DETERMINATION OF THE STEREOCHEMISTRY OF TRITIUM LABEL IN SULBACTAM OBTAINED BY ISOTOPE EXCHANGE OF PENICILLANIC ACID (R)-SULPHOXIDE BENZYL ESTER

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SUMMARY

Sulbactam, a β-lactamase inhibitor, has been prepared in tritiated (170 mCi $mmol^{-1}$) form by the highly stereospecific isotope exchange procedure involving penicillanic acid (R)-sulphoxide benzyl ester. The stereochemistry of the tritiation has been established by ^3H n.m.r. in conjunction with a ^1H NOESY spectrum of sulbactam.

**Key words:** Sulbactam, tritiation, hydrogen isotope exchange

INTRODUCTION

In the treatment of clinical infections the prevalence of resistant β-lactamase producing bacteria continues to be of major concern.[1-3] Several approaches have been taken to develop antimicrobial agents that can overcome this resistance, including drugs that act primarily as β-lactamase inhibitors. Sulbactam (1), a semi-synthetic penicillanic acid sulphone is a β-lactamase inhibitor that when combined with other β-lactam antibiotics can greatly enhance the antibacterial activity.

(1)
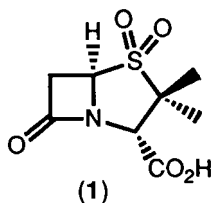
In our search for a suitably radiolabelled form of sulbactam (1) for tracer studies, the possibility of carrying out a catalytic dehalogenation[4] on 6,6-dibromopenicillic acid was considered, but rejected, on the grounds that some of the label at C-6 would be lost following lactam ring opening when sulbactam (1) inactivates the lactamase enzyme.[6] A more attractive alternative, which should lead to specific labelling in the C-2 methyl position(s) was suggested by the findings of Cooper[5] and Knowles[6]. Thus, Cooper *et al.* found that heating (*ca* 80°C) a solution of the penicillic sulphoxide methyl ester (β-sulphoxide)(2a) in benzene containing a large excess of D_2O for 24 h gave a product in which the deuterium was located only in the β-methyl group.[5] Similar treatment of phthalimidopenicillin α-sulphoxide ester (2b) (α-sulphoxide thermodynamically more stable than the β-isomer[7]) gave deuterium incorporation only in the α-methyl group, identification of the methyl signals being achieved by nuclear Overhauser effects (n.O.e)[7]- the high field methyl signal (δ 1.45) being associated with the 2α group and the low field (δ 1.54) the 2β methyl group. These results were interpreted in terms of a thermal equilibration between the sulphoxide (2) and the sulphenic acid (3) so that when it is established in the presence of D_2O exchange and consequent deuterium incorporation into the methyl group(s) can occur. All the available evidence suggests that no isomerization takes place.[6]

Based on the above observations, Knowles et al. found that similar treatment of the penicillanic acid β-sulphoxide benzyl ester (2c) resulted in the incorporation of deuterium in the 2β methyl group.[5] In this case the site of

(2a) R^1 = PhOCH$_2$CONH, R^2 = Me   (3)

