# The Microbial Metabolism of Penicillin V Sulphoxide and its Possible Relevance to the Mode of Action of Penicillin

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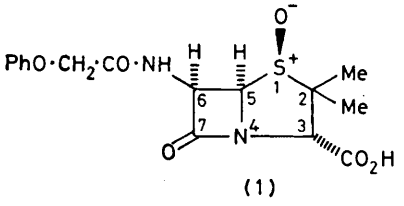
*Summary* Extracellular enzymes of bacteria and streptomycetes rapidly convert two molecules of penicillin V sulphoxide into one molecule of an unstable metabolite, probably (**5**); the implications of this finding for the mode of action of penicillin are discussed.

PENICILLIN-METABOLISING enzymes are known to catalyse hydrolysis of the β-lactam N(4)–C(7) and C(5)–C(6) bonds and also various *N*-acyl side chains,[1] while in the degradation of anhydropenicillin V to (±)-phenoxyacetylthioglycylvaline[2] both rings of this derivative are cleaved. Penicillin V β-sulphoxide (**1**) is now shown to undergo a novel transformation by various bacteria (*e.g., Bacillus megaterium* and *Streptomyces venezuelae*) leading to an unstable metabolite (PSM).



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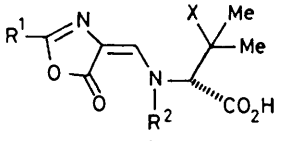
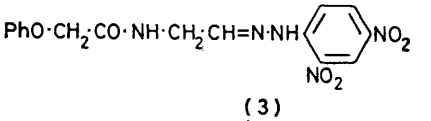


| | X | $R^1$ | $R^2$ |
|---|---|---|---|
| (2) | SH | $Ph{\cdot}CH_2$ | H |
| (6) | SOH | $PhO{\cdot}CH_2$ | $PhO{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO$ |



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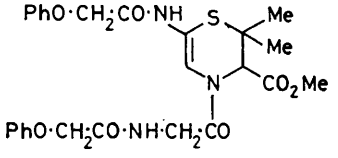

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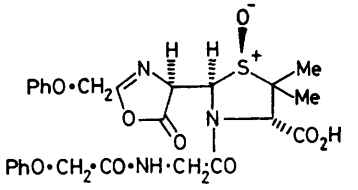

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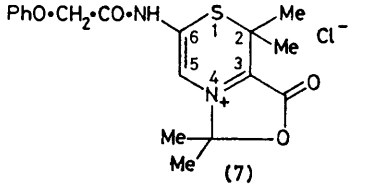

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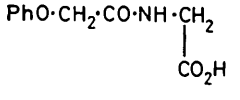

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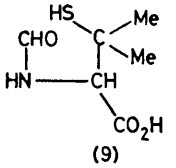

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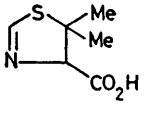


(10)

