Isomerisation of Benzyl 6-Phenoxyacetoxypenicillanates and their *S*-Oxides

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The preparation of benzyl 6α- and 6β-phenoxyacetoxypenicillanate and their (*S*)- and (*R*)-*S*-oxides is described. No epimerisation was observed when benzyl 6α- or 6β-phenoxyacetoxypenicillanate or benzyl 6α- or 6β-trimethylsilyloxypenicillanate was treated with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN). Benzyl 6β-phenoxyacetoxypenicillanate (*S*)-*S*-oxide was transformed into the 6α-isomer with DBN as catalyst; the converse reaction was not observed. With both isomers in these experiments, the phenoxyacetoxy side chain was hydrolysed to a large extent.

THE reaction of 6β-aminopenicillanic acid with nitrous acid yields 6α-hydroxypenicillanic acid, from which 6α-phenoxyacetoxypenicillanic acid has been prepared.[1] In order to study the influence of the replacement of the amide side chain of penicillin by an ester group on chemical and biological properties, it was necessary to prepare also 6β-phenoxyacetoxypenicillanic acid. As treatment of some penicillin derivatives with base is reported to give a mixture of 6α- and 6β-isomers,[2-6] we thought that a similar conversion would be possible with penicillanic acid derivatives having an ester group on C-6.

Treatment of benzyl 6α-phenoxyacetoxypenicillanate (IV) with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN), a base which had given excellent results with penicillin esters,[6,7] left the starting material unchanged, perhaps because the isomerisation equilibrium did not favour the 6β-isomer. As 6β-phenoxyacetoxypenicillanic acid had been prepared in the meantime by another method,[8] we tried to repeat this preparation. Oxidation of benzyl 6α-hydroxypenicillanate (I) with di-isopropylcarbodiimide–dimethyl sulphoxide in the presence of orthophosphoric acid or pyridinium trifluoroacetate [9] gave a mixture which contained about 50% of benzyl 6-oxopenicillanate (V), the remainder being starting material along with several other products. The desired product (V) could not be purified by chromatography on silica gel, which caused partial decomposition. However when benzyl 6α-hydroxypenicillanate (I) was oxidized with dimethyl sulphoxide–acetic anhydride,[10] benzyl

