Preparation of 6-*epi*-Phenoxymethyl- and 6-*epi*-Benzyl-penicillin

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Base-catalysed epimerisation at position 6 of *N*-trimethylsilyl derivatives of phenoxymethylpenicillin benzyl ester in the presence of triethylamine and DBN has been investigated. When triethylamine was used as catalyst a 1,4-thiazepine was obtained in addition to a mixture of the penicillin ester with natural configuration and its 6-epimer. No 1,4-thiazepine was formed when DBN was used as an epimerisation catalyst. The method was also extended to the trimethylsilyl esters of phenoxymethyl- and benzyl-penicillin. The 6-epimers of both penicillins were isolated in *ca.* 70% yield.

BASE-CATALYSED epimerisation of penicillanic acid derivatives has been reported in several communications.[1-5] The process occurs when a diacylamino-, an acylalkylamino-, a trialkylammonio-, or an arylmethyleneamino-substituent is present at the position 6. Attempts to isomerise penicillins with a secondary amide side chain failed. It has been stated [4] that the first proton to be removed by base is that of the secondary amide function, and that the proximity of the resulting negative charge prevents the loss of a second proton at position 6. We have briefly reported [6] a method for the epimerisation of penicillins with a secondary amide side chain, *e.g.*, phenoxymethyl- and benzyl-penicillin. The method is based on *N*-silylation of the amide group with silyl-exchange reagents such as *NO*-bis(trimethylsilyl)acetamide (BSA), followed by treatment with an organic base.

Phenoxymethylpenicillin benzyl ester (Icx) was *N*-silylated with BSA in dichloromethane solution and treated with triethylamine for 24 h at room temperature. On t.l.c. of the reaction mixture, two spots were detected (R_F 0·39 and 0·78). The compound with R_F 0·39, which was insoluble in ether, was isolated in 19·5% yield and identified as (3*S*)-benzyl 2,3,4,7-tetrahydro-2,2-dimethyl-7-oxo-6-phenoxyacetamido-1,4-thiazepine-3-carboxylate (V) on the basis of comparison of its analytical and spectral data with those of the thiazepine prepared from methyl 6-phthalimidopenicillanate.[7,8] The ether-soluble fraction (58%) was a mixture of (IIcx) and (Icx) in the ratio of 7 : 3 (determined by n.m.r.). The mixture could not be separated into its components by thin-layer or column chromatography. When the 6-epimer (IIcx), which had been prepared by benzylation

[1] S. Wolfe and W. S. Lee, *Chem. Comm.*, 1968, 242.
[2] D. A. Johnson, D. Mania, C. A. Panetta, and H. H. Silvestri, *Tetrahedron Letters*, 1968, 1903.
[3] D. A. Johnson and D. Mania, *Tetrahedron Letters*, 1969, 267.
[4] J. P. Clayton, J. H. C. Nayler, R. Southgate, and E. R. Stove, *Chem. Comm.*, 1969, 130.
[5] J. R. Jackson and R. J. Stoodley, *Chem. Comm.*, 1971, 647.
[6] A. Vlietinck, E. Roets, P. Claes, and H. Vanderhaeghe, *Tetrahedron Letters*, 1972, 285.
[7] O. K. J. Kovacs, B. Ekström, and B. Sjöberg, *Tetrahedron Letters*, 1969, 1683.
[8] J. P. Clayton, R. Southgate, B. G. Ramsey, and R. J. Stoodley, *J. Chem. Soc.* (*C*), 1970, 2089.

of (IIax), was treated with BSA and triethylamine, the thiazepine (V) could be isolated in 18% yield and the remaining product (64% yield) contained a 7 : 3 mixture of (IIcx) and (Icx). These results indicate that the same equilibrium mixture was obtained from either (Icx) or (IIcx). The triethylamine-catalysed isomerisation of (Icx), *N*-silylated with BSA, was also studied in dimethylformamide solution (Table). After a short reaction time (2·5 h) 16% yield of the thiazepine (V) and 64% yield of the mixture of (IIcx) and (Icx) (ratio 43 : 57) was obtained. Longer reaction times (24 and 48 h) gave more (30%) of the thiazepine and different ratios (75 : 25 and 95 : 5) of (IIcx) and (Icx). All these experiments were carried out in the presence of 5 equiv. of triethylamine. When (Icx) was isomerised for 24 h in the presence of 10 equiv. of the catalyst, 48% of (V) and only 30% of the mixture of (IIcx) and (Icx) (ratio 98 : 2) was obtained. During prolonged reaction or with larger amounts of base, the increased formation of the thiazepine (V) occurs at the expense of (Icx).

