β-LACTAM ANTIBIOTICS. II

STRUCTURE-ACTIVITY RELATIONSHIPS OF 6-[α-(α′-UREIDO-ACYLAMINO) ACYLAMINO] PENICILLANIC ACIDS

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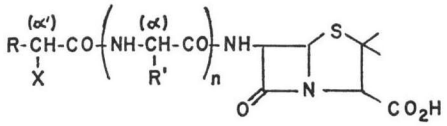
(Received for publication July 13, 1976)

The influence on the structure-activity relationships (S.A.R.) of the stereochemistry and various alkyl, aryl, aralkyl and heterocyclic substituents at the two chiral centres in the dipeptide side-chain of a new series of penicillins was examined. In many cases the effects of these changes had a pronounced influence on the degree of activity against Gram-positive and especially Gram-negative bacteria. Several compounds indicated that the size, shape and spatial disposition of a substituent were the parameters of importance in influencing activity, rather than its lipophilic or electronic character. The most active homologues in the series provided broad-spectrum penicillins which in terms of their *in vitro* antibacterial properties showed improvements over certain of the marketed penicillins. Thus 6-[D-α(α′-ureidoacyl-amino)acylamino]penicillanic acids were found which had a carbenicillin-like profile, with improvements against *Pseudomonas aeruginosa, Klebsiella aerogenes*, sensitive and β-lactamase-producing Gram-positive cocci.

The aim of finding a penicillin with improved broad-spectrum activity, especially against the typically more penicillin resistant Gram-negative bacteria such as *Pseudomonas* and *Klebsiella*, continues to attract attention. The increasing usage of broad-spectrum β-lactam antibiotics by the clinician in conjunction with the reports of a steady increase in recent years of infections caused by *Pseudomonas aeruginosa* have contributed to the continued interest in this objective.[1,2] One of the earliest fruitful chemical approaches to finding broad-spectrum, anti-pseudomonas penicillins consisted of coupling di-substituted acetic acids, in which one of the substituents was acidic, to the 6-amino group of the penicillin nucleus (6-amino penicillanic acid or 6-APA). Penicillins such as carbenicillin (**Ia**; R=Ph; X=CO_2H), suncillin (**Ia**; R=Ph; X=$NHSO_3H$) and sulbenicillin (**Ia**; R=Ph; X=SO_3H) testify to the success of this approach.[3]

Formula 1.



Ia n=0 **Ib** n=1

In an earlier paper attention was drawn to the observation that when these same di-substituted acetyl side-chains were attached to the amino group of D-α-amino benzylpenicillin (ampicillin) the penicillins produced (**Ib**; R=R′=Ph; X=CO_2H/$NHSO_3H$/SO_3H), had a similar antibacterial spectrum of activity to that of the lower homologues (**Ia**), including anti-pseudomonas activity.[4] It was therefore decided to investigate penicillins of structural type (**Ib**) in more detail with a view to finding a penicillin superior to the marketed anti-pseudomonas penicillins. As a result of this

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investigation a more comprehensive analysis of the *in vitro* structure-activity relationships for this class of penicillins can be attempted. This paper describes the effects of various alkyl, aryl, aralkyl and heterocyclic substituents and stereochemistry at the two chiral centres (α and α') in the side-chain of 6-[α-(α'-ureido acylamino) acylamino] penicillanic acids (**Ib**; X=$NHCONH_2$; R and R′ variable). The consequences of replacing the ureido group at the α'-carbon atom in **Ib** by other 'functional' groups will be presented in part III of this series.

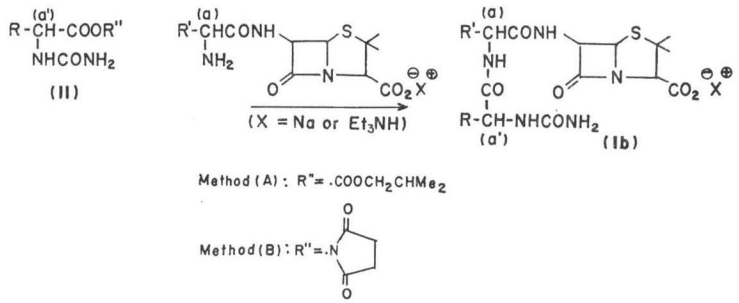
Experimental

Synthetic Methods

All melting points were taken on a Büchi melting point apparatus and are uncorrected. I.R. spectra were obtained on a Perkin Elmer 457 infrared grating spectrophotometer. N.M.R. spectra were recorded on a Varian A–60 spectrometer using tetramethylsilane as an internal standard.

