Substituted Penicillanic Acid 1,1-Dioxides as β-Lactam Inhibitors: Studies on 6-Benzylidene- and Hydroxybenzylpenam Sulphones

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The preparation of the two epimers of (6*R*)-6-(α-hydroxybenzyl)penicillanic acid and the corresponding sulphones is described. The absolute configuration about the hydroxy substituted carbon atom has been elucidated by an investigation of the dehydration to the corresponding 6-benzylidenepenicillanic acids. (α*R*,6*R*)-6-(α-Hydroxybenzyl)penicillanic acid 1,1-dioxide (**15**) is a powerful inhibitor of class C β-lactamases such as that isolated from *Pseudomonas aeruginosa*. Some comments are made on the possible mode of action of these types of inhibitor.

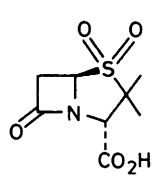
Because of the widespread incidence of antibacterial resistance to the β-lactam antibiotics caused by β-lactamase formation there is a growing interest in the development of effective β-lactamase inhibitors.[1] Studies in this area can also provide information on the mode of action of the β-lactamases.[2]

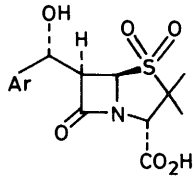
Penicillanic acid sulphone (**1**) (CP-45 899) has been studied extensively as a β-lactamase inhibitor.[3] For example, with the RTEM β-lactamase enzyme from *Escherichia coli* it has been shown to react as a branched-chain inhibitor.[4] However, these studies also showed that considerable turnover (enzyme-catalysed hydrolysis) of the substrate occurs before irreversible inactivation is effected. As part of our current interest in the side-chain modification of β-lactam antibiotics,[5] the incorporation of hydroxyarylalkyl substituents into the penicillanic acid sulphone has been studied. Although it is known that hydroxyalkyl substituents can be introduced into β-lactam antibiotics, in some cases without detriment to biological activity, few studies involving the introduction of arylalkyl substituents have been reported.[6] This omission is surprising considering that many of the natural substrates of the β-lactamases contain pendant aromatic groups and that the sulphones of penicillins with acylamido side-chains can behave as β-lactamase inhibitors.[7]

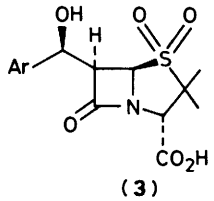
The principal targets in this work were hydroxybenzyl derivatives of the types (**2**) and (**3**). It was hoped that these targets might show greater selectivity and affinity than the parent sulphone (**1**). The route to these compounds commenced with benzhydryl 6,6-dibromopenicillanate (**4**), itself readily prepared from 6β-aminopenicillanic acid.[8]

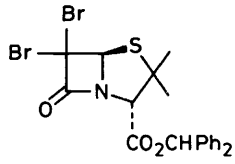
Metallation of the dibromide (**4**), by exchange with methylmagnesium bromide, followed by reaction with benzaldehyde, afforded a mixture of the hydroxybenzyl adducts (**5**). Stereoselective reduction of this mixture with tributyltin hydride gave, as the principal products, two isomeric alcohols in the ratio 5:3. That these alcohols both had the (6*R*)-configuration was evident from their ^{1}H n.m.r. spectra, which indicated 5-H to 6-H coupling constants of 5 and 4.5 Hz for the major and minor alcohols, respectively, reflecting *cis*-substitution of the β-lactam ring. Oxidation of the two alcohols with *m*-chloroperbenzoic acid (2.2 equiv.) afforded the corresponding major and minor sulphones.

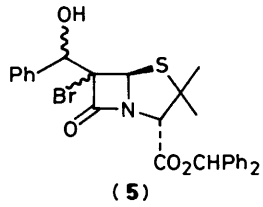
The assignment of the stereochemistry about the carbinol centre in the sulphides and sulphones was tackled chemically. Whilst dehydration has been used as a chemical means for verifying the alcohol configurations in the α-hydroxyethyl isomers, which are characteristic of the thienamycin compounds,[9] it was recognised that care had to be taken, in the current series, not to form benzylic carbonium ions and thus lose the stereochemical integrity of these systems. Dehydration of the major alcohol sulphide, with thionyl chloride and


(**1**)


(**2**) Ar = aromatic ring


(**3**)


(**4**)


(**5**)

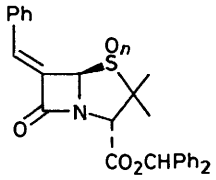
triethylamine in dichloromethane, afforded two olefins, in the ratio 3:1, which were identified as the (*Z*)-benzylidene derivative (**6**) and the (*E*)-isomer (**8**) respectively, whereas the minor alcohol, upon similar treatment, afforded the same olefins in a 1:1 ratio. By contrast, elimination of water from the sulphones proceeded much more cleanly, the major alcohol sulphone producing only the (*Z*)-olefin (**7**), the minor alcohol a 1:5 ratio of olefins (**7**) and (**9**). Assignment of configuration to the benzylidene derivatives was made on the basis of their ^{1}H n.m.r. spectroscopic properties.[10] For the (*Z*)-olefins the vinylic proton occurs at lower fields (δ 7.0—7.4), whilst in the (*E*)-series this proton occurs at δ 6.5—6.8. Furthermore, in the (*Z*)-olefins the aromatic protons appear as a broad band between δ 7.2—7.6, whilst in the (*E*)-isomers they occur as two groups of signals, the *ortho*-protons appearing at δ 7.8—8.0 (Table).

Assuming no prior epimerisation about position 6 and an *E*2-like elimination process, these results may be accounted for by assigning to the major alcohols the (α*R*)-configuration [structures (**10**) and (**11**)] and to the minor alcohols the (α-*S*)-configuration [structures (**12**) and (**13**)]. No evidence for epimerisation was obtained by treating the alcohols with triethylamine alone under the reaction conditions used in the

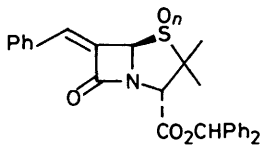
Table. ^{1}H N.m.r. chemical shifts of benzylidene derivatives[a]

(*Z*)-Series	α-H	Aromatic	(*E*)-Series	α-H	Aromatic
(6)	7.03	7.3—7.4	(8)	6.57	7.3—7.4 (3 H), 7.8—7.9 (2 H)
(25)	7.05	7.25—7.4	(23)	6.82	7.3—7.4 (3 H), 7.8—8.0 (2 H)
(7)	7.41	7.35—7.4	(9)	6.81	7.3—7.5 (3 H), 7.9—8.0 (2 H)
(26)	7.09	7.2—7.6	(24)	6.80	7.25—7.55 (3 H), 7.8—8.0 (2 H)

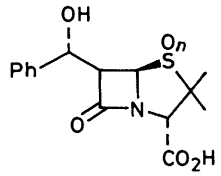
[a] Spectra observed at 90 MHz in $CDCl_3$ with $SiMe_4$ as internal reference; δ_4 values in p.p.m.



