DRUG DESIGN

edited by E. J. Ariëns

Volume III

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DEPARTMENT OF PHARMACOLOGY
UNIVERSITY OF NIJMEGEN
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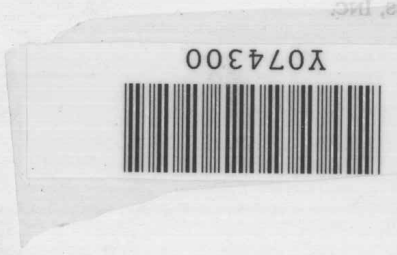

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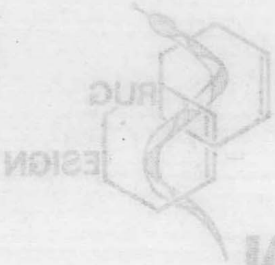

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Preface

Drug design requires cooperation of researchers in fundamental and applied science. Researchers in the drug industry play a most important role in this field. They must convert the requirements for specific bioactive compounds of medicine, agriculture, and of everyday life into workable principles, recognize the potentials arising from basic studies, and integrate them in research and development programs having definite restricted goals. Hopefully the volumes of this treatise will help by presenting surveys of our knowledge on and insight into the pharmacology and medicinal chemistry of various groups of bioactive compounds and by indicating or outlining research programs that have led to or may lead to specific objectives. Volume IV which will be devoted chiefly to the design of drug application forms is in preparation.

The fact that a number of industrial investigators have been willing to contribute to this series of volumes is greatly appreciated.

E. J. ARIËNS

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Contents

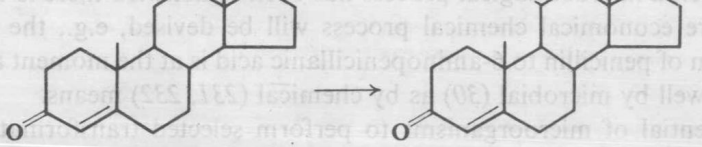
Chapter 1 Microbial Conversion as a Tool in the Preparation of Drugs

R. Beukers, A. F. Marx, and M. H. J. Zuidweg

I. Introduction

> In the course of a program designed to study microbial transformations of steroids and sterols, it was discovered that the triterpenoid acid, eburicoic acid, was converted by the fungus *Glomerella fusarioides* to 3,4-seco-$\Delta^{8,24(28)}$-eburicadien-4-ol-3,21-dioic acid, a *new compound with antibacterial properties* (*251*).
>
> In the course of a routine screening procedure several microorganisms were found to oxidize the 4-methyl group of Miracil D. A preferred microorganism is *Aspergillus sclerotiorum*. Conversion products were the 4-hydroxymethyl analog (Hycanthone), the 4-carboxaldehyde and the corresponding carboxylic acid (*359*).*

The quotations above are from only two of a large number of reports that illustrate the use of microorganisms in the preparation of new compounds with biological activity. Such publications have appeared since 1952, when Peterson and Murray discovered that a strain of the fungus *Rhizopus arrhizus* could be used to convert progesterone into 11α-hydroxyprogesterone in a 50% yield (*336*). From the latter substance cortisone may be synthesized in



good yield. The introduction of an 11α-hydroxyl group is chemically very difficult. The synthesis of cortisone used at that time started with substances with a 12-oxygen function, cholic acid or hecogenine (*113*), and required many reaction steps with a moderate overall yield.

The discovery of Peterson and Murray has initiated an extensive screening

for other microorganisms with similar activity. At the moment the mold *Rhizopus nigricans* is used for this hydroxylation with a yield of over 90%.

Screening programs have since been extended to obtain other valuable microbial conversions leading to products already known or to new compounds with useful biological activity. A series of papers dealing with the conversion of 19-nortestosterone may serve as an illustration of a study to discover new drugs (*43*, *116–120*, *257*).

The utilization of microorganisms to carry out a certain transformation may have decisive advantages when compared with the chemical methods.