The majority of the 6-[α-(α'-ureidoacylamino) acylamino] penicillanic acids (**Ib**) were synthesised by reaction of either the isobutoxy formic anhydride of the side-chain acid (Method A) or the N-succinimido ester of the α-ureido acid (Method B) and alkali or amine salt of the appropriate α-aminopenicillin (Scheme 1.). One representative example of each of these coupling procedures is given below and details of the syntheses of the other penicillins in this series have been reported in the

Scheme 1.



patent literature.[5] Whereas the isobutoxy formic anhydrides of the side-chain acids are too unstable to isolate and store, the N-succinimido esters of these acids are usually sufficiently stable to be isolated and purified by crystallisation. Occasionally formation of the 'mixed' anhydride is incomplete and results in reaction of excess isobutoxy chloroformate with aminopenicillin to produce N-isobutyl-carbonylamino penicillins. This side-reaction was partly responsible for the lower yields obtained by Method A (20~60%) compared with Method B (70~90%). No significant racemisation of the side-chains (**II**) was observed using either synthetic procedure.

All the penicillins used in the tests for antibacterial activity were substantially pure by various criteria. Purity was assessed by N.M.R. and I.R. spectroscopic analysis, biochromatographic evidence and other physical data such as hydroxylamine and/or iodometric assay.

N-Carbamoyl-D-phenylalanine

To a suspension of D-phenylalanine (3.3 g) in water (50 ml) was added potassium cyanate (1.62 g) and the mixture heated at 80°C until a clear solution was obtained. After cooling and acidification with 5 N hydrochloric acid to pH 1.5, the product (2.8 g, 67%) was removed by filtration, washed with water and dried. N-Carbamoyl-D-phenylalanine had m.p. 181~182°C (Found: C, 57.4; H, 6.0; N, 13.4%. $C_{10}H_{12}N_2O_3$ requires C, 57.7; H, 5.8; N, 13.4%), ν_{max} (nujol) 3480, 3320, 1690, 1635, 1560, 1300, 1255 and 1155 cm^{-1}, δ [$(CD_3)_2SO$] 2.96 (2H, m, $C\underline{H}_2$), 4.43 (1H, m, $C\underline{H}$), 5.68 (1H, s, $N\underline{H}$), 6.23 (2H, d, $N\underline{H}_2$) and 7.28 (5H, s, $C_6\underline{H}_5$).

N-Carbamoyl-D-tryptophan

Prepared in quantitative yield from D-tryptophan, N-carbamoyl-D-tryptophan had m.p. 199~

200°C (Found: C, 58.3; H, 5.4; N, 16.7%. $C_{12}H_{13}N_3O_3$ requires C, 58.3; H, 5.3: N, 17.0%), ν_{max} (KBr) 3390, 3235, 1698, 1633, 1535, 1457, 1344, 1253, 1066, 752, 555 and 516 cm^{-1}, δ [$(CD_3)_2SO$] 3.15 (2H, d, $C\underline{H}_2$), 4.48 (1H, q, C$\underline{H}$), 5.68 (1H, s, N$\underline{H}$), 6.27 (2H, d, $N\underline{H}_2$), 6.8~7.7 (5H, m, indolyl C$\underline{H}$), 10.91 (1H, s, N$\underline{H}$).

D-α-(D-2-Ureido-3-phenylpropionamido)-(3'-thienyl)acetamidopenicillanic acid (**8**)