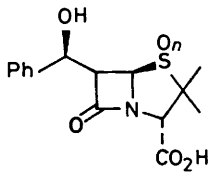
(6) *n* = 0
(7) *n* = 2



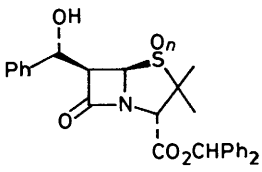
(8) *n* = 0
(9) *n* = 2



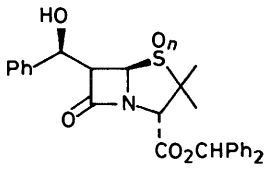
(14) *n* = 0
(15) *n* = 2



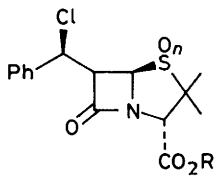
(16) *n* = 0
(17) *n* = 2



(10) *n* = 0
(11) *n* = 2



(12) *n* = 0
(13) *n* = 2



(18) *n* = 0, R = $CHPh_2$
(19) *n* = 2, R = $CHPh_2$
(20) *n* = 1, R = $CHPh_2$
(21) *n* = 0, R = H
(22) *n* = 2, R = H

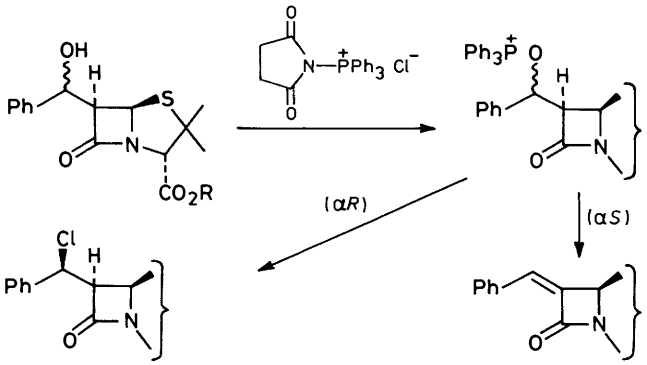
dehydrations. The benzylidene products were also configurationally stable to the reaction conditions. The formation of olefinic mixtures by dehydration of the alcohols (**10**)—(**13**) with thionyl chloride and triethylamine probably reflects competition from some *E*1-type elimination processes from the benzylic centre.

Removal of the ester protecting groups from the alcohols (**10**)—(**13**) could be effected with trifluoroacetic acid in trifluoroethanol.[11] The acids thus liberated, compounds (**14**)—(**17**), were converted into their potassium salts by exchange with potassium 2-ethylhexanoate. In order to confirm that no undesirable chemical changes had occurred during the deprotection sequence, samples of the potassium salts were re-acidified and then re-esterified with diphenyldiazomethane. In all cases, clean formation of the corresponding benzhydryl esters was observed.

Attempted deprotection of the benzylidene derivatives (**6**)—(**9**) failed; in each case loss of the benzylidene group and the formation of complex mixtures of products were observed. In order to obtain the free acids of the benzylidene derivatives (**6**)—(**9**), therefore, alternative routes were sought. Dehydration of the alcohol acids (**14**)—(**17**) with the thionyl chloride–triethylamine reagent was not possible because of the onset of other processes, such as the anhydropenicillin rearrangement.[12] A direct Mitsunobu reaction, which has been utilised as a means of dehydration,[13] was ruled out because of competition between dehydration and intermolecular ester formation. Use of the mild chlorinating agent, triphenylphosphine–*N*-chlorosuccinimide,[14] however, proved to be successful. With the major alcohol sulphide (**10**), the latter reagent afforded a single chloride (**18**) in which the configuration about the benzylic position must be inverted with respect to that in its parent alcohol.[14] The chloride (**18**) could be oxidised with *m*-chloroperbenzoic acid into the corresponding sulphone (**19**), although the yield of the latter product was low owing to the sluggish oxidation of the intermediate sulphoxides (**20**) under these reaction conditions.

A different reaction was observed between the minor alcohol sulphide (**12**) and the triphenylphosphine–*N*-chlorosuccinimide reagent as this afforded, directly, the (*E*)-benzylidene derivative (**8**). With the hydroxy sulphones (**11**) and (**13**) and the latter reagent, direct formation of the corresponding benzylidene derivatives, (**7**) and (**9**), was observed. The configuration of the benzylidene products are as expected for *E*2-type eliminations initiated by formation of the triphenylphosphonium derivative (Scheme 1) followed by loss of the 6α-proton; again no evidence for epimerisation about position 6 could be obtained.

The formation of the chloride (**18**) from the sulphide (**10**) requires explanation. A study of molecular models indicates



Scheme 1.

that the phenyl group of the benzyl function predominantly accommodates an area of space away from the sulphur-substituted carbon (Figure); for the (αR)-alcohol (**10**) (Figure, *a*)

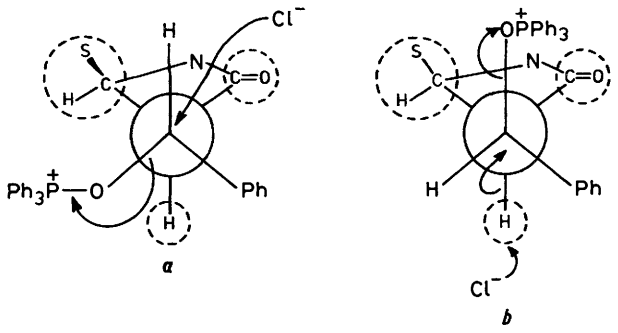


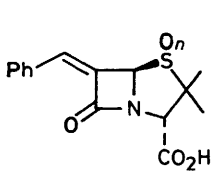
Figure. Attack by chloride ion on (αR)- and (αS)-alcohols (**10**) and (**12**) (*a* and *b*) respectively

chloride attack has relatively unhindered access to the rear of the α-carbon atom, whilst for the (αS)-epimer (**12**) (Figure, b) the leaving group can readily adopt a *trans*-periplanar conformation with respect to the proton at position 6, hence allowing direct elimination to give the (*E*)-benzylidene derivative (**8**). In the sulphone series, in which the 6α-hydrogen has greater acidity,[15] elimination is observed from both the alcohols (**11**) and (**13**).