Microbial transformations, which are essentially enzyme reactions, are very selective in nature, often specific and well suited to obtain special conformations. A microbial process, if available, can be superior to a chemical one when a modification of only one of several similar substituents is required or when a stereospecific conversion is wanted. The number of reaction steps may be much smaller and in some cases a combination of two or more reactions can be performed by the same organism. The conversion takes place under very mild conditions and may have a high yield.

Chemical processes, on the other hand, are as a rule easier to handle and require less complicated equipment. The fermentation technique is quite expensive, implying the necessity of a high yield of conversion and a minimal production of side products. The concentration of the substrate, for the same reason, is bound to a certain minimal limit.

The way from the discovery of an organism suitable to carry out the desired transformation to an industrial process can be very long and tedious. Most cases never even reach the pilot plant, mainly because the process is not economical. The organism that is selected initially often has not the right combination of properties. A selection or mutation to strains with improved qualities is then unavoidable. A number of procedures are available (*153*).

Even when a microbiological process has been established there is the risk that a more economical chemical process will be devised, e.g., the partial degradation of penicillin to 6-aminopenicillanic acid is at the moment accomplished as well by microbial (*30*) as by chemical (*231*, *232*) means.

The potential of microorganisms to perform selected transformations is almost unlimited. No wonder the use of microbial systems to achieve desired changes has a very long history. Records dating from 3,000 B.C. are available to show that ancient civilizations, unknowingly, made use of yeasts to convert the sugar moiety of certain plant materials into ethanol in the production of intoxicating liquors. The scientific explanation of these processes had to wait till 1857, when Pasteur published his famous paper on the nature of fermentations (*330*).

Following this discovery, attempts to obtain desirable changes in substrate molecules have been made by many investigators.

Boutroux in 1880 succeeded in a conversion of glucose into gluconic acid (*46*), Brown obtained propionic acid from *n*-propanol, and fructose from mannitol (*52*). In 1896 Bertrand discovered the important conversion of sorbitol into sorbose (*37*) and in 1898 the formation of dihydroxyacetone from glycerol (*38*, *39*). Most of these conversions are still being applied on an industrial scale. Numerous microbial transformations have since been described (*464*).

The discovery of the antibiotics initiated a rapid development of the fermentation industry. When it was then found that in certain cases microorganisms could be used to accomplish valuable transformations the application on large scale proved feasible.

This chapter will deal with those microbial transformations that have been used in the preparation of drugs or closely related substances. It will be impossible to give a complete compilation. The most important or interesting conversions will be mentioned, arranged according to the chemical type of the reaction (*464*), and illustrated by some examples and recent publications. Only those reactions will be considered that represent a relatively small change in the substrate molecule. The product of the conversion must have been isolated and identified. This implies that hypothetical intermediates in degradative or biosynthetic pathways will not be mentioned.

A number of reviews on microbial conversions have been included in the list of references (*9*, *71*, *74*, *145*, *197*, *222*, *223*, *298*, *349*, *420*, *425*, *459*, *500*, *501*, *505*).

II. Practical Aspects of Microbial Transformations

A. Requirements

A microbial transformation proceeds as a result of the catalytic action of the biocatalysts, i.e., the enzymes. In many cases the combined enzyme activities of a microorganism lead to a complete breakdown of the substrate. An organism is suited to yield a particular product if two requirements are fulfilled: (a) the presence of the enzyme or enzymes catalyzing the desired transformation and, (b) the absence or suppression of the activity of enzymes catalyzing further conversion of the product. Various possibilities exist to prevent the unwanted reactions: inhibition of certain enzymes by special agents, chemical modification of the substrate or mutation of the organism. Examples of each possibility will be given in Section III,B.

The majority of enzymes catalyzing transformations are found inside the cell or bound to the outer membrane. This applies in particular for those enzymes that possess a requirement for a cofactor, i.e., enzymes catalyzing