Structure Elucidation of Fusarin C, a Mutagen produced by *Fusarium moniliforme*

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The assignment of structure (**1**) to fusarin C, a mutagen isolated from cultures of *Fusarium moniliforme* is based on a detailed study of its high-field ^1H and ^{13}C n.m.r. spectra and *X*-ray crystallography of the 8*Z* isomer of (**1**) which defined the substitution pattern and relative configuration of the 2-pyrrolidone moiety; nuclear Overhauser enhancement experiments indicate that the 2*E*,4*E*,6*E*,8*E*,10*E* polyene chromophore of (**1**) exists in solution as an equilibrium between two conformers with s-*cis* and s-*trans* topology of the C-5–C-6 single bond.

Fusarium moniliforme Sheldon occurs world-wide on a great variety of plant hosts and is one of the most prevalent fungi on maize (*Zea Mays* L.).[1] An isolate of *F. moniliforme* (strain MRC 826), obtained from maize in an area of the Transkei, Southern Africa with a high oesophageal cancer rate,[2] was found to be not only highly toxic and able to induce leukoencephalomalacia in horses,[3] but also mutagenic.[4] The main mutagen isolated in the present study appears to be identical to fusarin C on the basis of the limited reported spectroscopic data[5] and this name is therefore retained for this metabolite.

High resolution mass spectrometric analysis of the molecular ion, *m/z* 431.1970, of fusarin C (**1**) gave the molecular formula as $C_{23}H_{29}NO_7$; the metabolite had λ_{max} (MeOH) 358 nm (ε 32 000) and ν_{max} ($CHCl_3$) 1720, 1630, and 1590 cm^{-1}. The ^1H and ^{13}C n.m.r. data for fusarin C are collated in Table 1. Selective population inversion (SPI)[6] experiments established the two- and three-bond (C,H) connectivity pattern for fusarin C (**1**) but did not allow us to differentiate between the substituted 2-pyrrolidone in (**1**) and that shown in (**2**). The structure elucidation of two related non-mutagenic metabolites, fusarin A (**3**) ($C_{23}H_{29}NO_6$) and D

Table 1. N.m.r. data for fusarin C (**1**).[a]

Carbon atom	δ_C[b]/p.p.m.	^1J(CH)/Hz	δ_H[c]	J(HH)/Hz
1	16.15 Qd	127.3	1.773 dd	7.2, 1.4
2	140.33 Dm	157.5	6.957 qd	7.2, 1.1
3	130.81 S	—		
4	126.67 Dm	157.5	6.071 qd	1.4, 1.1
5	137.81 S	—		
6	140.99Dm	151.3	6.302 sbr.	
7	135.42 S	—		
8	149.15 Dm	157.0	6.790 d	15.0
9	123.79 D—	154.4	6.670 dd	15.0, 11.0
10	145.73 Dm	154.6	7.492 dbr.	11.0
11	133.90 S	—		
12	190.17 Sm	—		
13	62.17 Sd	—		
14	64.15 Dd	197.1	4.061 d[d]	2.1
15	85.92 S	—		
17	170.27 S	—		
18	36.27 T—	128.5	2.059 ddd	14.6, 6.0, 3.7
			2.113 ddd	14.6, 8.3, 4.1
19	58.77 Tt	144.0	4.050 ddd	11.1, 8.3, 3.7
			3.935 ddd	11.1, 6.0, 4.1
20	167.77 S	—		
21	52.07 Q—	146.7	3.715 s	
22	18.91 Qdd	127.1	1.729 d	1.4
23	14.28 Qdd	127.5	2.091 d	1.3
24	11.55 Qd	128.6	1.981 d	1.3

[a] Recorded on a Bruker WM-500 n.m.r. spectrometer. [b] Relative to internal Me_4Si; solvent CD_2Cl_2. Capital letters refer to the pattern resulting from directly bonded (C,H) couplings [^1J(CH)] and small letters to that from (C,H) couplings over more than one bond [$^{>1}J$(CH)]. S = singlet, D or d = doublet, T or t = triplet, Q = quartet, and m = multiplet. [c] Relative to internal Me_4Si; solvent CD_2Cl_2. s = singlet, d = doublet, q = quartet, and br. = broad. The chemical shifts of the N-16 proton (δ 7.01) and the two hydroxy protons (δ 2.85 and 5.10) are concentration dependent. [d] Doublet due to coupling to the N-16 proton.

(**4**) ($C_{23}H_{29}NO_7$), based on a similar detailed study of their high-field ^1H and ^{13}C n.m.r. spectra to be reported in a full paper, confirmed the presence of the substituted 2-pyrrolidone (**1**) in fusarin C. In the event additional evidence for the structure (**1**) was provided by *X*-ray crystallography of an isomer of fusarin C.

Prolonged exposure of fusarin C to u.v. light as well as high temperatures results in extensive decomposition.[4] Irradiation of a dichloromethane solution of (**1**) at 366 nm for 5 min, however results in the formation of a mixture of four compounds, which includes starting material. H.p.l.c. of the mixture on silica gel using dichloromethane–methanol (95 : 5 v/v) as eluant resulted in the isolation of three isomers of fusarin C, which, on the basis of their ^1H n.m.r. chemical shifts and (H,H) coupling constants, are assigned the 8*Z* {10-H [δ 7.675 (ddq, *J* 10.9, 1.3, 1.3 Hz)], 9-H [δ 6.469 (dd, *J* 11.0, 11.0 Hz)], and 8-H [δ 6.400 (dbr., *J* 11.0 Hz)]}, the 6*Z* {10-H [δ 7.473 (dm, *J* 11.2 Hz)], 9-H [δ 6.716 (dd, *J* 15.0, 11.2 Hz)], and 8-H [δ 7.465 (d, *J* 15.0 Hz)]}, and the 10*Z* {10-H [δ 6.560 (d, *J* 11.7 Hz)], 9-H [δ 7.157 (dd, *J* 14.9, 11.7 Hz)], and 8-H [δ 6.567 (d, *J* 14.9 Hz)]} stereochemistries, respectively.

