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STRUCTURAL IDENTITY AND STEREOCHEMICAL REVISION OF POLYOXIMIC ACID

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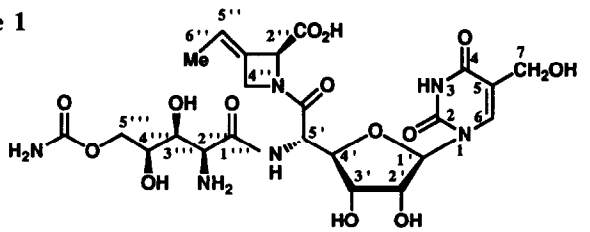
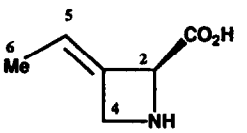
Abstract: The stereochemistry of the exocyclic double bond in polyoximic acid has been revised to *cis*-3-ethylidene-L-azetidine-2-carboxylic acid, based on the single crystal X-ray analysis of the racemic naturally-derived acid, on NMR studies of polyoxin A, and of synthetic samples of *cis*- and *trans*-polyoximic acids.

The isolation and characterization of the polyoxin group of nucleoside antibiotics was described by Isono and co-workers over twenty years ago.[1] In total, fifteen polyoxins, designated as A to O, and having closely related structures were reported. Their structures were the subject of chemical and spectroscopic studies,[2] which among other, unveiled the presence of polyoximic acid **1** as a unique amino acid component in polyoxins A, F, H, and K. The structures and configuration of polyoxin A and polyoximic acid **1** as proposed in 1969 are depicted below.

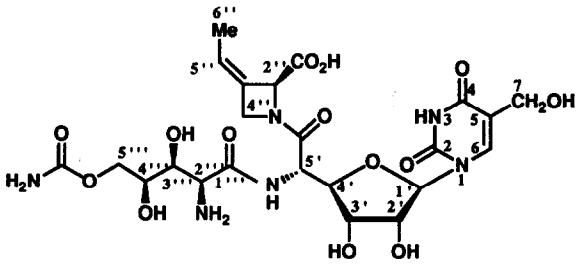
Figure 1



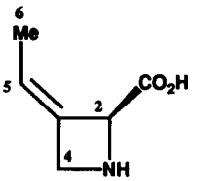
Polyoxin A (original structure)



***1*, *trans*-Polyoximic Acid**



Polyoxin A (revised structure)



***2*, *cis*-Polyoximic Acid**

Mild alkaline or acidic hydrolysis of polyoxin A led to racemic polyoximic acid, (3-ethylidene-azetidine-2-carboxylic acid), the structure and stereochemistry of which was assigned based on its 60 MHz N.M.R. spectrum in D_2O, and by n.O.e studies.[2] The absolute configuration of the amino acid was deduced from the observation of a positive Cotton effect of the N-dithiocarbethoxy dihydropolyoximic acid,[2] and comparisons with the analogous derivative prepared from L-azetidine-2-carboxylic acid.

As a result of our interest in the total synthesis of polyoximic acid in optically pure form,[3] we have discovered a discrepancy in the ^1H NMR spectrum of our synthetic *trans*-3-ethylidene-L-azetidine-2-carboxylic acid[4,5] and that of the authentic amino acid[2] (Figure 2). Intrigued by this, we determined the single crystal structure of the amino acid obtained by acidic hydrolysis of polyoxin A. Quite surprisingly, the stereochemistry of the ethylidene group was found to be *cis* (Figure 3).[6] We then redirected our synthesis plan, and completed an unambiguous total synthesis of the enantiomerically pure *cis*-isomer.[3] The synthetic product was found to be identical to the racemic naturally-derived amino acid in all respects including an X-ray powder diffraction diagram. Its NMR spectrum in CD_3OD is shown in Figure 2. Control experiments were conducted to show that the *cis*- and *trans*- acids were not interconverted during acidic treatment. It is interesting that the vinylic proton and the methyl protons in the *trans*-isomer are shifted downfield and upfield respectively, compared to the signals of the *cis*-isomer. This pattern was also found in D_2O and DMSO-d_6.

