Enzymes in organic synthesis
The Ciba Foundation is an international scientific and educational charity. It was established in 1974 by the Swiss chemical and pharmaceutical company of CIBA Limited—now CIBA-GEIGY Limited. The Foundation operates independently in London under English trust law.

The Ciba Foundation exists to promote international cooperation in biological, medical and chemical research. It organizes about eight international multidisciplinary symposia each year on topics that seem ready for discussion by a small group of research workers. The papers and discussions are published in the Ciba Foundation symposium series. The Foundation also holds many shorter meetings (not published), organized by the Foundation itself or by outside scientific organizations. The staff always welcome suggestions for future meetings.

The Foundation’s house at 41 Portland Place, London, W1N 4BN, provides facilities for all the meetings. Its library, open seven days a week to any graduate in science or medicine, also provides information on scientific meetings throughout the world and answers general enquiries on biomedical and chemical subjects. Scientists from any part of the world may stay in the house during working visits to London.
Enzymes in organic synthesis

Ciba Foundation symposium 111

1985

Pitman
London
Contents

Symposium on Organic synthesis using enzymes, held at the Ciba Foundation, London, 15–17 May 1984

Editors: Ruth Porter (Organizer) and Sarah Clark

A. R. Battersby  Chairman’s introduction  1

J. B. Jones  An illustrative example of a synthetically useful enzyme: horse liver alcohol dehydrogenase  3  
Discussion  14

A. R. Battersby  Enzymic synthesis of labelled chiral substances  22  
Discussion  29

S. M. Roberts  Enzyme-controlled reactions giving alkanols of use in the synthesis of biologically active molecules  31  
Discussion  37

M. D. Scawen  Large-scale purification of enzymes  40  
Discussion  53

K. Mosbach  Immobilized enzymes in organic synthesis  57  
Discussion  66

General discussion I  Synthesis of 5,6-dihydroxycyclohexa-1,3-diene  71

G. M. Whitesides  Applications of cell-free enzymes in organic synthesis  76  
Discussion  90

H. Simon, H. Günther, J. Bader and S. Neumann  Chiral products from non-pyridine nucleotide-dependent reductases and methods for NAD(P)H regeneration  97  
Discussion  108
C. Fuganti and P. Grasselli  Stereochemistry and synthetic applications of products of fermentation of α,β-unsaturated aromatic aldehydes by baker's yeast  112
Discussion  125

Y-F. Wang, C-S. Chen, G. Girdaukas and C. J. Sih  Extending the applicability of esterases of low enantioselectivity in asymmetric synthesis  128
Discussion  139

W. R. Abraham, H. M. R. Hoffmann, K. Kieslich, G. Reng and B. Stumpf  Microbial transformations of some monoterpenoids and sesquiterpenoids  146
Discussion  157

General discussion II  Synthesis of tetrahydrofolate derivatives  161
Synthesis of optically active propylene oxides  165

M. Ohno  Creation of novel chiral synthons with enzymes: application to enantioselective synthesis of antibiotics  171
Discussion  183

J. Markussen and A. Vølund  Kinetics of trypsin catalysis in the industrial conversion of porcine insulin to human insulin  188
Discussion  201

A. R. Fersht and G. P. Winter  Redesigning enzymes by site-directed mutagenesis  204
Discussion  213

E. T. Kaiser and C. Radziejewski  The design of new enzyme active sites for the catalysis of specific chemical reactions  219
Discussion  225

Final general discussion  NAD⁺ degradation by resting cells  231
Economic aspects  233
Applications of enzyme methodology  234

Index of contributors  239

Subject index  241

[Also presented at the symposium but not included here:
Participants

C. Abell  University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK

D. Arigoni  Laboratorium für Organische Chemie, ETH-Zentrum, Universitätstrasse 16, CH-8092 Zürich, Switzerland

A. R. Battersby (Chairman)  University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK

D. Bellus  Central Research Laboratories, CIBA-GEIGY AG, CH-4002 Basel, Switzerland

Sir John Cornforth  The School of Molecular Sciences, University of Sussex, Falmer, Brighton, Sussex BN1 9QJ, UK