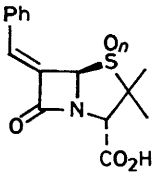
Deprotection of the chlorosulphone (**19**) gave the acid (**22**) which could be dehydrochlorinated with 1,5-diazabicyclo-[4.3.0]non-5-ene (DBN) at room temperature to produce, as the major product, the (*E*)-benzylidene acid (**24**) contaminated with a small amount of the (*Z*)-isomer (**26**), ratio >9:1 respectively.

Dehydrations involving the triphenylphosphine–*N*-chlorosuccinimide reagent could also be effected, without too much loss of material, on the hydroxy acids. Thus, treatment of the minor alcohol sulphide (**16**) with the reagent at room temperature for 16 h gave the (*E*)-benzylidene sulphide (**23**), whilst the major sulphone (**11**) produced the (*Z*)-benzylidene sulphone (**26**). The remaining isomer of the four possible benzylidene derivatives (**23**)—(**26**), the (*Z*)-benzylidene sulphide (**25**), could be generated by dehydrochlorination of the chlorosulphide (**21**), giving the (*Z*)-isomer as the major product. The formation of the acid (**25**) may be explained by assuming an *E*1-type elimination process; DBN-catalysed dehydrochlorination of the corresponding chloro sulphone (**22**), in which the 6α-proton is expected to be more acidic than in the case of the sulphide, follows an *E*2-type reaction process. Participation of *E*1*cb*-type elimination processes have been discounted in these reactions since no evidence for these has been observed. Control experiments on the starting alcohols or the chlorides afforded no epimers at position 6. The benzylidene products were also configurationally stable to the bases used in their formation.

The potassium salts of the hydroxy acids (**14**)—(**17**) and the benzylidene acids (**23**)—(**26**) have been examined for β-lactamase activity. Of these materials the hydroxy acid (**15**) has proven to be a highly effective inhibitor against class C β-lactamases,[1] such as the enzyme from *Pseudomonas aeruginosa*.* Kinetic evidence suggests that the inhibitory action is



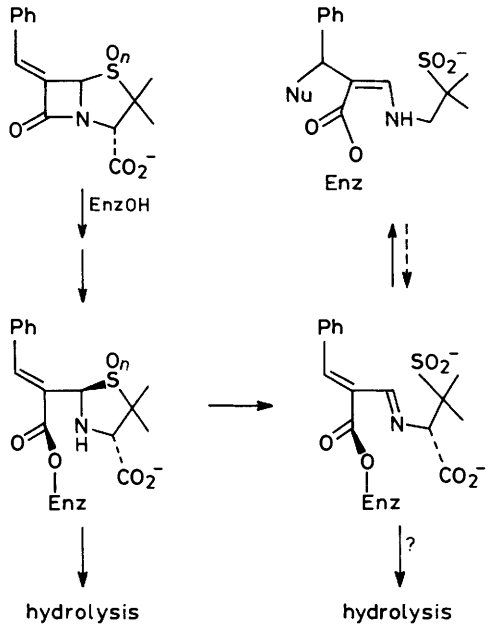
(**23**) *n* = 0
(**24**) *n* = 2



(**25**) *n* = 0
(**26**) *n* = 2

accompanied by only a small amount of turnover and that the hydroxybenzyl sulphone (**15**) acts as a high-affinity substrate. Inhibition of the enzyme is also irreversible, with no recovery of enzyme activity over several hours at pH 8.0. A slow recovery of enzyme activity is observed under more acidic conditions (pH 5.3). Irreversible inactivation is associated with the appearance of increased u.v. absorbance at *ca.* $\lambda_{max.}$ 280 nm; a similar absorption maximum appears on treatment of the alcohol (**15**) with alcoholic base. This absorption is characteristic of the β-aminoacrylate chromophore and could be due to the development of such a system after opening of the β-lactam ring.

The benzylidene sulphones (**24**) and (**26**) also behave as inactivators of the *Ps. aeruginosa* β-lactamase. Whilst the (*Z*)-isomer (**26**) is about twice as active as the (*E*)-isomer (**24**), it is only half as active as the hydroxybenzyl sulphone (**15**). Interestingly, inactivation of the enzyme by the benzylidene derivatives also results in the formation of a new chromophore, with $\lambda_{max.}$ between 270—290 nm, again suggesting the opening of the β-lactam ring with the possible formation of the β-aminoacrylate system. The route to this chromophore must involve the addition of a nucleophile to the benzylidene group and, in the micro-environment of the active site of the enzyme, this nucleophile can be either water or a functional group associated with the enzyme (Scheme 2). Further studies on the chemistry of these inhibitors are required.



Scheme 2. EnzOH = enzyme; Nu = water or group on enzyme

Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. I.r. spectra were recorded on a Perkin-Elmer 297

* We thank Dr. S. G. Waley and his colleagues, Sir William Dunn School of Pathology, University of Oxford, for carrying out these assays. Details on the assays of the alcohol (**15**) will be presented elsewhere.

spectrophotometer, either on film or for solids, in $CHCl_3$ solution. U.v. spectra were taken in ethanol solutions on a Pye-Unicam PU 8800 spectrophotometer. ^1H N.m.r. spectra were recorded on either a Perkin-Elmer R32 (90 MHz) instrument or a Jeol FX90Q (90 MHz) spectrometer for solutions in deuteriochloroform (tetramethylsilane as internal reference) or as stated. Mass spectra were recorded on a Kratos MS25 instrument with accurate mass measurements being carried out on an AEI-Kratos MS9/50 instrument.

