# Demethoxyviridin and Demethoxyviridiol : New Fungal Metabolites

By **David C. Aldridge** and **W. Brian Turner**,* Imperial Chemical Industries Ltd., Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG

Alexander J. Geddes * and **Bernard Sheldrick,** Astbury Department of Biophysics, The University, Leeds LS2 9JT

Demethoxyviridin (3) and demethoxyviridiol (4) have been isolated from an unidentified fungus. Their structures were deduced from their spectroscopic properties and that of demethoxyviridiol was confirmed by X-ray analysis.

WHILE screening fungi for biologically active molecules we found that extracts of the mycelium of the unidentified fungus ACC 3199 contained two related metabolites, $C_{19}H_{14}O_5$ and $C_{19}H_{16}O_5$. Comparison of their spectra (see Experimental section) with those of the known fungal metabolites viridin (1) [1] and viridiol (2) [2] showed them to be the demethoxy-derivatives (3) and (4), respectively. Demethoxyviridin had previously been isolated from *Apiospora camptospora*.[3]
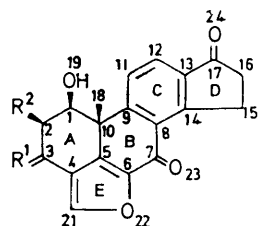
	R¹	R²
(1)	O	OMe
(2)	H,β-OH	OMe
(3)	O	H
(4)	H,β-OH	H

Although the gross structures of the compounds were clear from their spectroscopic properties, and the n.m.r. spectrum showed the *cis*-relation of the 1,3-diol system on ring A, models suggested that the alternative stereochemistry (5) for demethoxyviridiol could not be ruled out. The stereochemistry of viridin has been confirmed by X-ray analysis,[4] and we used this approach to define unambiguously the stereochemistry of demethoxyviridiol. The results confirm the stereochemistry shown in (4). The absolute stereochemistry of compounds (1)—(4) has not been defined, but the stereochemistry shown at C-10 corresponds to that of the related fungal metabolite wortmannin, whose absolute configuration is known.[5]

Two features of the n.m.r. spectra merit comment. First, Grove *et al.*[1] assigned signals at τ 6·2 and 7·1 in the spectrum of viridin to the 16- and 15-protons, respectively. We believe that these assignments should be reversed since the signal of the 3-protons in indanone appears at lower field than that of the 2-protons,[6] and this effect would be enhanced by the 7-oxo-group in the

viridin series. Secondly, there is long-range coupling (J 1 Hz) between H-21 and H-3 in demethoxyviridiol.

TABLE 1

Final positional parameters ($\times 10^5$) for the non-hydrogen atoms, with standard deviations in parentheses

Atom	x/a	y/b	z/c
C(1)	−07424(59)	46216(29)	64610(63)
C(2)	−17194(62)	50381(30)	77347(64)
C(3)	−18911(61)	44919(30)	94265(67)
C(4)	−05555(59)	36955(29)	99232(63)
C(5)	08555(55)	35850(28)	91451(59)
C(6)	15839(60)	27572(27)	95923(64)
C(7)	28038(59)	23414(27)	88026(64)
C(8)	31978(57)	28986(26)	73171(60)
C(9)	24423(56)	37640	68151(59)
C(10)	13319(57)	42307(26)	78764(60)
C(11)	28367(60)	42477(28)	53914(65)
C(12)	39458(64)	39087(30)	44626(69)
C(13)	46946(63)	30602(29)	49875(67)
C(14)	43425(61)	25558(26)	63756(65)
C(15)	53511(71)	16510(30)	66641(78)
C(16)	63785(83)	16793(34)	52162(92)
C(17)	59645(68)	25808(32)	42296(77)
C(18)	27088(60)	49617(29)	92374(64)
O(19)	−06283(52)	53123(22)	51913(50)
O(20)	−13587(89)	50081(33)	112119(70)
C(21)	−05585(68)	29244(31)	108861(73)
O(22)	07628(43)	23362(19)	107240(46)
O(23)	34369(47)	15716(18)	92241(50)
O(24)	65537(56)	28681(26)	30345(59)

