Introduction

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The two years since the publication of Volume 8a have been a period of consolidation for the business community. As large chemical and pharmaceutical companies have merged into ever larger companies with increasing focus on their primary mission (the "best of breed" concept), small companies specializing in biotransformations have prospered, by providing expertise on demand. The lack of familiarity of most synthetic chemists with biotransformations, has in fact worked to the advantage of these small companies. Increasing demands for enantiomerically pure materials will provide further fuel for this trend. Many medium sized, fine and speciality chemical manufacturers are adding biotransformations to their repertoire of skills by acquisitions or internal developments. Much of the impetus in the speciality area, is the demand for so called natural or "nature identical" products.

On the other hand the spectre of genetic modification (GM) has undoubtedly acted as a brake on the development of many new products and processes. It remains to be seen what the public and legislators will tolerate. Attempts to stop the growing of GM crops and banish foodstuffs from GM organisms from the human food chain, lie outside the ambit of biotransformations. But regulation of these activities may well have a "knock on effect". For example enzymes derived from GM organisms, are used in the beverage industry for dehazing and clarification. Will these be regulated? Hydrolytic enzymes from GM organisms are used in laundry. Will non-foodstuff uses escape regulation?

On the academic front, enantioselective reactions which were once the sole preserve of enzymes are now being encroached upon by abiotic catalysts. Two good examples are the Baeyer–Villiger reaction and ester formation/hydrolysis. Conversely the first enzymes and catalytic antibodies capable of catalyzing pericyclic processes such as the Diels–Alder reaction are now emerging.

Volume 8b commences with the cornerstone of organic synthesis: carbon–carbon bond formation. Although there is no enzymatic equivalent of the Grignard or organolithium reagent, aldol and benzoin condensations can be performed with exquisite stereochemical control. These reactions are constrained by a limited range of anionic components, but are fairly promiscuous with electrophiles. Lyases (Chapter 2) are involved in a galaxy of reactions, which includes just about every prosthetic group/cofactor known or unknown. In connection with the latter, it has been discovered in

the last year, that histidine ammonia-lyase which was long thought to contain a catalytically active dehydroalanine residue, actually contains an unprecedented 4-methylene-imidazol-5-one residue. This result throws doubt on the presence of the dehydroalanine residue in other enzymes, thought to contain it. Chapter 2 covers carboxy-lyases (many of which are involved in carbon–carbon bond formation), hydro-lyases (including the intricate biosynthesis of deoxysugars) and the ammonia-lyases many of which are involved in the biosynthesis of amino acids. Halocompounds (Chapter 3) have a reputation as un-natural compounds which are persistent and damaging to the environment or at best as exotic marine natural products. This reputation is unfounded. The majority of halocompounds in the environment originate from eological and natural sources. The fascinating topic of the biosynthesis of the ten known natural organofluorocompounds provides an insight into an element largely spurned by nature. The cleavage and formation of phosphate bonds (Chapter 4) is neglected in most biotransformations reviews. Yet for most phosphorylated and pyrophosphorylated cofactors the majority of the binding energy to the enzyme originates from this linkage. Whereas, the traditional organic chemist utilizes halosubstituents as nucleofuges, nature utilizes phosphates and their derivatives, because they can be selectively activated by carefully placed protonation sites.

Short polypeptide, RNA, and DNA sequences (<18 residues) are now routinely synthesized using automated machinery. In contrast, the synthesis of even a trisaccharide requires a dedicated team of specialists and although spectacular advances have been made, a general approach to the controlled synthesis of polysaccharides is still lacking. Simultaneous control of stereo- and regioselectivity have confounded conventional chemical approaches. Enzymatic glycosidation (Chapter 5) provides a powerful approach to escape this impasse.

The "Applications" superchapter covers selected industrial scale processes (commissioned by Dr. JÖRG PETERS) and the use of biotransformation products as intermediates for organic synthesis. Chapter 6 reviews general aspects of industrial biotransformations and banishes some myths about their limitations. The carbohydrate theme of chapter 5 is continued in chapter 7, which describes the development of the microbial oxidation of aminosorbitol, in the synthesis of 1-deoxynojirimycin, a key precursor of the anti-diabetes drug Miglitol®. This is an important example of a synthetic route, which is fast and efficient, because the biotransformation step removes the need to employ protecting groups to enforce regioselectivity.

The majority of amino acids are manufactured by fermentation, although there are also examples of amino acids produced employing isolated enzymes (e.g., L-*tert*. leucine). Developments in molecular biology and genome manipulation have enabled biosynthetic pathways to be optimized to previously unimaginable levels (Chapter 8). This area has been further promoted by the continuing demand for the dipeptide artificial sweetener, Aspartame.

Many of the important natural flavorings or fragrances are extracted in small amounts from exotic plants and the supply is affected by seasonal variations due to the weather, crop pests, and even political factors. Although many flavorings and fragrances can be synthesized via chemical routes, the resulting products cannot be marketed using the term "natural", since EU and US legislation has defined the term "natural": The product must be produced from natural starting materials applying physical, enzymatic, or microbial processes for their final production. These are ideal circumstances for the development of biotransformation routes, described in Chapter 9. For example, potential precursors of vanillin are found in numerous plants and even waste products. Biotransformations can convert these materials with low or even negative value (i.e., disposal costs) into valuable products.

Biotransformations have yielded a wealth of new routes to novel and known compounds. These have provided marvellous opportunities for synthetic organic chemists. Chapter 10 gives brief descriptions of an enormous range of biotransformations and describes applications of the products in the synthesis of natural products.

The final superchapter in Volume 8b provides a glimpse into two areas which are likely to play a major role in future developments in

biotransformations. Catalytic antibodies (Chapter 11) are literally artificial enzymes. They can be engineered to catalyze reactions which have no counterparts in the natural world. Sophisticated strategies for raising antibodies and screening techniques for identifying active catalysts, have already yielded catalysts of exquisite selectivity. Intrinsic activity and turnover continue to be limited, but in the best cases they are only a few orders of magnitude lower than conventional enzymes.

The final chapter deals with wholly synthetic enzymes. These may be constructed from the familiar amino acid building blocks of conventional enzymes and may even borrow design features, but the structures are, nevertheless, totally alien. For example, poly-leucine catalyzes the enantioselective epoxidation of enones by hydrogen peroxide and cyclic dipeptides catalyze the enantioselective addition of cyanide to aldehydes. This area will surely benefit from the ever continuing improvements in computer processing power and new software for molecular modeling.

All predictions of the future have one thing in common. They are wrong! But this does not absolve us from trying to discern avenues that are ripe for development. A Third Edition of this volume is likely to contain a wider range of reactions (possibly including pericyclic and photochemical processes), more engineered pathways and more site directed mutagenesis. Gene transfer and heterologous expression is now common place. Are there better recipient organisms than the ubiquitous *E. coli*? Enzymes from thermophiles were touted as the universal panacea for industrial processes at elevated temperatures, but their early promise has not been fulfilled. Why? Enzyme catalyzed reactions in organic solvents are highly desirable for hydrophobic substrates, but are currently thwarted by low activity. Is it possible to design and synthesize an enzyme which is soluble in organic solvents (e.g., a lipase mutant)? These proposals represent reasonable extensions of current technology. However, it is certain that the most spectacular advances will be made in areas, that cannot be predicted at present.

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