STUDIES ON 6α-SUBSTITUTED PENICILLINS

II. SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF 6β-(2-ARYL-2-SULFOACETAMIDO)-6α-METHOXY PENICILLANIC ACIDS

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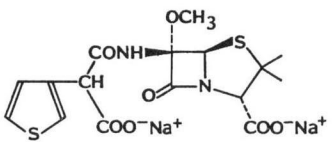
The synthesis and antibacterial activity of 6α-methoxysulbenicillin analogues (**2**) are described. Structure-activity studies of these derivatives bearing hydrophilic substituents in the phenyl ring led to the identification of disodium 6β-[D-2-(3,4-dihydroxyphenyl)-2-sulfoacetamido]-6α-methoxypenicillanate (**2m**) as a compound with potent activity against *Pseudomonas aeruginosa* including β-lactamase producing strains. Additional substitution of **2m** gave derivatives **2p**, **2q**, **2r**, with a further improvement in activity against Gram-negative bacteria.

A recent report[1] from these laboratories described the synthesis of novel penicillin derivatives containing substituents at the 6α-position. Of particular biological interest were those derivatives which combined a 6α-methoxy group with an acid function in the 6β-side chain (*e.g.*, **1**, **2**). These compounds showed *in vitro* activity against a broad range of Gram-negative bacteria; including β-lactamase producing strains, although activity against *Pseudomonas aeruginosa* and Gram-positive bacteria was poor. The results previously reported indicated that the introduction of an amino or hydroxy at the 4-position of the phenyl ring enhanced *in vitro* activity against strains of *P. aeruginosa*.



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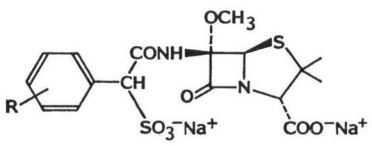

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These results prompted us to prepare a series of 6α-methoxy sulfopenicillins with hydrophilic substituents on the phenyl ring with a view to improving the activity against Gram-negative bacteria, in particular *P. aeruginosa*. Some of our results are presented in this paper.

Chemistry

The preparation of 6α-methoxysulfopenicillins (**2c**～**g**, **2j**～**r**, Table 1) is outlined below (Scheme 1). Acylation of benzyl 6β-amino-6α-methoxypenicillanate (**4**) with a sulfoacetyl chloride (**3**), prepared directly from the corresponding acetyl chloride and sulfur trioxide-dioxan complex[1],

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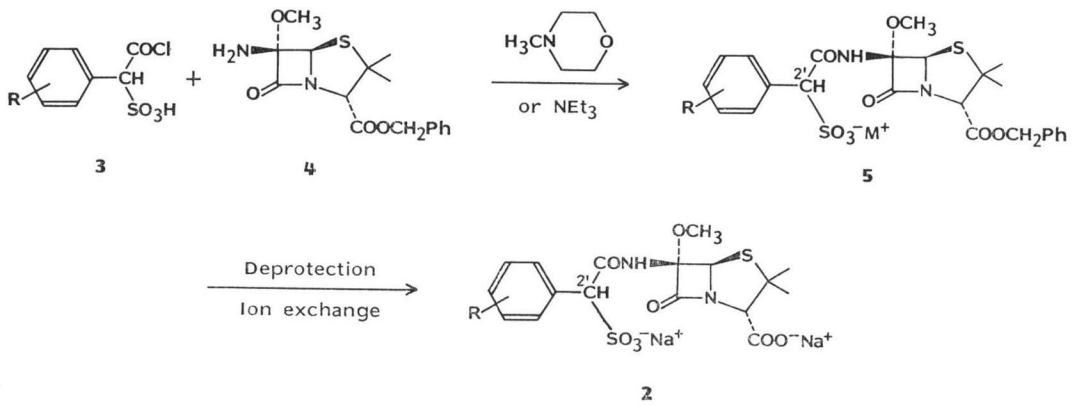
Scheme 1.



3 4 5 2

followed by removal of the protecting groups from the purified intermediate **5** readily provided the sulfopenicillins (**2**).

The protected penicillins (**5**) are formed as a mixture of diastereoisomers, epimeric in the side-chain at C-2′. However, diastereo-induction by the optically active penicillin nucleus **4** resulted in a predominance of the D-isomer in crude **5**. As it is well known in penicillin derivatives[2,3] that C-2′ D-isomers are generally the more antibacterially active diastereoisomers, every effort was made during the chromatographic purification of **5** to enhance the predominance of the C-2′ D-isomers. In certain cases the pure C-2′ D-isomer was obtained by crystallization following initial chromatographic purification.

