Enzymes as Catalysts in Organic Synthesis

edited by M. P. Schneider

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PREFACE

Enzymatic reactions can frequently be employed to effect transformations in organic syntheses that otherwise would be difficult to carry out. However, it is only recently that systematic attempts have been made by organic chemists to explore the scope and utility of such methods.

It ist now becoming widely appreciated that, compared with chemical methods, enzyme catalyzed processes often offer significant advantages including those of efficiency, chemoselectivity, regioselectivity, diastereo- and enantioselectivity. It is not surprising therefore that these reactions are coming into ever increasing prominence both on a laboratory and industrial scale for the preparation of chiral building blocks and the production of pharmaceuticals, agro- and fine chemicals. However, the ground rules for such applications are still sketchy and in this respect interdisciplinary, fundamental, research in academic laboratories of the scope and applicability of enzymatic methods is urgently needed.

In order to bring together scientists from various different backgrounds with an interest in this area, the present NATO Advanced Research Workshop was organized. It was attended by synthetic organic chemists, bioorganic chemists, biochemists and microbiologists from academic, government and industrial laboratories. A particular feature of this meeting was the main focus on the requirements of synthetic organic chemistry. The participating organic chemists were giving a broad view of the current state of organic synthesis against which the putative advantages of enzymatic methods would have to be measured.

The activities of the workshop consisted of lectures, poster sessions and round-table discussions. Important themes illustrated in the lectures included applications of hydrolytic enzymes and oxido-reductases (isolated and whole cell systems) for the preparation of enantiomerically pure chiral building blocks, the use of aldolases for enzymatic carbon-carbon bond formation, the preparation of chirally labelled compounds, co-factor recycling and the application of artificial co-factors, the application of microbial transformations in

the synthesis of natural products, the development of bioreactors using immobilized enzymes for the industrial production of chiral organic compounds in optically pure form (e. g. L-aminoacids) and for the treatment of byproducts from the food industry and chemical plants, and the industrial production of enzymes.

Recent achievements in enzyme engineering, the preparation and properties of semisynthetic enzymes and the possibilities of genetic engineering in producing enzymes tailored to specific purposes were reported.

The conclusion emerging from the workshop was clear: enzyme-catalyzed reactions play an important role both in academic and industrial laboratories, particularly for the preparation of enantiomerically pure building blocks needed in the synthéses of biologically active compounds. In the discussions frequently the different viewpoints of academic and industrial participants emerged. A particularly useful outcome of the meeting was therefore the clear underlining of the need for more basic research in academic laboratories and increased collaborations with industrial groups.

We are grateful to the NATO Scientific Affairs Division for funding a meeting which was intended

- to close a certain gap between a wealth of biochemical knowledge and its practical use in organic synthesis; and
- to amalgamate the diverse, yet complementary, viewpoints of different scientific disciplines in order to serve its main purpose: to promote an increased use of

Enzymes as Catalysts in Organic Synthesis.

M. Schneider

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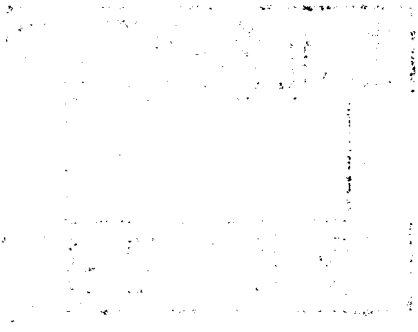

BAKER'S YEAST MEDIATED PREPARATION OF CARBOHYDRATE-LIKE CHIRAL SYNTHONS

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ABSTRACT: Baker's yeast fermenting on D-glucose converts C_6-C_3 α,β-unsaturated aromatic aldehydes into C_6-C_5, carbohydrate-like, (2S,3R) methyl diols (4), with yields of about 25%, as well as into products (2) and (3). Products (4) and homologous diols prepared by enantio- and stereoselective reduction of α-acetoxy ketones such as (75)-(77), can be used instead of natural carbohydrates as starting materials for the synthesis of optically active forms of natural products belonging to quite different structural classes.

A current approach[1] to the synthesis of enantiomerically pure forms of natural products and drugs relies on the use as starting materials of components of the collection of inexpensive, readily available optically active compounds produced by nature, called the 'pool of chirality', which includes, among others, carbohydrates, amino acids, hydroxyacids like tartaric, malic, lactic, and citramalic, alcohols like D-mannitol, and few terpenes. However, the present composition of the 'pool of chirality' is far from being satisfactory, the major drawback arising from the fact that most of the components are really available in only one enantiomeric form, a circumstance which dictates, when the absolute configuration of the target molecule is opposite to the one of the chosen starting material, chemical manipulation of the chiral center(s), usually through multistep, low-yield sequences.

