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Discovery and development of new β-lactam antibiotics

A.G. Brown

Beecham Pharmaceuticals Research Division, Brockham Park, Betchworth, Surrey. RH3 7AJ, England.

Abstract - The discovery of the various classes of β-lactam antibiotics is reviewed and the derived structures with clinical potential highlighted. In addition, the involvement of carbapenam intermediates in the biosynthesis of carbapenems is discussed. Novel non-β-lactam compounds detected via bioassays for β-lactams are listed.

It is well over 50 years since the discovery of penicillin; it is over 40 years since the recognition of its chemotherapeutic potential by the Florey School at Oxford in the early 1940's. Since the end of World War II commercial and academic interest and investment in β-lactam antibiotics has been high and has led to a wide array of antibacterial agents of immense chemotherapeutic value to man.

I would like to review some of the approaches used over the years to discover new β-lactam antibiotics and show how such compounds have resulted in new agents being made available to the clinician in order to combat bacterial infection. In general the discovery of new β-lactams from natural sources has prompted a complementary explosion in synthetic organic chemistry to provide analogous structures. With only a few exceptions it is not unreasonable to say that the detection and characterisation of new β-lactams from microorganisms has been the major source of inspiration for the design of new β-lactams with potential chemotherapeutic value.

The initial discovery of penicillin, while perhaps considered to be an excellent example of serendipity, has been rationalised (ref. 1). An agar plate inoculated with a Staphylococcus sp. became contaminated with a strain of Penicillum notatum. The plate was laid aside at room temperature; the P.notatum grew, and when the plate was eventually incubated, the growth of the Staphylococcus was affected because of the antibacterial substance (penicillin) which had diffused into the agar and so a typical zone of inhibition in the vicinity of the mould was obtained. Thus we have a screening organism (a Staphylococcus strain) and a random isolate (P.notatum) yielding a novel antibiotic substance, predominantly penicillin F, 1. The reinvestigation, development and large scale production studies by Florey and his colleagues in Oxford and their wartime collaboration with American associates and industrialists led to the large scale manufacture of penicillin G, 2 using P.chrysogenum. Other penicillin derivatives could be prepared by feeding certain precursors to the fermentation.

The detailed investigations of the discrepancy between bioassays and chemical assays resulted in Beecham scientists in the mid 1950's recognising 6-aminopenicillanic acid (6-APA), 3, as a precursor of penicillins during the fermentation of P.chrysogenum. These results prompted the preparation of 6-APA on a large scale, principally by the enzymatic deacylation of penicillin G. Chemical reacylation of 6-APA then led to the preparation and evaluation of many thousands of new penicillins by numerous pharmaceutical companies and the clinical use of compounds such as methicillin, ampicillin, amoxycillin, carbenicillin, ticarcillin, azlocillin, mezlocillin, piperacillin, mecillinam and flucloxacillin (ref. 2).

Previous to the discovery of 6-APA, studies at Oxford had uncovered the cephalosporin family of β-lactams following detailed examination of the antibacterial substances isolated from a strain of Cephalosporium acremonium supplied by Professor Brotzu. Brotzu had suggested that from microorganisms associated with sewage outflows one could expect to obtain compounds antagonistic to sewage bacteria. C.acremonium was such an organism and Abraham and his colleagues, in addition to finding penicillin N, 4, isolated cephalosporin C, 5 (ref. 3). It is worth remembering that this culture also gave the steroids of the cephalosporin P series, the first members of the fusidic acid class of antibiotics (ref. 4). Development of this discovery of cephalosporin C led to the clinical use of cephalothin, cephaloridine, cephalexin etc. - the so called first generation cephalosporins (ref. 5).