Hydroxyl groups were protected as their acetates which could be removed by mild hydrolysis of the intermediate **5** or sulfopenicillin (**2**). Amino groups were derived from nitro-substituents by hydrogenation concomitant with removal of the C-3 benzyl protecting group as already described[1]. Alkaline hydrolysis was employed as the method of deprotection to give **2k** and initially **2o**[4] which contain nitro groups. However an enzymatic method[5,6] was later adapted for simultaneous removal of acetates and the C-3 benzyl protecting group to provide **2o** without the racemisation seen at C-2′ with alkaline hydrolysis. Enzymic deprotection was the method of choice for the dihydroxy analogue **2m**.

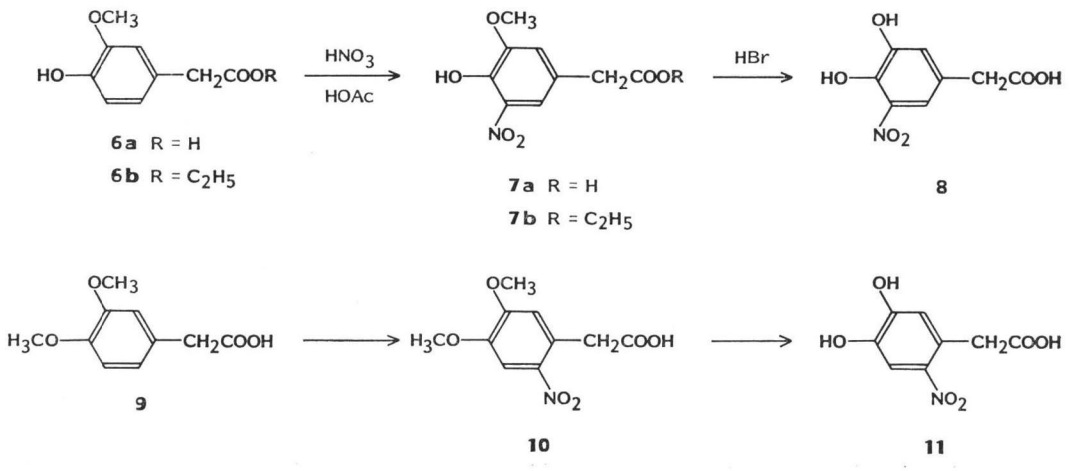
Derivatisation of **2c** with formic acetic anhydride or ethyl acetimidate at controlled pH readily gave **2h** and **2i**. Attempts to prepare the formamidine **6** (R=4-NHCH=NH) in a similar manner, with ethyl formimidate readily provided a new product (HPLC) but on standing this unexpectedly hydrolysed to **2h**. Further studies on this initial product showed that it rapidly decomposed above pH 7 but its formation from **2c** was very slow below pH 8.

Literature procedures were followed for the preparation of known substituted phenylacetic acids and syntheses were devised for the preparation of several new tri-substituted analogues which were required for our structure activity investigations.

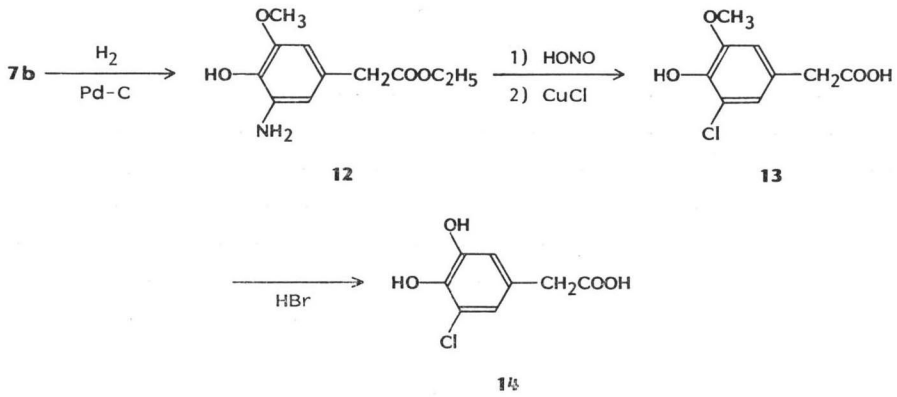
Nitration of homovanillic acid (**6a**) gave a mono-nitro derivative whose ^{1}H NMR aromatic coupling, J=3 Hz, suggested that it was the 5-nitro isomer (**7a**), whereas nitration[7] of homoveratric acid (**9**) gave the 6-isomer† (**10**), with singlets for the aromatic protons. Further confirmation of the struc-

† In the interest of clarity in the discussion, we have employed non-systematic numbering in the nomenclature of some tri-substituted phenylacetic acids.

Scheme 2.



Scheme 3.



ture of this nitration product was obtained by demethylation of **7** and **10** (Scheme 2) to the dihydroxy acids, **8** and **11**, which were non-identical (HPLC). It was later found that **8** was more readily obtained from ethyl homovanillate (**6b**), by nitration followed by treatment with hydrogen bromide in acetic acid.