(2b) R^1 =

(2c) R^1 = H, R^2 = CH$_2$C$_6$H$_5$
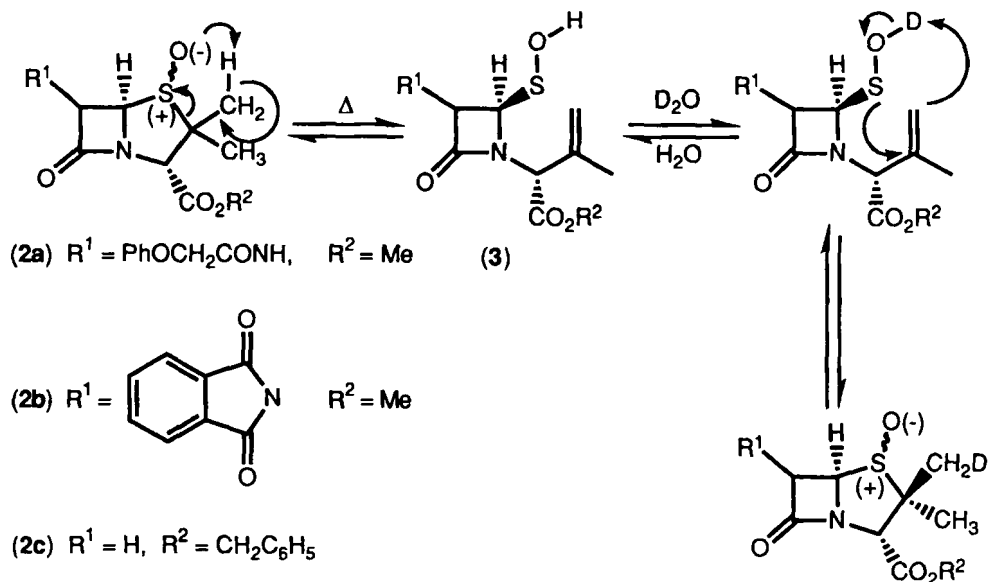
labelling was established by ^{1}H n.m.r. which showed a diminished intensity in the signal for the deshielded methyl group (δ1.32 in C$_6$D$_6$). These studies were then extended to the tritiation of the penicillanic acid β-sulphoxide benzyl ester (2c) *via* heating in the presence of HTO at 100°C. The resulting tritiated sulphoxide (5) was then converted to the *penicillanic acid sulphone* (7) by oxidation to the sulphone (6) and then debenzylation. However, the exact position and stereochemistry of the tritium label was not established in the above study. Nevertheless, the fact that the tritium label was localized in the penicillamine sulphinate portion was determined by degradation studies involving the formation of the 2,4-dinitrophenylhydrazone of acetaldehyde after base treatment of the sulphone (7).

Based on the above mechanism of labelling the incorporation of the label in the methyl position(s) and subsequent oxidation to the sulphone ensures that it is unlikely that the tritium label will be lost during subsequent tracer studies. In addition, the utilization of ^{3}H n.m.r.[8] to study the labelled material offers the potential to determine both the site and the

stereospecificity of the labelling (*vide infra*). Thus, [3]H n.m.r. spectroscopy would provide a more direct method of determining the position of the ^{3}H label than was available in the earlier study[6] of Knowles et al. Furthermore, the availability of the α-sulphoxide (4)[9] suggested the following synthetic scheme, based on equilibration to the thermodynamically more stable β-sulphoxide followed by an electrocyclic ring opening, exchange and then ring closure to the β-sulphoxide (5):-
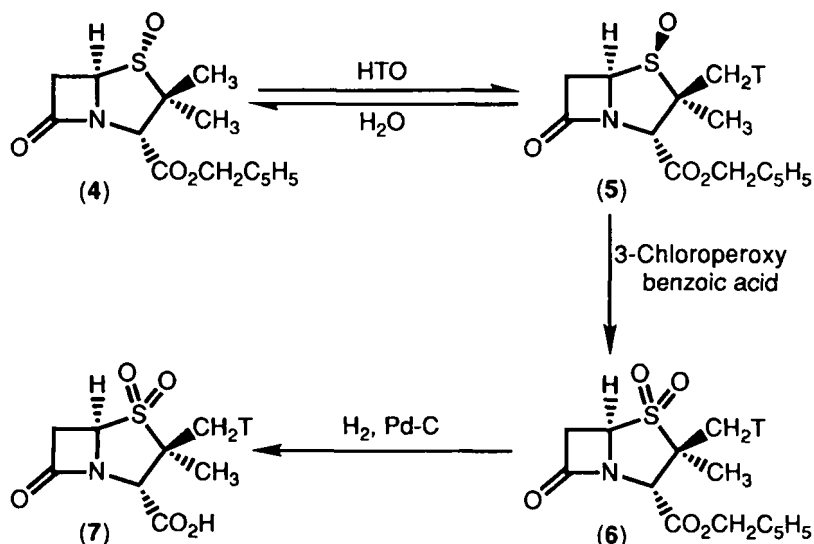
EXPERIMENTAL

