Penicillin Biosynthesis: Direct Biosynthetic Formation of Penicillin V and Penicillin G

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The enzyme isopenicillin N synthetase is able to convert directly the dipeptides, phenoxyacetylcysteinylvaline and phenylacetylcysteinylvaline into penicillin V and G respectively; these are however very slow compared with substrates of the α-aminoadipoyl or adipoylcysteinylvaline type.†

From our previous studies on structural variations of the α-aminoadipoyl† moiety in the natural substrate δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine ACV (**1a**) of isopenicillin N synthetase (IPNS) we concluded that a six carbon, or equivalent chain, terminating in a carboxy group as in (**1b**), was the minimum requirement for penicillin synthesis, at rates comparable to that of the natural substrate (**1a**).[1] Now with the greater availability of pure IPNS we have found that peptides containing aromatic and unsaturated moieties in place of the α-aminoadipoyl side chain can in fact be cyclised to the corresponding penicillins, albeit at very low rates.

Thus phenoxyacetyl-L-cysteinyl-D-valine (**1c**)[2] on incubation under normal‡ conditions with a highly purified sample of IPNS from *Cephalosporium acremonium* CO728[3] gave antibiotic activity against *Staphylococcus aureus* N.C.T.C. 6571, destroyed by β-lactamase I (*B. cereus*). In control experiments with denatured enzyme [sodium dodecyl sulphate (SDS), urea] no bioactive material was obtained. Acidification of the crude incubation mixture (pH 3) and extraction (EtOAc) followed by esterification (diazomethane) and preparative t.l.c. (20 × 20 cm, SiO_2 PF.254, Merck, EtOAc : CH_2Cl_2 1 : 9, R_f 0.5) gave penicillin V methyl ester (**2c**), with a g.c.–mass spectrum identical to an authentic sample.§ The product, as pencillin V methyl ester, was identified by the presence of *m/z* 174, as ion (**3**), and *m/z* 94 which corresponds to cleavage of phenol from the side chain. The conversion was 0.04%. Under similar conditions, LLD-ACV was converted into isopenicillin N in 100% yield. Incubation of the second substrate, phenylacetyl-L-cysteinyl-D-valine (**1d**), with IPNS as before gave a penicillinase-sensitive antibiotic (*S. aureus*), which after purification by the same method was shown to be penicillin G, as its methyl ester (**2d**), identical by g.c.–mass spectroscopy with authentic material (*m/z* 174 and 91). In this case the conversion was 0.12%.¶

Table 1. Michaelis constants at pH 7.7, 27 °C.

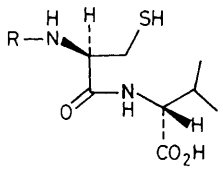
Substrate	K_m (mM)	$V_{max.}$ (μmol min^{-1})
LLD-ACV	0.16	1.64
Phenoxyacetyl CV	0.50	0.80×10^{-3}
Phenylacetyl CV	0.91	2.5×10^{-3}

† δ-(α-aminoadipoyl) = 5-amino-5-carboxypentanoyl.

‡ The peptide (1 mg) in NH_4HCO_3 50 mM solution (2 ml), ascorbate (50 mM, 100 μl), iron(II) sulphate (50 mM, 100 μl), catalase (Sigma, 10%, 50 μl), and dithiothreitol (100 mM, 100 μl) were stirred for 10 min (pH 7.7). IPNS (5.46 i.u. in 50 mM NH_4HCO_3 solution, 2.65 ml) was added and mixture divided into two vials and shaken (27 °C, 270 r.p.m.) for 25 min. Acetone (10 ml) was added and the supernatant collected after centrifugation.

§ G.c.–mass spectra were performed on a 12–120 Quadrapole Mass Spectrometer using an ACE 25m JOW DBI column, 60—240 °C, 15 °C min^{-1}.

¶ *Added in proof:* We have recently learned that Dr. Luengo (Univ. León, Spain) has observed enzymatic synthesis of penicillin G with extracts of *P. chrysogenum* and *C. acremonium*; J. M. Luengo, personal communication, and J. M. Luengo, M. T. Alemany, F. Salto, F. Ramos, M. J. López-Nietro, and J. F. Martin, *Biotechnology*, 1985, in the press.



