# Penicillin Biosynthesis. On the Stereochemistry of Carbon–Sulphur Bond Formation with Modified Substrates

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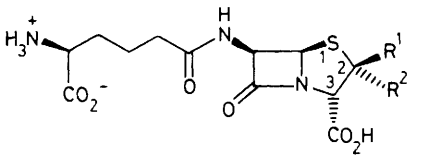
Enzymatic conversion of the two modified substrates (*S*-α-amino-δ-adipyl)-*S*-cysteinyl-(2*R*,3*R*)-3-$^2$H-α-aminobutyrate† (**2c**) and (*S*-α-amino-δ-adipyl)-*S*-cysteinyl-(2*R*,3*S*)-3-$^2$H-α-aminobutyrate† (**2d**) by the enzyme isopenicillin N synthetase gave from both precursors the same penam product, namely (2*S*)-2-deuterio-norisopenicillin N, indicating that this enzyme is capable of forming carbon–sulphur bonds by retention and also inversion pathways respectively.

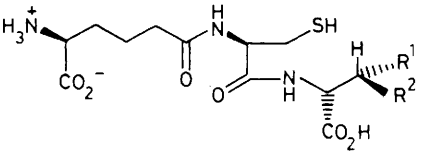
Formation of the carbon–sulphur bond at C(2) during the biosynthesis of isopenicillin N (**1a**) from the natural precursor (**2a**) has been shown to proceed with retention of configuration,[1] a result which was suggested to be in accord with a proposed radical cyclisation mechanism, Scheme 1, in which case ring closure was faster than rotation of the tert.-radical.[2] We argued that by reducing the substitution at the postulated radical, from tertiary to secondary, it might be then possible that rotation would compete with cyclisation and provide an alternative stereochemical result. Since we have shown[3] that the α-aminobutyryl peptide (**2b**) is indeed a substrate for the isopenicillin N synthetase enzyme we have been able to test this possibility, as follows. The two diastereotopic peptides, (**2c**) and (**2d**), derived from (2*R*,3*R*)-(2-amino-3-deuteriobutyrate) and (2*R*,3*S*)-(2-amino-3-deuteriobutyrate) respectively, were prepared by a known procedure[4] using labelled α-aminobutyric acid obtained by the procedure of Crout.[5] These were separately incubated (20 °C) with the purified isopenicillin N synthetase (MW = 37 000) obtained from *Cephalosporium acremonium*[6] (CW 19) in the presence of $FeSO_4$ (0.1 mM), dithiothretiol, (3 mM), ascorbate (1.5 mM), catalase, and oxygen for 5 h. The penam product (**1b**) was purified by paper electrophoresis (5 kV, 1 h, pH 3.5).

The $^1$H n.m.r. spectra ($D_2O$; 300 MHz) of the product from each precursor were *identical*, showing a singlet for the C(3)-H (δ 4.2, 1H) and also for the C(2)-$CH_3$ (δ 1.3). The product was oxidised ($NaIO_4$) to the β-sulphoxide[7] giving rise to a downfield shift (0.15 p.p.m.) of the C(2)-$CH_3$ resonance.



(**1**)

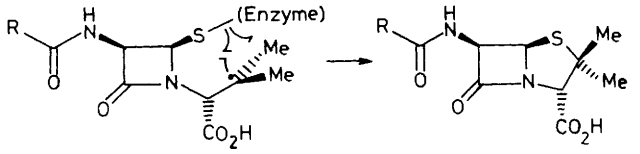
a; $R^1 = R^2 = Me$
b; $R^1 = Me, R^2 = {}^2H$
c; $R^1 = Me, R^2 = {}^1H$



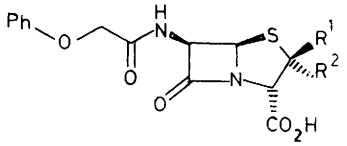
(**2**)

a; $R^1 = R^2 = Me$
b; $R^1 = Me, R^2 = H$
c; $R^1 = {}^2H, R^2 = Me$
d; $R^1 = Me, R^2 = {}^2H$

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



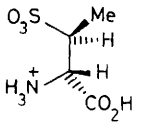
Scheme 1



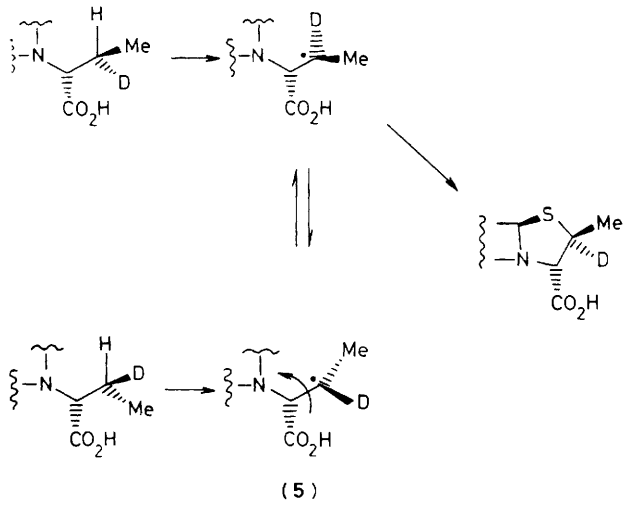
(3)

a; $R^1$ = Me, $R^2$ = H

b; $R^1$ = H, $R^2$ = Me



(4)



(5)

Scheme 2

A similar downfield shift (0.28) was noted in the β-sulphoxide derived from (**3a**) whereas that from (**3b**)[8] exhibited an upfield shift (0.18). Confirmation of the (2*S*)-assignment (**1b**) was obtained by oxidation ($HCO_3H$–$HCO_2H$) to norpenicillaminic acid which comigrated on paper electrophoresis (5 kV, 1.5 h, pH 1.8) with (**4**) obtained from (**3a**) and was readily distinguished from its isomer, obtained from (**3b**). The preferred formation of the (2*S*)-norisopenicillin N has been already observed in the non-deuteriated substrate (**2b**).[3b]

The formation of the same (2*S*)-norisopenicillin N from the two diastereoisomeric peptides (**2c**) and (**2d**) is in accord with our earlier postulate[2] of a radical mechanism for the C(2)–S bond formation, since rotation in the less sterically hindered intermediate (**5**), followed by an enzyme directed coupling, would explain our results, Scheme 2.[9] It is also evident that a substantial kinetic isotope effect is favouring the hydrogen removal since we could not detect any of the hydrogen isotopomer (**1c**) (⩽10%).

In conclusion, the observation of both retention and also inversion of configuration during C–S bond formation from diastereoisomeric precursors supports the radical mechanism proposed for this step in penicillin's biosynthesis. An alternative mechanism in which a double inverting displacement occurs, to give in the case of the valine peptide overall retention,[10] is not in accord with these results.

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## References

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9 Similar stereochemical results in quite unrelated enzymic reactions have been observed; *e.g.* the coenzyme $B_{12}$-dependent ethanolamine ammonia lyase: *cf.* P. Diziol, H. Haas, J. Rétey, S. W. Graves, and B. M. Babior, *Eur. J. Biochem.*, 1980, **106**, 211.
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