

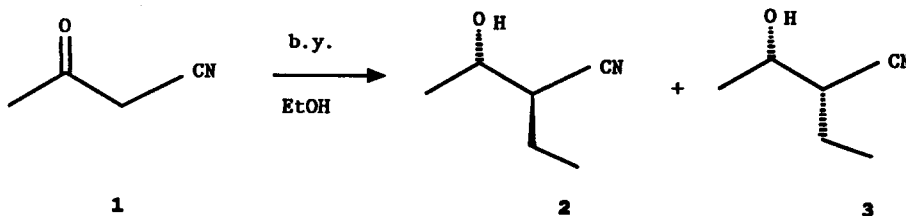
CHEMO-ENZYMATIC ALKYLATION OF ACTIVE METHYLENE COMPOUNDS

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Summary: Active methylene compounds undergo chemical condensation in water with aldehydes derived from yeast oxidation of the corresponding alcohols. Unsaturated compounds so obtained are then further reduced by yeast.

The use of baker's yeast (b.y.) as a reagent in organic synthesis has received much attention during the last decade.¹ Since the complex enzymatic system of yeast can catalyze many different reactions, more and more attention is devoted to transformations which are substrate specific, and this specificity is mainly achieved by selection of an appropriate starting material. By submitting new non conventional substrates to fermenting yeast, novel catalytic abilities are then encountered.^{2, 3} Among the various classes of reaction capabilities displayed by yeast, particularly attractive are reports on the b.y. catalyzed C-C bond formation, due to the general interest for this fundamental reaction in organic chemistry and to the scarcity of examples of this type of reactions in biocatalysis in general.^{4, 5} Among the multiple activities of b.y., three C-C bond forming reactions have been reported so far: acyloin condensation,⁶ sterol cyclization,⁷ and Michael type addition.⁸

Recently starting from cyanoacetone **1** in fermenting yeast, the two stereoisomeric alkylated products **2** and **3** have been obtained in high ee

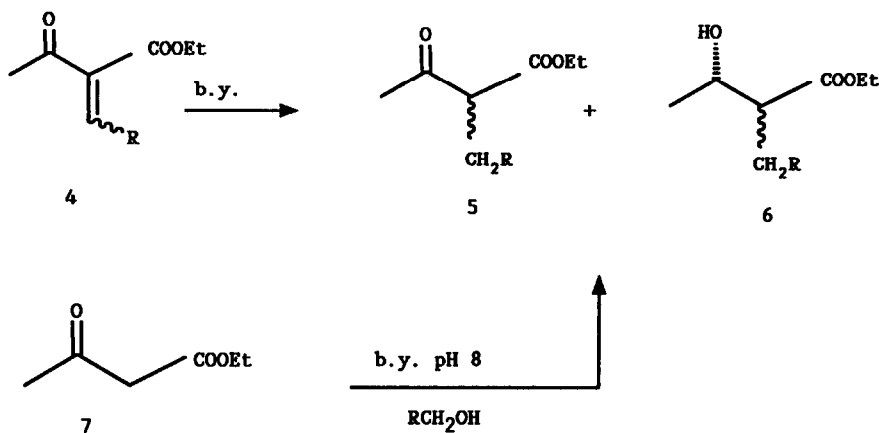


Scheme 1

(Scheme 1), and the results has been regarded by the Authors as the consequence of a novel C-C bond forming reaction catalyzed by b.y.⁹

We wish to report our own experimental observations on this subject, leading eventually to propose a mechanism for the reaction in Scheme 1.

We first examined the behaviour of compounds 4a,b prepared from 3-oxobutyrates and the corresponding aldehydes. B.y. reduction afforded as expected¹⁰ the mixture of the two diastereoisomeric β -hydroxybutanoates 6 in high optical purity (>97% ee) as determined by comparison of the MTPA esters obtained from 6 and from *rac* 6.¹¹ (Scheme 2). The isolation of the saturated ketone 5 in nearly racemic form at low conversions is similar



a R = Me

b R = Et

Scheme 2

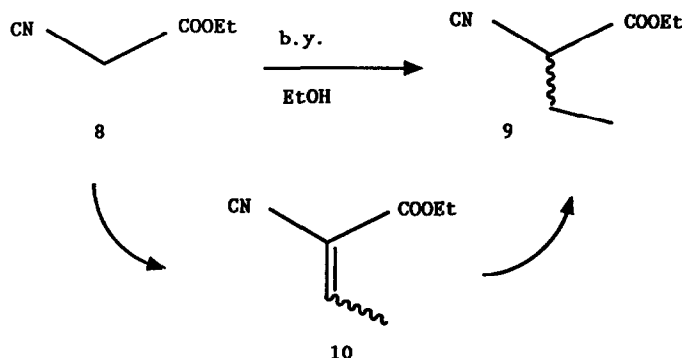
to the results obtained in the reported reaction of cyanoacetone. Since it is generally considered that the enantioselectivity of the b.y. reduction of prochiral trisubstituted double bonds is affected from the *E:Z* ratio of the starting alkene, the low *de* observed is expected.¹² Moreover, since saturation of the double bond occurs prior to carbonyl reduction, the intermediate α -substituted β -ketoesters are prone to undergo epimerization. Formation of the condensation product 4 was equally observed when ethyl acetoacetate was reacted with aldehydes in water in the presence of Na_2HPO_4 at pH 8.

