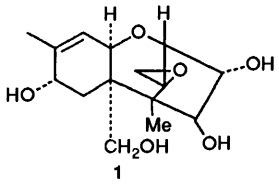
Metabolic Products of *Fusarium acuminatum*: Acuminatopyrone and Chlamydosporol

John Frederick Grove* and Peter B. Hitchcock
School of Molecular Sciences, University of Sussex, Brighton, Sussex BN1 9QJ, UK

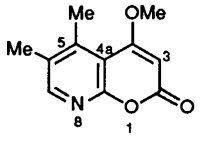
Two metabolic products of a non-toxic strain of *Fusarium acuminatum* are shown to be 4-methoxy-5,6-dimethyl-2*H*-pyrano[2,3-*b*]pyridin-2-one (acuminatopyrone) and *trans*-7,8-dihydro-7-hydroxy-4-methoxy-7,8-dimethyl-2*H*,5*H*-pyrano[4,3-*b*]pyran-2-one (chlamydosporol).

Although some strains of the soil saprophytic fungus *Fusarium acuminatum* are prolific producers of trichothecene mycotoxins, primarily esters of T-2 tetraol **1**,[1,2] other strains are non-toxic. This paper reports the isolation of two biologically inactive 2-pyrones, **2** and **4**, from one such strain.

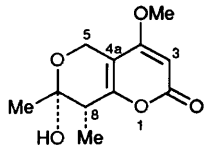
The pyrones were readily separated by column chromatography. The less polar compound **2** was basic and had the composition $C_{11}H_{11}NO_3$, with two C–Me groups (δ_H 2.33, 2.59; δ_C 11.65, 23.7) on a heteroaromatic ring system (λ_{max} 220, ~269, 277, 291, ~304 nm. log ε 4.40, 4.15, 4.20, 4.09, 3.87). It was optically inactive and was assigned the trivial name acuminatopyrone. The three oxygen atoms were contained in an OMe group (δ_H 4.00; δ_C 57.2) and in an ester or lactone ring (ν_{max} 1730 cm^{-1}). Treatment with boiling dilute hydrochloric acid removed these oxygen functions and created an additional C–Me group (δ_H 2.17), as part of a MeCO group (ν_{max} 1668 cm^{-1}; δ_C 203.85) in the neutral, $C_9H_{11}NO_2$, product. This must



1

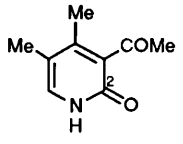


2



4

H⁺

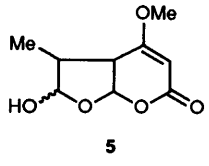


3

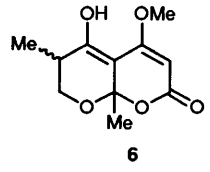
arise by decarboxylation of a masked β-keto acid function. The spectroscopic properties of the $C_9H_{11}NO_2$ compound were consistent with those expected for a trisubstituted pyridinone [NH: δ_H 12.7 (br); CO: ν 1638 cm^{-1}, δ_C 166.0] in which the free position (δ_C 8.70 was adjacent to the ring N atom. The UV spectrum, λ_{max} 305 nm log ε 3.69, by comparison with the known spectra of 3-acetyl-4,6-dimethylpyridin-2-one, λ_{max} 325 nm log ε 3.90,[3] and 3-acetyl-2,6-dimethylpyridin-4-one, λ_{max} 261 nm log ε 3.35,[4] indicated that the compound was 3-acetyl-4,5-dimethylpyridin-2-one **3**.

It follows that acuminatopyrone has the pyrano[2,3-*b*]pyridine structure **2** with the OMe substituent at the 4-position and the 3-position (δ_C 90.5)[5] unsubstituted (δ_H 5.60). The UV spectrum (see above) closely resembled that of the 4-hydroxy-5,7-dimethyl analogue.[6] Few pyrano[2,3-*b*]pyridin-2-ones have been described in the literature, and none from natural sources.

