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**Review**

# THE BIOSYNTHESIS OF SOME ß-LACTAM ANTIBIOTICS

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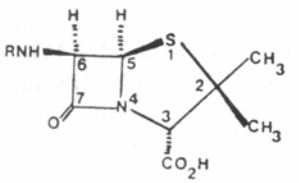
The biosynthesis of the B-lactam antibiotics with particular emphasis on developments of the past several years. Considerable progress has been made especially in recent years, in the elucidation of the detailed pathways taken for the biosynthesis of such compounds. However, major problems in this field remain unsolved. In particular the basic mechanism for the formation of bicyclic ring system remains unknown, although several proposed mechanisms have recently been shown to be untenable.

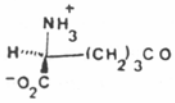
The discussion in this review will be limited to those naturally occurring B-lactam compounds which are derivatives of either the penicillin (penam) ring system Figure 1, or the cephalosporin (3-cephem) ring system, Figure 2.



1 a. R = $PhCO_2H$

b. R = $PhOCH_2CO$

c. R = H

d. R = 

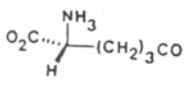
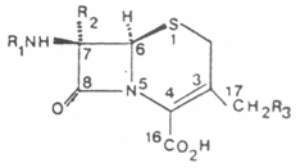
e. R = 

Fig.1



| 2 | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| a. | D-α-aminoadipyl | H | OAc |
| b. | D-α-aminoadipyl | H | OH |
| c. | H | H | OAc |
| d. | D-α-aminoadipyl | $OCH_3$ | OAc |
| e. | D-α-aminoadipyl | H | $OCONH_2$ |
| f. | D-α-aminoadipyl | $OCH_3$ | $OCONH_2$ |
| g. | D-α-aminoadipyl | H | H |

Fig.2

The penicillins, produced by various species of penicillium, especially P.chrysogenum, as well as other species of fungi (LEMKE and BRANNON, 1972), all possess the same bicyclic ring system, Figure 1, consisting of a B-lactam ring cis-fused to a thiazolidine ring. Over one hundred penicillins, differing only in the nature of an N-acyl side chain, can be produced by fermentations to which an appropriate side chain precursor has been added (BEHRENS, 1949). Aliphatic or aryl-substituted aliphatic carboxylic acids or analogues which can easily generate such acids in vivo constitute acceptable side-chain precursors. However, of the many penicillins producable biosynthetically,only benzylpenicillin (penicillin G) Figure 1a and phenoxymethylpenicillin (penicillin V) Figure 1b are great clinical utility (LEMKE and BRANNON, 1972). In the absence of a suitable side-chain precursor, fermentation of P.chrysogenum leads to the formation of a 6-aminopenicillanic acid Figure lc and isopenicillin N, Figure 1d (FLYNN *et. al*, 1962). The former compound (6-APA) is an important intermediate in the preparation of the so-called semi-synthetic penicillins, produced by acylation of the free amino group.

A close relative of isopenicillin N, namely penicillin N Figure 1e, was isolated (CRAWFORD *et al.*, 1952, ABRAHAM *el.* at, 1953, ABRAHAM and NEWTON, 1954) in 1952 from culture of *Cephalosporin* sp. which was isolated from seawater near outflow of a Sardinian Sewer. This antibiotic has subsequently been isolated from other related species (LEMKE and BRANNON, 1972), especially C. acremonium. No other penicillins are produced by species which synthesize penicillin N and side-chain precursors which are easily incorporated into penicillins by P. chrysogenum are not utilized (ARNSTEIN, 1958).

Later, a new antibiotic, Cephalosporin C was isolated (NEWTON and ABRAHAM, 1955, NEWTON and ABRAHAM, 1956, ABRAHAM and NEWTON, 1961) from C. acremonium, and shown to have structure Figure 2a (HODGKIN and MASLEN, 1961, ABRAHAM, 1962). Deacetylcephalosporin C Figure 2b, also is produced by C. acremonium (JEFFERY *et. al*, 1961, HUBER *et. al.*, 1972). These compounds were naturally produced examples of the 3-cephem system. The α--aminoadipyl side chain has the D-configuration in common with penicillin N. No other side-chain analogues have been produced biosynthetically, and 7-aminocephalosporanic acid (7-ACA) Figure 2c, is not formed by C. acremonium, in contrast to the biosynthetic abilities of P. chrysogenum. However, 7-ACA can be prepared chemically or biologically from cephalosporin C and serves as a precursor for the preparation of some valuable antibiotics (ADRIENS *et. al*, 1975). One of the problems in this approach has been the relatively low yield of cephalosporin C produced in cultures, as compared with the yields of penicillins by P. chrysogenum. This problem has therefore stimulated extensive research on the development of synthetic routes for converting the readily available penicillins into analogues having the 3-cephem ring system. This goal has now been accomplished (NAGARAJAN *et al*, 1971).

