SEMISYNTHETIC β-LACTAM ANTIBIOTICS. III

STRUCTURE-ACTIVITY RELATIONSHIPS OF α-SULFOPENICILLINS[1]

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Structure-activity relationships were examined for a number of α-sulfopenicillins and related compounds. Among the α-sulfopenicillins tested, the α-thienyl and α-(*p*-aminophenyl) homologues showed the greatest activity against *Pseudomonas aeruginosa*, and broad spectra comparable to that of α-sulfobenzylpenicillin (**1**). With the exception of these two homologues, any modification in position or structure of the α-sulfo or α-phenyl group of **1**, diminished activity. Relationships between antipseudomonal or antistaphylococcal activity and a negative charge in the acyl side chain are discussed.

Infections caused by *Pseudomonas aeruginosa* have progressively increased during the last decade[2,3]. Carbenicillin, the first of the antipseudomonal semisynthetic penicillins, has played a significant role in the therapy of pseudomonal infections[4]. Further research has led to the synthesis of other penicillins with improved antipseudomonal activities, *e.g.*, α-tetrazolylbenzylpenicillin[5], α-sulfoaminobenzylpenicillin[6], α-(substituted ureido)benzylpenicillins (α-guanylureido[8,9], α-furoylureido-[9]) and α-carboxy-3-thienylmethylpenicillin[10,11]. Among these new penicillins, α-sulfobenzylpenicillin (**1**) has recently been used clinically[1,12,13]. This paper reports the structure-activity relationships of 32 sulfopenicillins which include related compounds.

Materials and Methods

6-(α-Sulfoacylamido) penicillanic acids, α-methylsulfinyl- and α-methylsulfonylbenzylpenicillins were prepared by acylation of 6-aminopenicillanic acid (6-APA) with the appropriate acid chloride. α-Sulfamoylbenzylpenicillin[15] (compound **4**, Table 1) and 6-(*o*-carboxylbenzamido) penicillanic acid[16] (compound **31**, Table 5) were prepared according to the method described in the previous reports. N-(α-Sulfoacyl) derivatives of α- or *p*- aminobenzylpenicillin (compounds **28**~**30**, **32**) were synthesized by reacting D(−)-α-aminobenzylpenicillin or *p*-aminobenzylpenicillin with α-sulfophenylacetyl chloride, respectively. Products were chromatographically isolated as sodium salts in a manner similar to that described previously[1]. Penicillins used in the MIC test were the mixture of the D- and L-diastereoisomers and confirmed to be substantially pure by NMR analysis[14]. The detailed data on the synthesis and chemical properties of each penicillin will be reported elsewhere.

The minimal inhibitory concentration (MIC) of the penicillins was determined by the agar dilution method. Nutrient agar (pH 7.0) was used as the assay medium. The test organism was grown for 18~24 hours on nutrient agar and one loopful of a suspension

containing about 1 mg per ml of test organism was used as inoculum. MIC was determined after incubation at 37°C for 18 hours.

The eleven microorganisms listed in Table 6 were used in MIC tests for all 32 penicillins but data are given in the other tables only for representative species in order to facilitate comparison.

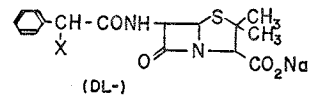
Result and Discussion

α-Sulfobenzylpenicillin (**1**), representative of the series studied, shows broad antibacterial activity against Gram-positive and Gram-negative bacteria including *P. aeruginosa*[1]. Thirty-two penicillins with the related structure to **1** have been synthesized[14] and tested for *in vitro* antibacterial activity. The MIC data for representative four species, *P. aeruginosa*, *Proteus vulgaris*, penicillin G-sensitive and resistant *Staphylococcus aureus* are listed in Tables 1~5. The activities of three typical penicillins in this series against eleven species of bacteria are described in Table 6.

Table 1 compares *in vitro* antibacterial activity of four benzylpenicillins bearing an α-sulfur-containing substituent. Modification of the sulfo group gave a penicillin with a narrow antibacterial spectrum as compared to **1**. Replacement of the sulfo hydroxyl group by a methyl or amino group, as with 3 (X=$-SO_2CH_3$) and 4(X=$-SO_2NH_2$), resulted in penicillins with enhanced antistaphylococcal activity and lowered effect against Gram-negative bacteria.

