The Structure and Biological Activity of Cercosporamide from *Cercosporidium henningsii*

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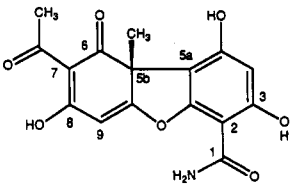
Received October 16, 1990

Summary: Cercosporamide, a potent antifungal agent and phytotoxin from the fungus *Cercosporidium henningsii*, was characterized as a heterodimer related to usnic acid.

Cassava (*Manihot esculenta*), a tuber of the spurge family Euphorbiaceae, is a primary root crop for human consumption in semitropical to tropical regions. The plant is extensively cultivated for domestic consumption in west tropical Africa, South America, and throughout the South Pacific regions and is an important export in the form of tapioca. "Brown leaf spot" is a major leaf disease of cassava caused by *Cercosporidium henningsii*.[2] Infection of leaves by *Cercosporidium henningsii* causes necrotic lesions—symptoms reminiscent of those caused by known phytotoxins. The development of these lesions in affected leaves generally results in leaf drop, leading to a direct loss in tuber yield. Few chemically directed phytotoxin studies on *C. henningsii* or other disease-causing pathogens of cassava have appeared[2] despite the importance of this plant as a food crop in less developed regions of the world where it is grown in soils of marginal quality and diseases can dramatically decrease yields. If the *C. henningsii* phytotoxins could be identified, they could be used in cassava tissue culture systems to find toxin-insensitive cassava biotypes. Our studies on *C. henningsii*, including the isolation and characterization of an unusual bioactive amide, are the subject of this report.

Two strains of *C. henningsii* were obtained from the CMI culture collection. Each strain was grown in shake culture for 3 weeks at 23 °C on an M-1-D medium[3] containing an extract of cassava leaves, and the different strains produced different phytotoxins. The Thailand strain produced citrinin (2),[4] which was identified by spectral studies. The African strain of *C. henningsii* produced the structurally novel phytotoxin cercosporamide (1). The structure of cercosporamide (1) was deduced by single-crystal X-ray diffraction analysis and 2D long range ^1H–^{13}C COSY[5] NMR techniques.



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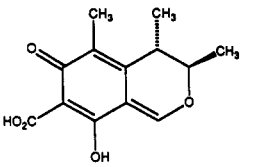

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The isolation of cercosporamide was exceptionally easy; the ethyl acetate extract of culture broth 1:1 (v/v) was evaporated under reduced pressure to yield a red precipitate which was quickly washed several times with cold ethyl acetate. This residue was dissolved in ethyl acetate and cooled to −5 °C to give crystals (mp 188–189 °C) of optically active ($[\alpha]_D$ = −26°) cercosporamide. A further recrystallization gave crystals suitable for single-crystal X-ray diffraction analysis.

Cercosporamide crystallized in the orthorhombic space group $P2_12_12_1$ (Z = 8) with the following unit cell parameters: a = 12.426 (2), b = 14.423 (2), and c = 16.597 (4) Å. A total of 1023 independent reflections (Cu Kα radiation, $2\theta \leq 114°$) were collected at room temperature, and 953 of these were judged observed ($|F_o| \geq 4\sigma(|F_o|)$). The structure was solved by direct methods using the MULTAN80 package of programs and refined using full-matrix least-squares techniques to standard discrepancy indices of R = 0.0516 and R_w = 0.0708 (see the supplementary material). The asymmetric unit contained two structurally identical but crystallographically distinct molecules, only one of which is shown in Figure 1.

The spectral data for cercosporamide were wholly consistent with 1. A molecular ion at m/z 331.0691 in the high-resolution EIMS indicated $C_{16}H_{13}N_1O_7$ (calcd 331.0692). In addition to the nonexchangeable protons, there were five D_2O exchangeable signals in the ^1H NMR spectrum (see the supplementary material). Two phenol

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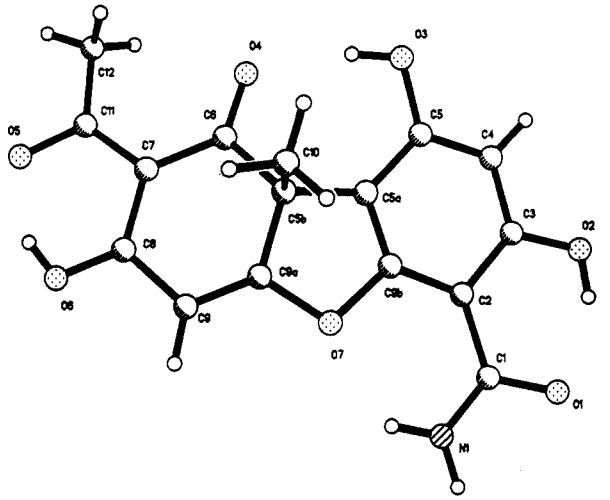