Recently several analogues of cephalosporin C, Figure 2d, Figure 2e and Figure 2f were isolated (BEHRENS *et. al*, 1948) from various species of Streptomyces. They differ from cephalosporin C having a 7α methoxy group and/or carbamoyl group in place of acetoxy group.

Biosynthetic studies on penicillin were started in the 1940s as a part of mas-

sive international collaborative efforts on the development of penicillin as an antibiotic (BEHRENS, 1949). The main objectives of biosynthetic work were the improvement o f the yield o f penicillin by fermentation methods and the possible production of new penicillins by adding appropriate precursors to the culture. Both objectives of this work were reached to some extent and a large number of new penicillins were produced having modified N-acyl side chains. However, penicillins structurally modified in the penam ring system were not obtained. The studies were conducted by observing the effect on penicillin yield of adding to the cultures various putative biosynthetic precursors (BEHRENS *et. al*., 1948, BEHRENS *et. al*., 1948, BEHRENS, 1948, HOCKENHULL *et. al.,* 1949). Unfortunately the results are rather misleading from the point o f view of elucidating the nature of the direct, in vivo precursors of penicillin. As pointed out by Behrens (SUS, 1950) stimulation of penicillin production may result, not from direct incorporation of the added nutrient, but through its inter action in some indirect manner with the microbial metabolism. For example, the addition of L-cysteine, now accepted as a direct precursor of penicillin, reduced the yield of penicillin in these studies. Similarly DL-valine did not stimulate penicillin production and higher concentrations of DL-valine were inhibitory, although valine is now accepted as a direct penicillin precursor. Thus the approach of observing the increase or decrease in penicillin yields in cultures of P. chrysogenum is not a valid method for evaluating biosynthetic precursors or intermediates although it has been used in a distressing number of investigations in this held.

Examination of the structures of both the penicillin and the cephalosporins suggests as a working hypothesis the assumption that their ring systems could be formed from the carbon skeletons of valine and cysteine as in Figure 3 and Figure 4. In both types of B-lactam antibiotic the cysteine part of the molecules has a configuration in common with that of L-cysteinc. In penicillin the valine part of the molecule has a configuration like that of D-valine whereas in the cephalosporins, the configuration all identity of the valine part is lost.

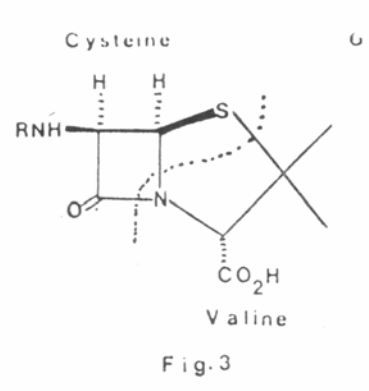



Fig. 3

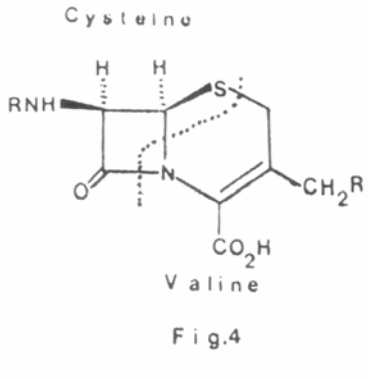



Fig.4

In an attempt to demonstrate the direct incorportion of valine as well as phenylacetyl derivatives into bezsylpenicillin,, Behrens *et al.* prepared deuterophenyl acetyl-15N-DL-valine Figure 5, having 41% of the phenyl group hydrogens replaced by deuterium and containing 32 atom per cent 15N.

Upon incubation of Figure 5 with P. chrysogenum, benzylpenicillin Figure 1a was isolated. The labelling results indicated that 92% of the benzyl group in Figure 1a had been formed from the added precursor, but very little of the 15N was incorporated. The use of Figure 5 as precursor is rather curious, since it is clear from the structure of Figure 1 that Figure 5 would have to under gosome kind of fragmentation, probably to phenylacetate and valine before either could be incorporated into benzylpenicillin. Thus the incorporation experiment could have been done simply by using a mixture of deuterated phenylacetic acid and 15N-valine.

