CHIMIA 50 (1996) Nr. 9 (September)

nates of the native wild-type apoenzyme (from *H. Holden, U. Wisconsin*) and modeling of important features at the active site suggest critical attributes that can be further modified to continue the rational design of this enzyme for bioremediation of pesticide contaminants and CWA stockpiles. OPH is one of the few enzymes which has been shown to be capable of hydrolyzing the P–S bond of various OP pesticides; however, it possesses a wide range of catalytic rates (0.0067–167 s^{-1}). Nonetheless, it has been possible to enhance the unique P–S bond hydrolysis of this enzyme by selecting specific changes in the amino acids bordering the active site. Thus, it appears that the capacity for further improvement is remarkable, and the opportunity for a variety of biotechnical applications from the development of transgenic soil fungi and plants to whole cell hydrolysis in slurry-bed bioreacter systems to air stream purging and development of neurotoxin-specific bioreactors (see references) is quite pronounced.

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Chimia 50 (1996) 431–432

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Biocatalysis and Process Integration in the Synthesis of Semi-synthetic Antibiotics

Alle Bruggink*

Introduction

The fine chemical industry is one of the industry segments where the impact of biocatalysis is felt most profoundly. Possible explanations are [1][2]:

- the need to replace traditional, stoichiometric processes in order to improve the product to waste ratio,
- the failure to translate chemocatalytic processes from petrochemicals to fine chemicals,
- the ready acceptance of enzymes by the organic chemist as part of his toolbox, whereas organic chemistry is still the dominating discipline in fine chemical industry [3],
- the low entry barrier, *i.e.* low investments, for new technologies in this small scale industry, facilitated by the fact that fine chemical companies become increasingly part of larger industrial conglomerates.

Synthesis of Semi-synthetic Antibiotics

The industrial production of semi-synthetic antibiotics, with a history of some 30 years, is an outstanding example of the development of biocatalysis. Cefalexin, with an annual consumption of almost 2000 t, the largest cephalosporin on the world market, serves as a useful illustration. The original synthesis (see *Fig.*) starting from benzaldehyde and fermentation of penicillin G was a ten-step process employing stoichiometric chemistry only and causing a waste stream of 30–40 kg per kg of end product. Often 4–6 different companies were involved to serve the chain from basic raw materials to bulk drug; nowadays one or two companies cover the full production column.

The *Chemferm* Process

In the *Chemferm* process (*Fig.*) only six steps are needed, whereby biocatalysis is involved in three of them [4]; a major improvement through the eyes of the organic chemist [5]. However, when it comes to the design of the production plant for the final coupling step, the engineers are faced with an equilibrium process requiring recycle of starting materials and handling of many solids:

- crystallization and isolation of the desired cefalexin,
- crystallization and recovery of excess 7-ADCA (= 7-aminodeacetoxycephalosporanic acid),
- crystallization and isolation of phenylglycine from undesired hydrolysis of both end product and side-chain precursor.

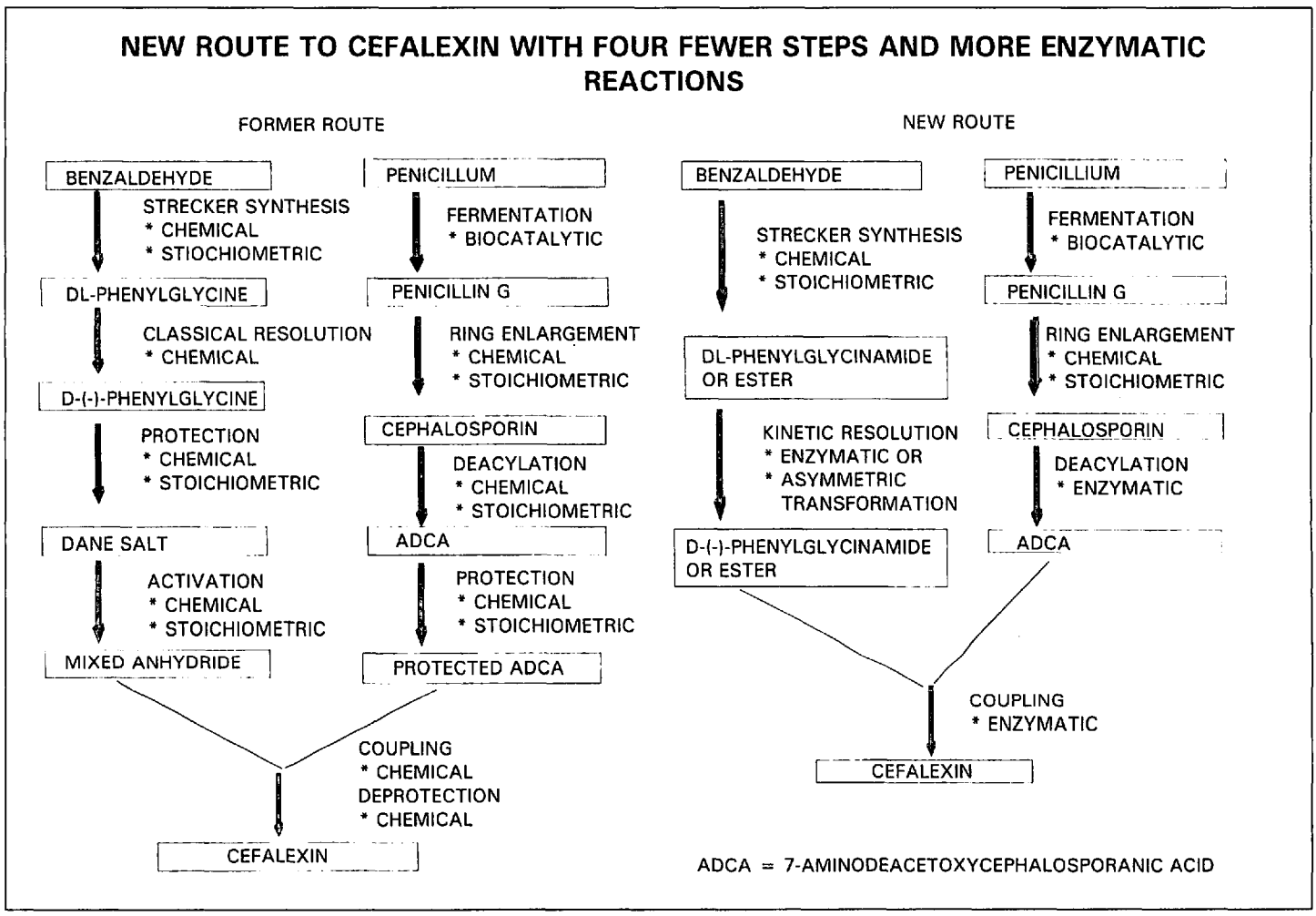
So far, in our developments at *Chemferm*, the environment has been the main winner. Only aqueous waste streams containing some simple inorganic salts are produced, whereas the traditional process releases methylene chloride and other solvents and needs stoichiometric amounts of silylating agents, *Dane*-salt-protected side chains and acylating promotors (such as pivaloyl chloride) which all end up as waste.

