Studies Related to Penicillins. Part 22.[1] Mechanistic Aspects of β-Elimination Reactions Involving Penicillanate 1,1-Dioxides

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In the presence of 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) (0.5 mol equiv.) and deuterium oxide, methyl benzylpenicillinate 1,1-dioxide (1a) was converted into its 3,6-dideuteriated derivative (3a), its 3,6-dideuteriated 6-epimer (3b), and (2*R*,3*S*)-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-4-oxo-3-phenylacetamido[3-^{2}H]azetidine-2-sulphinic acid (2b). Under similar conditions, methyl penicillanate 1,1-dioxide (1c) afforded its 3-deuteriated derivative (3c) which was then transformed into (2*R*)-(1-methoxycarbonyl-2-methylprop-1-enyl)-4-oxoazetidine-2-sulphinic acid (2c). The aforementioned results indicate that the β-elimination reactions occur by *E*1*cB* pathways, in which C-3 anionic intermediates, *i.e.* (4a) and (4b), are formed reversibly.

Methyl benzyl[2β-*methyl*-$^2H_3$]penicillinate 1,1-dioxide (7a) and methyl [2β-*methyl*-$^2H_3$]penicillanate 1,1-dioxide (7b) were transformed by DBN into the corresponding sulphinic acids, in which the deuterium label was located exclusively in the methyl group *anti* with respect to the methoxycarbonyl moiety, *i.e.* compounds (12a) and (12b). These findings suggest that the carbanionic intermediates (4a) and (4b) undergo the β-elimination reactions by way of the conformers (5a) and (5b).

p-Nitrobenzyl penicillanate 1,1-dioxide (13b) was converted into [3-^{2}H]penicillanic acid 1,1-dioxide (18a) by deuterium-exchange and hydrogenolysis steps. The last mentioned compound was comparable to sulbactam (17a) in its ability to synergise the antibacterial activity of ampicillin against β-lactamase-producing bacteria.

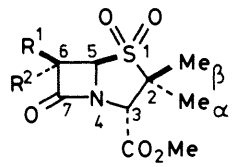
Recently, we showed [1] that penicillanate 1,1-dioxides, *e.g.* (1a), react with 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) to give azetidinone sulphinic acids, *e.g.* (2a). Initially, the base brought about an equilibration of the penicillinate (1a) with the 6-epimer (1b); the latter compound then isomerised to the sulphinic acid (2a). We now define the mechanism of the β-elimination reaction.

Results and Discussion

In principle, two pathways warrant consideration for the isomerisation of the sulphone (1b) to the sulphinic acid (2a): elimination by an *E*2 pathway and elimination by an *ElcB* mechanism. In the hope of distinguishing between these possibilities, the sulphone (1a) was treated with DBN (0.5 mol equiv.) in dichloromethane saturated with deuterium oxide. The recovered neutral product, which contained two components (t.l.c.), was fractionated by silica-gel chromatography. The first eluted material (26% yield) was clearly the 3,6-dideuteriated penicillinate (3a) on the basis of its spectroscopic properties. N.m.r. spectroscopy indicated that essentially total exchange of the 6α-hydrogen atom and 40% exchange of the 3β-hydrogen atom had occurred. The second eluted material (25% yield) was the 3,6-dideuteriated *epi*-penicillinate (3b). On the basis of n.m.r. spectroscopy, its 6β-hydrogen atom had been completely replaced by deuterium and its 3β-hydrogen atom had undergone 60% exchange. As expected, the acidic product was the 3-deuteriated sulphinic acid (2b); it was fully deuteriated at position 3 according to n.m.r. spectroscopy.

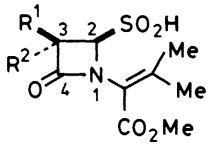
On the basis of the foregoing results, it is clear that the isomerisation reaction involves an *ElcB* process in which the anionic intermediate (4a) intervenes.

In theory, the anionic intermediate (4a) may undergo the β-elimination reaction by way of the conformers (5a) and/or (6) (assuming that the C⁻3-anion, in the pyramidal geometry, must be *anti*-periplanar with respect to the S-1–C-2 bond for the elimination †). To distinguish between these possibilities, it is necessary to know the fate of the geminal dimethyl group in the reorganisation. The geminal dimethyl groups of the



(1)

a; R^1 = $PhCH_2C(=O)NH$, R^2 = H
b; R^1 = H, R^2 = $PhCH_2C(=O)NH$
c; R^1 = R^2 = H
d; R^1 = NH_2, R^2 = H
e; R^1 = $PhCH_2OC(=O)NH$, R^2 = H
f; R^1 = H, R^2 = Cl

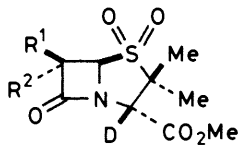


(2)

a; R^1 = H, R^2 = $PhCH_2C(=O)NH$
b; R^1 = D, R^2 = $PhCH_2C(=O)NH$
c; R^1 = R^2 = H

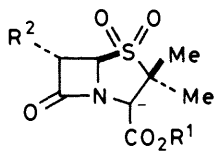
precursor (1a) [δ ($CDCl_3$) 1.37 and 1.56 [2] and the product (2a) [δ ($CDCl_3$) 2.00 and 2.23] [1] can be differentiated by n.m.r. spectroscopy. Accordingly, it should be possible to determine whether or not a stereoselective elimination occurs by employing a selectively deuteriated precursor, *e.g.* (7a).

† The planar geometry is considered to represent the energetically favoured arrangement of carbanions adjacent to CO_2R groups (D. J. Cram, 'Fundamentals of Carbanion Chemistry,' Academic Press, New York, 1965). However, the pyramidal geometry better illustrates the stereochemical features of the elimination reaction.