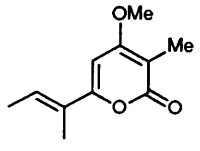
The more polar compound **4** was neutral and had the composition $C_{11}H_{14}O_5$. The UV spectrum, λ_{max} 285, ~292 nm log ε 4.04, 4.01, suggested an aromatic or heteroaromatic ring system. Six of the eleven carbon atoms were contained in C=O (ν_{max} 1710 cm^{-1}), MeCH (δ_H 1.33, 2.78, *J*/Hz 7), Me (δ_H 1.55), OMe (δ_H 3.81) and CH_2O (δ_H 4.42, 4.55 AB system *J*/Hz 15.0) groupings, excluding the presence of a benzene ring and suggesting the presence of a 2-pyrone. The ^{13}C NMR spectrum



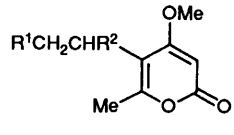
5



6



8



7

strongly supported a 4-methoxy-2-pyrone with an unsubstituted 3-position (δ_C 88.1, δ_H 5.44).[5] The above groupings, together with an OH (ν_{max} 3590 cm^{-1}) account for all five oxygen atoms in the molecule.

The hydrogen atoms of the CH_2O group were individually coupled (*J*/Hz 3.0, 1.8) to the methine hydrogen at δ_H 2.78. It was considered that these were homallylic couplings; thus the CH_2O and MeCH groups are at positions 5 and 6 (or 6 and 5) of the 4-methoxypyrone ring. Although the pyrone [4] had a sharp melting point and ran as a single spot on TLC, a solution in $CDCl_3$ contained two molecular species, in the ratio 2.4:1, giving closely similar ^1H and ^{13}C NMR spectra (see Experimental section). The data quoted above are for the more abundant (major) species. The NMR spectra of substituted cyclic hemiacetals, *e.g.* astepyrone **5**,[7] commonly show this phenomenon when both epimers are present in solution. The dihydropyrano[4,3-*b*]pyran-2-one structure **4** readily satisfies this condition (but see below). The chemical shift (δ_C 105.1) of C-4a favours this structure rather than the alternative pyrano-[3,4-*b*]pyran-2-one.

Both homoallylic couplings in the major species are (relatively) large indicating that the 8-H is pseudo-axial[8] with the bulky methyl groups *trans* and equatorial. In the minor species the homoallylic couplings are both small (*J*/Hz 1.7, 0.7), consistent with the 8-H being pseudo-equatorial. Molecular models show that this can be achieved by a conformational change in the dihydropyran ring without, necessarily, making a configurational change at the hemiketal centre.

Chlamydosporol **6**, a yellow oil, $[\alpha]_D$ −14°, of composition

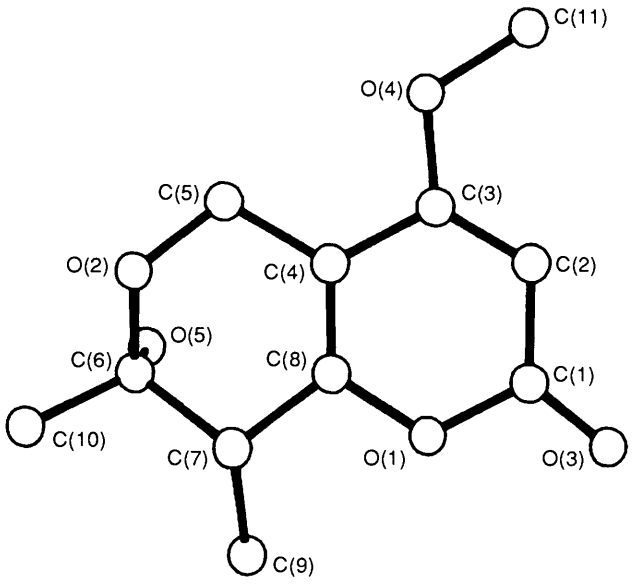


Fig. 1 X-ray molecular structure of chlamydosporol showing the crystallographic numbering scheme