Reduction of the nitro-ester **7b** followed by diazotisation of the crude amino ester **12** and a Sandmeyer reaction with copper(I) chloride produced, after deprotection, 5-chloro-3,4-dihydroxyphenyl acetic acid† (**14**) (Scheme 3).

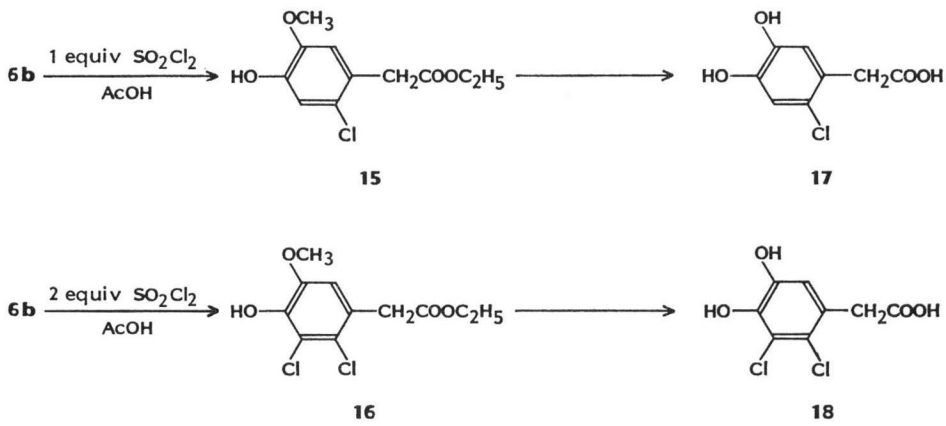
When chlorination of **6b** was examined, as a more direct route to **14**, the 6-chloro† isomer (**15**) was obtained together with a small amount of a dichloro compound (**16**) (Scheme 4). The structure of the major product was confirmed as **15** by deprotection to give the acid **17** which was shown to be different from **14** that had been previously synthesised (mixed mp, mixed ^{1}H NMR).

With 2 equivalents of sulfuryl chloride **16** became the major product. The chlorine substitution pattern was established when the same product, **18**, was obtained by deprotection of **16** or by treatment of **14** with 1 equivalent of sulfuryl chloride.

6-Chloro-3,4-dihydroxyphenylacetic acid† (**17**) was later prepared directly from 3,4-dihydroxy-

† See footnote on p. 1420.

Scheme 4.



phenylacetic acid with 1 equivalent of sulfuryl chloride. However, unlike **14**, further chlorination of **17** gave a mixture rather than the dichloro acid **18**.

Results and Discussion

In our previous paper[1] we identified 6α-methoxysulbenicillin (**2a**) as a β-lactamase stable derivative, active against Enterobacteriaceae and its 4-hydroxy (**2b**) and 4-amino (**2c**) analogues with slightly lower activity against the Enterobacteriaceae but more active against *P. aeruginosa*.

The *in vitro* antibacterial activity of substituted phenyl derivatives against selected organisms is shown in Table 1. As has been found in other series[2,3], the penicillins derived from side-chains with the D-stereochemistry, *e.g.*, **2c**, are more active than those from the L-stereoisomer, *e.g.*, **2d**. Acetate esters, used for the protection of phenolic hydroxyl groups during the preparation of the penicillins, were readily hydrolysed under the conditions of the *in vitro* testing. As a result the antibacterial activity of an acetoxy derivative, *e.g.*, **2e**, was essentially the same as its parent hydroxy compound, *e.g.*, **2b**. Several hydroxy analogues, therefore, were only tested as their acetates.

Interestingly, the moderate activity against ticarcillin-sensitive and -resistant strains of *P. aeruginosa* shown by **2b** was lost when the substituent was moved round the phenyl ring, **2f, g**. A reduction in activity against Gram-negative organisms was also observed when the 4-amino group was derivatised, **2h, i**.

Since the 4-amino, preferably underivatised, or 4-hydroxyl group appeared to be essential for activity against *P. aeruginosa* the possibility of improving this activity by the addition of a second substituent was examined. A number of di-substituted derivatives were synthesised, all containing an amino or hydroxyl group at the 4-position of the phenyl ring. The *in vitro* results, showed that when the second substituent was lipophilic, as exemplified by halogen or nitro, **2j** and **2k**, the activity was less than the mono-substituted derivatives, **2b** or **2c**. A second hydrophilic substituent was also examined. Thus the 3-amino-4-hydroxyphenyl analogue, **2l**, also resulted in a reduction of antibacterial activity, but the 3,4-dihydroxyphenyl analogue, **2m**, showed a greater level of activity than had been seen previously in this series against ampicillin-sensitive or -resistant strains of *Escherichia coli* and particularly against ticarcillin-sensitive or -resistant strains of *P. aeruginosa*, *vide infra*. However, against other members of the Enterobacteriaceae **2m** was only moderately active.