We then tried to reproduce the formation of the alkylated product in the following conditions: ethyl β -ketobutyrate 7 was treated with b.y.

at pH 8 due to Na_2HPO_4 , with 5% EtOH or n-PrOH during 48 hours at 30 °C. As expected compounds 6a-b respectively could be detected by GC/MS, although only in trace amounts, due to the strongly competing reduction of the carbonyl group.¹³ Peaks corresponding to the two diastereoisomeric hydroxy esters 6a were also detected in the gaschromatographic trace of the mixture obtained according to the procedure described in *Organic Synthesis* for the preparation of (S)-ethyl-3-hydroxy-butyrate.¹⁴ We consider that this set of findings strongly supports the mechanism of this kind of transformations which we suggest be active also in the reported alkylation of 1, involving the following steps:

- yeast oxidation of the alcohol to the aldehyde.¹⁵
- chemical condensation between the active methylene compound and the aldehyde with formation of the unsaturated carbonyl compound.
- double bond reduction by yeast to give the saturated product.

The results observed suggest that this mechanism should apply to other active methylene compounds provided that the intermediate unsaturated esters are reduced by b.y., and we checked this hypothesis by submitting to b.y. transformation ethyl cyanoacetate in which the absence of a reduceable carbonyl group avoids competition of the substrate for different enzymic systems in yeast. Indeed from compound 8, the alkylated pro-



duct 9 was isolated in chemical yields depending from the pH which regulates the condensation step: at starting pH of 6, only 6% of product 9 was obtained, whereas at starting pH 8,¹⁶ the observed yield of 9 was 60% as determined by GLC of the crude extract. Both the α -alkyl cyanoacetate from b.y. reduction of the synthetic precursor 10 and the one obtained from the direct feeding of 8 in fermenting yeast, are racemic as expected from the observation that synthetic 10 is a nearly 1:1 mixture of Z:E stereoisomers.¹⁷ Once again, formation of the condensation product 10 was observed reacting 8 with acetaldehyde in water at pH 8.

The contribution of Mrs. Rosanna Bernardi for GLC analysis is gratefully acknowledged.

REFERENCES

- 1 S. Servi, *Synthesis*, 1990, 1
- 2 G. Fronza, C. Fuganti, P. Grasselli, G. Poli and S. Servi, *J. Org. Chem.*, 1988, 53, 6153
- 3 G. Fronza, C. Fuganti, G. Pedrocchi-Fantoni and S. Servi, *Chem. Lett.* 1989, 2141
- 4 H.G. Crout and H. Cristen in *Modern Synthetic Methods*, 1989, vol. 5, R.Scheffold Ed. Springer Verlag 1-114
- 5 J.B. Jones, *Tetrahedron*, 1986, 42, 3351
- 6 C. Fuganti and P. Grasselli, *Chem. Ind. (London)*, 1977, 983
- 7 J.C. Medina, R. Guajardo and K.S. Kyler, *J. Am. Chem. Soc.*, 1989, 11, 2310
- 8 T. Kitazume and N. Ishikawa, *Chem. Lett.* 1984, 1815
- 9 T. Itoh, Y. Takagi and T. Fujisawa, *Tetrahedron Lett.* 1989, 53, 6153
- 10 The reduction of activated double bonds by b.y. is well documented. See section 7 in ref. 1.
- 11 The four diastereoisomers were separated by GC on a OV-1701, 25 m column at temperatures from 40 to 180 °C: **6a** rt (min) 36.5, **36.7**, 37.3, **37.7**. **6b** rt (min) 38.05, **38.34**, 39.07, **39.43**. The ratio of the stereoisomers (boldface) was 1:1.
- 12 M. Utaka, S. Konishi, T. Okubo, S. Tsuboi and A. Takeda, *Tetrahedron Lett.*, 1987, 28, 1447
- 13 100 g of commercial b.y. was incubated at 35 °C in the presence of 100 g of glucose and 2 g of Na₂HPO₄ in 1 L of water. After 1 h, compound 7 (1.5 mL) in 40 mL of EtOH or PrOH was added. The fermentation was continued for 48 h. **6a-b** were detected by GC in a 0.2% yield. The same observations were made when acetaldehyde or propionaldehyde were added instead of the corresponding alcohols.
- 14 D. Seebach, M.A. Sutter, R.H. Weber, M.F. Zueger, *Org. Synth.* 1985, 63, 1
- 15 C. Fuganti, D. Ghiringhelli and P. Grasselli, *Experientia*, 1978, 34, 297
- 16 100 g of commercial b.y. was incubated at 35 °C in the presence of 50 g of glucose and 2 g of Na₂HPO₄ in 1 L of water. After 1 h, compound 8 (2 mL) was added under stirring. Fermentation was continued for 72 h. GC analysis of the crude extract shows the presence of 60 % of **9**. During the fermentation the pH decreases markedly up to 4.5 in the first case and to 6 when the starting mixture was at pH 8.
- 17 As determined by GLC