Scorpinone: A New Natural Azaanthraquinone Produced by a *Bispora*-like Tropical Fungus

Ana Miljkovic,*,† Peter G. Mantle,† David J. Williams,‡ and Birgitte Rassing§

Departments of Biochemistry and Chemistry, Imperial College of Science, Technology and Medicine, London SW7 2AY, U.K., and Microbiology Department, Novo-Nordisk A/S, Bagsvaerd, Denmark

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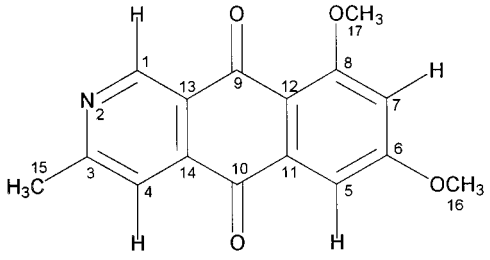
Scorpinone (**1**), 3-methyl-6,8-methoxy-2-aza-9,10-anthraquinone, has been isolated from the mycelium of a cultured sterile fungus of Caribbean origin. The structure was elucidated by X-ray crystallography, and 2D NMR spectral data have been assigned. The compound is one of very few known fungal azaanthraquinones.

Administration of radiolabeled biosynthetic intermediates to fungal fermentations has been a standard practice for obtaining qualitative and quantitative evidence concerning pathways of compounds with known structure. However, similar administration of putative biosynthetic precursors to near confluent biomass of a poorly studied fungus in batch fermentation may selectively reveal labeled products derived from particular precursors.[1] Such products are seen after autoradiography of thin-layer chromatograms of culture extract. Ideally, administration is usually delayed until most of the fungal biomass has been formed, to minimize competition by primary processes for intermediates of secondary pathways. Such methodology has been miniaturized to the 200 μL scale for the purpose of screening fungal isolates for the production of secondary metabolites containing particular structural units such as acetate, anthranilate, tryptophan, or one-carbon groups donated from methionine.[2] This novel miniaturized method has proven to be not only an innovative approach but also a powerful tool in its ability to reveal fungal secondary metabolic potential.[2] During screening of fungi freshly isolated from a Caribbean marine environment, notably revealing compounds radiolabeled from [^{14}C methyl] methionine, attention was focused on a relatively polar metabolite of a sterile fungus growing mainly in the form of black vegetative mycelium. Here we describe the isolation and structure elucidation of a new natural azaanthraquinone, designated scorpinone (**1**) on account of its molecular shape and ketone functions.



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The EIMS of **1** showed a molecular ion at *m/z* 283, the molecular formula of which was inferred as $C_{16}H_{13}NO_4$ from the HRMS data [*m/z* 283.0846, M+, −0.4 mmu]. The X-ray analysis showed compound **1** to have the 3-methyl-6,8-methoxy-2-aza-9,10-anthraquinone structure shown in Figure 1. The crystals contain two independent molecules in the asymmetric unit, both of which have identical conformations. The molecules are essentially planar, though in both cases there is a slight twisting of ring C with respect to rings A and B (which are coplanar to within 0.03 and 0.04 Å in molecules 1 and 2, respectively) such that O(7) and O(9) lie ca. 0.25 Å "above" and 0.20 Å "below" the plane of rings A and B (the respective deviations for molecule 2 are 0.12 and 0.18 Å). Examples of structurally characterized azaanthraquinones are rare,[3,4] with, to our knowledge, only two previous related systems having been reported. The patterns of bonding in the ring systems in the present structure do not differ significantly from those in the literature.

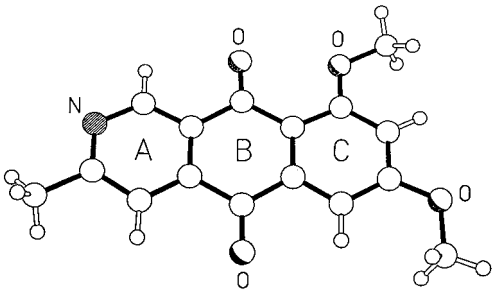

Figure 1. Perspective diagram of **1** from X-ray crystallographic data prepared by ORTEP.

The two independent molecules each pack, via a unit cell translation in the crystallographic *b* direction, to form continuous π stacks with partial overlap of all three ring systems (A with A, B with B, C with C). The degree of overlap for the two molecules differs, with, in one molecule, the centroid···centroid and mean interplanar spacings for rings B being 3.86 and 3.42 Å, respectively, while in the other the equivalent spacings are 3.86 and 3.53 Å. The planes of the stacks associated with the two molecules are inclined by ca. 26°. There is no evidence of any intermolecular hydrogen bonding interactions.

Complete assignment of ^{1}H and ^{13}C NMR data was achieved through DEPT experiments and pulse field gradient HMBC experiments in $CDCl_3$. Numbering of the structural positions follows the system used for 8-*O*-methylbostrycoidin.[5] The two carbonyls were typically evident as low-intensity ^{13}C signals at δ 180.5 and 183.5, and OCH_3 signals were sufficiently resolved in the spec-

* To whom correspondence should be addressed. Tel: +44-20-7848-4794. Fax: +44-20-484-4800. E-mail: ana.miljkovic@kcl.ac.uk.
† Department of Biochemistry, Imperial College, London.
‡ Department of Chemistry, Imperial College, London.
§ Novo-Nordisk. Denmark.