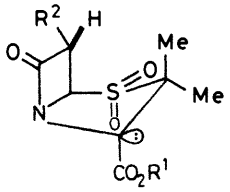
(3)

a ; R^1 = $PhCH_2C(=O)NH$, R^2 = D
b ; R^1 = D, R^2 = $PhCH_2C(=O)NH$
c ; R^1 = R^2 = H



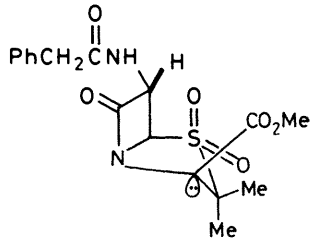
(4)

a ; R^1 = Me, R^2 = $PhCH_2C(=O)NH$
b ; R^1 = Me, R^2 = H



(5)

a ; R^1 = Me, R^2 = $PhCH_2C(=O)NH$
b ; R^1 = Me, R^2 = H



(6)

It is known that oxidation of methyl benzylpenicillinate (8a) with sodium periodate leads to the thermodynamically favoured β-sulphoxide (9a) which, in refluxing benzene, equilibrates with the sulphenic acid (10a).[3] When heated in benzene saturated with deuterium oxide, the sulphoxide (9a)[4] was converted into the deuteriated sulphoxide (11a). Oxidation of compound (11a) with potassium permanganate in aqueous acetic acid afforded the deuteriated sulphone (7a), in which, on the basis of n.m.r. spectroscopy ($CDCl_3$), the 2α-methyl group (δ 1.32) was undeuteriated and the 2β-methyl group (δ 1.51) was 82% deuteriated.

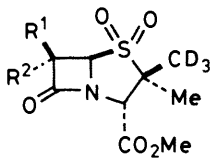
When treated with DBN, the deuteriated sulphone (7a) underwent the usual β-elimination reaction. By n.m.r. spectroscopy ($CDCl_3$), the derived sulphinic acid contained 81% deuterium in the high-field methyl group (δ 2.00); the low-field methyl group (δ 2.23) was undeuteriated. This result clearly established that the β-elimination reaction had occurred in a highly stereocontrolled manner.

Although it may reasonably be inferred that the high-field signal should be attributed to the methyl group *anti* to the methoxycarbonyl group,[5] it was desirable to place this supposition on a firmer basis. This was achieved by 360-MHz n.O.e.-difference spectroscopy on the sulphinic acid (2a). Thus irradiation of the signal at δ 2.00 caused a 2% enhancement of the one-proton double doublet at δ 5.24 [attributed to the 3-proton of (2a)] and a 1% enhancement of the one-proton doublet at δ 4.66 [attributed to the 2-proton of (2a)]; no corresponding effects were observed when the signal at δ 2.23 was irradiated. Clearly, only the methyl group *anti* with respect to the methoxycarbonyl group can be involved in these relaxation processes. The sulphinic acid derived from the sulphone (7a) must therefore possess the structure (12a).

Evidently, the isomerisation of the sulphone (1b) to the sulphinic acid (2a) involves an *E1cB* elimination in which the carbanionic intermediate (4a) adopts the geometry (5a).

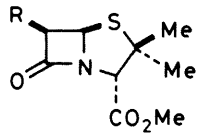
To examine the generality of the aforementioned conclusion, the behaviour of the sulphone (1c) was investigated. The crystalline sulphone (1c), prepared in 89% yield from the salt (13a) by the action of iodomethane in *N*,*N*-dimethylformamide (DMF), reacted with DBN to give the sulphinic acid (2c). Although only a moderate yield (42%) of the sulphinic acid (2c) was realised, this result probably reflects an unfavourable partition coefficient of the acid between water and ethyl acetate (the extracting solvent in the work-up procedure), rather than a poor β-elimination reaction. Thus the addition of iodomethane to a dichloromethane solution of the sulphone (1c), which had been pre-treated with DBN, resulted in the isolation of the methyl sulphone (14) in 78% yield. Methylations of the foregoing type have been reported previously.[1]

When treated briefly with DBN in dichloromethane saturated with deuterium oxide, the sulphone (1c) was converted into its 3-deuteriated derivative (3c). On the basis of



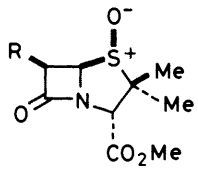
(7)

a ; $R^1 = PhCH_2C(=O)NH$, $R^2 = H$
b ; $R^1 = R^2 = H$
c ; $R^1 = H$, $R^2 = Cl$
d ; $R^1 = NH_2$, $R^2 = H$
e ; $R^1 = PhCH_2O(C=O)NH$, $R^2 = H$



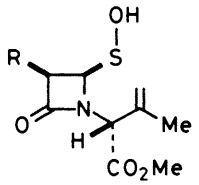
(8)

a ; R = $PhCH_2C(=O)NH$
b ; R = $PhCH_2OC(=O)NH$



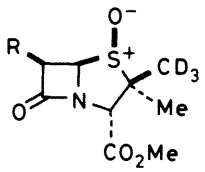
(9)

a ; R = $PhCH_2C(=O)NH$
b ; R = $PhCH_2OC(=O)NH$



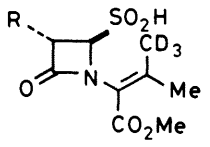
(10)

a ; R = $PhCH_2C(=O)NH$
b ; R = $PhCH_2OC(=O)NH$



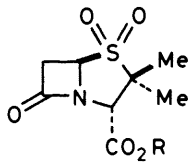
(11)

a ; R = $PhCH_2C(=O)NH$
b ; R = $PhCH_2OC(=O)NH$



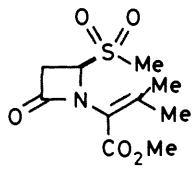
(12)

a ; R = $PhCH_2C(=O)NH$
b ; R = H

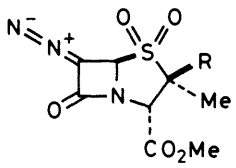


(13)

a ; R = Na
b ; R = $p\text{-}O_2NC_6H_4CH_2$

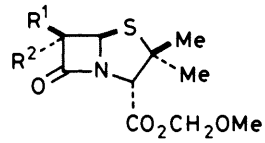


(14)



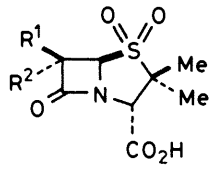
(15)

a ; R = CD_3
b ; R = Me



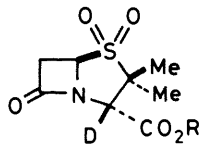
(16)

a ; $R^1 = NH_2$, $R^2 = H$
b ; $R^1 = H$, $R^2 = Cl$



(17)

a ; $R^1 = R^2 = H$
b ; $R^1 = D$, $R^2 = H$
c ; $R^1 = H$, $R^2 = D$
d ; $R^1 = R^2 = D$



(18)

a ; R = H
b ; R = $p\text{-}O_2NC_6H_4CH_2$

n.m.r. spectroscopy, the sample contained 83% deuterium at position 3. The foregoing experiment was repeated and left until the starting sulphone had been depleted; work-up afforded the sulphinic acid (2c) which contained no detectable deuterium. Clearly, the carbanionic intermediate (4b) intervenes in the conversion of the sulphone (1c) into the sulphinic acid (2c) and, evidently, the 6-hydrogen atoms of the sulphone (1c) are much less acidic than those of the sulphones (1a) and (1b).

