The Stereochemistry of the Incorporation of the Methyl Groups of 'Chiral Methyl Valine' into Methylene Groups in Cephalosporin C

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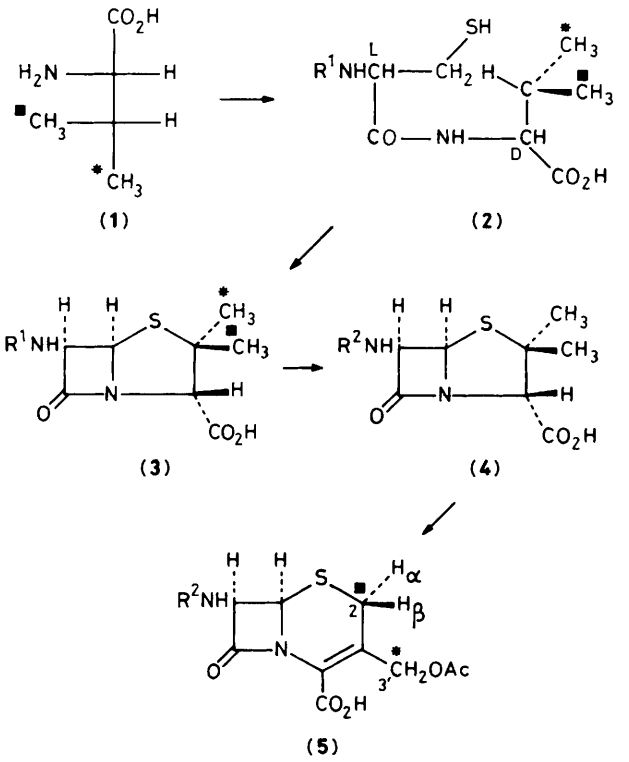
Analysis by tritium n.m.r. has indicated that the incorporation of the *pro-S* methyl group of 'chiral methyl valine' [as (**1**)] into the C-3′ exocyclic methylene group of cephalosporin C (**5**) in *Cephalosporium acremonium* occurs with at least 70% stereospecificity overall; in contrast, the tritium n.m.r. spectra obtained after the incorporation of *pro-R* methyl groups of opposite configurations into the C-2 methylene group of cephalosporin C were virtually identical, indicating that at some stage there is a common stereochemically identical intermediate.

It is generally accepted that incorporation of valine (**1**) into cephalosporin C (**5**) proceeds by way of the tripeptide (L-α-amino-δ-adipyl)-L-cysteinyl-D-valine† (**2**), isopenicillin N (**3**), and penicillin N (**4**) (Scheme 1).[1] Substitution of hydrogen by sulphur at C-3 of the original valine (**1**) residue during generation of the penam system is stereospecific and takes place with overall retention of configuration.[2] The subsequent ring expansion to the dihydrothiazine ring of the cephem system is also stereospecific, with the overall consequence that the 4-*pro-S* methyl group of valine (**1**) becomes the exocyclic methylene (C-3′) and the 4-*pro-R* methyl group becomes the endocyclic methylene group C-2 (indicated by * and ■, respectively, in Scheme 1).[2a,b,3] In order to obtain further insight into these key processes in cephalosporin C biosynthesis, we undertook an investigation of the stereochemistry of the methyl–methylene transformations.

We have described the synthesis of 'chiral methyl valine' that is labelled stereospecifically in both diastereoisotopic methyl groups.[4a,b] As the configurations of the methyl groups produced by our procedure are rigorously linked to the configuration of the prochiral isopropyl methine centre, we proposed that such labelled valines could be used directly in biosynthetic experiments.[4b] The requirement that the biosynthetic system should operate stereospecifically with respect to the transformations affecting the prochiral centre with which the related *pro*,prochiral centres are configurationally linked, is satisfied in the biosynthesis of cephalosporin C.



Scheme 1. R^1 = L-α-amino-δ-adipyl, R^2 = D-α-amino-δ-adipyl.