M. D. Edge  Chemistry I Department, Pharmaceuticals Division, Imperial Chemical Industries PLC, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, UK

A. R. Fersht  Department of Chemistry, Imperial College of Science & Technology, South Kensington, London SW7 2AY, UK

C. Fuganti  Dipartimento di Chimica, Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133 Milano, Italy

O. Ghisalba  Central Research Laboratories, CIBA-GEIGY AG, CH-4002 Basel, Switzerland

B. T. Golding  Department of Organic Chemistry, The University, Newcastle upon Tyne NE1 7RU, UK

J. T. Johansen  Carlsberg Biotechnology Ltd, 16 Tagensvej, DK-2200 Copenhagen N, Denmark

J. B. Jones  Department of Chemistry, University of Toronto, Lash Miller Chemical Laboratories, 80 St George Street, Toronto, Ontario M5S 1A1, Canada
E. T. Kaiser  Laboratory of Bioorganic Chemistry and Biochemistry, The Rockefeller University, 1230 York Avenue, New York, New York 10021-6399, USA

K. Kieslich  Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-3300 Braunschweig-Stöckheim, FRG

J. R. Knowles  Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138, USA

J. Markussen  Novo Research Institute, Novo Alle, DK-2880 Bagsvaerd, Denmark

K. Mosbach  Pure and Applied Biochemistry, Chemical Center, University of Lund, PO Box 740, S-220 07 Lund, Sweden

M. Ohno  Faculty of Pharmaceutical Sciences, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

S. M. Roberts  Department of Microbiological Chemistry, Glaxo Group Research Limited, Greenford Road, Greenford, Middlesex UB6 OHE, UK

M. D. Scawen  Public Health Laboratory Service, Centre for Applied Microbiology and Research, Microbial Technology Laboratory, Porton Down, Salisbury, Wiltshire SP4 OJG, UK

C. J. Sih  Center for Health Sciences, School of Pharmacy, University of Wisconsin Madison, 425 North Charter Street, Madison, Wisconsin 53706, USA

H. Simon  Lehrstuhl für Organische Chemie und Biochemie der Technischen Universität München, Lichtenbergstrasse 4, 8046 Garching, FRG

C. J. Suckling  Department of Pure & Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL, UK

S. C. Taylor  Corporate Bioscience and Colloid Laboratory, Imperial Chemical Industries PLC, PO Box 11, The Heath, Runcorn, Cheshire WA7 4QE, UK

G. M. Whitesides  Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge, Massachusetts 02138, USA
Chairman’s introduction

A. R. BATTERSBY

University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK


There are times in the development of any subject when a meeting for assessment, discussion and criticism is particularly appropriate. For the topic of this symposium, it seems to me that the timing is just right. We are not so early in the use of enzymes for organic synthesis that the key problems for the future cannot be usefully discussed (or even seen). Nor is the development so far advanced that we are at the tidying-up stage. Quite a number of people urged the Ciba Foundation to hold this meeting; they are all present and notable among them are Bryan Jones, George Whitesides, Jeremy Knowles and Duilio Arigoni. As far as I know, this is the first international meeting dealing with enzymes for synthesis but, for a topic so obviously important industrially and academically, it will clearly not be the last.

Enzymes are catalysts which achieve amazing rate enhancements for the reactions they promote. This aspect will certainly be in our minds during the meeting but I suspect that two other characteristics of enzymes will be even more to the fore. These are their abilities to catalyse (a) regiospecific and (b) stereospecific reactions. We will be exploring areas of synthesis where enzymic methods, not only those based on isolated enzymes but also those involving whole microorganisms, have clear advantages over non-enzymic approaches. So we need to look into sources of enzymes and how to isolate, handle and possibly stabilize them. Further, since some important enzymic processes involve cofactors, the problems of effective (and economically feasible) methods for cofactor regeneration must be faced.

