X-Ray Structures of Phlebiakauranol and Phlebianorkauranol, Highly Oxygenated Antibacterial Metabolites of *Phlebia strigosozonata*

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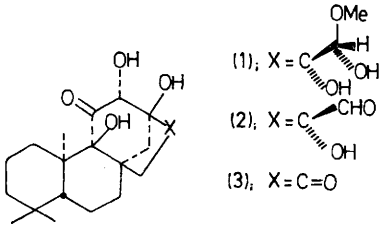
Summary Two new antibacterial metabolites, phlebiakauranol (**1**) and phlebianorkauranol (**3**) have been isolated from *Phlebia strigosozonata* and shown to have highly oxygenated kaurane structures.

THE basidiomycete *Phlebia strigosozonata* produces two closely related, colourless, antibacterial metabolites in addition to the pigment phlebiarubrone,[1] and we have assigned highly oxygenated kaurane structures to both. The metabolites, which are present in the culture liquid as well as the mycelium of the fungal culture, were separated by countercurrent distribution. Fractions were selected on the basis of their antibacterial activity as well as their behaviour with alkali.† Both metabolites were active against our test strain of *Staphylococcus aureus* in a dilution of 16 μg/ml.



Phlebiakauranol (**1**), m.p. 208—213° (from MeOH) was optically active [c.d. (MeOH) 323 nm, $\Delta\epsilon$ + 110·6°] and mass spectrometry indicated the empirical formula $C_{20}H_{30}O_6$ (*m/e* 366·2040) while elemental analysis indicated the formula $C_{20}H_{30}O_6 \cdot 2MeOH$. Phlebiakauranol had no u.v. absorption above 210 nm,† its i.r. spectrum ($CHCl_3$) showed a strong OH absorption at 3600 cm^{-1} and a broad CO band at 1700 cm^{-1}, and its n.m.r. spectrum was complex but suggested a diterpenoid structure [δ 0·80, 0·84, and 1·38 (each s. Me) and 3·25 (6H, initially attributed to 2 MeOH of solvation)]. The δ 3—4 region was obscured by OH resonances and attempted D_2O exchange caused gel formation. No peaks were present above δ 4. Prolonged heating of phlebiakauranol (**1**) at 65 °C under reduced pressure caused loss of MeOH and appearance of an aldehydic singlet at δ 9·57. The broad singlet at δ 3·78 became more readily visible, and a poorly resolved peak at δ 4·00 in the original sample disappeared. The apparent complexity of phlebiakauranol and the small amounts available prompted a single crystal *X*-ray diffraction analysis.

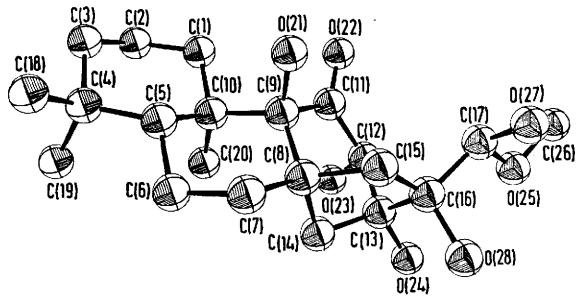


FIGURE. A perspective drawing of the *X*-ray diffraction structure of phlebiakauranol (**1**). Hydrogens are omitted and absolute stereochemistry is not implied.

Single crystals from MeOH belong to the common monoclinic space group $P2_1$ with a = 6·992(1), b = 12·535(1), c = 13·001(1) Å, β = 97·694(6)°. A total of 1503 unique diffraction maxima with $\theta \leqslant 55°$ were recorded using a fully automated four-circle diffractometer and monochromated Cu-K_α (1·5418 Å) *X*-rays. After Lorentz,

† On basification both phlebiakauranol (**1**) and phlebianorkauranol (**3**) rearrange, producing a strong u.v. absorption at 326 nm. Acidification causes a shift to 287 nm. As yet we have been unable to identify the products of these rearrangements.

polarization, and background corrections, 1328 reflections were judged observed [$|F_o| \geqslant 3\sigma(|F_o|)$]. The structure was solved by a multisolution tangent formula approach coupled with the recycling of a plausible molecular fragment.[2] Full-matrix, least-squares refinement with anisotropic temperature factors for C and O and isotropic temperature factors for H converged to $R = 0{\cdot}034$ for the observed reflections.[3] The final X-ray model is shown in the Figure.

