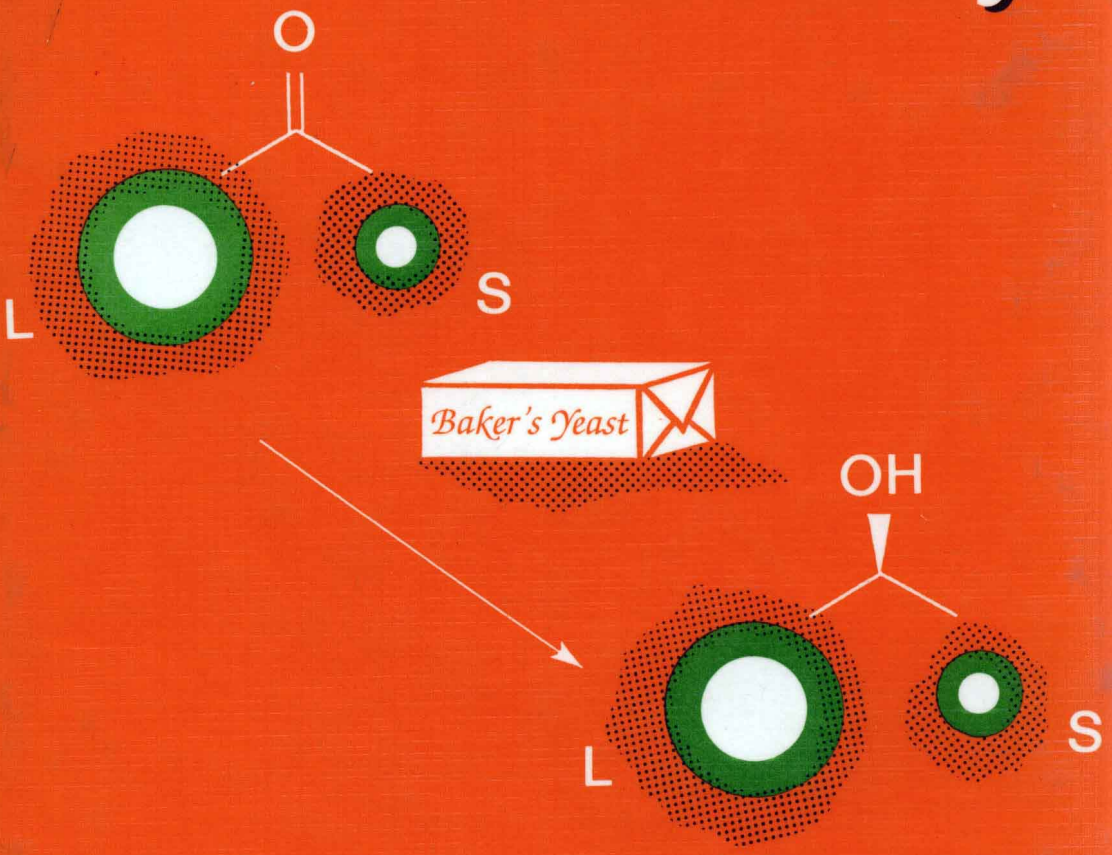
Kurt Faber

Bio-transformations in Organic Chemistry



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Biotransformations in Organic Chemistry

With 31 Figures, 238 Schemes, and 18 Tables

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Preface

The use of natural catalysts - enzymes - for the transformation of non-natural man-made organic compounds is not at all new: they have been used for more than one hundred years, employed either as whole cells, cell organelles or isolated enzymes [1]. Certainly, the object of most of the early research was totally different from that of the present day. Thus the elucidation of biochemical pathways and enzyme mechanisms was in the foreground of the reasearch some decades ago. It was mainly during the 1980s that the enormous potential of applying natural catalysts to transform non-natural organic compounds was recognized. What started as a trend in the late 1970s could almost be called a fashion in synthetic organic chemistry in the 1990s. Although the early euphoria during the 'gold rush' in this field seems to have eased somewhat, there is still no limit to be seen for the future development of such methods. As a result of this extensive, recent research, there have been an estimated 5000 papers published on the subject [2]. To collate these data as a kind of 'super-review' would clearly be an impossible task and, furthermore, such a hypothetical book would be unpalatable for the non-expert.

