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PIGMENTS FROM *FUSARIUM MONILIFORME* SHELDON

STRUCTURE AND ^{13}C NUCLEAR MAGNETIC RESONANCE ASSIGNMENTS OF AN AZAANTHRAQUINONE AND THREE NAPHTHOQUINONES

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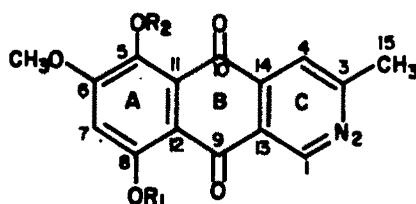
Abstract—Physico-chemical techniques established the novel structure of 8-O-methylbostrycoidin, the main pigment from *Fusarium moniliforme* as 6,8-dimethoxy-5-hydroxy-3-methyl-2-azaanthraquinone. The structures of three biogenetically related naphthoquinones were elucidated by comparison of spectroscopic data.

F. moniliforme is a major parasite of several Gramineae.[1,2] This fungus is wide-spread in both humid and subhumid temperate zones and extending into subtropical and tropical areas throughout the world.[1] This paper relates the isolation and identification of four dark-red pigments from the cultivation of *F. moniliforme* on solid media. This coloration is typical of cultures of *F. moniliforme*.[1]

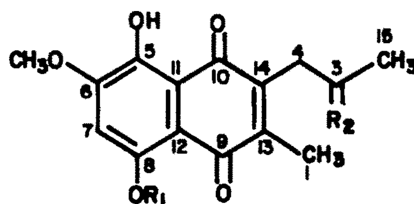
The toxinogenic strain of *F. moniliforme* (MRC 602) was isolated from mouldy ears of maize in the Transkei. Cultures on autoclaved maize kernels were incubated at 25° for 21 days, dried overnight at 45° and ground to a fine meal. Prolonged extraction of this mouldered material with chloroform–methanol gave a lipidic dark-red nontoxic fraction. This fraction was purified by solvent partition, partition and adsorption chromatography and yielded four new pigments,† viz. the 8-O-methyl derivatives of bostrycoidin (1a),[3] javanicin (2a),[4] solaniol (2c)[5] and fusarubin (3a).[4] Bikaverin,[6] a benzoxanthentrione pigment from *F. moniliforme* (= *Giberella fujikuroi*) and other *Fusarium* species was not produced by strain MRC 602.

8-O-Methylbostrycoidin (1b). 8-O-Methylbostrycoidin (1b) is the major pigment from cultures of this strain of *F. moniliforme*. The structure of this metabolite (1b), $C_{16}H_{13}NO_5$, m.p. 215–216° was established by physical methods, particularly ^1H and ^{13}C NMR spectroscopy. Its electronic spectral data, viz. λ_{max} (log ϵ) 247.5 (4.50), 318 (3.92), and 480 nm (3.83); λ_{max} (MeOH–acidic) 227 (4.32), 262 (4.20), 310 (3.88), and 510 nm (3.73); λ_{max} (MeOH-alkaline) 259 (4.44), 306 (3.82) and 546 nm (4.0) are consistent with the conjugated chromophore as found in the proposed structure. Treatment of 8-O-methylbostrycoidin with Zn in HOAc led to a change in the color from dark-red to light pink, however, upon removal of the Zn by filtration, the material was readily oxidized to starting

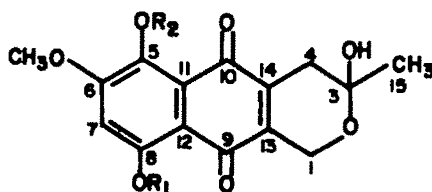
†The present numbering system for these compounds was chosen for direct comparison of the corresponding carbon atoms in the different pigments.



