# Preparation and Deoxygenation of 6-*epi*-Penicillin *S*-Oxides

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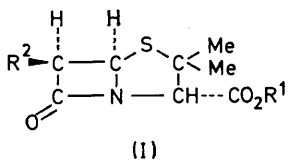
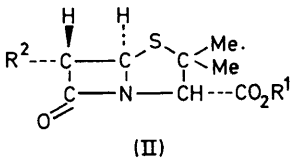
6-*epi*-Phenoxymethyl- and 6-*epi*-benzylpenicillin and their benzyl esters have been prepared by deoxygenation of the corresponding 6-*epi*-*S*-oxides. The latter are obtained by epimerisation of the sulphoxides with normal configuration. Various reaction conditions were investigated for this purpose.

THE 6-epimer of benzylpenicillin (IIay) has been prepared from 6-*epi*-aminopenicillanic acid, which was obtained from hetacillin, a penicillin which is not readily available.[1] As the epimerisation of penicillin *S*-oxide has been described,[2] we thought that deoxygenation of the 6-epimer of benzyl- or phenoxymethylpenicillin *S*-oxide would be a more easily applicable procedure.

The 6-epimer of phenoxymethylpenicillin *S*-oxide trichloroethyl ester has been obtained by treating the ester of normal configuration with *NO*-bis(trimethylsilyl)acetamide (BSA) in dichloromethane.[2] When the same procedure was applied to the benzyl ester of phenoxymethylpenicillin *S*-oxide (IIIcx), the 6-epimer (IVcx) was isolated in 55% yield. As



(I)



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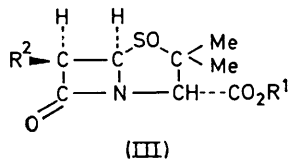

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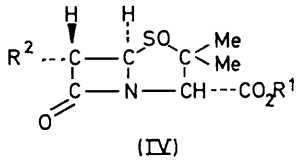


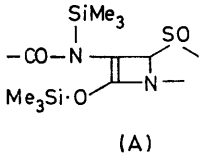
(IV)

a ; $R^1$ = H
b ; $R^1$ = Me
c ; $R^1$ = $CH_2Ph$

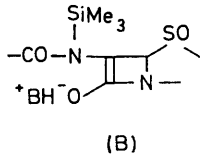
x ; $R^2$ = PhO·$CH_2$·CO·NH
y ; $R^2$ = $PhCH_2$·CO·NH

several alkaline substances catalyse the epimerisation of penicillins, it seemed appropriate to examine their influence on the isomerisation reaction. We used 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), which has been reported to be the most effective of the organic bases.[3] When DBN was added to a dichloromethane solution of (IIIcx), which had been treated with BSA, a 2 : 8 mixture of epimers (IIIcx) and (IVcx) (as determined by n.m.r.) was obtained after 10 min at 0°. From this mixture, the epimer (IVcx) was isolated in 73% yield. Equilibration of (IIIcx) in dichloromethane with DBN alone gave a 4 : 6 mixture of (IIIcx) and (IVcx). The same relative amounts of (IIIcx) and (IVcx) were obtained by treating the 6-epimer (IVcx) with BSA and DBN or with DBN alone. Phenoxymethylpenicillin *S*-oxide (IIIax) was also epimerised with BSA and DBN. After hydrolysis of the intermediate trimethylsilyl ester, 6-*epi*-phenoxymethylpenicillin sulphoxide (IVax) was isolated in 68% yield.

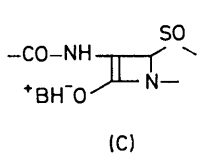
When the benzyl ester of benzylpenicillin *S*-oxide (IIIcy) was treated with BSA alone under the conditions described for (IIIcx), no 6-epimer was obtained. This means either that epimerisation did not occur, or that the 6-epimer was formed and decomposed during the reaction. The latter explanation was suggested by the observation that the 6-epimer (IVcy) decomposed when kept for several days in dichloromethane



(A)



(B)



(C)

at room temperature. Addition of BSA and DBN to a solution of (IIIcy) caused rapid equilibration and again a 2 : 8 mixture of (IIIcy) and (IVcy) was obtained, from which (IVcy) was isolated in 65% yield. Epimerisation of (IIIcy) with DBN alone gave a 4 : 6 mixture. After reaction of the trimethylsilyl ester of benzylpenicillin *S*-oxide (IIIay) with BSA and DBN, the 6-epimer (IVay) was obtained in 87% yield.

The epimerisation by BSA has been explained in terms of the formation of an intermediate silyl enol ether (A).[2] During the isomerisation with BSA and DBN, another intermediate is probably formed. The enolate (B), which has been proposed as intermediate for the isomerisation of some penicillins,[4] would explain the reaction, although an ion pair also could be the transition state of the reaction.[3] A similar enolate (C), with an unsilylated side chain, would be the intermediate in the isomerisation catalysed by DBN alone. In this case, the acidity of the C-6 proton would be enhanced by the sulphoxide system. Penicillins with a secondary amide side chain cannot be isomerised with base.[4] Similarly cephalothin *S*-oxide, but not cephalothin itself, could be epimerised with triethylamine.[5] The side chain also has a distinct influence upon the equilibrium mixture. With a silylated side chain a 2 : 8 mixture of normal and *epi*-forms was obtained, whereas a free secondary amide gave a 4 : 6 mixture.

