Structure and Absolute Configuration of the Asticolorins, Toxic Metabolites from *Aspergillus multicolor*

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The structure elucidation of the asticolorins A—C, toxic metabolites isolated from cultures of *Aspergillus multicolor*, is based on *X*-ray crystallography of asticolorin A (**1**) and on a detailed study of their ^1H and ^{13}C n.m.r. spectra; the chirality of the C-29 secondary hydroxy group present in asticolorin A, as determined by the method of Horeau, established the absolute configuration.

Asticolorin A (**1**), a mycotoxin isolated from toxic extracts of whole maize cultures of *Aspergillus multicolor*, strain MRC 638, crystallised from acetone as monoclinic crystals, m.p. >320 °C, and showed $[\alpha]_D^{20}$ −120.5° (*c* 0.20, acetone) and $\lambda_{max.}$ (MeOH) 225 (ε 81 370), 263 (28 080), 290 (23 290), 304 (12 660), and 316 nm (20 480). Field desorption mass spectrometry gave the molecular ion as *m/z* 538 ($C_{33}H_{30}O_7$) whereas elemental analysis proved the empirical formula as $2C_{33}H_{30}O_7 \cdot 3MeCOMe \cdot H_2O$. The presence of both acetone and water as solvent of crystallisation in the crystals was confirmed by *X*-ray crystallography.

Crystal data: monoclinic, space group $P2_1$, a = 13.381(6), b = 24.855(9), c = 9.965(5) Å, β = 99.28(1)°, Z = 4, and D_c = 1.32 g cm^{-3}. Intensity measurements were made at 22 °C with Mo-K_α radiation (λ = 0.7107 Å; graphite monochromator) on a four-circle diffractometer in the ω–2θ mode with $3 \leq \theta \leq 27°$. A total of 6513 unique reflections were measured of which 1293 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[1] by re-entering the part of the structure that was found in one solution as a correctly orientated but wrongly positioned entity. The structure was refined by blocked-matrix least-squares techniques using the SHELX[2] computer program with $1/\sigma_F^2$ weights. The hydrogen atoms were included in the refinement in calculated positions. Convergence, with anisotropic thermal parameters for all non-hydrogen atoms and a common isotropic thermal parameter for the hydrogen atoms, was reached at R_w = 0.069 (R = 0.090) using all data. The difference electron density map based on the final atomic parameters showed no maxima greater than 0.32 e Å^{-3}. The resulting structure as well as the relative configuration are illustrated in Figure 1.‡

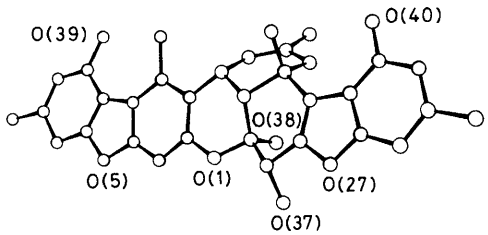
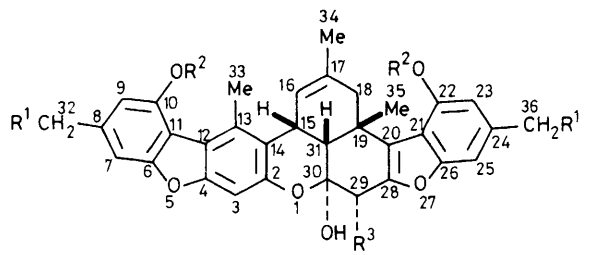


Figure 1. Perspective view of the crystal structure of asticolorin A (**1**).

The asymmetric unit as shown in Figure 2 contains 93 non-hydrogen atoms present as two asticolorin A, one water, and three acetone molecules. The four solvent molecules are linked to the four phenolic hydroxy groups by intermolecular hydrogen bonds with interoxygen distances of less than 2.8 Å. Furthermore the oxygen atom of the water molecule is also 2.77 Å removed from the oxygen atom of the C-30 hydroxy group of an asticolorin A molecule in the other asymmetric unit.

With the relative configuration of asticolorin A (**1**) available, the chirality of the C-29 hydroxy group and thus the absolute configuration of the molecule was determined by the method of Horeau.[3] Methylation of asticolorin A with methyl iodide and potassium carbonate in acetone gave the dimethyl ether (**2**), $2C_{35}H_{34}O_7 \cdot C_6H_6$, m.p. 258—260 °C. Esterification of (**2**) with racemic α-phenylbutyric anhydride and 4-dimethylaminopyridine proceeded smoothly, leading quantitatively to the 29-*O*-α-phenylbutyrate. The recovered α-phenylbutyric acid had $[\alpha]_D^{20}$ −21.0° (*c* 7.4, benzene). Asticolorin A must therefore have the 29*S* configuration[3,4] and consequently the absolute configuration as depicted in (**1**).

The structure elucidation of the related metabolite, asticolorin B (**3**), m.p. >320 °C (M^+, 536; $C_{33}H_{28}O_7$) is based on the high-field ^1H and ^{13}C n.m.r. data (Bruker WM-500 spectrometer) of these metabolites. It was evident that the C-29 secondary hydroxy function present in asticolorin A (**1**) (29-H: δ_H 5.079d, J 5.2 Hz; C-29: δ_C 70.75) is replaced by a carbonyl group in asticolorin B (**3**) [$\nu_{max.}$ (KBr) 1665 cm^{-1}; δ_C 180.84].



(**1**) $R^1 = R^2 = H$, $R^3 = OH$

(**2**) $R^1 = H$, $R^2 = Me$, $R^3 = OH$

(**3**) $R^1 = R^2 = H$, $R^3 = =O$

(**4**) $R^1 = OH$, $R^2 = H$, $R^3 = =O$

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

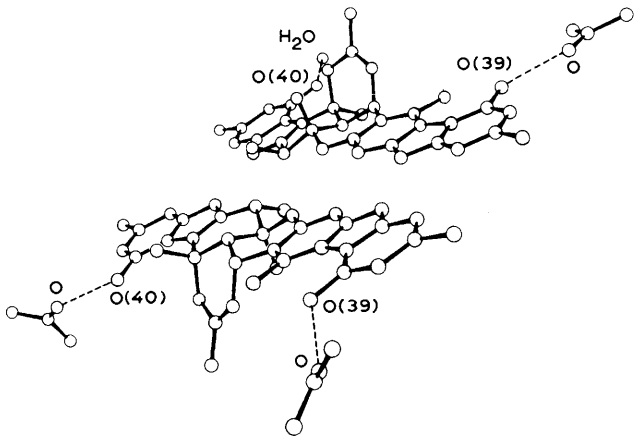

Figure 2. Perspective view of the asymmetric unit showing the location of the two asticolorin A molecules and the acetone and water of crystallisation.

In order to study the biosynthesis of the asticolorins, cultures of *A. multicolor* were grown on a yeast extract–sucrose medium. Initially good yields of both asticolorin A and B were obtained but eventually only a single new metabolite, asticolorin C (**4**) $2C_{33}H_{28}O_9 \cdot CHCl_3$, m.p. >320 °C ($M^+$, 568), was produced. The n.m.r. data for (**4**) indicated that both the C-8 and C-24 methyl groups (δ_H 2.380, 2.343; δ_C 21.60, 21.16) present in asticolorin B (**3**), are replaced by hydroxymethyl functions (δ_H 4.543, 4.572; δ_C 62.73, 62.65) in asticolorin C (**4**).

The asticolorins, a new type of mycotoxin, are derived biosynthetically from mevalonate and four molecules of orsellinic acid by oxidative phenol coupling.[5]

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