N-Carbamoyl-D-phenylalanine (1.04 g, 0.005 M) suspended in dry acetone (30 ml) at −10°C was treated with triethylamine (0.71 ml) and isobutyl chloroformate (0.69 g, 0.005 M) and stirred at −10°C for 30 minutes. D-α-Amino(3-thienyl)acetamido penicillanic acid (1.78 g, 0.005 M) in water (30 ml) was treated with triethylamine to give a clear solution (pH 8.4) and then diluted with acetone (30 ml) and cooled to 0°C. The mixed anhydride solution, chilled to −40°C, was filtered through Celite into the stirred penicillin solution and the mixture allowed to warm slowly to room temperature over 1 hour. After evaporation of the acetone under reduced pressure, the aqueous layer was acidified to pH 2 under ethyl acetate. The required product (1.6 g, 62%) crystallised on evaporation of the ethyl acetate solution m.p. 175~177°C (dec) (Found: C, 52.3; H, 5.0; N, 12.5; S, 11.4%. $C_{24}H_{27}N_5O_6S$ requires C, 52.8; H, 5.0; N, 12.8; S, 11.8%), ν_{max} (KBr) 3360, 1750, 1650, 1525, and 704 cm^{-1}, δ [$(CD_3)_2SO$] 1.48 (3H, s, $C\underline{H}_3$), 1.58 (3H, s, $C\underline{H}_3$), 2.92 (2H, m, $C\underline{H}_2$), 4.28 (1H, s, C3–$\underline{H}$), 4.61 (1H, m, $CH_2C\underline{H}$), 5.3~6.1 (5H, β-lactams, $CON\underline{H}_2$, C$\underline{H}$), 7.37 (8H, m, $C_6\underline{H}_5$, $C_4\underline{H}_3S$), 6.26, 8.58 and 9.07 (3×1H, d, CON$\underline{H}$), hydroxylamine assay 100% (penicillin G), biochromatogram (butanol-ethanol-water) single zone Rf 0.40.

D-α-[D-2-Ureido-3(3'-indolyl)propionamido]phenylacetamidopenicillanic acid (**38**)

Dicyclohexylcarbodiimide (1.13 g, 0.0055 M) was added to a stirred solution of N-carbamoyl-D-tryptophan (1.24 g, 0.005 M) and N-hydroxysuccinimide (0.58 g, 0.005 M) in D.M.F. (15 ml) and acetone (10 ml) at 0°C. The mixture was stirred for 1 hour at 0~5°C and then left overnight in the refrigerator. A solution of D-α-aminophenylacetamidopenicillanic acid (1.68 g, 0.0048 M) in acetone (25 ml), chloroform (25 ml) with triethylamine (*ca* 0.7 ml) chilled to 0°C and the N-succinimido ester filtered in through Celite. After stirring for 1/2 hour at 0°C the solution was allowed to reach ambient temperatures. The crystalline triethylammonium salt of the required penicillin (2.4 g; 72%) was removed by filtration and dried, m.p. 200~203°C (dec.) (Found: C, 59.4; H, 6.7; N, 14.4; S, 4.6%. $C_{34}H_{45}N_7O_6S$ requires C, 60.1; H, 6.7; N, 14.4; S, 4.7%). ν_{max} (KBr) 3350, 1767, 1660, 1640, 1610, 1530, 1458, 1392 and 749 cm^{-1}, δ [$(CD_3)_2SO$] 1.11 (9H, t, $(C\underline{H}_3CH_2)_3$), 2.7~3.2 (6H, m, $(CH_3C\underline{H}_2)_3$), 1.42 (3H, s, $C\underline{H}_3$), 1.53 (3H, s, $C\underline{H}_3$), 2.7~3.2 (2H, m, $C\underline{H}_2CH$), 4.59 (1H, m, $CH_2C\underline{H}$), 4.00 (1H, s, C3–$\underline{H}$), 5.3~5.9 (5H, β-lactams, $C\underline{H}CON\underline{H}_2$), 6.9~7.7 (10H, m, C_6H_5, indolyl C$\underline{H}$), 6.85, 8.53 and 8.97 (3×1H, d, CON$\underline{H}$), 10.84 (1H, br., N$\underline{H}$), hydroxylamine assay 94% (penicillin G), biochromatogram (butanol-ethanol-water) single zone Rf 0.30.

The penicillin free acid was prepared by acidification (pH 2) of an aqueous solution of the triethylammonium salt and collection of the product by filtration.

Cultures

The cultures examined were either clinical isolates or the standard reference strains *Staphylococcus aureus* NCTC 6571 (Oxford) and *Pseudomonas aeruginosa* NCTC 10662.

Minimum Inhibitory Concentrations

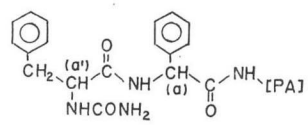
Antibacterial activity was determined by serial dilution of the compounds in nutrient agar (Blood agar Base, Oxoid) incorporating 5% (v/v) defibrinated horse blood (Wellcome).