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[2] G. E. Gutowski, *Tetrahedron Letters*, 1970, 1779.
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[4] J. P. Clayton, J. H. C. Nayler, R. Southgate, and E. R. Stove, *Chem. Comm.*, 1969, 130.
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It has been shown that the sulphoxide groups in phenoxymethyl- and benzyl-penicillin *S*-oxides have the *S*-configuration.[6,7] During the isomerisation of the benzyl and trimethylsilyl esters of these penicillins we observed the formation of only one product. The oxidation of the benzyl ester of 6-*epi*-phenoxymethylpenicillin (IIIcx) with *m*-chloroperbenzoic acid gave the same sulphoxide (IVcx) as had been obtained by isomerisation. Oxidation of the sodium salt of 6-*epi*-phenoxymethylpenicillin (IIax) with sodium periodate gave the sulphoxide (IVax), which was also obtained by hydrogenolysis of the benzyl ester (IVcx) and by isomerisation of the product with normal configuration (IIIax). It has been reported that base-catalysed epimerisation of the (*R*)-oxide of methyl phthalimidopenicillinate occurs without change of the configuration of the sulphoxide function.[8] For this reason we assume that the epipenicillin *S*-oxides have kept the *S*-configuration of the starting products, although further work is necessary to prove this.

Deoxygenation of the sulphoxide function to a sulphide group has been described for cephalosporin *S*-oxide esters [9,10] and penicillin *S*-oxide esters.[11-13] Most of the reagents described here were applied to phenoxymethylpenicillin *S*-oxide benzyl ester (IIIcx). Only the reaction of (IIIcx) with phosphorus tribromide in dimethylformamide at 0° for 20 min [11,12] gave a satisfactory yield (52%) of (Icx). Phosphorus trichloride gave a lower yield, and the other reagents caused extensive degradation. Reduction of the 6-*epi*-*S*-oxide (IVcx) under the same conditions gave a practically quantitative conversion into the 6-*epi*-penicillin ester (IIcx). To get a similar result with the *S*-oxide of the normal series (IIIcx), it was necessary to use a larger amount of phosphorus tribromide (8 mol. equiv. instead of 4) and a reaction time of 45 min. Catalytic hydrogenolysis of (IIcx) under conditions which gave a satisfactory (60%) yield with the product of normal configuration, gave a low yield of 6-*epi*-phenoxymethylpenicillin (IIax).

Catalytic debenzylation of the 6-*epi*-*S*-oxide (IVcx) could be effected in better yield (75%). Deoxygenation of the *epi*-*S*-oxide acid (IVax) was also achieved with phosphorus tribromide in dimethylformamide at −18° for 30 s. By this procedure we were able to isolate the 6-*epi*-penicillin (IIax) as its potassium salt in 76% yield. The overall yield of the conversion (IVcx) ⟶ (IIax) was 41% (*cf.* 20% in the former sequence). The most efficient method of preparation of 6-*epi*-phenoxymethylpenicillin by use of the sulphoxide intermediate, is the sequence (Iax) ⟶ (IIIax) ⟶ (IVax) ⟶ (IIax). This method, however, still is inferior to direct epimerisation of phenoxymethylpenicillin.[14,15]

The epipenicillins are highly soluble in organic solvents. The potassium salt of (IIax) can be dissolved in dichloromethane or butan-2-one, from which it can be precipitated with petroleum or cyclohexane.

Deoxygenation of 6-*epi*-benzylpenicillin *S*-oxide benzyl ester (IVcy) with 4 or 8 mol. equiv. of phosphorus tribromide was incomplete. The desired product (IIcy) was isolated in 52% yield after column chromatography. Deoxygenation was also effected with the free acid (IVay) in about the same yield to produce 6-*epi*-benzylpenicillin (IIay). The physical constants were in agreement with those reported for the product prepared from 6-*epi*-aminopenicillanic acid.[1]

Although the most efficient method for the preparation of 6-*epi*-phenoxymethyl- and benzyl-penicillin was subsequently found to be the isomerisation of the silyl derivatives, the epimerisation of the sulphoxides remains of interest because it shows the influence of changes in various parts of the molecule on the equilibrium mixture. The deoxygenation of sulphoxides may in fact be applicable to other reaction sequences.

## EXPERIMENTAL

M.p.s were determined with a Büchi–Tottoli apparatus and are corrected. Solvents were evaporated off under reduced pressure below 30° (bath). Microanalyses were performed by A. Bernhardt (5251 Elbach über Engelskirchen, West Germany). For t.l.c. we used Merck precoated silica gel F-254 plates, in benzene–acetone (80 : 20) for esters and in acetone–acetic acid (95 : 5) for free acids and their salts. Spots were located by u.v. illumination and exposure to iodine vapour. Column chromatography was performed over silica gel (Merck; 0·05—0·2 mm). Petroleum refers to the fraction of boiling range 40—60°. I.r. spectra were run on a Perkin-Elmer 257 spectrometer for KBr discs, unless otherwise stated. Mass spectra were recorded on an A.E.I. MS12 apparatus. N.m.r. spectra were taken on a Varian A60 or XL100 spectrometer with tetramethylsilane (TMS), hexamethyldisiloxane (HMDS), or the sodium 2,2-dimethyl-2-silapentane-5-sulphonate (DSSA) as internal standard. The first-order coupling constants are the measured peak spacings.

*Phenoxymethylpenicillin S-Oxide* (IIIax).—(*a*) Phenoxymethylpenicillin potassium salt (Iax) was oxidised with sodium periodate [16] to the corresponding sulphoxide (IIIax) (82%), m.p. (from dry acetone) 167—168° (decomp.), $[\alpha]_D^{25}$ +174° (*c* 0·5 in acetone), $R_F$ 0·55, $\nu_{max.}$ 3322, 1678, 1530 (amide), 1795 (β-lactam), 1728 ($CO_2H$), and 1020 (S=O) $cm^{-1}$, δ [$(CD_3)_2SO$; HMDS] 1·23 (s, $CH_3$), 1·61

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(s, $CH_3$), 4·39 (s, 3-H), 4·62 (s, $CH_2$), 5·44 (d, *J* 4·5 Hz, 5-H), 5·95 (dd, *J* 4·5 and 10 Hz, 6-H), 6·75—7·53 (m, Ph), and 8·20 (d, *J* 10 Hz, NH).