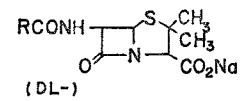
Since the spatial sizes of sulfamoyl and methylsulfonyl moieties do not differ much from that of the sulfo group[7], the activity against penicillin G-resistant *Staphylococcus* of **1** may be explained not by the steric hindrance concept but by the electrostatic blocking due to the presence of the negatively charged sulfo group. The β-lactam of **1** appears to be located near the electron-negative charge and protected from enzymatic hydrolysis with β-lactamase by electrostatic repulsion. The explanation for the antistaphylococcal activity appears to be right for other com-

Table 1. Antibacterial activity of benzylpenicillins with a sulfur-containing α-substituent



No.	X	MIC (μg/ml)			
		Ps. aeruginosa IFO 3448	*Pr. vulgaris* IFO 3045	*S. aureus* FDA 209 P	*S. aureus* No. 87
1	$-SO_3Na$	20	2	0.5	5
2	$-SOCH_3$	>100	50	<0.2	10
3	$-SO_2CH_3$	>100	>100	<0.2	>100
4	$-SO_2NH_2$	>100	10	<0.2	>100

Table 2. Antibacterial activity of 6-(sulfoacylamido) penicillanic acid



No.	R	MIC (μg/ml)			
		Ps. aeruginosa IFO 3448	*Pr.vulgaris* IFO 3045	*S. aureus* FDA 209 P	*S. aureus* No. 87
1		20	2	0.5	5
5		100	1	1	50
6		100	50	2	2
7		50	2	0.5	5
8		100	50	0.5	100

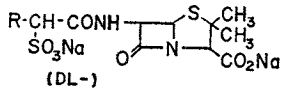
pounds listed in Tables 2~5. Influence of the configuration of the acyl side chain (D- and L-) on activity was already reported[1].

Table 2 shows MIC values for four new sulfopenicillins where the location of the phenyl or sulfo group changes from the α- to an other position. Changing the position of the sulfo or phenyl group from the α- to β-position diminished antibacterial activities, particularly against *P. aeruginosa* (**5** and **6**). Similarly, introduction of a sulfo group into the phenyl ring instead of the α-position eliminated antipseudomonal effect, as with **8**, an isomer of α-sulfo-(p-chlorophenyl)-methylpenicillin (**7**).

Since the positions of both sulfo and phenyl groups greatly influenced the antipseudomonal activity of **1**, enhancing activity is expected when these groups are coincidently located at the α-position of the 6-acyl side chain. Therefore, we prepared and tested other penicillins bearing α-sulfo and a second α-substituent. Table 3 shows the activity of ten α-sulfo-α-alkylmethylpenicillins with alkyl group containing from zero to ten carbon atoms. The alkyl analogues except the lower members (**9**, **10**, **11**) were almost equally active against *Staph aureus*. In contrast with this behavior to Gram-positive bacteria, variation of the chain length of alkyl group greatly affected the activity against Gram-negative bacteria. The alkyl homologues with the broadest spectrum was α-sulfo-*n*-pentylpenicillin (R=C_4H_9-, **13**). The *in vitro* potency of **13** was 2 times greater than that of **1** against penicillin G-resistant *Staph. aureus* but 2~5 times less than that of **1** against *P. vulgaris* and *P. aeruginosa*.

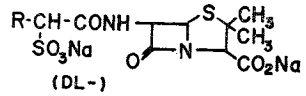
Increasing or decreasing the carbon number

Table 3. Antibacterial activity of 6-(α-sulfo-α-alkylacetamido) penicillanic acid



No.	R	MIC (μg/ml)			
		Ps. aeruginosa IFO 3448	*Pr. vulgaris* IFO 3045	*S. aureus* FDA 209 P	*S. aureus* No. 87
1		20	2	0.5	5
9	H-	>100	>100	50	100
10	CH_3-	>100	>100	20	50
11	CH_3CH_2-	100	20	5	10
12	$CH_3(CH_2)_2$-	50	20	0.5	5
13	$CH_3(CH_2)_3$-	50	10	0.5	2
14	$CH_3(CH_2)_4$-	100	10	0.5	1
15	$CH_3(CH_2)_5$-	>100	50	0.5	2
16	$CH_3(CH_2)_6$-	>100	>100	1	2
17	$CH_3(CH_2)_9$-	>100	>100	0.5	2
18		>100	10	1	5