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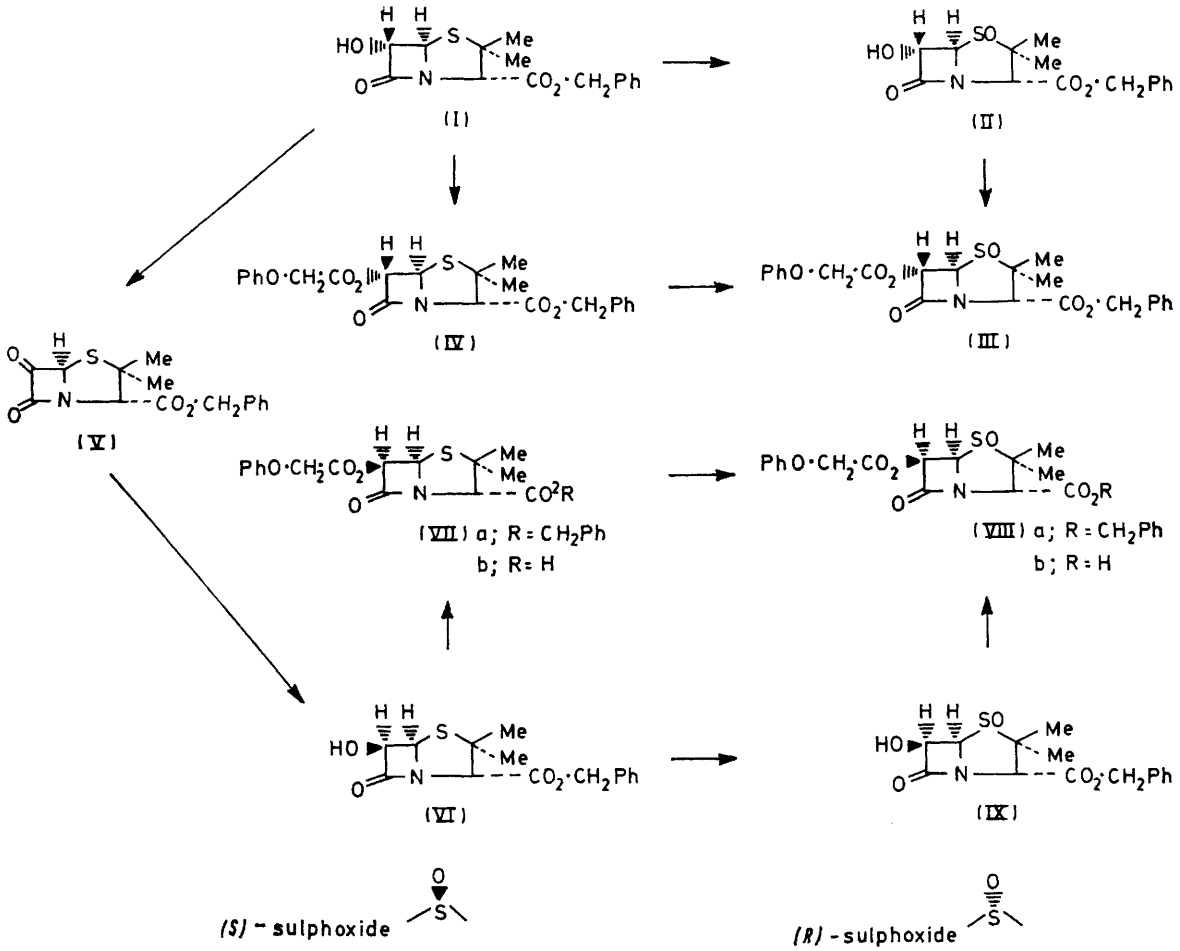
6-oxopenicillanate (V) was obtained as a crystalline product in excellent yield. Compound (V) has been described before as an oil.[8,11] Reduction of (V) with sodium borohydride gave benzyl 6β-hydroxypenicillanate (VI), which was transformed into benzyl 6β-phenoxyacetoxypenicillanate (VIIa) as described by Lo and Sheehan.[8] When the reaction mixture obtained after reduction with sodium borohydride was acidified with hydrochloric acid, some β-chloropenicillanate was obtained. This compound was identical with the product obtained by treatment of benzyl 6α-chloropenicillanate with DBN.[12]

Treatment of both benzyl 6α- and 6β-phenoxy-

when benzyl 6α- or 6β-trimethylsilyloxypenicillanate was treated with DBN.

In the penicillin series, it has been observed that epimerisation occurs more readily with sulphoxides,[14,15] probably owing to the increased acidity of the C-6 proton in these derivatives. Benzyl 6α-phenoxyacetoxypenicillanate (*S*)-*S*-oxide (*S*)-(III) could be prepared by the reaction of (IV) with *m*-chloroperbenzoic acid, or by



phenoxyacetylation of benzyl 6α-hydroxypenicillanate (*S*)-*S*-oxide (*S*)-(II), obtained from (I) by treatment with peroxy-acid. We were unable to prepare benzyl 6β-phenoxyacetoxypenicillanate *S*-oxide (VIIIa) from (VIIa) with peroxy-acid, because a complex mixture

After the solution had been kept overnight at −20 °C, only (*S*)-(II) could be isolated. Apparently, the 6β-isomer (*S*)-(VIIIa) was transformed completely into the 6α-isomer (*S*)-(III), which by hydrolysis of the ester side chain gave (*S*)-(II). When benzyl 6α-phenoxyacetoxypenicillanate (*S*)-*S*-oxide (*S*)-(III) was treated with DBN, no epimerisation took place. Prolonged treatment led only to hydrolysis of the ester side chain and formation of (*S*)-(II). These results prove that the C-6 proton of the sulphoxide is sufficiently acidic for base-catalysed epimerisation to occur, but that with a phenoxyacetoxy side chain, the equilibrium is shifted in favour of the 6α-isomer.

