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TETRAHEDRON: *ASYMMETRY*

Baker's yeast mediated biohydrogenation of unsaturated compounds containing a methylene group: enantioselective preparation of 2-methyl alkanols from 2-substituted acrolein acetals

Patrizia Ferraboschi,[a] Shahrzad Reza-Elahi,[a] Elisa Verza [a] and Enzo Santaniello [b,*]

[a]*Dipartmento di Chimica e Biochimica Medica, Università degli Studi di Milano, Italy*
[b]*Dipartmento di Scienze Precliniche LITA Vialba, Università degli Studi di Milano, Italy*

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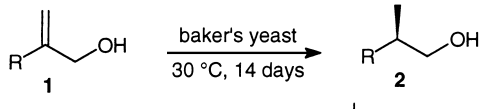
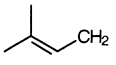
Abstract

The baker's yeast mediated biohydrogenation of unsaturated compounds containing a methylene group may constitute an enantioselective biocatalytic approach to the preparation of 2-methyl-1-alkanols, as exemplified by the reduction of the compounds **8a**–**d** to 90–98% enantiomerically pure alcohols **2a**–**d**.

Among biocatalysts available for organic synthesis,[1] a special role has been recognized for microorganisms that may be regarded as readily available reagents for the preparation of enantiomerically pure compounds.[2] Baker's yeast (*Saccharomyces cerevisiae*) is well suited to this kind of application, since it is commonly accessible and its utilization does not require any special care or skill in microbiology.[3] The reducing capabilities of baker's yeast have been extensively exploited, the biohydrogenation of double bonds constituting an interesting reaction that may proceed with high enantioselectivity.[4] In this context, we have recently shown that the baker's yeast biohydrogenation of 2-substituted allyl alcohols **1** can be used for the preparation of alcohols **2** of high enantiomeric excess (ee).[5]



a. R = $PhCH_2$ b. R = 

c. R = $PhCH_2OCH_2$ d. R = Ph

The above results could be considered as a special example of the recognized capability of baker's yeast to mediate the biohydrogenation of other unsaturated alcohols such as compounds **3** to the corresponding

* Corresponding author. E-mail: enzo.santaniello@unimi.it

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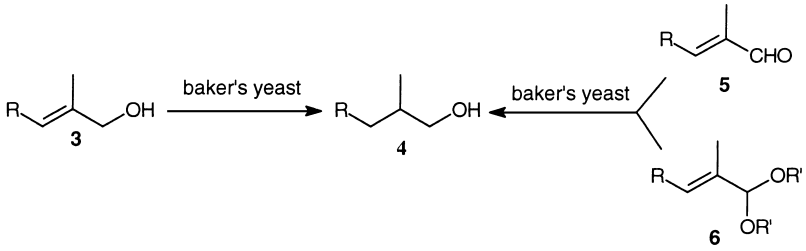
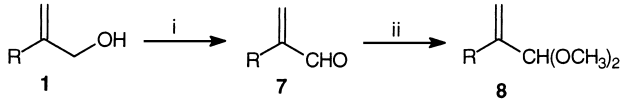
saturated alcohols **4**.[6] It has been proposed that the real substrate for the addition of hydrogens is the aldehyde **5** that can be also used as the substrate of the biohydrogenation,[7] although sometimes a possible inhibition of the enzymatic system(s) by the latter compounds may occur.[8]



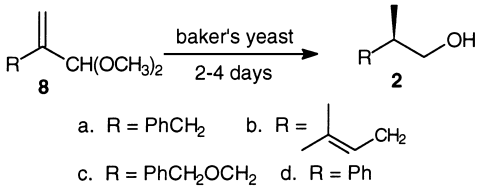
A controlled release of aldehyde **5** into the reaction medium can be achieved using unsaturated acetals **6** as substrates, since these compounds are slowly hydrolyzed during the fermentation of the yeast.[9] In this way a convenient biohydrogenation to the final saturated alcohol **4** can be realized. Starting from all the above considerations, we have extended our preliminary observations on the reduction of 2-substituted allyl alcohols **1a**–**c**[5] and planned to use the unsaturated aldehydes **7** or the corresponding dimethyl acetals **8**[10] as substrates for the baker's yeast mediated biohydrogenation.



Experimental conditions: i. $MnO_2/CHCl_3$, r.t., 16h; ii. $CH(OCH_3)_3/CH_3OH$, NH_4Cl, reflux, overnight.