The Petri dishes were dried at 55°C and inoculated with 0.001 ml of an undiluted 24-hour broth culture of the test strain by means of a replicating device (Dynatech Laboratories), an inoculum of about 10^5 cells per spot. Minimum inhibitory concentrations (MIC) were recorded after incubation for 18 hours at 37°C; the MIC value being taken as the lowest concentration preventing visible growth.

Results and Discussion

The absolute configuration at the asymmetric carbon atom in the side-chain of the clinically available and related penicillins of type **Ia** (Formula I) has been observed to have a pronounced

Table 1. Effect of stereochemistry at the α- and α'-carbon atoms on the antibacterial activity of 6-[α-(α'-ureido-β-phenylpropionamido)phenylacetamido] penicillanic acid



No.	Configuration α	Configuration α'	MIC (μg/ml) Gram-positive organisms *S. aureus*	*S. aureus**	*St. faecalis*	*St. pyogenes*	Gram-negative organisms *E. coli*	*S. typhi*	*Ps. aeruginosa*	*P. mirabilis*	*P. morganii**	*K. aerogenes**
1	D	D	0.25	25	2.5	0.02	5.0	5.0	12.5	5.0	5.0	125
2	D	DL	0.5	50	2.5	0.02	12.5	12.5	25	12.5	25	125
3	D	L	0.25	250	5.0	0.25	125	125	250	125	125	>500
4	L	D	0.5	125	25	0.05	500	250	>500	250	>500	>500
5	L	DL	0.5	50	12.5	0.05	>500	>500	>500	>500	>500	>500
6	L	L	1.25	50	25	0.12	>500	500	>500	500	>500	>500
Ticarcillin			1.25	25	25	0.5	2.5	5.0	12.5	2.5	5.0	250

* β-Lactamase-producing strains

effect on antibacterial activity.[6] In practise the R-configuration, which is usually synonymous with D-stereochemistry in penicillins **Ia**, has been found to be more active than the S-epimer by a factor of four or more. The results in Table 1 clearly demonstrate that at both of the chiral centres in the side-chain of a penicillin of type **Ib** (where R′=Ph; R=$PhCH_2$; X=$-NHCONH_2$) the absolute configuration again played a decisive part in determining antibacterial potency. At both of the dissymmetric centres the D- (=R) configurations gave rise to maximal activity, especially against Gram-negative organisms. In fact in sharp contrast to the good level of activity against a range of Gram-negative bacteria, including *Pseudomonas*, observed for the D,D-stereoisomer (**1**), the L,L-stereoisomer (**6**) is devoid of any significant activity against these organisms. The D,L-stereoisomer (**3**) and the L,D-stereoisomer (**4**) have intermediary activities as expected, although the steric disposition of substituents around the α-carbon atom would appear to have a slightly more pronounced effect than at the α'-carbon atom.

The operation of such a marked stereoselective effect in this penicillin molecule could be due to a number of reasons. The main considerations are:—

(a) Stereoselective microbial biotransformations of the stereoisomer

(b) Differences in active transport into the bacterium *via* the oligopeptide permease system.

(c) Differences in passive transport into the bacterium

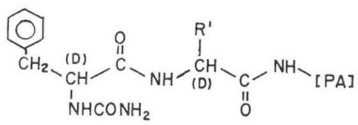
(d) Differences in the free-energy changes involved in the substrate-receptor interactions.

It is not possible to comment on microbial metabolism but active transport *via* the oligopeptide permease would be very unlikely due to the molecular size and absence of a basic α-amino group in these molecules.[7] The stereoisomers **1** and **6** could conceivably have different lipid/water

partition coefficients (P) which would produce differences in their penetration rates into the bacterium and binding to the receptor(s). In fact the log P values (between octanol and water) for these two compounds were found to be almost identical, *viz*, 1.6 and 1.7 for **1** and **6** respectively. This difference is much too small to account for the large difference in antibacterial activity in terms of transport or receptor binding differences. A 'steric' explanation involving a preferred alignment or orientation of the substituents attached to the α and α' centres in **Ib** in relation to its interaction with the receptor would seem to be more plausible. This is a common phenomenon in structure-activity relationships (S.A.R.) and due to our lack of precise knowledge of the receptor involved it is not possible to account for this 'steric' effect. None of the currently available quantitative mathematical models for S.A.R., such as HANSCH analysis, have been able to deal with differences in antipodes or diastereomers.[8] For example, although the antibacterial activities of chloramphenicol derivatives have received considerable attention it is not yet possible to offer any explanation why the naturally occurring D-*threo* stereoisomer should be 99-times more potent than the other three stereoisomers![8,9]