The assignment of configuration to the sulphoxides was based on aromatic solvent-induced shifts (ASIS). This method has been used for various penicillin *S*-oxides.[16,17] The n.m.r. data for the (*S*)-*S*-oxide (*S*)-(VIIIa) were obtained from the pure product, whereas those of the (*R*)-isomer were deduced from the spectrum of the *RS*-mixture. Because the same reaction with (IV) yielded only the (*S*)-*S*-oxide, it was necessary to apply another method. Oxidation of (IV) with ozone gave a 1 : 1 mixture of (*R*)- and (*S*)-*S*-oxides (III), from which the n.m.r. data for the (*R*)-*S*-oxide could be obtained.

In the (*S*)-*S*-oxides, H-5 and the 2α-methyl group are

TABLE 1

Solvent shifts and net ASIS * for benzyl 6α-phenoxyacetoxypenicillanate and its *S*-oxides

		3-H	5-H	6-H	2β-Me	2α-Me
(IV)	$\delta(CDCl_3)$	4.58	5.70	5.33	1.57	1.39
	$\delta(C_6D_6)$	4.47	5.60	5.27	1.15	1.10
	$\Delta_1(CDCl_3 - C_6D_6)$	+0.11	+0.10	+0.06	+0.42	+0.29
(*S*)-(III)	$\delta(CDCl_3)$	4.55	4.97	5.87	1.62	1.07
	$\delta(C_6D_6)$	4.67	4.46	6.12	1.35	0.62
	$\Delta_2(CDCl_3 - C_6D_6)$	−0.12	+0.51	−0.25	+0.27	+0.45
	$\Delta_2 - \Delta_1$	−0.23	+0.41	−0.31	−0.15	+0.16
(*R*)-(III)	$\delta(CDCl_3)$	4.50	4.80	6.00	1.40	1.31
	$\delta(C_6D_6)$	4.37	4.84	5.76	0.86	1.10
	$\Delta_3(CDCl_3 - C_6D_6)$	+0.13	−0.04	+0.24	+0.54	+0.21
	$\Delta_3 - \Delta_1$	+0.02	−0.14	+0.18	+0.12	−0.08

* Net ASIS = ASIS(sulphoxide) − ASIS(sulphide) in p.p.m.

TABLE 2

Solvent shifts and net ASIS * for benzyl 6β-phenoxyacetoxypenicillanate and its *S*-oxides

		3-H	5-H	6-H	2β-Me	2α-Me
(VIIa)	$\delta(CDCl_3)$	4.55	5.64	5.80	1.58	1.40
	$\delta(C_6D_6)$	4.48	5.35	5.35	1.30	1.15
	$\Delta_1(CDCl_3 - C_6D_6)$	+0.07	+0.29	+0.45	+0.28	+0.25
(*S*)-(VIIIa)	$\delta(CDCl_3)$	4.71	5.02	5.79	1.59	1.03
	$\delta(C_6D_6)$	4.18	4.52	5.27	1.32	0.58
	$\Delta_2(CDCl_3 - C_6D_6)$	+0.53	+0.50	+0.52	+0.27	+0.45
	$\Delta_2 - \Delta_1$	+0.46	+0.21	+0.07	−0.01	+0.20
(*R*)-(VIIIa)	$\delta(CDCl_3)$	4.42	4.85	6.18	1.45	1.26
	$\delta(C_6D_6)$	4.32	4.67	5.59	1.32	1.05
	$\Delta_3(CDCl_3 - C_6D_6)$	+0.10	+0.18	+0.59	+0.13	+0.21
	$\Delta_3 - \Delta_1$	+0.03	−0.11	+0.14	−0.15	−0.04

* See Table 1.

