Synthesis of Chirally Labelled Cysteines and the Steric Origin of C(5) in Penicillin Biosynthesis

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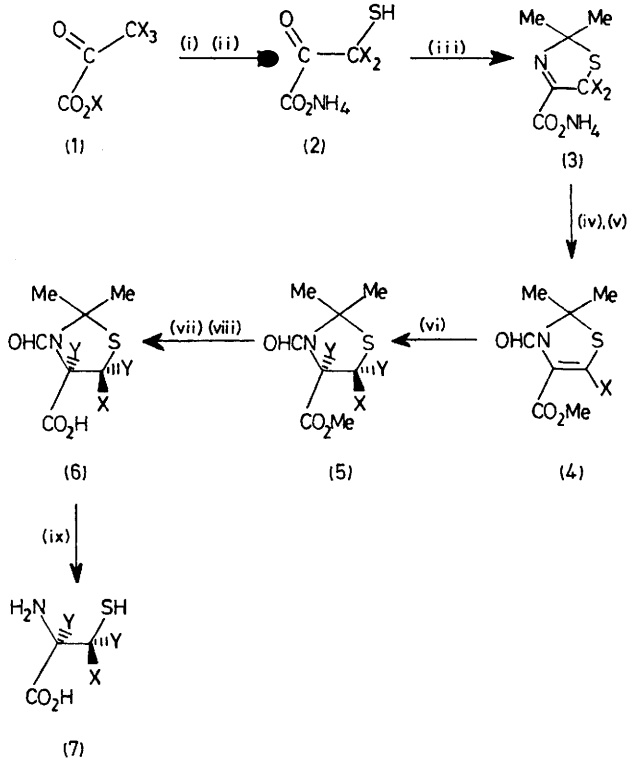
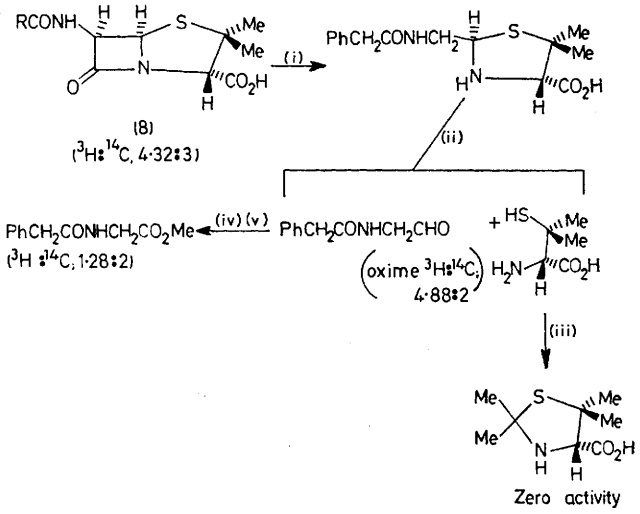
Summary The (2*R*, 3*R*)-[2,3-$^3H_2$]- and (2*R*, 3*S*)-[3-^3H]-forms of cysteine have been synthesised and used to show that the incorporation of C-3 of cyst(e)ine into penicillin G proceeds with overall retention of stereochemistry.

RECENTLY the studies of Arnstein[1] on the biosynthesis of penicillin have been extended by work on the stereochemistry of incorporation of the isopropyl group of L-valine into penicillins.[2] The stereochemical origin of C-5 of penicillins (**8**), which is known to be derived from the β-carbon atom of L-cyst(e)ine,[1c] is still unknown. In an attempt to clarify this we have synthesised (2*R*,3*R*)-[2,3-$^3H_2$]-cysteine and (2*R*,3*S*)-[3-^3H]-cysteine and fed these compounds to cultures of *Penicillium chrysogenum*.



SCHEME 1. (i) Br_2; (ii) NH_4SH; (iii) NH_3–acetone; (iv) Me_2SO_4; (v) HCO_2COMe; (vi) Y_2, 10% Pd–C; (vii) NaOH; (viii) strychnine; (ix) N-HCl.

The synthesis of the labelled cysteines is outlined in Scheme 1, using pyruvic acid (**1**, X = H) as the starting material for the synthesis of (2*R*,3*R*)-[2,3-$^3H_2$]-cysteine; and [$^3H_4$]-pyruvic acid (**1**, X=^{3}H) as the starting material for the synthesis of (2*R*,3*S*)-[3-^3H]-cysteine. The 3-thiazoline (**3**) was synthesised by adaptation of known methods[3] and this compound was converted to the *N*-formyl-4-thiazoline (**4**) by reaction with formic–acetic anhydride. Catalytic hydrogenation of the thiazoline (**4**, X=^{3}H) yielded the racemic thiazolidine (**5**, X=^{3}H, Y=H). The L-thiazolidine (**5**, X=Y=H) was synthesised from L-cysteine[4] and this compound could be hydrolysed to the acid, (**6**, X=Y=H) and thence to L-cysteine without racemisation. The racemic thiazolidine (**5**, X=^{3}H, Y=H) was therefore hydrolysed to the acid (**6**, X=^{3}H, Y=H) which was resolved by repeated recrystallisation of the strychnine salt from ethyl acetate. Hydrolysis of the L-isomer yielded (2*R*,3*S*)-[3-^3H]-cysteine.



SCHEME 2. (i) H_2SO_4; (ii) $HgCl_2$; (iii) acetone; (iv) Ag_2O; (v) CH_2N_2.