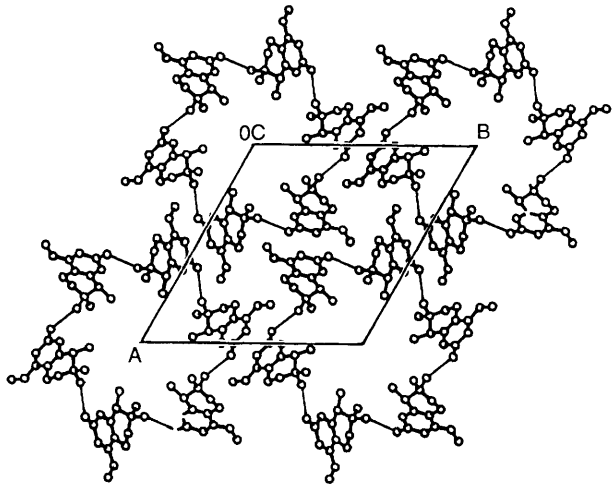


Fig. 2 Crystal packing of chlamydosporol as seen in projection down the *c* axis

Table 1 Fractional atomic coordinates ($\times 10^4$)

	x	*y*	*z*
O(1)	24(4)	6 356(4)	4 269(11)
O(2)	1 745(4)	7 164(4)	9 096(10)
O(3)	−605(4)	5 607(4)	1 457(11)
O(4)	1 898(4)	5 711(4)	4 265(11)
O(5)	2 068(5)	8 326(5)	6 976(12)
C(1)	−21(6)	5 810(6)	2 691(16)
C(2)	624(6)	5 573(6)	2 646(15)
C(3)	1 241(6)	5 878(6)	4 131(15)
C(4)	1 258(6)	6 438(6)	5 792(14)
C(5)	1 917(6)	6 744(7)	7 491(16)
C(6)	1 468(7)	7 736(7)	8 404(16)
C(7)	593(6)	7 240(6)	7 282(17)
C(8)	665(6)	6 651(6)	5 760(15)
C(9)	297(7)	7 817(7)	6 278(20)
C(10)	1 431(8)	8 183(8)	10 356(20)
C(11)	1 953(7)	5 173(7)	2 607(19)

$C_{11}H_{14}O_5$ stated to be a mixture of epimers at position 6, has recently been obtained from a strain of *F. chlamydosporum* grown on a rice medium.[9] From a comparison of the spectroscopic properties, particularly the NMR spectra, of chlamydosporol and the pyrone **4** it was clear that they were identical. Accordingly, we undertook the X-ray crystallographic examination of the pyrone which showed structure **4** to be correct. The major species present in $CDCl_3$ solution has the same configuration as that of the solid crystallised from benzene.

Table 2 Intramolecular distances (Å) and angles (°) with estimated standard deviations in parentheses

O(1)–C(1)	1.384(13)	O(1)–C(8)	1.383(12)
O(2)–C(5)	1.401(14)	O(2)–C(6)	1.42(2)
O(3)–C(1)	1.216(13)	O(4)–C(3)	1.36(2)
O(4)–C(11)	1.47(2)	O(5)–C(6)	1.409(11)
C(1)–C(2)	1.42(2)	C(2)–C(3)	1.354(14)
C(3)–C(4)	1.456(15)	C(4)–C(5)	1.498(14)
C(4)–C(8)	1.30(2)	C(6)–C(7)	1.544(14)
C(6)–C(10)	1.51(2)	C(7)–C(8)	1.49(2)
C(7)–C(9)	1.52(2)		
C(1)–O(1)–C(8)	121.4(9)	C(5)–O(2)–C(6)	114.3(8)
C(3)–O(4)–C(11)	116.7(8)	O(1)–C(1)–O(3)	115(1)
O(1)–C(1)–C(2)	117.4(9)	O(3)–C(1)–C(2)	127(1)
C(1)–C(2)–C(3)	119(1)	O(4)–C(3)–C(2)	126(1)
O(4)–C(3)–C(4)	112.6(8)	C(2)–C(3)–C(4)	122(1)
C(3)–C(4)–C(5)	122(1)	C(3)–C(4)–C(8)	117.0(9)
C(5)–C(4)–C(8)	121(1)	O(2)–C(5)–C(4)	113(1)
O(2)–C(6)–O(5)	110(1)	O(2)–C(6)–C(7)	110.6(8)
O(2)–C(6)–C(10)	104(1)	O(5)–C(6)–C(7)	107.8(8)
O(5)–C(6)–C(10)	111.2(8)	C(7)–C(6)–C(10)	113(1)
C(6)–C(7)–C(8)	107(1)	C(6)–C(7)–C(9)	113.9(9)
C(8)–C(7)–C(9)	112.8(9)	O(1)–C(8)–C(4)	123(1)
O(1)–C(8)–C(7)	112(1)	C(4)–C(8)–C(7)	124.9(9)