TABLE 3

Solvent shifts and net ASIS * for benzyl 6β-phenoxyacetamidopenicillanate † and its *S*-oxides †

		3-H	5-H	6-H	2β-Me	2α-Me
Phenoxymethyl-penicillin benzyl ester (A)	$\delta(CDCl_3)$	4.49	5.56	5.70	1.55	1.40
	$\delta(C_6D_6)$	4.45	5.20	5.52	1.25	1.17
	$\Delta_1(CDCl_3 - C_6D_6)$	+0.05	+0.36	+0.18	+0.30	+0.23
(*S*)-*S*-Oxide (B)	$\delta(CDCl_3)$	4.69	5.00	6.07	1.64	1.05
	$\delta(C_6D_6)$	4.25	3.76	5.58	1.05	0.46
	$\Delta_2(CDCl_3 - C_6D_6)$	+0.64	+1.24	+0.29	+0.59	+0.59
	$\Delta_2 - \Delta_1$	+0.59	+0.88	+0.09	+0.29	+0.36
(*R*)-*S*-Oxide (C)	$\delta(CDCl_3)$	4.43	4.75	5.52	1.63	1.22
	$\delta(C_6D_6)$	4.43	4.46	4.84	1.24	1.00
	$\Delta_3(CDCl_3 - C_6D_6)$	0.00	+0.29	+0.68	+0.39	+0.22
	$\Delta_3 - \Delta_1$	−0.05	−0.07	+0.50	+0.09	−0.01

* See Table 1. † These compounds were prepared by previously described methods: (A), [1,7] (B),[14] (C).[16]

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shielded [0.41 and 0.16 p.p.m. for (III) (Table 1) and 0.21 and 0.20 p.p.m. for (VIIIa) (Table 2)]; similar observations have been made for the (*S*)-*S*-oxides of penicillanates with amide side chains.[16,17] In the (*R*)-*S*-oxide (*R*)-(III) H-6 and the 2β-methyl group are shielded,

by esterification of 6α-hydroxypenicillanic acid, obtained from 6-aminopenicillanic acid,[1] or by treatment of benzyl 6-diazopenicillanate, obtained from benzyl *N*-nitroso-phenylacetamidopenicillanate, with perchloric acid in water–acetone.[20]

(b) *From benzyl 6α-hydroxypenicillanate* (S)-S-*oxide* (*S*)-(II). To a solution of benzyl 6α-hydroxypenicillanate (*S*)-*S*-oxide (1.29 g, 4 mmol) in dichloromethane (40 ml) were added phenoxyacetyl chloride (1.03 g, 6 mmol) and, with cooling and stirring, triethylamine (0.56 ml, 4 mmol). The mixture was kept for 3 h at room temperature. After being washed twice with potassium hydrogen carbonate (M; 10 ml) and ice–water (2 × 20 ml), the organic layer was evaporated to leave a yellow glass. This crystallised after addition of dry ether (25 ml) to give benzyl 6α-phenoxyacetoxypenicillanate (*S*)-*S*-oxide (1.48 g, 81%), m.p. 153—155° (decomp.).

Benzyl 6α-Phenoxyacetoxypenicillanate (R)- *and* (S)-S-*Oxides* (III).—Benzyl 6α-phenoxyacetoxypenicillanate (100 mg) was dissolved in a mixture of acetone (30 ml) and water (20 ml) at 0 °C. Ozone was bubbled through the solution at 0 °C for 1 h. Evaporation of the acetone and lyophilisation of the remaining aqueous phase gave a white powder (105 mg) which consisted of a mixture of the (*R*)- and (*S*)-*S*-oxides (1 : 1 by n.m.r.), R_F values 0.68 (*S*) and 0.61 (*R*). The n.m.r. spectrum of the (*R*)-*S*-oxide (as deduced from the spectrum of the mixture) showed δ ($CDCl_3$) 1.31 (s, CH_3), 1.40 (s, CH_3), 4.50 (s, 3-H), 4.67 (s, OCH_2), 4.80 (d, *J* 1.5 Hz, 5-H), 5.15 (AB, CH_2), and 6.00 (d, *J* 1.5 Hz, 6-H).