Table 4. Antibacterial activity of 6-(α-sulfo-α-arylacetamido) penicillanic acid



No.	R	MIC (μg/ml)			
		Ps. aeruginosa IFO 3448	*Pr. vulgaris* IFO 3045	*S. aureus* FDA 209 P	*S. aureus* No. 87
1		20	2	0.5	5
19		10	5	0.2	10
20		20	2	0.5	10
7		50	2	0.5	5
21		100	10	0.2	5
22		>100	5	0.5	5
23		100	20	0.5	5
24		>100	10	1	12.5
25		5	5	1	5
26		50	5	0.5	2
27		10	2	1	5

from the optimal number of four tended to progressively narrow the antibacterial spectrum. Replacement of phenyl with an alkyl group, even with the optimal alkyl chain length, generally led to reduced activity against Gram-negative bacteria. Consequently, we focused our attention on the α-sulfopenicillins having a phenyl or another aryl radical in the α-position.

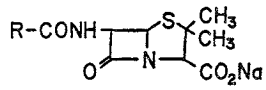
Table 4 compares the activities of ten α-sulfopenicillins containing a substituted phenyl ring or a thienyl group at the α-position. Introducing a substituent into the phenyl group resulted in a penicillin with lower antipseudomonal activity than **1**, except for *o*- and *p*-fluoro-and *p*-amino analogues (**19**, **20** and **25**). A similar decrease in activity caused by introduction of a substituent has been noted for α-amino-[18], α-azido-[18] and α-sulfoaminobenzylpenicillins[6]. The absence of such a decrease with *o*- and *p*-fluoro compounds probably relates to the spatial size of the fluorine atom which is relatively small.

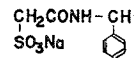
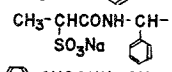
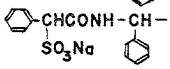
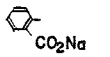
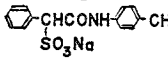
Most striking was the penicillin obtained when an amino group was introduced into the *p*-position of the benzene ring. MIC data in Table 4 show that, of the 23 α-sulfopenicillins, maximum antipseudomonal activity was obtained with compound **25**, which has a broad spectrum like **1**. It is remarkable that introduction of a *p*-amino group did not lead to a decreased activity; this seems to be due to an additive effect of the *p*-amino and the α-sulfo groups.

When the benzene ring was replaced by thiophene, broad spectrum activity comparable or slightly superior to that of **1** was retained. A similar relationship has been found in other series of semisynthetic penicillins[6,11,17,18]. On the other hand, replacement by naphthalene reduced antipseudomonal activity (**26**) and this suggests that when the size of the aromatic ring is increased, Gram-negative activity falls.

Table 5 shows the MIC values for three sulfoacylated ampicillins (**28**~**30**) and two other compounds. Generally, hitherto known antipseudomonal penicillins are also active against penicillin G-resistant strains of *Staphylococcus*[1,4,6,11]. The correlation

Table 5. Antibacterial activity of sulfoacyl derivatives of aminobenzylpenicillin



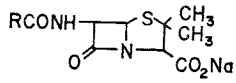
No.	R	MIC (μg/ml)			
		Ps. aeruginosa IFO 3448	*Pr. vulgaris* IFO 3045	*S. aureus* FDA 209 P	*S. aureus* No. 87
1		20	2	0.5	5
28		10	2	0.2	>100
29		20	< 0.2	0.5	50
30		20	1	0.2	>100
31		>100	50	2	5
32		50	2	1	20

between antipseudomonal and antistaphylococcal activity (penicillin G-resistant) no longer holds with these penicillins. Each compound showed good antipseudomonal effect but reduced activity against penicillin G-resistant *Staphylococci*. Antipseudomonal effects probably depend upon the existence of an anionized sulfo group in the α-sulfoacylamido moiety. Since the β-lactam carbon is located far from the negative charge of the sulfo group (they are eight bonds apart) in **28**~**30**, the negative charge presumably cannot help protect it from hydrolysis by β-lactamase. The antistaphylococcal activity of compound **31** can be explained in the same way. Although compounds **28**~**30** belong to a different type of penicillin, these antipseudomonal effects may be contrasted with PRICE's view that the effect of α-sulfoaminobenzylpenicillin against *P. aeruginosa* decreases with increase in the distance of the negative charge of the sulfoamino group from the α-carbon of the acyl side chain[6]. Compound **32**, prepared by reacting *p*-aminobenzylpenicillin with α-sulfophenylacetyl chloride, unexpectedly inhibited *P. aeruginosa*.