TABLE 2

Final positional parameters ($\times 10^4$) and isotropic temperature factors ($\times 10^3$) for the hydrogen atoms; standard deviations in parentheses

Atom	x/a	y/b	z/c	U
H C(1)	−1733(77)	4011(37)	5494(85)	46(18)
H' C(2)	−0714(65)	5611(29)	8528(67)	18(13)
H" C(2)	−3230(61)	5219(31)	6615(68)	18(12)
H C(3)	−3442(61)	4271(29)	8858(67)	16(12)
H C(11)	2336(54)	4787(26)	5177(62)	4(10)
H C(12)	4251(65)	4248(31)	3511(71)	19(13)
H' C(15)	4266(58)	1144(27)	6210(61)	2(10)
H" C(15)	6388(67)	1572(34)	8204(73)	22(14)
H' C(16)	5696(80)	1155(38)	3973(82)	47(17)
H" C(16)	8030(73)	1565(35)	6224(81)	44(17)
H' C(18)	2120(63)	5219(32)	9920(69)	20(13)
H" C(18)	3926(59)	4743(30)	9953(68)	13(12)
H"' C(18)	2717(71)	5529(34)	8133(77)	26(16)
H O(19)	−1363(94)	5133(49)	3826(103)	71(24)
H C(21)	−1164(74)	2678(34)	11790(81)	32(14)

The atomic co-ordinates, bond lengths, bond angles, and torsion angles for demethoxyviridiol (4) are listed in Tables 1—5. The bond lengths and angles show no

[1] J. F. Grove, P. McCloskey, and J. S. Moffatt, *J. Chem. Soc. (C)*, 1966, 743.

[2] J. S. Moffatt, J. D. Bu'Lock, and T. H. Yuen, *Chem. Comm.*, 1969, 839.

[3] B. F. Burrows, personal communication.

[4] S. Neidle, D. Rogers, and M. B. Hursthouse, *J.C.S. Perkin II*, 1972, 760.

[5] T. J. Petcher, H.-P. Weber, and Z. Kis, *J.C.S. Chem. Comm.*, 1972, 1061; J. MacMillan, T. J. Simpson, and S. K. Yeboah, *ibid.*, 1063.

[6] E. Lustig and E. P. Ragelis, *J. Org. Chem.*, 1964, **32**, 1398.

TABLE 3

Bond lengths (Å) with estimated standard deviations in parentheses

C(1)–C(2)	1·541(8)	C(8)–C(9)	1·416(4)
C(1)–C(10)	1·566(5)	C(8)–C(14)	1·406(7)
C(1)–O(19)	1·425(6)	C(9)–C(10)	1·529(7)
C(2)–C(3)	1·535(7)	C(9)–C(11)	1·401(7)
C(3)–C(4)	1·511(6)	C(10)–C(18)	1·550(5)
C(3)–O(20)	1·421(5)	C(11)–C(12)	1·375(8)
C(4)–C(5)	1·405(7)	C(12)–C(13)	1·392(6)
C(4)–C(21)	1·366(7)	C(13)–C(14)	1·380(7)
C(5)–C(6)	1·354(6)	C(13)–C(17)	1·478(8)
C(5)–C(10)	1·491(7)	C(14)–C(15)	1·539(6)
C(6)–C(7)	1·418(7)	C(15)–C(16)	1·548(10)
C(6)–O(22)	1·378(6)	C(16)–C(17)	1·516(7)
C(7)–C(8)	1·498(7)	C(17)–O(24)	1·212(8)
C(7)–O(23)	1·250(5)	C(21)–O(22)	1·375(6)

significant deviations from their accepted values with the possible exception of the angle C(3)–C(4)–C(21) (131·57°). This large value shows the considerable strain involved in linking rings A and B through ring E.