By the mid-1960's, with the widespread use of penicillins and cephalosporins, problems of resistance associated with bacteria capable of producing a variety of β-lactamases became a major concern in β-lactam therapy. This was despite the use of the penicillinase stable methicillin and the isoxazolyl penicillins against Staphylococcus aureus. The preparation of penicillins and cephalosporins with high stability to β-lactamases now became a target. A major advance in the development of such compounds came around 1970 with the discovery of the cephamycins from Streptomyces spp. in Lilly and Merck screening programmes, although the details of the methods used have not been published (ref. 6). The cephamycins are 7α-methoxycephalosporins and possess a D-α-aminoadipoyl side chain; cephamycin A 6, B 7, and C 8, are the most commonly encountered examples. The fact that a 7α-methoxy substituent introduced increased β-lactamase stability into a cephalosporin sparked off a high effort on the chemistry of penicillins and cephalosporins. One result was the identification of cefoxitin 9 as a clinically useful compound (ref. 7).

Modification of the penicillin nucleus at the corresponding 6α-position was studied in parallel, though early results indicated that 6α-methoxy penicillins lacked useful activity (ref. 8). More recently, however, 6α-methoxy ticarcillin (temocillin) 10, has been investigated in depth. Temocillin has good activity against a variety of Gram-negative bacteria and shows pronounced stability towards β-lactamases derived from these organisms; it is inactive against Gram-positive bacteria. Temocillin is unique among penicillins in that it has a very prolonged serum half-life (5.5 hrs) in humans following i.m. dosing (ref. 9).

As the cephamycins are derived by methoxylation of a cephalosporin, in order to find an alternative process to the chemical preparation of 6-methoxy ticarcillin 10, we examined the possible microbial transformation of various penicillin substrates into methoxy penicillins. From these studies we identified 7α-hydroxycephalosporin C 11 as an intermediate in the enzymatic methoxylation of cephalosporin C, 5, to yield 12. In addition we demonstrated a low conversion of penicillin N, 4, into 6α-methoxy penicillin N, 13, using cell free extracts of S.clavuligerus, S.lipmanii and S.wadayamensis. The isolation of the 6α-hydroxy compound 14 was not achieved, but the ready conversion of synthetic 6α-hydroxy penicillin N 14 and other 6α-hydroxy substituted penicillins to the 6α-methoxy derivative was observed. The 2β-acetoxymethyl derivative 15 was also methoxylated giving 16. These experiments indicated that enzymatic methoxylation was a two step process, the oxygenation step being more specific than the methylation of the intermediate hydroxy compound (ref. 10).

Around 1967 a screen for β-lactamase inhibitors was devised in Beecham laboratories (ref 11). Application of this screen led to the detection of a series of potent β-lactamase inhibitors from a number of strains of S.olivaceus. These inhibitors were characterised as the carbapenem derivatives (olivanic acids) 17, 18, and 19 (ref 12). Early on in these investigations, in order to establish whether the activity produced by S.olivaceus was indeed a cephamycin, the cephamycin producing Streptomyces cultures deposited in the ATCC by Merck and Lilly were purchased and screened. One culture, S.clavuligerus yielded a β-lactamase inhibitor with different properties to the olivanic acids and the cephamycins. This inhibitor was isolated and characterised as clavulanic acid 20 (ref. 13). Subsequently other groups have reported clavulanic acid from other Streptomyces spp. and identified further clavam derivatives 21 to 27 from S.clavuligerus mutants and other Streptomyces spp. These compounds demonstrated weak antifungal activity, particularly against plant pathogens (ref. 14). It should be noted that the absolute stereochemistry of clavulanic acid at C3 and C5 is as in the naturally occurring penicillins, while that at C5 in the clavams 21 to 27 is the opposite configuration.

The β-lactamse inhibitory activity of clavulanic acid along with its useful pharmacokinetic properties have resulted in the development of Augmentin* (potassium clavulanate + amoxycillin 28) for oral and parenteral use and Timentin* (potassium clavulanate + ticarcillin 29) for parenteral use again a wide range of bacterial infections, and in particular those caused by organisms producing plasmid and certain chromosomally mediated β-lactamases (ref. 15).