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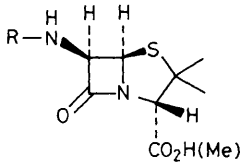

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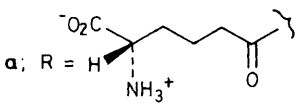

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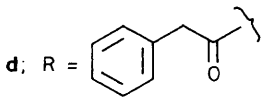

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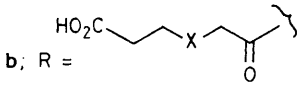

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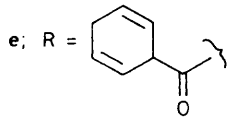

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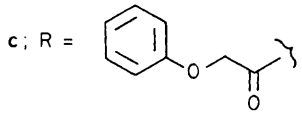

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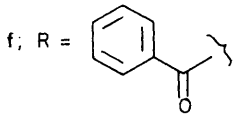

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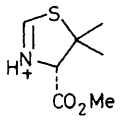

(3)

Similarly cyclohexa-2,5-dienoyl-L-cysteinyl-D-valine (**1e**), prepared by sodium/liquid ammonia reduction of *N*-benzoyl-L-cysteinyl-*S*-benzyl-D-valine benzyl ester, gave a pencillinase-sensitive antibiotic (*S. aureus*) shown after derivatisation by g.c.–mass spectroscopy to be phenyl penicillin methyl ester (**2f**), in 0.20% yield.[4] The presence of ion (**3**), along with *m/z* 105 and 77, confirmed the presence of (**2e**) by comparison with authentic material. It is possible that the pencillin (**2e**) may be present but no attempt was made to identify it. The aromatisation of (**2e**) to (**2f**) must occur after cyclisation since incubation of benzoyl-L-cysteinyl-D-valine (**1f**) gave no bioactive material (*S. aureus*).

The origin of the much less efficient conversion of these peptides, as compared with the natural substrate, was evaluated by determination of the Michaelis constants (K_m) and maximum velocity ($V_{max.}$) parameters, Table 1. ‖ Apparently the major influence is on the $V_{max.}$ parameter suggesting that the catalytic event, as opposed to binding, is sensitive to the absence of the δ-carboxy group, which substantially depresses the conversion rates.

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References

1 J. E. Baldwin, E. P. Abraham, R. M. Adlington, G. A. Bahadur, B. Chakravarti, B. P. Domayne-Hayman, L. D. Field, S. L. Flitsch, G. S. Jayatilake, A. Spakovskis, H-H. Ting, N. J. Turner, R. L. White, and J. J. Usher, *J. Chem. Soc., Chem. Commun.*, 1984, 1225; 'Recent Advances in the Chemistry of β-Lactam Antibiotics,' Third International Symposium, 1984, Cambridge, eds. A. G. Brown and S. M. Roberts, p. 62.

2 Prepared either by standard methods, see J. E. Baldwin, S. R. Herchen, B. L. Johnson, M. Jung, J. J. Usher, and T. Wan, *J. Chem. Soc., Perkin Trans. 1*, 1981, 2253 or by *N*-acylation of cystine, followed by ethyl 1,2-dihydro-2-ethoxy-1-quinoline carboxylate (EEDQ) coupling with valine benzhydryl ester and deprotection with trifluoroacetic acid (TFA) to give the dipeptides as the disulphides in 57% overall yield.

3 C-P. Pang, B. Chakravarti, R. M. Adlington, H-H. Ting, R. L. White, G. S. Jayatilake, J. E. Baldwin, and E. P. Abraham, *Biochem. J.*, 1984, **222**, 789.

4 Note the antibioactivity (*S. aureus*) of (**2f**) is only 10% of that of penicillin G, *cf.* K. E. Price, *Adv. Appl. Microbiol.*, 1969, **11**, 17.

‖ Conditions were: enzyme (1.64 i.u. in 50 mM NH_4HCO_3 solution, 330 μl), peptide (10 mM, aq., 10, 15, 30, 60 μl, made up to 100 μl with 50 mM NH_4HCO_3 solution), ascorbate (50 mM, 20 μl), iron(II) sulphate (5 mM, 20 μl), catalase (Sigma, 10%, 10 μl), and dithiothreitol (100 mM, 20 μl) to pH 7.7 and incubated at 27 °C under saturating oxygen. Samples (100 μl) were withdrawn at 2 min intervals and directly bioassayed against *S. aureus*. Experiments where the samples were initially quenched with acetone and the acetone evaporated gave the same bioassay result as those directly added to the plate. The kinetic parameters were determined from the Eadie–Hofstee and Lineweaver–Burk plots.