The effects of a number of changes in the R′ substituent at the α-carbon atom in **Ib** (R=$PhCH_2$;

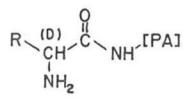
Table 2. Effect of substitution at the α-carbon atom on the antibacterial activity of 6-[D-α-(D-α'-ureido-β-phenylpropionamido)acylamino] penicillanic acid



No.	R′	MIC (μg/ml)									
		Gram-positive organisms				Gram-negative organisms					
		S. aureus	*S. aureus**	*St. faecalis*	*St. pyogenes*	*E. coli*	*S. typhi*	*Ps. aeruginosa*	*P. mirabilis*	*P. morganii**	*K. aerogenes**
1		0.25	25	2.5	0.02	5.0	5.0	12.5	5.0	5.0	125
7		0.5	25	5.0	0.12	12.5	12.5	25	12.5	25	125
8		0.25	12.5	1.25	0.02	5.0	2.5	12.5	5.0	5.0	25
9		0.25	125	2.5	0.12	25	5.0	500	5.0	250	500
10		0.5	25	5.0	0.12	25	25	50	12.5	25	125
11		0.25	25	1.25	0.12	500	250	500	500	500	500
12	$CH_3CH_2CH_2$-	0.5	25	2.5	0.05	25	25	125	25	500	500
13	H	0.5	250	250	0.12	125	50	1000	125	1000	500
14		0.5	12.5	1.25	0.02	2.5	5.0	12.5	1.25	25	50
Ticarcillin		1.25	25	25	0.5	2.5	5.0	12.5	2.5	5.0	250

* β-Lactamase-producing strains

Table 3. Effect of substitution at the α-carbon atom on the antibacterial activity of 6-(D-α-aminoacylamino) penicillanic acid



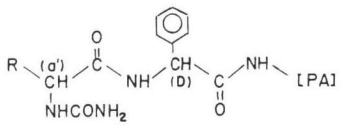
No.	R	MIC (μg/ml)									
		Gram-positive organisms				Gram-negative organisms					
		S. aureus	*S. aureus**	*St. faecalis*	*St. pyogenes*	*E. coli*	*S. typhi*	*Ps. aeruginosa*	*P. mirabilis*	*P. morganii**	*K. aerogenes**
15		0.12	500	1.25	0.02	5.0	1.25	>500	1.25	>500	125
16		0.12	500	2.5	0.05	5.0	1.25	>500	1.25	125	125
17		0.12	250	2.5	0.02	2.5	1.25	500	1.25	50	50
18		0.05	250	2.5	0.02	5.0	1.25	>500	1.25	50	50
19		0.12	500	1.25	0.02	5.0	2.5	>500	2.5	125	125
20		0.25	125	2.5	0.05	50	12.5	>500	25	250	—
21	$CH_3CH_2CH_2$–	0.25	50	2.5	0.12	5.0	2.5	>500	5.0	125	125
22	H	1.25	250	5.0	—	50	25	>500	—	—	—
Ticarcillin		1.25	25	25	0.5	2.5	5.0	12.5	2.5	5.0	250

* β-Lactamase-producing strains

X=$NHCONH_2$; $\alpha=\alpha'=$D) are presented in Table 2. When R′ is a phenyl (**1**) or a bioisosteric 3-thienyl ring (**8**) maximal activity is achieved. Loss of aromaticity as in the case when R′ is cyclohexa-1,4-dienyl ring (**7**) resulted in a slight loss of activity whereas replacing the 3-thienyl substituent (**8**) by the isomeric 2-thienyl (**9**) ring caused a substantial loss in potency, especially against *Ps. aeruginosa* and β-lactamase-producing strains of *Staphylococcus* and *Proteus*. The greatest effects occurred when R′ is a proton (**13**) or a benzyl group (**11**) which resulted in a loss of any significant activity against any of the Gram-negative organisms in both cases. The last two results roughly parallel the effects of these two substituents in the parent α-amino penicillins (**20** and **22**) shown in Table 3, from which the dipeptide penicillins (**11** and **13**) are derived. However the activities in the case of the parent α-amino penicillins (**17** and **18**), which are equipotent, do not correspond with the striking differences mentioned for the 3- and 2-thienyl substituents in the higher homologues (**8** and **9** respectively).