The atomic numbering scheme adopted for crystallographic purposes is shown in Fig. 1. The crystal packing is shown in Fig. 2 in projection down the *c* axis. The six molecules related by the $\bar{3}$ inversion centre are linked by hydrogen bonds O(3)···H′–O(5)′, where symmetry element′ is $-1 + y, y - x, 1 - z$, and the O(3)···O(5)′ distance is 2.88(1) Å. It can be seen that there are channels in the crystal structure along the line $x = 0, y = 0$ and benzene solvate molecules disorder within these channels. Atomic coordinates are listed in Table 1, and intramolecular distances and angles in Table 2.

The assignment of structure **6** to chlamydosporol resulted from a misinterpretation of the ^1H NMR spectrum in which the couplings between the CH_2O and MeCH groups were ascribed to vicinal couplings.[9] Our material was optically inactive in conformity with the space group $P\bar{3}$.

In the open (keto) form of the hemiketal, the structure of chlamydosporol **4** is unexceptional. Naturally occurring 5,6-disubstituted 4-methoxy-2-pyrones are not uncommon, *e.g.* the *Macrophoma commelinae* metabolites **7** (R^1, R^2 = H or OH);[10] nor is the branched four-carbon side chain unusual, *e.g.* nectriapyrone **8**.[11]

Experimental

M.p.s were taken on a Kofler hot stage apparatus and are corrected. ^1H and ^{13}C NMR spectra were obtained at 500 and 125.77 MHz respectively in $CDCl_3$ with $SiMe_4$ as internal standard. *J* values are given in Hz. Molecular weights were taken from the mass spectra. Unless stated otherwise, IR spectra were obtained on mulls in Nujol and, in analytical TLC, Merck silica gel 60 F_{254} was used with chloroform–methanol (49:1). UV spectra were determined on solutions in methanol.

Fermentations with Fusarium acuminatum *C.M.I. 35098.*—Conical culture flasks containing glucose–ammonium nitrate medium[12] (200 cm^3) were inoculated and incubated at 25 °C for 11 d. The fermentation was harvested and the culture filtrate

(20 dm^3, pH 5.3) was adjusted to pH 7.0 and extracted with chloroform. The gummy product (860 mg), in benzene, was chromatographed in UV light on a column of neutral aluminium oxide, Brockmann Grade II, (30 g) made up in benzene. Elution of a great fluorescent band with benzene (600 cm^3) furnished a solid (316 mg) which, after three recrystallisations from benzene or ethyl acetate formed prisms, m.p. 203–204 °C decomp., R_F 0.38 of *4-methoxy-5,6-dimethyl-2H-pyrano[2,3-b]pyridin-2-one* (*acuminatopyrone*) **2** (Found: C, 64.4; H, 5.4; N, 7.0; OMe, 14.3% *M* 205.0743. $C_{11}H_{11}NO_3$ requires C, 64.4; H, 5.4; N, 6.8; OMe, 15.2%; *M* 205.0739); ν_{max}/cm^{-1} OH absent, 3097 (=CH), 1730, 1613 and 846; λ_{max}/nm 220, ~269, 277, 291 and ~304 log ε 4.40, 4.15, 4.20, 4.09 and 3.87 respectively; δ_H 2.33 (s, 3 H), 2.59 (s, 3 H), 4.00 (s, 3 H, OMe), 5.60 (s, 3-H) and 8.82 (s, 7-H); δ_C 11.65 (Me), 23.7 (Me), 57.2 (OMe), 90.5 (C-3), 110.8 (C-4a), 119.2 (C-6), 143.0 (C-7), 157.5, 161.9, 162.5 and 166.95; *m/z* 205 (100), 177 (95), 162 (60), 147 (40) and 135 (35); $[\alpha]^{20}_{589-365}0$ (*c* 0.0922 in methanol).

Acuminatopyrone was soluble in dilute hydrochloric acid from which it was precipitated by the addition of sodium hydroxide. There was no uptake of hydrogen on attempted microhydrogenation in acetic acid in the presence of a palladium catalyst. It did not give a precipitate with 2,4-dinitrophenylhydrazine.