1a $R_1 = R_2 = H$
1b $R_1 = CH_3$, $R_2 = H$
1c $R_1 = CH_3$, $R_2 = COCH_3$



2a $R_1 = H$, $R_2 = O$
2b $R_1 = CH_3$, $R_2 = O$
2c $R_1 = H$, $R_2 = H,OH$
2d $R_1 = CH_3$, $R_2 = H,OH$



3a $R_1 = R_2 = H$
3b $R_1 = CH_3$, $R_2 = H$
3c $R_1 = CH_3$, $R_2 = COCH_3$

material, indicating that the metabolite is a quinone; this supposition is in accordance with the IR spectrum of **1b** ν_{max} 1640 and 1591 cm^{-1}. 8-O-Methylbostrycoidin forms blue alkali-metal salts and exhibits strong chelating properties with e.g. Ca^{2+} and Mg^{2+}. Acetylation of **1b** gave a fluorescent light-yellow monoacetate (**1c**), $C_{18}H_{15}NO_6$, m.p. 254–246°; λ_{max} 236.5 (4.49), 263 (4.08), 287 (4.09) and 406 nm (3.75); ν_{max} 1768, 1664 and 1593 cm^{-1}.

The ^{1}H and ^{13}C NMR data as given in Table 1 and 2, respectively, have been obtained from the completely assigned ^{1}H and natural-abundance ^{13}C NMR spectra of **1b** and **1c**. The ^{13}C assignments are derived from coupled (Fig. 1a), proton noise decoupled (p.n.d.), heteronuclear selective population inversion (SPI)[7] and selective proton decoupled ^{13}C NMR spectra.

The three C signals derived from the Me and OMe groups were deduced from chemical shift considerations and the value of directly bonded (C, H) couplings. The OMe signal in 5-methoxy-1,4-napthoquinone is found at lower field than in its 6-methoxy analogue.[8] These results have been used in assigning the OMe resonances to a specific C atom as given in Table 2. The proton resonances $\delta = 9.44$, 7.85 and 6.86 were correlated by SPI experiments with the methine signals at δ(C) = 149.6, δ(C) = 116.9 and δ(C) = 104.2, respectively. The observed chemical shifts (^{1}H and ^{13}C) and ^1J(CH) of C-1–H and C-4–H are similar to those of the corresponding positions in isoquinoline.[9,10] The two low-field carbon resonances at $\delta = 188.7$ and $\delta = 179.0$, characteristic of anthraquinonoidal oxo C atoms in a similar environment,[8,11] were assigned to C-10 and C-9, respectively. On acetylation of **1b** the C-10 resonance showed the expected shift to high field [δ(**1c**) − δ(**1b**) = −6.5 ppm].

The substitution pattern of ring A of 8-O-methylbostrycoidin (**1b**) was determined from carbon-proton couplings in aromatic systems,[12] couplings from a H-bonded phenolic proton,[11] and the effect of acetylation on ^{13}C chemical shifts.[13] Application of a selective π pulse ($H_2 = 5$ Hz) to low field (~5 Hz) of the OH-proton resonance (Fig. 1c) affected the ^{13}C resonances at $\delta = 155.1$ [C-6, ^3J(C-6, OH) = 6.4 Hz], $\delta = 148.9$ [C-5, ^2J (C-5, OH) = 4.5 Hz], and $\delta = 115.4$ [C-11, ^3J(C-11, OH) = 4.5 Hz]. When the selective π pulse was applied to low field of the C-7 proton resonance (Fig. 1b), the signals at $\delta = 156.0$ [C-8, ^2J(C-8, 7-H) = 4.3 Hz), $\delta = 155.1$ [C-6, ^2J (C-6, 7-H) = 4.3 Hz], $\delta = 148.9$ [C-5, ^3J(C-5, 7-H) = 6.9 Hz], and $\delta = 110.9$ [C-12, ^3J(C-12, 7-H) = 5.7 Hz] were modified. Selective decoupling of the OMe-protons simplified the resonances at $\delta = 156.0$ (C-8) and $\delta = 155.5$ (C-6).