This is the case of the synthesis of the 2,3,6-trideoxy-3-amino hexoses of the L series present in the therapeutically important anthracycline glycosides adriamycin[2] and its 4'-epimer,[3] which can be realized either from inexpensive hexoses of the D series,[4] but with a critical inversion of configuration at position 5 at some stage of the sequence, or from the rather rare 6-deoxy sugar L-rhamnose.[5]

Furthermore, the choice of types of chirality is rather poor, those

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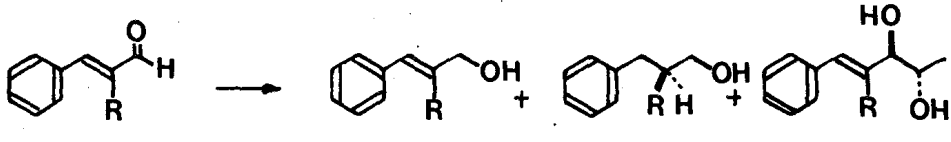
of the type R,R^1CHX, where X= oxygen or nitrogen functions, being particularly abundant, whereas those of the type R,R^1,R^2CH and $R,R^1,R^2C(OH)$, quite frequent, for example, amongst the insect pheromones, occur rather rarely.

Consequently, there is at present an interest in expanding the composition of the 'pool of chirality' and new chiral products are expected to be accessible from the microbial transformations of non-conventional substrates. Microbes are indeed capable of performing a variety of transformations of non-conventional substrates using enzymes either of the primary or of the secondary metabolism.[6] From a practical point of view, we would expect to be synthetically useful those transformations of non-conventional substrates leading to optically active products performed by microorganisms commercially available at low cost, possessing large quantities of enzymes (usually of the primary metabolism), and showing a large substrate specificity, while mantaining a precise reaction stereospecificity.

Baker's yeast meets most of the above requirements, and its ability to transform stereoselectively non-conventional substrates into chiral products using its constitutive enzymes has long been recognized.[7] However, apart from (R) phenylacetylcarbinol, obtained in an acyloin-type condensation[8] from benzaldehyde and 'statu nascendi' acetaldehyde, most of the optically active products obtained up to now by this means contain chiral centres of the type R,R^1,R^2CH and/or R,R^1CHOH, which arise by formal addition of hydrogen onto (activated) double bonds and/or carbonyl carbon of the precursors. These processes occur without modification of the original carbon framework, and are now being mimicked in non-enzymic asymmetric synthesis.[9]

In the reawakening of interest for the microbially aided preparation of synthetically useful chiral products, our observation[10] that baker's yeast fermenting on D-glucose converts aromatic α,β-unsaturated aldehydes (1) into C_6-C_5 (2S,3R) 2,3-methyldiols (4), with yields of 25%, as well as into products (2) and (3) (equation 1) is particularly fruitful from a synthetic point of view.



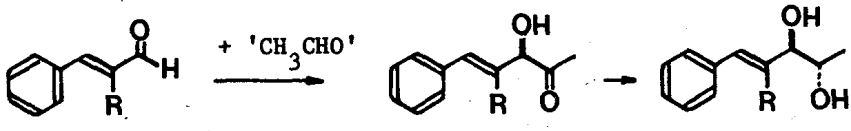
(1) (2) (3) (4)

R= H, Me, Br

equation 1

Indeed, whereas the production from[11] (1) of (2) and (3) falls amongst the known baker's yeast capacities, the formation of the methyldiols (4), which contain two more carbon atoms than the precursor aldehydes and two adjacent chiral centres of the type R,R^1CHOH, is new and, to our knowledge, without equivalent in non-enzymic asymmetric synthesis.

The formation of the (2S,3R) diols from α,β-unsaturated aromatic aldehydes by fermenting baker's yeast can be viewed as the overall consequence of a complex aldehyde condensation-reduction involving two distinct chemical operations. (i) Addition of a C_2 unit equivalent of acetaldehyde onto the si face of the carbonyl carbon of the α-position unsaturated aldehydes forms (R) α-hydroxyketones, and (ii) reduction of the latter intermediates on the re face of the carbonyl gives rise to the diols actually isolated (equation 2).



equation 2

Under suitable experimental conditions,[12] (R) hydroxyketones can be obtained as sole transformation products of the aldehydes by actively fermenting baker's yeast.