To define the stereochemical consequences of the β-elimination reaction, efforts were directed towards the derivation of the deuteriated sulphone (7b). It was hoped that this compound would be available from the deuteriated sulphone (7a) by way of the intermediates (15a) and (7c). However, in preliminary experiments, attempts to transform the sulphone (1a) into the diazopenicillanate (15b) by the action of dinitrogen tetraoxide [6] were unrewarding. The possibility of converting the deuteriated sulphone (7a) into the deuteriated aminopenicillanate (7d) and thence the deuteriated chloropenicillanate (7c) was also considered. However, efforts to prepare the aminopenicillanate (1d) from the sulphone (1a) by the action of phosphorus(v) chloride and methanol [7] were unsatisfactory.

The aminopenicillanate (1d) was prepared in quantitative yield by hydrogenolysis of the previously reported benzyloxycarbonyl derivative (1e).[1] Surprisingly, when treated with sodium nitrite in dilute hydrochloric acid, the aminopenicillanate (1d) afforded the diazo derivative (15b), isolated as a slightly impure syrup in *ca.* 50% yield after silica-gel chromatography. Under corresponding conditions, the aminopenicillanate (16a) afforded the chloropenicillanate (16b).[8] The conversion of the diazopenicillanate (15b) into the chloropenicillanate (1f) (90% yield) was effected by using a mixture of 6M-hydrochloric acid and dichloromethane [it was necessary to conduct the reaction on a small scale (0.5 mmol) to achieve a good yield]. The chloropenicillanate (1f) was transformed into the penicillanate (1c) in almost quantitative yield by using zinc in acetic acid.

To complete the feasibility study, it only remained to demonstrate that the penicillinate (8b) could be oxidised to the sulphoxide (9b), which, in turn, could be oxidised to the sulphone (1e). Treatment of the penicillinate (8b) [1] with sodium periodate in aqueous methanol gave a single crystalline sulphoxide (81% yield) which, on the basis of analogy,[3] was assigned the stereostructure (9b). The conversion of the sulph-

oxide (9b) into the sulphone (1e) was readily achieved in 87% yield by the action of potassium permanganate in aqueous acetic acid.

When heated in benzene saturated with deuterium oxide, the sulphoxide (9b) was converted into the deuteriated sulphoxide (11b) by way of the intermediate (10b). On the basis of n.m.r. spectroscopy ($CDCl_3$), the 2β-methyl group (δ 1.67) contained 85% deuterium and the 2α-methyl group (δ 1.20) was undeuteriated. The deuteriated sulphoxide (11b) was transformed, by way of the compounds (7e), (7d),* (15a), and (7c) into the deuteriated sulphone (7b). By n.m.r. spectroscopy ($CDCl_3$), the last described material contained 80% deuterium in the 2β-methyl group (δ 1.76) and no deuterium in the 2α-methyl group (δ 1.46). Clearly, as expected, there was no loss in the stereochemical integrity of the geminal dimethyl group during the (11b) ⟶ (7b) transformation.

When treated with DBN, the deuteriated sulphone (7b) underwent the β-elimination reaction. On the basis of n.m.r. spectroscopy ($CDCl_3$), the derived sulphinic acid contained 82% deuterium in the high-field methyl group (δ 2.01) and no deuterium in the low-field methyl group (δ 2.24). By analogy with the deuteriated sulphinic acid (12a), the signal at δ 2.01 was assigned to the methyl group *anti* to the methoxycarbonyl group. Therefore, the deuteriated sulphinic acid was considered to possess the structure (12b).

On the basis of the foregoing results it is evident that the isomerisation of the sulphone (1c) to the sulphinic acid (2c) involves the reversible formation of the carbanionic intermediate (4b) which adopts the geometry (5b) for the elimination reaction.

Sulbactam (17a) is a potent β-lactamase inhibitor [9] which synergises the action of ampicillin against resistant bacteria.[10] Knowles and his co-workers,[11] in studying the mechanism of the inhibition reaction, prepared the deuteriated penicillanic acids (17b—d). Interestingly, compounds (17b) and (17d) showed an acceleration [2.5-fold for (17b) and 3-fold for (17d)] in the rate of their β-lactamase inactivation compared with the undeuteriated material (17a). Accordingly, it was deemed worthwhile to make use of the deuterium-exchange results, uncovered in the present investigation, to prepare the 3-deuteriated penicillanic acid (18a).

When treated briefly with DBN in dichloromethane saturated with deuterium oxide, the sulphone (13b) [1] was converted into its 3-deuteriated derivative (18b) (97% yield after SiO_2 chromatography). On the basis of n.m.r. spectroscopy, the sample contained 90% deuterium at position 3. Hydrogenolysis of compound (18b) over palladium gave the 3-deuteriated penicillanic acid (18a) (95% yield) which contained 90% deuterium at position 3.

In synergy tests, the compound (18a) was comparable to sulbactam (17a) in its ability to increase the antibacterial activity of ampicillin against β-lactamase-producing bacteria.

Experimental

For general experimental details, see Part 20.[12] Dry solvents, referred to in the ensuing experiments, were prepared as follows: benzene was stored over sodium wire; DMF was distilled under reduced pressure from calcium hydride and stored over molecular sieves (Type 4A). Ether refers to diethyl ether, light petroleum refers to that fraction boiling in the range 40—60 °C.