Table 1. *In vitro* activity of 6α-methoxysulbenicillin derivatives (**2a~r**).

2	R	C-2′ D:L	MIC (μg/ml)[a]										
			E.c. NCTC 10418	*E.c.* JT4[b]	*P.a.* NCTC 10662	*P.a.* Dalgleish[c]	*K.p.* A	*S.m.* US32	*E.cl.* N1	*P.m.* C977	*P.m.* 889[b]	*S.a.* Oxford	*S.p.* CN10
a	H	D	5	10	>100	>100	1	10	2.5	2.5	5	>100	50
b	4-OH	7:3	25	25	50	50	5	50	10	25	10	>100	25
c	4-NH_2	D	5	10	25	25	2.5	25	5	5	5	>100	25
d	4-NH_2	L	25	50	50	50	25	100	25	25	50	100	10
e	4-OAc	8:1	25	25	50	50	10	50	25	25	25	>100	25
f	3-OAc	3:2	25	50	>100	>100	10	50	25	25	25	>100	10
g	2-OAc	4:1	100	100	>100	>100	25	>100	100	50	100	>100	>100
h	4-NHCHO	9:1	25	50	50	50	10	100	25	25	25	>100	>100
i	4-NHC(=NH)CH_3	9:1	10	10	25	25	10	25	50	25	25	>100	5
j	3-Cl, 4-NH_2	4:1	50	25	100	100	5	100	10	10	10	>100	10
k	3-NO_2, 4-OH	4:1	100	>100	>100	>100	10	100	10	5	2.5	>100	>100
l	3-NH_2, 4-OH	3:1	50	100	100	100	25	100	50	50	50	>100	50
m	3-OH, 4-OH	D	2.5	2.5	2.5	2.5	5	25	25	25	25	>100	25
n	5-NH_2, 3-OH, 4-OH†	2:1	100	25	50	50	>100	>100	>100	>100	>100	>100	100
o	5-NO_2, 3-OH, 4-OH	D	5	2.5	5	2.5	10	50	50	25	50	>100	50
p	5-Cl, 3-OAc, 4-OAc†	D	0.5	0.25	1	0.5	5	10	10	10	10	>100	25
q	6-Cl, 3-OAc, 4-OAc†	D	2.5	0.5	5	2.5	5	5	5	10	5	>100	5
r	5-Cl, 6-Cl, 3-OAc, 4-OAc†	D	2.5	1	2.5	2.5	2.5	10	1	10	5	>100	25

Test organisms and abbreviations: *E.c.* NCTC 10418; *Escherichia coli* NCTC 10418, *E.c.* JT4; *Escherichia coli* JT4, *P.a.* NCTC 10662; *Pseudomonas aeruginosa* NCTC 10662, *P.a.* Dalgleish; *Pseudomonas aeruginosa* Dalgleish, *K.p.* A; *Klebsiella pneumoniae* A, *S.m.* US32; *Serratia marcescens* US32, *E.cl.* N1; *Enterobacter cloacae* N1, *P.m.* C977; *Proteus mirabilis* C977, *P.m.* 889; *Proteus mirabilis* 889, *S.a.* Oxford; *Staphylococcus aureus* Oxford, *S.p.* CN10; *Streptococcus pyogenes* CN10.

[a] Determined by serial dilution in nutrient agar containing 5% defibrinated horse blood, inoculum 0.001 μl of an overnight broth culture (approximately 10^6 cfu).

[b] Ampicillin-resistant.

[c] Ticarcillin-resistant.

† See footnote on p. 1420.

Table 2. *In vitro* activity of the dihydroxyphenyl analogue, **2m**, against selected strains of *P. aeruginosa* (MIC, μg/ml).

P. aeruginosa strain	**2m**	Ticarcillin	Piperacillin	Cefsulodin
P. aeruginosa NCTC 10662	5	10	5	1
P. aeruginosa ACTC 27853	2.5	10	2.5	1
P. aeruginosa Pu 21p	5	10	5	2.5
P. aeruginosa RPS66	5	125	50	10
P. aeruginosa RPS69	5	125	25	5
P. aeruginosa R17	5	125	125	25
P. aeruginosa 10662 (pip)	2.5	125	125	25
P. aeruginosa Dalgleish	2.5	>1,000	125	50
P. aeruginosa FR4	5	>1,000	250	100

The increased activity seen with **2m** prompted the preparation of a derivative, **2n**, bearing three hydrophilic groups. This proved disappointing, having much reduced activity compared to **2m**. However, in contrast to the di-substituted analogues, the introduction of a lipophilic substituent into **2m** was beneficial. Although a 5-nitro group, **2o**, had only a marginal effect on the *in vitro* activity, a 5-chloro† group, **2p**, significantly increased activity against *E. coli* and *P. aeruginosa*. Substitution of **2m** with a 6-chloro† group retained activity against *E. coli* and *P. aeruginosa* but improved activity against the remaining Enterobacteriaceae tested. Unusually, compound **2r**, a hybrid analogue of the two previous derivatives also combined the improvements in activity seen with those two compounds.