Tritiation of α-sulphoxide (4)

100 mg of α-sulphoxide (4) was placed in a thick walled glass tube and sufficient toluene to dissolve it. Tritiated water (50 Ci ml^{-1}, 3μl) was added and the tube evacuated and flame sealed. The tube was then kept at 90°C for 12 h. After cooling the contents were extracted into toluene, washed with water, dried over anhydrous sodium sulphate and the solvent removed by freeze drying. Some 94 mg of the labelled compound (5), total activity 32 mCi was recovered. [1]H and [3]H n.m.r. spectroscopy confirmed that *the compound had not decomposed and that it was labelled in one of the methyl groups.*

Oxidation of tritiated β-sulphoxide (5)

94 mg of tritiated β-sulphoxide (5) was dissolved in chloroform (1 ml) and cooled to 0°C in an ice bath prior to adding a solution of 3-chloroperoxybenzoic acid (80 mg) in chloroform (1 ml). The mixture was stirred for 15 min at 0°C, allowed to warm to room temperature and stirred for an additional 4 h. The solvent was evaporated under reduced pressure and ethyl acetate (2 ml) added to the residue. This solution was then washed with three successive portions of saturated aqueous sodium bicarbonate solution (2 ml), then with distilled water (2 ml) and finally with saturated aqueous sodium chloride solution (2 ml) before being dried over anhydrous magnesium sulphate. Evaporation of the solvent provided the sulphone (6) as an oil. This compound is considerably less polar ($R_f \approx 0.8$) on silica gel thin layer, eluted with 2:1 ethyl acetate:hexane than is the starting sulphoxide (5)($R_f \approx 0.2$). The crude product also contains some 3-chlorobenzoic acid ($R_f \approx 0.3$). After column chromatography using the above solvent system and removal of the solvent some 34 mg of crude sulphone (6) was obtained at a total activity of 12 mCi.

Debenzylation of sulphone (6)

Compound (6) (34 mg.) was combined with acetone (6.6 ml), water (3.4 ml) and 10% palladium on charcoal catalyst (100 mg) and the mixture shaken in a Parr hydrogenation apparatus under 60 psi of hydrogen for 30 min. At the end of this period the catalyst was removed by filtration and the organic solvent evaporated under reduced pressure. The aqueous residue was then extracted with successive portions of ethyl acetate. The extracts were combined and dried over anhydrous sodium sulphate before removal of the solvent under reduced pressure. Some 18 mg of product (7) with a radiochemical purity of >98% (as determined by radio-t.l.c. and ^{3}H n.m.r.) and a total radioactivity of 7 mCi was obtained.

^{3}H n.m.r Analysis

^{1}H and ^{3}H n.m.r. spectra were recorded at 300.13 MHz and 320.13 MHz, respectively, on a Bruker AC-300 n.m.r. spectrometer fitted with a 5 mm dual ^{1}H/^{3}H probe and computer switchable selective amplifiers.

Approximately 100 μl of a d_6-DMSO solution of the tritiated sulbactam (7) together with the internal reference (tetramethylsilane, TMS) were injected into a 3 mm microsample tube (Wilmad SK-1374B). The sample was frozen in liquid nitrogen, the tube evacuated and sealed just below the filling extension. The tube was fitted with PTFE spacer rings and inserted into a standard 5 mm n.m.r. tube, closed with a serum cap (Wilmad 521-5). The annular space round the inner tube was filled with CCl_4. Such doubly enclosed sample offers the maximum degree of safety.