The signals at $\delta = 149.6$ and $\delta = 163.9$ were allocated to the C atoms adjacent to the N group in ring C, viz. C-1 and C-3, respectively. Selective inversion of a C-1 proton transition (Fig. 1d) established a coupling of 12.4 Hz between this proton and C-3 and influenced the resonances at $\delta = 125.5$ [C-13, ^2J(C-13, 1-H) = 6.5 Hz] and $\delta = 137.4$ [C-14, ^3J(C-13, 1-H) = 5.4 Hz]. Selective inversion of a C-4 proton transition affected the resonances at $\delta = 188.7$ [C-10, ^3J(C-10, 4-H) = 4.5 Hz], $\delta = 163.9$ [C-3, ^2J (C-3, 4-H) = 2.6 Hz], $\delta = 125.5$ [C-13, ^3J(C-13, 4-H) = 4.4 Hz], and $\delta = 25.1$ [C-15, ^3J(C-15, 4-H) = 2.2 Hz]. The magnitude of these observed couplings corresponds remarkably well with those reported for isoquinoline.[10]

The intensity gain which can be obtained with progressively saturated SPI experiments[7] for quaternary C atoms is clearly illustrated in Fig. 1b–d. The signals resulting from the quaternary C atoms (e.g. those from C-11 and C-12) could hardly be detected under the rapid pulsing condition employed in these experiments. Selective pulsing of a transition of a proton which couples to quaternary C atoms results in a spectacular enhancement of the intensities of these C resonances.

From the foregoing data it is evident that this new

Table 1. ^{1}H NMR data of bostrycoidin (**1a**), 8-O-methylbostrycoidin (**1b**), 5-O-acetyl-8-O-methylbostrycoidin (**1c**), 8-O-methyljavanicin (**2b**), 8-O-methylsolaniol (**2d**), 8-O-methylfusarubin (**3b**) and 5-O-acetyl-8-O-methylfusarubin (**3c**)

Proton	Compound[a]						
	1a[b]	1b	1c	2b	2d	3b[c]	3c
1	9.47	9.44	9.38	2.11	2.12	4.66 ABX$_2$	4.69 ABX$_2$
3					4.18 ABX	J(AB)=18Hz	J(AB)=18Hz
4	7.91	7.85	7.73	3.78	2.62	2.50 J(AX)=J(BX)	2.45 J(AX)=J(BX)
					2.80	2.78 =2.7 Hz	2.80 = 2.7 Hz
7	6.70	6.86	6.88	6.74	6.49	6.85	6.73
15	2.78	2.76	2.72	2.31	1.36d J=7 Hz	1.56	1.58
OCH$_3$	4.00	4.05	3.98	3.99	3.87	4.01	3.93
OCH$_3$		4.05	4.06	4.02	3.92	4.05	3.98
COCH$_3$			2.44				2.41
OH	13.10	13.19		13.02	13.22	13.16	
OH	13.38				3.41 d J=5 Hz		

[a]Proton chemical shifts are relative to internal $(CH_3)_4Si$ in $CDCl_3$. All resonances are singlets except otherwise indicated.

[b]From Ref. 3.

[c]In 1:1 $CDCl_3$ and $(CD_3)_2SO$.

Table 2. ^{13}C NMR chemical shifts, directly bonded [$^1J(CH)$] and over more than one bond [$^{>1}L(CH)$] carbon-13-proton coupling constant of 8-O-methylbostrycoidin (1b), 5-O-acetyl-8-O-methylbostrycoidin (1c), 8-O-methyljavanicin (2b), 8-O-methylsolaniol (2d), 8-O-methylfusarubin (3b) and 5-O-acetyl-8-O-methylfusarubin (3c)