Following a suggestion by Hockenhull (HOCKENHULL *et. al*, 1949), Sus (SUS, 1950) and later Stevens et al. (STEVENS *et. al*, 1954) investigated the possibility that the thioether Figure 6 might be a penicillin precursor. In the experiments

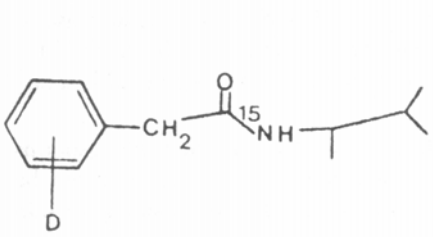



Fig.5

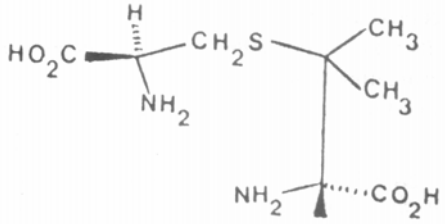



Fig.6

by Stevens (STEVENS *et. al*, 1954), the pure isomer Figure 6 formed from L-cysteine and D-penicillinamine was synthesized. Its ability to serve as a precursor was tested by observing the resultant depression in the utilization of L-cysteine-35S added to the medium at the same time. In fact, no depression of the utilization of 35S-L-cysteine was observed. The results are inconclusive, since it was taken up by the mycelium. Also it is clear that Figure 6 would have been incoporated intact it been take up.

Shimi and [man (SHIMI and IMAN, 1966), (SHIMI and IMAN, 1968) proposed an alternative pathway for the biosynthesis of the thiazolidine ring of penicillin. In a study of the degradation of penicillin by washed mycelial mats of P. chrysogenum or a penicillin-producing strain of Aspergillus flavus, it was observed that benzylpenicillin was degraded to phenylacetic acid, cystcine and valine, the latter apparently being further metabolized to glycine and acetone or isopropanol. It was observed (SHIMI and IMAN, 1968) that the addition of glycine and acetone or isopropanol to washed pre-formed mat's of P. chrysogenum resulted in the formation of significant quantities of valine. Furthermore, the production of benzylpenicillin by these mycelial mats was markedly stimulated by the addition of acetone, isopropanol or glycine, even more that by added cysteine or valine. The authors therefore suggested that incorporation of exogenous valine into penicillin might occur by cleavage of the

added valine into glycine and acetone followed by reassembly of these components as the D-valine part of the thiazolidine ring, Figure 7 -- Figure 8. Unfortunately the above experiments were not performed with labelled additives and therefore it cannot be concluded that the acetone and glycline were directly incorporated into valine or into the "valine-derived" part of the penicillin molecule without prior metabolic transformation.

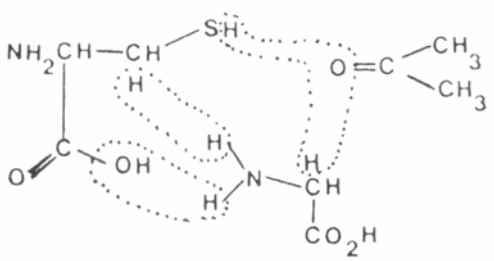


Fig. 7

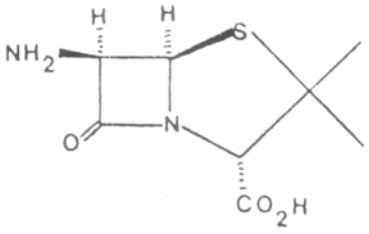


Fig. 8

Warren *et al.*, (Warren, *et. al.,* 1967) later found that Cephalosporin C. biosynthesis was not depressed by addition to the cultures of D-valine, but that the synthesis of penicillin N was depressed by D-valine. As earlier observed in P. chrysogenum by Stevens (Stevens and DELONG, 1958), the uptake of L-valine by Cephalosporium sp. was rapid but uptake of D-valine or a-kctoisovalcric acid was slow. The observations supported the view that the biosynthesis of both antibiotics in Cephalosporium sp. occured directly from L-valine rather than from D-valine.

In some other recent studies, the questions of the retention or loss of the C-2, C-3 and methyl group hydrogens of valine in biosynthesis of penicillin and cephalosporin C have been examined. Bycroft *et al.*, (Bycroft *et. at,* 1975) showed, in a double label experiment with 2-3H- D- pr L-valine, mixed with (U-l4C)-1-vallineor 1-$^{14}$C-D-valine respectively, that the C-2 hydrogen of both L and D-valine is eliminated. The total incorporation of 14C into benzylpenicillin was remarkably high (37%) and moreover was essentially identical whether D or L-14C-valine was used as precursor. However, the rate of absorption of these precursors differed in that L-valine was rapidly absorbed, whereas D-valine was rather slowly absorbed. The directly incorporated into penicillin but may first be converted to L-valine, either via a-ketoisovaleric acid or via an alternative pathway not involving C-N cleavage.

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