A sample of chiral methyl valine (**6**) having 4-*pro-S* and 4-*pro-R* methyl groups of *R* and *S* configuration, respectively,[4] was incorporated into cephalosporin C by the use of a suspension of washed cells of *Cephalosporium acremonium*.‡§ The ^1H and ^3H (broad-band proton decoupled) n.m.r. spectra of the derived cephalosporin C are shown in Figure 1(a) and (b), respectively. In the ^3H n.m.r. spectrum, the region corresponding to the exocyclic methylene group C-3′ of cephalosporin C showed a major singlet at δ 4.4 p.p.m. and a minor singlet at δ 4.6 p.p.m. An undecoupled spectrum was obtained, the signal-to-noise ratio of which only permitted observation of the signal at δ 4.4 p.p.m., which, however, remained as an apparent singlet. This signal, therefore, must have arisen from a CDT species. This result is as expected for a stereospecific conversion in which a normal deuterium isotope effect operated, leading to a predominance of the CDT species produced by cleavage of a C–H bond, over the corresponding CHT species produced by cleavage of a C–D bond. In the region corresponding to the endocyclic methylene group, singlets at δ 3.4 and 3.1 p.p.m. were observed of approximately 2:1 relative intensity.

A corresponding experiment was carried out with a valine sample (**7**) containing methyl groups with the opposite configurations [*pro-S*,(*S*), *pro-R*,(*R*)].[4]§¶ The proton undecoupled ^3H n.m.r. spectrum of the cephalosporin C produced is shown in Figure 1(c). (The broad-band proton decoupled spectrum was essentially identical.) This spectrum shows a labelling of the exocyclic methylene group of cephalosporin C complementary to that found in the first experiment, consisting of an apparent singlet, attributable to a CDT group at δ 4.6 p.p.m. (In this experiment, no related minor signal at δ 4.4 p.p.m. could be seen.) It is estimated that if any of the CHT

† α-Amino-δ-adipyl = 5-amino-5-carboxypentanoyl.

‡ Administered as an equimolar mixture of (2*S*,3*S*,4*R*)- and (2*S*,3*R*,4*S*)-[4-^2H,^3H]valine. Activity administered = 108 mCi; activity in cephalosporin C = 250 μCi.

§ Approximately 60% of all molecules were also labelled with tritium at C-3.

¶ Administered as an equimolar mixture of (2*S*,3*S*,4*S*)-, (2*R*,3*S*,4*S*)-, (2*S*,3*R*,4*R*)-, and (2*R*,3*R*,4*R*)-[4-^2H,^3H]valine. Activity administered = 1.45 Ci; activity in cephalosporin C = 1.2 mCi.

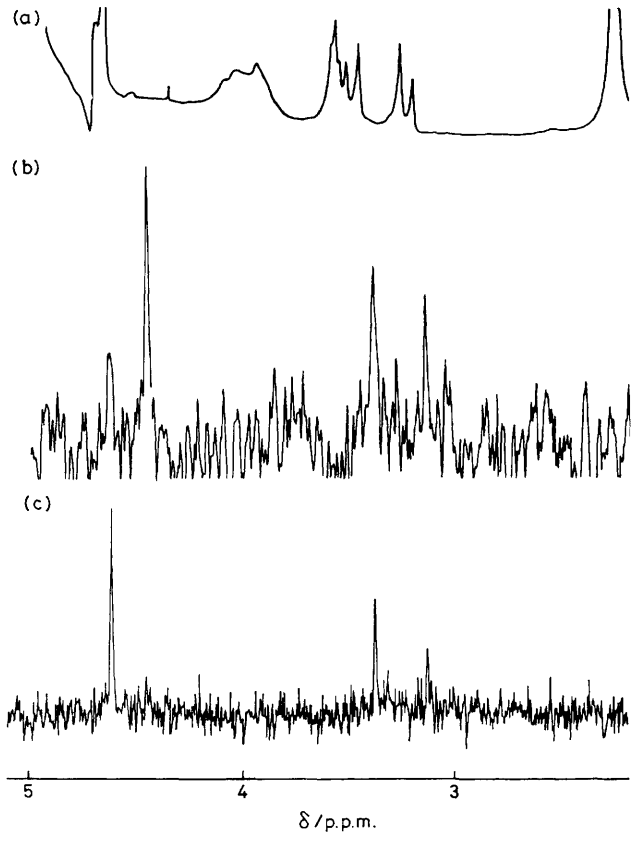
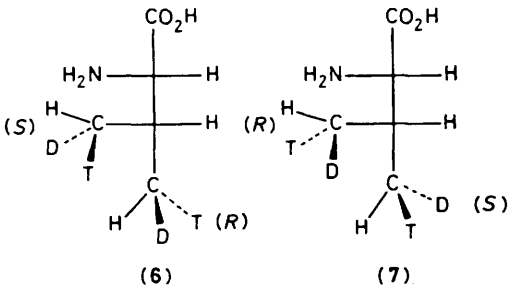