The 3,4-dihydroxyphenyl derivative (**2m**) was further evaluated against a number of strains of *P. aeruginosa* (Table 2). Against the ticarcillin-sensitive strains the 6α-methoxysulfopenicillin (**2m**) showed a similar level of activity to piperacillin and cefsulodin. However, against those strains showing resistance to ticarcillin **2m** showed no increase in MIC whereas piperacillin and cefsulodin were both much less active. Thus **2m** showed a narrow range of MIC values, 2.5~10 μg/ml, against the wide range of strains of *P. aeruginosa* tested.

In conclusion this series of substituted phenyl 6α-methoxysulfopenicillins are β-lactamase stable antibacterial agents. The substituents on the phenyl ring have a profound affect on the spectrum and level of activity achieved. The 3,4-dihydroxyphenyl group (**2m**) in particular increases activity specifically against *E. coli* and *P. aeruginosa*. Further functionalisation of **2m** with a 5-chloro† group (**2p**) improves this still further while a 6-chloro†, substituent, **2q**, affected the remaining Gram-negative organisms tested. The improvements seen in these two derivatives were also seen in the tetra-substituted analogue, disodium 6β-[D-2-(3,4-diacetoxy-5,6-dichlorophenyl)-2-sulfoacetamido]-6α-methoxypenicillanate† (**2r**).

Experimental

IR spectra were recorded on a Perkin Elmer 197,457 or 983 machine for 0.4% w/w sample in a 300-mg KBr disc, unless otherwise stated. ^{1}H NMR spectra were recorded at 60 MHz on a Varian EM 360, at 90 MHz on a Perkin Elmer R32 and at 250 MHz on a Brucker WM250 instrument, for solutions in [$(CD_3)_2CO$], with TMS as internal standard, unless otherwise stated.

Solutions were dried over anhydrous magnesium sulfate and solvents were removed by evaporation under reduced pressure using a rotary evaporator.

Hydrogenation of benzyl-protecting groups was performed in the presence of an equal weight of 10% palladium on carbon at atmospheric temperature and pressure, followed by HPLC and the catalyst

† See footnote on p. 1420.

removed by filtration through Celite.

Compounds used for antibacterial testing were all essentially single materials, or pairs of stereoisomers, analysed by reverse phase HPLC, Waters Associates Inc. μBondapak C-18 column eluted with MeOH in 0.05 M ammonium acetate (pH 4.5) or with acetonitrile in 0.05 M sodium acetate (pH 5.0) and detected by UV absorption at 240 nm. Where possible the identity of the final products was confirmed by positive ion fast atom bombardment mass spectrometry[1].

General Procedures for the Preparation of Penicillins **2c**~**r**

See Table 3.

Acylation of Hydroxyphenylacetic Acids

i) The hydroxyphenylacetic acid (25 mmol) in acetic anhydride (100 ml) and pyridine (1 ml) was stirred overnight then evaporated to dryness. The residue was heated under reflux in H_2O (100 ml) for 0.25 hour, cooled, acidified to pH 2.5 and extracted with EtOAc (3×50 ml). The extracts were washed with saturated brine, dried and evaporated.

ii) The hydroxyphenylacetic acid (10 mmol) in dichloromethane (50 ml) was treated with triethylamine (10 mmol+10 mmol per OH group) and 4-dimethylaminopyridine (0.1~0.5 mmol) then cooled in ice and acetic anhydride or isobutyryl chloride (10 mmol per OH group) added dropwise. After 0.5 hour the solution was washed with dilute HCl (25 ml) and H_2O (25 ml), dried and evaporated.

2-(Substituted-phenyl)-2-sulfoacetyl Chlorides (**3**)

The acetic acid (10 mmol) in dichloromethane (50 ml) was stirred for 1 hour with oxalyl chloride (1.0 ml) and DMF (10 μl). The solution was degassed *in vacuo*, cooled in ice and treated with 1 M sulfur trioxide-dioxan in 1,2-dichloroethane (10 ml). The solution was allowed to warm to *ca.* 20°C overnight and was taken to contain 10 mmol of **3**.