The unusual hemiacetal structure (**1**) is fully consistent with the spectral and chemical data. The low shift of the hemiacetal carbon proton (δ 4·00) could be due to shielding by the C(11) carbonyl. Heating (**1**) *in vacuo* converts it into phlebiakauranol aldehyde (**2**). Phlebiakauranol may be an artifact derived from the aldehyde (**2**) or its hydrate. Phlebianorkauranol (**3**), m.p. 218—228° (from EtOAc), has a c.d. maximum at 312 nm, $\Delta\epsilon$ +92·1°. Elemental analysis and high-resolution mass spectrometry indicated a molecular formula of $C_{19}H_{28}O_5$, and its i.r. spectrum was nearly identical to that of (**1**) with the addition of a CO band at 1740 cm^{-1}. Single crystal X-ray diffraction showed that (**3**) belonged to the monoclinic space group C_2 with $a = 12{\cdot}951(6)$, $b = 7{\cdot}313(3)$, $c = 17{\cdot}884(8)$ Å, $\beta = 94{\cdot}83(4)°$. Full-matrix least-squares refinement with anisotropic temperature factors for C and O and isotropic H atoms converged to $R = 0{\cdot}036$ for the 993 observed reflections.[3] The structure is similar to that of (**1**).

The n.m.r. spectrum of (**3**) was similar to that of (**1**) but had a doublet (J 2·5 Hz) at δ 3·62, which we assign to the acyloin proton on C(12) split by the bridge proton on C(14).[4] Such coupling was not visible in (**2**) perhaps owing to slight conformational differences which distort the W planarity of these protons.[5]

Phlebianorkauranol (**3**) on warming with Ac_2O (60—70°C, 72 h) forms a monoacetate, m.p. 210—213; m/e 378·2063. As anticipated the acyloin proton shifts to δ 5·00 and remains a doublet.

Kauranes have apparently not been isolated previously from Basidiomycetes, and since they are biogenetic precursors of gibberellins,[6] we are investigating whether gibberellins may also be present. It has been recently reported that crude extracts from several Basidiomycete sporophores have giberellin-like activity but no pure compounds have been isolated.[7]

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[1] T. C. McMorris and M. Anchel, *Tetrahedron*, 1967, **23**, 3985.
[2] P. Main, M. Woolfson, and G. Germain, MULTAN, Department of Physics, University of York, 1971.
[3] The following library of crystallographic programs was used: C. R. Hubbard, C. O. Quicksall, and R. A. Jacobson, 'The Fast Fourier Algorithm and the Programs ALFF, ALFFDP, ALFFT, and FRIEDEL,' U.S.A.E.C. Report IS-2625, Iowa State University Institute for Atomic Research, Ames, Iowa, 1971; W. R. Busing, K. O. Martin, and H. A. Levy, 'A Fortran Crystallographic Least Squares Program,' U.S.A.E.C. Report ORNL-TM-305, and C. Johnson, 'ORTEP, A Fortran Thermal-Ellipsoid Plot Program,' U.S.A. E. C. Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, Tenn., 1965.
[4] A. Rassat, C. W. Jefford, J. M. Lehn, and B. Waegell, *Tetrahedron Letters*, 1964, 232.
[5] L. M. Jackman and S. Sternhell, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' Pergamon Press, Oxford 1969, p. 334.
[6] W. B. Turner, 'Fungal Metabolites,' Academic Press, London, 1971, ch. 6.
[7] G. F. Pegg, *Trans. Brit. Mycological Soc.*, 1973, **61**, 277.