The point of this volume is to provide a condensed introduction to this field: The text is written from an organic chemist´s viewpoint in order to encourage more 'pure' organic chemists of any level, to take a deep breath and leap over the gap between the 'biochemical' sciences and 'classic organic chemistry' and to make them consider biochemical methods as an additional tool when they are planning the synthesis of an important target molecule. This book may serve as a guide for updating a dusty organic-chemistry curriculum into which biochemical methods should be incorporated. The wide arsenal of classic synthetic methods has not changed radically but it has been significantly widened and enriched due to the appearance of biochemical methods. Certainly, biochemical methods are not superior in a general sense - they are no panacea -, but they definitely represent a powerful synthetic tool to complement other methodology in modern synthetic organic chemistry.

In this book, the main stream of novel developments in biotransformations, which in many cases have already had some impact in organic chemistry, are put to the fore. Other cases, possessing great potential but still having to show their reliability, are mentioned more briefly. The literature covered by this volume extends to the end of 1991. Special credit, however, is given to some very old' papers as well to acknowledge the appearance of novel concepts. References are selected according to the philosophy that 'more is not always better'. Generally, I have attempted to sort out the most useful references from the pack, in order to avoid writing a book with the charm of a telephone directory! Thus special emphasis is placed on reviews and books,which are often mentioned during the early paragraphs of each chapter to facilitate rapid access to a specific field if desired.

I wish to express my deep gratitude to Stanley M. Roberts for undergoing the laborious task of correcting the manuscript of this book and for raising numerous helpful questions and hints. Special thanks also go to Kurt Königsberger for his untiring support in setting up and maintaining a biotransformation database, which served as an indispensable tool for keeping abreast with the literature.

I shall certainly be pleased to receive comments, suggestions and criticism from readers for incorporation in future editions.

Graz, 1992 K. Faber

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Contents

1 Introduction and Background Information

1.1 Introduction

Any exponents of classical organic chemistry will probably hesitate to consider a biochemical solution for one of their synthetic problems. This would be due, very often, to the fact, that biological systems would have to be handled. When growth and maintainance of whole microorganisms is concerned, such hesitation is probably justified: In order to save endless frustrations a close collaboration with a biochemist is highly recommended to set up and use fermentation systems [1]. On the other hand isolated enzymes (which may be obtained increasingly easily from commercial sources either in a crude or partially purified form) can be handled like any other chemical catalyst [2]. Due to the enormous complexity of biochemical reactions compared to the repertoire of classical organic reactions, it follows that most of the methods described will have a strong empirical aspect. This 'black box' approach may not entirely satisfy the scientific purists, but as organic chemists are prone to be rather pragmatists, they may accept that the understanding of a biochemical reaction mechanism is not a *conditio sine qua non* for the success of a biotransformation. In other words, a lack of understanding of biochemical reactions should never deter us from using them, if their usefulness has been established. Notwithstanding, it is undoubtedly an advantage to have an acquaintance with basic biochemistry, and with enzymology, in particular.

1.2 Common Prejudices Against Enzymes

If one uses enzymes for the transformation of non-natural organic compounds, the following prejudices are frequently encountered:

- *'Enzymes are sensitive'.*

This is certainly true for most enzymes if one thinks of applying boiling water, but that also holds for most organic reagents, e.g. butyl lithium. If certain precautions are met, enzymes can be remarkably stable. Some

candidates even can tolerate hostile environments such as temperatures greater than 100 °C and pressures beyond 200 bar [3].

- *'Enzymes are expensive'.*

Some are, but others can be very cheap if they are produced on a reasonable scale. Considering the higher catalytic power of enzymes compared to chemical catalysts, the overall efficiency of a process may be better even if a rather expensive enzyme is required. Moreover, enzymes can be reused if they are immobilized. It should be emphasized that for most chemical reactions relatively crude and thus reasonably priced enzyme preparations are adequate.

- *'Enzymes are only active on their natural substrates'.*

This statement is certainly true for some enzymes, but it is definetely false for the majority of them. The fact, that nature has now developed its own peculiar catalysts over 3 x 10^9 years does not necessarily imply that they are designed to work only on their natural target molecules. As a matter of fact, many enzymes are capable of accepting non-natural substrates of an unrelated structural type and can convert them often exhibiting the same high specificities as for the natural counterparts. It seems to be a general trend, that the more complex the enzyme´s mechanism, the narrower the limit for the acceptability of 'foreign' substrates. It is a remarkable paradox that many enzymes display high specificities for a specific type of reaction while accepting a wide variety of substrate structures.