The 8*Z* isomer of fusarin C crystallised from dichloromethane as orthorhombic crystals (m.p. 170—172 °C), space group $P2_12_12_1$ with a = 17.285(8), b = 16.544(7), c = 7.899(5) Å, Z = 4, D_c = 1.27, and D_m = 1.22 g cm^{-3}. Intensity measurements were made with Cu-K_α radiation (λ = 1.5418 Å; graphite monochromator) on a Philips PW 1100 four-circle diffractometer in the ω–2θ mode with $3 \leq \theta \leq 66°$. A total of 2282 unique reflections were measured of which 430 were regarded as unobserved with $I < 2\sigma(I)$. The measured reflections were corrected for background and Lorentz–polarization effects only. Accurate cell parameters were obtained by least-squares techniques from the diffractometer settings for 25 reflections. The structure was solved using MULTAN 78[7] and refined by blocked-matrix least-squares techniques using the SHELX[8] computer programme with $1/\sigma^2$ weights. The positions of all the hydrogen atoms could be determined from difference Fourier maps and their parameters were included in the refinement. Convergence, with anisotropic thermal parameters for all non-hydrogen atoms and a common isotropic thermal parameter for the hydrogen atoms was reached at R = 0.056 (R_w = 0.042) using all data. The difference electron density map based on the final atomic parameters showed no maxima greater than 0.20 e Å^{-3}.†

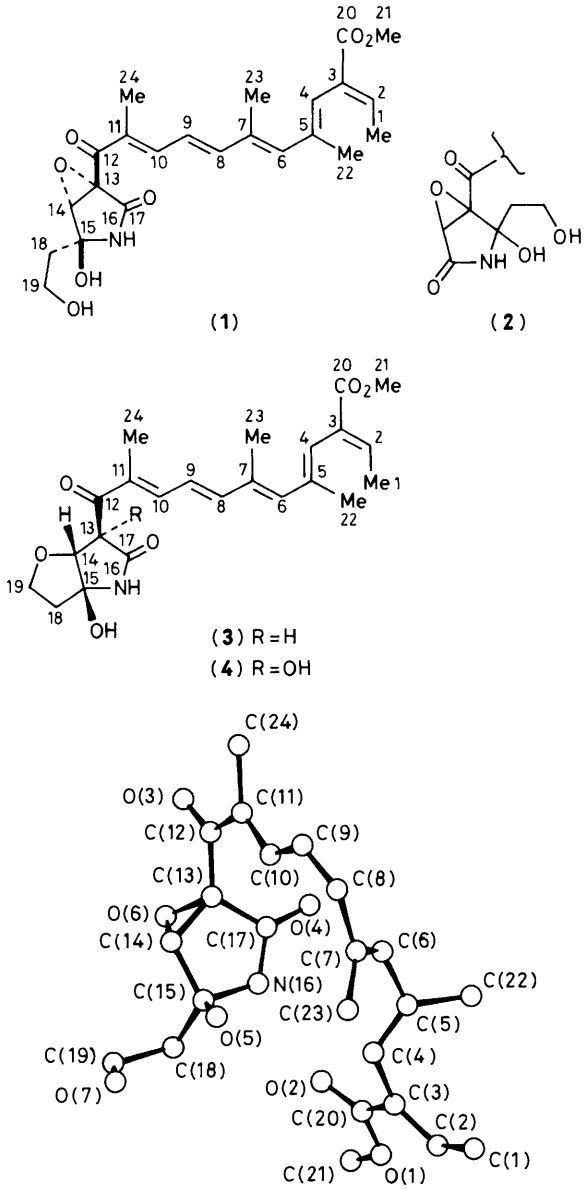

Figure 1. Perspective drawing of the 8*Z* isomer of fusarin C.

† The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Rd., Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

The resulting structure as well as the relative configuration are illustrated in Figure 1. The conformation of the polyene chromophore deserves particular attention. The torsional angles, ϕ, for the constituent atoms of this moiety indicate that a high degree of distortion *i.e.* deviation from planarity exists about the C-3–C-4 (ϕ 67.5°), C-5–C-6 (ϕ 55.8°), and C-7–C-8 (ϕ 51.3°) single bonds. It is evident that in the crystal the 2*E*,4*E*,6*E*,8*Z*,10*E* polyene chromophore exists as the distorted 3-s-*cis*,5-s-*cis*,7-s-*trans*,9-s-*trans*,11-s-*trans* conformer. This conformation also leads to intramolecular hydrogen bonding between the N-16 proton and O-2 of the methoxycarbonyl group (1.964 Å). In addition the oxygen atoms of the two hydroxy groups are 2.688 Å apart and the respective hydrogen atoms are aligned in the direction of the opposing oxygen atom.

In contrast, homonuclear ^{1}H-{^{1}H} nuclear Overhauser enhancement experiments indicate that the 2*E*,4*E*,6*E*,8*E*,10*E* conjugated polyene in fusarin C (**1**) exists in solution as an equilibrium between two conformers with s-*cis* and s-*trans* topology of the C-5–C-6 single bond. It is of interest to note that 11-*cis*-retinal, the chromophore of the visual pigment rhodopsin, is believed to exist in solution as an equilibrium mixture of twisted 12-s-*cis* and 12-s-*trans* conformers.[9]

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