OXOID

Volume 4 No 2 September 1983

culture

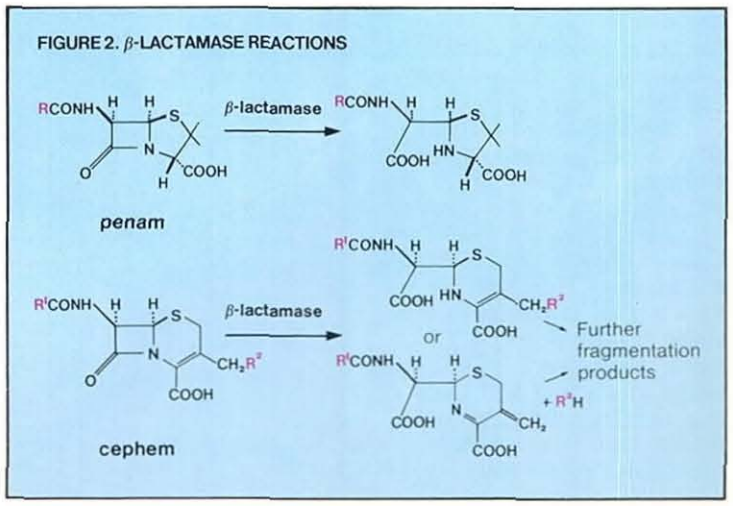
Published as a service to microbiology
by Oxoid Limited

The β-lactam antibiotics

Allan G Brown PhD, FRSC and **Christopher Reading** M.I. Biol., Beecham Pharmaceuticals Research Division, Chemotherapeutic Research Centre, Brockham Park, Betchworth, Surrey, England.

It is over 50 years since the publication of Fleming's original work on penicillin. In that time a variety of naturally occurring β-lactam structural types have been reported, particularly in the last twelve years or so.[1,2] It is also over 40 years since the first detailed description of the enzyme β-lactamase, which was soon followed by the recognition of its involvement in the development of resistance by bacteria to β-lactam antibiotics.[3] The following review describes and illustrates the various β-lactam structural types that have been identified and developed over these years. Their susceptibility or resistance to the major classes of β-lactamases that have been recognised as contributing to the problems of resistance are discussed for certain representative examples. Related topics such as penetration and binding to target site enzymes will be mentioned where appropriate but are not discussed in depth. Certain β-lactams are also potent inhibitors of β-lactamases and their structures and effects on the microbiological properties of certain penicillins and cephalosporins are mentioned.

Naturally occurring β-lactam structures have generally been defined and classified by a trivial nomenclature (e.g. as penicillins, cephalosporins, cephamycins, clavulanic acid, olivanic acid, thienamycin, the nocardicins and the monobactams), the name of a compound usually relating to the producing organism and a chemical feature of the new compound.[4] More recently, β-lactams have been categorised by a further system based on a defined parent β-lactam skeleton (**Figure 1**), i.e. penicillins and cephalosporins have been derived from the penam (**1**) and cephem (**2**) nuclei respectively, clavulanic acid from the clavam nucleus (**3**), while thienamycin and the olivanic acids are called carbapenems (**4**); other β-lactam antibiotics are based on the penem (**5**), oxacephem (**6**), and monobactam (**7**) ring systems.



β-Lactamases and resistance

β-Lactamases hydrolyse the cyclic amide bond of penicillins, cephalosporins and related compounds to produce antibacterially inactive degradation products (**Figure 2**). Of the β-lactamases produced by Gram-positive organisms, only the staphylococcal enzyme has clinical relevance. The β-lactamases from Gram-negative bacteria are a diverse group of enzymes for which Richmond and Sykes[5] first produced a comprehensive classification system which was later modified.[6,7] These classifications are based on the ability of the enzyme to hydrolyse a range of β-lactam substrates (substrate profile) and on the genetic origin of the enzyme (plasmidal or chromosomal).

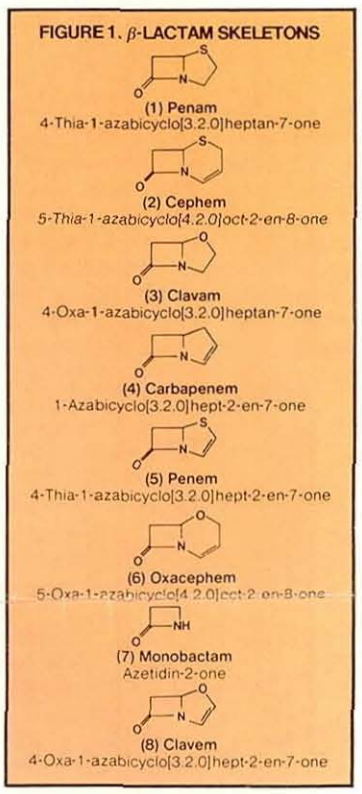
The targets for β-lactam antibiotics, the penicillin binding proteins (**PBPs**), are located on the inner cytoplasmic membrane of the Gram-negative cell wall. The outer membrane of this wall limits the rate of entry of some β-lactams into the periplasmic space between the two membranes. β-Lactamase in the periplasmic space hydrolyses the low concentrations of incoming antibiotic with an efficiency depending on the V_{max} and K_m values of the enzyme for that particular substrate. A high value of K_m/V_{max} or a low value of V_{max}/K_m will indicate good stability at the low concentrations which are likely to be obtained in the periplasm.[8,9] Hence, these measurements serve as a better guide than just V_{max} rates when considering the role of β-lactamase in the resistance of clinical bacteria. Alternatively, stability data obtained at low substrate concentrations provides a more relevant measurement than V_{max} values. Unfortunately, much of the published data has been obtained for substrate profile comparisons at concentrations which give maximum rates of hydrolysis.

PENAMS (Figure 3)

Penicillins are defined as N-acylated derivatives of 6β-aminopenicillanic acid (**9:** 6-APA), which is a derivative of the penam nucleus (**Figure 1:1**); this is a bicyclic ring system derived by the fusion of azetidinone and thiazolidine rings. **Figure 3** lists a number of chemotherapeutically useful penicillins. The type of acylamino side chain present in these lactamase but resistance has emerged by changes in intrinsic penicillins varies considerably.

Early penicillins were benzylpenicillin (**10**) and phenoxymethylpenicillin (**11**) which have activity primarily against Gram-positive cocci. These penicillins are susceptible to virtually all bacterial β-lactamases although a β-lactamase from *E. coli* was recently shown to have little activity against benzylpenicillin.[10] The introduction in 1960 of 2,6-dimethoxyphenyl penicillin, methicillin (**12**), followed by isoxazolyl penicillins such as cloxacillin (**13**) and flucloxacillin (**14**) which were stable to staphylococcal β-lactamase, eliminated the clinical relevance of β-lactamase-mediated resistance in these organisms. It is interesting that after twenty years, staphylococci have not evolved a new β-sensitivity (methicillin-resistant staphylococci). Although methicillin and isoxazolyl penicillins have high resistance to many Gram-negative β-lactamases they have little antibacterial activity against the organisms which produce them. They have, however, proved useful in the characterisation of β-lactamases; enzymes which hydrolyse alkoxyaryl and isoxazolyl penicillins include the plasmid mediated OXA types and PSE-2, as well as chromosomal enzymes from Klebsiella (Class IV), Class Ic cephalosporinases and the unusual cephalosporinase from *E. coli* JD41.