Figure 1. A computer-generated perspective drawing of the final X-ray model of cercosporamide (1). The absolute configuration shown is arbitrary.

protons were shifted downfield to δ 10.50 and 13.28 due to hydrogen bonding with adjacent carbonyl groups. Slowly exchangeable amide protons between δ 6.54 and 7.12 were clearly observed in 2D NOESY.[5]

The lowest shifted signal at δ 18.80 suggested the presence of a keto–enol tautomer. This was verified by ^{13}C NMR (125 MHz) signals at δ 200.76, 104.60, and 190.89, appropriate for a tautomeric system. These spectral data and the approximately equal bond lengths (~1.45 Å) determined by X-ray analysis for C7–C8, C8–C9, and C8–C11 indicate that cercosporamide exists, both in solution and in the solid state, as a tautomeric mixture. Further assignments of the ^{13}C NMR spectrum were performed with 2D long-range ^{1}H–^{13}C COSY (see the supplementary material). An aromatic proton at δ 6.10 on C4 has cross peaks in 2D ^{1}H–^{13}C COSY with C5 at 156.5 and C3 at 164.7 by a two-bond coupling, and C2 at δ 93.0 by a three-bond coupling. Similarly, an olefinic proton at δ 6.00 on C9 has cross peaks with C9a at δ 177.7 by a two-bond coupling and C5b at 57.9 and C7 at 104.5 by a three-bond coupling.

Cercosporamide is related to the well-known fungal metabolite usnic acid, and they presumably share an oxidative coupling biosynthesis.[7] But while usnic acid and known related metabolites arise from the coupling of two identical units, cercosporamide unites two different units. The biological activity of cercosporamide is also noteworthy. In leaf puncture wound tests[8] (2 μL of a 0.1 mg/mL solution) cercosporamide produces lesions on cassava, corn (*Zea mays*), and purslane (*Portulaca oleracea*). Since cercosporamide has no effect on dandelion (*Taraxacum offinale*), tomato (*Lycopersicon esculentum*), and cucumber (*Cucumis sativus*), it is a host-selective toxin. In a cassava protoplast assay cercosporamide exhibited an LD_{50} of 20 μM at 2 h in a flow cytometry assay system.[9] Cercosporamide was also tested against various human pathogenic yeasts, dermatophytes, and opportunistic fungi employing an in vitro minimum concentration test[10] that gave values as low as 1 μg/mL. Inhibition of serine/threonine kinases, including protein kinase C (IC_{50} 1.6 μM) and myosin light chain kinase (IC_{50} 13 μM) was also observed.

Acknowledgment. J.C. and G.S. thank the NSF (DMB86-07347) for financial support. Appreciation is expressed to J. Sears, Montana State University, for gathering MS data, to G. Gray of the Beckman Instrument Company for doing COSY and NOESY experiments, to J. Uzawa for collecting ^{1}H NMR data, to David Loebenberg for antifungal assays, and to Robert Bishop for PKC/MLCK assays. The culture of *C. henningsii* used in this work was obtained from the Commonwealth Mycological Institute, London, England.

Supplementary Material Available: Experimental procedures, spectral assignments, tables of fractional coordinates, thermal parameters, and interatomic distances and angles for 1 and spectral data for 1 and 2 (9 pages). Ordering information is given on any current masthead page.

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Enantioselective Transformation of Propargyl Esters to Dihydrofurans

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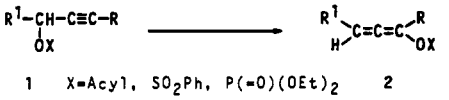
Summary: Transformation of enantiomerically enriched propargyl esters **5** into dihydrofurans **6** with complete enantiospecificity is achieved by Ag(I)-catalyzed rearrangement and cyclization, and the sequence is successfully applied to the enantioselective synthesis of an antitumor protective and hypolipidemic antibiotic, (*S*)-(−)-ascofuranone.

Silver(I)-mediated rearrangement of propargyl esters **1** has been known to give allenic products **2** as shown in Scheme I.[1] Racemization, however, took place during the transformation of enantiomerically enriched esters **1** into **2**.[2] Previously, we proposed allenic species **2** (R = C(OH)R^2_2) as reaction intermediates of silver(I)-mediated conversion of the 2-butyne-1,4-diol derivatives **5** into dihydrofurans **6**.[3] If cyclization of **2** (R = C(OH)R^2_2) into **6** was much faster than the racemization, chiral products might be produced with stereospecificity.[4] Herein we

Scheme I



1 X=Acyl, SO_2Ph, P(=O)(OEt)$_2$ 2

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