Isomerisation of phenoxymethylpenicillin benzyl ester (Icx) in DMF

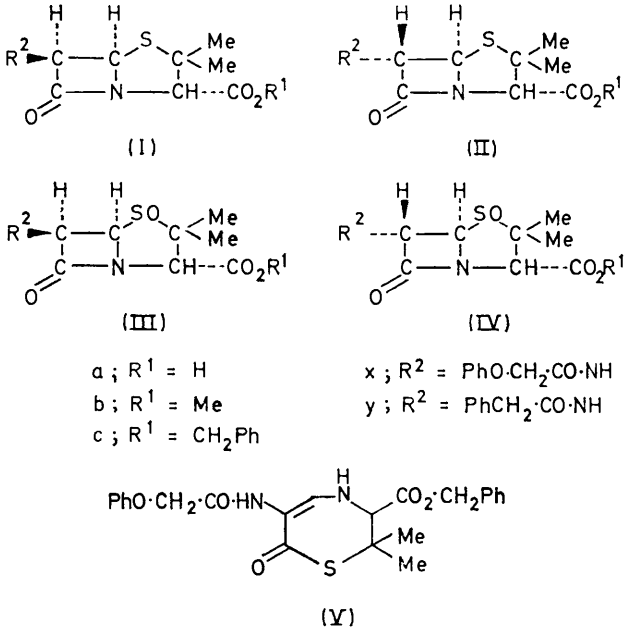
Expt. no.	Equiv. Et_3N	Reaction time (h)	% (V)	% Mixture of (Icx) and (IIcx)	Ratio * (IIcx) : (Icx)	Ratio † (V) : (IIcx) : (Icx)
1	5	2·5	16	64	43 : 57	20 : 34·5 : 45·5
2	5	24	30	52	75 : 25	36·5 : 47·5 : 16
3	5	48	30	40	95 : 5	43 : 54 : 3
4	10	24	48	30	98 : 2	61·5 : 37·5 : 1

* Determined by n.m.r. † Calculated from %(V), % mixture and ratio of (IIcx) and (Icx) in the mixture.

It has been reported [5] that the rearrangement to 1,4-thiazepines does not occur when strong bases such as 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) are used. When a solution of (Icx) in dichloromethane was treated with 2·5 mol. equiv. of BSA and 1 mol. equiv. of DBN for 10 min at room temperature, no rearrangement to (V) occurred, and a 3 : 1 mixture of (IIcx) and (Icx) was obtained. The two epimers could not be separated as such by chromatography but separation was achieved after oxidation to the sulphoxides (IIIcx) and (IVcx) with *m*-chloroperbenzoic acid. The relative amounts isolated were in agreement with the ratio determined by n.m.r., and the physical constants of (IVcx) were identical with those observed for the same product obtained by epimerisation of the sulphoxide (IIIcx).[9]

When (Icx) was *N*-silylated with BSA and kept at room temperature for 24 h in dimethylformamide (DMF), no isomerisation was observed. On the other hand, equilibration of the methyl ester of 6-*epi*-phenoxymethylpenicillin (IIbx) with BSA and DBN also gave a 3 : 1 mixture of (IIbx) and (Ibx).

This method was also applied to the phenoxymethylpenicillin acid (Iax). By treatment with BSA, both amide and carboxy-groups were transformed into trimethylsilyl derivatives. Addition of DBN gave, after 15 min, a 3 : 1 mixture of epimers. The penicillins were isolated from the mixture, as their potassium salts, and were taken up in dry acetone. The potassium salt of (Iax) crystallised in 17% yield, and the epimer (IIax) was obtained from the filtrate in 70% yield. A small amount of penicillin with natural configuration could be removed from (IIax) by treatment with penicillinase, which does not hydrolyse the 6-*epi*-penicillins. The 6-*epi*-penicillin (IIax) was transformed into the methyl ester (IIbx). The physical constants of this compound were different from those published for the product obtained by total synthesis.[10] However, the penicillin obtained by total synthesis was racemic, being prepared from DL-penicillamine. When the method of Bose *et al.* was performed with D-penicillamine,[11] the methyl ester produced had the same physical constants as (IIbx). Thus the 5,6-*trans*-penicillin of Bose *et al.* is indeed the 6-epimer.



Benzylpenicillin (Iay) was epimerised in the same way and 6-*epi*-benzylpenicillin (IIay) was obtained, having the same physical constants as the product obtained from 6-*epi*-aminopenicillanic acid.[3] Except for optical rotation, physical constants of the methyl ester (IIby) were in agreement with those published. It appears that the rotation reported here is correct and that an error was made in recording the original

[9] P. Claes, A. Vlietinck, E. Roets, H. Vanderhaeghe, and S. Toppet, preceding paper.

[10] A. K. Bose, C. Spiegelman, and M. S. Manhas, *J. Amer. Chem. Soc.*, 1968, **90**, 4506.

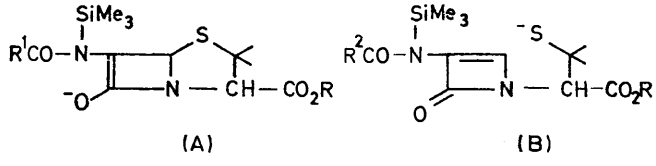
[11] J. Thomis and H. Vanderhaeghe, unpublished results.

data.[12] The antimicrobial activity of (IIay) against *Micrococcus pyogenes* var. *aureus* was only 0·01% of that observed for (Iay); this is in agreement with the results reported by Johnson *et al.*[3] but the activity given by Sawai *et al.*[13] is much greater.