TABLE 4

Bond angles (°) and their estimated standard deviations in parentheses

C(2)–C(1)–C(10)	110·60(34)	C(8)–C(9)–C(11)	119·40(43)
C(2)–C(1)–O(19)	105·25(36)	C(10)–C(9)–C(11)	117·52(26)
C(10)–C(1)–O(19)	112·84(38)	C(1)–C(10)–C(5)	102·98(35)
C(1)–C(2)–C(3)	118·97(38)	C(1)–C(10)–C(9)	116·31(34)
C(2)–C(3)–C(4)	110·55(44)	C(1)–C(10)–C(18)	110·39(32)
C(2)–C(3)–O(20)	110·35(40)	C(5)–C(10)–C(9)	109·45(32)
C(4)–C(3)–O(20)	109·01(35)	C(5)–C(10)–C(18)	110·22(34)
C(3)–C(4)–C(5)	122·21(42)	C(9)–C(10)–C(18)	107·40(35)
C(3)–C(4)–C(21)	131·57(49)	C(9)–C(11)–C(12)	121·96(38)
C(5)–C(4)–C(21)	105·71(42)	C(11)–C(12)–C(13)	117·94(49)
C(4)–C(5)–C(6)	107·97(43)	C(12)–C(13)–C(14)	122·31(50)
C(4)–C(5)–C(10)	126·20(38)	C(12)–C(13)–C(17)	126·96(50)
C(6)–C(5)–C(10)	125·58(46)	C(14)–C(13)–C(17)	110·72(40)
C(5)–C(6)–C(7)	125·39(46)	C(8)–C(14)–C(13)	119·85(38)
C(5)–C(6)–C(22)	109·79(43)	C(8)–C(14)–C(15)	129·05(45)
C(7)–C(6)–O(22)	124·35(37)	C(13)–C(14)–C(15)	111·10(47)
C(6)–C(7)–C(8)	113·58(36)	C(14)–C(15)–C(16)	103·87(42)
C(6)–C(7)–O(23)	124·02(47)	C(15)–C(16)–C(17)	106·69(48)
C(8)–C(7)–O(23)	122·34(47)	C(13)–C(17)–C(16)	107·59(51)
C(7)–C(8)–C(9)	122·06(44)	C(13)–C(17)–O(24)	125·39(45)
C(7)–C(8)–C(14)	119·42(36)	C(16)–C(17)–O(24)	127·01(55)
C(9)–C(8)–C(14)	118·52(42)	C(4)–C(21)–O(22)	110·77(50)
C(8)–C(9)–C(10)	122·92(39)	C(6)–O(22)–C(21)	105·68(35)

The only significant difference in bond lengths between viridin (1)[4] and demethoxyviridiol (4) lies in the C(3)–C(4) distance [1·441 in (1) and 1·511 Å in (4)], and the only significant difference in bond angles is in that at C(1) [C(2)–C(1)–C(10) 114·8 in (1) and 110·6° in (4)]. All the other corresponding bond lengths and angles lie within three standard deviations of each other.

In the solid state both compounds have rings C, D, and E flat, rings C and D co-planar, atoms C(5), C(6), C(8), and C(9) co-planar, and ring A buckled. However, whereas in viridin the molecule is ' bent ' about a line joining C(10) and C(7), this is hardly apparent in demethoxyviridiol. Thus C(10) and C(7) are displaced from the least squares best plane through C(5), C(6), C(8), and C(9) by 0·304 and 0·108 Å, respectively, in viridin and by 0·138 and 0·019 Å in demethoxyviridiol. In fact ring B is essentially flat in demethoxyviridiol with the exception of C(10) which is 0·078 Å out of plane. A possible reason for this difference in conformation is

the difference in intermolecular hydrogen bonding. In viridin there is an O–H · · · O bond between O(24) and O(19), which is absent in demethoxyviridiol, while in

TABLE 5

Torsion angles (°)