The first broad spectrum peni-



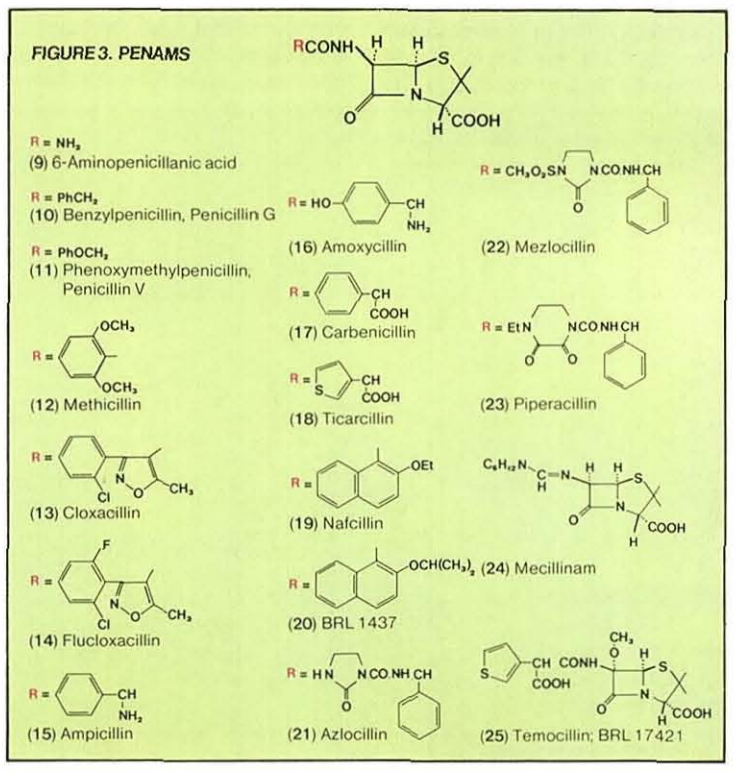


Table 1. The hydrolysis of penicillins by β-lactamase.

Producing organism	Ref.	Enzyme 5, 6, 7, Class.	Rates of hydrolysis (V_{max}) relative to benzylpenicillin (= 100)					
			Benzylpenicillin	Methicillin	Cloxacillin	Ampicillin	Carbenicillin	Cephaloridine
Enterobacter cloacae P99	7	Ia	100	30	0	0	0	6600
Escherichia coli 255	33	Ib	100	-	<2	<1	<1	270
Pseudomonas GN 918	7	Id*	100	<5	<5	10	<5	770
Morganella morganii	7, 33	I*	100	<5	<5	<5	<5	682-1000
Proteus vulgaris	33	Ic* (cefuroximases)	100	-	<1	107	14	714
Bacteroides fragilis	34,37	Ic (cefuroximases)	100	125-165	<3-80	30-60	16	3125-13800
Proteus mirabilis C889	35	II penicillinase	100	-	8	198	102	5
Klebsiella E70	35	IV (Broad spectrum chromosomal)	100	-	<5	191	18	55
Klebsiella 1082E	39	IV (Broad spectrum chromosomal)	100	25	8	83	12	83
Branhamella catarrhalis	36	IV (Broad spectrum chromosomal)	100	-	-	115	95	12.5
	38	TEM-1	100	0	0	106	10	76
	38	TEM-2	100	0	0	107	10	74
	38	SHV-1	100	<2	<2	212	8	56
	38	OXA-1	100	332	190	382	30	30
Gram-negative plasmid-mediated β-lactamase	38	OXA-2	100	23	200	179	15	37
	38	OXA-3	100	29	350	178	10	44
	38	PSE-1	100	<2	<2	90	97	18
	38	PSE-2	100	803	371	267	121	32
	38	PSE-3	100	-	3	101	253	10
	38	PSE-4	100	16	<2	88	150	40
*Staphylococcus** *aureus* Russell	35	plasmid-mediated gram positive penicillinase*	100	>1	>1	233	38	>1

* Inducible

Table 2. The hydrolysis of mezlocillin and azlocillin by Pseudomonas and Klebsiella NCTC 8172 β-lactamases

Incubation time (h)	Residual antibiotic (%)					
	Pseudomonal β-lactamase (Class I)		Klebsiella β-lactamase (Class IV)			
	Azlocillin	Ticarcillin	Mezlocillin	Ampicillin	Cefazolin	Carbenicillin
0	85	100	100	100	100	100
0.25	70	100	70	58	100	100
0.5	48	100	32	32	100	100
1.0	0	100	0	0	100	100

Substrates at 50μg/ml. Data from reference 41.

cillins were the orally administered α-aminopenicillin, ampicillin (**15**), and the parenteral α-carboxypenicillin, carbenicillin (**17**). These and the later analogues, amoxycillin (**16**) and ticarcillin (**18**), have little stability to plasmidal or Class II chromosomal enzymes (**Table 1**). Whilst α-aminopenicillins **15** and **16** are substrates for broad spectrum chromosomal enzymes (Class IV), the α-carboxypenicillins, such as **17**, are relatively stable to Class IV Klebsiella β-lactamases (**Table 2**). Like most semi-synthetic penicillins, **15** and **16** are hydrolysed by Class Ic cephalosporinases (cefuroximases) from *Proteus vulgaris*, Bacteroides and *Pseudomonas cepacii*.

Cephalosporinases of Class Ia, b and d have little effect on semi-synthetic penicillins, with α-carboxypenicillins being the most stable (**Table 1**). This stability extends the antibacterial spectrum of the α-carboxypenicillins to cover Indole-positive Proteus, Enterobacter, Serratia and Pseudo-

Table 3. The hydrolysis of mezlocillin and piperacillin by various β-lactamases; hydrolysis rate relative to benzylpenicillin (100).

Strain studied	β-Lactamase; Richmond and Sykes group.	Benzyl-penicillin	Ampicillin	Mezlocillin	Piperacillin
1. *E. cloacae* 1051E	P99 Group I	100	0	0	15
2. *E. coli* 1541E	D31	100	0	0	4
3. *Bact. fragilis* 1781	Group I	100	92	304	82
4. *E. coli* 1193E	TEM-1 Group III	100	127	92	77
5. *K. pneumoniae* 1976E	SHV-1 Group III	100	189	133	147
6. *K. aerogenes*	K1 Group IV	100	67	60	25
7. *E. coli* 2138E	OXA-1	100	403	116	52

Data from Reference 40 – penicillin concentration 50μg/ml

monas organisms producing Class I enzymes, as well as to Klebsiella which produce Class IV β-lactamases. The high stability of semi-synthetic penicillins, such as cloxacillin, methicillin and α-carboxypenicillins, combined with their high affinity for Class I cephalosporinases, allows them to act as competitive inhibitors of cephalosporinases whence moderately stable acyl-enzyme intermediates are often formed. In some cases, even the α-aminopenicillins are capable of acting in this way. The spectrum of potent β-lactamase inhibitory activity is extended to cover TEM and Klebsiella enzymes by the alkoxy substituted naphthylpenicillins, nafcillin (**19**) and BRL 1437 (**20**). The limited or poor synergistic activity of these semi-synthetic penicillins in antibacterial tests may reflect their poor permeability and thus their inability to inhibit periplasmic β-lactamase.