Benzyl 6-Oxopenicillanate (V).—(a) *Oxidation with dimethyl sulphoxide–acetic anhydride.* Benzyl 6α-hydroxypenicillanate (3.07 g, 10 mmol) was dissolved in dimethyl sulphoxide (20 ml). Acetic anhydride (5 ml) was added. The solution was stored at room temperature for 48 h, then poured into a mixture of ice–water (50 ml) and potassium hydrogen carbonate (M; 50 ml). The suspension was extracted with benzene (3 × 50 ml) and the organic layer was washed with ice–water (5 × 50 ml), dried (Na_2SO_4), and evaporated. The yellow glass became crystalline; yield 2.82 g (92%), m.p. 52°, $[\alpha]_D^{20}$ +183° (*c* 0.5 in C_6H_6), R_F 0.58, *m/e* 305, $\nu_{max.}$ 1 833 (C=O), 1 779 (β-lactam), 1 745, 1 201 (ester), and 760 and 705 cm^{-1} (phenyl), δ($CDCl_3$), 1.45 (s, CH_3), 1.50 (s, CH_3), 4.80 (s, 3-H), 5.22 (s, CH_2), 5.76 (s, 5-H), and 7.37 (s, C_6H_5). The product contained a trace of benzyl 6α-acetoxypenicillanate (t.l.c. and mass spectrum).

(b) *Oxidation with dimethyl sulphoxide, carbodi-imide, and orthophosphoric acid.* Benzyl 6α-hydroxypenicillanate (614 mg, 2 mmol) was dissolved in dimethyl sulphoxide (6 ml). Anhydrous orthophosphoric acid (98 mg, 1 mmol) and dimmol). After 17 h the excess of carbodi-imide was decomposed and, by extraction with benzene, a yellow glass was obtained. The product had a purity of about 50% and could not be purified by column chromatography.

Benzyl 6β-Hydroxypenicillanate (VI).—(a) *Reduction with borohydride.* Benzyl 6-oxopenicillanate (2.8 g, 9.2 mmol) was dissolved in methanol–ethanol (280 ml; 1 : 1) and cooled to 0 °C. A solution of sodium borohydride (0.25 g) in ethanol–water (360 ml; 1 : 1) was added with stirring at 0 °C. After 2 min the mixture was acidified with phosphoric acid (20%; 90 ml) to pH 2.0. The suspension was extracted with dichloromethane (2 × 100 ml) and the extracts were washed with 5% potassium hydrogen carbonate (100 ml) then water (100 ml), dried (Na_2SO_4), and evaporated to leave a yellow oil (2.51 g). Recrystallisation from benzene–pentane gave benzyl 6β-hydroxypenicillanate (1.27 g, 45%), m.p. 97°, $[\alpha]_D^{20}$ +220° (*c* 0.5 in MeOH), R_F 0.70, *m/e* 307, $\nu_{max.}$ 3 460 (OH), 1 770 (β-lactam), 1 730, 1 208, 1 180 (ester), 760, and 702 cm^{-1} (phenyl), δ($CDCl_3$), 1.42 (s, CH_3), 1.61 (s, CH_3), 3.03br (OH), 4.50 (s, 3-H), 5.12 (d, *J* 4 Hz, 6-H), 5.18 (s, CH_2), 5.57 (d, *J* 4 Hz, 5-H), and 7.37 (s, C_6H_5). By recrystallisation from carbon tetrachloride another crystalline form was obtained, m.p. 111—113°, $\nu_{max.}$ 3 320 (OH), 1 740 (β-lactam and ester), 1 205, 1 180 (ester), 1 452, 747, and 692 cm^{-1} (phenyl).

