Verrucosidin, a Tremorgen from *Penicillium verrucosum* var *cyclopium*

Leo T. Burka,* Maya Ganguli, and Benjamin J. Wilson

Department of Chemistry and Center in Environmental Toxicology, Vanderbilt University, Nashville, Tennessee 37235, U.S.A.

The structure of a nitrogen-free fungal neurotoxin, verrucosidin, isolated from *Penicillium verrucosum* var *cyclopium*, has been established as (**1**) by chemical, spectroscopic, and *X*-ray crystallographic methods.

The structure of the tremorgenic mycotoxin, herein named verrucosidin, isolated earlier[1] from the fungus *Penicillium verrucosum* var *cyclopium*, has now been established as (**1**). The neurotoxin is unusual in that it contains no nitrogen, while almost all other fungal tremorgens with known structures contain an alkaloidal element.[2]

The compound crystallizes from ether as colourless plates, m.p. 90—91 °C, $[\alpha]_D^{26}$ +92.4° (*c* 0.25, MeOH). The empirical formula, $C_{24}H_{32}O_6$, was determined by mass spectrometry (*m/z* 416) and by elemental analysis. The i.r. spectrum revealed a C=O stretch at 1700 cm^{-1} and strong C–O–C absorption in the 1240—845 cm^{-1} region; no other signals for oxygen-containing functional groups were present. The ^1H and ^{13}C n.m.r. spectra† revealed that more than one third of the carbon atoms were methyl groups: 3 bonded to quaternary sp^3 carbon, 4 to quaternary sp^2 carbon, and one each to a methine sp^3 carbon and to an oxygen atom. The presence of a 6-substituted 4-methoxy-3,5-dimethylpyrone moiety was suspected suggesting a possible structural relationship to citreoviridin (**2**),[3,4] and associated mycotoxins.[3,5,6] In fact the five ^{13}C n.m.r. signls of the pyrone unit of (**2**) bear a close relationship to those of (**1**) except that the signal at δ 88.2 p.p.m. for unmethylated C-2 of (**2**) is replaced by one at 110.0 (or 110.6 p.p.m.) for methylated C-2 of (**1**) (Table 1). A major difference, however, is that the u.v. spectrum of (**1**) [λ_{max} 294 nm (ε 13 000) and 241 nm (ε 21 000)] is consistent with an isolated pyrone, whereas extended conjugation in (**2**) leads to a maximum at 388 nm (ε 48 000).

The $C_{16}H_{23}O_3$ fragment attached to C-6 of the pyrone contains the two trisubstituted double bonds, 6 of the methyl groups, and three oxygen atoms, all of which must be present as epoxides or other cyclic ethers. Degradation of (**1**) with KOH (0.5 M in H_2O–MeOH, 1 : 1) gave two fragments, one a carboxylic acid, $C_{10}H_{14}O_4$, for which the structure is not yet conclusively known and the other an aldehyde, $C_{14}H_{20}O_3$ (**3**). The u.v., i.r., and ^1H and ^{13}C n.m.r. spectra‡ of (**3**) established that the aldehyde was present as a 5-substituted-2,4-dimethylpenta-2,4-dienal unit. The remainder of the structure of (**3**) was tentatively assigned by comparison with (**2**). The linkage between the pyrone and side-chain fragments was assigned as an epoxidized alkene which isolates the pyrone chromophore from the diene.

Confirmation of structure (**1**) and assignments of the relative configurations of the six chiral centres was obtained by *X*-ray crystallography. The compound crystallizes in the orthorhombic space group $P2_12_12_1$ with a = 5.7971(8), b = 11.912(2), c = 33.634(5) Å, Z = 4, D_c = 1.19 g/cm^3. Intensity data were collected on a Nicolet R3m/E Crystallographic System equipped with a copper-target *X*-ray tube, a graphite-crystal monochromator, and an automatic attenuator. Data were collected using the ω scan method with a variable scan rate ranging from 3.91 to 29.30°/min. One set of data was

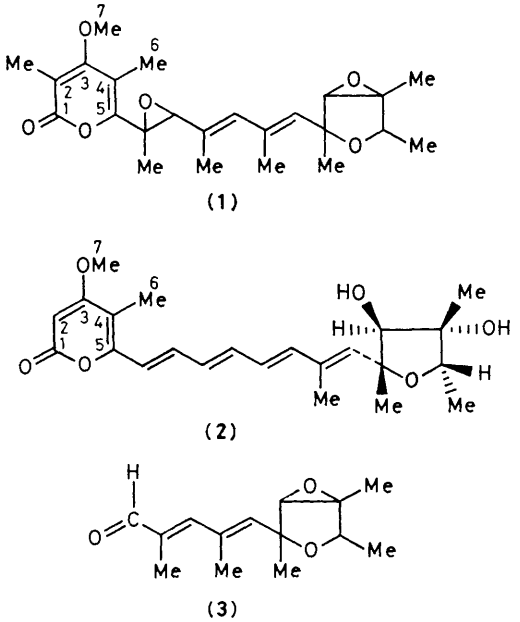

Table 1. ^{13}C N.m.r. chemical shifts (δ in p.p.m.) of the pyrone moiety of verrucosidin (**1**) and citreoviridin (**2**).