Benzyl 6β-[2-(Substituted-phenyl)-2-sulfoacetamido]-6α-methoxypenicillanates (**5**)

The solution of **3** (10 mmol) was added dropwise to benzyl 6β-amino-6α-methoxypenicillanate (**4**, 3.36 g, 10 mmol) in dichloromethane (40 ml) containing triethylamine (5 ml) or *N*-methylmorpholine (5 ml), cooled in ice. The solution was allowed to warm over 1 hour, washed with dilute HCl (25 ml) and H_2O (50 ml), dried and evaporated to dryness. Chromatography of the residue on silica gel eluting with MeOH in $CHCl_3$ provided the title compound. The required C-2′ D-diastereoisomers were eluted more strongly in later fractions and, where possible, were obtained pure by crystallization[8].

Disodium 6β-[2-(Substituted-phenyl)-2-sulfoacetamido]-6α-methoxypenicillanates (**6**) (See Table 3)

Method A: The benzyl ester in aq MeOH was hydrogenated in the presence of an equal weight of 10% palladium on carbon, filtered through Celite and passed through a column of Amberlite IR-120 (Na^+) to give the disodium salts. The title compounds were isolated by freeze drying following chromatography on Sephadex G-25 or XAD-2 where necessary.

Method B: **2c** in H_2O at 0°C was treated with excess acetic formic anhydride at pH 6.5 then chromatographed on Diaion HP-20 and freeze dried to furnish **2h**. Similarly ethyl acetimidate at pH 8.5 provided **2i**.

Method C: **5k** in aq $NaHCO_3$ was maintained at pH 10 with 1 M NaOH for 3.5 hours, chromatographed on Diaion HP-20 and freeze dried.

Method D: As Method C at pH 9.5 for 1.5 hours followed by Method A.

Method E: The acetoxy-substituted phenylpenicillin ester (0.4 g) in H_2O (15 ml) containing Alcalase (Subtilisin, Carlsberg) bound to Mitsubishi WK10S resin (8 g) was stirred at pH 7.5 and 37°C. When the reaction was complete, HPLC, the mixture was filtered, adjusted to pH 5.5~6, and chromatographed on Diaion HP-20, XAD-2 or Sephadex G-25 to provide the dihydroxypenicillins.

3,4-Dihydroxy-5-nitrophenylacetic Acid (**8**)

4-Hydroxy-3-methoxy-5-nitrophenylacetic Acid (**7a**): Homovanillic acid (**6a**, 1.82 g, 10 mmol) in acetic acid (15 ml) was treated dropwise with conc nitric acid (0.5 ml) in acetic acid (1 ml) at 10~15°C. After 0.5 hour the mixture was poured onto iced water (100 ml) and the precipitate of **7a** collected: MP 215~217°C, 1.24 g (55%); IR ν_{max} (Nujol) cm^{-1} 1700; ^1H NMR (60 MHz, DMSO-d_6) δ 3.61 (2H, s),

Table 3. 6α-Methoxysulfopenicillins (**2c~r**)

	5		2[a]			
	R	Yield (%)	R	D : L[b]	Method	Yield (%)
c	4-NO_2	27	4-NH_2	D	A	87
d	4-NO_2	8	4-NH_2	L	A	81
e	4-OAc	30	4-OAc	8 : 1	A	69
f	3-OAc	10	3-OAc	3 : 2	A	57
g	2-OAc	19	2-OAc	4 : 1	A	56
h			4-NHCHO	9 : 1	B	78
i			4-NHC(=NH)CH_3	9 : 1	B	19
j	3-Cl, 4-NO_2	24	3-Cl, 4-NH_2	4 : 1	A	69
k	3-NO_2, 4-OAc	25	3-NO_2, 4-OH	4 : 1	C	36
l			3-NH_2, 4-OH	3 : 1	D	32
m	3-OAc, 4-OAc	8	3-OH, 4-OH	D	E	67
n	3-OAc, 4-OAc, 5-NO_2	33	3-OH, 4-OH, 5-NH_2^+	2 : 1	A	27
o		9	3-OH, 4-OH, 5-NO_2	D	E	40
p	3-OAc, 4-OAc, 5-Cl[†]	16	3-OAc, 4-OAc, 5-Cl[†]	D	A	79
q	3-OAc, 4-OAc, 6-Cl[†]	5	3-OAc, 4-OAc, 6-Cl[†]	D	A	48
r	3-OAc, 4-OAc, 5-Cl, 6-Cl[†]	7	3-OAc, 4-OAc, 5-Cl, 6-Cl[†]	D	A	59

[a] All **2** had ν_{max} (KBr) cm^{-1} 1770~1760 (β-lactam), 1685~1670 (CONH), 1212~1200, 1045~1036 (SO_3^-).
[b] Estimated by HPLC and/or NMR.
[c] In D,L-mixtures the shifts for the minor isomer, where different, are in parentheses.
[†] See footnote on p. 1420.