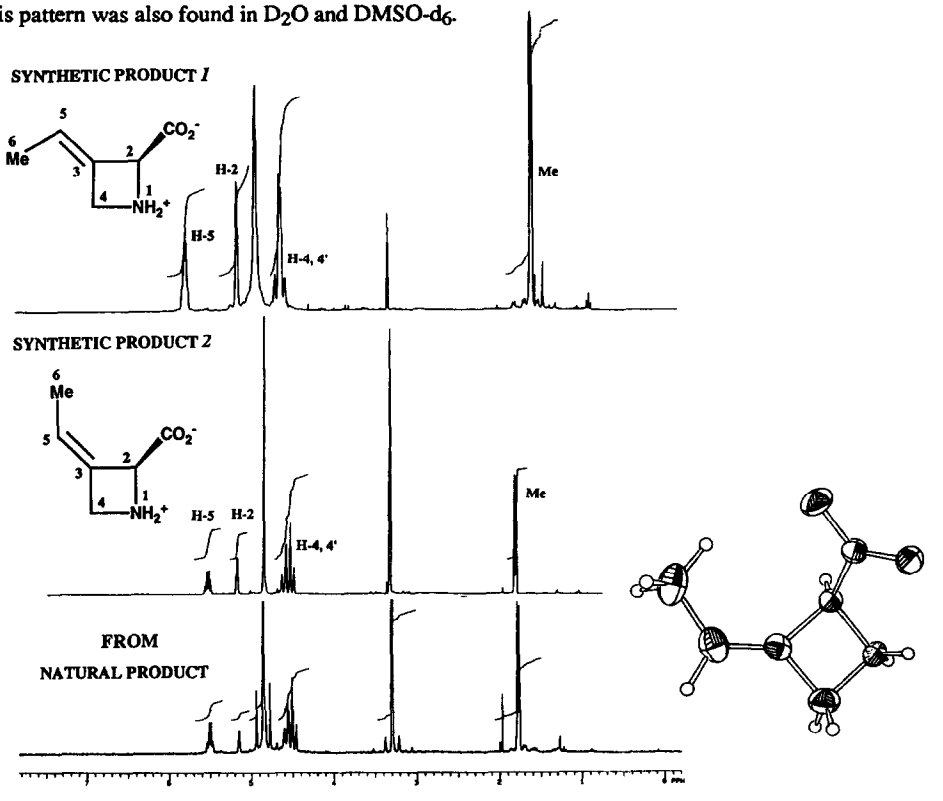


Figure 2. 300 MHz ^1H NMR (CD_3OD) spectra of synthetic *trans* 1, synthetic *cis*-2, and racemic polyoximic acid obtained by acid hydrolysis (ref. 2). ORTEP drawing of the crystal structure of rac. polyoximic acid.

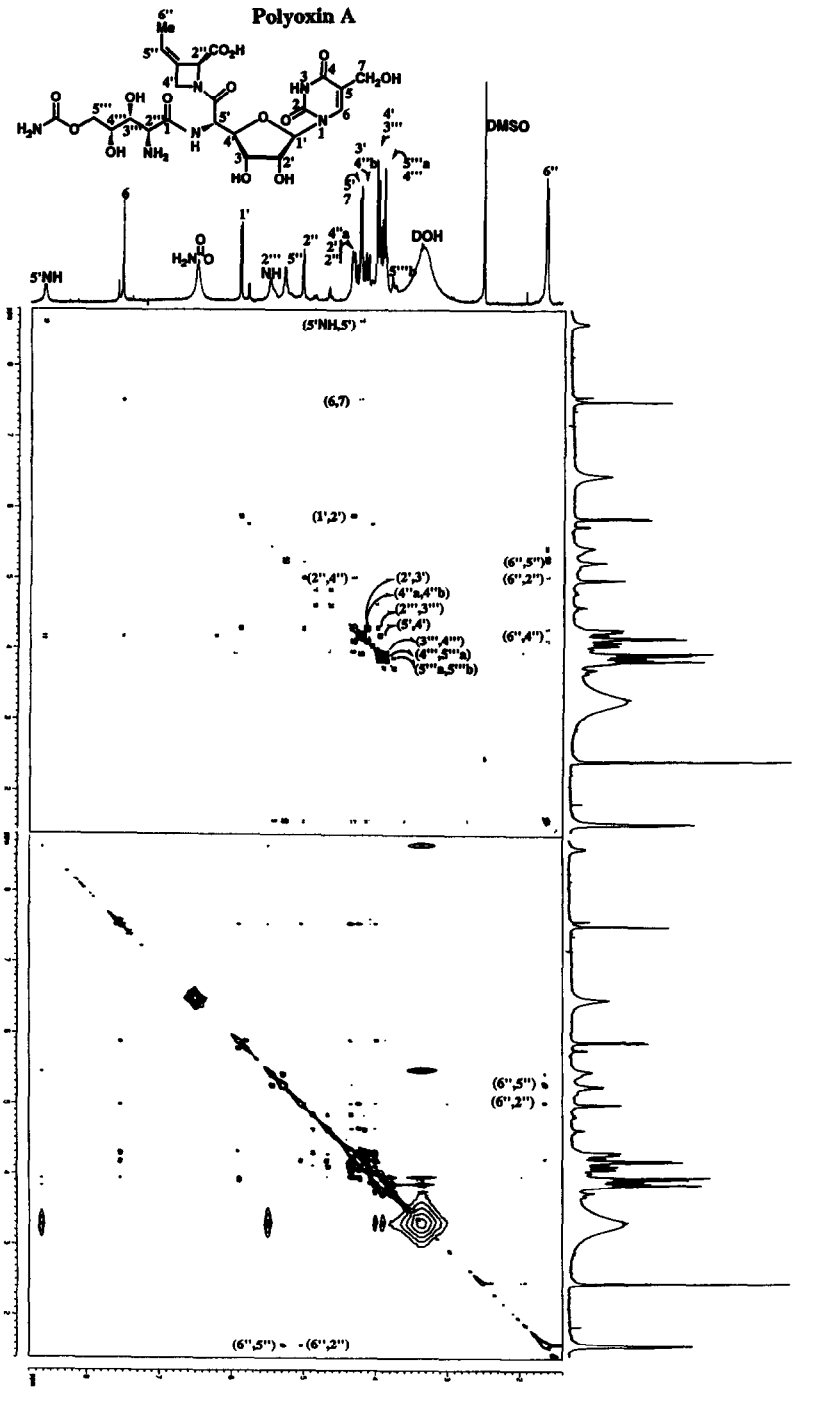


Figure 3. (top). 2D COSY NMR spectrum of natural polyoxin A in DMSO-d_6 at 400 MHz