A wider variety of substituent effects were studied at the α'-carbon atom in **Ib** (R′=Ph; X=$NHCONH_2$; $\alpha=$D) and are presented in Table 4. Unlike substitution at the α-carbon atom the direct attachment of an aromatic group, such as a phenyl ring, at the α'-carbon (**23**) did not result in maximal broad-spectrum activity. In fact compound **23** was devoid of anti-pseudomonas activity.

Table 4. Effect of substitution at the α'-carbon atom on the antibacterial activity of 6-[D-α-(α'-ureidoacylamino)phenylacetamido]penicillanic acid

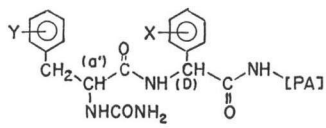


No.	R	α'	MIC (μg/ml)									
			Gram-positive organisms				Gram-negative organisms					
			S. aureus	*S. aureus**	*St. faecalis*	*St. pyogenes*	*E. coli*	*S. typhi*	*Ps. aeruginosa*	*P. mirabilis*	*P. morganii**	*K. aerogenes**
1		D	0.25	25	2.5	0.02	5.0	5.0	12.5	5.0	5.0	125
23		D	0.12	500	1.25	<0.03	12.5	12.5	500	25	125	1000
24		D	0.5	50	1.25	0.05	5.0	5.0	50	2.5	12.5	50
25		DL	0.5	250	5.0	0.05	50	12.5	125	12.5	500	500
26	CH_3-	D	0.25	125	1.25	0.12	12.5	5.0	50	1.25	250	125
27	$CH_3(CH_2)_3-$	D	2.5	50	2.5	0.12	12.5	5.0	50	12.5	25	125
28	$CH_3(CH_2)_5-$	D	0.5	50	5.0	0.25	12.5	12.5	25	12.5	12.5	125
29	$CH_3CH_2\underset{\displaystyle CH_3}{\underset{|}{C}}H-$	DL	1.25	125	5.0	0.25	125	125	250	50	125	500
30	$\begin{matrix}CH_3\\CH_3\end{matrix}\!\!>\!CH-$	D	1.25	50	2.5	0.12	25	25	500	5.0	125	500
31		D	1.25	250	2.5	0.05	125	25	1000	12.5	250	1000
32	$CH_3S(CH_2)_2-$	D	0.5	125	2.5	0.05	25	12.5	50	5.0	25	1000
33	$(CH_3)_2CHCH_2-$	D	0.5	125	25	0.12	25	25	50	5.0	12.5	250
34	$CH_2=CHCH_2-$	D	1.25	125	1.25	0.05	25	5.0	125	5.0	500	250
35	NH_2COCH_2-	D	0.5	50	5.0	0.12	12.5	5.0	25	1.25	50	50
36	$H_2NCO(CH_2)_2-$	D	0.12	250	2.5	0.05	12.5	1.25	125	2.5	125	
37	$PhCH_2OCH_2-$	DL	1.25	250	2.5	0.12	50	25	250	25	25	250
38		D	0.25	12.5	2.5	0.05	5.0	2.5	50	5.0	5.0	12.5
39		DL	0.5	125	2.5	0.12	25	12.5	50	5.0	250	1000
40		D	0.25	250	2.5	0.05	5.0	1.25	50	5.0	50	25
41	H	—	0.5	500	5.0	0.12	12.5	5.0	250	2.5	250	500
42		DL	0.5	250	2.5	0.12	125	50	250	125	125	500
43		D	0.25	12.5	2.5	0.05	12.5	12.5	25	5.0	12.5	125
Ticarcillin			1.25	25	25	0.5	2.5	5.0	12.5	2.5	5.0	125