When the reaction mixture after reduction was acidified with hydrochloric acid, a mixture of benzyl 6β-chloro- and 6β-hydroxypenicillanate was obtained. The products were separated by chromatography on silica gel (20 g) in a gradient of dichloromethane to dichloromethane–ether (4 : 1). Fractions (5 ml) 12—17 yielded benzyl 6β-chloropenicillanate (96 mg, 3%), as an oil, *m/e* 325 and 327, R_F 0.80, $\nu_{max.}$ 1 789 (β-lactam), 1 743, 1 203, 1 181 (ester), 745, and 695 cm^{-1} (phenyl), δ($CDCl_3$) 1.36 (s, CH_3), 1.57 (2, CH_3), 4.45 (s, 3-H), 5.19 (s, CH_2), 5.22 (d, *J* 4 Hz, 5-H), 5.50 (d, *J* 4 Hz, 6-H), and 7.28 (s, C_6H_5). Fractions 21—44 yielded, after crystallisation from benzene–pentane, benzyl 6β-hydroxypenicillanate (1.12 g, 40%), m.p. 97°. On t.l.c. on silica gel with benzene–acetone (8 : 2) the R_F value of the 6α-hydroxy-derivative (I) was 0.67. The R_F values with the system CH_2Cl_2–Et_2O (50 : 1) were 0.21 for (VI) and 0.10 for (I).

(b) *Reduction with zinc borodeuteride.* Reduction of benzyl 6-oxopenicillanate in ether with 4 mol. equiv. of zinc borohydride gave benzyl 6β-hydroxypenicillanate (45%). The reaction performed with zinc borodeuteride

3-H), 4.73 (s, O·CH_2·C_6H_5), 5.20 (s, CH_2·C_6H_5), 5.64 (d, *J* 4 Hz, 5-H), 5.80 (d, *J* 4 Hz, 6-H), 6.70—7.50 (m, Ph), and 7.36 (s, Ph). The peak at δ 5.80 was absent in the spectrum of the product prepared by phenoxyacetylation of benzyl 6β-hydroxypenicillanate deuteriated at C-6.

Benzyl 6β-Hydroxypenicillanate (S)-S-*Oxide* (*S*)-(IX).—Benzyl 6β-hydroxypenicillanate (307 mg, 1 mmol) was dissolved in dichloromethane (5 ml) and cooled to 0 °C. A solution of *m*-chloroperbenzoic acid (85%; 203 mg, 1 mmol) in dichloromethane (5 ml) was added dropwise in 30 min to the stirred mixture. Stirring was continued for 30 min at 0 °C. The resulting suspension was then diluted with dichloromethane (50 ml), washed with potassium hydrogen carbonate (M; 2 × 10 ml) and water (2 × 10 ml), dried (Na_2SO_4), and evaporated to leave an oil, which was dissolved in benzene (10 ml). The solution was freeze-dried yielding a glass (235 mg, 72%) R_F 0.47 *m/e* 323 n.m.r. to consist of benzyl 6β-phenoxyacetoxypenicillanate (*S*)- and (*R*)-*S*-oxides in the ratio 2 : 1. The two isomers had the same R_F 0.70 on t.l.c. in benzene–acetone (8 : 2), but in dichloromethane–ethyl acetate (9 : 1) the (*S*)-*S*-oxide had R_F 0.68 and the (*R*)-*S*-oxide 0.57; $\nu_{max.}$ 1 800 (β-lactam), 1 752, 1 180 (ester), 1 600, 1 592, 755, 692 (phenyl), and 1 068 cm^{-1} (S=O); for the (*R*)-*S*-oxide δ($CDCl_3$) 1.26 (s, CH_3), 1.45 (s, CH_3), 4.42 (s, 3-H), 4.71 (s, O·CH_2·C_6H_5), 4.85 (d, *J* 4 Hz, 5-H), 5.20 (AB, CH_2·C_6H_5), 6.18 (d, *J* 4 Hz, 6-H), 7.30 (s, C_6H_5), and 6.70—7.40 (m, C_6H_5).

Attempted Isomerisation of Benzyl 6-Hydroxypenicillanate.—Benzyl 6β-hydroxypenicillanate (92 mg, 0.30 mmol) was dissolved in dichloromethane (10 ml) and *NO*-bistrimethylsilylacetamide (0.2 ml, 0.96 mmol) was added. The solution was kept for 2 h at room temperature and then evaporated to leave an oil. A solution of this in hexane

(*S*)-*S*-oxides in the ratio of 1 : 1 (n.m.r.) as well as benzyl 6α- and 6β-hydroxypenicillanate *S*-oxides. After reaction overnight at −20 °C the orange mixture was separated by column chromatography on silica gel (20 g) in a gradient of benzene to benzene–acetone 9 : 1. Only benzyl 6α-hydroxypenicillanate *S*-oxide (71 mg, 21%) was isolated. Benzyl 6β-phenoxyacetoxypenicillanate (*S*)-*S*-oxide in dichloromethane at 0 °C was rapidly destroyed in the presence of DBN.

