Reduction of Carboxylates to Alkanols catalysed by *Colletotrichum gloeosporoides*

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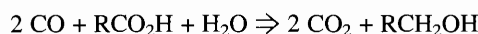
Sodium carboxylates (C_6–C_{10}) are effectively reduced to the corresponding alcohols by growing cultures of *Colletotricum gloeosporoides* in a preparatively useful way; intermediacy of the aldehydes is established by deuterium incorporation experiments; the unusual reducing capacity is proposed as an useful procedure for the preparation of alcohols of value as aroma components.

Applications of enzymes as specific and chiral catalysts in synthetic organic chemistry are now well established, with utilization of oxidoreductases for C=X ⇒ CHXH conversions being one of the best documented areas of their exploitation.[1–3]

However, very few examples of enzymic reduction of carboxylic acids in a non-activated form in aqueous solution to alcohols have been reported, generally related to the inactivation of xenobiotics.[4] The most notable example[5,6] regards *Clostridium thermoaceticum* and *Clostridium formicoaceticum*, which are able to perform the reduction of a variety of carboxylic acids *via* an intermediary aldehyde at the expense of formate or even more effectively with carbon monoxide. In the latter case the reaction proceeds according to eqn. (1).



$$2\,CO + RCO_2H + H_2O \Rightarrow 2\,CO_2 + RCH_2OH$$

This operation however is not a natural function of the enzymatic machinery of the organism, requiring instead artificial cofactors for it to take place.

The reduction of the carboxyl function in microorganisms is an endogenous function which proceeds *via* the formation of activated thioesters of relatively low redox potential. While the reduction of acetyl-S-CoA to acetaldehyde requires −0.41 V, the corresponding reduction of acetate to acetaldehyde has a redox potential of −0.60 V.[7] The cofactors of common reductases have a redox potential of −0.320 V, large enough for the reduction of aldehydes or ketones ($E_0 \approx 0.1$–0.2 V). However the reduction of natural carboxylates to alcohols is seldom observed since they are preferentially degraded to C_2 units for energy supply. *Clostridium acetobutylicum* however reduces butyrate to *n*-butanol in acetone–butanol fermentation. Since *C. acetobutylicum* belongs to the strictly anaerobic genus *Clostridium* it has enormous reduction capacities. Application in biocatalysis is however restricted owing to the fact that the enzyme production in terms of space/time yield is very low.

In this context it is of interest that C_6–C_{10} natural fatty acids added as their sodium salts to a growing culture of the fungus *Colletotrichum gloeosporoides* CBS 193.32 are effectively directly reduced to the corresponding alcohols in high yields. The compounds employed are given in Fig. 1.

Thus carboxylic acids **1–6** are reduced to the corresponding alcohols on 24 h incubation in 70% yields at concentrations up to 2 g l^{-1}.† Products of β-oxidation, the main metabolic operation active on this type of compound in microbial metabolism, is only observed with **3** as a substrate with heptan-2-one, a typical β-oxidation product being obtained. 2-Methyl hexanoic acid **2** is reduced by *C. gloeosporoides* to 2-methylhexan-1-ol with an efficiency comparable with that of hexanoic acid. However, the mode of the enzymic reduction is not sensitive to the stereochemistry of the carbon atom at position 2. Indeed, multidimensional GLC analysis of 2-methylhexan-1-ol obtained from 2-methylhexanoic acid at *ca.* 50% conversion and comparison with a sample of racemic