C(10)–C(1)–C(2)–C(3)	−52·1
O(19)–C(1)–C(2)–C(3)	23·7
C(1)–C(2)–C(3)–C(4)	15·2
C(1)–C(2)–C(3)–O(20)	135·9
C(2)–C(3)–C(4)–C(5)	9·7
O(20)–C(3)–C(4)–C(5)	−111·7
C(2)–C(3)–C(4)–C(21)	−160·8
O(20)–C(3)–C(4)–C(21)	77·7
C(3)–C(4)–C(5)–C(6)	−170·5
C(21)–C(4)–C(5)–C(6)	2·2
C(3)–C(4)–C(5)–C(10)	4·1
C(21)–C(4)–C(5)–C(10)	176·8
C(4)–C(5)–C(6)–C(7)	169·5
C(10)–C(5)–C(6)–C(7)	−5·1
C(4)–C(5)–C(6)–O(22)	−2·9
C(10)–C(5)–C(6)–O(22)	−177·5
C(5)–C(6)–C(7)–C(8)	−1·9
O(22)–C(6)–C(7)–C(8)	169·4
C(5)–C(6)–C(7)–O(23)	−179·1
O(22)–C(6)–C(7)–O(23)	−7·8
C(6)–C(7)–C(8)–C(9)	1·4
O(23)–C(7)–C(8)–C(9)	178·7
C(6)–C(7)–C(8)–C(14)	−178·2
O(23)–C(7)–C(8)–C(14)	−1·0
C(7)–C(8)–C(9)–C(10)	5·7
C(14)–C(8)–C(9)–C(10)	−174·6
C(7)–C(8)–C(9)–C(11)	−179·0
C(14)–C(8)–C(9)–C(11)	0·6
C(8)–C(9)–C(10)–C(1)	−127·2
C(11)–C(9)–C(10)–C(1)	57·5
C(8)–C(9)–C(10)–C(5)	−11·0
C(11)–C(9)–C(10)–C(5)	173·6
C(8)–C(9)–C(10)–C(18)	108·6
C(11)–C(9)–C(10)–C(18)	−66·7
C(5)–C(10)–C(1)–C(2)	58·1
C(9)–C(10)–C(1)–C(2)	177·8
C(18)–C(10)–C(1)–C(2)	−59·5
C(5)–C(10)–C(1)–O(19)	−54·7
C(9)–C(10)–C(1)–O(19)	65·0
C(18)–C(10)–C(1)–O(19)	−172·3
C(9)–C(10)–C(5)–C(4)	−162·7
C(1)–C(10)–C(5)–C(4)	−38·4
C(9)–C(10)–C(5)–C(6)	11·0
C(1)–C(10)–C(5)–C(6)	135·3
C(18)–C(10)–C(5)–C(4)	79·4
C(18)–C(10)–C(5)–C(6)	−106·9
C(8)–C(9)–C(11)–C(12)	−0·2
C(10)–C(9)–C(11)–C(12)	175·3
C(9)–C(11)–C(12)–C(13)	−0·4
C(11)–C(12)–C(13)–C(14)	0·7
C(11)–C(12)–C(13)–C(17)	−178·0
C(12)–C(13)–C(14)–C(8)	−0·8
C(17)–C(13)–C(14)–C(8)	178·6
C(12)–C(13)–C(14)–C(15)	−179·8
C(17)–C(13)–C(14)–C(15)	−1·0
C(8)–C(14)–C(15)–C(16)	−179·8
C(13)–C(14)–C(15)–C(16)	−0·2
C(14)–C(15)–C(16)–C(17)	1·3
C(15)–C(16)–C(17)–C(13)	−1·9
C(15)–C(16)–C(17)–O(24)	178·1
C(16)–C(17)–C(13)–C(12)	−179·4
O(24)–C(17)–C(13)–C(12)	0·6
C(16)–C(17)–C(13)–C(14)	1·8
O(24)–C(17)–C(13)–C(14)	−178·2
C(13)–C(14)–C(7)–C(9)	−0·4
C(15)–C(14)–C(8)–C(9)	179·1
C(13)–C(14)–C(8)–C(7)	179·3
C(15)–C(14)–C(8)–C(7)	−1·2
C(3)–C(4)–C(21)–O(22)	171·0
C(5)–C(4)–C(21)–O(22)	−0·7
C(5)–C(6)–O(22)–C(21)	2·4
C(7)–C(6)–O(22)–C(21)	−170·1
C(4)–C(21)–O(22)–C(6)	−1·0