Further elution of the column with benzene–methanol (200:1; 400 cm^3) brought off a blue fluorescent band which afforded a solid (52 mg). Recrystallisation from ethyl acetate gave prisms, m.p. 172–174 °C, R_F 0.49 [in chloroform–methanol (9:1)], of trans-*7,8-dihydro-7-hydroxy-4-methoxy-7,8-dimethyl-2H,5H-pyrano[4,3-b]pyran-2-one* (*chlamydosporol*) **4** Found: C, 58.8; H, 6.0%; *M* 226. $C_{11}H_{14}O_5$ requires C, 58.4; H, 6.2%; *M* 226); ν_{max}/cm^{-1} 3355 (OH), 3090 (=CH), 1698, 1645 and 1566 [$\nu(CHCl_3)/cm^{-1}$ 3590, 3380, 1710, 1660 and 1570]; λ_{max}/nm 285, ~292; log ε 4.04 and 4.01; *m/z* 226 (15), 208, 193, 184, 166 (100); $[\alpha]^{20}_{589-436}0 \pm 1$ (*c* 0.0775 in methanol); δ_H major species: 1.33 (d, *J* 7.2, 8-Me), 1.55 (s, 7-Me), 2.24 (s, OH), 2.78 (ddq, *J* 1.8, 3.0, 7.2, 8-H), 3.81 (s, OMe), 4.42 (dd, *J* 15.0, 1.8, 5-H) 4.55 (dd, *J* 15.0, 3.0, 5-H), 5.44 (s, 3-H) minor species: 1.25 (d, *J* 7.0, 8-Me), 1.49 (s, 7-Me), 2.39 (s, OH), 2.59 (dq, *J* 1.7, 7.0, 8-H), 3.81 (s, OMe), 4.46 (dd, *J* 15.0, 0.7, 5-H), 4.51 (dd, *J* 15.0, 1.7, 5-H) and 5.44 (s, 3-H); δ_C (*a*, assignments may be reversed) major species: 15.8 (8-Me), 26.4 (7-Me), 40.0 (C-8), 56.0 (OMe), 56.2 (C-5), 88.1 (C-3), 97.7 (C-7), 105.1 (C-4a), 158.8 (C-2)[a], 164.4 (C-6)[a] and 168.2 (C-4) minor species: 11.3 (8-Me), 27.1 (7-Me), 39.9 (C-8), 56.0 (OMe), 56.6 (C-5), 87.8 (C-3), 97.1 (C-7), 105.9 (C-4a), 157.4 (C-2)[a], 164.4 (C-6)[a] and 168.2 (C-4).

Chlamydosporol gave no colouration with iron(III) chloride.

3-Acetyl-4,5-dimethylpyridin-2(1H)-one **3**.—Acuminatopyrone (10 mg) in hydrochloric acid (2 mol dm^{-3}; 1 cm^3) was heated at 100 °C for 8 h. The solution was neutralised with sodium hydroxide (2 mol dm^{-3}) and extracted with ethyl acetate. The solid product (8 mg) was crystallised from ethyl acetate giving the *pyridin-2-one* **3** as felted needles, R_F 0.16, which sublimed without melting at 195–200 °C (Found: C, 65.4; H, 6.9; N, 8.7%; *M* 165. $C_9H_{11}NO_2$ requires C, 65.4; H, 6.7; N, 8.5% *M* 165); ν_{max}/cm^{-1} 3200 (br, NH), 1668, 1638 and 1520; λ_{max}/nm 225, 258, 264 and 305; log ε 4.35, 3.98, 3.98 and 3.69 respectively; δ_H 2.17 (s, 3 H), 2.52 (s, 3 H), 2.66 (s, 3 H), 8.70 (s, 6-H), 12.7 (br, NH); δ_C 10.2, 23.05, 26.2, 89.7, 115.2, 120.1, 149.1 (C-6), 166.0 (C-2) and 203.85 (COMe); *m/z* 165 (90), 150 (100), 122 (82) and 94 (8).

The pyridin-2-one was soluble in water, and the solution as neutral to the Universal Indicator. It gave no colour with iron(III) chloride.