3.88 (3H, s), 7.19, 7.37 (2H, 2×d, J=3 Hz).

Anal Calcd for $C_9H_9NO_6$: C 47.58, H 3.99, N 6.17.
Found: C 47.65, H 3.89, N 5.89.

Ethyl 4-Hydroxy-3-methoxy-5-nitrophenylacetic Acid (**7b**): Nitration of ethyl homovanillate (**6b**) as described for **6a** provided **7b**: MP 67~68°C from EtOAc - cyclohexane, 56% yield; IR ν_{max} (Nujol) cm^{-1} 1725, 1645, 1540; ^1H NMR (60 MHz, $CDCl_3$) δ 1.29 (3H, t, J=8 Hz), 3.63 (2H, s), 4.00 (3H, s), 4.22 (2H, q, J=8 Hz), 7.21, 7.67 (2H, 2×d, J=2 Hz), 10.81 (1H, br s).

Anal Calcd for $C_{11}H_{13}NO_6$: C 51.76, H 5.13, N 5.49.
Found: C 51.78, H 4.99, N 5.35.

8: **7a** (5.28 g) (or **7b**) in acetic acid (20 ml) and 48% hydrobromic acid (15 ml) was heated under

and their intermediate C_3 benzyl esters (**5**).

2[a]								
^{1}H NMR[c] (DMSO-d_6 or D_2O, ppm)								FAB-MS (m/z, M+H)
2×2CH$_3$	OCH$_3$	3-H	2′-H	5-H	Aromatic protons	CONH	Other	
1.32, 1.37	3.25	3.90	4.42	5.29	6.3~7.3	9.30		504
1.37, 1.41	3.40	3.97	4.17	5.55	6.3~7.3	9.32		504
1.30, 1.35	3.40	3.89 (3.96)	4.76 (4.44)	5.29 (5.35)	6.9~7.6	9.34	2.22 (Ac)	547
1.36	3.40	3.39 (4.00)	4.80 (4.50)	5.31 (5.38)	6.9~7.5	9.38	2.22 (Ac)	547
1.25, 1.34	3.55	4.21 (4.29)	5.40 (5.28)	5.49 (5.60)	7.1~8.1	(D_2O)	2.37 (Ac)	547
1.28, 1.34	3.43	3.89 (3.96)	4.68 (4.47)	5.30 (5.36)	7.3~7.6	9.33	8.25, 10.17 (NHCHO)	532
1.33	3.40	3.94 (3.98)	4.79 (4.48)	5.25 (5.30)	7.0~7.6	9.35	2.23 (CH$_3$)	501 (Diacid)
1.2~1.6	3.39	3.90 (3.97)	4.54 (4.22)	5.30 (5.34)	6.6~7.4	9.26		—
1.30, 1.35	3.41	3.92 (3.95)	4.76 (4.40)	5.25 (5.32)	6.8~8.0	9.31		—
1.37	3.35	3.92 (3.98)	4.35 (4.12)	5.29 (5.34)	6.4~6.8	9.30 (9.22)		—
1.38	3.38	3.92	4.48	5.31	6.5~6.9	9.30		521
1.40	3.32	3.93 (4.00)	4.17 (4.07)	5.28	6.18, 6.29 (2×d, J=2 Hz)			536
1.33	3.38	3.90	4.38	5.29	6.81~7.20 (2×d, J=2 Hz)	9.25		—
1.25, 1.30	3.39	3.89	4.88	5.27	7.30, 7.59 (2×d, J=2 Hz)	9.27	2.25, 2.30 (2×Ac)	639
1.35	3.40	3.95	5.21	5.26	7.38, 7.68 (2×s)	9.40	2.35 (2×Ac)	—
1.15, 1.28	3.50	4.17	5.42	5.74	7.95	(D_2O)	2.29, 2.33 (2×Ac)	673

reflux for 5 hours while a slow stream of hydrogen bromide was passed through the mixture. The mixture was poured into H_2O and extracted with EtOAc (3×50 ml). The extracts were washed with H_2O, dried and evaporated to give **8**: MP 172~174°C from EtOAc - cyclohexane, 4.24 g (86%); IR ν_{max} (Nujol) cm^{-1} 3370, 1690, 1540, 1350; ^{1}H NMR (60 MHz) δ 3.68 (2H, s), 7.30, 7.60 (2H, 2×d, J=2 Hz), 9.71 (3H, br s).

Anal Calcd for $C_8H_7NO_6$: C 45.08, H 3.31, N 6.57.
Found: C 45.03, H 3.22, N 6.40.