Figure 1. (a) ^1H and (b) ^3H (broad-band proton decoupled) n.m.r. spectra of cephalosporin C derived from chiral methyl valine (**6**); (c) ^3H (undecoupled) n.m.r. spectrum of cephalosporin C derived from chiral methyl valine (**7**). The ^1H n.m.r. spectrum was determined at 300 MHz, the ^3H spectra at 320 MHz for solutions of the sodium salt in D_2O.

species had been present, the corresponding doublet would have been discernible if the intensities of the components of the doublet had exceeded 20% of the intensity of the signal due to the CDT species. The results with respect to the exocyclic C-3′ methylene group are therefore entirely in accord with the expectation of a stereospecific oxidation with a stereospecificity exceeding 70%. Since the signals due to this methylene group have not been assigned, it is not possible at present to state whether this result corresponds to retention or inversion of configuration.

The two results with respect to the endocyclic methylene group were remarkable for the resemblance between the corresponding ^3H n.m.r. signals. The absence of proton coupling in the signals in the undecoupled spectra showed that the only discernible species formed were CβDαT and CαDβT. The signals from the cephalosporin C obtained after incorporation of chiral methyl valine with a *pro-R* methyl group of the *S* configuration indicated that the ratio $\alpha T:\beta T$ was not less than 1 or greater than 2.** After the incorporation of the more highly labelled *pro-R*, (*R*)-valine (**7**) the corresponding signals showed clearly that this ratio was greater than 1 but less than 2. It is thus evident that a reversal of the configuration of the valine methyl group does not result in a reversal of the relative amounts of ^3H incorporated into the α and β positions. It can be concluded therefore that the conversion of the *pro-R* chiral



methyl group of the penicillin N generated from chiral methyl valine, into the endocyclic methylene group of cephalosporin C, is accompanied by a deuterium isotope effect that is sufficiently large for the product to be formed mainly by loss of protium rather than of deuterium or tritium.†† It also follows that during the ring expansion reaction, an intermediate with the same stereochemistry is formed regardless of the configuration of the methyl groups in the precursor.

The results described above have important implications for the biochemical mechanism involved in the generation of the dihydrothiazine ring of cephalosporin C, particularly if an $\alpha T:\beta T$ ratio of up to 2:1 is confirmed by future experiments, and raise questions relating to the 'racemisation' of a valine methyl group. The present results also illustrate how the configurational linking of a *pro*,prochiral centre with a prochiral centre, coupled with the observation of a biosynthetic transformation by tritium n.m.r.,[4b] permit simultaneous observation of stereochemical events at two different centres, events that in the present study are revealed to be of a fundamentally different kind.

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** The signals due to the C-2 protons of cephalosporin C (**5**) were assigned by nuclear Overhauser effect experiments, first by L. D. Field and J. E. Baldwin (personal communication) and subsequently by us.

†† The spectrum shown in Figure 1(b) was obtained with proton decoupling. It is assumed that the isotope effects associated with C–H bond cleavage in the two incorporation experiments were essentially the same and that therefore the product formed in both incorporation experiments arose mainly by loss of protium.