Spectra were acquired with an automated routine which collected ^{1}H and ^{3}H data under standard conditions and stored the free induction decay without user intervention. Data were collected into 32K of 24 bit words (including zero filling) using 30 degree flip angle pulses and acquisition times of 3.4 seconds and 1.6 seconds for ^{1}H and ^{3}H, respectively, with spectral widths of 15 ppm and 12 ppm.

RESULTS AND DISCUSSION

The tritiated sulbactam (7) had a specific activity of 170 mCi mmol^{-1}.

The ^{1}H n.m.r. spectrum of sulbactam (7) is given in Figure 1a; the corresponding ^{3}H n.m.r. spectrum (^{1}H decoupled) shows that only one of the methyl groups has been tritiated (Figure 1b). A ^{1}H NOESY spectrum showed an n.O.e. between the singlet at δ3.77 due to the H-3β proton and the methyl singlet at δ1.46 which was assigned to the C-2β methyl. This latter chemical shift is the same as in the ^{3}H n.m.r. spectrum, consequently, the tritium has been introduced stereospecifically into this group. It will be interesting to see whether such stereospecificity is retained in structurally
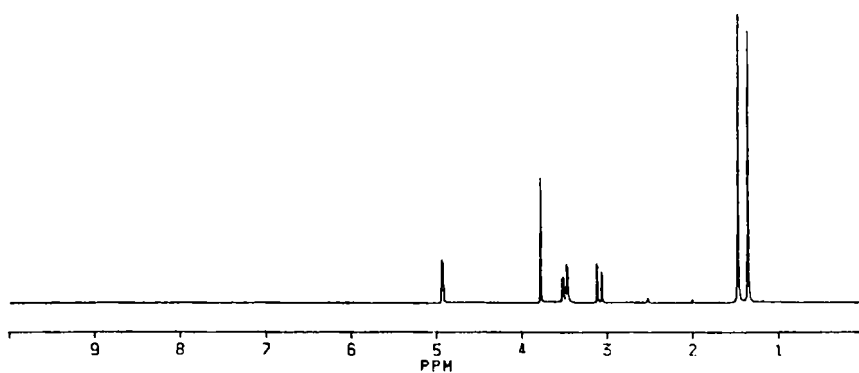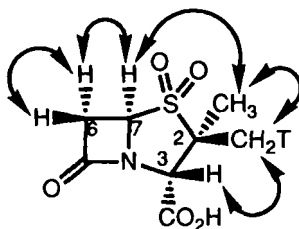
(a) [1]H n.m.r. Spectrum of Tritiated Sulbactam (7)
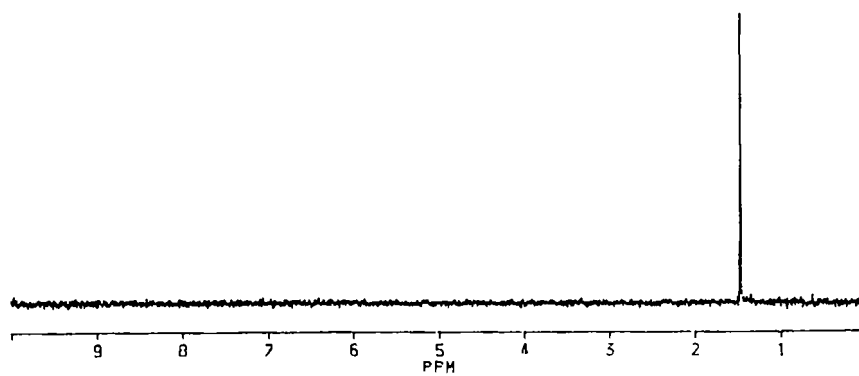
(b) [3]H n.m.r ([1]H Decoupled)

FIGURE 1

modified compounds. An n.O.e was also observed between the double doublet at $\delta 4.91$ due to H-7α and the methyl singlet at $\delta 1.34$, so that the latter can be assigned to the C-2α methyl. Other observed n.O.e's are indicated with the connectivities shown.

ACKNOWLEDGEMENTS

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