Though a wide range of enzymes can be isolated from living systems of one sort or another, my guess is that there will be great opportunities for using genetic methods to produce enzymes that are modified to affect some feature of the mode of action or even to tailor the enzyme for a specific purpose. Nature’s enzymes, though marvellous, have developed within a set of ‘rules’, e.g. that
they must be water soluble, must work well in physiological pH and temperature ranges and, in many cases, must not fall out of the cell. We should consider changing the rules, and in the last two contributions to this symposium we will be looking along these lines to the future. I hope that during the next three days we will be able to sort out the strengths and weaknesses of the enzymic approach to various synthetic problems and perhaps in some cases to point the way for fresh developments.
An illustrative example of a synthetically useful enzyme: horse liver alcohol dehydrogenase

J. BRYAN JONES

Department of Chemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A1

Abstract. The enzymes of most general and continuing value in organic synthesis are those that can operate stereospecifically on a wide range of structurally varied substrates. Horse liver alcohol dehydrogenase (HLADH, EC 1.1.1.1), a commercially available NAD(H)-dependent oxidoreductase that catalyses $\text{C}==\text{O} \rightleftharpoons \text{CH(OH)}$ interconversions, is such an enzyme. The scope of applications of enzymes endowed with this degree of versatility is illustrated by the preparative-scale use of HLADH to catalyse specific oxidoreductions of a broad spectrum of alcohol and ketone substrates. Examples are given of enantiomeric distinctions, of discrimination between enantiotopic groups in substrates possessing prochiral centres or in meso compounds, of regiospecific transformations and of combinations of various specificities. The value of the enzyme-derived products as chiral synthetic precursors of molecules of current interest is demonstrated.

1985 Enzymes in organic synthesis. Pitman, London (Ciba Foundation symposium 111) p 3-21

Why are enzymes such intrinsically attractive catalysts for organic synthesis? There are many reasons. For example, with one or two exceptions, such as the Diels–Alder reaction, there are enzyme-catalysed equivalents for most organic reactions. Also, enzymes are very efficient catalysts: reactions can be effected under mild conditions so that problems such as epimerization, isomerization, rearrangement and racemization, to which many sensitive molecules are prone, may be avoided. Most importantly, enzymes are highly selective and specific in their catalyses. It is this property that provides their most notable advantage from the organic chemist’s viewpoint, particularly with respect to asymmetric synthesis. Of the six main enzyme groups of the International Union of Biochemistry classification (International Union of Biochemistry 1979), the oxidoreductases (group 1), the hydrolases (group 3) and the lyases (group 4) represent the most generally useful enzyme types at the present time.

Many enzymes require coenzymes in order to be catalytically active. Coenzymes are expensive and it is seldom economically feasible, nor chemically desirable, to provide them in the stoichiometric amounts formally
required. Instead, catalytic amounts of the required coenzyme are used in conjunction with an inexpensive process for continuously regenerating the active form. This subject has received considerable attention (Walt et al 1984 and references therein, Wong et al 1983 and references therein) and is addressed further in this symposium (Whitesides, Simon et al and Mosbach, this volume). Problems still remain, but for the most widely used enzymes, coenzyme-recycling systems are now efficient enough to be used economically in kilogram-scale syntheses.

For an enzyme to be accepted into routine use in synthesis it must be readily, preferably commercially, available. It should accept a broad structural range of substrates, but retain the ability to operate stereospecifically in each individual transformation. While these two specificity criteria are somewhat antithetical, they are satisfied by a number of enzymes. It is also useful to have guidelines that permit prediction of stereospecificity. Knowledge of the mechanism of catalysis can be helpful, for avoiding inhibitors for example. Above all, the experimental procedure for the preparative reactions should be straightforward and not require sophisticated biochemical equipment. Horse liver alcohol dehydrogenase (HLADH, EC 1.1.1.1) satisfies most of these criteria and will be used to illustrate the scope of synthetic applicability of a versatile enzyme.