The isolation of the benzylpenicillin acid (Iay) as its ether complex is a delicate operation.[14] We have therefore developed a modification which permits the use of a salt of (Iay). Since BSA will not silylate salts of carboxylic acids,[15] chlorotrimethylsilane was used for this step, and BSA was employed for silylation of the amide function. Epimerisation with DBN gave an equilibrium mixture from which the 6-epimer was isolated in 72% yield. The method was also applied to (Iax).

The DBN-catalysed isomerisation of penicillins with a silylated amide side-chain generally gave 1 : 3 ratio of normal to *epi*-penicillins. These relative amounts are similar to the 2 : 8 ratio observed with penicillin *S*-oxides having a silylated amide function.[9] With penicillin *S*-oxides having a free amide group, a ratio of 4 : 6 was obtained.[9] A practically quantitative conversion of phthalimidopenicillanic derivatives into the 6-epimers has been reported.[5] A similar high conversion was also obtained when the methyl ester of hetacillin was treated with DBN.[16] These data show the influence of the structure of the side chain upon the equilibrium in the base-catalysed epimerisation of penicillins.

The formation of the same equilibrium mixture, starting either from the normal or from the *epi*-penicillin, can be explained in terms of an intermediate enolate (A) or ion pair.[4,17] When a weaker base, such as triethylamine, is used, an intermediate enethiolate (B)



is formed. In this intermediate, the nucleophilic attack of the thiol function on the carbonyl group of the β-lactam, yields the thiazepine (V). We observed the formation of thiazepine (V) when starting either from the normal or from the *epi*-penicillin. This result is in agreement with the observation of Kovacs *et al.*,[7] but differs from those of other authors.[17,18] Our results, obtained during the triethylamine-catalysed isomerisation in dimethylformamide solution, seem to indicate that formation of the thiazepine is faster with the normal penicillin than with the 6-epimer. The fact that most authors [7,17,18] have used phthalimidopenicillanic derivatives in their isomerisation, where the equilibrium is shifted almost completely to the 6-epimer, may explain why the formation of the thiazepine from the 6-epimer was not always observed. For further information concerning the intermediates during the isomerisation of penicillin, the kinetics of the transformation of penicillin into the 6-epimer and the thiazepine should be studied with various side chains and in various solvents.

Direct isomerisation of penicillins is apparently a more convenient method for the preparation of 6-*epi*-penicillins than the deoxygenation of 6-*epi*-sulphoxides.[9] It is possible to separate the salts of *epi*- and normal penicillins making use of their greatly different solubilities. This is not true for the esters, which cannot be separated by chromatography either. The esters of *epi*-penicillins must be prepared by esterification of the acids or by deoxygenation of 6-*epi*-sulphoxide esters.

EXPERIMENTAL

General experimental details are given in the preceding paper.[9] The biotape method described by Cole [19] was used for microbiological assay. The percentage of penicillin with natural configuration in some preparations of 6-*epi*-penicillins was determined by the penicillinase method, which is based on the observation that 6-*epi*-penicillins are penicillinase-resistant. A stirred solution of an alkali salt of the epimeric mixture (0·2 mmol) in carbon dioxide-free water (15 ml), placed in a closed vessel, was accurately adjusted to pH 7 with 0·1N-NaOH using a Radiometer pH-stat apparatus (Titrator type TTT11, pH meter type pH M26, Titrigraph type SBR2, 2·5 ml autoburette type ABU12). An aqueous penicillinase (Penase Leo lot 80096) solution (1 ml; containing 20,000 units ml^{-1}), accurately adjusted to pH 7, was added, and the solution was kept at constant pH (7) by means of the pH-stat. The percentage of penicillin with natural configuration was calculated from the amount of base consumed. All determinations were carried out at 20°. The purity of a benzylpenicillin potassium salt standard assayed by this procedure was in agreement with the value obtained by the alcalimetric method.[20] Penicillin free acids were dissolved in acetone (1 ml), neutralised to the extent of about 90% with NaOH (0·1N), diluted with carbon dioxide-free water (14 ml) and further neutralised as described for the penicillin salts.

Epimerisation of Phenoxymethylpenicillin Benzyl Ester (Icx).—(a) *With BSA and triethylamine in dichloromethane.* A solution of (Icx) (4·4 g, 10 mmol) prepared according to Hauser *et al.*[21] in anhydrous CH_2Cl_2 (10 ml) was treated with BSA (3·06 ml, 12·5 mmol) and stirred for 1 h at room temperature. Freshly distilled triethylamine (7 ml, 50 mmol) was added and the mixture was stored at room temperature for 24 h. It was poured into a mixture of ice–water (150 ml) and HOAc (2N; 25 ml). The suspension was extracted with CH_2Cl_2 (4 × 50 ml). The combined

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[13] T. Sawai, T. Saito, and S. Mitsuhashi, *J. Antibiotics*, 1970, **23**, 488.
[14] N. T. Trenner and R. P. Buhs, *J. Amer. Chem. Soc.*, 1948, **70**, 2897.
[15] J. F. Klebe, H. Finkbeiner, and D. M. White, *J. Amer. Chem. Soc.*, 1966, **88**, 3390.
[16] E. Roets, unpublished results.
[17] B. D. Ramsay and R. J. Stoodley, *Chem. Comm.*, 1971, 450.
[18] S. Wolfe, W. S. Lee, and R. S. Misra, *Chem. Comm.*, 1970, 1067.
[19] M. Cole, *Biochem. J.*, 1969, **115**, 757.
[20] Brit. Pharmacopoeia, 1968, p. 229.
[21] D. Hauser and M. P. Sigg, *Helv. Chim. Acta*, 1967, **50**, 1327.