* β-Lactamase-producing strains

Maximal broad-spectrum activity was achieved by attaching the aromatic or heteroaromatic rings *via* a methylene bridge to the α'-carbon atom (**1**, **38**, **39** and **40**). A good level of activity against the Gram-negative organisms was also obtained for compounds having a straight chain alkyl substituent at the α'-position (**26**, **27** and **28**). However when one of the two hydrogen atoms in the methylene adjacent to the α'-centre was replaced by a further carbon atom substitution (**29**, **30** and **31**) the activity against the Gram-negative organisms was substantially reduced. Increasing the alkylene bridge between the phenyl ring and the α'-carbon atom from methylene (**1**) to ethylene (**25**) resulted in a significant reduction in stability to staphylococcal β-lactamase and activity against *E. coli*, *Ps. aeruginosa* and *P. morganii*, even allowing for the expected two-fold increase in activity for compound **25** if it had the preferred D-configuration at the α'-position. When R is a proton (**41**) the broad-spectrum activity was ampicillin-like, *viz.*, lacking both stability to staphylococcal β-lactamase and clinically useful activity against *Ps. aeruginosa* and *P. morganii*. Compound **38**, derived from D-tryptophan, was of special interest in view of its improved activity against *K. aerogenes*, which is an organism usually sensitive to cephalosporins but characteristically resistant to penicillins. These substituent effects strongly suggest that the size and shape rather than lipophilicity are the important

Table 5. Effect of ring substitution on the antibacterial activity of 6-[D-α-(α'-ureido-β-phenylpropionamido) phenylacetamido]penicillanic acid



No.	X	Y	α'	MIC (μg/ml)									
				Gram-positive organisms				Gram-negative organisms					
				S. aureus	*S. aureus**	*St. faecalis*	*St. pyogenes*	*E. coli*	*S. typhi*	*Ps. aeruginosa*	*P. mirabilis*	*P. morganii**	*K. aerogenes**
1	H	H	D	0.25	25	2.5	0.02	5.0	5.0	12.5	5.0	5.0	125
14	4-OH	H	D	0.5	12.5	1.25	0.02	2.5	5.0	12.5	1.25	25	50
43	H	4-OH	D	0.25	12.5	2.5	0.05	12.5	12.5	25	5.0	12.5	125
44	4-OH	4-OH	D	0.5	12.5	2.5	0.02	12.5	5.0	25	5.0	500	125
45	H	4-F	DL	0.5	25	5.0	0.12	12.5	12.5	50	12.5	12.5	500
46	4-OH	4-F	DL	1.25	12.5	2.5	0.05	12.5	12.5	125	12.5	50	500
47	H	4-Cl	DL	0.5	50	2.5	0.05	12.5	25	125	25	25	500
48	4-OH	4-Cl	DL	0.5	12.5	2.5	0.02	12.5	12.5	50	12.5	12.5	1000
49	H	4-NO_2	DL	0.5	25	5.0	0.12	25	25	125	25	25	250
50	4-OH	4-NO_2	DL	0.5	12.5	1.25	0.02	12.5	5.0	25	50	125	250
51	H	3-OH	DL	0.5	50	2.5	0.05	12.5	5.0	50	5.0	25	250
42	H	4-OCH_3	L	0.5	250	2.5	0.12	125	50	250	125	125	500
52	4-$OCOCH_3$	H	D	12.5	125	12.5	0.2	12.5	12.5	25	12.5	125	125
Ticarcillin				1.25	25	25	0.5	2.5	5.0	12.5	2.5	5.0	250

* β-Lactamase-producing strains

Table 6. Comparison of the antibacterial activities of the two most potent penicillins in this series with some standard penicillins and cephalosporins

Penicillins or cephalosporins	MIC (μg/ml)									
	Gram-positive organisms				Gram-negative organisms					
	S. aureus	*S. aureus**	*St. faecalis*	*St. pyogenes*	*E. coli*	*S. typhi*	*Ps. aeruginosa*	*P. mirabilis*	*P. morganii**	*K. aerogenes**
Compound **38**	0.25	12.5	2.5	0.05	5.0	2.5	50	5.0	5.0	12.5
Compound **14**	0.5	12.5	1.25	0.02	2.5	5.0	12.5	1.25	5.0	50
Ampicillin	0.12	500	1.25	0.02	5.0	1.25	>500	1.25	>500	125
Carbenicillin	1.25	25	50	0.5	2.5	2.5	50	1.25	1.25	250
Ticarcillin	1.25	25	25	0.5	2.5	5.0	12.5	2.5	5.0	250
Cephalexin	1.25	5.0	125	0.5	12.5	5.0	>500	12.5	>500	5.0
Cephalothin	0.12	0.5	25	0.05	12.5	5.0	>500	5.0	500	5.0

* β-Lactamase-producing strains

attributes in the side-chains.