When aldehyde **7** (R=$PhCH_2$) was the substrate, with a low yeast/substrate ratio (5 g/mmol) only the unsaturated alcohol **1a** was obtained. Using a 20 g/mmol ratio, modest yields of the nearly enantiomerically pure 2-methyl alkanols **2a** were obtained (20% yield, 98% ee) but a slow addition of an ethanolic solution of the substrate was required (96 h). The product of the biohydrogenation was accompanied by 10% of unsaturated alcohol **1a**. Similar results were also obtained from the aldehydes **7b**,**c**. For the acetals **8a**–**d** a few experimental conditions were tested to establish the most satisfactory protocol in terms of yields of saturated alcohol and enantioselectivity. A yeast/substrate ratio of 20 g/mmol gave the highest yield (60–83%) in the biohydrogenation products and minimized the formation of unsaturated alcohols. For **8a** and **8b** a ratio of 95/5 and 82/18 of saturated **2a**,**b**/unsaturated **1a**,**b** was obtained, whereas only traces of the unsaturated alcohol **1c** were formed from **8c**. In the above incubation conditions, the formation of the saturated acids was minimized to about 5% in every case.[11]

The best experimental protocol consisted of the sequential addition of 5–6 portions of substrate to the fixed amount of yeast at 5–10 hour intervals.[12] As for the bioreduction of the unsaturated alcohols **1a**–**c**,[5] the configurations were established as *R* for the alcohols obtained from substrates **8a**,**b** and *S* for the alcohol **1c**.[13] Furthermore, the alcohol **2b** showed a 90% ee and nearly enantiomerically pure **2a** and **2c** (>98% ees) could be prepared in this way.[14]



The most interesting result was obtained from the acetal **8d** that yielded in 48 hours the (*R*)-alcohol **2d** in 82% yield and >98% ee[15] and only 2.5% of the unsaturated alcohol **1d**. In this case, the slow release of the intermediate aldehyde circumvented the problems associated with the direct biohydrogenation of

the unsaturated alcohol **1d** that could not be transformed into the corresponding saturated alkanol **2d**.[5a] It should also be noted that compound **2d** could not be obtained in an enantiomerically pure form by the enzymatic resolution of (*RS*)-**1d**. In fact, the *Pseudomonas cepacia* lipase-catalyzed transesterification afforded a nearly racemic product under the same conditions that allowed a highly enantioselective resolution of a long series of similar 2-methyl-1-alkanols.[16] In conclusion, we have shown that substrates containing a methylene moiety such as the acetals **8a**–**c** are nicely biohydrogenated to the corresponding saturated compounds, i.e. 2-methyl alkanols **2a**–**c**, faster than the alcohols (2–4 versus 14 days). Finally, results from the acetal **8d** show that the biotransformation may be successful even in the case where the corresponding alcohol fails to react.

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10. The alcohols **1a** and **1b** were prepared according to Ref. 5a; the synthesis of alcohol **1c** was carried out as described in Ref. 5b. Oxidation to the aldehydes **7a**–**c** with MnO_2 in chloroform at room temperature (16 h) gave 55–65% of isolated products. Reaction of the aldehydes with trimethyl orthoformate in methanol in the presence of NH_4Cl at reflux overnight afforded the acetals **8a**–**c** (73–83% of isolated products).
11. The biohydrogenation of aldehydes **7** and acetals **8** constantly afforded variable amounts of the unsaturated alcohols **1** that are the products of bioreduction of the substrates and require longer times (14 days, see Ref. 5a) for their biohydrogenation to the alcohols **2**. Prolonged incubation with fermenting baker's yeast leads also to the formation of the saturated acids. This oxidative mechanism of baker's yeast has already been observed for other substrates, see: Sato, T.; Hanayama, K.; Fujisawa, T. *Tetrahedron Lett.* **1988**, *29*, 2197–2200.
12. Typically, to a solution of sucrose (15.4 g) in water (280 ml) baker's yeast (31 g) was added. The suspension was kept at 30°C, under vigorous stirring (0.5 h), then a solution of **8a** (0.3 g, 1.56 mmol) in ethanol (3 ml) was added over 3 days (five additions). The reaction progress was monitored by GLC (HP-5, oven temperature 130°C). The mixture was filtered through a Celite pad; the aqueous solution was extracted with diethyl ether (3×100 ml) and after usual work-up, the alcohol **2a** (0.14 g, 60%) was obtained from silica gel column chromatography (1/20) by elution with hexane/ethyl acetate (7/3).
13. The stereochemical outcome is the same for all three substrates, the difference in the configuration being only a consequence of the priorities of the groups.

14. The enantiomeric excess of the alcohols **2a**–**c** was established by ^{1}H NMR (500 MHz) analysis of the corresponding (*R*)-MTPA esters, comparing the resonances of the MTPA ester from the (*RS*)-alcohol and the same derivative of the products from the biotransformation, as described in Ref. 5a and b.
15. The *R* configuration of (+)-alcohol **2d** was established by comparison with the published values of specific rotation: Suzuki, K.; Katayama, E.; Matsumoto, T.; Tsuchihashi, G. *Tetrahedron Lett.* **1984**, *25*, 3715–3718. The ee was determined by ^{1}H NMR (500 MHz) analysis of (*R*)-MTPA esters of (*RS*)-**2d** (obtained by $NaBH_4$ reduction of 2-propionaldehyde) and of (*R*)-**2d**. Only the decoupling technique allowed a significant spectrum to be obtained: by irradiation of methyl group signal (a doublet centered at 1.268 ppm) of the (*RS*)-derivative the proton at C-2 showed two triplets centered at 3.157 and 3.174 ppm. In the same derivative from the (*R*)-alcohol **2d** only the triplet at 3.157 was present.
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