Carbon atom	1b			1c		2b	2d	3b	3c	
	δ^a	$^1J(CH)$ Hz	$^{>1}J(CH)$ Hz	δ^a	$\delta(1c)-\delta(1b)$	δ^a	δ^a	δ^b	δ^a	$\delta(3c)-\delta(3b)$
1	149.6D	185.5	-	149.2		13.9	13.9	57.7	58.0	
3	163.9Sdqd	-	12.4;6.3;2.6	163.8		203.1	67.2	93.1	94.2	
4	116.9Dq	167.5	3.6	117.1		41.1	36.7	31.7	31.9	
5	148.9Sdd	-	6.9;4.5	133.1	-15.8	147.9	147.4	147.6	133.0	-14.9
6	155.1Sdqn	-	6.4;4.5	160.2[c]	5.1	155.1[c]	154.7[c]	155.0[c]	159.4[c]	4.4
7	104.2D	158.4	-	102.1	- 2.1	102.9	101.8	103.0	100.9	- 2.1
8	156.0Sqn	-	4.3	157.6[c]	1.6	155.6[c]	155.0[c]	155.3[c]	157.5[c]	2.2
9	179.0S	-	-	179.9		181.2	181.3	179.0	180.9	1.9
10	188.7Sd	-	4.5	182.2	- 6.5	189.4	190.5	188.7	182.6	- 6.1
11	115.4Sd	-	4.5	126.6	11.2	114.3	114.1	113.7	125.1	11.4
12	110.9Sd	-	5.7	114.0	3.1	110.6	109.9	108.9	112.4	3.5
13	125.5Sdd	-	6.5;4.4	124.9		150.0	149.6	144.6	141.9	- 2.7
14	137.4Sd	-	5.4	138.2		137.8	141.4	136.8	137.8	
15	25.1Qd	127.6	2.2	25.2		30.2	23.9	28.3	28.9	
6-OCH_3	56.4Q	145.8	-	56.4		56.4	56.2	56.1	56.2	
8-OCH_3	57.0Q	145.1	-	56.8		57.0	56.5	56.4	56.5	
C = O				168.9					169.1	
CH_3				20.7					20.7	

[a] Relative to internal $(CH_3)_4Si$. Solvent $CDCl_3$. Measured from internal $CDCl_3$ and corrected by using the expression $\delta[(CH_3)_4Si] = \delta[CDCl_3] + 77.0$. Capital letters refer to the pattern resulting from directly bonded protons and small letters to (C, H) couplings over more than one bond. S = singlet, D or d = doublet, Q or q = quartet, qn = quintet.

[b] Relative to internal $(CH_3)_4Si$. Solvent 1:1 $CDCl_3$ and $(CD_3)_2SO$. Measured from internal $(CD_3)_2SO$ and corrected by using the expression $\delta[(CH_3)_4Si] = \delta[(CD_3)_2SO] + 39.7$.

[c] May be interchanged.