The acylureido penicillins, such as azlocillin (**21**), mezlocillin (**22**) and piperacillin (**23**), are derivatives of ampicillin and are unstable to staphylococcal and Gram-negative β-lactamases (**Table 3**). These compounds, however, have an antibacterial spectrum similar to the α-carboxypenicillins, but they are more active despite their poorer stability to β-lactamases, including those produced by Pseudomonas and Klebsiella (**Table 2**). This instability is seen when minimum inhibitory concentration (MIC) values are obtained using high inocula, but the clinical relevance of this effect has yet to be determined. Acylureido-penicillins have very high affinity for PBP-3 and their ability to rapidly inhibit septation at low concentrations may explain their activity despite having poor β-lactamase stability.

Mecillinam (**24**), a 6-β-amidinopenicillanic acid derivative, is an unusual structural type amongst commercially available β-lactams. Its activity is limited to the Enterobacteriaceae and it is similar to the semi-synthetic penicillins in terms of β-lactamase stability. It is relatively stable to cephalosporinases and has lower V_{max} values than ampicillin against TEM and Class IV β-lactamases. Having a K_m value significantly higher than that of ampicillin for TEM β-lactamase it is more stable at low concentrations and, as a result, is more active than ampicillin against Enterobacteriaceae which produce moderate amounts of this plasmid-mediated β-lactamase. Mecillinam specifically inhibits PBP-2 and produces osmotically stable round cells. This specific action on cell wall biosynthesis produces a synergistic effect when mecillinam is combined with normal penicillins and cephalosporins.

Until recently it seemed unlikely that a penicillin would be obtained with stability to a broad range of Gram-negative β-lactamases and yet retaining sufficient intrinsic antibacterial activity to make it therapeutically useful. The introduction of temocillin (**25:** BRL 17421) has provided a penicillin with useful chemotherapeutic properties and high stability to virtually all bacterial β-lactamases. Temocillin is a 6α-methoxy penicillin and its stability compared to other α-methoxy-β-lactams will be discussed later.

CEPHEMS (Figure 4)

Cephalosporins are N-acylated derivatives of 7β-aminocephalosporanic acid (**26:** 7-ACA) which is derived from the cephem nucleus (**Figure 1:2**); a cephem is the bicyclic ring system obtained by the fusion of an azetidinone with a dihydrothiazine ring. Representative samples are listed in **Figure 4**. In addition to variations being made in the structure of cephalosporins at C-7, further modifications at C-3 can lead to dramatic changes in chemotherapeutic properties. Thus, the acyl side-chain of cephalosporins can vary from α-aminoadipoyl, as in cephalosporin C (**27**), through the 2-thienylacetyl substituent of cephaloridine (**28**) and cephalothin (**29**), the α-amino-, α-hydroxy-, and α-sulphono-phenacetyl groups of cephalexin (**30**), cefamandole (**31**) and cefsulodin (**32**), and the acylureido function of cefoperazone (**33**), to the α-oximino-α-aminothiazole and α-furan units of cefotaxime (**34**), ceftazidime (**35**) and cefuroxime (**36**). Likewise, the C-3 substituent can be varied considerably in these compounds, as seen in **Figure 4**.

The term cephalosporinase was introduced by Abraham and Newton for the cephalosporin C hydrolysing enzyme they detected in crude extracts of *Bacillus cereus*. Most chromosomally mediated enzymes in the Gram-negative bacteria are cephalosporinases. The three earliest cephalosporins, cephalothin (**29**) and cephaloridine (**28**) both used parenterally, and the orally administered cephalexin (**30**) have comparable antibacterial properties. Cephaloridine and cephalothin are readily hydrolysed by Class I cephalosporinases, Class IV enzymes and plasmidal β-lactamases (**Table 4**), but like most cephalosporins are relatively stable to staphylococcal β-lactamase. Cephalexin is relatively stable to plasmid and Class IV enzymes (**Table 4**) having low V_{max} values and low affinity. Even cephaloridine can have reasonable MIC values against strains producing moderate amounts of TEM β-lactamase because it has very poor affinity for this β-lactamase. Attempts to improve the activity of cephaloridine resulted in cefazolin (**37**) with better intrinsic Gram-negative activity but little increased resistance to β-lactamase (**Tables 4** and **5**). Further improvements in antibacterial activity were obtained with ceforanide (**38**) and cefotiam (**39**). Lower V_{max} values for some enzymes, as seen for cefotiam in Tables 4 and 5, are offset by higher affinity and more efficient hydrolysis at lower concentrations and so this compound cannot be considered as any more stable than cefazolin.

Orally absorbed cephalosporins, such as cephalexin (**30**), have acylamino side-chains similar to those of amoxycillin and ampicillin and stable substituents at C-3, e.g. a methyl group. Additions to this group since the advent of cephalexin have all had similar antibacterial activity and poor stability to cephalosporinases, with cephaloglycin (**40**), cefatriazine (**41**) and cefaclor (**42**) having marginally improved intrinsic antibacterial activity. Little has been achieved therefore in the search for an orally active β-lactamase stable cephalosporin.

Good stability against some Class I enzymes was first achieved with cefuroxime (**36**) and cefamandole (**31**), as shown in **Tables 4** and **5**. Cefuroxime, however, has significantly better stability against broad spectrum β-lactamases of the TEM and Class IV types than does cefamandole. They have similar intrinsic antibacterial activity and their spectra are extended, compared to earlier cephalosporins, to cover strains of Haemophilus, Neisseria, *E. coli*, Klebsiella and Enterobacter, but despite exhibiting stability to the Sabath and Abraham enzyme (Id), they lack activity against Pseudomonas. Enzymes which fall into the Ic category of the Richmond and Sykes classification hydrolyse *cefuroxime and cefamandole* prompting Mitsuhashi[11] to term this group 'cefuroximases'.

