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THE STRUCTURE OF LEPTOSPHAERIN

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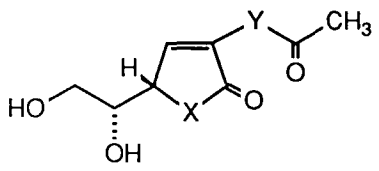
Summary. The structure and relative configuration of leptosphaerin, a metabolite of the marine ascomycete Leptosphaeria oraemaris (Linder) was established as **2** by spectral analysis and elimination of an alternative structure **1** through synthesis.

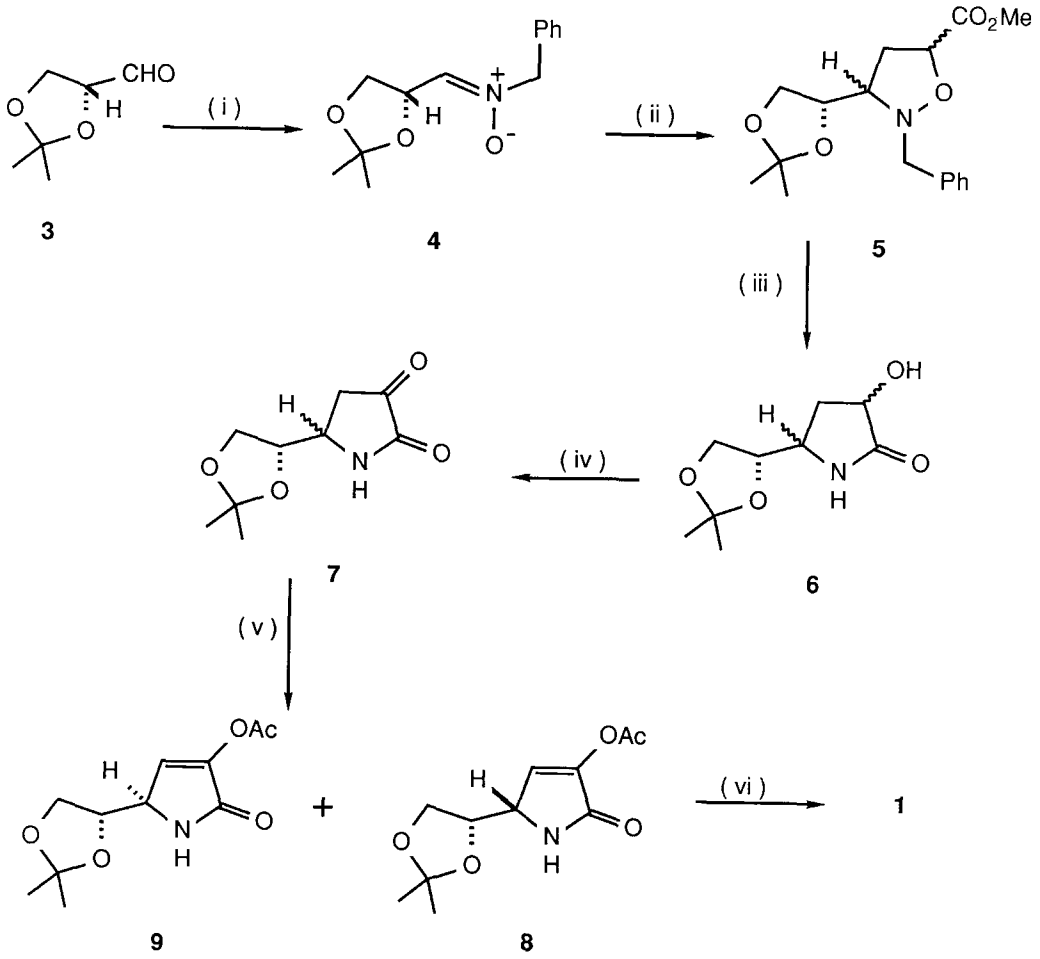
Previous studies have led to the isolation and identification of several metabolites from the marine ascomycete Leptosphaeria oraemaris (Linder).[1] Further examination of extracts of L. oraemaris grown in liquid culture afforded a new crystalline substance (mp 189.5-190.5°C, $C_8H_{11}NO_2$) which we have named leptosphaerin.

The ultraviolet spectrum of leptosphaerin (λ_{max} 246 nm), in combination with its ^1H NMR spectrum which displayed a one-proton signal at δ 7.51, suggested an $\alpha\beta$-unsaturated carbonyl chromophore bearing a β hydrogen. The NMR spectrum also revealed that three exchangeable protons were present, while the infrared spectrum gave evidence for both OH and NH groups. The nature of the hydroxyl functions was made clear when the NMR spectrum of leptosphaerin was



1 X=NH, Y=O
2 X=O, Y=NH

measured in d_6-dimethyl sulfoxide.[2] This solvent permitted observation of OH protons coupled to vicinal CH protons and established the presence of a primary alcohol from a triplet at δ 4.80. After cooling the sample to 10°C, a secondary alcohol function became apparent from a doublet at δ 5.26. Although the coupling between the corresponding carbon-bound protons could not be fully resolved, it was evident from double resonance experiments that the CH_2OH and CHOH units were directly connected. A proton which appeared as a double doublet at δ 5.12 was shown to be coupled to the proton at δ 7.51 by irradiation of the former. This result also confirmed that the proton at δ 5.12 was vicinal to the CHOH function. With the allocation of a three-proton singlet at δ 2.14 to an acetyl group, the ^1H NMR data suggested either 1 or 2 as the gross structure of leptosphaerin.