(*b*) A suspension of phenoxymethylpenicillin potassium salt (Iax) (11·65 g, 30 mmol) in anhydrous $CH_2Cl_2$ (60 ml) was treated with chlorotrimethylsilane (3·78 ml, 30 mmol) and stirred for 30 min at room temperature. The solution was chilled at 0° and a suspension of *m*-chlorobenzoic acid (85%; 6·08 g, 30 mmol) in anhydrous $CH_2Cl_2$ (60 ml) was added during 30 min. The mixture was stirred for 2 h at room temperature, poured into ice–water (300 ml), acidified (to pH 2·2) with $H_3PO_4$ (40%) and extracted six times with EtOAc (250 ml). The combined organic layer was washed twice with ice–water (100 ml), dried ($Na_2SO_4$), and evaporated until crystallisation occurred, yielding the sulphoxide (IIIax) 10·64 g, 97%), m.p. 157—158° (decomp.). Recrystallisation from anhydrous hot acetone afforded material (9·5 g, 87%) of m.p. 162—164° (decomp.), $[\alpha]_D^{20}$ +176° (*c* 0·5 in $Me_2CO$). Evaporation of the filtrate and crystallisation of the residue from the water gave *m*-chlorobenzoic acid (3·5 g), m.p. 158°.

*Phenoxymethylpenicillin* S-*Oxide Benzyl Ester* (IIIcx). —Freshly distilled triethylamine (6·97 ml, 50 mmol) and freshly distilled benzyl bromide (7·02 ml, 58 mmol) were added to a solution of phenoxymethylpenicillin *S*-oxide (IIIax) (18·3 g, 48·7 mmol) in anhydrous dimethylformamide (DMF). The mixture was stirred for 4 h, poured into ice–water (500 ml), and extracted with $CH_2Cl_2$ (5 × 50 ml). The combined organic layer was successively washed with $NaHCO_3$ (0·05M), HCl (0·05M), and water, dried ($Na_2SO_4$), and evaporated to a yellow oil, which was triturated with anhydrous ether (150 ml) to give the crystalline ester (IIIcx) (17·2 g, 83%), m.p. 126·5—127·5° (decomp.), $[\alpha]_D^{25}$ +142° (*c* 1 in $Me_2CO$). Recrystallisation from boiling methanol increased the m.p. to 128—129°, but the rotation was unchanged; *m/e* 456, $R_F$ 0·58, $\nu_{max.}$ 3352, 1685, 1518 (amide), 1791 (β-lactam), 1752, 1208 (ester), and 1020 (S=O) $cm^{-1}$, δ ($CDCl_3$; TMS) 1·05 (s, $CH_3$), 1·64 (s, $CH_3$), 4·51 (s, $O{\cdot}CH_2{\cdot}CO$), 4·69 (s, 3-H), 5·00 (d, *J* 4·5 Hz, 5-H), 5·22 (AB pattern, $CH_2$), 6·07 (dd, *J* 4·5 and 10·5 Hz, 6-H), 6·81—7·38 (m, Ph), and 8·25 (d, *J* 10·5 Hz, NH). The doublet at 8·25 disappeared and the double doublet at 6·07 collapsed to a doublet on addition of NaOD.

6-epi-*Phenoxymethylpenicillin* S-*Oxide Benzyl Ester* (IVcx).—(a) *Epimerisation with BSA*. Phenoxymethylpenicillin *S*-oxide benzyl ester (IIIcx) (7 g, 15·3 mmol) was thoroughly dried and dissolved in anhydrous $CH_2Cl_2$ (75 ml). BSA (9·3 ml, 38·05 mmol) was added and the mixture was stored under nitrogen at room temperature.[2] Samples taken at regular intervals were analysed by t.l.c. Chromatograms showed the appearance of the epimer (IVcx) ($R_F$ 0·36) and a decrease in intensity of the starting compound (IIIcx). T.l.c. also showed the presence of side-products (at the origin), which were not further investigated. After 10 days all the starting material had disappeared and the solvent was evaporated off to leave a reddish oil, which was triturated with anhydrous ether (60 ml). The crystalline precipitate (4·04 g) was recrystallised from hot methanol yielding the epimer (IVcx) (3·84 g, 55%), m.p. 153·5—155° (decomp.), $[\alpha]_D^{20}$ +220° (*c* 0·5 in $Me_2CO$), *m/e* 456, $R_F$ 0·36, $\nu_{max.}$ 3300, 1688, 1528 (amide), 1784 (β-lactam), 1755, 1210 (ester), and 1038 (S=O) $cm^{-1}$, δ ($CDCl_3$; TMS), 1·05 (s, $CH_3$), 1·59 (s, $CH_3$), 4·55 (s, 3-H and $O{\cdot}CH_2{\cdot}CO$), 5·08 (d, *J* 1·5 Hz, 5-H), 5·21 (AB pattern, $CH_2$), 5·45 (dd, *J* 1·5 and 8 Hz, 6-H), 6·85—7·44 (m, Ph), and 7·71 (d, *J* 8 Hz, NH).