Cefonicid (**43**) is structurally related to cefamandole but is reported to have better stability to *B. fragilis* β-lactamase.[12] It also has lower V_{max} values than cefamandole with TEM β-lactamase, but its higher affinity probably results in similar stability at physiological concentrations. The Class I stability of cefuroxime is achieved despite a very high affinity for this type of enzyme. The K_i values of cefuroxime approach those of cloxacillin and helps explain why cefuroxime

FIGURE 4. CEPHEMS

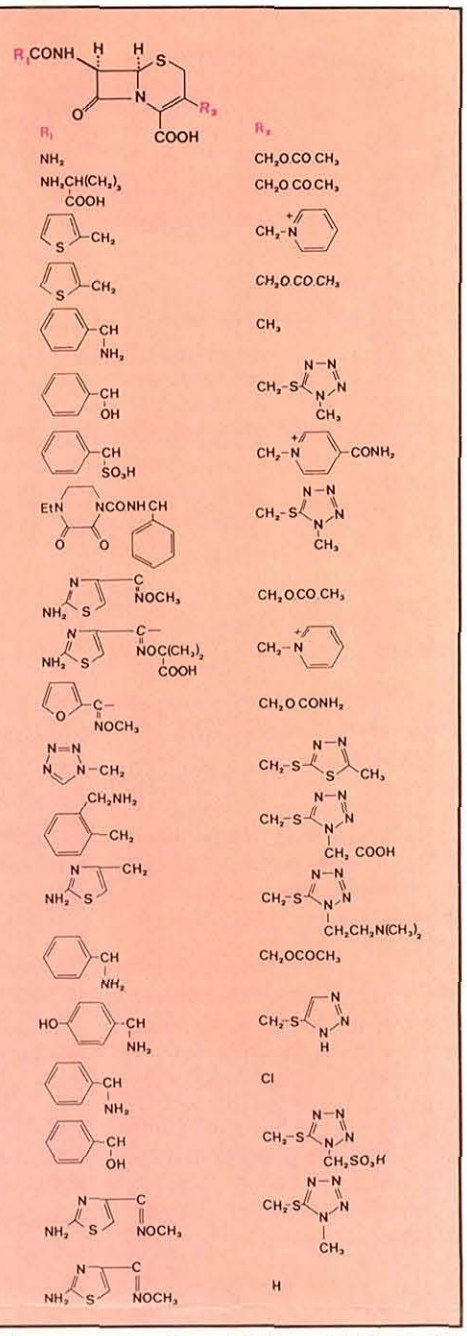
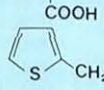
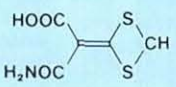
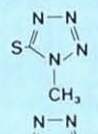
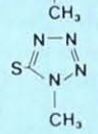


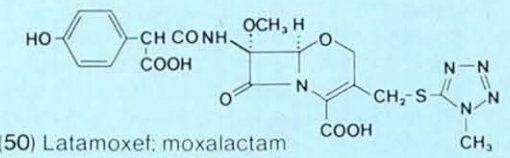
Table 4. Comparison of the relative rates of hydrolysis of cephalosporins by clinical β-lactamases.

Producing organism	Enzyme type	Relative rates of hydrolysis														
		cephaloridine*	cephalothin*	cephalexin*	cefazolin*	cefamandole*	cefuroxime*	cefoxitin*	cefoperazone	cefotiam	cefsulodin	cefmenoxime	cefotaxime	ceftizoxime	ceftazidime	cefmetazole	latamoxef
Enterobacter cloacae P99	Ia	100	12.5	45	0.1	0	0	0	1	7	<1	<1	3	<1	<1	<1	<1
Citrobacter intermedius	1	100	-	-	-	-	-	-	32	38	8	8	15	1	<1	<1	<1
Pseudomonas aeruginosa	Id	100	60	70	110	0	0	0.1	0	14	15	0	15	1	<1	<1	0
Bacteroides fragilis	Ic (STH4)	100	-	-	-	-	-	-	37	22	14	<1	15	1	1	0	<1
Klebsiella pneumoniae	IV (K1)	100	68	0.5	75	60	1.3	0	3	22	4	7	7	<1	3	<1	<1
Plasmid mediated in Gram-negative bacteria	TEM-1	100	25	0.5	15	20	0.1	0	54	5	2	<1	<1	<1	0	<1	0
	TEM-2	100	-	-	-	-	-	-	61	7	3	1	0	1	0	1	0
	SHV-1	100	-	-	-	-	-	-	73	3	6	1	0	<1	0	0	0
	OXA-1	100	-	-	-	-	-	-	22	25	2	85	22	122	7	0	0
	OXA-2	100	-	-	-	-	-	-	80	-	3	3	0	2	0	0	0
	OXA-3	100	-	-	-	-	-	-	47	-	9	6	0	6	0	0	0
	PSE-1	100	-	-	-	-	-	-	16	8	47	247	27	0	0	20	0
	PSE-2	100	-	-	-	-	-	-	165	258	32	264	16	44	30	12	11
	PSE-3	100	-	-	-	-	-	-	225	285	7	0	<1	36	8	0	8
	PSE-4	100	-	-	-	-	-	-	4	2	0	3	1	0	2	0	0
Staphylococcus Aureus PC-1	Gram-positive penicillinase	100	-	-	-	-	-	-	62	0	38	39	0	0	30	0	0

Data from references 42 and 43*

FIGURE 5.
7α-METHOXYCEPHEMS AND OXACEPHEMS

	R_1	R_2
(46) Cephamycin C	$NH_2CH(CH_2)_3$ COOH	$OCONH_2$
(47) Cefoxitin		$OCONH_2$
(48) Cefotetan		
(49) Cefmetazole	$N{\equiv}CCH_2SCH_2$	



(50) Latamoxef; moxalactam

is an inhibitor of these β-lactamases. The α-methoxyimino group in the cefuroxime side chain is the structural feature which is thought to be responsible for its β-lactamase stability.

Further α-methoxyimino cephalosporins include cefotaxime (**34**), cefmenoxime (**44**) and ceftizoxime (**45**). In these compounds the furyl ring in the 7-acylamino side chain of cefuroxime is replaced by the 5-aminothiazol-3-yl group which had been shown earlier to give good intrinsic antibacterial activity in cefotiam.

These compounds are extremely active against the Enterobacteriaceae but only have marginal activity against Pseudomonas. Like cefuroxime they are stable to Class I enzymes, excluding Ic, (**Tables 4** and **5**) and are also hydrolysed to some extent by plasmid-mediated enzymes. As shown in Table 4, OXA-1 and 2 hydrolyse these α-methoxyimino cephalosporins under V_{max} conditions with ceftizoxime appearing to have the best stability overall.

Cephalosporins with activity against Pseudomonas have only been obtained recently. Cefsulodin (**32**) has antibacterial activity limited to virtually only Pseudomonas, despite having a wider range of β-lactamase stability (**Tables 4** and **5**). It is hydrolysed by cefuroximases and the PSE group, which is reflected in its MIC values against carbenicillin-resistant Pseudomonas strains producing PSE types. Cefoperazone (**33**) is structurally quite different from cefsulodin having a side chain very similar to that of the acylureido penicillin, piperacillin. This antibiotic has poorer stability to Class I enzymes than, say, cefuroxime and is hydrolysed readily by plasmid enzymes. Despite broad spectrum intrinsic activity, which includes Pseudomonas, it performs poorly against many β-lactamase-producing bacteria.