The effects of a number of substitutions in each of the phenyl rings in **Ib** (R′=Ph; R=$PhCH_2$; X=$NHCONH_2$; α=D) are illustrated in Table 5. The presence of an hydroxyl group in one or both of the phenyl rings (**14**, **43** and **44**) preserved the good level of broad-spectrum activity of the parent penicillin (**1**). No improvements in activity were achieved by any of the other substituents and in fact a reduction in activity particularly against *Ps. aeruginosa* and *P. morganii* often occurred.

A comparison is made in Table 6 of the *in vitro* activities of two of the more potent penicillins in this series (**38** and **14**) with some of the well-established broad-spectrum penicillins and cephalosporins in clinical practice. Carbenicillin has a broader spectrum of activity than ampicillin, and the newer penicillins are of interest in view of their improved activity against β-lactamase producing staphylococci, *Pseudomonas* and *Klebsiella* compared to carbenicillin. Ticarcillin (**Ia**; R=3-thienyl; X=CO_2H), which has been marketed recently, has improved anti-pseudomonas activity over carbenicillin and compound **14** is as active as ticarcillin *in vitro* against this important organism.[3] Cephalexin and cephalothin have good activity against *E. coli, S. typhi, P. mirabilis* and *Klebsiella* plus good stability to staphylococcal β-lactamase. Some of the newer cephalosporins undergoing clinical evaluation, such as cefazolin, cephamandole and cefuroxime, are of interest in view of their improved intrinsic activity against a number of Gram-negative organisms in addition to greater stability to those strains which produce β-lactamases.[10] All these cephalosporins, however, are devoid of any anti-pseudomonas activity.

In conclusion, penicillins of type **Ib** have been found which compare favourably in their *in vitro* activities to penicillins (and cephalosporins) in clinical practice and represent an advance over the penicillins of type **Ib** previously reported.[4,11] Antibacterial potency was highly dependent on the nature of the substituents and the stereochemistry at the two chiral centres in the side-chain of these penicillins.

References

1) HERSH, E. M.; G. P. BODEY, B. A. NIES & E. J. FREIREICH: Causes of death in acute leukemia. J. Am. Med. Assoc. 193: 105~109, 1965
2) SMITH, C. B. & M. FINLAND: Carbenicillin; activity *in vitro* and absorption and excretion in normal young men. Appl. Microbiol. 16: 1753~1760, 1963
3) ROLINSON, G. N. & R. SUTHERLAND: Semisynthetic penicillins. Adv. in Pharm. Chem. 11: 151~220, 1973
4) FERRES, H.; M. J. BASKER & P. J. O'HANLON: β-Lactam antibiotics. I. Comparative structure-activity relationships of 6-acylamino penicillanic acid derivatives and their 6-(D-α-acylaminophenyl-acetamido)penicillanic acid analogues. J. Antibiotics 27: 922~930, 1974
5) FERRES, H.; D. J. BEST & A. V. KEMMENOE: Aminoacyl dipeptide penicillins. British Patent 21203/73, 1973
6) NAYLER, J. H. C.: Advances in penicillins. Adv. in Drug Res. 7: 1~105, 1973
7) FICKEL, T. E. & C. GILVARG: Transport of impermeant substances in *E. coli* by way of oligopeptide permease. Nature, New Biol. 241: 161~163, 1973
8) TUTE, M. S.: Principles and practise of HANSCH analysis: A guide to structure-activity correlation for the medicinal chemist. Adv. in Drug Res. 6: 1~77, 1971
9) GARROD, L. P. & F. O'GRADY: Antibiotic and Chemotherapy. Chapter VIII. Chloramphenicol. pp. 132~146, Churchill Livingstone, 1972
10) O'CALLAGHAN, C. H.: Classification of cephalosporins by their antibacterial and pharmacokinetic properties. J. Antimicr. Chemoth. 1: 1~12, 1975
11) HATT, B. W.; P. M. NEWSOME & H. SMITH: Penicillins. British Patent 1180745, 1970