(b) *Epimerisation with BSA and DBN*. A solution of (IIIcx) (1·37 g, 3 mmol) in anhydrous $CH_2Cl_2$ (15 ml) was treated with BSA (0·98 ml, 4 mmol), stirred for 60 min at room temperature, and then cooled to 0°. DBN (0·36 ml, 3 mmol) in anhydrous $CH_2Cl_2$ (5 ml) was added at once and stirring was continued for 10 min at 0°. The mixture was then shaken with HOAc (2N; 1·5 ml) diluted with $CH_2Cl_2$ (10 ml), washed twice with water, dried ($Na_2SO_4$), and evaporated to a brown oil. Trituration of the residue with dry benzene (20 ml) yielded the epimer (IVcx) (1 g, 73%), identical with that described under (a). The filtrate was chromatographed over silica gel (5·0 g) with benzene–acetone (90 : 10) to yield crystalline starting material (IIIcx) (205 mg, 15%). After evaporation the filtrate afforded an amorphous solid (150 mg) consisting mainly of the two epimers, with traces of degradation products. The n.m.r. spectrum of the crude reaction mixture (97%) showed a ratio of 8 : 2 for (IVcx) and (IIIcx).

(c) *Epimerisation with DBN*. DBN (0·24 ml, 2 mmol) in anhydrous $CH_2Cl_2$ (1 ml) was added to a solution of (IIIcx) (913 mg, 2 mmol) in anhydrous $CH_2Cl_2$ (10 ml), cooled to 0°. The solution was stirred for 10 min at 0°, shaken with HOAc (N; 2 ml), diluted with $CH_2Cl_2$ (10 ml), washed twice with water (10 ml), dried ($Na_2SO_4$), and evaporated to a brown oil. Trituration with anhydrous ether (10 ml) yielded a mixture (841 mg, 92%) of the two epimers (IVcx) and (IIIcx) in a ratio of 6 : 4 (as determined by n.m.r.). The crude reaction mixture (500 mg) was crystallised from anhydrous benzene (25 ml) yielding (IVcx) (265 mg, 49%) in a single fraction. Evaporation of the filtrate to an oil, which was then crystallised from boiling methanol, yielded starting material (175 mg, 32%). Evaporation of the filtrate gave an amorphous solid (80 mg) which consisted mainly of a mixture of the two epimers together with side products.

(d) *Oxidation of* 6-epi-*Phenoxymethylpenicillin benzyl ester* (IIcx). The benzyl ester (IIcx) was oxidised with *m*-chloroperbenzoic acid in $CH_2Cl_2$ solution. The sulphoxide (IVcx) was obtained in 80% yield, m.p. 157—158° (decomp.), $[\alpha]_D^{20}$ +220° (*c* 0·5 in $Me_2CO$), *m/e* 456, and was identical with the compound prepared by epimerisation of (IIIcx).

*Epimerisation of* 6-epi-*Phenoxymethylpenicillin* S-*Oxide Benzyl Ester* (IVcx).—(a) *Isomerisation with BSA and DBN*. The *epi*-sulphoxide (IVcx) (685 mg, 1·5 mmol) was equilibrated in the presence of BSA (0·47 ml, 2 mmol) and DBN (0·18 ml, 1·5 mmol) as described under (b) for (IIIcx). The reaction was stopped after 10 min by addition of HOAc (2N; 0·75 ml). After dilution with $CH_2Cl_2$ (5 ml) the organic layer was washed with water, dried ($Na_2SO_4$), and evaporated to a brown oil, which was a mixture of (IVcx) and (IIIcx) in a ratio of 8 : 2 (n.m.r.). Trituration with anhydrous ether (10 ml) yielded 513 mg (75%) of the mixture. The filtrate contained mainly side products. The mixture (300 mg) was crystallised from anhydrous benzene, yielding the epimer (IVcx) (232 mg, 58%). Evaporation of the filtrate and crystallisation from boiling methanol yielded (IIIcx) (68 mg, 16%).

(b) *Isomerisation with DBN*. The *epi*-sulphoxide (IVcx) (228 mg, 0·5 mmol) was equilibrated with DBN (0·06 ml, 0·5 mmol) as described under (c) for (IIIcx). The reaction

was stopped after 10 min by addition of HOAc (N; 0·5 ml). The organic layer was diluted with $CH_2Cl_2$ (5 ml), washed with water, dried ($Na_2SO_4$), and evaporated to a yellow oil, which consisted of (IVcx) and (IIIcx) in a ratio of 6 : 4 (n.m.r.). The oil was crystallised from anhydrous benzene (2 ml), yielding starting material (IVcx) (104·5 mg, 46%). The filtrate was evaporated and crystallised from boiling methanol, yielding (IIIcx) (81 mg, 36%), m.p. 123·5—124·5° (decomp.), $[\alpha]_D^{20}$ +142° (*c* 0·5 in $Me_2CO$), *m/e* 456.