A novel modification of the α-methoxyimino substituent was achieved by replacing the methyl group with 2,2-dimethylacetic acid, as in ceftazidime (**35**). This cephalosporin, at present under development, is an exceptionally broad spectrum Gram-negative antibacterial with excellent activity against Pseudomonas. It is highly stable to the cephalosporinases, including the Ic type enzyme from *Proteus vulgaris*, but low rates of hydrolysis by *B. fragilis* Ψ-lactamase may be responsible for the reduced activity against this anaerobe. Low rates of hydrolysis have been reported with OXA-1, Klebsiella K1 and PSE-2,-3 and staphylococcal β-lactamases (**Table 4**).

7α-METHOXY-CEPHEMS, OXACEPHEMS (Figure 5) and 6α-METHOXY-PENICILLINS

The discovery of cephamycin C (**46**), a 7α-methoxy-cephem, provided a cephalosporin with stability to a wide range of Gram-negative β-lactamases. The marketed analogue, cefoxitin (**47**), has moderate antibacterial activity and its spectrum includes Klebsiella, Indole-positive Proteus, Citrobacter, *E. coli* and Bacteroides. Activity against Enterobacter and Pseudomonas is poor although cefoxitin is relatively stable to their β-lactamases. This spectrum reflects its good β-lactamase stability (**Tables 4** and **5**) which includes stability to the Ic enzymes and helps explain its unusual activity against Bacteroides.

The 7α-methoxy group is the structural feature which gives good stability, its presence affecting the reactivity of the β-lactam ring towards β-lactamases. The 7α-methoxy group does not necessarily reduce affinity as cefoxitin has low K_i values for Class I enzymes and acts as an inhibitor. The 7α-methoxy substituent actually reduces the intrinsic antibacterial activity but achieves a balance between activity and resistance to β-lactamase. The methoxy group in the α-methoxyimino cephalosporins perhaps mimics the methoxy group of the cephamycins and explains the stability of this series and that of ceftazidime. Newer cephamycins such as cefotetan (**48**) and cefmetazole (**49**) have spectra and stability similar to cefoxitin but are reported 2-4 times more active (**Table 4**).

By replacing the sulphur atom of cephem (**Figure 1:2**) with oxygen, a series of analogues called the oxacephems (**Figure 1:6**) have been synthesised. The most notable example of this series is latamoxef (**50:** moxalactam) which possesses a 7α-methoxy group. This β-lactam is significantly more active than the cephamycins and shares their high stability to a wide range of β-lactamase types (**Table 4**) with improvements over cefoxitin in stability to some Class I enzymes (**Figure 8**). Latamoxef, like the new cephalosporins, has relatively poor activity against staphylococci but like the cephamycins has some activity against Bacteroides, with a spectrum extended to include Pseudomonas. Latamoxef has better β-lactamase inhibitory activity than cefoxitin against Class I enzymes and like cefoxitin can react to form relatively stable enzyme-inhibitor complexes.

Temocillin (**25:** BRL 17421) is an α-carboxy penicillin with a 6α-methoxy substituent.[13] This compound is highly stable to β-lactamases even when compared to cefoxitin (**Table 6**); only an inducible enzyme from Providencia has been shown to slowly hydrolyse this β-lactam but the organism was still sensitive to the antibiotic. Temocillin is active against the Enterobacteriaceae and β-lactamase-producing strains of Haemophilus and Neisseria, but lacks activity against Gram-positive organisms and Bacteroides. Temocillin, unlike the cephamycins and latamoxef, has poor affinity for β-lactamases and has no β-lactamase inhibitory activity. This poor affinity may explain its greater stability at low concentrations to Class I enzymes when compared to the 7α-methoxy-cephems.

Table 5. The hydrolysis of cephalosporins by Class 1 β-lactamases. V_{max} rates relative to cephaloridine (100). Data from reference 11

β-lactamase produced by:	cephaloridine	cefazolin	cephalexin	cefotiam	cefamandole	cefoperazone	cefsulodin	cefuroxime	cefotaxime	ceftizoxime	cefoxitin	benzylpenicillin	ampicillin
Cephalosporinases													
E. coli	100	311	29	82	5	4	<1	<1	<1	<1	<1	63	<1
E. cloacae	100	100	14	29	<1	1	<1	<1	<1	<1	<1	12	<1
C. freundii	100	116	80	8	<1	1	<1	<1	<1	<1	<1	3	<1
S. marcescens	100	198	6	16	<1	2	<1	<1	<1	<1	<1	3	<1
P. rettgeri	100	99	8	4	<1	<1	<1	<1	<1	<1	<1	3	<1
M. morganii	100	20	4	8	<1	<1	<1	<1	<1	<1	<1	16	<1
P. aeruginosa	100	222	64	12	<1	5	5	<1	<1	<1	<1	29	<1
Cefuroximase (Ic)													
P. vulgaris	100	387	274	222	278	15	9	1140	84	<1	<1	20	12
P. cepacia	100	156	58	161	452	10	161	239	174	29	<1	161	323
B. fragilis	100	60	<1	8	6	7	3	50	7	2	<1	3	<1

Table 6. The β-lactamase stability of temocillin, a 6α-methoxy substituted penicillin, in comparison to cefuroxime, cefotaxime and cefoxitin.

Source of β-lactamase	Substrate remaining after 1 h (%)[a]			
	cefoxitin	cefuroxime	cefotaxime	BRL 17421
Plasmid mediated:				
TEM-1	100	22	0	100
TEM-2	100	0	0	100
PSE-4 (pMG19)	100	60	47	100
OXA-1 (pGN238)	100	0	0	100
OXA-3 (P57b)	85	100	17	100
SHV-1 (pR1010)	100	34	0	100
HMS-1 (pR997)	100	65	34	100
Chromosomally mediated:				
Escherichia coli (5)[b]	47-100[c]	0-100	0-70	100
Enterobacter spp (10)	0-100	0-75	0-85	100
Citrobacter spp (5)	100	100	18-100	100
Serratia marcescens (3)	100	0-32	0-17	100
Pseudomonas aeruginosa (5)	32-100	24-100	32-100	100
Providencia spp (10)	90-100	0-100	0-100	0-100
Proteus spp (6)	80-100	0-100	0-100	100
Klebsiella pneumoniae (4)	100	0-100	0-8	100
Miscellaneous:				
Acinetobacter calcoaceticus (5)	100	75-100	30-100	100
Bacteroides fragilis (3)	100	0	0	100
Staphylococcus aureus (2)	100	100	100	100

* [a] Substrate concentration, 200μg/ml, temperature 37°C, pH 7.0, same enzyme concentration for all substrates.

[b] Number of strains tested.

[c] Range of results for strains tested. Data from reference 44.