Crystal Data for Chlamydosporol.—$C_{11}H_{14}O_5$, M = 226.2, trigonal, space group $P\bar{3}$ (no. 147), $a = b$ = 17.962(9), c = 6.426(4) Å, U = 1795.5 $Å^3$, Z = 6, D_{calc} = 1.26 gcm^{-3}, $F(000)$ = 720. Monochromated Mo-Kα radiation, λ = 0.710 69 Å, μ = 0.9 cm^{-1}.

Crystallographic Analysis of Chlamydosporol.—The compound was crystallised from benzene. Data were collected using a crystal *ca.* 0.2 × 0.15 × 0.10 mm on an Enraf–Nonius CAD4 diffractometer in the θ–2θ mode with $\Delta\theta = (0.8 + 0.35 \tan\theta)°$ and a maximum scan time of one minute. A total of 1654 unique reflections were measured for $2 < \theta < 23°$ and $+h, -k, +l$ and 799 reflections with $|F^2| > 3\sigma(F^2)$ were used in the refinement, where $\sigma(F^2) = [\sigma^2(I) + (0.04I)^2]^{1/2}/Lp$. No correction was made for absorption.

The structure was solved by direct methods (SHELXS-86)[13] and non-hydrogen atoms refined anisotropically by full matrix least squares. Hydrogen atoms, except for the hydroxy H atom which could not be located, were held fixed at calculated positions with $U_{iso} = 1.3U_{eq}$ for the parent atom. With weights of $w = 1/\sigma^2(F)$ the refinement converged at R = 0.092, R' = 0.137, 145 variables, S = 4.4, $(\Delta/\sigma)_{max}$ = 0.01. A final difference map revealed several peaks of up to 0.9 e $Å^{-3}$ near the line $x = 0$, $y = 0$, which are presumably due to extensively disordered benzene solvate, but which could not be rationally modelled. Programs from the Enraf-Nonius SDP-Plus package were run on a microVax II computer.*

Acknowledgements

We thank A. M. Greenway for the mass spectra and Dr. A. Avent for the NMR spectra.

References

1 J. F. Grove, *Nat. Prod. Rep.*, 1988, **5**, 187.
2 A. Visconti, C. J. Mirocha, A. Logrieco, A. Bottalico and M. Solfrizzo, *J. Agric. Food Chem.*, 1989, **37**, 1348.
3 C. Bonsall and J. Hill, *J. Chem. Soc. C*, 1967, 1836.
4 P. Caramella and A. Querci, *Synthesis*, 1972, 46.
5 W. V. Turner and W. H. Pirkle, *J. Org. Chem.*, 1974, **39**, 1935.
6 E. Abignente, P. De Caprariis and M. L. Stein, *Farmaco Ed. Sci.*, 1975, **30**, 992.
7 K. Arai, T. Yoshimura, Y. Itatani and Y. Yamamoto, *Chem. Pharm. Bull.*, 1983, **31**, 925.
8 D. W. Cameron, D. G. I. Kingston, N. Sheppard and Lord Todd, *J. Chem. Soc.*, 1964, 98.
9 M. E. Savard, J. D. Miller, B. Salleh and R. N. Strange, *Mycopathologia*, 1990, **110**, 177.
10 S. Shimizu, I. Sakurai and Y. Yamamoto, *Chem. Pharm. Bull.*, 1983, **31**, 3781.
11 M. S. R. Nair and S. T. Carey, *Tetrahedron Lett.*, 1975, 1655.
12 P. W. Brian, A. W. Dawkins, J. F. Grove, H. G. Hemming, D. Lowe and G. L. F. Norris, *J. Exp. Bot.*, 1961, **12**, 1.
13 G. M. Sheldrick in *Crystallographic Computing 3*, eds. G. M. Sheldrick, C. Kruger and R. Goddard, Oxford Univ. Press, 1985, pp. 175–189.

* Tables of hydrogen atom co-ordinates and anisotropic temperature factors are available from the Cambridge Crystallographic Data Centre. For details of CCDC deposition scheme see Instructions for Authors, *J. Chem. Soc., Perkin Trans. 1*, 1991, Issue 1.

Paper 0/05288D
Received 26*th November* 1990
Accepted 13*th December* 1990