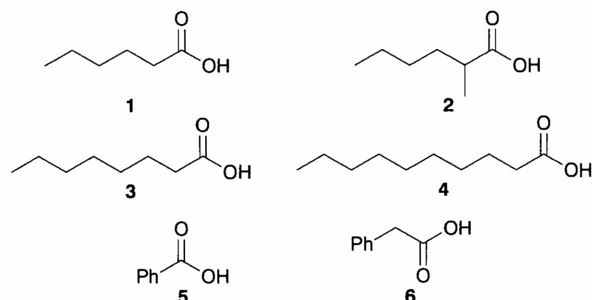


Fig. 1

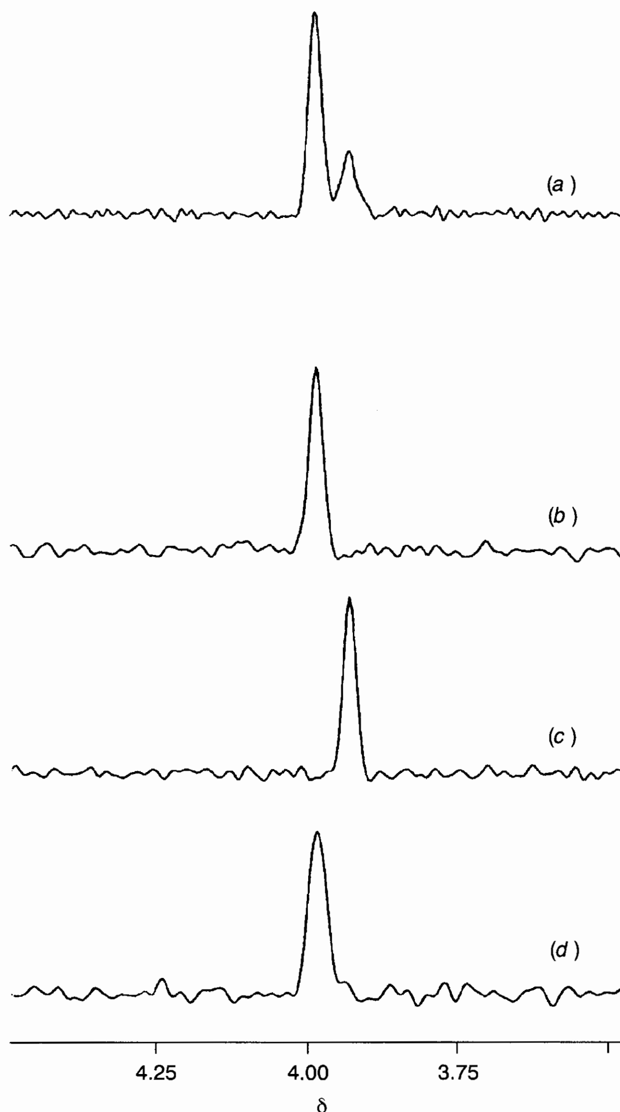
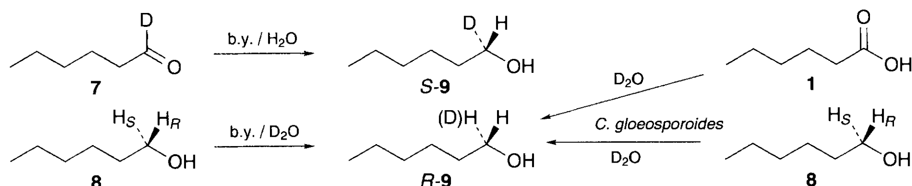


Fig. 2 ^{2}H NMR spectra of *S*- and *R*-**9** from different origin. (*a*) Incubation of **1** in D_2O in the presence of baker's yeast (b.y.); (*b*) incubation of **8** in D_2O; (*c*) incubation of **7** with b.y.; (*d*) incubation of **8** in D_2O in the presence of b.y.



Scheme 1

2-methylhexanol indicated no enantiomeric enrichment. While the fact that benzoic and phenylacetic acid are not utilized as a carbon source, being only reduced to the corresponding primary alcohol, is not surprising since they can be considered as end products in their metabolic pathway, the reduction of the mid-chain fatty acids to the corresponding alcohols is unexpected.

In order to gain an insight into the mechanism of the reduction, biotransformation of hexanal in D_2O and deuteriated hexanal in H_2O were effected with *C. gloeosporoides* and baker's yeast (b.y.) affording the deuteriated alcohols **9**. The high-resolution ^2H NMR spectra of the corresponding (+)-MTPA esters were examined. The deuterium spectra (61 MHz) were performed under proton broad-band decoupling to remove all heteronuclear coupling constants and reduce the linewidth of deuterium signals. The resulting labelled (*R*)- and (*S*)-[1-^{2}H]-hexan-1-ols were assigned in the following way:[8] [1-^{2}H]-hexan-1-al **7** was incubated with b.y. giving as expected the carbinol of (*S*) configuration [(*S*)-**9**, Fig. 2(*c*), deuterium signal at δ 3.92], whereas the (*R*)-alcohol [(*R*)-**9**] was obtained by incubation of hexan-1-ol **8** in D_2O in the presence of yeast [Fig. 2(*d*) deuterium signal at δ 3.98]. In the first case the chiral carbinol is produced as a consequence of an enantioselective hydride addition onto the aldehydic carbon (Scheme 1).

The (*R*)-carbinol is instead obtained after the enantiospecific D^- equivalent delivery from NAD^{2}H formed *in situ* by the known exchange reaction with the medium mediated by lipoyl dehydrogenase (EC 1.5.4.3) of yeast.[9] A parallel experiment was similarly performed with *C. gloeosporoides*. Incubation of **8** in D_2O gives enantiomerically pure (*R*)-**9** [Fig. 2(*b*)] in perfect analogy with the results obtained in b.y. incubation, supporting an identical mechanism. From the reduction of the carboxylic acid, a compound containing deuterium at both positions with a ratio H_R over H_S of 3 : 1 is obtained [Fig. 2(*a*)]. These results indicate that while H_R is introduced in the reduction of the hypothetic intermediate aldehyde, H_S must derive from a direct reduction of the carboxylate mediated by the same deuteriated cofactor active in the aldehyde reduction. The reason for the incomplete deuteriation of H_S of (*R*)-**9** is due to the fact that what we obtain in the first step of the reduction is the product of a kinetically controlled reaction.

The significance of this rare catalytic activity is enhanced by the preparative value of the bioreduction leading to a group of natural alkanols useful as aroma components as alternative to extraction from botanical sources where they are present at trace levels. The products obtained in this procedure are *natural* by any means and legislation, thus deserving consumer preference.[10]

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Footnote

† The microorganism was grown on a CZYE medium (Biolife) in a shaken flask at 30 °C for 4 h. After this time the sodium carboxylate dissolved in a minimum amount of water was added and the flask was shaken for additional 24 h. The mixture was then extracted with CH_2Cl_2 and analysed by GC using heptan-1-ol as internal standard.

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