CARBAPENEMS (Figure 6)

A carbapenem (**Figure 1:4**) is the ring system obtained by replacing the sulphur atom of a penem by a (CH_2) group; this β-lactam type is illustrated by the thienamycin (**51**) and olivanic acid families.[14] The analogous saturated ring is called a carbapenam. It is a notable feature of the carbapenems in both naturally occurring and synthetic series that the substituents at C-6 and C-2 are considerably different from those of the penam (penicillin) and cephem (cephalosporin) classes of β-lactams. Of the carbapenems a thienamycin derivative, MK 0787 (**52:** N-formimidoylthienamycin), is the most interesting chemotherapeutically, though it is not without problems of metabolic instability.[15]

Thienamycin is highly stable to most β-lactamases and its 6α-hydroxyethyl side chain is believed to mimic the 6α-methoxy group of the cephamycins by sterically hindering hydrolysis. However, it is not completely stable and a penicillinase from *Pseudomonas maltophilia* hydrolyses the derivative MK 0787.[16] *Bacillus cereus* II β-lactamase has been reported also to hydrolyse some carbapenems.[47] Whilst thienamycin and MK 0787 have some moderate inhibitory activity against Class I enzymes the sulphated olivanic acids MM 4550, MM 17880 and MM 13902 (**53**) are extremely potent inhibitors of a wide range of β-lactamases [14] for which they have high affinity, interacting to form relatively stable enzyme-inhibitor complexes.[17]

Stereochemical differences at C-6 and C-8, and the sulphate ester group, influence enzyme inhibitory properties. Similarly, stereochemistry influences the antibacterial activity of carbapenems. Furthermore, acetylation of the 2-cysteamine side chain of thienamycin reduces activity against Pseudomonas in particular. Thienamycin and MK 0787 are extremely potent broad spectrum β-lactam antibiotics, which includes activity against Pseudomonas and Gram-positive bacteria. The olivanic acids, such as MM 13902 (**53**), have good antibacterial activity but are generally less active than thienamycin and have no useful effect on Pseudomonas.

The β-lactam ring of carbapenems is hydrolysed by the mammalian

FIGURE 6. CARBAPENEMS, PENEMS AND MONOBACTAMS

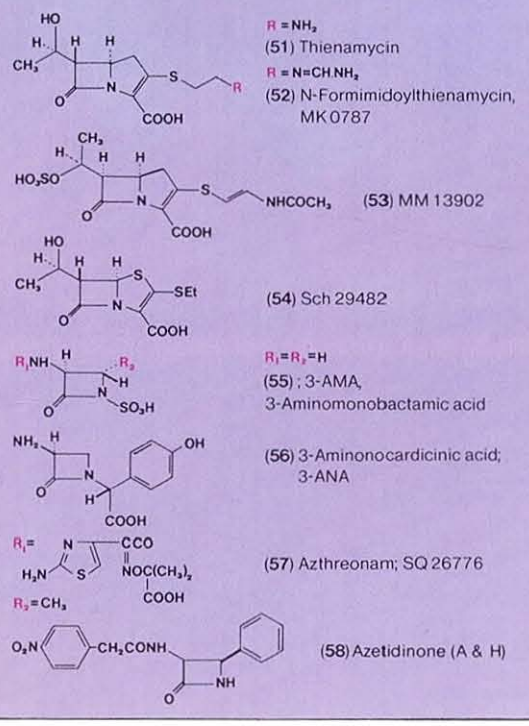
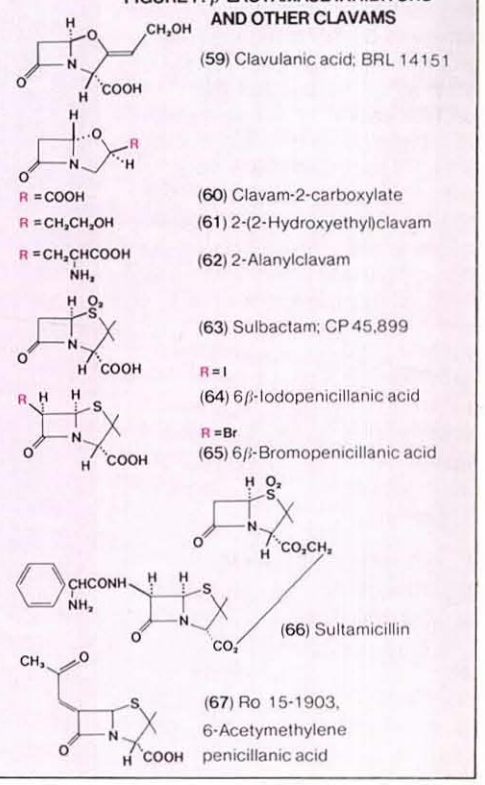


FIGURE 7. β-LACTAMASE INHIBITORS AND OTHER CLAVAMS



renal dipeptidase, dehydropeptidase I, to yield an antibacterially inactive degradation product.[18] It is ironic that β-lactams with such high stability to bacterial β-lactamases should prove susceptible to a mammalian β-lactamase which has no effect on traditional penicillins, cephalosporins, or clavams. The co-administration of specific, non-β-lactam inhibitors of this enzyme with MK 0787 is being considered.

PENEMS

The penem nucleus (**Figure 1:5**) is an unsaturated analogue of the penam ring system. So far, no naturally occurring penem derivative has been reported, though many synthetic examples have been prepared and one, Sch 29482 (**54**), has been investigated in some depth;[19] this compound is the result of trying to combine the chemical and microbiological features of the penicillin and thienamycin families (**Figure 6**).

Like the carbapenems, Sch 29482 is highly stable to nearly all β-lactamases, including the Ic enzymes. Slight hydrolysis by TEM β-lactamase has been reported[20] and also by *Ps. maltophilia* β-lactamase, which hydrolyses carbapenems. Though similar to cefotaxime in spectrum and lacking Pseudomonas activity, it has the distinction of being an oral antibiotic unlike all other β-lactamase-stable β-lactams which are administered parenterally. It is highly serum bound and MIC values increase dramatically in 50% serum, which may suggest some instability. The clinical relevance of these properties remains to be seen. A major drawback with Sch 29482 is that it is metabolised to ethyl mercaptan.

MONOBACTAMS, and other azetidinone derivatives (Figure 6)

Azetidin-2-one (**Figure 1:7**) derivatives were known before the discovery of penicillin. However, despite considerable study it is only recently that naturally occurring azetidinones with potentially useful properties have been isolated from bacterial species and Nocardia. The monobactams[21] and the nocardicins[22] are N-acyl derivatives of 3-amino-monobactamic acid (**55:** 3-AMA) and 3-aminonocardicinic acid (**56:** 3-ANA), respectively, and therefore closely resemble the penicillins and cephalosporins; 3α-methoxy derivatives are also known, though not in the nocardicin family. Azthreonam (**57**) is an example of a monobactam with potentially useful chemotherapeutic properties.