*Deoxygenation of Phenoxymethylpenicillin* S-*Oxide Benzyl Ester* (IIIcx).—$PBr_3$ (0·38 ml, 4 mmol) was added to a solution of (IIIcx) (456 mg, 1 mmol.) in anhydrous DMF (50 ml), cooled to 0°. The mixture was stirred for 20 min at 0° and poured into a cooled solution of $NaHCO_3$ (2 g) in water (100 ml). The suspension was extracted twice with EtOAc (50 ml). Extracts were washed with water, dried ($Na_2SO_4$), evaporated to dryness and chromatographed over silica gel (1·5 g) with benzene–EtOAc (90 : 10) eluant. Fractions (7 ml) 11—15 contained (Icx) (230 mg, 52%), which was crystallised from ether–petroleum; m.p. 74—76°, $[\alpha]_D^{20}$ +144° (*c* 1 in MeCO), *m/e* 440, $R_F$ 0·78, $\nu_{max.}$ 3360, 1690, 1520 (amide), 1790 (β-lactam), 1758, and 1202 (ester) $cm^{-1}$, δ ($CDCl_3$; TMS) 1·40 (s, $CH_3$), 1·55 (s, $CH_3$), 4·49 (s, 3-H), 4·54 (s, $O{\cdot}CH_2{\cdot}CO$), 5·18 (s, $CH_2$), 5·56 (d, *J* 4 Hz, 5-H), 5·70 (dd, *J* 4 and 9 Hz, 6-H), 6·84—7·38 (m, Ph). Physical constants and spectral data were identical with those obtained for (Icx) prepared by benzylation of phenoxymethylpenicillin.[17] Fractions 17—20 yielded starting material (IIIcx) (135 mg, 13%). Reduction of (IIIcx) (456 mg, 1 mmol) under more severe conditions (0·76 ml $PBr_3$ at 0° for 45 min) yielded (Icx). (405 mg, 92%). In this case column chromatography was not necessary for isolation.

6-epi-*Phenoxymethylpenicillin Benzyl Ester* (IIcx).—(a) *From the sulphoxide* (IVcx). The sulphoxide (IVcx) (2·28 g, 5 mmol) was deoxygenated in the presence of $PBr_3$ (1·9 ml, 20 mmol) as described for (IIIcx). The residual oil obtained on evaporation of the EtOAc extracts was dissolved in benzene (75 ml) and freeze-dried, yielding an amorphous powder (2·15 g, 98%), m.p. 82—86° with sintering at 42°, $[\alpha]_D^{20}$ +172° (*c* 1 in $Me_2CO$), *m/e* 440, $R_F$ 0·79, $\nu_{max.}$ 3340, 1680, 1520 (amide), 1778 (β-lactam), 1745, and 1205 (ester) $cm^{-1}$ δ ($CDCl_3$; TMS) 1·36 (s, $CH_3$), 1·55 (s, $CH_3$), 4·50 (s, $O{\cdot}CH_2{\cdot}CO$), 4·54 (s, 3-H), 5·14 (s, $CH_2$), 5·22 (dd, *J* 1·5 and 9 Hz, 6-H), 5·26 (d, *J* 1·5 Hz, 5-H), 6·86—7·38 (m, Ph), and 7·69 (d, *J* 9 Hz, NH).

(b) *By esterification of* (IIax). A solution of (IIax) potassium salt (3·3 g,8·5 mmol) in anhydrous DMF (75 ml) was treated with freshly distilled benzyl bromide (2·42 ml, 20 mmol) and stirred at room temperature for 4 h. The mixture was poured into ice–water (150 ml), extracted with $CH_2Cl_2$ (3 × 50 ml), dried ($Na_2SO_4$), and evaporated to an oil, which was shaken with petroleum (3 × 50 ml), dissolved in dry benzene (50 ml), and freeze-dried, yielding an amorphous powder (3·86 g), which was chromatographed over silica gel (40 g) using a gradient of benzene changing to benzene–acetone as eluant. Fractions (10 ml) 67—105 were evaporated to near dryness; the product was dissolved in dry benzene (25 ml) and freeze-dried, yielding (IIcx) (2·12 g, 57%), which was not obtained in a crystalline state; m.p. 82—84° with sintering at 42°, $[\alpha]_D^{20}$ 171° (*c* 0·5 in $Me_2CO$), *m/e* 440.

6-epi-*Phenoxymethylpenicillin* (IIax).—(a) *Hydrogenolysis of the benzyl ester* (IIcx). A solution of (IIcx) (2 g, 4·55 mmol) in EtOAc (10 ml) was hydrogenated over 10% Pd–C (2 g) for 5 h at room temperature and at a pressure of 3 kg $cm^{-2}$. The catalyst was filtered off and washed with EtOAc (5 × 20 ml). The combined filtrates were concentrated to 25 ml and water (25 ml) was added. The cooled and stirred mixture was adjusted to pH 7 with 0·2N-KOH. Freeze-drying of the aqueous layer yielded the *potassium salt* of (IIax) as a hygroscopic and amorphous powder (360 mg, 20%). Less hygroscopic material was obtained by precipitation with petroleum from a concentrated solution of (IIax) in $CH_2Cl_2$; m.p. 159—161° (decomp.), $[\alpha]_D^{20}$ +194° (*c* 1 in $H_2O$), $R_F$ 0·77. Analysis (Found: C, 49·9; H, 4·5; N, 7·15. $C_{16}H_{17}KN_2O_5S$ requires C, 49·45; H, 4·4; N, 7·2%), $\nu_{max.}$ 3740—3100 (hydrate), 3320, 1665, 1530 (amide), 1760 (β-lactam), 1600, and 1395 ($CO_2^-$) $cm^{-1}$, δ ($D_2O$; DSSA) 1·52 (s, $CH_3$), 1·58 (s, $CH_3$), 4·34 (s, 3-H), 4·54 (s, $CH_2$), 4·88 (d, *J* 1·5 Hz, 6-H), 5·30 (d, *J* 1·5 Hz, 5-H), and 6·81—7·51 (m, Ph). Evaporation of the dried ($Na_2SO_4$) EtOAc layer afforded starting material (IIax) (1·1 g, 55%), which was debenzylated in an identical way, yielding (IIax) (260 mg) (total yield 35%).