Reaction of the Sulphone (1a) *with DBN–Deuterium Oxide.*—To a stirred solution of the sulphone (1a) [13] (0.400 g, 1.05 mmol) in deuteriochloroform (2 cm^3) was added deuterium oxide (0.1 cm^3) followed by a solution of DBN (0.062 g, 0.5 mmol) in deuteriochloroform (0.5 cm^3). After 10 min the mixture was diluted with ethyl acetate and the solution was washed with dilute hydrochloric acid. The organic layer was extracted with sodium hydrogen carbonate solution. Evaporation of the dried ($MgSO_4$) organic layer left a syrup which contained two components (t.l.c.). These were fractionated by silica-gel chromatography [EtOAc–light petroleum (1 : 2) as eluant].

The first eluted material (0.106 g, 26%) was *methyl benzyl-*[3,6-$^2H_2$]*penicillinate* 1,1-*dioxide* (3a). On the basis of n.m.r. spectroscopy, the sample was 40% deuteriated at position 3 and fully deuteriated at position 6 [δ ($CDCl_3$) 1.35 and 1.55 (each 3 H, s, together CMe_2), 3.62 (2 H, s, PhCH_2C=CO), 3.81 (3 H, s, OMe), 4.50 (0.6 H, s, 3-H), 4.73 (1 H, s, 5-H), 6.91 (1 H, br s, NH), and 7.27 (5 H, s, Ph)]. The sample, after recrystallisation from dichloromethane–ether, showed m.p. 173—174 °C [lit.,[13] for (1a) 173—174 °C]; $[\alpha]_D$ +184° (0.2% in EtOH) {(1a) showed $[\alpha]_D$ +194° (0.4% in EtOH)}.

The second eluted material (0.097 g, 24%) was *methyl benzyl*-6-epi-[3,6-$^2H_2$]*penicillinate* 1,1-*dioxide* (3b). On the basis of n.m.r. spectroscopy, the sample was 60% deuteriated at position 3 and fully deuteriated at position 6 [δ ($CDCl_3$) 1.39 and 1.57 (each 3 H, s, together CMe_2), 3.59 (2 H, s, PhCH_2C=O), 3.77 (3 H, s, OMe), 4.36 (0.4 H, s, 3-H), 4.81 (1 H, s, 5-H), 6.77 (1 H, br s, NH), and 7.27 (5 H, s, Ph)]. The sample, after recrystallisation from dichloromethane–ether showed m.p. 118—120 °C [lit.,[2] for (1b) 124—125 °C]; $[\alpha]_D$ +176° (0.2% in EtOH) {(1b) showed $[\alpha]_D$ +176° (0.5% in EtOH)}.

The sodium hydrogen carbonate layer was acidified with dilute hydrochloric acid and extracted with ethyl acetate. Evaporation of the dried ($MgSO_4$) organic phase left (2R,3S)-1-(1-*methoxycarbonyl*-2-*methylprop*-1-*enyl*)-4-*oxo*-3-*phenylacetamido*[3-^2H]*azetidine*-2-*sulphinic acid* (2b) (0.120 g, 30%). On the basis of n.m.r. spectroscopy, the sample was fully deuteriated at position 3 [δ ($CDCl_3$) 2.00 and 2.23 (each 3 H, s, together CMe_2), 3.62 (2 H, s, PhCH_2C=O), 3.71 (3 H, s, OMe), 4.68 (1 H, s, 2-H), 7.25 (5 H, s, Ph), 7.99 (1 H, br s, NH), and 9.50 (1 H, br s, SO_2H)]. The sample, after recrystallisation from chloroform–ether, showed m.p. 129—131 °C [lit.,[1] for (2a) 136—137 °C]; $[\alpha]_D$ −171° (0.7 in $CHCl_3$) {lit.,[1] for (2a) $[\alpha]_D$ −176° ($CHCl_3$)}.

Thermolysis of the Sulphoxide (9a) *in the Presence of Deuterium Oxide.*—A solution of the sulphoxide (9a) [4] (4.00 g) in a mixture of dry benzene (150 cm^3) and deuterium oxide (1 cm^3) was heated at just below reflux. From time to time (*ca.* every 1.5 d), the deuterium oxide was replaced with a fresh sample. After *ca.* 1 week the mixture was evaporated. After the addition of tetrachloromethane and re-evaporation the residue was recrystallised from methanol–ether to give *methyl benzyl*[2β-methyl-$^2H_3$]*penicillinate* 1β-*oxide* (11a) (3.15 g, 79%); m.p. 131—132 °C [lit.,[4] for (9a) 123 °C]; $[\alpha]_D$ +214° (0.9% in $CHCl_3$) {(9a) showed $[\alpha]_D$ +231° (1% in $CHCl_3$)}. On the basis of n.m.r. spectroscopy, the sample was 85% deuteriated in the 2β-methyl group [δ ($CDCl_3$) 1.15 (3 H, s, CMe_α), 1.68 (0.45 H br s, CMe_β), 3.60 (2 H, s, PhCH_2C=O), 3.80 (3 H, s, OMe), 4.62 (1 H, s, 3-H), 5.00 (1 H, d, *J* 4 Hz, 5-H), 6.00 (1 H, dd, *J* 10 and 4 Hz, 6-H), 7.10 (1 H, br d, *J* 10 Hz, NH), and 7.25 (5 H, s, Ph)].

Reaction of the Deuteriated Sulphoxide (11a) *with Potassium Permanganate* (*With E. Perrone*).—A solution of potassium permanganate (0.150 g, 0.95 mmol) in water (1.5 cm^3) was

* When treated with phenylacetyl chloride and triethylamine in dichloromethane, the deuteriated aminopenicillanate (7d) was converted into the deuteriated penicillinate (7a), confirming that the sulphoxide precursor was indeed the β-sulphoxide (9b).

added slowly to a stirred solution of the deuteriated sulphoxide (11a) (0.300 g, 0.82 mmol) in 4 : 1 acetic acid–water (3 cm^3) at 0 °C. After 2 h the mixture was decolourised by the addition of hydrogen peroxide solution and partitioned between dichloromethane and sodium hydrogen carbonate solution. Evaporation of the dried ($MgSO_4$) organic layer and recrystallisation of the residue from chloroform–light petroleum gave *methyl benzyl*[2β-methyl-$^2H_3$]*penicillinate* 1,1-*dioxide* (7a) (0.270 g, 86%); m.p. 148—150 °C (decomp.) [lit.,[13] for (1a) 173—174 °C]; $[\alpha]_D$ +179° (0.7% in EtOH) {(1a) showed $[\alpha]_D$ +194° (0.4% in EtOH)}. On the basis of n.m.r. spectroscopy, the sample contained 82% deuterium in the 2β-methyl group [δ ($CDCl_3$) *inter alia* 1.32 (3 H, s, CMe_α) and 1.51 (0.54 H, br s, CMe_β], 3.56 (2 H, s, PhCH_2C=O), 3.72 (3 H, s, OMe), 4.48 (1 H, s, 3-H), 4.70 (1 H, d, *J* 4 Hz, 5-H), 6.03 (1 H, dd, *J* 10 and 4 Hz, 6-H), 6.93 (1 H, br d, *J* 10 Hz, NH), and 7.18 (5 H, s, Ph)].