The synthetic azetidinone (**58**) prepared by Allen and Hanbury (British Patent 1201,720) is a progressive inhibitor of staphylococcal β-lactamase only and has no antibacterial activity. Nocardicins have weak *in vitro* antibacterial activity with stability to staphylococcal and some *E. coli* β-lactamases,[23] but apart from this little data is available.

Most of the naturally occurring monobactams have a 3-α-methoxy group analogous to that found in the bicyclic cephamycins which confers high stability to a number of β-lactamases, whilst the desmethoxy analogues are substrates. Azthreonam is a prospective commercial development of these natural products but it lacks the 3-α-methoxy group.

Stability to β-lactamase and antibacterial activity have been attained by attaching the ceftazidime side chain to C-3 of the monobactam nucleus. Substituents at C-4 also influence β-lactamase stability and the 4β-methyl analogue of azthreonam (4α-methyl) is even more stable than azthreonam to some enzymes. Azthreonam is very stable to most Class I enzymes but not Ic types, though *Proteus vulgaris* strains remain susceptible. It has good stability to most plasmid-mediated enzymes but is slowly hydrolysed by PSE-2 and the Class IV Klebsiella K1 β-lactamase. Like cefoxitin and latamoxef it has a high affinity for, and is a progressive inhibitor of, Class I enzymes; the inhibition being achieved by the formation of moderately stable acyl intermediates (half-life = 6.8h with *E. cloacae* P99 enzyme). Azthreonam (**57**) has a narrow antibacterial spectrum being limited to aerobic Gram-negative bacteria. It has some activity against Pseudomonas, although it is inferior to ceftazidime and MK 0787. It appears particularly active against Serratia, Morganella, Providencia and Proteus strains, being reported as better than ceftazidime *in vitro*.

β-LACTAMASE INHIBITORS (Fig 7)

Clavam (**Figure 1:3**) is the parent ring system found in the potent β-lactamase inhibitor, clavulanic acid (**59**), isolated from *Streptomyces clavuligerus*;[24] it can be considered as the bicyclic ring system formed by the fusion of an azetidinone and an oxazolidine. Simpler derivatives of this nucleus, e.g. clavam-2-carboxylate (**60**), 2-(2-hydroxyethyl) clavam (**61**) and 2-alanylclavam (**62**), are produced by Streptomyces spp but have an absolute stereochemistry at C-5 which is opposite to that of clavulanic acid.[25] Clavem (**8**) is related to clavam (**3**) as penem (**5**) is to penam (**1**) - see **Figure 1**; only synthetic examples of (**8**) are known.

Clavulanic acid is a potent inhibitor of all plasmid-mediated enzymes including staphylococcal β-lactamase.[26] It inhibits chromosomal enzymes of Class II, e.g. *Proteus mirabilis*, and broad spectrum enzymes (Class IV) such as are found in Klebsiella and Branhamella. It also inhibits cephalosporinases of Class Ic (cefuroximases) but does not have significant activity against other members of the large Class I group of Gram-negative β-lactamases. An oral formulation of amoxycillin and potassium clavulanate is now in clinical use.[27] It extends the spectrum of the widely used β-lactam antibiotic amoxycillin to cover β-lactamase-producing staphylococci, TEM-producing Haemophilus, *Neisseria gonorrhoea*, E. coli and other members of the Enterobacteriaceae possessing plasmid-mediated β-lactamases. In addition, strains of *K. aerogenes*, *P. mirabilis*, *P. vulgaris*, *Branhamella catarrhalis* and Bacteroides spp, which produce chromosomal enzymes and are not susceptible to amoxycillin, are readily inhibited by the combination. A formulation of the α-carboxy penicillin ticarcillin and potassium clavulanate is under development.[28] Ticarcillin is used for the treatment of serious Gram-negative and Gram-positive infections and its formulation with clavulanic acid will extend its spectrum to cover strains producing plasmid-mediated β-lactamases or Class II penicillinases. Ticarcillin, of course, already has intrinsic resistance to most Class I cephalosporinases and to Class IV β-lactamases from Klebsiella.

The β-lactamase inhibitors, penicillanic acid sulphone (**63:** sulbactam; CP 45,899), 6β-iodo and 6β-bromopenicillanic acids, (**64**) and (**65**) respectively, are examples of simple derivatives of the penam nucleus.[29,30] Sulbactam is generally less active as a β-lactamase inhibitor than clavulanic acid, especially against TEM β-lactamase. Its spectrum, however, includes some moderate activity against Class I enzymes and this is reflected in formulations of sulbactam and ampicillin where some activity has been obtained against Enterobacter and Serratia spp producing Class I enzymes. The oral pro-drug, sultamicillin (**66**), is under development and comprises ampicillin (**15**) covalently linked by an ester bond to sulbactam (**63**) which on hydrolysis in the blood releases the separate components.[31]

A formulation of sulbactam with cefoperazone (**33**) is also under consideration. Cefoperazone, as discussed earlier, has good broad spectrum intrinsic activity but is β-lactamase labile.

The 6β-halo penicillanic acids, **64** and **65** have similar potency and spectra to clavulanic acid, but like sulbactam they have some moderate activity against cell-free preparations of Class I cephalosporinases, yet this has not been evident in antibacterial synergy tests.[46] A new inhibitor, 6-acetylmethylene penicillanic acid (**67:** Ro 15-1903), has been described recently.[32] It inhibits Class I enzymes to the same extent as sulbactam and was also reported to be significantly more active than clavulanic acid against plasmid-mediated, Class IV and staphylococcal β-lactamases when preincubated with cell-free enzyme preparations. The *in vitro* and *in vivo* antibacterial results for this compound combined with ampicillin were disappointing in comparison to its reported inhibitory activity.

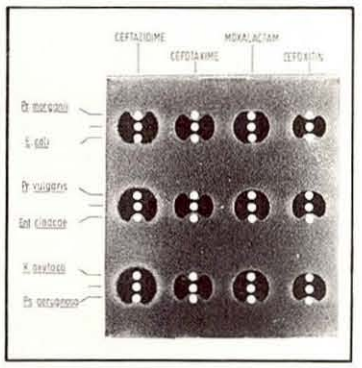


FIGURE 8. Comparison of the β-lactamase stability of α-methoxyimino and 6α-methoxy cephalosporins using Masuda's double disc test. Central disc contains 30μg of antibiotic flanked by two discs containing 30μl of β-lactamase from various Gram-negative organisms. Data from reference 45.

Conclusion

As mentioned earlier, the nomenclature used in this short review is part trivial, part systematic. This is because the use of fully systematic nomenclature for these β-lactams is rather complex and cumbersome, as illustrated for the parent penam, cephem, clavam, oxacephem and carbapenem rings in **Figure 1**. Significant progress has been made toward solving the problem of β-lactamase mediated resistance either by structural modifications of existing β-lactam series, by the discovery of novel β-lactam structures, or by the use of β-lactamase inhibitors. The adaptability of the bacterial population may, however, provide further challenges to the medicinal chemist in the years ahead either by the development of alternative resistance mechanisms or by the selection of strains which produce β-lactamases suited to these new β-lactams. The inhibitors and stable compounds of today may be the substrates of tomorrow.