Catalytic tritiation of the thiazoline (**4**, X=H) yielded the racemic thiazolidine (**5**, X=H, Y=^{3}H) which was converted to the acid (**6**, X=H, Y=^{3}H) and thence to (2*R*,3*R*)-[2,3-$^3H_2$]-cysteine as above. There was very little loss of activity on degrading the acid (**6**, X=H, Y=^{3}H) to cysteine and this was taken to indicate that little indiscriminate tritium exchange had occurred during catalytic tritiation. There is ample precedent for assuming that the catalytic reduction steps in both syntheses would proceed with *cis*-stereochemistry.[5]

The two stereoselectively labelled cysteines were mixed with L-[*U*-^{14}C]-cysteine and fed separately to *Penicillium chrysogenum*. L-[3,3,3′,3′-$^3H_4$,*U*-^{14}C]-Cystine was also fed to *P. chrysogenum* and penicillin G was isolated from each of the experiments as the *N*-ethyl-piperidinium salt, and crystallised to constant activity. The results, which are summarised in the table, indicate poor retention of tritium from (2*R*,3*S*)-[3-^3H]-cysteine, the expected retention of tritium from L-[3,3,3′,3′-$^3H_4$]-cystine, and good retention of tritium from (2*R*,3*R*)-[2,3-$^3H_2$]-cysteine. The penicillin G obtained from the experiments in which the latter compound was fed to *P. chrysogenum* was degraded as outlined in

TABLE

Incorporation of L-cyst(e)ine into penicillin G in *P. chrysogenum*

Experiment	Labelling pattern in L-cyst(e)ine	% Incorporation of ^{14}C	Ratio $^{3}H:^{14}C$ fed	Ratio $^{3}H:^{14}C$ incorporated	Retention of ^{3}H in penicillin G /%
1	(2*R*,3*S*)-[3-^{3}H,*U*-^{14}C]	1·98	2·46:1	0·35:1	14
2	(2*R*,3*S*)-[3-^{3}H,*U*-^{14}C]	1·63	2·46:1	0·31:1	13
3	[3,3,3′,3′-$^{3}H_4$,*U*-^{14}C]	4·3	1·9:1	0·80:1	42
4	[3,3,3′,3′-$^{3}H_4$,*U*-^{14}C]	2·4	1·9:1	0·78:1	41
5	(2*R*,3*R*)-[2,3-$^{3}H_2$,*U*-^{14}C]	3·04	2·5:1	1·44:1	58
6	(2*R*,3*R*)-[2,3-$^{3}H_2$,*U*-^{14}C]	1·54	2·5:1	1·48:1	59

Scheme 2. Although levels of activity were not sufficient to allow us to separate the phenylacetyl and the glycine portions, it was evident that incorporation of tritium at C-5 was high, and the indications were that loss of tritium from C-6 accounted for much of the reduced activity in the penicillin. This is not an unusual feature of incorporation of [α-^{3}H]-amino acids.[6,7]

Although we have made the assumption that the reduction steps in our syntheses occur with *cis*-stereochemistry, the incorporation figures do indicate stereospecificity of a high order. Since there can be no rational mechanism for reduction of the thiazoline (**4**) with *trans*-stereospecificity, we can conclude that C-3 of L-cyst(e)ine is incorporated into C-5 of penicillin with overall retention of stereochemistry. These experiments may, therefore, have a bearing on considerations of the mechanism of the oxidation-cyclisation sequences in penicillin biosynthesis.

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[1] (a) H. R. V. Arnstein and P. T. Grant, *Biochem. J.*, 1954, **57**, 353, 360; (b) H. R. V. Arnstein and M. E. Clubb, *ibid.*, 1957, **65**, 618; (c) H. R. V. Arnstein and J. C. Crawhall, *ibid.*,1957, **67**, 180; (d) H. R. V. Arnstein and D. Morris, *ibid.*, 1960, **76**, 323.

[2] J. E. Baldwin, J. Loliger, W. Rastetter, N. Neuss, L. L. Huckstep, and N. De La Higuera, *J. Amer. Chem. Soc.*, 1973, **95**, 3796; N. Neuss, C. H. Nash, J. E. Baldwin, P. A. Lemke, and J. B. Grutzner, *ibid.*, 1973, **95**, 3797; H. Kluender, C. H. Bradley, C. J. Sih, P. Fawcett, and E. P. Abraham, *ibid.*, 1973, **95**, 6149; H. Kluender, F. Huang, A. Fritzberg, H. Schnoes, C. J. Sih, P. Fawcett, and E. P. Abraham, *ibid.*, 1974, **96**, 4054.

[3] F. Asinger, M. Thiel, H. Sedlak, O. Hampel, and R. Sowada, *Annalen*, 1958, **615**, 84; (see also R. B. Woodward, Fr. P. 1491658, 1967).

[4] J. C. Sheehan and D. H. Yang, *J. Amer. Chem. Soc.*, 1958, **80**, 1158.

[5] See for example T. Tchen and H. Van Milligan, *J. Amer. Chem. Soc.*, 1960, **82**, 4115; G. W. Kirby and J. Michael, *Chem. Comm.*, 1971, 415; R. H. Wrightman, J. Staunton, A. R. Battersby, and K. R. Hanson, *J.C.S. Perkin* 1, 1972, 2355; G. W. Kirby and M. J. Varley, *J.C.S. Chem. Comm.*, 1974, 833.

[6] See for example E. A. Evans, 'Tritium and its Compounds,' Butterworths, London, 1966, pp. 185, 358 and 403.

[7] *Added in proof:* B. W. Bycroft, C. M. Wels, K. Corbett, and D. A. Lowe (*J.C.S. Chem. Comm.*, 1975, 123) have recently re-examined the question of origin of C-6 in penicillin biosynthesis by feeding L-[U-^{14}C, α-^{3}H]cystine to a high-producing strain of *P. chrysogenum*. These workers note a smaller, but significant reduction of isotopic ratio at C-6.