- *'Enzymes work only in their natural environment'.*

It is generally true that an enzyme displays its highest catalytic power in water, which in turn represents something of a nightmare for the organic chemist if it is there the solvent of choice. Quite recently, some noteworthy rules for conducting biotransformations in organic media have been delineated. Although the activity is usually lower in such an environment, many other advantages can be accrued thus making many processes more effective by using biocatalysts in organic solvents [4].

1.3 Advantages and Disadvantages of Biocatalysts

1.3.1 Advantages of Biocatalysts

- *Enzymes are very efficient catalysts.*

Typically the rates of enzyme-mediated processes are accelerated, compared to those of the corresponding nonenzymatic reactions, by a factor of 10^8. The acceleration may even exceed a value of 10^{12}, which is far above what chemical catalysts are capable of achieving. Generally chemical catalysts are employed in

concentrations of a mole fraction of 0.1 - 1% whereas most enzymatic reactions can be performed at sufficient rates with a mole fraction of 10^{-3} - 10^{-4}% of catalyst, which clearly makes them more effective by some orders of magnitude.

- *Enzymes are environmentally acceptable.*

Unlike heavy metals, for instance, biocatalysts are completely degraded in the environment.

- *Enzymes act under mild conditions.*

Enzymes act in a pH range of about 5-8, typically around 7, and in a temperature range of 20 - 40 °C, preferably at around 30 °C. This minimizes problems of undesired side-reactions such as decomposition, isomerisation, racemisation and rearrangement, which often plague traditional methodology.

- *Enzymes are not bound to their natural rôle.*

They exhibit a high substrate tolerance by accepting a large variety of man-made unnatural substances and they are not specifically required to work in water. If advantageous for a process, the aqueous medium can often be replaced by an organic solvent.

- *Enzymes can catalyse a broad spectrum of reactions.*

Like all catalysts, enzymes only accelerate a reaction, but they have no impact on the position of the thermodynamic equilibrium of the reaction. Thus, in principle, some enzyme-catalysed reactions can be run in both directions.

There is an enzyme-catalysed process equivalent to almost every type of organic reaction [5]: for example

Hydrolysis-synthesis of esters [6], amides [7], lactones [8], acid anhydrides [9], epoxides [10] and nitriles [11].

Oxidation-reduction of alkanes [12], alkenes [13], aromatics [14], alcohols [15], aldehydes and ketones [16, 17], sulphides and sulphoxides [18].

Addition-elimination of water [19], ammonia [20], hydrogen cyanide [21].

Halogenation and dehalogenation [22], alkylation and dealkylation [23], isomerisation [24], acyloin- [25] and aldol reactions [26]. Even Michael-additions have been reported [27].

Some major exceptions, where equivalent reaction types cannot be found in nature are the Diels-Alder reaction and the Cope-rearrangement, although [3,3]-sigmatropic rearrangements such as the Claisen-rearrangement are known. On the other hand, some biocatalysts can accomplish reactions impossible to emulate in organic chemistry e.g. the selective functionalization of ostensibly non-activated positions in organic molecules.

Enzymes display three major types of selectivities:

- *Chemoselectivity*

Since the purpose of an enzyme is to act on a single type of functional group, other sensitive functionalities, which would normally react to a certain extent under chemical catalysis, survive. For instance, enzymatic ester hydrolysis does not show any propensity for acetal-cleavage.

- *Regioselectivity and Diastereoselectivity*

Due to their complex three-dimensional structure, enzymes may distinguish between functional groups, which are chemically situated in different regions of the same substrate molecule [28].