(b) *Deoxygenation of* 6-epi-*phenoxymethylpenicillin* S-*oxide* (IVax). A solution of (IVax) (1·8 g, 4·9 mmol) in anhydrous DMF (40 ml) was cooled to −18° and treated with $PBr_3$ (3·81 ml, 40 mmol) for 30 s. The yellow mixture was poured into an ice-cooled aqueous solution (360 ml) of $(NH_4)_2HPO_4$ (35·6 g). The solution was covered with EtOAc (200 ml) and acidified (pH 2) at 0° with 40% $H_3PO_4$. After separation the aqueous layer was extracted EtOAc (2 × 100 ml). The combined organic layer was washed with ice–water (50 ml). After addition of ice–water (50 ml) the mixture was neutralised (pH 7) with N-KOH. The aqueous layer was washed with ether (2 × 25 ml), evaporated to remove the ether, and freeze-dried, yielding 6-*epi*-phenoxymethylpenicillin potassium salt (IIax) (1·45 g, 76%), having the same physical properties as the product described under (a).

6-epi-*Phenoxymethylpenicillin* S-*Oxide* (IVax).—(a) *Hydrogenolysis of the benzyl ester* (IVcx). A solution of (IVcx) (1·5 g, 3·3 mmol) in EtOAc (200 ml) was hydrogenated over 10% Pd–C (1·5 g) for 3 h at 3 kg $cm^{-2}$. The catalyst was filtered off and washed with EtOAc (20 ml) and acetone (5 × 20 ml). The combined filtrates were evaporated and the product was suspended in $CH_2Cl_2$ (20 ml). Water (40 ml) was added and the stirred suspension was neutralised (pH 7·0 with KOH (0·25N). The organic layer was dried ($Na_2SO_4$) and evaporated, and, upon crystallisation from hot methanol, the residual oil afforded starting material (IVcx) (350 mg, 23%). The aqueous layer was concentrated to 15 ml, cooled, and acidified (pH 2·5) with $H_3PO_4$ (40%). The precipitate was filtered off and dissolved in acetone (120 ml). Gradual addition of water (20 ml) gave the crystalline *product* (IVax) (900 mg, 75%), m.p. 158—160° (decomp.), $[\alpha]_D^{20}$ +264° (*c* 0·5 in $Me_2CO$), $R_F$ 0·58 (Found: C, 52·35; H, 4·9; N, 7·65. $C_{16}H_{18}N_2O_6S$ requires C, 52·45; H, 4·95; N, 7·65%), $\nu_{max.}$ 3575, 3518, 3250, 1680, 1550 (amide), 1768 (β-lactam), 1729 ($CO_2H$), and 990 (S=O) $cm^{-1}$, δ [$(CD_3)_2SO$; HMDS] 1·25 (s, $CH_3$), 1·59 (s, $CH_3$), 4·24 (s, 3-H), 4·59 (s, $CH_2$), 5·18 (dd, *J* 1·5 and 9 Hz, 6-H), 5·38 (d, *J* 1·5 Hz, 5-H), 6·89—7·45 (m, Ph), and 9·25 (d, *J* 9 Hz, NH).

(b) *Oxidation of* (IIax). 6-*epi*-Phenoxymethylpenicillin potassium salt (IIax) was oxidised with sodium periodate [16]

[17] D. Hauser and H. P. Sigg, *Helv. Chem. Acta*, 1967, **50**, 1327.

to the corresponding sulphoxide (IVax) (62%), m.p. 161—163° (decomp.), $[\alpha]_D^{20}$ +263° (*c* 0·5 in $Me_2CO$), identical with the compound described under (a).

(c) *Epimerisation of phenoxymethylpenicillin S-Oxide* (IIIax) *with BSA and DBN*. A solution of (IIIax) (1·83 g, 5 mmol) in anhydrous $CH_2Cl_2$ (25 ml) was treated with BSA (3·67 ml, 15 mmol), stirred for 30 min at room temperature, and then cooled to 0°. DBN (0·6 ml, 5 mmol) in anhydrous $CH_2Cl_2$ (2·5 ml) was added at once and stirring was continued for 10 min at 0°. The mixture was then quenched with HOAc (2N; 2·5 ml), diluted with water (20 ml) and evaporated to remove the organic solvent. Finally, it was chilled to 0° and acidified (pH 2·2) with $H_3PO_4$ (40%). The precipitate was centrifuged off and washed with water, yielding a mixture (1·62 g, 88%) of (IVax) and (IIIax) in a ratio of 9 : 1 (n.m.r.). The mixture was crystallised from anhydrous acetone, yielding (IVax) (1·25 g, 68%) in three fractions, m.p. 161·5—162·5° (decomp.), $[\alpha]_D^{20}$ +262° (*c* 0·5 in $Me_2CO$). The filtrate was evaporated; the residue was triturated with anhydrous ether, and the precipitate was crystallised from hot acetone, yielding a mixture (293 mg, 16%) of (IVax) and (IIIax) in a ratio 1 : 1 (n.m.r.).

*Benzylpenicillin S-Oxide* (IIIay).—Benzylpenicillin (Iay) sodium salt (10·68 g, 30 mmol) was oxidised as described for the sodium salt of (Iax) [method (b)] yielding, after crystallisation from EtOAc, crystalline (IIIay) acid (10·4 g, 99%), m.p. 140—142° (decomp.). The compound was sufficiently pure for subsequent reactions and it could be purified by chromatography over silica gel using a gradient of acetone changing to acetone–HOAc (99 : 5) as eluant. Fractions containing (IIIay) were evaporated to dryness and the product was crystallised from anhydrous ether, yielding 87% pure (IIIay), m.p. 143·5—144·5° (decomp.), $[\alpha]_D^{20}$ +207° (*c* 0·5 in $Me_2CO$), $R_F$ 0·56, $\nu_{max.}$ 3380, 1632, 1512 (amide), 1790 (β-lactam), 1745 ($CO_2H$), and 1018 (S=O) $cm^{-1}$, δ [$(CD_3)_2SO$; HMDS] 1·20 (s, $CH_3$), 1·58 (s, $CH_3$), 3·62 (s, $CH_2$), 4·34 (s, 3-H), 5·42 (d, *J* 4·5 Hz, 5-H), 5·80 (dd, *J* 4·5 and 9 Hz, 6-H), 7·32 (s, Ph), and 7·94 (d, *J* 9 Hz, NH).