the latter compound the O(20)–O(19) separation of 2·75 Å suggests that there might be a hydrogen bond between those two atoms in adjacent molecules.

EXPERIMENTAL

Isolation of the Metabolites.—The unidentified fungus ACC 3199 was grown as surface culture in Thompson vessels each containing 1 l of a Raulin–Thom medium. After 340 h the mycelium from 90 vessels was combined, dried, and extracted with chloroform (Soxhlet). The chloroform was removed and the residue (72·3 g) was washed with light petroleum leaving a solid (38·3 g) which was deposited on silica gel (Hopkin and Williams MFC; 350 ml) by evaporation of a solution in acetone. The silica gel was placed on a column of silica gel (1·4 l) made up in benzene. Elution with benzene (3 l), benzene–chloroform (9 : 1, 1 l; 3 : 1, 2 l; and 1 : 1, 6 l) and with chloroform (2 l) gave mixtures which were discarded. Further elution with chloroform (5 l) gave fractions (9·0 g) which were combined, treated with charcoal in acetone, and crystallised from acetone–light petroleum to give *demethoxyviridin* (3). Elution with acetone (2 l) gave fractions (22·3 g) which were treated with charcoal in acetone and crystallised from acetone to give *demethoxyviridiol* (4).

Demethoxyviridin forms prisms of variable m.p. from acetone, acetone–light petroleum, ethyl acetate, or aqueous methanol. The m.p. is either in the range 145—160° or in the range 220—240°, with decomposition in each case. Elemental analyses, too, varied over a wide range and we suspect that the crystals are solvated, probably hydrated. For example a sample crystallised from acetone had m.p. 230—240° (Found: C, 69·9; H, 4·5; H_2O, 1·6%; *m/e* 322·0836. $C_{19}H_{14}O_5,0\cdot29H_2O$ requires C, 69·7; H, 4·5; H_2O, 1·6%; *M*, 322·0841). The compound also gives two different solid-state i.r. spectra which cannot be correlated with the m.p.s: $\nu_{max.}$ (Nujol) (form I) 3370m, 1695s, 1675s, 1627m, 1590m, and 1532s; (form II) 3420m, 1699s, 1674s, 1653s, 1624m, 1586m, and 1530m cm⁻¹; $\lambda_{max.}$ (MeOH) 243 (ε 27,050) and 303 nm (11,600); $\lambda_{max.}$ (MeOH–NaOH) 295 (ε 46,700), 372 (6200), and 458 nm (9200); $\lambda_{max.}$ (MeOH–HCl) 273 (ε 45,000) and 390 nm (6450). Both forms give the same n.m.r. spectrum: τ [$(CD_3)_2SO$] 1·25 (s, H-21), 1·41 and 2·16 (AB system, *J* 8 Hz, H-11 and -12), 3·91 (d, (*J* 6 Hz, OH), 5·67 (m, *J* 6, 5·5, and 10 Hz, becoming dd, *J* 5·5 and 10 Hz, in presence of D_2O, H-1), 6·46 (m, 15-H_2), 6·91 (dd, *J* 10 and 18 Hz) and 7·28 (dd, *J* 5·5 and 18 Hz, 2-H_2), 7·39 (m, 16-H_2), and 8·45 (s, CH_3).