Reaction of the Deuteriated Sulphone (7a) *with DBN* (*With E. Perrone*).—DBN (0.073 g, 0.59 mmol) was added to a solution of the deuteriated sulphone (7a) (0.200 g, 0.52 mmol) in dichloromethane (20 cm^3). After 20 min the mixture was washed with dilute hydrochloric acid and then extracted with sodium hydrogen carbonate solution. The extract was acidified with dilute hydrochloric acid and extracted with dichloromethane. Evaporation of the dried ($MgSO_4$) organic layer and recrystallisation of the residue from chloroform–ether gave (2R,3S)-1-{(Z)-[methyl-$^2H_3$]-1-*methoxycarbonyl*-2-*methylprop*-1-*enyl*}-4-*oxo*-3-*phenylacetamidoazetidine*-2-*sulphinic acid* (12a) (0.100 g, 50%), m.p. 143 °C [lit.,[1] for (2a) 136—137 °C]. On the basis of n.m.r. spectroscopy, the sample contained 81% deuterium in the high-field methyl group [δ ($CDCl_3$) *inter alia* 2.00 (0.57 H, br s, CMe), 2.23 (3 H, s, CMe), 3.65 (2 H, s, PhCH_2C=O), 3.73 (3 H, s, OMe), 4.63 (1 H, d, *J* 2 Hz, 2-H), 5.18 (1 H, dd, *J* 5 and 1.5 Hz, 3-H), 7.25 (5 H, s, Ph), 7.80 (1 H, br d, *J* 5 Hz, NH), and 8.50 (1 H, br s, SO_2H)].

Reaction of the Salt (13a) *with Iodomethane*.—Iodomethane (2.25 g, 15.8 mmol) was added to a stirred suspension of the salt (13a) (1.31 g, 5.1 mmol) in dry DMF (10 cm^3). After 18 h the mixture was diluted with ethyl acetate and washed successively with water (2 ×) and brine. Evaporation of the dried ($MgSO_4$) organic phase left *methyl penicillanate* 1,1-*dioxide* (1c) (1.13 g, 89%), m.p. 105—107 °C (from $CHCl_3$–Et_2O); $[\alpha]_D$ +221° (0.9% in EtOH); $\nu_{max.}$ (KBr) *inter alia* 1 810 (β-lactam C=O) and 1 755 cm^{-1} (ester C=O); $\lambda_{max.}$ (EtOH) 213 (ε 260) and 225sh nm (100); δ ($CDCl_3$) 1.41 and 1.61 (each 3 H, s, together CMe_2), 3.49 (2 H, d, separation 3.5 Hz, 6-H_2), 3.82 (3 H, s, OMe), 4.42 (1 H, s, 3-H), and 4.68 (1 H, t, separation 3.5 Hz, 5-H); *m/z inter alia* 183 ($M^+ - O_2S$) and 83 (base peak) (Found: C, 43.6; H, 5.15; N, 5.6. $C_9H_{13}NO_5S$ requires C, 43.7; H, 5.25; N, 5.65%).

Reaction of the Sulphones (1c) *and* (7b) *with DBN*.—(*a*) A 30% solution of DBN in deuteriochloroform was added dropwise to a solution of the sulphone (1c) (0.500 g, 2.02 mmol) in deuteriochloroform (1.5 cm^3) until the starting material had disappeared (n.m.r. spectroscopy). The mixture was diluted with ethyl acetate and washed with dilute hydrochloric acid. The organic layer was then extracted with sodium hydrogen carbonate solution. Acidification of the extract with dilute hydrochloric acid was followed by extraction with ethyl acetate. Evaporation of the dried ($MgSO_4$) organic layer left (2*R*)-1-(1-methoxycarbonyl-2-methylprop-1-enyl)-4-oxoazetidine-2-sulphinic acid (2c) (0.210 g, 42%); $[\alpha]_D$ −31° (0.8% in EtOH); $\nu_{max.}$ (film) *inter alia* 3 380br (OH), 1 775 (β-lactam C=O), and 1 740 cm^{-1} (ester C=O); $\lambda_{max.}$ (EtOH) 223 nm (ε 400); δ ($CDCl_3$), 2.02 and 2.24 (each 3 H, s, together CMe_2), 3.29 (2 H, d, separation 3.5 Hz, 3-H_2), 3.83 (3 H, s, OMe), 4.78 (1 H, t, separation 3.5 Hz, 2-H), and 7.10 (1 H, br s, SO_2H) (addition of D_2O caused the signal at δ 7.10 to disappear); *m/z inter alia* 183 ($M^+ - O_2S$), 182 ($M^+ - HO_2S$), and 70 (base peak).

(*b*) The foregoing reaction was repeated with the deuteriated sulphone (7b) (0.130 g, 0.52 mmol). Work-up afforded (2*R*)-{(*Z*)-[*methyl*-$^2H_3$]-1-methoxycarbonyl-2-methylprop-1-enyl)}-4-oxoazetidine-2-sulphinic acid (12b) (0.047 g, 36%). On the basis of n.m.r. spectroscopy, the sample was 82% deuteriated in the high-field methyl group [δ ($CDCl_3$) *inter alia* 2.01 (0.54 H, br s, CMe) and 2.24 (3 H, s, CMe)].

