Aurovertin B, a Metabolite of *Calcarisporium arbuscula*

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Summary The structure of the fungal metabolite aurovertin B is proposed, based on its chemical properties and both ^{13}C and ^{1}H n.m.r. spectral data.

THE aurovertins are a group of toxic metabolites isolated from the fungus *Calcarisporium arbuscula* (Preuss).[1] They are potent inhibitors of ATP-synthesis and ATP-hydrolysis catalysed by mitochondrial enzyme systems.[2] We now

report evidence in support of structure (**1**) (or its enantiomer) for aurovertin B, the major component of the mixture of aurovertins produced by our strain of the organism.

The metabolites were separated by t.l.c. on silica gel and (**1**) crystallised from acetone as pale yellow needles [m.p. 164—167° ($C_{25}H_{32}O_8$, M^+ 460·2098); $[\alpha]_D^{20}$ — 50·6° (EtOH); λ_{max} (EtOH) 372 (ϵ 34,400), 360sh, 276, and 270 (28,200) nm]. Treatment with cold conc. H_2SO_4 gave a red colouration (λ_{max} 546 nm), a characteristic of the aurovertins. The i.r. spectrum of (**1**) included absorptions at 1610 and 1530 cm^{-1} (KBr) consistent with the presence of an α-pyrone unit. Alkaline hydrolysis resulted in the loss of one acetate group, while a diacetate was produced on acetylation with acetic anhydride–pyridine. Hydrogenation (5% Pd–C) gave a non-crystalline hexahydro-derivative (**2**) [M^+ 466; λ_{max} (EtOH) 288 (8,200) nm; ν_{max} (KBr) 1718, 1645, and 1565 cm^{-1}], retaining i.r. absorptions attributed to the α-pyrone, and an octahydro-derivative (**3**) [M^+ 468; λ_{max} (EtOH) 235 nm].

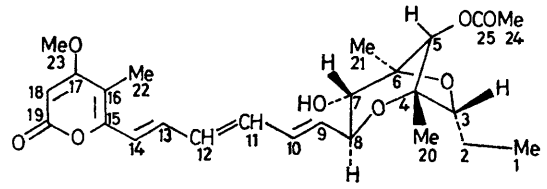
Structural evidence was deduced mainly from the ^{13}C n.m.r. and ^1H n.m.r. spectra of (**1**) and its derivatives (Table). ^{13}C N.m.r. data obtained from proton-noise-decoupled, single-frequency proton-decoupled, and undecoupled spectra, confirmed 25 carbon atoms present in the following groups: six –*C*H=C, four each of *C*H_3–C and >*C*H–O, two C>*C*–O, one each of –*C*H_2–, *C*H_3–O, and *C*H_3–*C*(=O)–O together with the five sp^2 carbons of a 4-oxy-α-pyrone ring.[3] The ^1H n.m.r. spectra confirmed the assignments and, with homonuclear decoupling, established the main structural units.

TABLE. N.m.r. data for aurovertin B and derivatives. Chemical shifts (p.p.m.)[a]

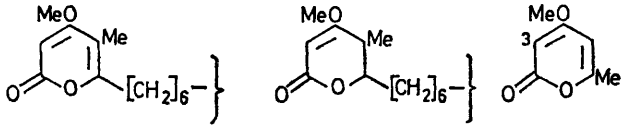
	^{13}C N.m.r.[b]	^1H N.m.r.[c]			
	(**1**)	(**1**)	(*J*/Hz)	(**2**)	(**3**)
C–1	11·8	1·05t	(7·5)	1·05	1·07
C-2	20·1	1·62m		1·61	1·64
C-3	85·4	3·89q	(8;5)	3·85	3·90
C-4	82·7†	—		—	—
C-5	80·8	4·74s		4·70	4·75
C-6	83·3†	—		—	—
C-7	76·2	3·24t	(8)	3·10	3·14
C-8	77·5	4·11q	(8;6)	3·50	3·5
C-9	119·4	5·89q	(14;6)	e	e
C-10	131·3‡	d		e	e
C-11	131·8‡	d		e	e
C-12	135·7‡	d		e	e
C-13	137·0‡	7·14q	(14;10)	e	e
C-14	134·7‡	d		2·49	e
C-15	154·3	—		—	4·35[f]
C-16	108·0	—		—	2·29[g]
C-17	163·6	—		—	—
C-18	88·7	5·45s		5·41	5·08
C-19	169·8	—		—	—
C-20	15·0	1·16s		1·12	1·13
C-21	16·4	1·24s		1·23	1·24
C-22	8·9	1·94s		1·86	1·11[h]
C-23	56·2	3·80s		3·81	3·74
C-24	20·7	2·14s		2·13	2·14
C-25	170·4	—		—	—