The identification of clavulanic acid 20 as a potent β-lactamase inhibitor prompted its extensive modification (ref. 16) and a number of syntheses of clavams as potential inhibitors (ref. 17); the parent β-lactam was also prepared (ref. 18). Analogues of clavulanic acid which have been reported include sulbactam 30 (ref. 19), 6β-bromo- and 6β-iodopenicillanic acids 31 and 32 (ref. 20), 6α-chloropenicillanic acid sulphone 33 (ref. 21), 2β-chloromethylpenicillanic acid sulphone 34 (ref. 22), YTR-830 35 (ref. 23), 6-methoxymethylene 36 and 6-acetylmethylenepenicillanic acid 37 (refs. 24,25) and various substituted alkylidenepenems 38 (ref. 26). Of these alternative inhibitors sulbactam has been developed furthest, either in combination with ampicillin, or cefoperazone (as Sulperazone), or as the mutual pro-drug with ampicillin (sultamicillin; Unasyn) (refs. 19, 27).

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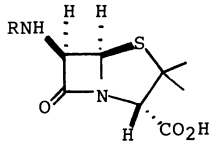
* Augmentin and Timentin are Trademarks of Beecham Group p.l.c.

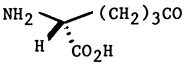
Structures 1–38

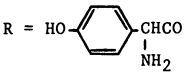


1 Penicillin F; R = $CH_3CH_2CH{=}CHCH_2CO$

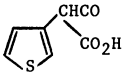
2 Penicillin G; R = $PhCH_2CO$

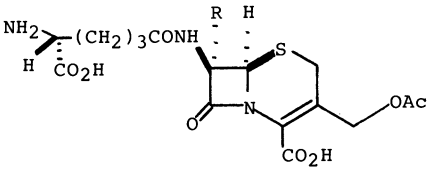
3 6-APA; R = H

4 Penicillin N; R = 



28 Amoxicillin; R =

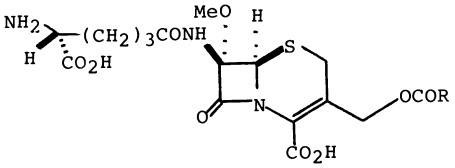
29 Ticarcillin; R = 



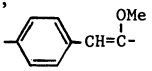
5 Cephalosporin C; R = H

11 R = OH

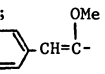
12 R = OMe



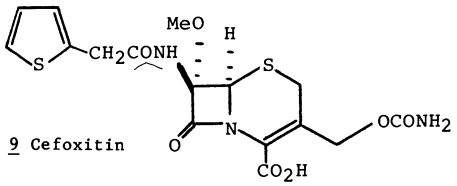
6 Cephamycin A;

R = 

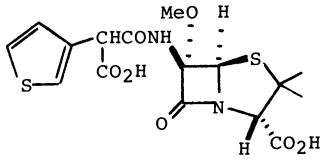
7 Cephamycin B;