Thin-layer chromatography (t.l.c.) was carried out on Kieselgel $GF60_{254}$ and column chromatography through Merck silica gel G60. Solvents were usually distilled and dried before use; light petroleum refers to the fraction of boiling range 40—60 °C and ether refers to diethyl ether throughout. Solvent ratios are described as ratios of volumes before mixing.

Benzhydryl 6,6-Dibromopenicillanate (**4**).—6-Aminopenicillanic acid was converted into dibromopenicillanic acid by diazotisation in the presence of bromine, as described by Volkmann *et al.*[8] The acid was esterified with diphenyldiazomethane in ether to give the title ester.

Preparation of the Benzaldehyde Adducts (**5**).—The ester (**4**) (5.0 g, 9.5 mmol) in dry THF (100 ml) at −70 °C under nitrogen was treated, dropwise, with a solution of methylmagnesium bromide in diethyl ether (3M; 4 ml, 12 mmol), and the resulting yellow solution stirred for 15 min before freshly distilled benzaldehyde (3 ml, 30 mmol) in THF (10 ml) was added, maintaining the temperature at −70 °C. After a further 15 min saturated aqueous ammonium chloride (5 ml) was added and the reaction mixture partitioned between ethyl acetate and water. The organic phase was washed with water and brine, then dried and the solvents removed under reduced pressure. The crude product was subjected to column chromatography through SiO_2 (200 g) with 1:4 ethyl acetate–light petroleum, to afford the α-hydroxybenzyl adducts (**5**) (4.5 g) as a mixture of isomers, which were carried on to the next stage without separation.

Reduction of the Benzaldehyde Adducts (**5**).—A solution of the adducts (4.5 g) in benzene (50 ml), tributyltin hydride (2 ml), and azoisobutyronitrile (5 mg) was heated to reflux for 2 h. The solvent was removed under reduced pressure and the residue subjected to column chromatography through SiO_2 using 1:3 ethyl acetate–light petroleum as eluant. Initially eluted was the minor alcohol, *benzhydryl* (αS,3S,5R,6R)-6-(α-*hydroxybenzyl*)-*penicillanate* (**12**) (1.2 g, 31%), m.p. 141—143 °C, $[\alpha]_D^{25}$ + 127° (*c* 0.1, $CHCl_3$), $\nu_{max.}$ 3 550, 1 780, and 1 750 cm^{-1}; δ 1.25 (3 H, s, Me), 1.68 (3 H, s, Me), 2.71 (1 H, d, *J* 4 Hz, exch. with D_2O, OH), 3.60 (1 H, dd, *J* 4.5, 11 Hz, 6-H), 4.50 (1 H, s, 3-H), 5.02 (1 H, dd, *J* 4, 11 Hz, α-H), 5.48 (1 H, d, *J* 4.5 Hz, 5-H), 6.94 (1 H, s, Ph_2CH), and 7.2—7.5 (15 H, m, aromatic H) (Found: C, 71.0; H, 5.5; N, 3.0; S, 6.8. $C_{28}H_{27}NO_4S$ requires C, 71.0; H, 5.7; N, 3.0; S, 6.8%).

The more polar alcohol was *benzhydryl* (αR,3S,5R,6R)-6-(α-*hydroxybenzyl*)*penicillinate* (**10**) (2.2 g, 57%), m.p. 167—169 °C, $[\alpha]_D^{25}$ + 142° (*c* 0.1, $CHCl_3$), $\nu_{max.}$ 3 590, 1 770, and 1 750 cm^{-1}; δ 1.20 (3 H, s, Me), 1.68 (3 H, s, Me), 3.08 (1 H, d, *J* 3 Hz, exch. with D_2O, OH), 3.91 (1 H, dd, *J* 5, 10 Hz, 6-H), 4.57 (1 H, s, 3-H), 5.18 (1 H, dd, *J* 3, 10 Hz, α-H), 5.20 (1 H, d, *J* 5 Hz, 5-H), 6.94 (1 H, s, Ph_2CH), and 7.2—7.5 (15 H, m, aromatic H) (Found: C, 71.0; H, 5.6; N, 3.1; S, 6.7. $C_{28}H_{27}NO_4S$ requires C, 71.0; H, 5.5; N, 3.0; S, 6.8%).

Preparation of the Sulphones (**11**) *and* (**13**).—The hydroxy sulphides (**10**) and (**12**) were separately oxidised to their corresponding sulphones as follows. To the hydroxy sulphide (1.0 g, 2.1 mmol) in dichloromethane (20 ml) at 0 °C was added *m*-chloroperbenzoic acid (0.48 g, 2.3 mmol) in dichloromethane (5 ml) and the stirred solution allowed to warm to room temperature (30 min) before a further portion of *m*-chloroperbenzoic acid (0.48 g) in dichloromethane (5 ml) was added. The solution was stirred at room temperature for 12 h before an aqueous solution of sodium sulphite (1M; 20 ml) was added, and the organic layer was then separated and washed with 5% aqueous sodium hydrogen carbonate solution (3 ×), water, and brine. The organic layer was dried and the solvent removed under reduced pressure. The sulphones were purified by chromatography through silica gel, eluting with 1:6 ethyl acetate–light petroleum. *Benzhydryl* (αR,3S,5R,6R)-6-(α-*hydroxy*)*benzyl*)-*penicillanate* 1,1-*dioxide* (**11**) (90%) had m.p. 167—169 °C; $[\alpha]_D^{17}$ + 169° (*c* 0.2, $CHCl_3$); $\nu_{max.}$ 3 300, 1 780, 1 740, 1 300, and 1 110 cm^{-1}; δ 1.02 (3 H, s, Me), 1.56 (3 H, s, Me), 2.15 (1 H, br s, exch. with D_2O, OH), 4.15 (1 H, dd, *J* 5, 10 Hz, 6 H), 4.37 (1 H, d, *J* 5 Hz, 5-H), 4.59 (1 H, s, 3-H), 5.76 (1 H, d, *J* 10 Hz, α-H), 6.93 (1 H, s, Ph_2CH), and 7.2—7.5 (15 H, m, aromatic H) (Found: C, 66.4; H, 5.2; N, 3.0; S, 6.6. $C_{28}H_{27}NO_6S$ requires C, 66.5; H, 5.4; N, 2.8; S, 6.3%).