Demethoxyviridiol forms prisms, m.p. >300° (from acetone) (Found: C, 70·4; H, 4·9%; *m/e*, 324·1001. $C_{19}H_{16}O_5$ requires C, 70·5; H, 5·1%; *M*, 324·0997); $\nu_{max.}$ (Nujol) 3420m, 3360m, 1708s, 1658s, 1619m, 1590m, and 1545m cm⁻¹; $\lambda_{max.}$ (MeOH) 249 (ε 23,500) and 319 nm (11,400); $\lambda_{max.}$ (MeOH–NaOH) 292 (ε 38,700), 370 (4850), and 462 nm (10,100); $\lambda_{max.}$ (MeOH–HCl) 277 (ε 41,100)

and 394 (5250); τ [$(CD_3)_2SO$] 1·99 (s, H-21), 1·40 and 2·23 (AB system, *J* 8 Hz, H-11 and -12), 4·27 (d, *J* 6 Hz, OH), 4·49 (d, *J* 6 Hz, OH), 5·18 (m, becoming dd, *J* 6 and 8·5 Hz, in presence of D_2O, 3-H), 6·00 (m, *J* 6, 11, and 4·5 Hz, becoming dd, *J* 4·5 and 11 Hz in presence of D_2O), 6·46 (m, 15-H_2), 7·40 (m, 16-H_2), 7·3—7·9 (complex m, 2-H_2), and 8·50 (s, CH_3).

Crystal Data for Demethoxyviridiol.—$C_{19}H_{16}O_5$, *M* = 324. Monoclinic, *a* = 7·483(2), *b* = 15·146(6), *c* = 7·283(2) Å, β = 115·41(0·03)°, U = 751·73 Å³, D_c = 1·43, *Z* = 2. Space group $P2_1$. Graphite monochromatised Cu-K_α radiation, λ = 1·54051 Å.

Three-dimensional intensity data were collected from a single crystal by using an Enraf–Nonius CAD-4 diffractometer and measured by the moving crystal moving counter technique in the range $3° \leq \theta \leq 70°$. 1581 independent reflections were measured but 366 of these were very weak and were not used in the analysis. The intensities were corrected for Lorentz and polarisation factors but no correction was made for absorption. The structure was solved by direct methods using MULTAN.[7] The starting set of the successful solution contained three origin-defining reflections, five ' Sigma 1 ' reflections, and just one reflection whose phase was randomly chosen as $\pm \pi/4$ or $\pm 3\pi/4$. The normalised structure factors (*E* values) were calculated by using the spherically averaged molecular scattering factors for three molecular ' fragments ' of assumed planar geometry, *viz.* (1) C(7)–C(17) and O(24), (2) C(3)–C(6), C(21), and O(22), (3) C(1), C(2), and O(19), and normal scattering factors for the remaining atoms. The first electron-density map calculated using the phases produced by this successful starting set showed all the atoms in the molecule except for C(1), O(19), and O(20). Full matrix least-squares isotropic refinement reduced the *R* value to 0·299. At this stage a difference-Fourier synthesis revealed the remaining non-hydrogen atoms and further refinement with the inclusion of these atoms reduced the *R* value to 0·109. The structure was then refined anisotropically to an *R* value of 0·062, and another difference-Fourier synthesis then revealed all the hydrogen atoms. With the inclusion of these atoms the *R* value was reduced to 0·031 although HO(20) did not refine to a sensible position. This is not the hydrogen atom involved in the hydrogen bond between O(19) and O(20). When the 366 weak reflections were included in the calculations the *R* value was 0·040. Structure factors, thermal parameters, and equations of least-squares planes with deviations of atoms from the planes are available as Supplementary Publication No. 21317 (11 pp., 1 microfiche).*

⁷ G. Germain, P. Main, and M. M. Woolfson, *Acta Cryst.*, 1971, **A27**, 368.

* For details of Supplementary Publications, see Notice to Authors No. 7 in *J.C.S. Perkin I*, 1974, Index issue.