HLADH is a commercially available, NAD(H)-dependent alcohol dehydrogenase that catalyses CH(OH)\(\rightleftharpoons\)C=O oxidoreductions of a broad structural range of substrates of interest to the organic chemist. We overcome the high costs of the NAD(H) coenzymes by using the ethanol coupled-substrate regeneration method (Zagalak et al 1966) for reductions, and a flavin mononucleotide-recycling system for oxidations (Jones & Taylor 1976); we use very simple experimental procedures in each case. We generally do our preparative-scale reactions on 1–2 g of substrate, but it is easy to scale up to 20 g or higher if more material is required. Reaction times vary from a few hours in the most favourable cases to 2–3 weeks for the slowest substrates. Accurate predictions about the stereospecificity of the HLADH-catalysed oxidoreduction process can be made with the Prelog rule (Prelog 1964) for simple acyclic substrates, and with a cubic-space model of the enzyme’s active site for more complex acyclic and cyclic substrates (Jones & Jakovac 1982). All the HLADH-catalysed reactions discussed below are in accord with the predictions of these models.

**Exploiting enantiomeric stereospecificity**

The enantiomeric specificity of HLADH has been widely exploited for the resolution of racemic ketones and alcohols (Jones & Beck 1976). When
HORSE LIVER ALCOHOL DEHYDROGENASE

(a)  
\[
\begin{align*}
(\pm)-1 & \xrightarrow{\text{HLADH, pH} 7} (\pm)-3 \\
(\pm)-3 & \xrightarrow{\text{NADH-recycling}} 50\%\text{reduction} \\
(\pm)-5 & \xrightarrow{\text{HLADH, pH} 9} (\pm)-5 \\
(\pm)-5 & \xrightarrow{\text{NAD-recycling}} 50\%\text{oxidation}
\end{align*}
\]

(b)  
\[
\begin{align*}
(\pm)-6 & \xrightarrow{\text{HLADH, pH} 7} (\pm)-6 \\
(\pm)-6 & \xrightarrow{\text{NADH-recycling}} 100\%\text{reduction}
\end{align*}
\]

R = Me, Et, i-Pr

1. Chromatographic separation
2. Chemical oxidation

FIG. 1. Some stereospecific oxidoreductions and resolutions of racemates.

completely stereospecific, these reactions terminate automatically at the '50%-of-reaction' point when all the reactive enantiomer has been transformed. Resolving the racemic bridged bicyclic compounds 1, 3 and 5 (Fig. 1a) by traditional methods is very tedious. In contrast, the HLADH-catalysed reductions and oxidations shown in Fig. 1a give highly enantiomerically enriched materials in a few hours (Irwin & Jones 1976). Furthermore, the reductions of the carbonyl groups of the reactive enantiomers of (±)-1 and (±)-3 are diastereotopically specific for one face of the carbonyl group, and give the endo- and exo-alcohols (+)-2 and (−)-4 respectively. The formation of the exo isomer 4 on reduction of (±)-3 represents an additional bonus, since the exo-alcohol is the thermodynamically less stable epimer and is not easily obtainable by direct chemical reduction. The thermodynamic stabilities of the alcohol products (+)-2 and (−)-4 do not influence the specificity of the enzymic reduction step. The stereochemistry of the alcohol product is determined only by the orientation of the carbonyl group with respect to the
direction of hydride donation from the coenzyme in the favoured enzyme-substrate (ES) complex preceding the transition state.

HLADH quite happily accepts hetero-atoms such as O and S (but not N, which complexes the Zn$^{2+}$ atom at the active site) in its substrates. Reduction of (±)-6 proceeds smoothly but is enantioselective only, with the (+) enantiomer being transformed only a little faster than the (−) enantiomer (Fig. 1b). The immediate conclusion in such cases is often that enzymic resolution of the racemates is not feasible under these conditions. However, it must be remembered that while an enzyme may bind both enantiomers of a racemate to form productive ES complexes, it will still operate stereospecifically on each individual substrate stereoisomer. Thus in the HLADH-catalysed reduction of (±)-6, for which hydride delivery occurs to the Re-face of the carbonyl group of each enantiomer, the alcohol products (+)-7 and (+)-8 are diastereomeric and can be separated chromatographically. Their subsequent chemical oxidation then provides the pure ketone enantiomers, (+)-6 and (−)-6 respectively (Davies & Jones 1979).

