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# COMMUNICATIONS

## Assignment of the C-5 configuration of penicilloic acids

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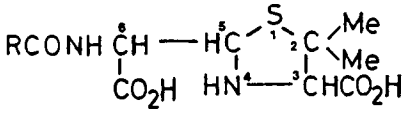
**Abstract**—Penicilloates prepared by the method of Munro et al (1978) were examined by nuclear magnetic resonance spectroscopy and optical rotation to assign the configuration at C-5. The configuration was 5*S* for carbenicillin and ticarcillin penicilloates and 5*R* for the other seven penicilloates examined. The chemical shift and coupling constants of 5-H and 6-H showed consistent differences between the isomers and the optical rotation of the 5*R*-isomers is consistently more positive than that of the corresponding 5*S*-isomer.

The epimerization at C-5 of penicilloic acids (I) in aqueous solution is well documented (Bird et al 1983; Branch et al 1987; Busson et al 1976; Carroll et al 1977; Degelaen et al 1979; Fong et al 1983; Ghebre-Sellassie et al 1984; Haginaka & Wakai 1985).



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Munro et al (1978) described the preparation of the penicilloates of eleven penicillins but the C-5 stereochemistry of the products was not assigned. Bird et al (1983) have shown that the penicilloates of amoxycillin and ampicillin prepared by this procedure have the 5*R*, 6*R* stereochemistry of the natural penicillins. However, Fong et al (1983) incorrectly assigned the 5*S*, 6*R* configuration, (the *β*-isomer in the terminology they used), to the amoxycillin penicilloic acid prepared in this way and stated (without any evidence) that epimerization occurs at C-6.

Recent work (Everett et al 1984, 1985; Haginaka & Wakai 1987) has shown that both C-5 diastereoisomers of the penicilloates of ampicillin, flucloxacillin and amoxycillin, are found as metabolites after administration of the penicillins to animals. The availability of reference samples of penicilloates of known stereochemistry is important for work of this type and for the identification of penicilloates formed as degradation products in stability studies of penicillins. Consequently the stereochemistry of the other nine penicilloates prepared by Munro et al (1978) has been assigned using $^1$H NMR and optical rotation measurements. Rotation data has also been obtained for amoxycillin and ampicillin penicilloic acids.

### Materials and methods

*Materials.* Deuterium oxide and sodium deuteroxide (30% w/w in deuterium oxide) were supplied by Fluorochem Ltd. The penicilloic acid or penicilloate samples were prepared by the method of Munro et al (1978). The penicillins were Beecham commercial samples.

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*Nuclear magnetic resonance (NMR) spectra.* $^1$H spectra were obtained on a Perkin Elmer R32 90MHz spectrometer at a probe temperature of about 35°C. A solution of tetramethylsilane, (TMS), in $CDCl_3$ in a sealed capillary tube was used to provide a lock and external reference signal. Chemical shifts are quoted downfield of internal DSS and were obtained by subtraction of 0·14 ppm from the measured shifts. This correction factor was determined from spectra of a sample run with internal DSS and with the external TMS reference.

Spectra were obtained on solutions containing about 20 mg of penicilloate or penicillin in 0·5 mL $D_2O$ adjusted to pD about 12 with NaOD. Spectra were obtained immediately after preparation of the solution and at intervals until no further significant change occurred.

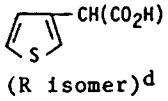
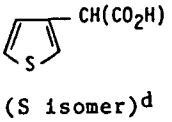
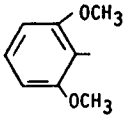
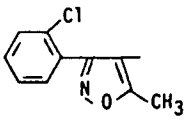
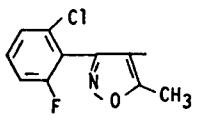
*Optical rotation.* Optical rotation was measured on a Perkin Elmer 141 polarimeter at 589 nm and 20°C using a 10 cm path length cell.

Solutions containing about 25 mg penicilloate in 1·5 mL $D_2O$ adjusted to pD about 12 with NaOD were used. The rotation was recorded immediately after sample preparation and at intervals until no further significant change occurred.