Benzhydryl (αS,3S,5R,6R)-6-(α-*hydroxybenzyl*)*penicillanate* 1,1-*dioxide* (**13**) (90%) had m.p. 143—145 °C, $[\alpha]^{D17}$ + 139° (*c* 0.2, $CHCl_3$), $\nu_{max.}$ 3 350, 1 780, 1 740, 1 310, and 1 100 cm^{-1}; δ 1.15 (3 H, s, Me), 1.60 (3 H, s, Me), 2.55 (1 H, d, *J* 4.5 Hz exch. with D_2O, OH), 4.17 (1 H, dd, *J* 5, 10 Hz, 6-H), 4.58 (1 H, s, 3-H), 4.72 (1 H, d, *J* 5 Hz, 5-H), 5.68 (1 H, dd, *J* 4, 5, 10 Hz, α-H), 6.97 (1 H, s, Ph_2CH), and 7.2—7.5 (15 H, m, aromatic H) (Found: C, 66.5; H, 5.3; N, 2.5; S, 6.6. $C_{28}H_{27}NO_6S$ requires C, 66.5; H, 5.4; N, 2.8; S, 6.3%).

General Method for Deprotection of the Benzhydryl Esters.—To a solution of the benzhydryl ester (1 mmol) in trifluoroethanol (4 ml) at 0 °C was added trifluoroacetic acid (0.5 ml) and the mixture stirred for 1 h whilst allowing the reaction temperature to rise to the ambient value. The solution was poured into 10% w/v aqueous sodium hydrogen carbonate and extracted with ethyl acetate. The aqueous phase was acidified with 1M-phosphoric acid and extracted with ethyl acetate. The organic extract was dried and evaporated under reduced pressure to afford the acid (yields 50—70%). In this manner were prepared the free acids of compounds (**14**)—(**17**).

The acids were converted into their potassium salts, for biological assay and storage, as follows. A solution of the acid in a minimum quantity of ethyl acetate–light petroleum (ratio *ca.* 1:2) was added to a concentrated solution of potassium 2-ethylhexanoate in the same solvent mixture. After mixing, a precipitate of the derived potassium salt separated out. The salt was collected by filtration and washed with a small portion of solvent before being dried and stored at 0 °C. The purity of the potassium salts was checked by t.l.c. and by reconverting a sample back into the corresponding benzhydryl ester and direct comparison with the authentic material. In all cases the potassium salts proved to be >90% pure. (αR,3S,5R,6R)-6-(α-*Hydroxybenzyl*)*penicillanic acid* (**14**), an amorphous solid, showed δ (^2H_2O) 1.44 (3 H, s, Me), 1.65 (3 H, s, Me), 4.34 (1 H, dd, *J* 4, 9 Hz, 6-H), 4.59 (1 H, s, 3-H), 4.93 (1 H, d, *J* 9 Hz, α-H), 5.27 (1 H, d, *J* 4 Hz, 5-H), and 7.2—7.4 (5 H, m, aromatic H) (Found: M^+ 307. $C_{15}H_{17}NO_4S$ requires *M* 307).

(αS,3S,5R,6R)-6-(α-*Hydroxybenzyl*)*penicillanic acid* (**16**), an amorphous solid showed δ ([$^2H_6$]acetone) 1.40 (3 H, s, Me), 1.56 (3 H, s, Me), 2.95 (1 H, dd, *J* 4, 10 Hz, 6-H), 4.46 (1 H, s, 3-H), 5.20 (1 H, d, *J* 10 Hz, α-H), 5.47 (1 H, d, *J* 4 Hz, 5-H), and 7.3—7.4 (5 H, m, aromatic H) (Found: M^+ 307, $C_{15}H_{17}NO_4S$ requires M^+ 307).

(αR,3S,5R,5R)-6-(α-*Hydroxybenzyl*)*penicillanic acid* 1,1-*dioxide* (**15**), an amorphous solid, showed δ ([$^2H_4$]methanol) 1.33 (3 H, s, Me), 1.52 (3 H, s, Me), 4.33 (1 H, dd, *J* 5, 10 Hz, 6-H), 4.41 (1 H, s, 3-H), 4.41 (1 H, d, *J* 5 Hz, 5-H), 5.33 (1 H, d, *J* 10.5 Hz,

α-H), and 7.2—7.5 (5 H, m, aromatic H) (Found: M^+ 339. $C_{17}H_{15}NO_6S$ requires M^+ 339). The potassium salt had m.p. 125 °C (decomp.).

(αS,3S,5R,6R)-6-(α-*Hydroxybenzyl*)*penicillanic acid* 1,1-*dioxide* (**17**), an amorphous solid, showed δ ([$^2H_4$]methanol) 1.47 (3 H, s, Me), 1.59 (3 H, s, Me), 4.15 (1 H, dd, *J* 5, 11 Hz, 6-H), 4.41 (1 H, s, 3-H), 4.94 (1 H, d, *J* 5 Hz, 5-H), 5.54 (1 H, d, *J* 11 Hz, α-H), and 7.2—7.5 (5 H, m, aromatic H) (Found: M^+ 339. $C_{17}H_{15}NO_6S$ requires M^+ 339). The potassium salt had m.p. 145—150 °C (decomp.).

Dehydration of Benzhydryl (αR,3S,5R,6R)-6-(α-*Hydroxybenzyl*)*penicillanate* 1,1-*Dioxide* (**11**).—The major hydroxy sulphone (1.01 g, 2 mmol) in dichloromethane (15 ml) was treated with triethylamine (0.82 g, 8 mmol) followed by thionyl chloride (0.48 g, 4 mmol) in dichloromethane (5 ml) at room temperature for 5 min before the mixture was heated to reflux for 2 h. The reaction mixture was cooled, washed with 1M-phosphoric acid (2 × 20 ml), water (10 ml), and brine (3 × 20 ml) then dried and the solvent removed under reduced pressure, to yield, as a crystallising oil, the (*Z*)-olefin (**7**). Flash chromatography through SiO_2, using 3:7 ethyl acetate–light petroleum as eluant, afforded *benzhydryl* (6Z)-*benzylidenepenicillanate* 1,1-*dioxide* (**7**) (0.84 g, 86%), m.p. 176—177 °C, $[\alpha]_D^{18}$ + 339° (*c* 0.2, $CHCl_3$), $\nu_{max.}$ 1 765, 1 750, 1 330, and 1 110 cm^{-1}; δ 1.22 (3 H, s, Me), 1.61 (3 H, s, Me), 4.58 (1 H, s, 3-H), 5.39 (1 H, d, *J* 1.4 Hz, 5-H), 7.00 (1 H, s, Ph_2CH), 7.3—7.4 (15 H, m, aromatic H), and 7.41 (1 H, d, *J* 1.4 Hz, α-H) (Found: C, 69.3; H, 5.2; N, 2.9; S, 6.8. $C_{28}H_{25}NO_5S$ requires C, 69.0; H, 5.2; N, 2.9; S, 6.6%).