Exploiting prochiral stereospecificity

While the ability of enzymes to discriminate between enantiomers is very important for the purposes of resolution and asymmetric synthesis, operating with racemic substrates is potentially a problem since only half the material is of the desired configuration. The residual ‘wrong’ enantiomer must be discarded unless its structure readily lends itself to recycling back to the starting racemate. The ability of enzymes to exert prochirally stereospecific control in their catalyses overcomes this problem because it permits direct asymmetric synthesis of chiral products from symmetric starting materials. HLADH is a powerful enzyme in this regard.

Enantiotopic- or diastereotopic-face specificity is manifest in many carbonyl-substrate reductions. In aldehyde reductions, for example, the Prelog rule predicts that the hydride equivalent from the NADH coenzyme will always be delivered to the Re-face of the carbonyl group. This permits the controlled synthesis of isotopically labelled alcohols of predetermined $S$ or $R$ chirality, such as 9 or 10 (Fig. 2a), from appropriately labelled aldehydes or coenzymes, for use as probes of the stereochemistry of biosynthetic pathways (Cornforth et al 1975, Battersby et al 1976).

Enantiotopic groups attached to prochiral centres can also be distinguished. In its oxidation mode, HLADH is enantiotopically selective for the pro-$S$ hydroxyethyl groups of 1,5-pentanediols substituted at C-3 (11, Fig. 2b). Furthermore, the initially formed hydroxyaldehydes (12) cyclize spontaneously in situ to the hemiacetals (13), which are themselves substrates of
HLADH and undergo further oxidation to give the (S)-lactones (14) directly (Irwin & Jones 1977a). This double oxidation opportunity represents a significant bonus and has been exploited extensively to obtain lactones from diols in a single-step reaction. Enantiotopically specific oxidation of glycerol to L-glyceraldehyde can also be effected (Bally & Leuthardt 1970).

In addition, enantiotopic-group specific reductions can be achieved. For the decalin diones 15 and 17 (Fig. 2c), HLADH-catalysed reductions are specific for the pro-R carbonyl groups and give the hydroxy decalones 16 and 18 respectively (Dodds & Jones 1982, Nakazaki et al 1982). Even with the

FIG. 2. Asymmetric synthesis via HLADH-mediated enantiotopic-face and enantiotopic-group distinctions.
more highly symmetric substrates 19 and 21, the stereospecificity of reduction is retained to give 20 and 22 as products.

*Meso* compounds are another attractive group of symmetric substrates; HLADH can distinguish between their enantiotopic groups to catalyse transformations of value in asymmetric synthesis. The structural range of *meso*-diols that undergo stereospecific HLADH-mediated oxidation is very broad, as exemplified in Fig. 3a. The enzyme is remarkable in its ability to discriminate between the enantiotopic hydroxyl groups of such diols, even when the structural variations between the acyclic, monocyclic and bridged bicyclic substrates are so diverse. For compounds 23–32, oxidation occurs in

![Chemical structures](image)

**FIG. 3.** Preparation of chiral synthons by enantiotopically specific transformations of *meso* compounds.
each case in the same sense in terms of absolute configuration, i.e. with pro-§
hydroxyl specificity for all the acyclic and carbocyclic diols and with pro-R
enantiotopic selection for the heterocyclic substrates 29-31 in which X = O
(Jacovac et al 1982, Ng et al 1984, Bridges et al 1984, Francis & Jones 1984,
Jakovac 1980).