R = 

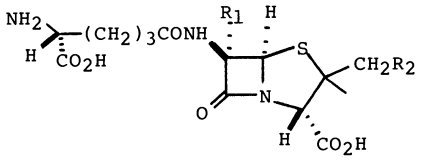
8 Cephamycin C; R = NH_2



9 Cefoxitin



10 Temocillin

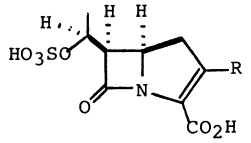


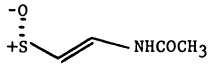
13 R_1 = OMe, R_2 = H

14 R_1 = OH, R_2 = H

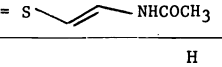
15 R_1 = H, R_2 = OAc

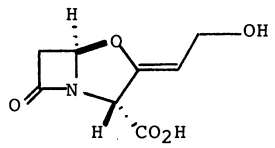
16 R_1 = OMe, R_2 = OAc



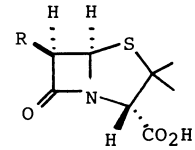
17 MM 4550; R = 

18 MM 13902; R = 

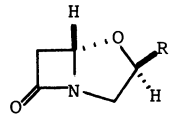
19 MM 17880; R = 



20 Clavulanic acid



31 R = Br 32 R = I



21 R = CO_2H

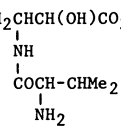
22 R = CH_2OH

23 R = CH_2OCHO

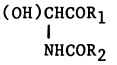
24 R = CH_2CH_2OH

25 R = 

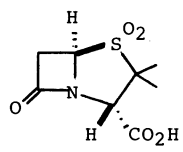
Clavalanine

26 R = 

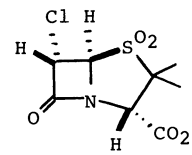
Valclavam

27 R = 

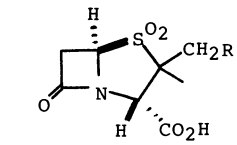
Clavamycins



30 Sulbactam

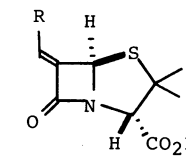


33



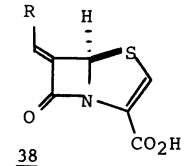
34 R = Cl

35 R = 



36 R = OMe

37 R = $COCH_3$



38

As mentioned above, the assay which led to the detection of clavulanic acid had also highlighted a number of strains of S.olivaceus as producers of β-lactamase inhibitory substances (ref. 11). Extensive fermentation, isolation and characterisation experiments resulted in the identification of the olivanic acid family of carbapenem β-lactams, the metabolites 17, 18, 19 and 39 to 42 (refs. 12,28). Complementary studies by Merck workers in a screening programme looking for inhibitors of cell wall synthesis revealed the thienamycin family of carbapenem β-lactams; the compounds 43 to 46 have been reported from S.cattleya (ref. 29), while the related olivanics (or epithienamycins) 39 to 42 were also isolated from S.fulvoviridis (ref. 30). To date over 40 carbapenem β-lactam antibiotics have been reported and include the PS-5 family (ref. 31), the carpetimycins (ref. 32), the asperenomycins (ref. 33) and the OA-6129 group (ref. 34). These carbapenems were detected in culture broths either by a variation on the β-lactamase inhibition screen described for the assay of clavulanic acid or by the use of β-lactam supersensitive strains of bacteria (vide infra).

The carbapenem β-lactams (in addition to clavulanic acid) were a milestone in affecting the traditional thoughts associated with structure/activity relationships prevailing in the penicillin and cephalosporin areas. Thus the β-lactams in the carbapenem series with trans-substituted β-lactam rings, plus R stereochemistry at C8 were generally the most stable (especially to the kidney enzyme, dehydropeptidase-I) and if the derivative was zwitterionic it usually demonstrated greater antibacterial activity than the corresponding N-acylated carbapenems. In general terms this meant compounds of the thienamycin type structure were more active than the olivanic acid equivalents. In terms of β-lactamase inhibitory properties, however, the olivanics, especially 17 to 19, were extremely potent, being more active than clavulanic acid on in vitro evaluation (ref. 35).

So far the only carbapenem to be developed to the clinic is thienamycin 43. In concentrated solution thienamycin self degrades via dimerisation. To overcome this problem the derviative N-formimidoyl thienamycin 47 has been produced (ref. 36). A feature of all naturally occurring carbapenems and derived derivatives is their ready degradation by the enzyme dehydropeptidase (DHP-I) which occurs in human kidney - the equivalent of a mammalian β-lactamase. To negate the effect of this enzyme on N-formimidoyl thienamycin 47 (Imipenem), Merck scientists have designed an inhibitor of DHP-I, called cilastatin 48; the product used clinically is called Primaxin and consists of a 1:1 mixture of 47 and 48. Chemotherapeutically, Primaxin is the most potent, broad spectrum β-lactam formulation available, with pronounced activity against Staphylococcus aureus and Pseudomonas aeruginosa strains; it is not orally absorbed (ref. 37,38).