- *Enantioselectivity*

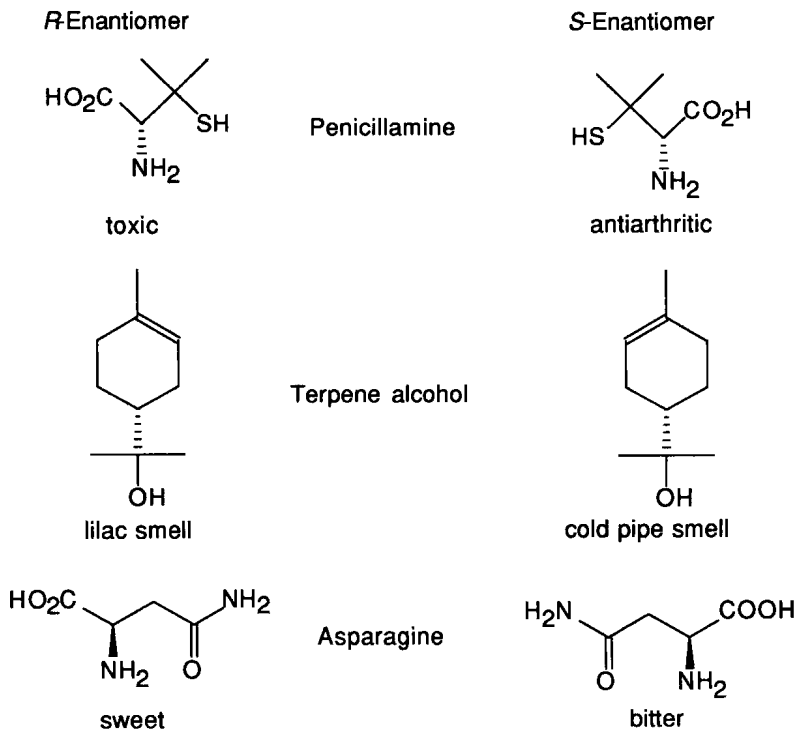
Last but not least, all enzymes are made from L-amino acids and thus are chiral catalysts. As a consequence, any type of chirality present in the substrate molecule is 'recognized' upon the formation of the enzyme-substrate complex. Thus a prochiral substrate may be transformed into an optically active product and both enantiomers of a racemic substrate may react at different rates, affording a kinetic resolution. These latter properties collectively constitute the 'specificity' of an enzyme and represent its most important feature for selective and asymmetric exploitation [29].

All the major biochemical events taking place in an organism are governed by enzymes. Since the majority of them are highly selective with respect to the chirality of a substrate, it is obvious that the enantiomers of a given bioactive compound such as a pharmaceutical or an agrochemical cause different biological effects. Consequently, they must be regarded as two distinct species. The isomer with the highest activity is denoted as the 'eutomer', whereas its enantiomeric counterpart possessing less or even undesired activities is termed as the 'distomer'. The range of effects derived from the distomer can extend from lower (although positive) activity, none or toxic events. The ratio of the activities of both enantiomers is defined as the 'eudismic ratio'. Some representative examples of different biological effects are given in Scheme 1.1.

As a consequence, racemates of pharmaceuticals and agrochemicals should be regarded with suspicion. Quite astonishingly, 88% of the 480 chiral synthetic drugs on the market were sold in racemic form in 1982 while the respective situation in the field of pesticides was even worse (92% racemic out of 480 chiral agents) [30]. Although at present most bioactive agents are still applied as racemates mainly due to economic reasons, this situation will

definitely change due to the legislation pressure. This leads to an increased need for enantiomerically pure compounds [31].

Scheme 1.1. Biological effects of enantiomers.



Unfortunately, less than 10% of organic compounds crystallize as a conglomerate (the remainder form racemic crystals) offering the possibility of separating their enantiomers by simple crystallisation techniques - such as by seeding a supersaturated solution of the racemate with crystals of one pure enantiomer.

The principle of asymmetric synthesis [32] makes use of enantiomerically pure auxiliary reagents which are used in catalytic or sometimes even stoicheometric amounts. They are often expensive and cannot be recovered in many cases.

Likewise, starting a synthesis with an enantiomerically pure compound which has been selected from the large stock of enantiomerically pure natural compounds [33] such as carbohydrates, amino acids, terpenes or steroids - the so-called 'chiral pool' -, has its limitations. According to a survey from 1984 [34] only about 10 - 20% of compounds are available from the chiral pool at an

affordable price (in the range of 100 - 250 US$ per kg). Considering the above mentioned problems with the alternative ways of obtaining enantiomerically pure compounds, it is obvious that enzymatic methods represent a valuable kit for the already existing toolbox available for the asymmetric synthesis of fine chemicals.

1.3.2 Disadvantages of Biocatalysts

There are certainly some drawbacks worthy of mention for a chemist using biocatalysts:

- *Enzymes are provided by Nature in only one enantiomeric form.*

Since there is no way of creating mirror-imaged enzymes from D-amino acids, it is impossible to invert an undesired direction of chirality of a given enzymatic reaction by choosing the 'other enantiomer' of the biocatalyst, a strategy which is possible if chiral chemical catalysts are involved. To gain access to the other enantiomeric product, one has to walk a long path in search of an enzyme with exactly the opposite stereochemical selectivities. However, this is sometimes possible.