organic layer was washed with ice–water (2 × 10 ml), dried (Na_2SO_4), and evaporated to 10 ml. Addition of anhydrous ether (50 ml) gave crystalline (3*S*)-benzyl 2,3,4,7-tetrahydro-2,2-dimethyl-7-oxo-6-phenoxyacetamido-1,4-thiazepine-3-carboxylate (V) (723 mg, 16%), m.p. 143—143·5°, $[\alpha]_D^{20}$ −225° (*c* 1 in Me_2CO), R_F 0·39, *m/e* 440, $\lambda_{max.}$ (MeOH) 315 (ε 7806) and 258 (6956), $\nu_{max.}$ 3365 (amine), 3315, 1665, 1535 (amide), 1742, 1220 (ester), 1630 (unsat. thiolactone), and 1568 (C=C) cm^{-1}, δ ($CDCl_3$; TMS) 1·42 (s, CH_3), 1·52 (s, CH_3), 4·33 (d, *J* 6 Hz, 3-H), 4·52 (s, O·CH_2·CO), 5·20 (s, CH_2Ph), 6·62br (NH), 6·86—7·37 (m, Ph), 8·01 (d, *J* 8 Hz, 5-H), and 8·45 (s, amide) (doublets at 4·33 and 8·01 collapsed to singlets when D_2O was added).

The filtrate was evaporated and the residue was dissolved in benzene and chromatographed over silica gel (50 g), using a gradient of benzene changing to benzene–acetone (80 : 20) as eluant. Fractions (10 ml) 56—94 were evaporated to a light yellow oil, dissolved in anhydrous benzene and freeze-dried to yield a mixture (2·57 g, 58%) of (IIcx) and (Icx) in the ratio 67 : 33 (by n.m.r.). Fractions 101—107 contained the thiazepine (V) (147 mg, 3·5%) (total yield 19·5%).

(b) *With BSA and triethylamine in DMF.* Solutions of (Icx) (1 mmol) in anhydrous DMF (1 ml) were silylated for 1 h with BSA (1·25 mmol) and epimerised in the presence of triethylamine (5 mmol) for 2·5, 24, and 48 h and in the presence of triethylamine (10 mmol) for 24 h. The mixtures were worked up as described under (a). Yields of (V) and ratios of (IIcx) to (Icx) are summarised in the Table. Almost pure 6-*epi*-phenoxymethylpenicillin benzyl-ester, m.p. 82—84° (with sintering at 41°), $[\alpha]_D^{20}$ +172° (*c* 1 in Me_2CO), R_F 0·79, *m/e* 440, was obtained in experiments 3 and 4. Physical and spectral data were identical with those reported for (IIcx) obtained by deoxygenation of the sulphoxide (IVcx).

(c) *With BSA and DBN in dichloromethane.* A solution of (Icx) (4·4 g, 10 mmol) in anhydrous CH_2Cl_2 (10 ml) was treated with BSA (6·125 ml, 25 mmol) and stirred at room temperature for 1 h. After cooling to 0°, DBN (1·2 ml, 10 mmol) was added and the solution was stirred for 10 min at room temperature. It was poured into a mixture of ice–water (50 ml) and HOAc (N; 10 ml). The suspension was extracted with CH_2Cl_2 (3 × 100 ml) and the combined organic layer was washed with ice–water (3 × 50 ml), dried (Na_2SO_4), and evaporated to a light yellow oil. This was taken up in CH_2Cl_2 (80 ml), cooled to 0°, and oxidised by dropwise addition, during 30 min, of *m*-chloroperbenzoic acid (85% pure; 2·02 g, 10 mmol) in CH_2Cl_2 (40 ml). The mixture was stirred for 30 min at 0°, washed with $NaHCO_3$ (N; 50 ml) and water, and dried (Na_2SO_4). It was evaporated to a yellow oil, which was triturated with anhydrous ether (50 ml) to give crystals (2·2 g, 48%) of 6-*epi*-phenoxymethylpenicillin *S*-oxide benzyl ester (IVcx). Recrystallisation from boiling methanol (10 ml) gave (IVcx) (1·47 g), m.p. 157—158° (decomp.), $[\alpha]_D^{20}$ +220° (*c* 0·5 in Me_2CO), R_F 0·36, *m/e* 456, identical with the product obtained by epimerisation of (IIIcx).[9] The filtrate was evaporated to dryness; the residue was dissolved in benzene (10 ml) and chromatographed over silica gel (50 g) using a gradient of benzene changing to benzene–acetone (80 : 20) as eluant. Fractions (10 ml) 54—73 were evaporated and the product was crystallised from boiling methanol, yielding (IIIcx) (740 mg, 16%), m.p. 128—129° (decomp.), $[\alpha]_D^{20}$ +142° (*c* 0·5 in Me_2CO), R_F 0·58, *m/e* 456.[9] Fractions 78—104 were evaporated and the product was crystallised from dry benzene, yielding (IVcx) (500 mg) (total yield 59%).