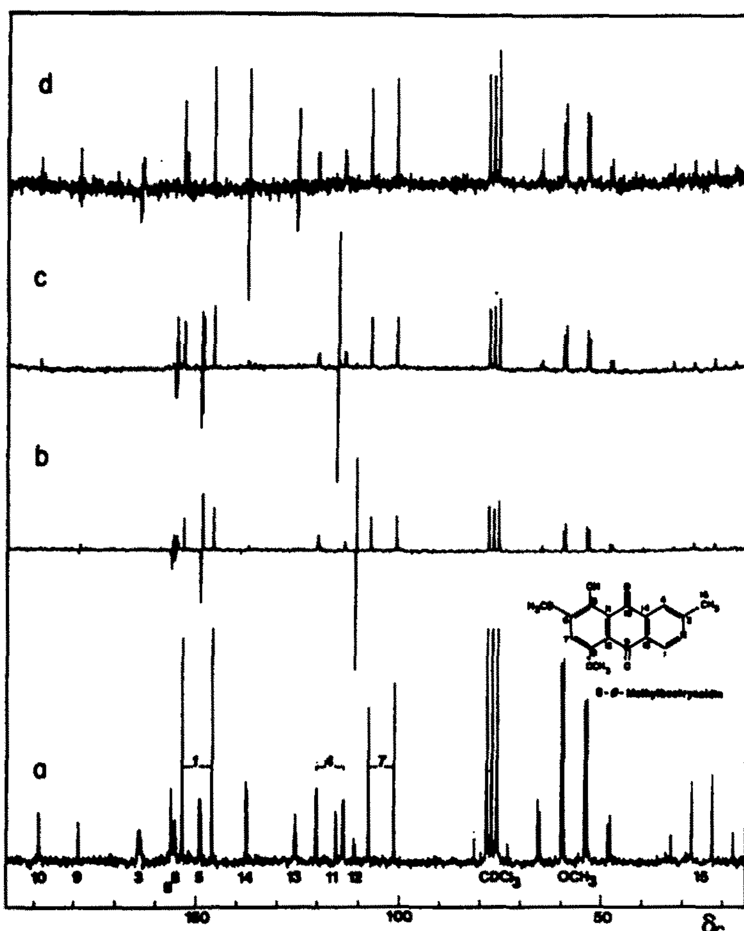


Fig. 1(a). The coupled n.O.e. 25.2 MHz ^{13}C NMR spectrum of 8-O-methylbostrycoidin (1b), spectral width: 5000 Hz; acquisition time: 0.8 sec; 90° pulse of 51 μsec; Results of SPI experiments, pulse delay: 1 sec; π pulse 0.094 sec. (b) Low field transition of 7-H selectively pulsed, transients: 5929. (c) Low field transition of the hydroxy-proton selectively pulsed, transients: 28 258. (d) Low field transition of 1-H selectively pulsed, transients: 6721.

pigment should be formulated as 6,8-dimethoxy-5-hydroxy-3-methyl-2-azaanthraquinone, therefore as 8-O-methylbostrycoidin. The UV spectra of bostrycoidin[14] and of 8-O-methylbostrycoidin are remarkably similar and differ substantially from the more complex spectra of their carbocyclic anthraquinone counterparts.[15] The ^{1}H NMR data of compounds 1a, 1b and 1c (Table 1) are in agreement with the structural assignments, the very low-field chemical shift of the 1-H being a distinctive feature of these compounds.

Bostrycoidin (1a), the only previously known azaanthraquinone, is a metabolite of *Fusarium bostrycoidin*[14] and of *Fusarium solani* D_2 purple.[3] Bostrycoidin exhibits antibiotic properties and strongly inhibits growth of the tubercle bacillus *in vitro*.

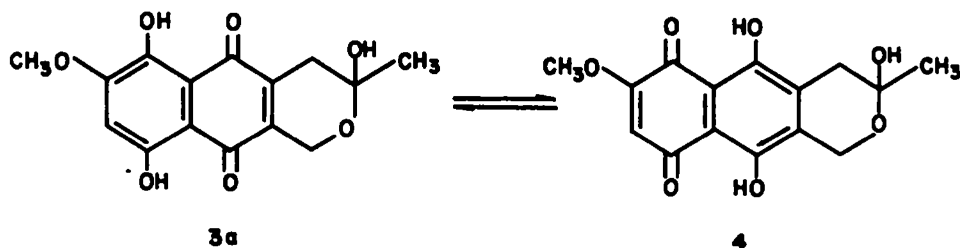
8-O-Methyljavanicin (2b), *8-O-methylsolaniol* (2d) *and 8-O-methylfusarubin* (3b). Cultures of *F. moniliforme* MRC 602 produced in addition to 8-O-bostrycoidin, three minor naphthoquinonoidal compounds, viz. 8-O-methyljavanicin (2b) $C_{16}H_{16}O_6$, m.p. 197–198°; 8-O-methylsolaniol (2d) $C_{16}H_{18}O_6$, m.p. 138–139°, and 8-O-methylfusarubin (3b) $C_{16}H_{16}O_7$, m.p. 152–154°.