[a] Relative to internal Me_4Si. [b] JEOL PFT-100 spectrometer at 25·15 MHz. [c] Varian HA-100 spectrometer. [d] Complex signal δ 6·2—6·5. [e] Envelope at δ 1·3—1·6. [f] Multiplet. [g] m(*J* 7; 3 Hz). [h] d(*J* 7 Hz).
†‡ These assignments may be reversed.

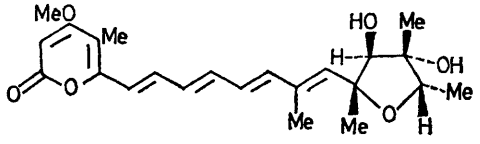
The pyrone substitution pattern was assigned as shown since (a) the ^{13}C n.m.r. spectrum of (**1**) shows olefinic carbon chemical shifts typical of a 4-oxy-α-pyrone;[3] (b) a nuclear Overhauser (n.o.e.) enhancement (18%) is observed between the methoxy-group and the hydrogen atom on the pyrone ring (δ 5·45); (c) the chemical shift of this methine hydrogen and the shielding effect of aromatic solvents are similar to those observed for 3-H of the model pyrone (**4**); (d) the u.v. and ^1H n.m.r. spectra of octahydro-aurovertin B (**3**) correlate with those of known dihydropyrones (*e.g.*, pestalotin[4]), although in (**3**) the C-22 methyl ^1H n.m.r. signal appears as a doublet (δ 1·11, $^3J_{16,22}$ 7 Hz) coupled to a multiplet at δ 2·29. Absorptions at δ 5·89—7·14 in (**1**), absent from the hydrogenation products, confirmed extension of the pyrone chromophore *via* a conjugated triene containing at least two *trans*-disubstituted double bonds ($^3J_{9,10}$ and $^3J_{13,14}$ 14 Hz).



(1)



(2) (3) (4)



(5)

It was found possible to explain the remaining spectral features of (**1**) only on the basis of a substituted 2,6-dioxabicyclo[3,2,1] octane system. Decoupling experiments established attachment of the chromophore and the carbon bearing the hydroxy-group to C-8, the coupling constant $^3J_{7,8}$ 8 Hz defining the *trans*-diaxial relationship of the protons. Stereochemistry at C-5 could be assigned since 5-H was deshielded (0·16 p.p.m.) on acetylation of the 7-hydroxy-group. The spectra also indicated an ethyl group attached to a group >CH–O which could only be C-3. This substituent was shown to exhibit hindered rotation about the C-2,3 bond, since irradiation of the methylene group collapsed the 3-H quartet to a singlet. Supported by the absence of long-range coupling between 3-H and 5-H this result suggested that the ethyl group occupies the 3-*endo* position. Finally, n.o.e. experiments (5-H : 7-H = 17% and 2-H_2 : 8-H = 18%) established the chair conformation of the six-membered ring and confirmed the relative stereochemistry at C-3 and C-5.

The close structural and biogenetic relationship of aurovertin B to citreoviridin (**5**)[5] has been confirmed by

comparison of the spectra and biological properties of the acetates of the metabolites and their hydrogenation products. In addition the structures of several other aurovertins including D (2-hydroxy-aurovertin B) have been elucidated and will be reported elsewhere.

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