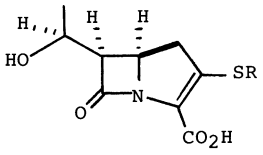
The discovery of the carbapenem β-lactams as potent antibacterial agents has inspired many organic chemists to tackle the total synthesis of this sytem. A major reason for this is the fact that S.cattleya, S.olivaceus and other carbapenem producing cultures do not yield large amounts of a single compound and fermentation titres have not been dramatically increased by extensive mutation programmes. Thienamycin, and hence N-formimidoyl thienamycin, are in fact prepared by total synthesis and not via fermentation (ref. 39). Analogues of thienamycin 43 and MM 13902 18 with greater metabolic stability have been the principal target. The Merck compound 49, for example, containing a 4β-methyl substituent appears to be the analogue with greatest potential as an alternative to thienamycin, being very much more stable to DHP-I, and therefore not requiring to be co-administered with a DHP-1 inhibitor yet still possessing potent antibacterial activity (ref. 38). Whether it is worth developing such a compound to the market-place will depend on the clinical effectiveness of Primaxin.

Analogue synthesis has also been extended by many groups to the preparation of penems corresponding to thienamycin and its derivatives. The Schering and Farmitalia companies have particularly concentrated on penem derivatives of the type 50 to 52 (ref. 40). Two Schering compounds, 50 and 51, while initially of some promise, have been abandoned for metabolism and/or toxicological problems.

Simultaneous to the announcement of the discovery of clavulanic acid and the carbapenems, the detection and characterisation of the 3-acylamino azetidinones of the nocardicin family e.g. nocardicin A 53, from Nocardia uniformis tsuyamanensis was described (ref. 41). The assay system used to detect these β-lactams involved the use of a mutant strain of Escherichia coli which was highly sensitive to a range of β-lactams. This assay coupled with in vitro tests to determine resistance to different β-lactamase types and effects on cell wall synthesis identified the nocardicins as members of a novel β-lactam family. No derivative of the nocardicins was found which was worth developing.

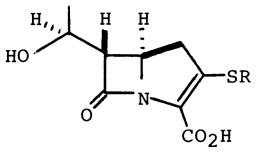
The use of a supersensitive strain of Pseudomonas aeruginosa (in conjunction with β-lactamases) to screen a wide variety of fungi, actinomycetes, yeasts and bacteria gave some new penicillins and cephalosporins, while the same test also yielded a number of carbapenems (ref. 42). Such as assay, however, was the basis on which the Takeda company found the 3-acylaminoazetidinones, sulfazecin 54 and isosulfazecin 55, from Pseudomonas

Structures 39–61



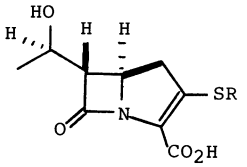
39 MM 22380; R = $NHCOCH_3$

40 MM 22382; R = $NHCOCH_3$



41 MM 22381; R = $NHCOCH_3$

42 MM 22383; R = $NHCOCH_3$

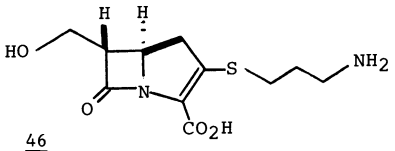


43 Thienamycin; R = NH_2

44 R = $NHCOCH_3$

45 R = $NHCOCH_3$

47 R = $N{=}CH{-}NH_2$



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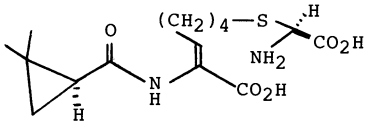
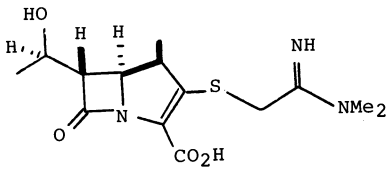