Experiments[13] designed to elucidate the substrate specificity of the sequence of equation 2 indicated that there is some tolerance by the enzymic system(s) involved as far as the structure of the aromatic aldehydes and the substituents in the α-position are concerned. However, α-ethyl and α-propyl cinnamaldehydes are not converted into the corresponding methyl diols. Furthermore, acetaldehyde is the only aldehyde accepted as second terminus of the reaction; when cinnamaldehyde was incubated with yeast in the presence of propionaldehyde or butyraldehyde, ((2S,3R) (7) was isolated as the sole transformation product. The failure of yeast to convert α-ethylcinnamaldehyde and propionaldehyde into the type of diols of equation 2 is probably due to the inability of these materials to be accepted as substrates by the condensing enzyme(s) (first part of equation 2). The substrate specificity of the second part of equation 2 is not as restricted, since the synthetic α-hydroxyketones derived from these aldehydes, or from other carbonyl compounds, are stereospecifically reduced by yeast (Figure 1).[14]

The (2S,3R) diols (7) and (8), obtained from cinnamaldehyde and α-methylcinnamaldehyde, respectively, have an absolute configuration matching that at positions 5 and 4 of 6-deoxy-L-sugars and they have been converted, in the work designed to establish their absolute configuration,[15] into L-amicetose (23) and L-olivomycose (24).

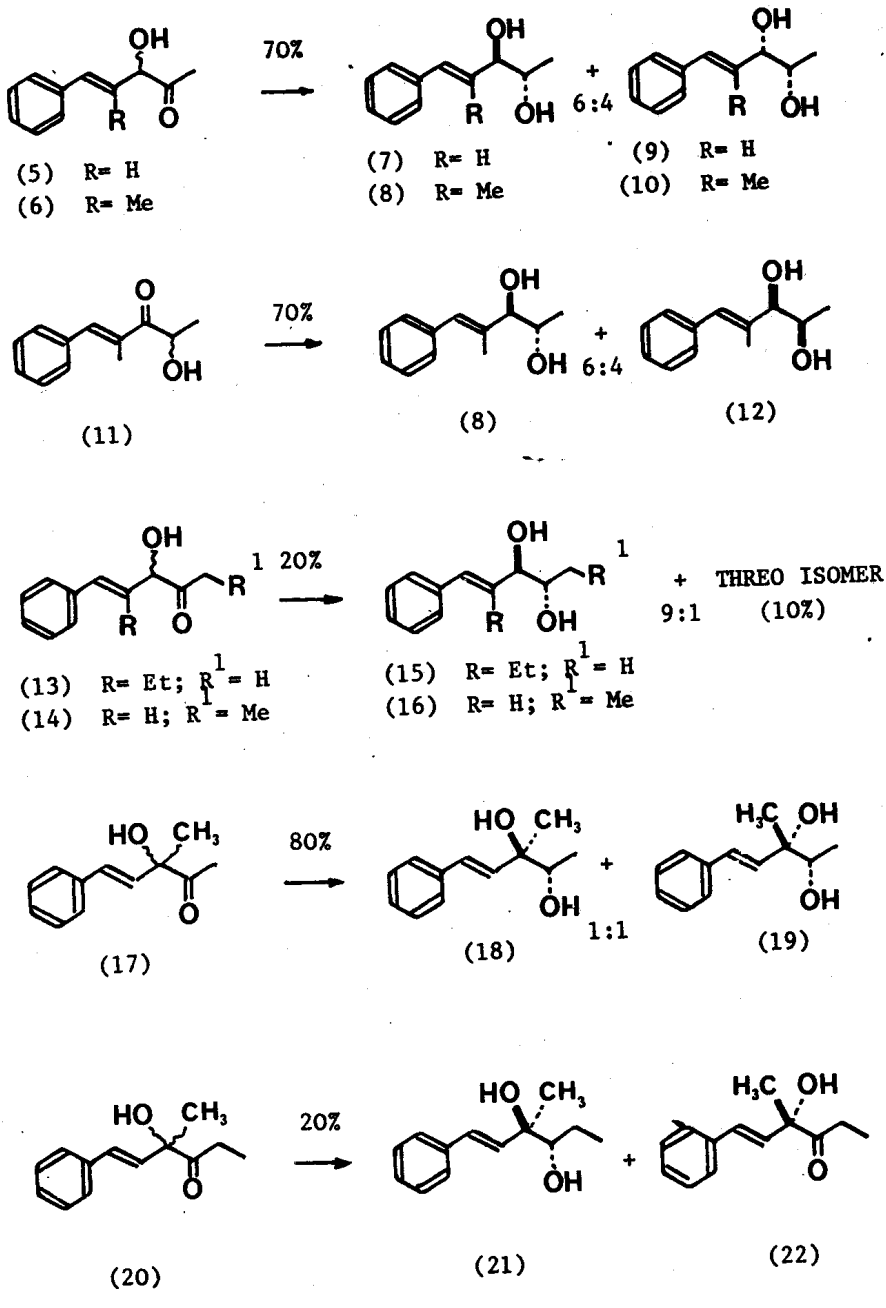


Figure 1: Conversion of racemic α-hydroxyketones to diols by baker's yeast