*Pure & Appl. Chem.*, Vol. 64, No. 8, pp. 1099–1101, 1992.
Printed In Great Britain.


# Minor synthetic capacities of baker's yeast towards unnatural substrates

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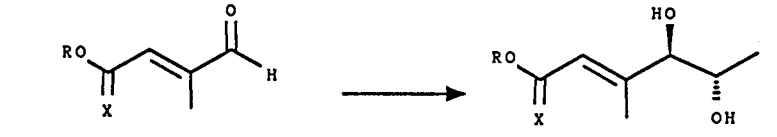
Abstract- Baker's yeast catalyzes numerous transformations on reactive substrates. α,β-Unsaturated aldehydes undergo water addition with the formation of chiral secondary alcohols, but sulfur nucleophiles (mercaptans) chemically add to the double bond with partial kinetic resolution observed in the secondary thiol obtained, following enantioselective reduction of the intermediate aldehydes. These sulfur nucleophiles however interact with the yeast metabolic pathway allowing the isolation of carbohydrate derived chiral intermediates. The use of deuterated precursors or performing the fermentation in $D_2O$ allows the isolation of chiral glycerol derivatives stereoselectively deuterated at different positions.

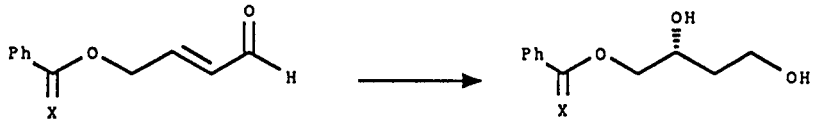
## INTRODUCTION

The ability of baker's yeast (b.y.) to effect stereoselective reductions of a variety of unsaturated compounds has been known for a long time (ref. 1-2) and has recently been exploited for the preparation of chiral compound in the enantiomerically pure form (ref. 3-4). However due to the complexity of the enzymic system at work in yeast cells, many reactions occur at the same time giving often mixtures of products derived from different catalytic capacities, and selectivity must be obtained by different metodologies including the use of irreversible inhibitors (ref. 5) or sometimes simply by substrate modifications. Variations in substrates structure can have a profound effect in the quality of the products. Typical examples of such a



1 R = Et, X = O
3 R = COPh, X = $H_2$

2 R = Et, X = O
4 R = COPh, X = $H_2$



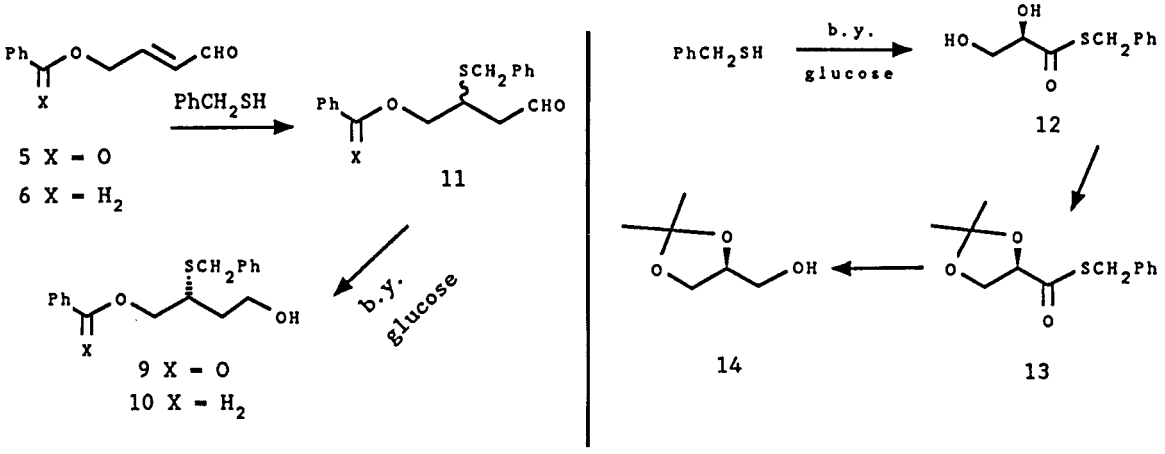
5 X = O
6 X = $H_2$

7 X = O
8 X = $H_2$

case are the reductions of 3-oxo-butanoates in which it is the substrate who selectively interacts with the enzymic system. We have been studying the transformation of α,β-unsaturated aldehydes and found that they can undergo in fermenting yeast double bond and carbonyl reduction and in some cases acyloin condensation. Due to the relevance of stereocontrolled C-C bond formation in organic synthesis, we explored a series of α,β-unsaturated aldehydes as possible substrates for the latter transformation and found that subtle variations in the structure of the substrate are responsible for completely different product formation (ref. 7).