48 Cilastatin



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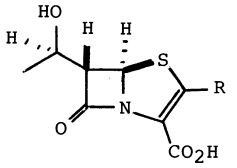
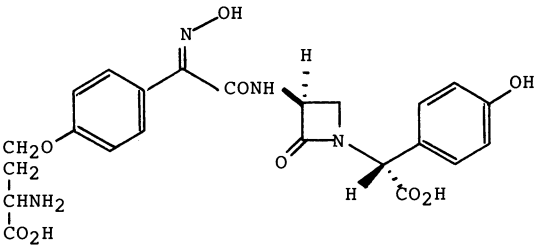


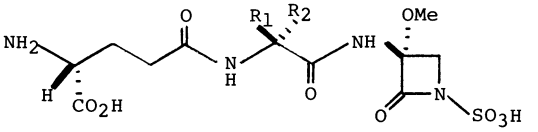
50 R = SEt

51 R = $SCH_2CH_2OCONH_2$

52 R = CH_2OCONH_2

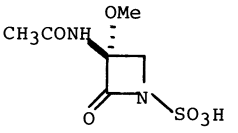


53 Nocardicin A

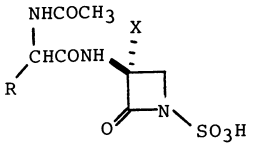


54 Sulfazecin, SQ 26,445; R_1 = H, R_2 = Me

55 Isosulfazecin; R_1 = Me, R_2 = H



56 SQ 26,180



57 R = $HO{-}C_6H_4{-}CH_2$; X = H

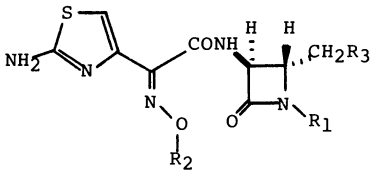
58 R = $C_6H_5{-}CH_2$; X = OMe

59 R = $HO_3SO{-}C_6H_4{-}CH(OH)$; X = OMe

60 R = $HO{-}C_6H_4{-}CH_2$; X = OMe

61 R = $HOSO_3{-}C_6H_4{-}CH(OSO_3H)$; X = OMe

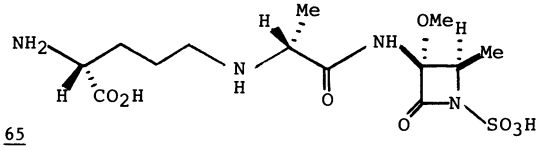
Structures 62–82



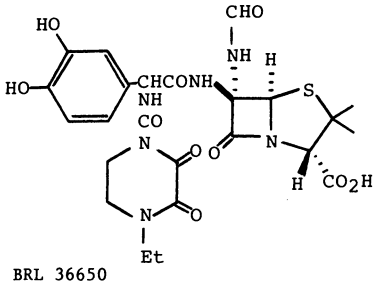
62 Aztreonam; $R_1 = SO_3H$, $R_2 = CMe_2CO_2H$, $R_3 = H$

63 Gloximonan; $R_1 = OCH_2CO_2CH_2CO_2Bu^t$, $R_2 = Me$, $R_3 = H$

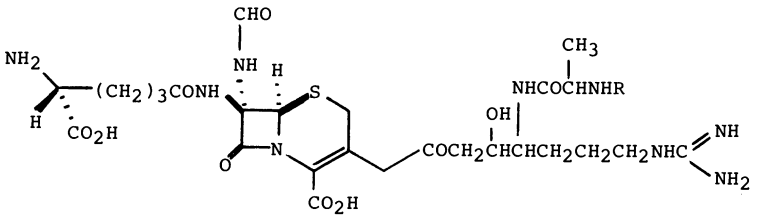
64 Carumonam; $R_1 = SO_3H$, $R_2 = CH_2CO_2H$, $R_3 = OCONH_2$