The isomer, *benzhydryl* (6E)-*benzylidenepenicillanate* 1,1-*dioxide* (**9**) showed m.p. 145—147 °C, $[\alpha]_D^{18.5}$ + 93°, (*c* 0.1, $CHCl_3$), $\nu_{max.}$ 1 750, 1 740, 1 660, 1 310, and 1 110 cm^{-1}; δ 1.19 (3 H, s, Me), 1.54 (3 H, s, Me), 4.56 (1 H, s, 3-H), 5.15 (1 H, d, *J* 0.8 Hz, 5-H), 6.81 (1 H, d, *J* 0.8 Hz, α-H), 7.0 (1 H, s, Ph_2CH), 7.3—7.5 (13 H, m, aromatic H), and 7.9—8.0 (2 H, m, aromatic H) (Found: C, 69.2; H, 5.0; N, 2.8; S, 6.8. $C_{28}H_{25}NO_5S$ requires C, 69.0; H, 5.2; N, 2.9; S, 6.6%).

Dehydration of Benzhydryl (αS,3S,5R,6R)-6-(α-*Hydroxybenzyl*)*penicillanate* 1,1-*Dioxide* (**13**).—In a similar manner to that described above, the minor hydroxy sulphone (1.01 g, 2 mmol) was dehydrated with triethylamine (0.82 g, 8 mmol) and thionyl chloride (0.48 g, 4 mmol) in dichloromethane (20 ml) at reflux for 4 h. After work-up the reaction produced a 5:1 ratio of the (*E*):(*Z*)-isomers (total yield 0.82 g, 85%).

Dehydration of the Sulphides (**10**) *and* (**12**).—Treatment of the (αR)-hydroxy sulphide (**10**) (50 mg, 0.1 mmol) with triethylamine (55 mg, 0.8 mmol) and thionyl chloride (25 mg, 0.2 mmol) in dichloromethane (4 ml) at reflux for 4 h and work-up in the usual manner, afforded a 1:3 ratio of the (*E*):(*Z*)-olefins. Column chromatography through SiO_2, using 1:4 ethyl acetate–light petroleum as eluant, gave, initially, *benzhydryl* (6E)-*benzylidenepenicillanate* (**8**) (8 mg, 17%), $[\alpha]_D$ + 92° (*c* 0.3, $CHCl_3$) $\nu_{max.}$ 1 740 cm^{-1}; δ 1.28 (3 H, s, Me), 1.57 (3 H, s, Me), 4.66 (1 H, s, 3-H), 5.80 (1 H, br s, 5-H), 6.57 (1 H, br s, α-H), 6.96 (1 H, s, Ph_2CH), 7.3—7.4 (13 H, m, aromatic H), and 7.9—8.0 (2 H, m, aromatic H) (Found: M^+ 455.155 26. $C_{28}H_{25}NO_3S$ requires M^+ 455.155 506).

Further elution, with 3:7 ethyl acetate–light petroleum, afforded the (6Z)-*benzylidene isomer* (**6**) (22 mg, 45%), m.p. 174—176 °C, $[\alpha]_D^{22}$ + 101° (*c* 0.2, $CHCl_3$), $\nu_{max.}$ 1 730 cm^{-1}; δ 1.28 (3 H, s, Me), 1.60 (3 H, s, Me), 4.67 (1 H, s, 3-H), 6.09 (1 H, d, *J* 0.9 Hz, 5-H), 7.02 (1 H, s, Ph_2CH), 7.03 (1 H, d, *J* 0.9 Hz, α-H), and 7.3—7.4 (15 H, m, aromatic H) (Found: C, 73.8; H, 5.6; N, 3.1; S, 7.2. $C_{28}H_{25}NO_3S$ requires C, 73.8; H, 5.5; N, 3.1; S, 7.0%).

Dehydration of the (αS)-hydroxy sulphide (**12**) in a similar manner, using the triethylamine–thionyl chloride reagent in dichloromethane, afforded the (*E*):(*Z*) benzylidene isomers, (**8**):(**6**), in a ratio of 5.5:4.5 respectively (overall yield, 93%).

Benzhydryl (αS,3S,5R,6R)-6-(α-*Chlorobenzyl*)*penicillanate* (**18**).—To a stirred solution of *N*-chlorosuccinimide (0.29 g, 2.25 mmol) in THF (5 ml) at room temperature was added, dropwise, a solution of triphenylphosphine (0.59 g, 2.25 mmol) in THF (5 ml). To the resulting suspension was added a solution of the (αR)-hydroxy sulphide (**10**) (0.75 g, 1.6 mmol) in THF (5 ml) and the mixture stirred for 1.5 h at room temperature by which time most of the suspension had dissipated. The solvent was removed under reduced pressure and the residue partitioned between ether and water (40 ml, 1:1). The organic phase was washed with water (2 × 30 ml) dried and evaporated. Column chromatography through SiO_2 using 3:7 ethyl acetate–light petroleum as eluant afforded the *title chloride* (**18**) (0.76 g, 97%), m.p. 129—131 °C, $[\alpha]_D^{24.5}$ + 248° (*c* 0.2, $CHCl_3$), $\nu_{max.}$ 1 760 and 1 715 cm^{-1}; δ 1.30 (3 H, s, Me), 1.68 (3 H, s, Me), 4.30 (1 H, dd, *J* 4, 12 Hz, 6-H), 4.50 (1 H, s, 3-H), 5.30 (1 H, d, *J* 12 Hz, α-H), 5.56 (1 H, d, *J* 4 Hz, 5-H), 6.92 (1 H, s, Ph_2CH), and 7.2—7.5 (15 H, m, aromatic H) (Found: C, 68.1; H, 5.2; N, 2.8; S, 6.6. $C_{28}H_{26}ClNO_3S$ requires C, 68.3; H, 5.3; N, 2.8; S, 6.5%).