The NOVO Process

An alternative process was developed by NOVO, acquired and further improved by *Chemferm*. Using β-naphthol as complexing agent, which surprisingly is compatible with the enzymatic condensation conditions, an almost quantitative yield of

*Correspondence: Prof. Dr. A. Bruggink[a][b])
[a]) *Chemferm, Industrial Pharmaceuticals*
P.O. Box 3933
NL-4800 DX Breda
[b]) University of Nijmegen
Department of Organic Chemistry
Toernooiveld
NL-6525 ED Nijmegen

CHIMIA 50 (1996) Nr. 9 (September)



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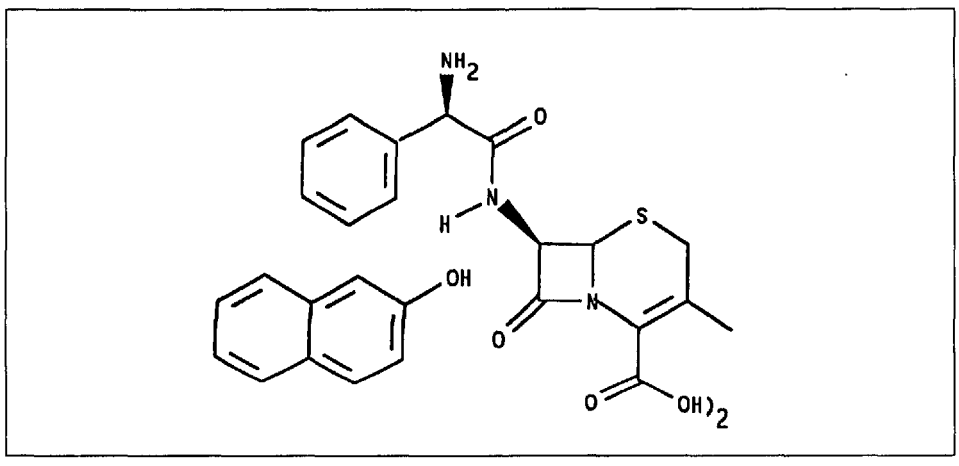

by eliminating organic waste. The overall production process, however, is more complicated, because of several recycle streams and a large number of solids to be handled.

A better understanding of enzyme action on a molecular level and a rational design of improved biocatalysts (including immobilization) are needed to reach more ideal processes where recycles are eliminated. Also, more adequate downstream processing including, *e.g.* selective absorption of end products through modern molecular recognition techniques, are required to achieve feasable processes at plant scale.

the 2:1 cefalexin-β-naphthol complex is obtained [6].

Again, from a synthetic point of view, an excellent process, but once more the chemical engineers have to solve a few tough separation and recycle problems:

- separation of phenylglycine from the cefalexin-β-naphthol complex,
- isolation of cefalexin, free of β-naphthol, from the complex,
- isolation and recycling of β-naphthol.

All this can be done, but a very careful optimization of all process parameters is required and extra investments in equipment for handling solids can not be avoided.

Conclusions

Integration of biocatalysis in the synthesis of s.s. antibiotics, *i.e.* cefalexin, greatly reduces the number of steps and is of considerable benefit to the environment

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