## RESULTS AND DISCUSSION

We found that while ethyl-β-methyl-γ-oxo-crotonate 1 can accept the $C_2$ unit coming from decarboxylation of pyruvate giving the corresponding diol 2 in 35% yields, the aldehyde 3 gives 4 in only 10% yields. Compound 5 differing from 3 for the substitution at the carbon atom α to the aldehydic group, affords on yeast transformation compound 7 arising from water addition across the double bond and subsequent carbonyl reduction in 25% yields (ref. 8). Compound 6 also behave in this way affording optically active 8. The synthetic potential of the two compounds 7 and 8 can be attributed to the fact that they can be considered as derived from unnatural malic acid. Attempts to apply this microbial hydroxylation to other α,β-unsaturated substrates failed and the structural feature required for such an enzymatic activity remained obscure. The possibility to extend the addition reaction using other nucleophiles as cosubstrates induced us to explore the behaviour of mercaptans with aldehydes 5 and 6 but following a rapid chemical addition only alcohols 9 and 10 deprived of optical activity were obtained. However, if the intermediate aldehydes 11




were submitted to b.y. reduction, at low conversion compounds 9 and 10 of ~50% ee were obtained (ref. 9). In the reaction mixture another product was present independently of the α,β-unsaturated aldehyde, and it was identified as the benzylthioglycerate depicted in 12. Its absolute configuration and enantiomeric purity was determined by trasforming it through 13 into (*S*)-2,2-dimethyl-1,3-dioxolan-4-methanol 14 of >98% ee. Despite the low amount of product obtainable (ref. 10) we found of interest the formation of the product for the following reasons: the thioglycerate 12 is obtained through a process amenable to an extraction in high enantiomeric excess; its formation is due to an interaction between an unnatural substrate and intermediates in a metabolic pathway allowing the capture of valuable intermediates; the use of deuterated precursors or deuterated solvent allows the obtainement of rare deuterated intermediates. In fact experiments with D-glucose (ref. 10) enriched with labelled material, besides supporting hypothesis on the formation of 12 give access to differently deuterated glycerol derivatives as shown in fig. 1: from 1 and 6 $^{13}$C-D-glucose fragments 12a and 12b differing slightly for the degree of dilution of the label were obtained. 6-Dideuterio glucose gave 12c with the methylene group showing the same dilution of deuterium at both hydrogen atoms. The deuterium from 1- and 2-deuterio glucose was found in 12e and 12d epimeric at C-3. These results suggest that benzyl mercaptan is inserted at some stage of the glycolytic pathway. The C3 unit can derive either from dihydroxyacetone-phosphate (DHAP) or from glyceraldehyde-3-phosphate (G3P), the two products which are in equilibrium with glucose in the presence of aldolase, as it is proved from incorporation of $^{13}$C both from 1-$^{13}$C-D-glucose and from 6-$^{13}$C-D-glucose. The other deuterium labelling experiments with the observed dilution values are in agreement with this scheme, taking into account the known interconversion of glucose and fructose where only one of the two C-1 hydrogen atoms in fructose is found in glucose (at C-2) (ref. 11). In particular the fact that dilution of fed material in 12d and 12e is higher than expected can be explained in terms of a multiple equilibrium involving glucose 6-P ⇆ fructose 6-P ⇆ mannose 6-P in which the hydrogen atom at $C_1$ moves from $C_1$ to $C_2$ and to water, being diluted. This was proved in the

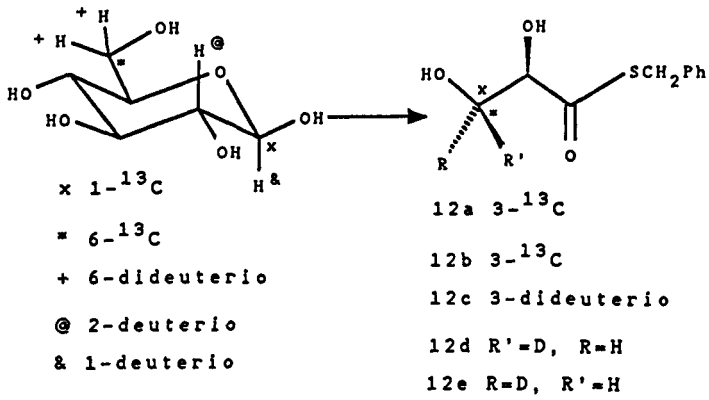
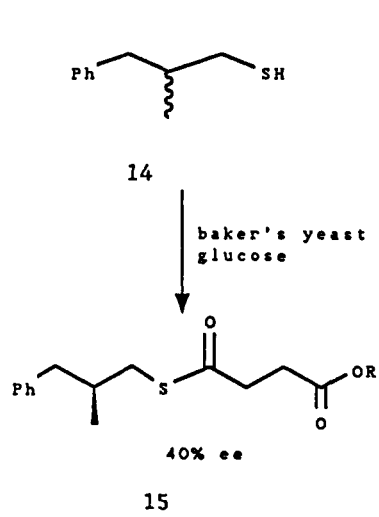



Fig. 1. Labelled benzylthioglycerate 12a-e obtained from benzyl mercaptan in fermenting yeast giving as nutrient D-glucose enriched with $^{13}C$ and D at different positions.




analysis of compound 13 obtained from b.y. transformation of benzyl mercaptan with D-glucose and D-mannose in $D_2O$ showing that in both cases the C 2 methine containes 100% D while C-3 contained 30 % of deuterium with opposite configurations consistent with their origin. Attempts to extend the possibility of insertion in the same glycolytic pathway by using different sulfur nucleophiles gave again unexpected products in minute amounts: in fact when racemic 2-methyl-3-phenyl-propanethiol 14 was slowly added to fermenting yeast, 15 of *S* absolute configuration and 40% ee was obtained presumably as the consequence of a reaction between the nucleophilic thiol and a succinate unit mediated by the enzyme succinate-thiokinase(ref. 12).

These examples show that almost every substrate has a different fate in complex enzymic systems like fermenting yeast and that insertion into known metabolic pathways with reactive compounds in the presence of deuterated cosubstrates, can give interesting labelled compounds. In fact the methodology of exploiting microbial reactions to prepare selectively labelled compound is currently increasingly used (ref. 13).

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