As in the diol oxidations shown in Fig. 2b, the enzyme readily catalyses the
oxidation of the hemiacetal tautomers of the initially formed hydroxyaldehydes,
and the lactone products 33-42 of the double oxidation are obtained
directly (Fig. 3a). Enantiotopic carbonyl groups of meso-dione substrates are
similarly distinguished by HLADH. Reduction of trans-decalin-2,6-dione
(43) occurs with complete stereospecificity for the pro-R carbonyl group to
give the hydroxy ketone 44 (Fig. 3b) (Dodds & Jones 1982).

Many chiral compounds of the types produced in the reactions shown in
Figs. 1-3 are valuable synthetic precursors of target molecules of current
interest. As illustrated in Fig. 4, chiral synthons readily obtainable from

![Chemical Structures]

**FIG. 4.** The lactone and alcohol products of HLADH-catalysed reactions are valuable precursors
of many target molecules of current interest.
HLADH-catalysed reactions can serve as valuable precursors of grandisol (45, Jones et al. 1982), of (+)-methyl chrysanthemate (46) and pyrethroids (Jakovac et al. 1982), of prostaglandins (Jakovac 1980) via 47 (Jones et al. 1974), of macrolides (Collum et al. 1980, Chen et al. 1981), and of (+)-4-twistanone (48, Dodds & Jones 1982, Nakazaki et al. 1982).

Exploiting combinations of specificity

A great additional advantage of enzymes is their ability to combine different specificities in a single step and thus achieve a degree of control presently unattainable in any other way.

Some examples of multiple specificity have already been given. In the reductions of (±)-1 and (±)-3 in Fig. 1, HLADH is enantiomerically selective for the (+)-ketone stereoisomers, and is also diastereotopically specific in its delivery of the hydride equivalent to the Si-face of the carbonyl group in (+)-1 and the Re-face of that in (+)-3. In Fig. 2c, the enzyme is enantiotopically specific both for the pro-R carbonyl groups of 15 and 17, and for the Re-faces of those carbonyl groups.

Regiospecificity combined with enantiomeric selectivity is another useful combination, as shown in Fig. 5a. Unless protecting groups are used, discrimination between unhindered primary and secondary alcohol functions in diols such as (±)-49 and (±)-50 is difficult to achieve in non-enzymic single-step reactions. However, with HLADH only those hydroxyl groups that can locate at the oxidoreduction site will be oxidized, so the primary and secondary functions can be discriminated on a regional basis. For 49, neither enantiomer can fit into the active site in a manner that positions the primary alcohol group at the enzyme’s oxidation site. Thus oxidation of the hydroxyethyl function does not occur. Furthermore, with the secondary alcohol function at the oxidation site, only the (+) enantiomer can form a productive ES complex (Jones & Jakovac 1982). This combination of regiospecificity and enantiomeric specificity results in the exclusive formation of the keto alcohol 51. The regional rather than chemical basis of the functional group selection is emphasized by the HLADH-catalysed oxidation of the cyclopentenyl diol (±)-50; in this case it is primary alcohol oxidation, also accompanied by enantiomeric selectivity, that is favoured. The initially formed hydroxyaldehyde 52 undergoes further oxidation, via (+)-53, in another enantiothermally selective process, to yield the Fried–Corey lactone (+)-54 of interest as a prostaglandin synthon (Partridge et al. 1973). The (−) enantiomer of 54, which is the precursor of natural prostaglandins, can be obtained by chemical oxidation of (−)-53 recovered from the HLADH-catalysed oxidation of (±)-53 (Irwin & Jones 1977b). The (+)-54 stereoisomer can serve as an intermediate for prostaglandin analogues.
FIG. 5. Remarkable combinations of regiospecificity, enantiomeric specificity and prochiral specificity can be achieved by enzymes in single-step reactions.

Analogous regiospecific carbonyl transformations are illustrated by the reductions of (±)-55 and (±)-56 (Fig. 5b). Here again, it would be difficult to achieve discrimination between the unprotected carbonyl functions in a chemical reduction. The enzyme, however, exhibits complete regiospecific preference for the cyclohexanone function in each case. Furthermore, the
reduction is concurrently both enantiomerically specific, and enantiotopically specific for the Re-faces of the carbonyl groups reduced (A. Krawczyk & J. B. Jones, unpublished work 1983).

