Phenoxyacetylthioglycyl-(±)-valine, a Fungal Metabolite of Anhydropenicillin V

By ROBERT THOMAS

(*Chemistry Department, University of Surrey, Guildford, Surrey*)

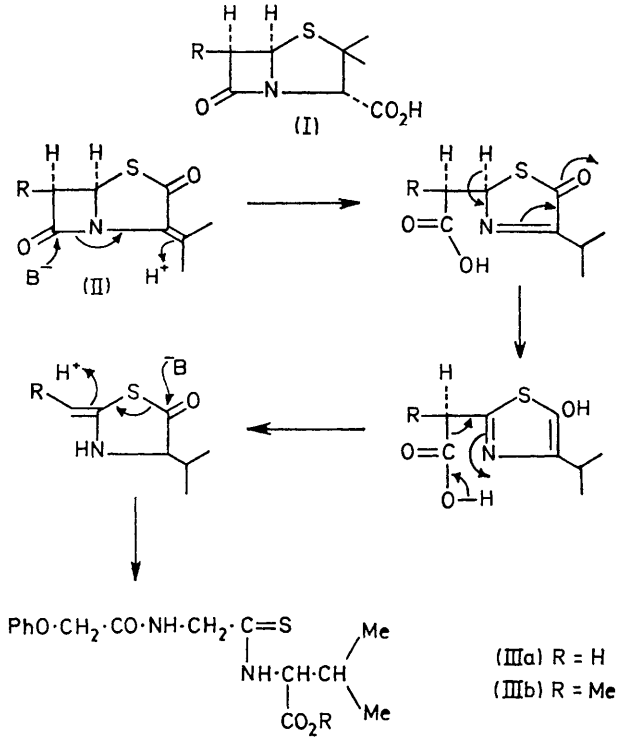
Summary Anhydropenicillin V is transformed by *Aspergillus niger, Penicillum chrysogenum*, and other fungi into the thiopeptide, phenoxyacetylthioglycyl-(±)-valine (IIIa); the simultaneous formation of trace amounts of penicillin V is probably due to a spontaneous rather than an enzyme-catalysed rearrangement.

PENICILLINS (I) can readily be rearranged into anhydropenicillins (II).[1] A partial reversal of this rearrangement indicated that an analogous process could constitute the thiazolidine cyclisation step in the biosynthesis of the penicillanic acid nucleus (I, R=H).[2]

Several fungi have now been shown to degrade anhydropenicillin V (II, R = phenoxyacetamido), with the subsequent accumulation of phenoxyacetylthioglycyl-(±)-valine (IIIa).

Anhydropenicillin V, prepared from penicillin V (I, R = phenoxyacetamido) by the general procedure of Wolfe *et al.*,[1] was isolated as a crystalline product, m.p. 124°; microanalytical and spectroscopic data were consistent with the expected structure (II, R = phenoxyacetamido). On incubating at sub-inhibitory levels for 24—72 h with a variety of micro-organisms, several fungi including *Aspergillus niger, A. nidulans*, and a non-penicillin producing

strain of *Penicillium chrysogenum* produced a low concentration of a common antibacterial substance. This was chromatographically indistinguishable from penicillin V.[3] However, while the bioactivity was present in culture filtrates it was also obtained in comparable yields in incubations with sterile media.



(I)

(II)

PhO·CH$_2$·CO·NH·CH$_2$·C(=S)–NH·CH(CO$_2$R)·CH(Me)$_2$

(IIIa) R = H
(IIIb) R = Me

Non-antibacterial metabolites capable of decolourising a starch–iodine spray reagent[4] or a NaN_3–I_2 mixture[5] were resolved by paper chromatography. A 3-day culture of *Aspergillus niger* when grown on a chemically defined medium,[3] metabolised anhydropenicillin V within 72 h with the formation of (IIIa) (16%), m.p. 128—129°.† Methylation with diazomethane yielded (IIIb) m.p. 115—116°.†

Strong absorption in the u.v. spectrum at λ_{max} 269 nm is consistent with a thioamidechromophore [*cf.* MeCS·NHMe, $\pi \rightarrow \pi^*$ transition, λ_{max} (EtOH) 261 nm (log ϵ 4·1)],[6] which appears to be present in $CDCl_3$ solution predominantly in the thione rather than the thiol form (n.m.r. evidence for the spin-coupled thioamide NH proton). As with normal amides, thioamides have been shown to possess planar conformations, indicating the partial sp^2 character of the carbon and nitrogen atoms, consequently (IIIa) may exist as the (Z) or (E) isomer.[7] The presence of a thioamide group could account for the observation that whereas (IIIa) readily decolourises the NaN_3–I_2 reagent,[5] (II) does not.

Supporting evidence for the thioglycylvaline component was obtained through the paper chromatographic characterisation of glycine and valine as the sole ninhydrin-detectable components of an acid hydrolysate of (IIIa). However, a hydrolysis of the parent anhydropenicillin V also generated these two amino-acids, allowing the possibility that the acidic degradation of (II) could involve the intermediate formation of (IIIa) or a closely related compound.

Anhydropenicillin V was not spontaneously converted to (IIIa) under the mildly acidic conditions of the *A. niger* fermentation, since on incubating for 72 h in sterile growth medium adjusted to pH 2, the only new product which could be detected, was a low level of a bioactive substance which corresponded chromatographically to penicillin V. The metabolic conversion of (II) to (IIIa) in comparatively non-acid producing fermentations was effected in good yields by the moulds *A. nidulans* and *P. chrysogenum*. A cell-free culture filtrate of *A. nidulans* was found to be ineffective, thus demonstrating the involvement of intracellular mycelial enzymes.

C.d. measurements of (IIIa) in methanol did not indicate any optical activity. This suggests that the valine chiral centre may be formed by a non-stereospecific process, or alternatively, that the optical inactivity may be a consequence of subsequent racemase activity. The metabolic process [(II) → (IIIa)] can be mechanistically rationalised as outlined in the Scheme.

I thank the Squibb Institute for Medical Research for financial support, Mr. and Mrs. D. Adam for experimental assistance, Mr. J. P. Bloxsidge for n.m.r. measurements, and Dr. A. I. Cohen and Mr. J. Delderfield for the determination of mass spectra.

(*Received, 7th February* 1972; *Com.* 191.)

† Satisfactory spectroscopic and microanalytical data were obtained for all new compounds.

[1] S. Wolfe, J. C. Godfrey, C. T. Holdrege, and Y. G. Perron, *Canad. J. Chem.*, 1968, **46**, 2549.
[2] S. Wolfe, R. N. Bassett, S. M. Caldwell, and F. I. Wasson, *J. Amer. Chem. Soc.*, 1969, **91**, 7205.
[3] E. Albu and R. Thomas, Biochem. J., 1963, **87**, 648.
[4] R. Thomas, *Nature*, 1961, **80**, 234.
[5] E. Chargaff, C. Levine, and C. Green, *J. Biol. Chem.*, 1948, **175**, 67; W. Walter, *Ann. Chem.*, 1960, **633**, 35.
[6] M. J. Jansen, *Rec. Trav. Chim.*, 1960, **79**, 454.
[7] W. Walter and J. Voss in 'The Chemistry of Amides,' ed. J. Zabicky, Interscience–Wiley, London, 1970, ch. 8, p. 386, and references therein.