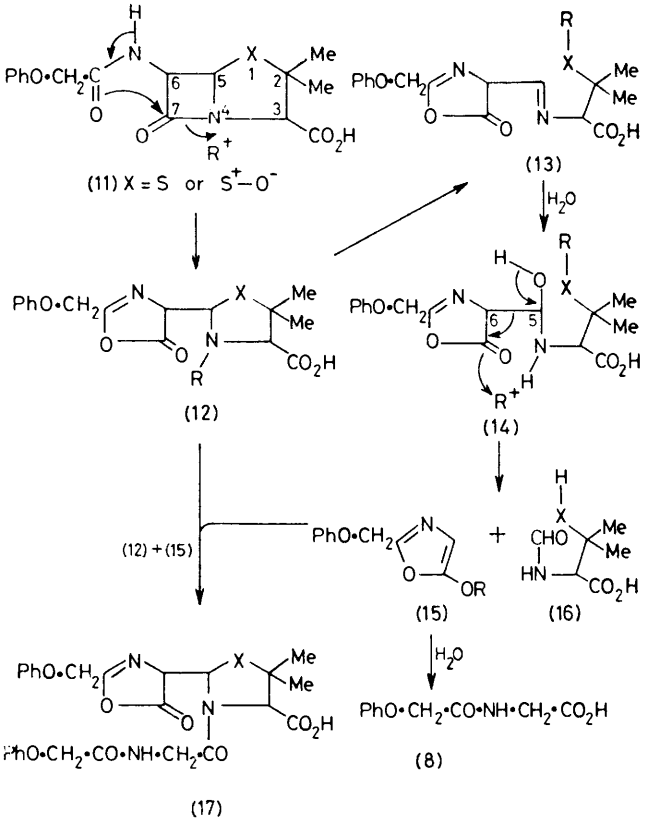
PSM appears in $CHCl_3$ extracts of the acidified filtrate as the major starch–iodine decolorising component[4] ($R_f$ 0·7—0·8), following ether–water chromatography on pH 5·7 buffered paper.[5] The moderately intense i.r. band at 1780 $cm^{-1}$, which is lost on dissolution in MeOH, may correspond to an oxazolone ring, *cf.* benzylpenicillenic acid (**2**),[6] $\nu_{max}$ 1780 $cm^{-1}$. In n.m.r. spectra (60 MHz, $CDCl_3$) of fresh extracts, the ratio of the relative integrals of the phenoxyacetamido and *C*-methyl protons approximates to twice that of the parent sulphoxide, while a broad doublet ($\tau$ 5·74, $J$ 5·0 Hz), collapsing to a broad singlet on $D_2O$ exchange, is indicative of a glycine unit. With 2,4-dinitrophenylhydrazine, PSM forms phenoxymethylpenilloaldehyde 2,4-dinitrophenylhydrazone (**3**), m.p. 190 °C, and on sequential treatment with acetyl chloride in acetone (90 min at 25 °C) and $CH_2N_2$ it yields an ester, $C_{26}H_{29}O_7N_3S$, m.p. 174 °C. The probable structure (**4**) is based on spectroscopic and degradative data; *e.g.* mass spectrum *m/e* 527 ($M^+$), 493 ($M - H_2S$), 336 ($M$ − $PhOCH_2$-$CONHCH$ : CO); n.m.r. (90 MHz, $CDCl_3$) $\tau$ 2·47 (1 H, br tr, $J$ *ca.* 5 Hz, $NHCH_2$), 2·59—3·10 (*ca.* 10 H, m, 2 phenyl), 4·84 (1 H, s, 5-*H*), 5·45 [5 H, br s, 3-*H* and 2 $CH_2OPh$, 3-*H*, and $CH_2$ are resolved in $(CD_3)_2CO$], 5·58 and 5·61 (each 1 H, ABX m, $J_{AB}$ *ca.* 18 Hz, $J_{AX/BX}$ *ca.* 5 Hz, $CH_2NH$), 6·25 (3 H, s, OMe), and 8·40 and 8·53 (each 3 H, 2 s, $CMe_2$); i.r. $\nu_{max}$ (Nujol) 1745 $cm^{-1}$ (ester); c.d. measurements showed zero rotation. Glycine was identified as the major amino acid formed on hydrolysis with 6N HCl (105 °C; 16 h). These data suggest a number of related structures

*S. venezuelae* NCIB 8231 culture filtrates effect complete degradation of the β-sulphoxide (50 mg/60 ml) on incubating for 20 min at 27 °C. The extracellular enzyme, which is inactivated on autoclaving, is readily adsorbed on bentonite (1 g/60 ml) from which it is regenerated on suspending in nutrient broth at pH 7·5. Penicillin V α-sulphoxide[3] is similarly degraded to PSM, but at a slower rate, whereas 7-phenoxyacetamidodeacetoxycephalosporanic acid β-sulphoxide is essentially unchanged under these conditions.

for PSM based on (**5**) or a degradation product resulting from elimination of $H_2O$ from the thiazolidine sulphoxide unit, hydrolysis of the oxazolinone ring, or decarboxylation. A feasible pathway for the ring expansion of (**5**) to (**4**) involves formation of the sulphenic acid (**6**) corresponding to the penicillenic acid (**2**) with loss of $CO_2$, as previously proposed to account for the novel degradation of (**1**) to the 1,4-thiazinium chloride (**7**),[7] which is obtained under parallel conditions.