Less pure (IIIay) was also prepared, in 75% yield, by method (a). The same material (IIIay), m.p. 160—162°, $[\alpha]_D$ +235° ($CHCl_3$) has been prepared by $NaIO_4$ oxidation of (Iay),[12] and material with m.p. 142—143° has been obtained by hydrogenolysis of the benzyl ester.[18]

*Benzylpenicillin S-Oxide Benzyl Ester* (IIIcy).—Benzylpenicillin *S*-oxide (IIIay) triethylammonium salt in DMF was esterified with benzyl bromide as described for (IIIcx) to give the corresponding benzyl ester (IIIcy) (78%), m.p. (methanol) 151—153° (decomp.), $[\alpha]_D^{20}$ +204° (*c* 0·5 in $Me_2CO$), *m/e* 440, $R_F$ 0·57, $\nu_{max.}$ 3395, 3360, 1680, 1510 (amide), 1780 (β-lactam), 1752, 1738, 1208 (ester), and 1020 (S=O) $cm^{-1}$, δ ($CDCl_3$; TMS) 1·03 (s, $CH_3$), 1·62 (s, $CH_3$), 3·58 (s, $CH_2$·CO), 4·67 (s, 3-H), 4·97 (d, *J* 4·5 Hz, 5-H), 5·25 (AB pattern, $CH_2$·O), 6·05 (dd, *J* 4·5 and 10 Hz, 6-H), 7·17 (d, *J* 10 Hz, NH), 7·35 (s, $C_6H_5$·$CH_2$·CO), and 7·43 (s, $C_6H_5CH_2O$) (lit., m.p. 146—148° for the product obtained by oxidation of the benzyl ester [18,19]).

6-epi-*Benzylpenicillin S-Oxide Benzyl Ester* (IVcy).—(a) *Epimerisation with BSA and DBN*. The benzyl ester (IIIcy) (4·4 g, 10 mmol) was epimerised in anhydrous $CH_2Cl_2$ with BSA (3·06 ml, 12·5 mmol) and DBN (1·2 ml, 10 mmol) as described for (IIIcx). The obtained reddish oil (94%), which consisted of the two epimers (IVcy) and (IIIcy) in a ratio 8 : 2 (n.m.r.), was chromatographed over silica gel (50 g) using a gradient of benzene changing to benzene–acetone (80 : 20) as eluant. Fractions (10 ml) 75—91 were evaporated to dryness; the product was triturated with ether (20 ml) and crystallised from anhydrous methanol (10 ml), yielding starting material (IIIcy) (420 mg, 9·5%), m.p. 145—148° (decomp.), $[\alpha]_D^{20}$ +175° (*c* 0·5 in $Me_2CO$), *m/e* 440. Fractions 94—120 were evaporated to dryness and the product was triturated with n-pentane (50 ml), yielding (IVcy) as an amorphous powder (2·86 g, 65%), m.p. 122—124° (decomp.) with sintering at 65°, $[\alpha]_D^{20}$ +228° (*c* 0·5 in $Me_2CO$), *m/e* 440, $R_F$ 0·30, $\nu_{max.}$ 3280, 1665, 1520 (amide), 1785 (β-lactam), 1750, 1210 (ester), and 1048 (S=O) $cm^{-1}$, δ ($CDCl_3$·, TMS) 1·02 (s, $CH_3$), 1·56 (s, $CH_3$), 3·59 (s, $CH_2$·CO), 4·50 (s, 3-H), 5·08 (d, *J* 2 Hz, 5-H), 5·22 (AB pattern, $CH_2$·O), 5·22 (dd, *J* 2 and 8 Hz, 6-H), 7·17 (d, *J* 8 Hz, NH), 7·34 (s, $C_6H_5$·$CH_2$·CO), 7·42 (s, $C_6H_5$·$CH_2$·O).

(b) *Epimerisation with DBN*. Compound (IIIcy) (1·32 g, 3 mmol) was epimerised with DBN as described for (IIIcx). The reaction was stopped by addition of N-HOAc. The residue obtained on evaporation of the $CH_2Cl_2$ layer was freeze-dried from benzene, yielding a 6 : 4 mixture (1·27 g) of (IVcy) and (IIIcy) (n.m.r.). This product (1 g) was chromatographed on a column of silica gel (25 g), using benzene changing to benzene–acetone (80 : 20) as eluant. Fractions 64—96 gave (IIIcy) (0·41 g, 39%); (IVcy) (0·47 g, 45%) was isolated from fractions 108—140.