### Results and discussion

The initial NMR spectra of all the penicilloate samples gave resonances indicating the presence of a single penicilloate isomer at C5. For all the samples except carbenicillin and ticarcillin penicilloates these resonances occurred at the same chemical shift as the resonance seen in the initial spectra of the corresponding penicillin samples which were hydrolysed in-situ to pencilloates by addition of NaOD. This confirms that these isolated penicilloates have the 3*S*, 5*R*, 6*R* configuration of the parent penicillins. Repeat spectra as the solutions aged showed a decrease in the initial penicilloate signals and progressive increase of a new set of signals. These spectra showed no deuterium exchange of the protons at positions 3, 5 or 6, so the spectral change is assigned to isomerization at C-5 by cleavage and re-formation of the C-S bond, as occurs with other penicilloates (Bird et al 1983; Ghebre-Sellassie et al 1984). The spectra of the carbenicillin and ticarcillin penicilloate samples were different from those obtained from carbenicillin or ticarcillin hydrolysed in-situ and did not change significantly as the solutions aged. However, the spectra of the solutions obtained by in-situ hydrolysis did change as the solutions aged, with signals appearing at the same chemical shifts as were found in the spectra of the carbenicillin or ticarcillin penicilloate samples. Thus the carbenicillin and ticarcillin penicilloate samples have the 5*S* configuration. The spectra of the in-situ hydrolysed penicillins showed that conversion of the 5*R* penicilloate to the 5*S* isomer occurred much more rapidly for carbenicillin and ticarcillin than for the other penicillins. This is consistent with the isolated penicilloates having the 5*S* configuration because a

Table 1. $^1$H chemical shifts and specific optical rotation of sodium penicilloates.

| Parent penicillin | R in I | C-5[c] | Chemical shift, ppm[a] C-2 methyls | 3-H | 5-H and 6-H | Specific optical rotation[b] Initial | After 24 h |
|---|---|---|---|---|---|---|---|
| Benzyl | $C_6H_5CH_2$ | *R* | 1·5, 1·2 | 3·4 | 5·0, 4·2 | +127 | +61 |
| | | *S* | 1·5, 1·0 | 3·4 | 5·0, 4·7 | | |
| Phenoxymethyl | $C_6H_5OCH_2$ | *R* | 1·5, 1·2 | 3·4 | 5·0, 4·3 | +115 | +79[e] |
| | | *S* | 1·6, 1·1 | 3·4 | 5·1, 4·8 | | |
| Phenethicillin | $C_6H_5OCH(CH_3)$ (*R* isomer)[d] | *R* | 1·4, 1·2 | 3·2 | 5·1, 4·2 | +116 | +77 |
| | | *S* | 1·5, 0·8 | 3·3 | 5·0, 4·7 | | |
| Phenethicillin | $C_6H_5OCH(CH_3)$ (*S* isomer)[d] | *R* | 1·5, 1·2 | 3·5 | 5·1, 4·2 | | |
| | | *S* | 1·6, 1·2 | 3·4 | 5·1, 4·7 | | |
| Propicillin | $C_6H_5OCH(C_2H_5)$ (*R* isomer)[d] | *R* | 1·4, 1·2 | 3·3 | 5·0, 4·2 | +96 | +70 |
| | | *S* | 1·5, 0·8 | 3·3 | 5·0, 4·7 | | |
| Propicillin | $C_6H_5OCH(C_2H_5)$ (*S* isomer)[d] | *R* | 1·5, 1·2 | 3·5 | 5·1, 4·2 | | |
| | | *S* | 1·6, 1·3 | 3·4 | 5·1, 4·7 | | |
| Carbenicillin | $C_6H_5CH(CO_2H)$ (*R* isomer)[d] | *R* | 1·2, 1·2 | 3·4 | 5·0, 4·2 | +1 | +9 |
| | | *S* | 1·5, 1·0 | 3·4 | 5·0, 4·7 | | |
| Carbenicillin | $C_6H_5CH(CO_2H)$ (*S* isomer)[d] | *R* | 1·5, 1·4 | 3·4 | 5·0, 4·2 | | |
| | | *S* | 1·5, 1·1 | 3·4 | 5·0, 4·8 | | |
| Ticarcillin |  (R isomer)[d] | *R* | 1·2, 1·2 | 3·4 | 5·0, 4·1 | — | — |
| | | *S* | 1·5, 1·0 | 3·4 | 5·0, 4·7 | | |
| Ticarcillin |  (S isomer)[d] | *R* | 1·5, 1·4 | 3·4 | 5·1, 4·2 | | |
| | | *S* | 1·5, 1·1 | 3·4 | 5·0, 4·7 | | |
| Methicillin |  | *R* | 1·6, 1·2 | 3·6 | 5·0, 4·4 | +102 | +46[f] |
| | | *S* | 1·6, 1·2 | 3·4 | 5·1, 5·0 | | |
| Cloxacillin |  | *R* | 1·5, 1·2 | 3·3 | 4·8, 4·2 | +80 | +48 |
| | | *S* | 1·5, 1·1 | 3·3 | 4·9, 4·7 | | |
| Flucloxacillin |  | *R* | 1·5, 1·2 | 3·3 | 4·9, 4·2 | +73 | +46 |
| | | *S* | 1·5, 1·1 | 3·3 | 4·9, 4·7 | | |

