Antibiotics 13285 A1 and A2: Novel Cepham and Penam Metabolites from a *Streptomyces* Species

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The norpenicillin N (**1b**) and the isomeric cepham (**2**) have been isolated from a *Streptomyces* species and identified from ^{1}H n.m.r. spectroscopy and detection of D-α-aminoadipic acid following acid hydrolysis.

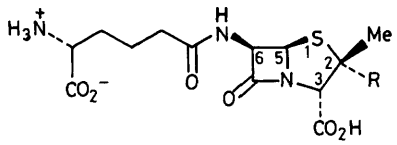
In the course of our work directed towards the discovery of novel β-lactam antibiotics from micro-organisms, we have isolated two new compounds which are close relatives of the penicillins and cephalosporins.

A species of *Streptomyces*, isolated from a soil sample and numbered ACC 13285 in our collection, when grown in a number of different media, produces several β-lactam antibiotics. The major component is believed to be penicillin N (**1a**) but a complex, 13285A, with very similar properties to penicillin N, was also detected.

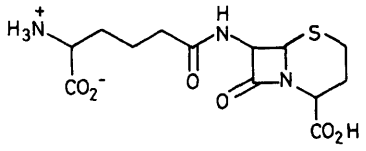
ACC 13285 was grown in a glycerol–peptone based medium† for 4—5 days and the culture broth (180 l) centrifuged to remove the cells. The antibiotics were concentrated from the liquor by sequential chromatography on Diaion HP20 (Mitsubishi Kasei), Amberlite IRA68 (Rhom and Haas), and silica gel columns. The 13285A complex was separated from the penicillin N on reverse-phase silica gel and further purified on Sephadex G25 (Pharmacia Fine Chemicals). The components of 13285A were separated on microcrystalline cellulose and two compounds, 13285 A1 (5 mg) and A2 (10 mg), were obtained substantially pure by further chromatography on reverse-phase silica gel using an ion-pair reagent (tetra-n-butylammonium bromide in phosphate buffer).

The ^{1}H n.m.r. spectrum of 13285 A2 shows signals consistent with the presence of a 'penicillin-like' β-lactam and an α-aminoadipyl moiety. However, the penicillin *gem*-dimethyl groups are replaced by a single methyl group which appears as a doublet and is coupled to an additional proton at δ 4.37. This in turn is coupled to a 'typical' penicillin proton, 3-H, at δ 4.6 and suggests the norpenicillin structure (**1b**) for

† ACC 13285 was grown in flasks with shaking at 250 r.p.m. at 28 °C, or in fermenters, in a medium containing the following constituents dissolved in deionised H_2O and adjusted to pH 7: glycerol (3.0%), peptone oxoid bact. L37 (2.0), calcium carbonate (0.1), potassium dihydrogen phosphate (0.024), magnesium sulphate heptahydrate (0.02), and minor element concentrate (0.1).



(1) **a**; R = Me
b; R = H



(2)

13285 A2. A *trans*-relationship between 2-H and 3-H (*J* 3.6 Hz) was confirmed by a nuclear Overhauser experiment in which irradiation of the methyl doublet gave signal enhancements of 23% for 2-H and 17% for 3-H but with no effect on the signal for 5-H. Thus the relative stereochemistry of the nucleus is that of a typical natural penam. The D-absolute configuration of the single chiral centre in the side-chain was established by acid hydrolysis of 13285 A2, leucylation of the resulting α-aminoadipic acid,[1] and analysis on an ion-exchange resin.‡ Conditions and comparative data for this sequence were first established using authentic samples of D- and L-α-aminoadipic acids and the corresponding penams, penicillin N and isopenicillin N.

The ^{1}H n.m.r. spectrum of 13285 A1, although less well defined, also shows the β-lactam and α-aminoadipyl signals of a typical penicillin–cephalosporin but has no obvious penam methyl or cephem C-2 or C-3′ methylene groups. The presence of a single proton double doublet at δ 4.63 and a two proton multiplet at δ 2.93 suggested the cepham structure (**2**) as a possibility consistent with the available data.

Recent publications[2] contain reports of the *in vitro* biosynthesis of penicillin and cephalosporin analogues from modified tripeptide substrates using a highly purified fungal enzyme, isopenicillin N synthetase. In particular (L-α-amino-δ-adipyl)-L-cysteinyl-D-(α-aminobutyrate) is converted into norisopenicillin N and the corresponding cepham.[3] N.m.r. spectra of these compounds are indistinguishable from those of the 13285 A2 and A1 compounds and differ from our metabolites only in the stereochemistry of the α-aminoadipyl side-chain.

The isolation of 13285 A1 and A2 from a species of *Streptomyces* constitutes a first time discovery of such β-lactams from an intact organism.

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‡ Ultropak-8 column (Na^+ form) in an LKB 4400 amino-acid analyser.

References

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