The spectro-analytical parameters of the three compounds indicated a close structural relationship among these naphthoquinones. 8-O-Methylfusarubin (3b) failed to give a molecular ion in its mass spectrum upon electron ionisation. The compound was, therefore, converted into a mono-acetate (3c) which exhibited the appropriate molecular ion in its mass spectrum.

The ^{1}H and ^{13}C NMR data of compounds 2b, 2d, 3b, and 3c as collated in Tables 1 and 2 confirmed the proposed structures of these naphthoquinones. The assignment of the ^{13}C signals are based on a comparison of the ^{13}C data with those of 8-O-methylbostrycoidin and on the previously mentioned assignment techniques. The assignment of the C-13 and C-14 resonances, although tentative, is based on the following observation. The ^{13}C data of 2b and 2d showed a bigger chemical shift difference for the C resonances assigned to C-14 than for those assigned to C-13. This phenomenon is in accordance with the structures of 2b and 2d.

The naphthazarines, e.g. fusarubin (3a) exist as two distinct tautomers (3a-4) in solution, the equilibrium composition being governed by well-known electronic factors. The tautomerism is prevented in the foregoing compound by the blocking of the C-8-phenolic OH-group and the compounds are, therefore, present in the form 2b, 2d and 3b only.

It is important to note that cultures of *Fusarium moniliforme* var *subglutinans* (MRC 604) contained 8-O-methylbostrycoidin (1b) and 8-O-methylfusarubin (3b) as the two major pigments. *F. moniliforme* (MRC 602)



and *F. moniliforme* var. *subglutinans* (MRC 604) do not produce the mycotoxin, moniliformin.[16] The relationship between the production of moniliformin by these fungi and the production of pigments is under investigation.

EXPERIMENTAL

M.ps were determined on a Kofler hot-stage apparatus. UV absorptions were measured (for solns in MeOH) on a Unicam SP 800 spectrometer. IR spectra (for solns in $CHCl_3$) on a Perkin-Elmer 237 spectrometer, and mass spectra on an A.E.I. MS9 double-focusing spectrometer. ^1H NMR spectra were recorded with a Varian EM-390 spectrometer (90 MHz; Me_4Si as lock signal and internal reference) and 25.2 MHz ^{13}C NMR spectra were recorded with a Varian XL-100-15 Fourier transform spectrometer equipped with a 16 K Varian 620i computer and a gated gyrocode decoupler.

Isolation of the pigments. Fusarium moniliforme (strain MRC 602) was grown in bulk on sterilized maize meal at 25° for 21 days. The dried mouldy maize (15 kg) was extracted with $CHCl_3$ over a period of 2 days and the solvent removed under reduced pres. The residue (1.4 kg) was partitioned between hexane (5 l.) and aq. 90% MeOH (5 l.). The aq. MeOH soln was concd and the residue partitioned between $CHCl_3$ (4 l.) and H_2O (4 l.). Evaporation of the $CHCl_3$ phase gave a residue (60 g) containing the pigments.

The foregoing residue (60 g) was chromatographed on formamide-impregnated cellulose powder (1.5 kg) packed in hexane. Hexane (8 l.) eluted lipids. Elution of the column with C_6H_6 (10 l.) gave a red-coloured fraction A (17 g), while elution with C_6H_6–CH_3COOEt(1:1) yielded another pigment-containing fraction B (6 g).

Fraction A (17 g) was further fractionated and purified by column chromatography on Merck Si gel Type H (1.6 kg) using $CHCl_3$–MeOH (96:4). The column was developed under 1 kg/cm^2 pres., 15 ml fractions were collected. Appropriate fractions were combined to yield 8-O-*methyljavanicin* (40 mg) and 8-O-*methylbostrycoidin* (1.2 g).

Fraction B (6 g) was purified as for fraction A on Merck Si gel, Type H (500 g). Elution with $CHCl_3$–MeOH (98:2) gave a pigment-containing material designated fraction C (710 mg). Elution of the column with $CHCl_3$–MeOH (95:5) yielded 8-O-*methylfusarubin* (380 mg).