(bottom). 2D NOESY NMR spectrum in DMSO-d_6 at 400 MHz.

In order to gain further insight into the stereochemical identity of the double bond in the polyoximic acid portion in polyoxin A itself, we carried out extensive ^1H NMR studies at 400 MHz.[7] The one dimensional NMR spectrum of polyoxin A is shown in Figure 3, where the 6"-methyl, 5"-vinylic and 2"-α-carboxamide protons are clearly distinguishable. These correspond in pattern and in chemical shift differences to the *cis*- rather than the *trans*-polyoximic acid moiety.

Proton connectivities by the J-correlated spectroscopy (COSY) technique located the "intact" polyoximic acid moiety (Figure 3, top). Next, we carried out 2-dimensional n.O.e. correlated spectroscopy experiment (NOESY) in order to secure the *cis*-orientation of the ethylidene group vis-a-vis the carboxamide group (Figure 3, bottom). Positive enhancements were observed between the ethylidene methyl group and the α-carboxamide hydrogen, which are concurrent with a *cis*-type geometry. These were independently corroborated by n.O.e. studies on the *cis*- and *trans*- polyoximic acids in D_2O and DMSO-d_6.

Thus, our efforts to synthesize polyoximic acid some 25 years after its isolation and structure determination, has led to a reassignment of the geometry of the double bond originally designated as being *trans*. The structural and stereochemical identity of this diminutive yet intriguing amino acid is thus secured through the combined powers of synthesis, NMR spectroscopy and X-ray crystallography.

Acknowledgments

We thank NSERCC, FCAR and Ciba-Geigy Agro for generous financial assistance. We thank Dr. Michel Simard for the X-ray structural analysis.

References and Notes

1. a. Isolation and characterization, Suzuki, S.; Isono, K.; Nagatsu, J.; Mizutani, T.; Kawashima, Y.; Mizuno, T. *J. Antibiotics*, Ser. A, **1965**, *18*, 131; Isono, K.; Kobinata, K.; Suzuki, S. *Agr. Biol. Chem.*, **1968**, *32*, 792 and previous papers in this series; b. Biosynthesis, Isono, K., Funayama, S.; Suhadolnik, R.J. *Biochemistry*, **1975**, *14*, 2992.
2. Isono, K.; Asahi, K.; Suzuki, S. *J. Am. Chem. Soc.*, **1969**, *91*, 7490.
3. Hanessian, S.; Fu, J.M.; Chiara, J.-L.; Di Fabio, R.; following paper in this issue.
4. For a recent synthesis of the DL-*trans* isomer, see Emmer, G. *Tetrahedron*, **1992**, *48*, 7165.
5. For earlier synthetic studies, see a. Baumann, H.; Duthaler, R.O. *Helv. Chim. Acta*, **1988**, *71*, 1025; b. Baumann, H.; Duthaler, R.O. *ibid*, **1988**, *71*, 1035.
6. Space group and cell dimensions: Monoclinic, P21/c a = 7.223(3), b = 10.472(4), c = 9.185(5) Å,β = 100.97(4)°, Volume = 648.7(5) $Å^3$; crystal dimensions: 0.23 x 0.28 x 0.38 mm. The structure was solved by direct method using MULTAN-80 and difference Fourier synthesis using NRCVAX.
7. All NMR experiments were performed in 5mm o.d. sample tubes at room probe temperature (22 °C) and the samples were degassed by bubbling argon. A Brüker AMX 400 MHz spectrometer was used for 2D experiments and a Varian VXR 300 MHz spectrometer for 1D n.O.e. irradiation. For 2D experiments, double quantum J-correlated spectroscopy sequence and 2D n.O.e. correlated spectroscopy sequence with quadrature detection and time proportional phase increment in both domains were used. The sample concentration of polyoxin A was 8 mM in DMSO-d_6. The 1D n.O.e. experiment was done in DMSO-d_6 and in D_2O with sample concentrations of *cis*- or *trans*- polyoximic acid in the range of 300-500 mM. Chemical shifts were measured in ppm with the solvent peak as reference (2.5 ppm for DMSO-d_6, 4.8 ppm for D_2O, 3.3 ppm for CD_3OD). The relaxation delay was 2 s for 2D experiments and 3 s for 1D n.O.e. and the mixing time for 2D n.O.e. correlated spectroscopy was 600 ms.

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