Prognosis

While HLADH, and other alcohol dehydrogenases (Jones & Beck 1976), are clearly remarkable in their versatility, they are by no means unique in this regard. The broad synthetic applicabilities of other enzymes, especially esterases (cf. Ohno, this volume), are already documented. With the field expanding so rapidly, and with aware synthetic chemists adopting enzymic methods with increasing enthusiasm, the rate of addition to the list of ‘useful enzymes’ continues to increase. However, enzymes of broad specificity can never satisfy all the demands that will be made of them. Thus, when a particular transformation of a given substrate cannot be accomplished by a versatile enzyme, an appropriate alternative enzyme or microorganism should be identified. This can be done by searching the literature for an example of a reaction in which an analogous structure is transformed in the desired way, as is done for more traditional chemical reagents. The ‘database’ of enzymes and microorganisms is now immense and constitutes an enormous, and as yet largely untapped, addition to the arsenal of the synthetic chemist. Recent reviews of the synthetic applications of enzymes provide leading references (Suckling & Wood 1979, Fischli 1980, Jones 1980, 1985, Whitesides & Wong 1983).

Acknowledgements

The generous support of the Natural Sciences and Research Council of Canada, the Atkinson Foundation of Toronto, and Hoffmann-La Roche (Nutley) is gratefully acknowledged.

REFERENCES

HORSE LIVER ALCOHOL DEHYDROGENASE

Francis CJ, Jones JB 1984 Can J Chem, in press
Nakazaki M, Chikamatsu H, Taniguchi M 1982 Horse liver alcohol dehydrogenase (HLADH)
mediated chemicoenzymatic asymmetric synthesis of \((+)-\text{twistanone from cis-decalin-2,7-dione. Chem Lett, p 1761-1764}\)

Ng GSY, Yuan LC, Jakovac IJ, Jones JB 1984 Enzymes in organic synthesis. 29. Preparations of enantiomerically pure \(\text{cis-2,3- and 2,4-dimethyl lactones via horse liver alcohol dehydrogenase-catalyzed oxidations. Tetrahedron 40:1235-1243}\)


Zagalak B, Frey PA, Karabatsos GL, Abeles RH 1966 The stereochemistry of the conversion of \(\text{D and L 1,2-propanediols to propionaldehyde. J Biol Chem 241:3028-3031}\)

DISCUSSION

Cornforth: You have spoken many times of 100% enantiomeric excess (ee) (e.g. Jakovac et al 1982, Bridges et al 1984), and I am sure that for preparative purposes that is correct, but have you explored the precision of that 100%?

Jones: I used 100% because that is the convention many chemists adopt. We measure the enantiomeric excesses by nuclear magnetic resonance (NMR) or by gas chromatography; the precision is 97% for NMR and 99% for gas chromatography. Dave Evans (personal communication 1983) now claims that one can be accurate to one part in 300 with capillary chromatography and certainly that is our initial experience also.

Taylor: Is the level of specificity shown by the horse liver alcohol dehydrogenase (HLADH) also seen in microbial enzymes?

Jones: Microbial enzymes tend to be more specific than the mammalian
enzymes. HLADH is a particularly tolerant enzyme; yeast alcohol dehydrogenase does not have anywhere near the breadth of specificity but, as Charlie Sih has demonstrated, there are fatty acid synthetases in yeast that do have quite broad specificities.

Taylor: Presumably, if you wanted to operate on a slightly larger scale, microbial enzymes would be much more favourable.

Jones: That's right. If you wanted to operate on a large scale, perhaps industrially, you would have to use a fermentation process to solve the cofactor problem, and you would want the specific enzyme or microorganism that did the job on your particular substrate. You would then not be very concerned about the breadth of specificity of the enzyme.

Mosbach: Do you use the pure EE enzyme, the SS isozyme (which takes steroids as substrates) or hybrid ES?