65



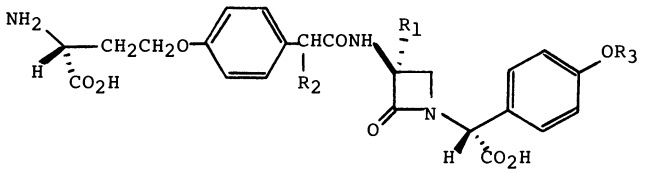
66 BRL 36650



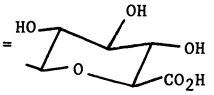
67 Chitinovorin A; R = H

68 Chitinovorin B; R = L-Ala

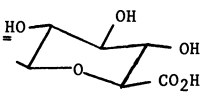
69 Cephabacin F_3; R = L-Ala-L-Ala etc.



70 Formadicin A; $R_1 = NHCHO$, $R_2 = OH$, $R_3 =$

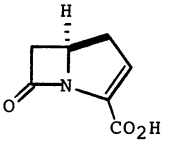


71 Formadicin B; $R_1 = H$, $R_2 = NHCHO$, $R_3 =$

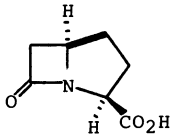


72 Formadicin C; $R_1 = NHCHO$, $R_2 = OH$, $R_3 = H$

73 Formadicin D; $R_1 = H$, $R_2 = NHCHO$, $R_3 = H$



74



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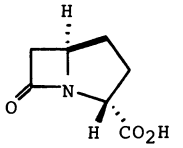
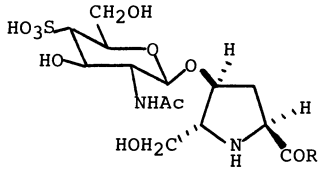


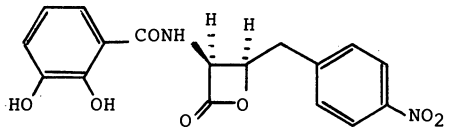
76



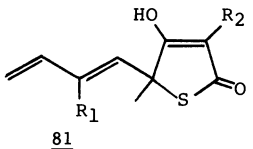
77 Bulgecin A; R = $NHCH_2CH_2SO_3H$

78 Bulgecin B; R = $NHCH_2CH_2CO_2H$

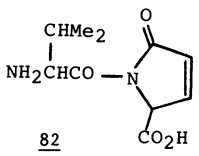
79 Bulgecin C; R = OH



80 Obafluorin



81



82

acidophila, and Ps.mesoacidophila (ref. 43). The Squibb company also discovered the azetidinone 54, but in addition have detected many other examples of related azetidinones, now called monobactams. For example, compounds 56 and 61 were found as part of a concentrated programme to screen bacteria for β-lactams using a β-lactamase induction assay based on a β-lactam supersensitive strain of Bacillus licheniformis. Compounds were derived from species of Pseudomonas, Gluconobacter acetobacter, Agrobacterium, Chromobacterium and Flexibacter (ref. 44). The basic β-lactam ring system was found to be readily synthesised and as the result of an intensive programme a large number of synthetic monobactams have been evaluated by Squibb, Takeda and others. So far the monobactam, aztreonam (SQ 26,776) 62 is marketed for use against certain Gram-negative bacteria; other examples such as gloximonan (SQ 82,531) 63 and carumonan (Ro-17,2301 AM 1080) 64 have been examined in some detail (ref. 45).