6-epi-*Benzylpenicillin Benzyl Ester* (IIcy).—(a) *Deoxygenation of* 6-epi-*benzylpenicillin S-oxide benzyl ester* (IVay). The ester (IVcy) (1·32 g, 3 mmol) was treated with $PBr_3$ (1·4 g, 12 mmol) as described for (IIIcx). The brown oil obtained on evaporation of the organic layer was a mixture of (IIcy) and the starting material (IVcy) in a ratio of 1 : 1 (t.l.c.). The oil was chromatographed over silica gel (15 g) using a gradient of benzene changing to benzene–acetone (80 : 20) as eluant. Fractions (5 ml) 42—55 were evaporated to an oil which was dissolved in anhydrous benzene and freeze-dried, yielding (IIcy) (652 mg, 52%) as an amorphous powder, m.p. 75—80° with sintering at 36°, $[\alpha]_D^{20}$ +149° (*c* 0·5 in $Me_2CO$), *m/e* 424 $R_F$ 0·75, $\nu_{max.}$ 3295, 1658, 1520 (amide), 1777 (β-lactam), 1745, and 1220 (ester) $cm^{-1}$, δ ($CDCl_3$; TMS), 1·36 (s, $CH_3$), 1·55 (s, $CH_3$), 3·58 (s, $CH_2$·CO), 4·49 (s, 3-H), 5·03 (dd, *J* 1·5 and 9 Hz, 6-H), 5·13 (d, *J* 1·5 Hz, 5-H), 5·14 (s, $CH_2$·O), 6·76 (d, *J* 9 Hz, NH), 7·29 (s, $C_6H_5$·$CH_2$·CO), 7·34 (s, $C_6H_5$·$CH_2$·O). The yield was not improved by using 8 mol. equiv. of $PBr_3$ and a reaction time of 45 min at 0°.

(b) *Esterification of* (IIay). The potassium salt of (IIay) (1·18 g, 3 mmol) was dissolved in anhydrous DMF (30 ml), treated with benzyl bromide (1·21 ml, 10 mmol) and stirred for 4 h at room temperature. The mixture was poured into ice–water (100 ml) and extracted with ether (3 × 100 ml). The combined organic layer was washed with water (3 × 50 ml), dried ($Na_2SO_4$), and evaporated to a light yellow oil, which was shaken with n-pentane (3 × 75 ml) to remove the excess of benzyl bromide. Finally, the oil was dissolved in anhydrous benzene and freeze-dried, yielding a yellow oil (1·21 g, 91%). Chromatography of the oil over silica gel, in a manner similar to that described in the previous section, yielded, after freeze-drying, (IIcy) (973 mg, 73%) as an amorphous solid, m.p. 75—80° with sintering at 38°, $[\alpha]_D^{20}$ +151° (*c* 0·5 in $Me_2CO$). The other

[18] A. W. Chow, N. M. Hall, and J. R. E. Hoover, *J. Org. Chem.*, 1962, **27**, 1381.

[19] C. Cavallito and J. Harley, *J. Org. Chem.*, 1950, **15**, 815.

physical constants were identical with those described under (a).

6-epi-*Benzylpenicillin* S-*Oxide* (IVay).—The sulphoxide (IIIay) (4·375 g, 12·5 mmol) was epimerised with BSA (9·02 ml, 37·5 mmol) and DBN (1·5 ml, 12·5 mmol) as described for (IIIax). The crude mixture obtained by acidification of the aqueous layer was thoroughly washed with EtOAc to remove the starting material (IIIay), and was twice washed with ether (50 ml), yielding (IVay) (3·8 g, 87%), m.p. 123—125° (decomp.), $[\alpha]_D^{20}$ +241° (*c* 0·5 in $Me_2CO$), $R_F$ 0·57, $\nu_{max.}$ 3555, 3495, 3285, 1672, and 1550 (amide), 1768 (β-lactam), 1730 ($CO_2H$), and 990 (S=O) $cm^{-1}$, δ [$(CD_3)_2SO$; HMDS], 1·22 (s, $CH_3$), 1·57 (s, $CH_3$), 3·54 (s, $CH_2$), 4·26 (s, 3-H), 5·09 (dd, *J* 1·5 and 9 Hz, 6-H), 5·34 (d, *J* 1·5 Hz, 5-H), 6·10—6·88 ($CO_2H$), 7·32 (s, Ph), and 9·23 (d, *J* 9 Hz, NH). The combined filtrates afforded a mixture (290 mg) containing mainly (IIIay) and (IVay) with side products.

6-epi-*Benzylpenicillin* S-*Oxide Methyl Ester* (IVby).—A solution of 6-*epi*-benzylpenicillin *S*-oxide (IVay) (0·5 g, 1·41 mmol) in anhydrous acetone (20 ml) was cooled to 0° and treated dropwise with ethereal $CH_2N_2$ until a yellow colour persisted and no more gas was evolved. The solution was evaporated to a yellow oil, which was crystallised from hot anhydrous benzene (10 ml), yielding the ester (IVby) (315 mg, 61%), m.p. 121—123° (decomp.), $[\alpha]_D^{20}$ +238° (*c* 0·5 in $Me_2CO$), *m/e* 364 $R_F$ 0·21, $\nu_{max.}$ 3522, 3478, 3310, 1670, 1564 (amide), 1780 (β-lactam), 1740, 1230 (ester), and 1045 (S=O) $cm^{-1}$, δ ($CDCl_3$; TMS), 1·17 (s, $CH_3$), 1·63 (s, $CH_3$), 3·57 (s, $CH_2$), 3·74 (s, $OCH_3$), 4·43 (s, 3-H), 5·05 (d, *J* 2 Hz, 5-H), 5·10 (dd, *J* 2 and 8 Hz, 6-H), 7·19 (d, *J* 8 Hz, NH), and 7·27 (s, Ph).

6-epi-*Benzylpenicillin* (IIay).—The sulphoxide (IVay) (1·052 g, 3 mmol) was deoxygenated as described for (IVax), yielding after freeze-drying potassium salt of (IVay) (529 mg, 47%), m.p. 151—154° (decomp.), $[\alpha]_D^{20}$ +197° (*c* 0·5 in $H_2O$), $R_F$ 0·75. The spectral data are given in the following paper.

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