Equilibration of 6-epi-*Phenoxymethylpenicillin Benzyl Ester* (IIcx).—A solution of (IIcx) (440·5 g, 1 mmol) in anhydrous CH_2Cl_2 (1 ml) was *N*-silylated with BSA and epimerised with triethylamine, as described for (Icx), under (a). The mixture yielded the 1,4-thiazepine (V) (80 mg, 18%) and a mixture (282 mg, 64%) of (IIcx) and (Icx) in the ratio of 7 : 3 (by n.m.r.).

6-epi-*Phenoxymethylpenicillin* (IIax) *Potassium Salt.*—*Method* (*a*).—Phenoxymethylpenicillin acid (35·04 g, 100 mmol) was thoroughly dried and suspended in anhydrous CH_2Cl_2 (100 ml). BSA (61·25 ml, 250 mmol) was added and the mixture was stirred for 1 h at room temperature. The solution was chilled to 0°, treated with DBN (15·42 ml, 125 mmol) in anhydrous CH_2Cl_2 (25 ml), stirred for 15 min at room temperature, then poured into H_3PO_4 (5N; 25 ml) in ice–water (750 ml) to give a green suspension. This was covered wth cold ether (750 ml) and acidified (pH 2·3) with H_3PO_4 (40%). The aqueous layer was extracted with ice-cold ether (2 × 500 ml) (the green gum which appeared at the interface was discarded). The combined organic layer was washed with ice–water (2 × 100 ml), stirred with ice–water (300 ml), and adjusted to pH 7 with N-KOH. The aqueous layer was evaporated *in vacuo* to remove the ether and freeze-dried to yield an amorphous powder (34·42 g, 89%) consisting of a mixture of the potassium salts of the two epimers (IIax) and (Iax) in the ratio 3 : 1 (as determined by n.m.r. and confirmed by the penicillinase method). The mixture was dissolved in anhydrous acetone (1 l) and stirred for 1 h at room temperature. The phenoxymethylpenicillin (Iax) potassium salt crystallised; the potassium salt of the 6-epimer remained in solution. After storage overnight, crystals were collected and crystallised from water–acetone, yielding the potassium salt of (Iax) (6·35 g, 16%), m.p. 240° (decomp.), $[\alpha]_D^{20}$ +222° (*c* 0·5 in H_2O), R_F 0·66, $\nu_{max.}$ 3375, 1680, 1500 (amide), 1772 (β-lactam), 1610, and 1395 (CO_2^-) cm^{-1}, δ (D_2O; DSSA) 1·51 (s, CH_3), 4·25 (s, 3-H), 4·55 (s, CH_2), 5·51 (d, *J* 4 Hz, 5-H), 5·58 (d, *J* 4 Hz, 6-H), and 6·81—7·51 (m, Ph).

The filtrate was evaporated to a foam, which was dissolved in anhydrous CH_2Cl_2 (200 ml); the solution was dried (Na_2SO_4) and concentrated to a small volume, and the product was precipitated with petroleum (b.p. 40—60°) yielding the potassium salt of (IIax) as an amorphous solid (29·0 g, 74%), m.p. 162—164° (decomp.), $[\alpha]_D^{20}$ +191° (*c* 1 in H_2O). The product contained 3·3% phenoxymethylpenicillin potassium salt (as determined by the penicillinase method). The latter was removed by treatment with penicillinase as follows. The crude product (22·6 g) was dissolved in water (100 ml) and treated at room temperature with a solution of 50,000 units of penicillinase in water (2 ml). The pH was kept constant (7) by means of a pH-stat apparatus. After 10 min all the penicillin with natural configuration was converted into its penicilloic acid, and the 6-epimer was isolated as its potassium salt, as described previously, yielding (IIax) (20·61 g, 68%), m.p. 169—171° (decomp.), $[\alpha]_D^{20}$ +195° (*c* 0·5 in H_2O), R_F 0·77 (Found: C, 49·4; H, 4·5; N, 7·3. Calc. for $C_{16}H_{17}KN_2O_5S$: C, 49·45; H, 4·4; N, 7·3%); see ref. 9 for spectral data.

Method (*b*). A suspension of (Iax) potassium salt (3·88 g, 10 mmol) in anhydrous CH_2Cl_2 (20 ml) was treated with chlorotrimethylsilane (1·27 ml, 10 mmol) and stirred for

30 min at room temperature. When BSA (3·06 ml, 12·5 mmol) was added the mixture turned into a gelatinous mass, which was stirred for 15 min at room temperature, then chilled to 0°. A solution of DBN (1·2 ml, 10 mmol) in anhydrous CH_2Cl_2 (4 ml) was added, and the suspension was stirred for another 15 min at room temperature. The mixture was worked up as described in the previous section, yielding, after freeze-drying, an amorphous solid (3·66 g, 95%) consisting of a mixture of the two epimeric potassium salts (IIax) and (Iax) in the ratio 3 : 1 (by n.m.r.). The proportion of (Iax) determined by the penicillinase method was 19·7%. Separation of the two epimers carried out as described under (a) afforded (Iax) potassium salt (755 mg, 19%) and (IIax) potassium salt (2·79 g, 71%). The m.p. of (IIax) potassium salt was 165—169° (decomp.) and the rotation ($[\alpha]_D^{20}$) +197° (*c* 0·5 in H_2O). The product contained 0·61% of (Iax) (as determined by the penicillinase method).

