The Structure of the Antibiotic Ostreogrycin B3

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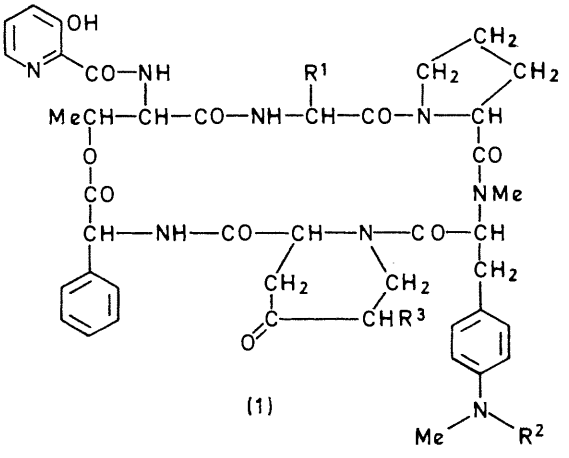
Summary Ostreogrycin B3 has been shown to contain a 5-hydroxy-4-oxo-pipecolyl residue in place of the 4-oxopipecolyl residue present in ostreogrycin B.

OSTREOGRYCIN B3 is one of three minor peptide antibiotics B1,† B2,† and B3, isolated in conjunction with the principal peptide antibiotic ostreogrycin B† from cultures of *Streptomyces ostreogriseus*.[1] The structures of ostreogrycins B (**1**; R^1 = Et; R^2 = Me, R^3 = H), B1 (**1**; R^1 = Me, R^2 = Me, R^3 = H), and B2 (**1**; R^1 = Et, R^2 = H, R^3 = H) have been confirmed by a study of their mass spectra. They show molecular ions with *m/e* 867, 853, and 853, respectively, which are in agreement with the empirical formulae $C_{45}H_{54}N_8O_{10}$, $C_{44}H_{52}N_8O_{10}$, and $C_{44}H_{52}N_8O_{10}$, but fragments from which information concerning the amino-acid sequence could be obtained did not occur above *m/e* 300. The previously suggested structures were also confirmed by a study of the respective n.m.r. spectra. A new amino-acyl fragment which is destroyed on acid hydrolysis[1b] is shown to be present in ostreogrycin B3.

Ostreogrycin B3, the most polar of the peptide antibiotics as shown by countercurrent distribution, is a colourless crystalline compound m.p. 215°, $[\alpha]_D^{25}$ − 57° (MeOH), having the molecular formula $C_{45}H_{54}N_8O_{11}$, in agreement with the value obtained for the molecular ion, *m/e* 883 (calc. 882·95). The u.v. spectrum of B3 was almost identical with that of ostreogrycin B. The i.r. spectrum showed the expected absorption due to amide N–H and C=O and to lactone C=O stretching frequencies and, like the other antibiotics, it exhibited an absorption maximum in the ketone carbonyl region at 1717 cm^{-1}. Further, the spectrum showed stronger absorption at 3500 cm^{-1} than did the other antibiotics and this was ascribed to the presence of an additional hydroxy-group.

Acid hydrolysis of ostreogrycin B3 yielded the components 3-hydroxypicolinic acid (HyPic), threonine, α-aminobutyric acid, proline, *p*-dimethylamino-*N*-methylphenylalanine (DmaMePhe), and phenylglycine, which were identified by comparison with the hydrolysis products of ostreogrycin B by means of two-dimensional t.l.c. on cellulose and by their elution times in an amino-acid analyser. Molar equivalents of the amino-acids were shown to be present. But whereas ostreogrycin B yielded 4-oxopipecolic acid on hydrolysis, the hydrolysate of ostreogrycin B3 was devoid of this amino-acid and no other significant ninhydrin-active material was present. Instead there appeared on chromatograms only spots which fluoresced blue and green, respectively, on irradiation with u.v. light. These materials, which were absent from the chromatograms of ostreogrycin B, were shown to be present in the hydrolysate of B3 in only minor amounts. Determination of the amino-acid sequence by partial hydrolysis showed the arrangement HyPic-Thr-Abu-Pro-DmaMePhe, while phenylglycine was shown to be *C*-terminal and involved in the lactone linkage. The unaccounted fragment, $C_6H_7NO_3$, must therefore be positioned between the *p*-dimethylamino-*N*-methylphenylalanine and the phenylglycine in place of the 4-oxopipecolyl group, $C_6H_7NO_2$, present in ostreogrycin B. Despite the destruction of this aminoacyl fragment on acid hydrolysis, the simplest hypothesis was that it consisted of a variation on the structure of 4-oxopipecolic acid and contained both the keto- and hydroxy-functions. Comparison of the n.m.r. spectra of all the peptides confirmed the presence of the structural features in B3 already known from the hydrolysis products. In addition, it could be deduced, albeit with little confidence, that a further N–CH_2– group was present in the missing fragment.



(1)

Oxidation of ostreogrycin B3 with periodate, followed by acid hydrolysis of the resulting product and examination of the hydrolysate by means of an amino-acid analyser, showed the presence of 0·85 molar equivalents of aspartic acid. Oxidative cleavage of an acyloin system in pipecolic acid in such a manner as to yield aspartic acid after hydrolysis requires cleavage of the nitrogen–carbon bond in the system –$NHCH_2$·CHO to give the free amino-group. Presumably this takes place by a mechanism involving prototropic rearrangement to an imine. Support for this suggestion was obtained by periodate oxidation of B3

† Antibiotics which are identical with or closely related in structure to members of the ostreogrycin complex are: Ostreogrycin B, vernamycin Bα,[2] pristinamycin 1A,[3] PA114B,[1] Mickamycin B.[4] Ostreogrycin B1, vernamycin Bδ,[2] pristinamycin 1C.[3] Ostreogrycin B2, vernamycin Bβ,[2] pristinamycin 1B.[3]

followed by sodium borohydride reduction of the oxidation product and then acid hydrolysis of the reduction product which yielded *N*-(2-hydroxyethyl)aspartic acid which was shown to be identical with an authentic sample by t.l.c. and by its elution time in an amino-acid analyser. These results suggested the presence of a 5-hydroxy-4-oxopipecolyl residue in the antibiotic and consequently the structure (**1**; R^1 = Et, R^2 = Me, R^3 = OH) for ostreogrycin B3.

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