Jones: We use the enzyme as it comes from Sigma because that is the enzyme that chemists are going to use. I have been criticized by biochemists for using an impure product, but since the mixture behaves consistently from year to year it does not really matter. I think the EE isozyme dominates because when the enzyme is purified, most of the SS disappears.

Mosbach: True, but the activity towards steroids comes from the SS isozyme.

Jones: Yes, but we never use steroids as substrates. Another reason why we believe we have the EE form is that the model we use to predict the stereospecificity of all our reactions is based on the results of C.-I. Bränden in Sweden, who has done the X-ray structure of the EE isozyme (Eklund et al 1976).

Suckling: You mentioned factors that should be considered if an enzyme is to be used in synthesis, but you underplayed the importance of the predictability of the reaction. I think that most people would like to think clearly about the outcome of a reaction before throwing in a substrate that might have taken quite a while to make.

Jones: I originally thought that too, and we spent a lot of time developing our cubic-space model of the HLADH active site (Jones & Jakovac 1982). This works well for specificity predictions and is simpler to use than the diamond lattice model of Prelog (1964). But I found that most chemists haven't the slightest interest in it. Instead, they would rather do a half-day experiment to see if the reaction works.

Arigoni: In principle, a model like the one you have developed should be able to predict the stereochemistry of a reaction. With your reaction, some of the products are known compounds but others are new. Would you claim that once you have established the absolute configuration of one product, you can assign configurations to the rest, or would you rather check each case separately? Specifically, you have obtained several β-substituted valerolactones by selective oxidation of the corresponding diols (Fig. 2b, p 7); how were the absolute configurations of these determined?
Jones: They were done painfully, by chemical degradation. In fact, all the absolute configurations I reported have been determined chemically.

Arigoni: Are there any cases where the experimental outcome is the opposite of that predicted by your model?

Jones: Not at the moment, but there is a good reason for that: my students will now not attempt a reaction unless they can see from the model that it is going to work. When there is some ambiguity, they tend to select the substrate in which they have most confidence, so the model becomes self-reinforcing. The model does not seem to work very well for the acyclic examples, the 3-substituted pentanediols. For these, there are so many possible conformations in the active site area in the model that I cannot predict with confidence why the pro-S group should be transformed rather than the pro-R or vice versa. In cases where we do make predictions we may well find in the future that some of these are not fulfilled, and if so we will have to modify the model.

Arigoni: I think that people have taken for granted that, with both yeast and liver alcohol dehydrogenases, if the substrate is an aldehyde the enzyme always gives the same sort of stereochemical result. In at least one case this is not correct, so I would cast a word of caution on this bad habit of extrapolating from other people’s results. The methyl ester of ω-hydroxylauric acid is oxidized by the yeast enzyme with exclusive removal of the pro-R hydrogen; with the liver enzyme the same hydrogen is labilized preferentially but, on equilibration, the pro-S hydrogen is labilized as well, albeit with a somewhat reduced rate (Gautier 1980).

Jones: It is true that a fundamental precept of the model is the pro-R hydrogen extraction of a primary alcohol, but the X-ray structure does bear this out by showing that the binding site is more open on one side than the other. That suggests that an aldehyde should bind in an unique way.

There is also a problem with 2-butanol for which the stereospecificity is just marginally the wrong way (Dutler 1977). Both enantiomers are transformed although this should not happen according to the model.

Sih: The mechanism for alcohol dehydrogenase may vary. Usually, NAD⁺ or NADH binds first and then the substrate comes in, but it has been shown that in some cases the substrate may bind before the pyridine nucleotide. If this happens, and there is a lot of NAD⁺ or NADH available, the commitment to catalysis increases and both R and S forms of the substrate are oxidized or reduced (Sih & Chen 1984). In other words, with this second mechanism, enantioselectivity depends on the concentration of the pyridine nucleotide. At low concentrations there is good enantioselection but at high concentrations there is poor enantioselection.

Arigoni: Are you referring to a specific oxidation, for example of primary alcohols?

Sih: No, I was talking in a general sense.