6-epi-*Phenoxymethylpenicillin* (IIax) *Acid*.—The potassium salt of (IIax), purified by treatment with penicillinase (2 g, 5·14 mmol), was dissolved in ice–water (150 ml), covered with ether (300 ml) and acidified to pH 2·2 with H_3PO_4 (40%). The ethereal solution of the free acid thus obtained was evaporated to dryness; the residue was dissolved in acetone (1 ml) and treated with water (20 ml), yielding a crystalline precipitate (890 mg, 49%) of (IIax) *acid*, m.p. 155—157° (decomp.), $[\alpha]_D^{20}$ +222° (*c* 0·5 in Me_2CO) (Found: C, 54·85; H, 5·3; N, 7·9. $C_{16}H_{18}N_2O_5S$ requires C, 54·85; H, 5·2; N, 8·0%), $\nu_{max.}$ 3415, 3320, 1640, 1533 (amide), 1780 (β-lactam), and 1745 (CO_2H) cm^{-1}. The phenoxymethylpenicillin acid prepared from the potassium salt had m.p. 129·5—130·5° (decomp.), $[\alpha]_D^{20}$ +174° (*c* 1 in Me_2CO), $\nu_{max.}$ 3335, 1660, 1530 (amide), 1755 (β-lactam), and 1738 (CO_2H) cm^{-1}.

6-epi-*Phenoxymethylpenicillin Methyl Ester* (IIbx).—A solution of (IIax) potassium salt (1·94 g, 5 mmol) in water (20 ml) was chilled to 0°, covered with ice-cold EtOAc (40 ml) and acidified (pH 2) with H_3PO_4 (40%). The organic layer was separated and the aqueous layer was extracted with EtOAc (2 × 20 ml). The combined organic layer was washed with ice–water (2 × 20 ml), dried (Na_2SO_4), and treated with ethereal CH_2N_2 at 0° until a yellow colour persisted and no more gas was evolved. The solution was evaporated to a foam, which was dissolved in anhydrous benzene (20 ml) and freeze-dried, yielding crude (IIbx) (1·42 g, 78%). T.l.c. showed traces of side products. The compound was chromatographed over silica gel (10 g) using a gradient of benzene changing to benzene–acetone (95 : 5) as eluant. Fractions (10 ml) 15—50 were evaporated and the residual oil was crystallised from EtOAc–n-pentane (1 : 10), yielding (IIbx) (1·189 g, 65%) in three crops, m.p. 84·5—87°, $[\alpha]_D^{20}$ +187·5° (*c* 0·5 in Me_2CO), R_F 0·69, *m/e* 364, $\nu_{max.}$ 3350, 1675, 1525 (amide), 1780, (β-lactam), 1745, and 1212 (ester) cm^{-1}, δ ($CDCl_3$; TMS) 1·44 (s, CH_3), 1·59 (s, CH_3), 3·77 (s, OCH_3), 4·52 (s, 3-H and O·CH_2·CO), 5·20 (dd, *J* 1·8 and 8 Hz, 6-H), 5·29 (d, *J* 1·8 Hz, 5-H), 6·82—7·35 (m, Ph), and 7·67br (d, *J* 8 Hz, NH).

Phenoxymethylpenicillin Methyl Ester (Icx).—This ester, prepared by reaction of (Iax) with CH_2N_2, and crystallised from EtOAc–n-hexane, had m.p. 67—69°, $[\alpha]_D^{20}$ +147° (*c* 1 in Me_2CO), R_F 0·69, *m/e* 364, $\nu_{max.}$ 3365, 1695, 1525 (amide), 1782 (β-lactam), 1740, and 1215 (ester) cm^{-1}, δ ($CDCl_3$; TMS) 1·47 (s. CH_3), 1·58 (s, CH_3), 3·75 (s, OCH_3), 4·45 (s, 3-H), 4·52 (s, O·CH_2·CO), 5·55 (d, *J* 4 Hz, 5-H), 5·68 (dd, *J* 4 and 8 Hz, 6-H), and 6·78—7·5 (m, Ph). Physical and spectral data were in agreement with those reported.[22,23]