Recent studies in our laboratories using a differential antibacterial assay based on β-lactam resistant and hypersensitive strains of an Actinetobacter sp. have led to the isolation and characterisation of the monobactam 65 as the homologue of 54 (ref. 46). As the monobactams so far reported appear to be derived via serine (ref. 47), no doubt the biosynthesis of the 4-methylazetidinone 65 can be considered to be formed from a threonine intermediate.

Following on from our interests in 6α-substituted penicillins with increased β-lactamase stability (vide supra), in excess of 60 6α-substituents have been examined for their effect on the activity and stability of the penicillin and cephalosporin nuclei. These studies identified the formamido group as being of special interest and in particular the 6α-formamido penicillin, BRL 36650 66, was found to have potent antibacterial activity against Gram-negative bacteria in addition to having pronounced stability to all types of β-lactamase (ref. 48). BRL 36650 was more active against Enterobacteriaceae species, especially those producing β-lactamase, than any other penicillin and similar in activity to the third generation cephalosporins (e.g. ceftazidime) and aztreonam; it was also highly effective against Pseudomonas aeruginosa, but had poor activity against Gram-positive organisms.

While these studies were in progress it became apparent that a number of groups had identified a range of cephalosporin derivatives whch contained a 7α-formamido function. These compounds, e.g. 67 to 69, were found using supersensitive strains of bacteria as the screening assay (ref. 49). The formamido function has also been reported in nocardicin analogues 70 to 73 not only as a substituent on the β-lactam/azetidinone ring, but also as a modification of the side-chain acylamino group (ref. 50). Whether any compounds containing a formamido function, be they penicillins (other than BRL 36650), cephalosporins or azetidinones (monobactams), with potential clinical utility will be developed remains to be seen.

Other β-lactams highlighted by the application of a supersensitive organism include the production of deacetylcephalosporin C from a Flexibacter sp. and the simple carbapenem 74 from Serratia and Erwinia spp. (ref. 51). This last result has led us to investigate the biosynthesis of the carbapenems. Limited studies by Merck have suggested that the parent ring system of carbapenems is derived from glutamic acid and acetate; the addition of further C1 units at C6 plus thiol addition at C3 followed by C6 and C3 side-chain oxidation then leads to the variety of carbapenems reported (ref. 52). Re-examination of the strain of Serratia producing carbapenem 74, using isotopically labelled ^{13}C has resulted in us reisolating 74 as its p-nitrobenzyl ester (pNB). In addition, however, we have isolated two carbapenam derivatives, 75 and 76, again as their pNB esters; the absolute configuration of these compounds has yet to be confirmed (ref. 53). One can therefore speculate that perhaps compound 76 is an early product of biosynthesis which is epimerised forming carbapenam 75, with ''natural'' relative stereochemistry at C3 and C5. It is then oxidised to the carbapenem (via a 2-hydroxycarbapenam); C6 alkylation/C3 thiol addition, plus subsequent oxidation will then lead to the other complex naturally occurring carbapenems. Further studies in this area are in progress.

Complementary to the above studies on naturally occurring β-lactams are the recent extensive studies of the Baldwin group at Oxford which have shown that isopenicillin N synthetase can accommodate certain tripeptide substrates to yield new fused β-lactams (ref. 54). Again, whether such studies can be undertaken on realistic a scale to be of use preparatively awaits investigation. The products, however, of such enzymatic reactions will doubtlessly prompt the organic chemist into the total and semi-synthesis of appropriate acylamino analogues.

From the above it is clear that the use of new methods designed to detect β-lactams continue to produce novel secondary metabolites containing a β-lactam ring. It is of interest to note also that such screens and related experiments have also thrown up a number of false positives which are of interest as antibacterial agents with unusual structures. For example the bulgecins 77 to 79 (ref. 55), β-lactone 80 (ref. 56), the thiolactomycins 81 (ref. 57) and the compound 82 (ref. 58) have been detected as spin-offs from the search for new β-lactams.

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