References

1. Flynn, E.H. (ed) (1972). *Cephalosporins and Penicillins: Chemistry and Biology*. Academic Press, New York.
2. Morin, R.B. and Gorman, M. (eds.) (1982). *Chemistry and Biology of β-Lactam Antibiotics*. Volumes 1-3. Academic Press, New York.
3. Hamilton-Miller, J.M.T. and Smith, J.T. (eds.) (1979). *Beta-Lactamases*. Academic Press, New York.
4. Brown, A.G. (1982). *J. Antimicrob. Chemother.*, **10**, 365-368.
5. Richmond, M.H. and Sykes, R.B. (1973). In: Rose, A.H. and Tempest, D.W. (eds.). *Advances in Microbial Physiology*, **Volume 9**, 31-88. Academic Press, New York.
6. Sykes, R.B. and Matthew, M. (1976). *J. Antimicrob. Chemother.*, **2**, 115-157.
7. Sykes, R.B. and Smith, J.T. (1979). In: Ref. 3, pp. 369-401.
8. Labia, R. (1974). *C.R. Acad. Sci. (Paris)*, **279D**, 109-112.
9. Pollock, M.R. (1965). *Biochem. J.*, **94**, 666-675.
10. Seibert, G. and Limbert, M. (1982). In: Periti, P. and Grassi, G.G. (eds.). *Current Chemotherapy*, 'Proc. 12th Int. Cong. Chemother. Florence, Italy 1981', **Volume 1**, ASM, Washington. pp 751-752.
11. Mitsuhashi, S. and Inoue, M. (1981). In: Mitsuhashi, S. (ed.) *Beta-Lactam Antibiotics*, Springer-Verlag, Berlin, pp. 41-58.
12. Mehta, R.J. *et al* (1981). *J. Antibiot.*, **34**, 202-205.
13. Slocombe, B. *et al* (1981). *Antimicrob. Agents Chemother.*, **20**, 38-46.
14. Brown, A.G. (1981). *J. Antimicrob. Chemother.*, **7**, 15-48.
15. Kropp, H. *et al* (1980). *Antimicrob. Agents Chemother.*, **17**, 993-1000.
16. Saino, Y. *et al* (1982). *Antimicrob. Agents Chemother.*, **22**, 564-570.
17. Easton, C.J. and Knowles, J.R. (1982). *Biochemistry*, **21**, 2857; and Charnas, R.L. and Knowles, J.R. (1981). *Biochemistry*, **20**, 2732.
18. Kropp, H. *et al* (1982). *Antimicrob. Agents Chemother.*, **22**, 62-70.
19. Ganguly, A.K. *et al* (1982). *J. Antimicrob. Chemother.*, **9**, Suppl C. 1-5.
20. Mitsuhashi, S. *et al* (1982). *J. Antimicrob. Chemother.*, **9**, Suppl. C. 91-95.
21. Sykes, R.B. *et al* (1981). *J. Antimicrob. Chemother.*, **8**, Suppl. E. 1-16.
22. Aoki, H. *et al* (1976). *J. Antibiot.*, **29**, 492-500.
23. Aoki, H. *et al* (1977). *J. Antibiot.*, **30**, S. 207-217.
24. Reading, C. and Cole, M. (1977). *Antimicrob. Agents Chemother.*, **11**, 852-857.
25. Brown, D., Evans, J.R. and Fletton, R.A. (1979). *J. Chem. Soc., Chem. Commun.* 282-3; Elson, S. and King, T.J., **unpublished results**; Wanning, M., Zähner, H., Krone, B. and Zeeck, A. (1981). *Tetrahedron Lett.*, **22**, 2539-40; Muller, J-C. *et al* (1983). *J. Antibiot.*, **36**, 217-225.
26. Reading, C. (1982). In: Leigh, D.A. and Robinson, O.P.W. (eds.) *Augmentin*, 5-21 Excerpta Medica, Amsterdam.
27. Rolinson, G.N. and Watson, A. (eds.). (1980). *Augmentin.*, and Leigh, D.A. and Robinson, O.P.W. (eds.) (1982). *Augmentin*, Excerpta Medica, Amsterdam.
28. Boon, R.J. *et al* (1981). *21st Interscience Conference on Antimicrobial Agents and Chemotherapy*. Abstract No. 442; and Staniforth, D.H., Jackson, D. and Horton, R. Abstract No. 443.
29. English, A.R. *et al* (1978). *Antimicrob. Agents Chemother.*, **14**, 414-419.
30. Daehne, von W. (1980). *J. Antibiot.*, **33**, 451-452.
31. Hartley, S. and Wise, R. (1982). *J. Antimicrob. Chemother.*, **10**, 49-55.
32. Arisawa, M. and Theu, R.L. (1982). *J. Antibiot.*, **35**, 1578-1583.
33. Sawai, T., Kauno, M. and Tsukamoto, K. (1982). *J. Bact.*, **152**, 567-571.
34. Simpson, I.N., Page, C.D. and Harper, P.B. (1982). *J. Antimicrob. Chemother.*, **9**, 29-45.
35. Farmer, T. and Reading, C. (1981). *Biochem. J.*, **199**, 779-787.
36. Farmer, T. and Reading, C. (1982). *Antimocrob. Agents Chemother.*, **21**, 506-508.
37. Britz, L.M. and Wilkinson, R.G. (1978). *Antimicrob. Agents Chemother.*, **13**, 373-382.
38. Matthew, M., Hedges, R.W. and Smith, J.T. (1979). *J. Bact.*, **138**, 657-662.
39. Marshall, M.J. *et al* (1972). *Applied Microbiol.*, **23**, 765-769.
40. Elwell, A. and Wise, R. (1982). *J. Antimicrob. Chemother.*, **10**, 560-561.
41. Basker, M.J. *et al* (1979). *Infection*, **7**, 67-73.
42. Simpson, I.N. *et al* (1982). *J. Antimicrob. Chemother.*, **9**, 357-368.
43. Richmond, M.H. and Wotten, S. (1976). *Antimicrob. Agents Chemother.*, **10**(2), 219-222.
44. Edmondson, R.A., Slocombe, B. and Cole, M. (1981). In: P. Periti and G.G. Grassi (eds.) *Current Chemother. and Immunother.*, 'Proc. 12th Int. Congr. Chemother', **1**, ASM, Washington, 321-322.
45. Labia, R. *et al* (1981). *J. Antimicrob. Chemother.*, **8**, Suppl. B. 141-146.
46. Coleman, K., Hunter, P.A. and Kenig, M. (1983). *13th International Congress of Chemotherapy, Vienna.*
47. Cassidy, P.J. (1981). *Developments in Industrial Microbiology*, **22**, 181-209.

Culture is published as a service to microbiology by Oxoid Limited, Wade Road, Basingstoke, Hampshire RG24 0PW, England.