Equilibration of 6-epi-*Phenoxymethylpenicillin Methyl Ester* (IIbx) *with BSA and DBN*.—A solution of (IIbx) (364·4 mg, 1 mmol) in anhydrous CH_2Cl_2 (1 ml) was chilled to 0°, treated with BSA (0·615 ml, 2·5 mmol) and stirred for 1 h at room temperature. DBN (0·12 ml, 1 mmol) in anhydrous CH_2Cl_2 was added to the cooled solution; the mixture was stirred for 15 min at room temperature and poured into a mixture of ice–water (5 ml) and HOAc (N; 1 ml). The suspension was extracted with CH_2Cl_2 (3 × 10 ml); the combined organic layer was washed twice with ice–water, dried (Na_2SO_4), and evaporated to a brown oil (385 mg) which consisted of (IIbx) and (Ibx) in the ratio 3 : 1 (n.m.r.). The mixture was oxidised with *m*-chloroperbenzoic acid as described earlier. After evaporation the resulting oil was crystallised from anhydrous ether, yielding (IVbx) (157 mg). The filtrate was chromatographed over silica gel (10 g), using a gradient of benzene changing to benzene–acetone (70 : 30) as eluant. Fractions (5 ml) 12—17 yielded (IIIbx) (47·5 mg, 13%), and fractions 21—32 yielded (IVbx) (47 mg, total yield 54%).

Phenoxymethylpenicillin S-*Oxide Methyl Ester* (IIIbx).—Esterification [24] of (IIIax) with CH_2N_2 and crystallisation from EtOAc–petroleum (b.p. 40—60°) gave (IIIbx), m.p. 127—128° (decomp.), $[\alpha]_D^{20}$ +168° (*c* 0·5 in Me_2CO), R_F 0·42, *m/e* 380, $\nu_{max.}$ 3395, 1695, 1508 (amide), 1792 (β-lactam), 1758, 1210 (ester), and 1035 (S=O) cm^{-1}, δ ($CDCl_3$; TMS) 1·20 (s, CH_3), 1·70 (s, CH_3), 3·79 (s, OCH_3), 4·53 (s, CH_2), 4·65 (s, 3-H), 5·04 (d, *J* 4·5 Hz, 5-H), 6·08 (dd, *J* 4·5 and 10 Hz, 6-H), 6·79—7·50 (m, Ph), and 8·23 (d, *J* 10 Hz, NH). Physical and spectral data were in agreement with those reported.[24]

6-epi-*Phenoxymethylpenicillin* S-*Oxide Methyl Ester* (IVbx).—A solution of (IIbx) (182·2 mg, 0·5 mmol) in CH_2Cl_2 (4 ml) was chilled to 0°, and a solution of *m*-chloroperbenzoic acid (85% purity; 105·5 mg, 0·52 mmol) in CH_2Cl_2 (2 ml) was added during 30 min. The mixture was stirred for 30 min at 0°, diluted with CH_2Cl_2, washed with $KHCO_3$ (0·5M; 2 × 10 ml) and water, dried (Na_2SO_4), and evaporated. The residue was triturated with anhydrous ether (10 ml), yielding crystals (136 mg, 72%). Recrystallisation from anhydrous CH_2Cl_2–ether yielded (IVbx) (80·4 mg, 42%), m.p. 125—127° (decomp.), $[\alpha]_D^{20}$ +235° (*c* 0·5 in Me_2CO), R_F 0·24, *m/e* 380, $\nu_{max.}$ 3255, 1675, 1530 (amide), 1782 (β-lactam), 1755, 1220 (ester), and 1052 (S=O) cm^{-1}. δ ($CDCl_3$; TMS), 1·17 (s, CH_3), 1·61 (s, CH_3), 3·72 (s, OCH_3), 4·45 (s, 3-H), 4·50 (s, O·CH_2·CO), 5·10 (d, *J* 2 Hz, 5-H), 5·40 (dd, *J* 2 and 8 Hz, 6-H), 6·77—7·40 (m, Ph), and 7·83 (d, *J* 8 Hz, NH).

6-epi-*Benzylpenicillin* (IIay) *Potassium Salt*.—*Method* (*a*). The di-isopropyl ether complex of benzylpenicillin (86% pure; 8·72 g, 17·2 mmol) [14] was thoroughly dried and dissolved in anhydrous CH_2Cl_2 (20 ml). BSA (14·7 ml, 60 mmol) was added and the mixture was stirred for 60 min at room temperature. The solution was chilled to 0°, treated with DBN (3·09 ml, 25 mmol) in anhydrous CH_2Cl_2

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(5 ml), stirred at room temperature for another 15 min, and worked up as described for (IIax) [method (a)]. After freeze-drying, an amorphous powder (6·7 g, 90%) was obtained which consisted of the two epimers (IIay) and (Iay) in the ratio 3 : 1 (as determined by n.m.r. and confirmed by the penicillinase method). Fractional crystallisation from anhydrous acetone (200 ml) and recrystallisation from acetone–water yielded (Iay) potassium salt (1·16 g. 18%), m.p. 210—212° (decomp.), $[\alpha]_D^{20}$ +279° (*c* 1 in H_2O), R_F 0·66, $\nu_{max.}$ 3370, 1670, 1492 (amide), 1778 (β-lactam), 1615, and 1395 (CO_2^-) cm^{-1}, δ (D_2O; DSSA) 1·50 (s, CH_3), 1·55 (s, CH_3), 3·61 (s, CH_2), 4·24 (s, 3-H), 5·42 (d, *J* 4 Hz, 5-H), 5·50 (d, *J* 4 Hz, 6-H), and 7·30 (s, Ph).