(a) Configuration. Shifts for *R*-isomer from spectra of fresh solutions and for S-isomer from additional lines in spectra of aged solutions. Identical shifts were obtained from the penicilloate samples and from the penicillins hydrolysed in-situ, except for carbenicillin and ticarcillin where the shifts are from the in-situ hydrolysis spectra. (b) All C-2 methyl and 3-H signals are singlets; all 5-H and 6-H signals are AB quartets with J 5 to 7Hz for *R*-isomers and about 3Hz for *S*-isomers. (c) Rotation of the penicilloate samples. The penicilloates of phenethicillin, propicillin and carbenicillin contained a mixture of the side-chain isomers. The penicilloates of amoxycillin and ampicillin gave rotations of 93 and 90 initially and 1 and 10 after 24 h, respectively. (d) Configuration at side-chain asymmetric centre, assigned after Bird et al (1982). (e) After 8·5 h. (f) After 72 h.

constant hydrolysis time was used in the preparative work for all the penicillins (Munro et al 1978).

Relevant chemical shifts of the penicilloate isomers are given in Table 1. These show one of the 5-H or 6-H resonances to be of similar shift in the two isomers, but the other signal is consistently about 0·5 ppm upfield in the 5*R*-isomers of its position in the 5*S*-isomers, with the *R*- and *S*-isomer shifts occurring in narrow ranges close to 4·2 and 4·7 ppm respectively.

The coupling constant $J_{5,6}$ was consistently about 3Hz for the *S*-isomers and 5 to 7Hz for the *R*-isomers. These results are consistent with those reported previously for ampicillin and amoxycillin penicilloates (Bird et al 1983) and provide a useful diagnostic tool for the C-5 configuration of penicilloates. The 3-H and C-2 methyl shifts show no consistent pattern between the 5*R*- and 5*S*-isomers. For 3-H this is contrary to the suggestion of Claes et al (1982) that this shift should be downfield in 5*R*-isomers compared with the 5*S*-isomers. The separation between the shifts of the two C-2 methyls is significantly lower in the 5*R*-isomers than in the 5*S*-isomers for most of the penicilloates but those of methicillin and the side-chain *S* isomers of phenethicillin and propicillin are exceptions.

Optical rotation measurements (see Table 1) showed a decrease with time for all the pencilloates except that from carbenicillin, which showed a small increase with time. This change is consistent with the suggestion of Claes et al (1982) that 5*R*-penicilloates have a more positive rotation than their 5*S*-isomers and the result obtained for carbenicillin penicilloate provides further confirmation that this sample is the 5*S*-isomer. Insufficient sample was available to obtain rotation measurements on the ticarcillin penicilloate sample. However, the very low value of $-22{\cdot}3°$ given by Munro et al (1978) is consistent with the 5*S* assignment for ticarcillin penicilloate.

We wish to thank Mr S. Woroniecki for supply of the penicilloate samples and Miss M. I. Tingley for help with optical rotation measurements.

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# Solute adsorption and concentration-dependent permeability in certain polymer films

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**Abstract**—Certain parameters (solute adsorption, permeability and swellability) of films of a cationic acrylatemethacrylate copolymer have been determined as functions of the solute (sulphacetamide sodium) concentrations. Both permeability coefficients and the free (unadsorbed fraction of solute increased almost proportionately during increase in solute concentration 1–5%, above 5%, further increase in the fraction of free solute was slight, while the permeability coefficients decreased slightly. The solute also reduced film swellability but the observed concentration-dependent permeability related more to the adsorption phenomenon.

Abdel-Aziz et al (1975) and Okor (1982) have indicated the potential of acrylatemethacrylate copolymer films in controlled release applications. Solutes permeate these films by a pore flow mechanism, (Okor & Anderson 1986a). In a preliminary report aimed at developing an ocular delivery system of sulphacetamide sodium, it was shown that the permeability coefficient versus concentration profile of the solute exhibited an initial increase followed by a decrease (Okor & Anderson 1986b). The suggestion was that electrostatic interaction of the ionic solute with cationic groups in the polymer structure caused the initial increase (the phenomenon being significant only at low solute concentrations), while the decrease was associated with the solute potential for reducing the aqueous swellability of the films. In the present report, physical adsorption of the solute by the polymer is presented as evidence for the proposed mechanism of solute-polymer electrostatic interaction.

## Materials and method

A hydrophilic, but water insoluble, acrylate methacrylate polymer containing small proportions of cationic (quaternary