(i) $PhCH_2NHOH$, $CHCl_3$, 79%; (ii) $CH_2{=}CHCO_2Me$, reflux, 97%; (iii) H_2, Pd/C, 51%; (iv) DMSO, DCC, H_3PO_4; (v) Ac_2O, pyr., 58% from **6**; (vi) $(CO_2H)_2$, H_2O.

Acetylation of leptosphaerin with acetic anhydride in pyridine afforded a substance containing two additional acetyl groups (δ 2.05 and 2.08). The ^{1}H NMR spectrum of this derivative exhibited an eight-line multiplet, which characterized the ABX spin system of a $AcOCH_2CH(OAc)$- subunit while the infrared spectrum confirmed the presence of an NH group from a band at 3360 cm^{-1} that had been partly obscured in the parent substance. The NH proton was identified in the NMR spectrum of the acetate from a broadened signal at δ 7.66 ($CDCl_3$) which was shifted to δ 10.12 in d_6-DMSO.

The ambiguity between expressions **1** and **2** for leptosphaerin was not removed by its infrared spectrum, the carbonyl region (1745, 1670, 1640 cm^{-1}) being compatible with either structure. In order to resolve this dichotomy, as well as establish the relative and absolute configuration of leptosphaerin, it was decided to investigate synthetic routes to **1** and **2**.

The acetonide **3**[3] of (R)-glyceraldehyde was condensed with N-benzylhydroxylamine[4] to give nitrone **4**,[5] which underwent cycloaddition with methyl acrylate in refluxing toluene to yield isoxazolidine **5**. Although the configuration at C3 and C5 of this adduct could not be assigned unequivocally,[6] this proved to be of no consequence for our purpose. Hydrogenolysis of **5** over palladium on carbon furnished the crystalline lactam [7] which, upon Moffatt oxidation,[8] gave the unstable ketolactam **7**. Acetylation of the latter in pyridine was accompanied by epimerization at the γ position of the lactam to furnish **8** and **9** (3:1). These were separated by chromatography and the major crystalline isomer identified as **8** from its ^{1}H NMR spectrum.[9] Exposure of **8** to aqueous oxalic acid gave **1** with chromatographic and spectral properties clearly different from those of leptosphaerin; the minor isomer **9** likewise failed to show any trace of leptosphaerin after hydrolysis.

With lactam **1** excluded as a candidate for leptosphaerin, the alternative α-acetamido-γ-lactone **2** became the logical assignment.[10] This was verified by a single crystal x-ray analysis[11] of leptosphaerin which confirmed structure **2** with the relative configuration shown (Figure 1). Leptosphaerin is thus a 2-aminohexose derivative[12] that has undergone substantial oxidative modification. A synthesis of **2** which establishes the absolute configuration of leptosphaerin is reported in the accompanying Letter.

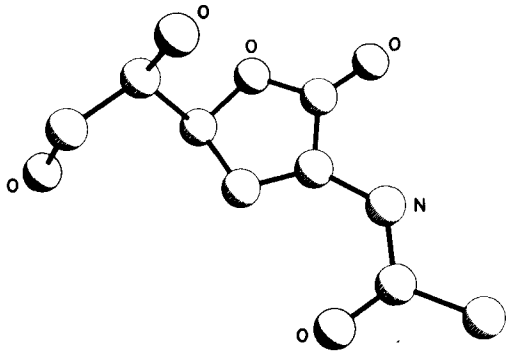


Figure 1. Single Crystal X-Ray Structure of Leptosphaerin

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(6) The vicinal coupling of 3 Hz between C3 and C1′ suggests that these centers bear a threo relationship (ie 3R)[5] and, on the basis of a general preference for an endo transition state in cycloadditions of this type (see Tufariello, J.J. in 1,3-Dipolar Cycloaddition Chemistry; Padwa, A., Ed.; Wiley: New York, 1984), C5 would be (R). A detailed stereochemical investigation of this 1,3-dipolar cycloaddition is in progress.

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(9) Vicinal H5-H1′ coupling was 7 Hz (see ref. 5).

(10) Lactone **2** has been claimed as one of the oxidation products of N-acetylmannosamine with bromine (Pravdic, N.; Fletcher, H.G. Carbohydr. Res. **1971**, 19, 339). While the reported properties of the oxidation product are in reasonable agreement with those of leptosphaerin, we experienced difficulty distinguishing the diastereomeric products in the mixture and could find no firm evidence for the presence of **2**.

(11) Leptosphaerin crystallized with orthorhombic symmetry and lattice constants of a = 4.8917(9), b = 7.657(2), and c = 24.119(3)Å. The systematic extinctions, crystal density, and presence of chirality were uniquely accommodated by space group $P2_12_12_1$ with one molecule forming the asymmetric unit. Full matrix least squares refinements with anistropic heavy atoms and fixed, isotropic hydrogens converged to a standard crystallographic residual of 0.0815.

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