)	*S*(94)[i]
(Phenylsulphonyl)acetone	*S*(72)		*S*(2)	*S*(94)[i]

[a] Culture IPV 2645. [b] Culture IPV 283. [c] Culture CBS 233.76. [d] Ref. 1. [e] Ref. 2. [f] M. Hirama, M. Shimizu, and M. Iwashita, *J. Chem. Soc., Chem. Commun.*, 1980, 587. [g] M. Bucciarelli, A. Forni, I. Moretti, and G. Torre, *J. Chem. Soc., Chem. Commun.*, 1978, 456. [h] B. Zhou, A. S. Gopalan, F. VanMiddlesworth, W. Shieh, and C. J. Sih, *J. Am. Chem. Soc.*, 1983, **105**, 5925. [i] S. Iriuchijima and N. Kojima, *Agric. Biol. Chem.*, 1978, **42**, 451.

In order to study the scope of this *Streptomyces* sp. (IPV 2645) we exposed it to several selected carbonyl compounds we had already found were reduced with *A. niger* and *G. candidum*. The results are listed in the Table, and are compared with the available data for reduction with Baker's yeast. Except for reduction of dithianylacetone the conditions were not optimized.

Analysis of the data reported in the Table shows that each micro-organism interacts in a different way with each substrate. This may be explained by the presence, in each micro-organism, of several oxidoreductases,[6] which generate secondary alcohols of opposite configuration. The configuration and the enantiomeric purity of the alcohol obtained depend on how many, and which, oxidoreductases operate on the carbonyl compound.

Noteworthy are the behaviour of ethyl and t-butyl 3-oxobutanoates: they are reduced to (*R*)- and (*S*)-3-hydroxybutanoate, respectively, by *Streptomyces* sp., and by fermenting Baker's yeast, with only small differences in the enantiomeric purity, while *A. niger* and *G. candidum* give an excess of the opposite enantiomers on changing from ethyl to t-butyl esters.

Our best success is represented by the production of (*R*)-DHP which is now available in gram quantity, but other interesting results are reported in the Table. Noteworthy is the action of *G. candidum* on trifluoroacetophenone and (phenylthio)acetone; in both cases the alcohols antipodal to those produced by Baker's yeast are obtained in good enantiomeric purity.

The alcohols isolated from the bioconversions have gas chromatography R_t values and i.r. and n.m.r. spectra identical with those of authentic samples. The enantiomeric composition of the alcohols was determined by esterification with (*S*)-1-phenylethyl isocyanate in the case of ethyl 4-chloro-3-hydroxybutanoate, (phenylthio)propan-2-ol, and (phenylsulphonyl)propan-2-ol, and with (+)-methoxy(trifluoromethyl)phenylacetyl chloride in all other cases, and comparing such derivatives with authentic samples by means of g.c. analysis.

Experimental

^{1}H N.m.r. spectra were recorded on a Varian XL 100/15 instrument in $CDCl_3$ with Me_4Si as internal standard. I.r. spectra were recorded on a Perkin-Elmer 137 Infracord spectrophotometer. Optical rotations were measured in a 1-dm cell on a Jasco DIP-181 polarimeter.

G.c. analyses were performed as follows: column A—25 m × 0.32 mm i.d. glass capillary column, coated with UC-W 982 (d_f 0.4 μm), using a C. Erba apparatus mod. 4160, and on-column injection system; carrier gas H_2, $\bar{u}$ 50 cm s^{-1}. Column B—25 m × 0.25 mm i.d. fused silica capillary column, coated with OV-1 (d_f 0.25 μm), using a Dani apparatus mod. 6500, and PTV injection system; carrier gas H_2, $\bar{u}$ 50 cm s^{-1}. Column C—2 m × 3 mm i.d. glass column, packed with 5% SP 1000 on 100/120 Supelcoport, using a Dani 3800 apparatus, and N_2 as carrier gas. R_t are in min.

The strains of *A. niger* (IPV 283), *G. candidum* (CBS 233.76), and *Streptomyces* sp. (IPV 2645) used for this study were maintained on malt–agar slopes.

General Procedure for Microbial Reduction.—Each microorganism was grown for the given time at 30 °C in shaken Erlenmeyer flasks (300 ml) containing the given culture medium (50 ml). The carbonyl compound (in standard procedure, 50 mg per flask), dissolved in ethyl alcohol (1 ml) or in dimethyl sulphoxide (0.5 ml), was added to the grown culture, and the incubation was continued for one further day. The resulting mixture was extracted twice with diethyl ether, the combined extracts were washed successively with aqueous sodium hydrogen carbonate and with aqueous sodium chloride, and were dried over sodium sulphate, and the ether was evaporated off. The composition of the crude residue was determined by g.c. analysis. For the determination of the enantiomeric composition of the secondary alcohol produced, the dried extract (*ca.* 1 mg) was added to a solution of (*S*)-1-phenylethyl isocyanate (5 mg) in toluene (0.1 ml) (the corresponding derivatives will be called PEC derivatives), or to a clear solution of (+)-methoxy (trifluoromethyl)phenylacetyl chloride (5 mg) in pyridine (0.1 ml) and tetrachloromethane (0.1 ml) (the corresponding derivatives will be called MTPA derivatives); after being kept at room temperature overnight the samples were analysed by g.c. Each reduction was performed, on two flasks, at least twice.