Fraction C (710 mg) was separated by chromatography on six 20 × 20 cm Merck pre-coated PLC plates Si gel F-254, thickness 2 mm. The plates were developed in $CHCl_3$–MeOH (94:6). 8-O-*methylsolaniol* (80 mg) was eluted from the plates.

On Si gel tlc plates in $CHCl_3$–MeOH (96:4) the above pigments appear as 8-O-*methylbostrycoidin*, 8-O-*methyljavanicin*, 8-O-*methylsolaniol* and 8-O-*methylfusarubin* at R_f 0.51, 0.44, 0.27, and 0.20, respectively.

8-O-*methylbostrycoidin* (1b) was crystallized from $CHCl_3$–MeOH and had m.p. 215–216°; ν_{max} 1641, 1591, 1311, 1265 cm^{-1}. UV and NMR data in text and Tables (Found: 63.75; H, 4.39; N, 4.63% and *m/e* 299.080 $C_{16}H_{13}NO_5$ requires: C, 64.20; H, 4.35; N, 4.68% and M$^+$ 299.083).

8-O-*Methyljavanicin* (2b) was crystallized from acetone and had m.p. 197–198°; λ_{max} 226, 282.5, 482, 510 and 550 (sh) nm (log ϵ 4.56, 4.04, 3.80, 3.75 and 3.80 respectively); ν_{max} 1620, 1470, 1438 and 1270 cm^{-1}. NMR data in Tables (Found: C, 63.31; H, 5.45% and *m/e* 304.097. $C_{16}H_{16}O_6$ requires: C, 63.15; H, 5.30% and M$^+$ 304.095).

8-O-*Methylsolaniol* (2d) was crystallized from MeOH and had m.p. 152–154°; λ_{max} 226, 285, 476, and 510 (sh) nm (log ϵ 4.49, 4.025, 3.82 and 3.69, respectively); ν_{max} 1628, 1470, 1435 and 1272 cm^{-1}. NMR data in Tables. (Found: *m/e* 306.111, $C_{16}H_{18}O_6$ requires: 306.110).

8-O-*Methylfusarubin* (3b) was crystallized from MeOH and had m.p. 138–139°; λ_{max} 226, 282.5, 484, 510 and 550 (sh) nm (log ϵ 4.48, 4.05, 3.83, 3.80 and 3.49, respectively); ν_{max} 1600 cm^{-1}. NMR data in Tables. (Found: C, 56.88; H, 5.39% $C_{16}H_{16}O_7 \cdot H_2O$ requires: C, 56.80; H, 5.33%).

The acetylation of 8-O-methylbostrycoidin. 8-O-Methylbostrycoidin (150 mg) in pyr–Ac_2O (1:1; 20 ml) was kept at 20° for 16 hr and poured onto ice. Extraction into $CHCl_3$ gave 5-O-acetyl-8-O-methylbostrycoidin (1c), m.p. 245–246° from acetone. It had ν_{max} 1768, 1664, 1594 and 1279 cm^{-1}. Its high resolution mass spectrum showed: *m/e* 341.089 (M$^+$, $C_{18}H_{15}NO_5$ requires: 341.089).

The acetylation of 8-O-methylfusarubin. 8-O-Methylfusarubin (100 mg) was acetylated as above for 2 hr to give 5-O-acetyl-8-O-methylfusarubin (120 mg). It had m.p. 199–201° from MeOH; λ_{max} 218, 269, 285 (sh), 415 nm (log ϵ 4.38, 4.10, 3.86 and 3.52, respectively); ν_{max} 1768, 1660, 1591 and 1270 cm^{-1}. (Found: C, 59.30; H, 5.03% and *m/e* 362.099. $C_{18}C_{18}O_8$ requires: C, 59.65; H, 4.97% and M$^+$ 362.100).

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