Reaction of the Sulphone (1c) *with DBN Followed by Iodomethane*.—To a stirred solution of the sulphone (1c) (0.200 g, 0.81 mmol) in dichloromethane (4 cm^3) was added DBN (0.143 g, 1.15 mmol) followed, after 15 min, by iodomethane (1.14 g, 8 mmol). After a further 1 h, the mixture was diluted with dichloromethane and washed in turn with dilute hydrochloric acid and brine. Evaporation of the organic phase and purification of the residue by silica-gel chromatography [EtOAc–light petroleum (1 : 1) as eluant] gave two fractions. The first eluted material (0.034 g, 17%) was the unchanged sulphone (1c), on the basis of n.m.r. spectroscopy. The second eluted material (0.164 g, 78%), isolated as a chromatographically homogeneous syrup, was *methyl* 3-*methyl*-2-[(2R)-2-*methylsulphonyl*-4-*oxoazetidin*-1-*yl*]*but*-2-*enoate* (14), $[\alpha]_D$ −68° (0.6% in EtOH); $\nu_{max.}$ (film) *inter alia* 1 775 (β-lactam C=O) and 1 725 cm^{-1} (ester C=O); $\lambda_{max.}$ (EtOH) 211 (ε 370) and 222sh nm (250); δ ($CDCl_3$) 2.10 and 2.25 (each 3 H, s, together CMe_2), 2.82 (3 H, s, SO_2Me), 3.36 (2 H, d, separation 4 Hz, CH_2C=O), 3.76 (3 H, m, OMe), and 5.08 (1 H, t, separation 4 Hz, NCHSO$_2$); *m/z inter alia* 261 (M^+) and 112 (base peak) (Found: M^+, 261.0669. $C_{10}H_{15}NO_5S$ requires *M*, 261.0671).

Reaction of the Sulphone (1c) *with DBN–Deuterium Oxide*. (*a*) A 20% solution of DBN in deuteriochloroform was added dropwise to a solution of the sulphone (1c) (0.300 g, 1.21 mmol) in a mixture of deuteriochloroform (1 cm^3) and deuterium oxide (0.15 cm^3). When the exchange of the 3-hydrogen atom was complete (n.m.r. spectroscopy) the mixture was diluted with dichloromethane and washed in turn with dilute hydrochloric acid and sodium hydrogen carbonate solution. Evaporation of the dried ($MgSO_4$) organic layer and purification of the product by silica-gel chromatography [EtOAc–light petroleum (1 : 1) as eluant] gave *methyl*[3-^2H]-*penicillanate* 1,1-*dioxide* (3c) (0.200 g, 73%). On the basis of n.m.r. spectroscopy, the material contained 83% deuterium at position 3 [δ ($CDCl_3$) 1.41 and 1.62 (each 3 H, s, together CMe_2), 3.50 (2 H, d, separation 3.5 Hz, 6-H_2), 3.83 (3 H, s, OMe), 4.42 (0.17 H, s, 3-H), and 4.69 (1 H, t, separation 3.5 Hz, 5-H)]. The sample, recrystallised from chloroform–ether, showed m.p. 95—99 °C, $[\alpha]_D$ +196° (1% in EtOH).

Reaction of the Benzyloxypenicillinates (1e) *and* (7e) *with Hydrogen–Palladium*.—(*a*) A stirred mixture of the benzyloxypenicillinate (1e)[1] (1.44 g, 3.64 mmol), ethyl acetate (30 cm^3), and 10% palladium–charcoal (1.50 g) was hydrogenated at 1 atmosphere until gas uptake had ceased (*ca.* 2 h). The mixture was filtered through Celite and the filtrate was evaporated to leave methyl 6β-aminopenicillanate 1,1-dioxide (1d) (0.950 g, 100%) as a chromatographically homogeneous syrup; $[\alpha]_D$ +213° (0.4% in EtOH); $\nu_{max.}$ (film) *inter alia* 3 420 (NH), 1 800 (β-lactam C=O), and 1 755 cm^{-1} (ester C=O); $\lambda_{max.}$ (EtOH) 218 (ε 2 300), 223sh (2 100), and 274 nm (600); δ ($CDCl_3$) 1.40 and 1.60 (each 3 H, s, together CMe_2), 2.38 (2 H, br s, NH_2), 3.83 (3 H, s, OMe), 4.47 (1 H, s, 3-H), and 4.70 and 4.86 (each 1 H, d, *J* 4 Hz, 5- and 6-H) (addition of D_2O

caused the signal at δ 2.38 to disappear); *m/z inter alia* 114, 82, and 64 (O_2S^+).

(*b*) The foregoing reaction was repeated with the deuteriated benzyloxypenicillinate (7e) (0.960 g, 2.41 mmol). Work-up as before gave methyl 6β-amino-[2β-*methyl*-$^2H_3$]penicillanate 1,1-dioxide (7d) (0.640 g, 100%); $[\alpha]_D$ +203° (0.6% in EtOH). On the basis of n.m.r. spectroscopy, the sample was 83% deuteriated in the 2β-methyl group [δ ($CDCl_3$) *inter alia* 1.40 (3 H, s, CMe_α) and 1.60 (0.51 H, br s, CMe_β).

Reaction of the Aminopenicillanates (1d) *and* (7d) *with Nitrous Acid.*—(*a*) A solution of sodium nitrite (0.200 g, 2.9 mmol) in water (1 cm^3) was added dropwise to a stirred solution of the aminopenicillanate (1d) (0.400 g, 1.53 mmol) in methanol (5 cm^3) and 1M-hydrochloric acid (5 cm^3, 5 mmol) at 0 °C. After 15 min the mixture was extracted with ethyl acetate. After being washed with sodium hydrogen carbonate solution and brine, the organic layer was dried ($MgSO_4$) and evaporated. Purification of the product by silica-gel chromatography [EtOAc–light petroleum (1 : 1) as eluant] gave methyl 6-diazopenicillanate 1,1-dioxide (15b) (0.210 g, 50%) as a slightly impure pale-yellow syrup; $[\alpha]_D$ +60° (0.8% in EtOH); $\nu_{max.}$ (film) *inter alia* 2 120 (C=$\overset{+}{N}$=$\overset{-}{N}$) and 1 760br cm^{-1} (β-lactam and ester C=O); δ ($CDCl_3$) *inter alia* 1.39 and 1.58 (each 3 H, s, together CMe_2), 3.77 (3 H, s, OMe), 4.18 (1 H, s, 3-H), and 5.43 (1 H, s, 5-H); *m/z inter alia* 226 and 166 (base peak).