A. niger (IPV 283) was grown for 2 days on Czapek-Dox medium at 150 rev min^{-1}. *G. candidum* (CBS 233.76) was grown for 3 days at 150 rev min^{-1} on a medium containing glucose (50 g l^{-1}), yeast extract (10 g l^{-1}), and peptone (10 g l^{-1}) in deionized water, and adjusted to pH 7. *Streptomyces* sp. (IPV 2645) was grown for 1 day at 90 rev min^{-1} on a medium containing glucose (30 g l^{-1}), malt extract (10 g l^{-1}), and yeast extract (10 g l^{-1}) in deionized water, and adjusted to pH 7.

Production of (R)-1-(1,3-*Dithian*-2-*yl*)*propan*-2-*ol* (**3**).—A solution of (1,3-dithian-2-yl)acetone (**2**) (1.0 g) in dimethyl sulphoxide (5 ml) was distributed into 20 Erlenmeyer flasks, and was reduced and worked-up as described in the general procedure. Flash-chromatographic separation of the crude extract with hexane–ethyl acetate (60:40, v/v) yielded unchanged dithianylacetone (305 mg) and (*R*)-DHP (642 mg). The alcohol was 99% pure on g.c. The enantiomeric purity was 99%. $[\alpha]_D^{20}$ −24.8° (*c* 1 in chloroform).

Gas-chromatographic Analysis.—Dithianylacetone: the reduction products were analysed on column A, 4 min at 110 °C, then 1 °C min^{-1} to 130 °C; the MTPA derivatives were analysed on column A, 4 min at 170 °C, then 1 °C min^{-1} to 220 °C (R_t 33.0, 33.5), and column B, 1 min at 100 °C, 20 °C min^{-1} to 170 °C, 2 min at 170 °C, and finally 1 °C min^{-1} to 210 °C (R_t 28.0, 28.5). The MTPA derivative of (*S*)-DHP was eluted first.

Ethyl 3-oxobutanoate: the reduction products were analysed on column C, 4 min at 100 °C, then 5 °C min^{-1} to 215 °C; the MTPA derivatives were analysed on column A, 4 min at 120 °C, then 1 °C min^{-1} to 160 °C (R_t 33.4, 33.8). The MTPA derivative of ethyl (*R*)-3-hydroxybutanoate was eluted first.

t-Butyl 3-oxobutanoate: the reduction products were analysed on column C, 4 min at 110 °C, then 5 °C min^{-1} to 215 °C; the MTPA derivatives were analysed on column A, 4 min at 130 °C, then 1 °C min^{-1} to 170 °C (R_t 31.9, 32.2), and column B, 2 min at 80 °C, 20 °C min^{-1} to 135 °C, 2 min at 135 °C, and finally 1.5 °C min^{-1} to 180 °C (R_t 22.6, 22.8). The MTPA derivative of t-butyl (*R*)-3-hydroxybutanoate was eluted first.

2,2,2-Trifluoroacetophenone: the reduction products were analysed on column A, 4 min at 60 °C, then 1 °C min^{-1} to 75 °C; the MTPA derivatives were analysed on column A, 4 min at 120 °C, then 1 °C min^{-1} to 155 °C (R_t 26.0, 28.4). The MTPA derivative of (*R*)-1-phenyl-2,2,2-trifluoroethanol was eluted first.

Ethyl 4-chloro-3-oxobutanoate: the reduction products were analysed on column A, 4 min at 60 °C, then 2 °C min^{-1} to 100 °C; the PEC derivatives were analysed on column A, 4 min at 160 °C, then 1 °C min^{-1} to 200 °C (R_t 30.3, 30.8), and column B, 1 min at 80 °C, 20 °C min^{-1} to 160 °C, 2 min at 160 °C, and finally 1 °C min^{-1} to 190 °C (R_t 26.4, 26.9). The PEC derivative of ethyl (*S*)-4-chloro-3-hydroxybutanoate was eluted first.

(Phenylthio)acetone: the reduction products were analysed on column A, 4 min at 110 °C, then 1 °C min^{-1} to 130 °C; the PEC derivatives were analysed on column A, 4 min at 170 °C, then 1.5 °C min^{-1} to 210 °C (R_t 26.6, 27.7), and column B, 1 min at 130 °C, 20 °C min^{-1} to 170 °C, 2 min at 170 °C, and finally 1.5 °C min^{-1} to 220 °C (R_t 28.3, 29.4). The PEC derivative of (*R*)-1-(phenylthio)propan-2-ol was eluted first.

(Phenylsulphonyl)acetone: the reduction products were analysed on column A, 4 min at 130 °C, then 1 °C min^{-1} to 150 °C; the PEC derivatives were analysed on column A, 4 min at 180 °C, then 2.5 °C min^{-1} to 220 °C (R_t 29.4, 30.2), and column B, 1 min at 100 °C, 20 °C min^{-1} to 170 °C, 2 min at 170 °C, and finally 2 °C min^{-1} to 230 °C (R_t 31.6, 32.5). The PEC derivative of (*R*)-1-(phenylsulphonyl)propan-2-ol was eluted first.

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