The filtrate was evaporated until crystals appeared. The crystals were collected in two crops, yielding (IIay) *potassium salt* (4·70 g, 73%), m.p. 154—155° (decomp.), $[\alpha]_D^{20}$ +197° (*c* 0·5 in H_2O), R_F 0·75 (Found: C, 49·3; H, 4·85; N, 7·25. $C_{16}H_{17}KN_2O_4S,H_2O$ requires C, 49·2; H, 4·9; N, 7·15%), $\nu_{max.}$ 3740—3100 (hydrate), 3315, 1655, 1550 (amide), 1760 (β-lactam), 1595, and 1398 (CO_2^-) cm^{-1}, δ (D_2O; DSSA) 1·49 (s, CH_3), 1·56 (s, CH_3), 3·62 (s, CH_2), 4·30 (s, 3-H), 4·79 (d, *J* 1·6 Hz, 6-H), 5·24 (d, *J* 1·6 Hz, 5-H), and 7·34 (s, Ph). The penicillinase method showed the presence of 3·6% penicillin with natural configuration. The latter was removed by treatment with penicillinase.

Johnson *et al.*[3] give m.p. 153—154°, $[\alpha]_D$ +196·4 (*c* 1 in H_2O) for (IIay). The antimicrobial activity was 0·01% of that of (Iay) (K salt) against *Micrococcus pyogenes* var. *aureus* ATCC 6538P.

Method (*b*). A suspension of (Iay) potassium salt (3·72 g, 10 mmol) in anhydrous CH_2Cl_2 (20 ml) was treated as described for (IIax) [method (b)] yielding, after freeze-drying, an amorphous powder (3·54 g, 94%) consisting of a mixture of the two epimeric potassium salts (IIay) and (Iay) in a ratio of 3 : 1. These were separated as described earlier, yielding (Iay) potassium salt (670 mg, 18%), m.p. 208—210° (decomp.), $[\alpha]_D^{20}$ +284° (*c* 0·5 in H_2O). The potassium salt (IIay) was isolated in 72% yield (2·68 g), m.p. 154—156° (decomp.), $[\alpha]_D^{20}$ +201° (*c* 0·5 in H_2O). The product contained 2·02% (Iay), as determined by the penicillinase method.

6-epi-*Benzylpenicillin Methyl Ester* (IIby).—A solution of (IIay) potassium salt (931 mg, 2·5 mmol) in water (15 ml) was treated as described for (IIbx). The yellow oil obtained upon evaporation of the mixture was triturated with anhydrous ether (20 ml), yielding (IIby) (712 mg, 82%). Recrystallisation from EtOAc–n-pentane (1 : 10) afforded (IIby) (615 mg, 71%), m.p. 113—114°, $[\alpha]_D^{20}$ +191° (*c* 0·5 in Me_2CO), +196° (*c* 0·5 in $CHCl_3$) R_F 0·61, *m/e* 348, $\nu_{max.}$ (KBr) 3230, 1655, 1550 (amide), 1782 (β-lactam), 1740, and 1210 (ester) cm^{-1}, $\nu_{max.}$ (CH_2Cl_2) 3420, 1680, 1498 (amide), 1778 (β-lactam), and 1748 (ester) cm^{-1}, δ ($CDCl_3$; TMS) 1·42 (s, CH_3), 1·57 (s, CH_3), 3·57 (s, CH_2), 3·70 (s, OCH_3), 4·44 (s, 3-H), 5·01 (dd, *J* 1·8 and 8 Hz, 6-H), 5·12 (d, *J* 1·8 Hz, 5-H), 6·92 (d, *J* 8 Hz, NH), and 7·27 (s, Ph).

Benzylpenicillin Methyl Ester (Iby).—Esterification of the penicillin acid (Iay) with CH_2N_2 and crystallisation from EtOAc–n-hexane gave (Iby), m.p. 97—98°, $[\alpha]_D^{20}$ +246° (*c* 0·5 in Me_2CO), R_F 0·64, *m/e* 348, $\nu_{max.}$ (CH_2Cl_2) 3405, 1675, 1490 (amide), 1779 (β-lactam), and 1747 (ester) cm^{-1}, δ ($CDCl_3$; TMS) 1·45 (s, CH_3), 3·61 (s, CH_2), 3·73 (s, OCH_3), 4·37 (s, 3-H), 5·48 (d, *J* 4 Hz, 5-H), 5·64 (dd, *J* 4 and 8 Hz, 6-H), 6·35 (d, *J* 8 Hz, NH), and 7·30 (s, Ph). Physical data were in agreement with those reported.[22]

Equilibration of 6-epi-*Benzylpenicillin Methyl Ester* (IIby) *with BSA and DBN*.—A solution of (IIby) (522·6 mg, 1·5 mmol) in anhydrous CH_2Cl_2 (15 ml) was *N*-silylated with BSA (0·98 ml, 4 mmol) and treated with DBN (0·18 ml, 1·5 mmol), as described for (IIbx), yielding a yellow oil (466 mg, 89%) which consisted of (IIby) and (Iby) in the ratio 3 : 1 (by n.m.r.). The mixture could not be separated into its components.

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