5-Chloro-3,4-dihydroxyphenylacetic Acid† (**14**)

5-Chloro-4-hydroxy-3-methoxyphenylacetic Acid† (**13**): **7b** (10.4 g) in EtOH (60 ml) and THF

† See footnote on p. 1420.

(60 ml) was hydrogenated in the presence of 10% palladium on carbon (1 g) for 1.5 hours. The filtered solution was evaporated to dryness and the crude amino ester (**12**) in 50% HCl (160 ml) treated dropwise, at 0°C, with sodium nitrite (3.04 g) in H_2O (40 ml). Cuprous chloride (4.0 g) in conc HCl (40 ml) was then added and the mixture heated at 45°C for 3.5 hours. The mixture was extracted with EtOAc (3×200 ml) and evaporation of the dried extracts gave **13**: MP 176~177°C from EtOAc - cyclohexane, 4.22 g (49%); ^{1}H NMR (60 MHz, DMSO-d_6) δ 3.50 (2H, s), 3.87 (3H, s), 6.90 (2H, s), 9.09 (1H, br s), 12.28 (1H, br s); MS *m/z* 216.0178 (M, calcd for $C_9H_9ClO_4$ 216.0189).

Anal Calcd for $C_9H_9ClO_4$: C 49.90, H 4.20, Cl 16.36.
Found: C 49.87, H 4.37, Cl 16.23.

14: Demethylation of **13** using the method described for the preparation of **8** gave the title compound: MP 166~168°C from EtOAc - cyclohexane, 89% yield; ^{1}H NMR (60 MHz) δ 3.60 (2H, s), 6.90 (2H, s), 7.60 (3H, br s); MS *m/z* 202.0029 (M, calcd for $C_8H_7ClO_4$ 202.0033).

Anal Calcd for $C_8H_7ClO_4$: C 47.43, H 3.48.
Found: C 47.57, H 3.53.

6-Chloro-3,4-dihydroxyphenylacetic Acid† (**17**)

i) Ethyl 6-Chloro-4-hydroxy-3-methoxyphenylacetate† (**15**): To **6b** (10.5 g, 50 mmol) in AcOH (50 ml) was added, dropwise, sulfuryl chloride (4.0 ml, 47.5 mmol) over 5 minutes. After 1 hour the reaction was poured into iced water (400 ml), the solid collected and crystallized from EtOAc - cyclohexane to provide **15**: MP 98~99°C, 8.09 g (66%); ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.27 (3H, t, *J*=7 Hz), 3.67 (2H, s), 3.82 (3H, s), 4.17 (2H, q, *J*=7 Hz), 5.83 (1H, br s), 6.75, 6.87 (2H, 2×s); MS *m/z* 244.0499 (M, calcd for $C_{11}H_{13}ClO_4$ 244.0503).

17: Demethylation of **15** using the method described for **8** gave **17**: MP 180~181°C from EtOAc - cyclohexane, 67% yield; ^{1}H NMR (60 MHz) δ 3.63 (2H, s), 6.88 (2H, s), 8.77 (3H, br s).

ii) **17**: Treatment of 3,4-dihydroxyphenylacetic acid with sulfuryl chloride by the method described for the preparation of **15** gave **17**, 94% yield.

Anal Calcd for $C_8H_7ClO_4$: C 47.43, H 3.48, Cl 17.50.
Found: C 47.51, H 3.59, Cl 17.44.

5,6-Dichloro-3,4-dihydroxyphenylacetic Acid† (**18**)

Ethyl 5,6-Dichloro-4-hydroxy-5-methoxyphenylacetate† (**16**): Prepared by chlorination of **6b** with **2** equivalents of sulfuryl chloride using the method described for **15**: MP 137.5~138.5°C from EtOAc - cyclohexane, 82% yield; ^{1}H NMR (60 MHz) δ 1.27 (3H, t, *J*=7 Hz), 3.72 (2H, s), 3.88 (3H, s), 4.15 (2H, q, *J*=7 Hz), 6.88 (1H, s), 8.00 (1H, br s); MS *m/z* 278.0114 (M, calcd for $C_{11}H_{12}Cl_2O_4$ 278.0112).

Anal Calcd for $C_{11}H_{12}Cl_2O_4$: C 47.33, H 4.33.
Found: C 47.34, H 4.36.

18: Prepared from **16** by the method described for the preparation of **8**: MP 187~189°C from EtOAc - cyclohexane, 87% yield; ^{1}H NMR (60 MHz) δ 3.70 (2H, s), 6.87 (1H, s), 8.00 (3H, br s); MS *m/z* 235.9636 (M, calcd for $C_8H_6Cl_2O_4$ 235.9644).

Anal Calcd for $C_8H_6Cl_2O_4$: C 40.54, H 2.55.
Found: C 40.40, H 2.54.

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