Benzhydryl (αS,3S,5R,6R)-6-(α-*Chlorobenzyl*)*penicillanate* 1,1-*Dioxide* (**19**).—*m*-Chloroperbenzoic acid (0.23 g, 1.3 mmol) in dichloromethane (5 ml) was added to the chloride (**18**) (0.66 g, 1.3 mmol) in dichloromethane (15 ml) at 0 °C. The solution was allowed to warm-up to room temperature and stirred for 30 min before another quantity of peracid (0.23 g, 1.3 mmol) was added, and the mixture was then heated to reflux for 4 h. The solution was cooled, and washed with aqueous sodium sulphite (2 × 20 ml), 10% w/v aqueous sodium hydrogen carbonate, water, and brine. The organic extract was dried and evaporated under reduced pressure and the residue chromatographed through SiO_2 using 3:7 ethyl acetate–light petroleum as eluant. The major product was the *title sulphone* (0.25 g, 36%), m.p. 179—181 °C, $[\alpha]_D^{24}$ + 160° (*c* 0.15, $CHCl_3$); $\nu_{max.}$ 1 800, 1 750, 1 325, and 1 120 cm^{-1}; δ 1.14 (3 H, s, Me), 1.58 (3 H, s, Me), 4.53 (1 H, s, 3-H), 4.49 (1 H, dd, *J* 5, 11 Hz, 6-H), 4.72 (1 H, d, *J* 5 Hz, 5-H), 5.96 (1 H, d, *J* 11 Hz, α-H), 6.94 (1 H, s, Ph_2CH), and 7.2—7.7 (15 H, m, aromatic H) (Found: C, 63.9; H, 5.0; N, 2.6; S, 6.3. $C_{28}H_{26}ClNO_5S$ requires C, 64.2; H, 5.0; N, 2.7; S, 6.1%).

(αS,3S,5R,6R)-6-(α-*Chlorobenzyl*)*penicillanic Acid* (**21**).—To a solution of the ester (**18**) (0.72 g, 1.5 mmol) in trifluoroethanol (7 ml) at 0 °C was added trifluoroacetic acid (2.2 ml) and the solution was stirred at 0 °C for 2 h and then poured into a mixture of ethyl acetate and 5% w/v aqueous sodium hydrogen carbonate. After work-up in the normal manner the *title acid* (0.25 g, 52%) was obtained as an amorphous solid, $\nu_{max.}$ 3 700—2 700, 1 770, 1 730, and 1 640 cm^{-1}; $\lambda_{max.}$ 202 (ε 13 900), 257 nm (5 200); δ 1.59 (3 H, s, Me), 1.70 (3 H, s, Me), 4.38 (1 H, s, 3-H), 4.46 (1 H, dd, *J* 5, 11 Hz, 6-H), 5.27 (1 H, d, *J* 11 Hz, α-H), 5.51 (1 H, d, *J* 5 Hz, 5-H), 6.8 (1 H, br s, CO_2H), and 7.25—7.6 (5 H, m, aromatic H) (Found: M^+ 325.0537. $C_{15}H_{16}{}^{35}ClNO_3S$ requires M^+ 325.053 937).

(αS,3S,5R,6R)-6-(α-*Chlorobenzyl*)*penicillanic Acid* 1,1-*Dioxide* (**22**).—The ester (**19**) (0.12 g, 0.23 mmol) in trifluoroethanol (3 ml) and trifluoroacetic acid (1 ml) was left for 2 h at 0 °C, then worked up in the normal manner to give the *title acid* (50 mg, 63%) as an amorphous foam, $\nu_{max.}$ 3 700—2 700, 1 790, 1 730, 1 635, 1 325, and 1 120 cm^{-1}; $\lambda_{max.}$ 202 (ε 11 400), 279 nm (12 300); δ 1.48 (3 H, s, Me), 1.63 (3 H, s, Me), 4.45 (1 H, dd, *J* 5, 11 Hz, 6-H), 4.43 (1 H, s, 3-H), 4.55 (1 H, br s, CO_2H), 4.78 (1 H, d, *J* 5, 11 Hz, 5-H), 5.94 (1 H, d, *J* 11 Hz, α-H), and 7.3—7.5 (5 H, m,

aromatic H) (Found: M^+ 357.044 19. $C_{15}H_{16}{}^{35}ClNO_5S$ requires M^+ 357.043 765).

Dehydrochlorination of the Chloro Sulphide (**21**).—The acid (0.25 g, 0.8 mmol) in dichloromethane (15 ml) was treated with DBN (0.3 g, 2.3 mmol) at room temperature for 2 h, then the solution was poured into 1M-phosphoric acid (70 ml) and extracted with ethyl acetate (2 × 20 ml). The organic extract was washed with water and brine, dried and the solvent removed under reduced pressure to afford an 8:1 mixture of the (*Z*):(*E*) acids, (**25**):(**23**) respectively (0.12 g, 54%), as an amorphous foam, $\nu_{max.}$ 3 750—2 400, 1 755, 1 725, and 1 640 cm^{-1}; $\lambda_{max.}$ 202 (ε 15 100), 278 nm (18 100); δ (major isomer) 1.61 (3 H, s, Me), 1.66 (3 H, s, Me), 4.48 (1 H, s, 3-H), 6.06 (1 H, s, 5-H), 7.05 (1 H, s, α-H), 7.25—7.4 (5 H, m, aromatic H), and 8.28 (1 H, br s, CO_2H) (Found: M^+ 289.077 21. $C_{15}H_{15}NO_3S$ requires M^+ 289.077 259).

Dehydrochlorination of the Chloro Sulphone (**22**).—The acid (26 mg, 0.08 mmol) in dichloromethane (5 ml) was treated with DBN (29 mg, 0.23 mmol) at room temperature for 2 h before work-up in the manner described above, to afford a mixture of the (*E*):(*Z*) benzylidene derivatives in a ratio of >9:1. The major olefin (**24**) showed $\nu_{max.}$ 3 750—2 700, 1 770, 1 740, 1 650, 1 325, and 1 120 cm^{-1}; δ 1.50 (3 H, s, Me), 1.61 (3 H, s, Me), 4.47 (1 H, s, 3-H), 5.16 (1 H, s, 5-H), 6.8 (1 H, s, α-H), 7.25—7.55 (3 H, m, aromatic H), 7.85—8.0 (2 H, m, aromatic H), and 8.28 (1 H, br s, CO_2H).