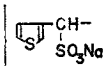
Although the penicillin nucleus is essential for antibacterial activity, the potency is controlled to a great extent by the nature of the side chain. The relative insensitivity of Gram-negative bacteria to most penicillins is probably due to their failure to penetrate to the site of the sensitive enzyme(s) in cell wall synthesis[19,20]. The components of the cell envelope in *P. aeruginosa*, *e.g.* exopolysaccharide as slime[21] and lipopolysaccharide in cell wall[22], are different from those of Enterobacteriaceae, and some of these components may be correlated with penetrability of antibiotics[23,24]. The nature of the penicillin side chain, partly at least, effects on the accessibility of the molecule for the active site in the bacterial cell[25,26].

Based on this cosideration and the experimental results, the following hypothesis is suggested. For activity of penicillin against *P. aeruginosa*, one requirement of the molecular moiety is that an amide group be situated in the α-position to the negatively charged atom or atomic group consisting of heteroatoms and/or the carbon with sp_2

Table 6. Antibacterial activity of semisynthetic penicillins



(DL-)

Test organism	MIC (μg/ml)			
				
Pseudomonas aeruginosa IFO 3038	15	5	10	20
Pseudomonas aeruginosa IFO 3448	20	5	10	50
Pseudomonas aeruginosa NCTC 10490	5	2	1	20
Escherichia coli NIHJ	2	5	5	1
Proteus vulgaris IFO 3045	2	5	2	5
Proteus morganii IFO 3168	5	2	2	5
Proteus mirabilis IFO 12255	1	5	2	1
Staphylococcus aureus FDA 209 P	0.5	1	1	0.2
Staphylococcus aureus No. 87 Pc-R	5	5	5	20
Bacillus subtilis PCI 219	0.2	0.5	0.5	0.2
Sarcina lutea PCI 1001	0.2	1	1	0.2

hybridized orbitals. The amide group in this case may or may not be directly attached to the 6-position of the penicillin nucleus. The orientation of the carbonyl group might be expected to be an important factor in the ability of the enzyme involved in cell-division and elongation to recognize the penicillin as their substrates[20] or in the ability of the molecule to penetrate the permeability barrier. Penicillins possessing the structure moiety; $-\underset{\underset{X^-}{|}}{CH}CONH-$ (X^- = negatively charged group) and an appropriate aryl or alkyl group at the α-position of the acyl side chain, will successfully reach the active sites of the enzymes in the pseudomonal cell membranes and finally inactivate the enzymes by the binding of the molecule.

Since an imido group which is flanked by two carbonyl groups or by a carbonyl and a guanyl group is relatively acidic*, α-furoylureido- and α-guanylureidobenzylpenicillin appear to fall under this category.

In conclusion, α-sulfopenicillins with α-phenyl (**1**), α-thienyl (**27**) and α-(*p*-aminophenyl) group (**25**), possessed the greatest *in vitro* activity among the 23 6-(α-sulfo-α-substituted) acetamidopenic illanic acids and related compounds examined. Table 6 shows the activities of these three typical sulfopenicillins against eight species of bacteria. These antibacterial spectra were essentially the same as that of **1**. In particular, the sodium salt of α-sulfobenzylpenicillin, which has the generic name sulbenicillin, has received extensive clinical evaluation as a broad and antipseudomonal antibiotic[12,13].

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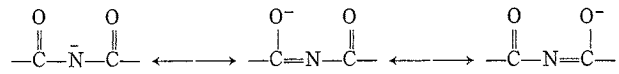
* Guanylurea is acidic, to much higher degree than in an amide, pK=6.15[27]. This is because the anion can be stabilized by resonance and the acyl group provides a larger orbital for electron delocalization.



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