Epimerisation of Benzyl 6α-Phenoxyacetoxypenicillanate (S)-S-*Oxide*.—Benzyl 6α-phenoxyacetoxypenicillanate (*S*)-*S*-oxide (150 mg, 0.328 mmol) was dissolved in dichloromethane (5 ml). The solution was cooled to −20 °C and DBN (0.04 ml, 0.33 mmol) was added. The solution became orange. The reaction was followed for 6 h by t.l.c. in benzene–acetone (8 : 2). No epimerisation took place but slow formation of benzyl 6α-hydroxypenicillanate (*S*)-*S*-oxide (R_F 0.28) and of several less polar products occurred. After 6 h the mixture was chromatographed over silica gel and benzyl 6α-hydroxypenicillanate (*S*)-*S*-oxide (35 mg, 35%) was isolated.

6β-*Phenoxyacetoxypenicillanic Acid* (S)-S-*Oxide* (*S*)-(VIIIb).—Benzyl 6β-phenoxyacetoxypenicillanate (*S*)-*S*-oxide (1 g) dissolved in ethyl acetate (50 ml) was hydrogenated over 10% palladium–charcoal (1 g) for 6 h at 3 kg cm^{-2}. The catalyst was filtered off and washed with ethyl acetate. The solution was hydrogenated again under the same conditions. The organic layer was evaporated to leave a colourless oil (0.328 g). Addition of ether (10 ml) afforded crystals of 6β-phenoxyacetoxypenicillanic acid (*S*)-*S*-oxide (0.266 g, 33%), m.p. 140—142° (decomp.), $[\alpha]_D^{18}$ +313° (*c* 0.5 in Me_2CO), R_F 0.56 in acetone–acetic acid (95 : 5), $\nu_{max.}$ 1 786 (β-lactam), 1 730 (CO_2H), 1 600, 1 590, 752, 691 (phenyl), and 996 cm^{-1} (S=O), δ(CD_3CN), 1.23 (s, CH_3), 1.65 (s, CH_3), 4.61 (s, 3-H), 4.79 (s, CH_2), 5.22 (d, *J* 4 Hz, 5-H), 5.92 (d, *J* 4 Hz, 6-H), and 6.70—7.50 (m, C_6H_5).

6β-*Phenoxyacetoxypenicillanic Acid* (VIIb).—6β-Phenoxyacetoxypenicillanic acid (*S*)-*S*-oxide (73.4 mg, 0.2 mmol) was dissolved in *NN*-dimethylformamide (2 ml) and cooled to −18 °C. Phosphorus tribromide (0.095 ml, 1 mmol) was added with stirring. After 30 s at −18 °C the yellow-green solution was poured into a cooled solution of sodium hydrogen carbonate (500 mg) in water (10 ml). The mixture was acidified with phosphoric acid (20%) to pH 2.0 and extracted with ethyl acetate (2 × 20 ml). The organic layer was washed with ice–water (2 × 20 ml) and brine (20 ml), dried (Na_2SO_4), and evaporated to leave a colourless oil (50 mg, 71%), $[\alpha]_D^{15}$ +146° (*c* 0.4 in Me_2CO), R_F 0.76 in acetone–acetic acid (95 : 5), $\nu_{max.}$ 1 775 (β-lactam), 1 740 (CO_2H), 1 600, 1 592, 752, and 689 cm^{-1} (phenyl), δ($CDCl_3$) 1.56 (s, CH_3), 1.61 (s, CH_3), 4.49 (s, 3-H), 4.73 (s, O·CH_2), 5.63 (d, *J* 4 Hz, 5-H), 5.81 (d, *J* 4 Hz, 6-H), 6.70—7.00 (m, C_6H_5), and 8.02br (CO_2H).

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