- *Enzymes require narrow operation parameters.*

The obvious advantage of working under mild reaction conditions can sometimes turn into a drawback: If a reaction proceeds only slowly under given parameters of temperature or pH, there is only a narrow scope for alteration. Elevated temperatures as well as extreme pH lead to deactivation of the protein as do high salt concentrations. The usual technique of lowering the reaction temperature in order to gain an increase in selectivity is of limited use with enzymatic transformations. The narrow temperature range for the operation of enzymes prevents radical changes, although positive effects from small changes have been reported [35]. Quite astonishingly, some of them remain catalytically active even in ice [36].

- *Enzymes display their highest catalytic activity in water.*

Due to its high boiling point and high heat of vaporization, water is usually the least desired solvent of choice for most organic reactions. Furthermore, the majority of organic compounds are only poorly soluble in aqueous media. Thus shifting enzymatic reactions from an aqueous to an organic medium would be highly desired, but the unavoidable price one has to pay is usually some loss of activity [37].

- *Enzymes are prone to inhibition phenomena.*

Many enzymatic reactions are prone to substrate or product inhibition, which causes the enzyme to cease to work at higher substrate and/or product

concentrations, a factor which limits the efficiency of the process. Whereas substrate inhibition can be circumvented comparatively easily by keeping the substrate concentration at a low level through continuous addition, product inhibition is a more complicated problem. The gradual removal of product by physical means is usually difficult as is the engagement of another step to the reaction sequence in order to effect a chemical removal of the product.

- *Enzymes may cause allergies.*

Enzymes may cause allergic reactions. However, this may be minimized if enzymes are regarded as chemicals and handled with the same care.

1.3.3 Isolated Enzymes versus Whole Cell Systems

The physical state of biocatalysts which are used for biotransformations can be very different. The final decision, as to whether one should use isolated, more or less purified enzymes or whole microorganisms - either in a free or immobilized form - depends on many factors, such as (i) the type of reaction, (ii) if there are cofactors to be recycled and (iii) the scale in which the biotransformation has to be performed. The general pros and cons of the situation are outlined in Table 1.1.

A whole section of biochemistry, microbiology and biochemical engineering - biotechnology- has led to the development of routes to a lot of speciality chemicals (ranging from amino acids to penicillins) starting from cheap carbon sources (such as carbohydrates), cocktail of salts and using viable whole cells. Such syntheses requiring a multitude of biochemical steps are usually referred to as 'fermentation' processes since they constitute de novo syntheses in a biological sense. In contrast, the majority of microbially mediated biotransformations, often starting from relatively complex organic molecules, makes use of only a single (or a few) biochemical synthetic step(s) by using (or rather 'abusing'!) the microbe's enzymatic potential to convert a non-natural organic compound into a desired product. To distinguish these latter processes from typical fermentations where a multitude of enzymes are involved, the term 'enzymation' is often used.

Table 1.1. Pros and cons of using isolated enzymes and whole cell systems.

System	Form	Pros	Cons
isolated enzymes	any	simple apparatus, simple work-up, better productivity due to higher concentration tolerance	cofactor recycling necessary
	dissolved in water	high enzyme activities	side reactions possible, lipophilic substrates insoluble, workup requires extraction
	suspended in organic solvents	easy to perform, easy work-up, lipophilic substrates soluble, enzyme recovery easy	low activities
	immobilized	enzyme recovery easy	loss of activity during immobilization
whole cells	any	expensive equipment, tedious work-up due to large volumes, low productivity due to lower concentration tolerance, low tolerance of organic solvents, side reactions likely due to metabolism	no cofactor recycling necessary
	growing culture	higher activities	large biomass, more by-products
	resting cells	workup easier, fewer by-products	lower activities
	immobilized cells	cell reuse possible	lower activities

1.4 Enzyme Properties and Nomenclature

The polyamide chain of an enzyme is kept in a three-dimensional structure - the one with the lowest ΔG [38] - believed to be determined by its primary sequence and called the 'primary structure'. For an organic chemist it may roughly be compared with a ball of yarn. Due to the natural aqueous environment, the hydrophilic polar groups such as -COOH, -OH, $-NH_2$, -SH,