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THE STRUCTURE OF GLOEOSPORONE, A NOVEL GERMINATION SELF-INHIBITOR FROM CONIDIA OF *Colletotrichum gloeosporioides*

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Abstract: Gloeosporone, a germination self-inhibitor from *Colletotrichum gloeosporioides,* has the constitution 4, as deduced from IR, ^{13}C and ^{1}H NMR, and MS data.

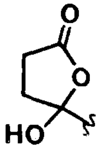
Conidia of *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *jussiaea* germinate poorly when sown in dense concentrations compared to diluted conidia. Aqueous exudates from dense conidial suspensions inhibit the germination of diluted conidia. Evaporation of such exudates, extraction with $CHCl_3$, and crystallization from hexane provides a pure metabolite, mp. 108-110°C, which has the same germination inhibiting effect. [2] We propose gloeosporone as the trivial name for this self-inhibitor. [3]

IR spectra show the presence of OH (3570, 3410 cm^{-1}), saturated ketone (1710), and γ-lactone functionality (1770). ^{13}C NMR confirms the presence of the two C=O's (209.0 and 174.4 ppm), and also shows that the molecule contains no additional unsaturation, but has one ketal or hemiketal carbon (99.0), two saturated $-\overset{|}{C}H-O$ carbons (74.4 and 73.5), 12 non-oxygenated CH_2's (40.4-21.2), and one CH_3 group (14.0). The non-isotopic ion of highest *m/z* in the high-resolution EI mass spectrum is $C_{18}H_{28}O_4^{+}$ (*m/z* 308.1989), and 309 is also the highest *m/z* ion in the CH_4 CI MS. [4] Yet gloeosporone itself cannot be $C_{18}H_{28}O_4$ because (a) the EI spectrum shows a $C_{13}H_{19}O_5$ ion (*m/z* 255.1225) and (b) the oxygen functions revealed above demand at least 5 oxygen atoms. [5] A molecular formula of $C_{18}H_{30}O_5$ thus becomes evident, the unseen molecular ion fragmenting (among other paths) by loss of H_2O to *m/z* 308 and by loss of C_5H_{11} to *m/z* 255. [6,7]

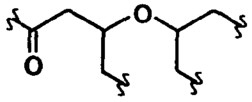
^{1}H NMR (90, 300, and 500 MHz with appropriate spin-decoupling) identifies the $-\overset{|}{C}H-O-$ environments as $-CH_2-C\mathit{H}(O-)-C\underline{H}_2-Z$ (H at 4.43, $\underline{H}$ at 2.04 and 2.73 ppm; $J_{\mathit{H}\underline{H}}$ 6.2 and 8.3, $J_{\underline{HH}}$

-18.7, J_{HH} 1.8 and 9.5 Hz) and $-CH_2C\mathit{H}(O-)-CH_2-$ (H at 5.06 ppm; J_{HH} 2.8, 5.7, 7.7, and 8.8 Hz), where Z is carbon bearing no hydrogen (either a C=O or the -O-C-O-), and also shows the presence of a $Z-CH_2-CH_2-Z$ system (2.44, 2.35, 2.27, 2.10 ppm; J_{gem}'s -14.8, -14.1 Hz; J_{vic}'s 3.7, 8.4, 3.4, 8.8 Hz). Only these three $Z-\underline{CH_2}$'s are attached to deshielding carbon (C=O or O-C-O), since all other ^1H resonances are upfield of 1.7 ppm. The CH_3 is on a CH_2 (t at 0.88 ppm).

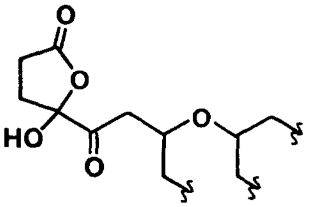
If the Z's flanking $-CH_2CH_2-$ were both C=O ($-C(=O)CH_2CH_2C(=O)O-$), gloeosporone could not be a γ-lactone; therefore that segment is $-O-C(O-)CH_2CH_2C(=O)-$ and the other C=O is the Z terminus of $-CH_2CH(O-)CH_2-$.[8] Upon addition of DMSO-d_6 to the $CDCl_3$ 300 MHz ^1H NMR solution, a broad 3.5 ppm resonance is replaced by a new sharp 5.37 ppm singlet which disappears upon addition of D_2O. This must be the O$\underline{H}$, which is therefore tertiary and part of O-C-O rather than a $-\overset{|}{C}H-O$. Hence the molecule contains $HO-\overset{|}{C}(O-)CH_2CH_2C(=O)-$ (called C=O A below), $-CH_2CH(O-)CH_2C(=O)-$ (C=O B), and $-CH_2CH(O-)CH_2-$, but only five oxygens. Consequently one of the disubstituted oxygens is duplicated in these three substructures (*i.e.*, an ether is present) and the other is the lactonic $C(=O)\underline{O}-$. Carbonyl B cannot be the lactone, because its γ-carbon carries no oxygen. Accordingly, B is the ketone and A the lactone. But for A to be the lactone it must connect to the ketal carbon; this reduces the oxygenated moieties of gloeosporone to 1 and 2:



1



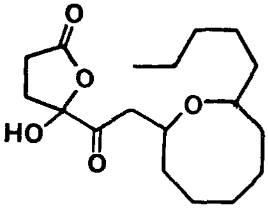
2



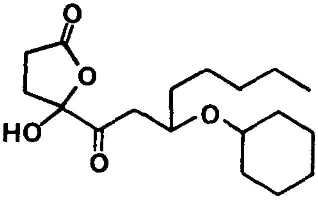
3

No non-protonated carbons remain, but the absence of additional ^1H resonance below 1.7 ppm prohibits the C=O of 2 from holding a second CH_2. Thus 1 and 2 are joined as 3. This clarifies the existence of a stable γ-lactol; such tautomeric cyclization of a γ,δ-diketo acid would not be surprising, with formation of the δ-keto-γ-lactol rather than the γ-keto-δ-lactol being the anticipated result.[9] Partial structure 3 also explains facile loss of H_2O in CI as well as EI MS (OH-protonated 3 should eject H_2O much more readily than a simple ROH_2^+), and the unusually low-field resonance of the DMSO-complexed O$\underline{H}$.[10]

Partial structure 3 needs but six CH_2's and the terminal CH_3 for completion. The keys to their location are in the MS. Ions from loss of C_5H_{11} are present, but those from loss of C_4H_9 or C_6H_{13} are not. Evidently a $(CH_2)_4CH_3$ segment is attached to a point of preferential fragmentation, which must be α to the ether oxygen of 3, with the three remaining CH_2's bridging the other two open positions of 3 to form either 4 or 5.



4



5

The presence of several series of large hydrocarbon ions, up to $C_{13}H_{24}$ (*m/z* 180.1873, 10% relative intensity), eliminates 5, which could beget nothing above C_7 hydrocarbons. Thus the constitution 4 is assigned to gloeosporone, with the relative and absolute configurations undefined.

A few additional points merit note here, even though their full discussion must be deferred to a full paper. First, reasonable mechanisms can be proposed for conversion of 4 to all of the 27 prominent ions which appear in the high-resolution EI MS. The most revealing are $C_4H_5O_3^+$, the base peak, (probably an ion with structure 1); $C_4H_7O_4^+$ (41%), which shows the contiguity of four of the five oxygens; and the $C_{13}H_{24}^+$ hydrocarbon ion mentioned above. Second, although to our knowledge the oxacane ring is unique among presently-known natural products, the oxygenation pattern of 4 is biogenetically unexceptional. Only the ketal carbon (C-4) is an oxidized site not directly compatible with a polyketide progenitor. It is even more amusing to note that a position corresponding to C-4 is exactly the one and only point of "extra" oxygenation in several macrodiolides, such as colletodiol,[11] produced by different *Colletotrichum* species.

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REFERENCES AND NOTES

1) a) Dept. of Chemistry, University of Arkansas. - b) Currently on leave at the Lab. für Organische Chemie, ETH Zürich; please correspond through the Arkansas address. - c) Dept. of Plant Pathology, University of Arkansas.

2) *A.R. Lax, G.E. Templeton,* and *W.L. Meyer,* Phytopathology, 74, 503 (1982) (Abstract); *A.R. Lax,* Ph.D. Dissertation, University of Arkansas, 1983.

3) Cf. *P.J. Allen* in "Physiological Plant Pathology", *R. Heitefuss* and *P.H. Williams,* eds., Springer Verlag, New York, 1976, pp 51-85.

4) CI and high-resolution EI MS data were obtained from the Midwest Center for Mass Spectrometry, Lincoln, NE, USA.

5) The $-\underline{O}H$ and $C(=O)\underline{O}-$ alone cannot satisfy the $-O-\underline{C}-O-$ and two $-\underline{C}H-O-$ carbons.

6) Formulas from which $C_{18}H_{28}O_4^+$ would be derived by loss of two or more H_2O's are incompatible with a C_{18} molecule containing only one methyl group and the oxygen functions detected by IR and NMR.

7) We have also recently obtained from *Prof. J. Seibl* (ETH) a low-resolution EI spectrum in which a very weak molecular ion (*m/z* 326) can indeed be discerned.

8) The Z terminus of $-CH_2CH(O-)CH_2Z$ cannot be the O-C-O or the same C=O that terminates ZCH_2CH_2Z, because a γ-lactone cannot be derived from such structures without also incorporating additional protons α to a C=O, which would be seen below 1.7 ppm.

9) *H.C. Brown, J.H. Brewster,* and *H. Shechter,* J. Am. Chem. Soc., 76, 467 (1954).

10) *O.L. Chapman* and *R.W. King,* J. Am. Chem. Soc., 86, 1256 (1964).

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