The conversion of (**1**) into a metabolite containing a phenoxyacetylglycyl substituent by extracellular enzymes of bacteria and streptomycetes, is analogous to the recently reported degradation of penicillin V to phenoxyacetylglycine (**8**) and either *N*-formylpenicillamine (**9**)[8] or the corresponding thiazoline (**10**),[9] by the cell wall peptidoglycan cross-linking enzymes of these micro-organisms. This indicates that penicillin V and its β-sulphoxide may undergo degradation by the same DD-carboxypeptidase-transpeptidase system through a common pathway, as outlined in the hypothetical Scheme. Cleavage of the thiazolidine ring of (**11**), *via* the elimination reaction (**12**) → (**13**),[10] may account for the ready degradation of the sulphoxide. This pathway implies the following. (i) The presence of two active sites, both of which may exhibit N(4)–C(7) β-lactamase activity, as in (**11**) → (**12**), although not necessarily requiring oxazolinone formation. These may correspond to the carboxy donor and amino acceptor sites which catalyse the D-alanyl-D-alanine cleavage and transfer reactions. (ii) Additional cleavages at the donor site of C(5)–S(1) *via* (**12**) → (**13**), and C(5)–C(6) *via* (**14**) → (**15**) with simultaneous release of *N*-formylpenicillamine (**16**). (iii) Formation of an enzyme-bound acylglycine derivative, *e.g.* (**15**), prior to displacement of the enzyme, either through hydrolysis (**15**) → (**8**) or amination, *e.g.* (**15**) → (**17**), corresponding to carboxypeptidase or transpeptidase activity respectively.

The uptake of penicillin at two enzyme sites offers increased scope for inhibitory mechanisms, one possible mode of action involving its degradation to metabolites closely resembling normal intermediates formed during the peptidoglycan cross-linking sequence. The Tipper–Strominger criteria for active site competition[11] leading to esterification of a serine residue,[12] although based on the conformational equivalence of 6-apa and D-alanyl-D-alanine, would appear to be more readily satisfied by structures such as the acylglycyl-D-alanine derivative (**17**) or related glycyl peptides.† The effectiveness of this mechanism would be enhanced by the direct synthesis of an inhibitor *in situ* at the active site, thereby ensuring prior access to the target enzyme. In addition, the presence of an oxazolinone substituent would facilitate covalent bond formation through interaction with adjacent nucleophilic centres on the enzyme, such as serine.




SCHEME. R = H or Enzyme.

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† It has recently been reported that release of the *Streptomyces* R61 DD-carboxypeptidase-transpeptidase from the benzylpenicilloyl–enzyme complex occurs after C(5)–C(6) cleavage with transfer of the phenylglycyl residue to $H_2O$ or an amino acceptor, *e.g.* D-alanine: A. Marquet, J.-M. Frère, J.-M. Ghuysen, and A. Loffet, *Biochem. J.*, 1979, **177**, 909.

[1] E. P. Abraham, 'Biosynthesis and Enzymic Hydrolysis of Penicillins and Cephalosporins' (E. R. Squibb Lectures on Chemistry of Natural Products), University of Tokyo Press, 1974, p. 38.
[2] R. Thomas, *J.C.S. Chem. Comm.*, 1972, 478.
[3] O. Spry, *J. Org. Chem.*, 1972, **37**, 795. A modified procedure involving ozonolysis of a solution (3·5%) of the K salt of penicillin V in $Me_2CO$–$H_2O$ (1 : 1) yielded (25%) crystalline α-sulphoxide, m.p. 157 °C (EtOAc).
[4] R. Thomas, *Nature*, 1961, **80**, 234.
[5] E. Albu and R. Thomas, *Biochem. J.*, 1963, **87**, 648.
[6] 'The Chemistry of Penicillins,' eds. H. T. Clarke, J. R. Johnson, and R. Robinson, Princeton University Press, 1949, p. 387.
[7] R. Thomas and D. J. Williams, *J.C.S. Chem. Comm.*, 1973, 226. (Analogous 1,4-thiazine structures have been described: T. S. Chou, W. A. Spitzer, D. E. Dorman, S. Kukolja, I. G. Wright, N. D. Jones, and M. O. Chaney, *J. Org. Chem.*, 1978, **43**, 3835; A. G. M. Barrett, *J.C.S. Perkin I*, 1979, 170.)
[8] J.-M. Ghuysen, *J. Gen. Microbiol.*, 1977, **101**, 13, and cited references.
[9] S. Hammarström and J. L. Strominger, *J. Biol. Chem.*, 1976, **251**, 7947.
[10] SH reagents are known to inhibit the release of *E. coli* D-alanine carboxypeptidase I-bound penicillin: S. J. Curtis and J. L. Strominger, *J. Biol. Chem.*, 1978, **253**, 2584.
[11] P. M. Blumberg and J. L. Strominger, *Bact. Rev.*, 1974, **38**, 291.
[12] J.-M. Frère, C. Duez, J.-M. Ghuysen, and J. Vanderkerckhove, *F.E.B.S. Letters*, 1976, **70**, 257; N. Georgopapadakou, S. Hammarström, and J. L. Strominger, *Proc. Nat. Acad. Sci. U.S.A.*, 1977, **74**, 1009.