(*b*) The foregoing reaction was repeated using the deuteriated aminopenicillanate (7d) (0.220 g, 0.83 mmol). Work-up as before, but omitting the chromatographic step, gave methyl 6-diazo[2β-*methyl*-$^2H_3$]penicillanate 1,1-dioxide (15a) (0.160 g) as a somewhat impure syrup. On the basis of n.m.r. spectroscopy, the sample was *ca.* 83% deuteriated in the 2β-methyl group [δ ($CDCl_3$) *inter alia* 1.39 (3 H, s, CMe_α) and 1.58 (0.5 H, br s, CMe_β)].

Reaction of the Diazopenicillanates (15b) *and* (15a) *with Hydrochloric Acid.*—(*a*) To a vigorously stirred solution of the diazopenicillanate (15b) (0.140 g, 0.51 mmol) in dichloromethane (3 cm^3) was added 6M-hydrochloric acid (0.5 cm^3). After 6 h the mixture was diluted with dichloromethane and washed with sodium hydrogen carbonate solution. Evaporation of the dried ($MgSO_4$) organic layer gave a crystalline residue (0.130 g, 90%) that was identical (n.m.r. spectroscopy) with methyl 6α-chloropenicillanate 1,1-dioxide (1f) [1] [δ ($CDCl_3$) 1.45 and 1.65 (each 3 H, s, together CMe_2), 3.85 (3 H, s, OMe), 4.43 (1 H, s, 3-H), and 4.65 and 5.15 (each 1 H, d, *J* 2 Hz, together 5- and 6-H)].

(*b*) The foregoing reaction was repeated with the crude deuteriated diazopenicillanate (15a) (0.100 g, *ca.* 0.36 mmol). Work-up as before gave methyl 6α-chloro[2β-*methyl*-$^2H_3$]-penicillanate 1,1-dioxide (7c) (0.090 g, *ca.* 87%). On the basis of n.m.r. spectroscopy, the sample contained 82% deuterium in the 2β-methyl group [δ ($CDCl_3$) *inter alia* 1.43 (3 H, s, CMe_α) and 1.64 (0.55 H, br s, CMe_β)].

Reaction of the Chloropenicillanates (1f) *and* (7c) *with Zinc–Acetic Acid.*—(*a*) A solution of the chloropenicillanate (1f) (0.130 g, 0.46 mmol) in acetic acid (1 cm^3) was added to a stirred suspension of zinc powder (1.00 g) in acetic acid (2 cm^3). After 2.5 h the mixture was diluted with dichloromethane and filtered. After being washed with water and sodium hydrogen carbonate solution, the filtrate was dried ($MgSO_4$) and evaporated to leave a crystalline residue (0.110 g, 96%) that was identical with the penicillanate (1c) (n.m.r. spectroscopy).

(*b*) The foregoing reaction was repeated with the deuteriated chloropenicillanate (7c) (0.100 g, 0.35 mmol). Work-up as before gave methyl [2β-*methyl*-$^2H_3$]penicillanate 1,1-dioxide (7b) (0.080 g, 91%). On the basis of n.m.r. spectroscopy, the sample contained 80% deuterium in the 2β-methyl group [δ ($CDCl_3$) *inter alia* 1.46 (3 H, s, CMe_α) and 1.76 (0.6 H, br, s, CMe_β)].

Reaction of the Benzyloxypenicillinate (8b) *with Sodium Periodate.*—To a stirred solution of the penicillinate (8b) [1] (10.0 g, 27.5 mmol) in methanol (300 cm^3) was added a solution of sodium periodate (23.5 g, 109.8 mmol) in water (300 cm^3). After 4 h the mixture was diluted with dichloromethane and washed with water. Evaporation of the dried ($MgSO_4$) organic layer gave *methyl benzyloxypenicillinate* 1β-*oxide* (9b) (8.40 g, 80%), m.p. 146—148 °C (from $CHCl_3$–Et_2O); $[\alpha]_D$ +188° (0.2% in EtOH); $\nu_{max.}$ (KBr) *inter alia* 3 400 (NH), 1 790 (β-lactam C=O), 1 750 (ester C=O), and 1 725 cm^{-1} (urethane C=O); $\lambda_{max.}$ (EtOH) 211 nm (ε 7 800); δ ($CDCl_3$) 1.20 and 1.67 (each 3 H, s, together CMe_2), 3.77 (3 H, s, OMe), 4.61 (1 H, s, 3-H), 4.98 (1 H, d, *J* 4.5 Hz, 5-H), 5.07 (2 H, s, PhCH_2O), 5.69 (1 H, dd, *J* 10 and 4.5 Hz, 6-H), 6.37 (1 H, br d, *J* 10 Hz, NH), and 7.28 (5 H, s, Ph) [addition of D_2O caused the signal at δ 6.37 to disappear and that at δ 5.69 to collapse to a d (*J* 4.5 Hz)]; *m/z inter alia* 380 (M^+), 362 ($M^+ - H_2O$), and 91 (C_7H_7, base peak) (Found: C, 53.4; H, 5.15; N, 7.25. $C_{17}H_{20}N_2O_6S$ requires C, 53.7; H, 5.25; N, 7.35%).

Thermolysis of the Sulphoxide (9b) *in the Presence of Deuterium Oxide.*—A solution of the sulphoxide (9b) (8.10 g, 21.3 mmol) in a mixture of dry benzene (400 cm^3) and deuterium oxide (3 cm^3) was heated at just below reflux. From time to time (*ca.* every day), the deuterium oxide was replaced with a fresh sample. After 4 d the mixture was washed with water, dried ($MgSO_4$), and evaporated to give *methyl benzyloxy*-[2β-methyl-$^2H_3$]*penicillinate* 1β-*oxide* (11b) (8.10 g, 100%). On the basis of n.m.r. spectroscopy, the material was 85% deuteriated in the 2β-methyl group [δ ($CDCl_3$) 1.20 (3 H, s, CMe_α) and 1.67 (0.44 H, br s, CMe_β)]. A sample, recrystallised from dichloromethane–ether, showed m.p. 138—139 °C, $[\alpha]_D$ +184° (1% in EtOH).