Dehydrations with Triphenylphosphine–N-Chlorosuccinimide.—(*a*) *Hydroxy sulphide* (**12**). A solution of the alcohol (1.0 g, 2.1 mmol) in THF (3 ml) was added to a stirred suspension of triphenylphosphine (0.83 g, 3.2 mmol) and *N*-chlorosuccinimide (0.42 g, 3.2 mmol) in THF (10 ml) at room temperature and the mixture stirred for a further 2 h before the solvent was removed under reduced pressure. The residue was partitioned between 1:1 water–ether (40 ml) and the organic layer washed with water (2 × 30 ml), dried and the solvent removed under reduced pressure. The residue, which showed (^1H n.m.r.) only the benzylidene product (**8**), was chromatographed through SiO_2 using 3:7 ethyl acetate–light petroleum as eluant, to give the (6E)-*benzylidene derivative* (**8**) (0.53 g, 55%), identical in its chromatographic behaviour with the material described above.

(*b*) *Hydroxy sulphone* (**11**). The major hydroxy sulphone (84 mg, 0.17 mmol) was dehydrated with triphenylphosphine (66 mg, 0.25 mmol) and *N*-chlorosuccinimide (33 mg, 0.25 mmol) in THF (5 ml) in the manner described above. After a reaction time of 2 h, work-up afforded, as the only product, the (6*Z*)-benzylidene sulphone (**7**) (96%), identical in its properties with the material described above.

(*c*) *Hydroxy sulphone* (**13**). The minor hydroxysulphone (0.1 g, 0.2 mmol) was dehydrated with triphenylphosphine (79 mg, 0.3 mmol) and *N*-chlorosuccinimide (40 mg, 0.3 mmol) in THF (6 ml) in the manner described above. After a reaction time of 1 h, work-up afforded, as the only product, the (6*E*)-benzylidene sulphone (**9**) (96 mg, 100%), m.p. 145—147 °C, identical in its properties with the material described above.

(*d*) *Hydroxy acid* (**16**). To the stirred complex of triphenylphosphine (0.70 g, 2.7 mmol) and *N*-chlorosuccinimide (0.36 g, 2.7 mmol) in THF (10 ml) was added a solution of the acid (0.41 g, 1.3 mmol) in THF (5 ml). The mixture was stirred at room temperature for 16 h and the solvent then removed under reduced pressure. The residue was partitioned between 1:1 water–ether (80 ml) and the organic layer extracted with 5% w/v aqueous sodium hydrogen carbonate. The aqueous solution was re-acidified, with 1M-phosphoric acid, before being extracted into ethyl acetate. The organic layer was washed with water and brine, dried, and the solvent removed under reduced pressure to afford (6E)-*benzylidenepenicillanic acid* (**23**) (0.10 g, 26%) as an amorphous foam, $\nu_{max.}$ 3 750—2 700, 1 755, 1 725, and 1 640 cm^{-1}; $\lambda_{max.}$ 255 (ε 5 100), 296 nm (7 800); δ 1.52 (3 H, s, Me), 1.63 (3 H, s, Me), 4.48 (1 H, s, 3-H), 5.16 (1 H, s, 5-H), 6.55 (1 H, br s, CO_2H), 6.82 (1 H, s, α-H), 7.3—7.7 (3 H, m, aromatic H), and 7.85—7.98 (2 H, m, aromatic H) (Found: M^+ 289.077. $C_{15}H_{15}NO_3S$ requires M^+ 289.077 259).

(*e*) *Sulphone acid* (**17**). The alcohol (83 mg, 0.25 mmol) was treated with the complex between triphenylphosphine (0.11 g, 0.4 mmol) and *N*-chlorosuccinimide (55 mg, 0.4 mmol) in THF (8 ml) at room temperature for 15 h. Work-up, in the manner described under (*d*), afforded (6E)-*benzylidenepenicillanic acid* 1,1-*dioxide* (**24**) (29 mg, 36%) as an amorphous foam, $[\alpha]_D{}^{23.5}$ + 97° (*c* 0.1 EtOH), $\nu_{max.}$ 3 750—2 700, 1 770, 1 740, 1 610, 1 325, and 1 120 cm^{-1}; $\lambda_{max.}$ 225 (ε 1 600), 288 nm (3 200); δ 1.50 (3 H, s, Me), 1.61 (3 H, s, Me), 4.47 (1 H, s, 3-H), 5.16 (1 H, s, 5-H), 6.80 (1 H, s, α-H), 7.25—7.55 (3 H, m, aromatic H), and 7.85—8.0 (2 H, m, aromatic H) (Found: $M^+ - CO_2$ 277.077 88. $C_{14}H_{15}NO_3S$ requires $[M^+]_D$ 277.077 259).

(*f*) *Sulphone acid* (**15**). The alcohol (0.16 g, 0.5 mmol) was treated with the complex between triphenylphosphine (0.21 g, 0.8 mmol) and *N*-chlorosuccinimide (0.11 g, 0.8 mmol) in THF (16 ml) at room temperature for 16 h. Work-up, in the manner described under (*d*), afforded the (6Z)-*benzylidenepenicillanic acid* 1,1-*dioxide* (**26**) (52 mg, 24%), as an amorphous foam, $\lambda_{max.}$ 3 700—2 700, 1 770, 1 730, 1 640, 1 320, and 1 115 cm^{-1}; $\lambda_{max.}$ 206 nm (ε 7 300), 284 nm (7 200); δ 1.65 (3 H, s, Me), 1.69 (3 H, s, Me), 4.63 (1 H, s, 3-H), 6.09 (1 H, s, 5-H), 7.09 (1 H, s, α-H), 7.2—7.6 (5 H, m, aromatic H), and 9.3 (1 H, br s, CO_2H) (Found: $M^+ - CO_2$ 277.077 57. $C_{14}H_{15}NO_5S$ requires M^+ 277.772 59).

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