	C-1	C-2	C-3	C-4	C-5	C-6	C-7
(**2**)	163.9	88.2	170.6	107.6	154.4	8.8	56.1
(**1**)	164.5[a]	110.0[b]	167.5[a]	110.6[b]	155.6	8.9	60.0

[a,b] Signals with the same letter may be interchanged.

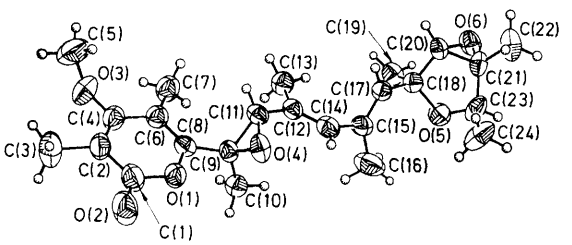

Figure 1. ORTEP view of the crystal structure of verrucosidin showing the crystallographic numbering scheme.

† ^1H N.m.r. ($CDCl_3$) δ 1.21 (3H, d, *J* 7 Hz), 1.42 (s), 1.44 (s), and 1.48 (s) (total of 9H), 1.93 (s) and 1.98 (s) (total of 6H), 2.05 (6H, s), 3.45 (1H, s), 3.50 (1H, s), 3.85 (3H, s), 4.12 (1H, q, *J* 7 Hz), 5.50 (1H, s), and 5.88 (1H, s); ^{13}C n.m.r. ($CDCl_3$) δ (off-resonance multiplicity) 8.9 (q), 10.0 (q), 13.5 (q), 15.0 (q), 15.2 (q), 18.2 (q), 18.5 (q), 21.6 (q), 60.0 (q), 60.3 (s), 64.4 (d), 67.1 (s), 67.1 (d), 76.4 (d), 79.7 (s), 110.0 (s), 110.6 (s), 127.7 (s), 131.1 (d), 132.7 (d), 134.2 (s), 155.6 (s), 164.5 (s), and 167.5 (s) p.p.m.

‡ λ_{max} (MeOH) 277 nm (ε 11 817) (α,β-unsaturated aldehyde); ν_{max} ($CDCl_3$) 2975, 2930, 1670 (O=CH–C=C–C=C), and 1610 cm^{-1}; ^1H n.m.r. ($CDCl_3$) δ 1.19 (3H, d, *J* 7 Hz), 1.45 (3H, s), 1.48 (3H, s), 1.95 (3H, s), 2.16 (3H, s), 3.44 (1H, s), 4.15 (1H, q, *J* 7 Hz), 5.91 (1H, s), 6.68 (1H, s), and 9.41 (1H, s, –CHO); ^{13}C n.m.r. ($CDCl_3$) δ 10.80 (q), 13.78 (q), 17.73 (q), 18.87 (q), 21.53 (q), 67.19 (d), 67.47 (s), 76.8 (d), 80.03 (s), 135.24 (s), 137.13 (s), 140.22 (d), 154.14 (d), and 195.80 (d) p.p.m.

collected for the (*hkl*) octant with $3° < 2\theta < 116°$. This set was used for the solution and preliminary refinement of the structure. A second set was collected for the $(-h, -k, -l)$ octant with $3° < 2\theta < 100°$ in an unsuccessful attempt to determine the absolute configuration of the compounds. All data were used in the final refinement. The structure was solved by the direct methods routine of the SHELXTL software package using default parameters. The structure was refined to final residuals of $R_1 = 5.8\%$ and $R_2 = 6.4\%$ for 2513 independent observed reflections with $I \geqslant 3\sigma(I)$. An ORTEP diagram of verrucosidin is shown in Figure 1.§

Verrucosidin appears to be a pyrone of polyketide origin in which all C_2 units except the starter unit bear a methyl substituent. The structure could arise by propionate (*i.e.*, methylmalonyl CoA) being employed to extend the polyketide chain; alternatively methylation may have occurred at each methylene position of a polyketide intermediate derived solely from acetate. A variety of such methylated species are observed among the naturally occurring analogues of (**2**).[3-5]

This research was supported by grants from the U.S.P.H.S. to Vanderbilt University. We thank Dr. C. F. Campana of Nicolet XRD Corporation for the *X*-ray determination, Ms. J. Doran for technical assistance, and Professor T. M. Harris for stimulating discussions.

Received, 9th February 1983; Com. 191

§ The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Rd., Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication,

References

1 B. J. Wilson, C. S. Byerly, and L. T. Burka, *J. Am. Vet. Med. Assoc.*, 1981, **179**, 480.
2 R. J. Cole in 'Mycotoxins in Human and Animal Health,' eds. J. V. Rodricks, C. W. Hasseltine, and M. A. Mehlman, Pathotox Publishers, Inc., Park Forest South, Illinois, 1977, pp. 583—595.
3 M. Niwa, T. Endo, S. Ogiso, H. Furukawa, and S. Yamamura, *Chem. Lett.*, 1981, 1285.
4 B. Franck and H. P. Gehrken, *Angew. Chem.*, 1980, **19**, 461.
5 S. Rebuffat, D. Davoust, L. Molho, and D. Molho, *Phytochemistry*, 1980, **19**, 427.
6 G. J. Kruger, P. S. Steyn, R. Vleggaar, and C. J. Rabie, *J. Chem. Soc., Chem. Commun.*, 1979, 441.