Reaction of the Sulphoxide (9b) *with Potassium Permanganate.*—(*a*) A solution of potassium permanganate (0.091 g, 0.38 mmol) in water (5 cm^3) was added during 1 h to a stirred ice-cooled solution of the sulphoxide (9b) (0.197 g, 0.52 mmol) in 4 : 1 acetic acid–water (15 cm^3). After a further 1 h the colour of the mixture was discharged by the passage of sulphur dioxide. The mixture was extracted with dichloromethane which was then washed in turn with water (2 ×) and sodium hydrogen carbonate solution. Evaporation of the dried ($MgSO_4$) organic layer gave methyl benzyloxypenicillinate 1,1-dioxide (1e) [1] (0.179 g, 87%) [δ ($CDCl_3$) 1.36 and 1.59 (each 3 H, s, together CMe_2), 3.81 (3 H, s, OMe), 4.48 (1 H, s, 3-H), 4.25 (1 H, d, *J* 4 Hz, 5-H), 5.13 (2 H, s, PhCH_2O), 5.82 (1 H, dd, *J* 11 and 4 Hz, 6-H), 6.15 (1 H, br d, *J* 11 Hz, NH), and 7.33 (5 H, s, Ph)]. The sample, recrystallised from chloroform–ether, showed m.p. 178—182 °C [lit.,[1] 184—186 °C].

(*b*) A solution of potassium permanganate (0.630 g, 3.99 mmol) in water (20 cm^3) was added during 1 h to a stirred ice-cooled solution of the deuteriated sulphoxide (11b) (1.40 g, 3.66 mmol) in 4 : 1 acetic acid–water (50 cm^3). Work-up after 1 h, as described in (*a*), gave *methyl benzyloxy*[2β-methyl-$^2H_3$]-*penicillinate* 1,1-*dioxide* (7e) (1.10 g, 75%). On the basis of n.m.r. spectroscopy, the material contained 84% deuterium in the 2β-methyl group [δ ($CDCl_3$) 1.35 (3 H, s, CMe_α) and 1.56 (0.48 H, br s, CMe_β)]. The sample, after recrystallisation from ethyl acetate–light petroleum, showed m.p. 191—193 °C

(decomp.) [lit.,[1] for (1e) 184—186 °C]; $[\alpha]_D$ +139° (1% in EtOH) {lit.,[1] for (1e) $[\alpha]_D$ +147° (EtOH)}.

Reaction of the Deuteriated Aminopenicillanate (7d) *with Phenylacetyl Chloride*.—To a stirred solution of the deuteriated aminopenicillanate (7d) (0.120 g, 0.45 mmol) in dichloromethane (2 cm^3) was added triethylamine (0.045 g, 0.45 mmol) and phenylacetyl chloride (0.077 g, 0.5 mmol). After 2 h the mixture was diluted with dichloromethane and washed with sodium hydrogen carbonate solution. Evaporation of the dried ($MgSO_4$) organic layer and purification of the product by silica-gel chromatogrpahy [EtOAc–light petroleum (1 : 2) as eluant] gave the deuteriated penicillinate (7a) (0.130 g, 75%) as a crystalline solid. On the basis of n.m.r. spectroscopy, the sample was 83% deuteriated in the 2β-methyl group [δ ($CDCl_3$) *inter alia* 1.36 (3 H, s, CMe_α) and 1.58 (0.51 H, br s, CMe_β)].

Reaction of the Sulphone (13b) *with DBN–Deuterium Oxide*.—A 20% solution of DBN in deuteriochloroform was added dropwise to a solution of the sulphone (13b) [1] (0.370 g, 1 mmol) in a mixture of deuteriochloroform (0.8 cm^3) and deuterium oxide (0.2 cm^3). When the exchange of the 3-proton was complete (n.m.r. spectroscopy), the mixture was diluted with dichloromethane and washed in turn with dilute hydrochloric acid and sodium hydrogen carbonate solution. Evaporation of the dried ($MgSO_4$) organic layer and purification of the product by silica-gel chromatography [EtOAc–light petroleum (1 : 2) as eluant] gave p-*nitrobenzyl*[3-^{2}H]*penicillanate* 1,1-*dioxide* (18b) (0.360 g, 98%). On the basis of n.m.r. spectroscopy, the material was 90% deuteriated at position 3 [δ ($CDCl_3$) 1.31 and 1.57 (each 3 H, s, together CMe_2), 3.48 (2 H, d, separation 4 Hz, 6-H_2), 4.45 (0.1 H, s, 3-H), 4.63 (1 H, t, separation 4 Hz, 5-H), 5.32 (2 H, s, OCH_2), and 7.55 and 8.50 (each 2 H, d, *J* 8 Hz, together C_6H_4)]. The sample, recrystallised from ethyl acetate–light petroleum, showed m.p. 144—146 °C (decomp.) [lit.,[1] for (13b) 149—151 °C]; $[\alpha]_D$ +147° (1% in CH_2Cl_2) {(13b) showed $[\alpha]_D$ +158° (1% in $CHCl_2$)}.

Reaction of the p-*Nitrobenzyl Penicillanate* (18b) *with Hydrogen–Palladium*.—A stirred mixture of the *p*-nitrobenzyl penicillanate (18b) (0.106 g, 0.29 mmol), ethyl acetate (5 cm^3), and 10% palladium–chracoal (0.200 g) was hydrogenated at 1 atmosphere until gas uptake had ceased (*ca.* 1 h). The mixture was filtered through Celite and the filtrate was washed in turn with dilute hydrochloric acid and brine. Evaporation of the dried ($MgSO_4$) organic layer gave [3-^{2}H]*penicillanic acid* 1,1-*dioxide* (18a) (0.064 g, 95%). On the basis of n.m.r. spectroscopy, the material was 90% deuteriated in the 3-position [δ ($CDCl_3$–CD_3SOCD_3) 1.43 and 1.56 (each 3 H, s, together CMe_2), 3.30 (1 H, dd, *J* 16 and 2 Hz, 6β-H), 3.65 (1 H, dd, *J* 16 and 4.5 Hz, 6α-H), 4.27 (0.1 H, s, 3-H), 4.87 (1 H, dd, *J* 4.5 and 2 Hz, 5-H), and 8.90 (1 H, br s, CO_2H)]. The sample, recrystallised from ethyl acetate–chloroform, showed m.p. 150—163 °C (decomp.) [lit.,[11] for (17a) 148 °C (decomp.)]; $[\alpha]_D$ +263° (0.2% in EtOH) {(17a) showed $[\alpha]_D$ +264° (0.2% in EtOH)}.

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