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trum obtained in $CDCl_3$ for HMBC correlation to establish their assignments within the structure. A B/E-linked scan MS experiment demonstrated that the molecular ion (*m*/*z* 283) lost 29 amu to give *m*/*z* 254 as the principal daughter ion, the loss being CHO according to accurate mass data for the relevant ions.

The new compound is one of a small group of fungal azaanthraquinones. They include bostrycoidin[6] and 8-*O*-methylbostrycoidin,[5] purple pigments of some *Fusarium* spp., which can naturally contribute prominent color to molded maize. 5-Deoxybostrycoidin is a minor metabolite of *Fusarium solani* (quoted as its teleomorph *Nectria haematococca* though probably not growing as such in the experimental conditions employed[7]), and its 6-*O*-demethyl analogue was produced by a mutant.[8] Either the latter is predictably a biosynthetic precursor of the former, or, as has been proposed,[9] the same inter-relationship occurs earlier before the nitrogen atom is added to a polyketide precursor. Tolypocladin was isolated from *Tolypocladium inflatum*.[10] The compound differs from bostrycoidin only in lacking any methoxy substituent.

Experiments on the biosynthesis of azaanthraquinones have not yet been performed. However, publications on azaanthraquinone synthesis have proposed the origin of the nitrogen from ammonia on the basis of reactions performed in somewhat extreme conditions.[9,11] Even a slow reaction at room temperature is difficult to accept as easily applicable to a biological system. Indeed, **1** has been found as a product of one particular synthetic reaction toward inserting a nitrogen atom into a selected structure.[11,12] In the more recent study,[11] partly assigned ^1H and ^{13}C NMR spectroscopic data for the product are similar to that of **1**, although the ^{13}C assignments for C-3 and C-8 should, by the present data, be reversed. Further, the idea[11] of azaanthraquinone biosynthesis being a way of "detoxifying" ammonia seems to ignore the fact that plant pathogenic fungi usually encounter a shortage of assimilable nitrogen in their natural environment. However, our discovery of **1** on account of methylation being involved in its biosynthesis[2] fits the predicted origin of *O*-methyl carbons 16 and 17 from *S*-adenosylmethionine.

Much of the ^1H NMR data for 8-*O*-methylbostrycoidin[5] is matched closely by that for **1**. However, there is less agreement concerning ^{13}C signal assignment for some of the quaternary carbons in the two compounds, which for **1** seems to have complementary support from the HMBC data. This notably involves positions 13 and 14 in 8-*O*-methylbostrycoidin,[5] where assignments of close numerical data might be reversed. Also, concerning positions 11 and 12 the present data may imply that the δ 115.4 signal in 8-*O*-methylbostrycoidin[5] belongs to the 12 position.

Adding to the metabolic profile of this fungus, caffeine was also isolated from cultivated mycelium, although characterized only by mass spectrometry[2] and never before regarded as a fungal metabolite. Recently, caffeine has been proven as a fungal metabolite of *Claviceps sorghicola*, in which it is the principle alkaloid,[13] adding credibility to its recognition as a metabolite in the Caribbean marine fungus.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Bruker 500 MHz spectrometer. Samples were internally referenced to tetramethylsilane (TMS). MS spectra were obtained on a Micromass autospec Q instrument in the EI mode. TLC was carried out on Camlab, SIL G-200 UV_{254} plates (2 mm thickness).

Organisms. The fungus was isolated from sediment collected in the inter-tidal zone around the Bahamas Islands. This sterile filamentous fungus with very occasional *Bispora*-like chlamydospores has been studied at the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. However, no mycological characteristic necessary for identification was found. Hence definitive identification was not possible. The fungus is deposited in the Novo Nordisk Culture Collection with NN 6654 culture number and is held specifically there in compliance with the Rio Convention.

Isolation. Mycelium of the fungus grown on potato dextrose agar was macerated in a sterile glass tissue homogenizer to give a finely divided suspension of inoculum for Czapek-Dox broth, supplemented with yeast extract (0.5%), 100 mL in each of 20 500 mL Erlenmeyer flasks. Cultures were incubated at 25 °C for two weeks on a rotary shaker (200 rpm and 10 cm eccentric throw). The black granular mycelium was separated by filtration, and excess surface moisture was removed and extracted with $CHCl_3$−MeOH (2:1). The solutes were applied in $CHCl_3$ to silica gel (GF_{254}) preparative (2 mm layer) plates and developed in toluene−ethyl acetate−formic acid (15:4:1). The yellow band (R_f 0.1) was excised and eluted in MeOH, and the solute was rechromatographed on precoated commercial plates (SIL G-100 UV_{254}, Camlab). The yellow band was eluted and the compound **1** crystallized (40 mg) from $CHCl_3$.

Compound **1** was also resolved by HPLC through a Separon SGX C18 reversed-phase column (150 × 5 mm) with 70% MeOH in water at 0.6 mL min^{-1} detected by a HP 1040M diode array detector with Chemstation software monitored at 280 nm and having a t_R of 14 min.

Scorpinone (1): yellow crystalline needles; mp 195 °C; UV (MeOH) λ_{max} 238, 282, 321 (sh), 405 nm; IR (film) ν_{max} 1595 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.42 (1H, sm H-1), 7.84 (1H, s, H-4), 7.43 (1H, d, J = 2.4 Hz, H-5), 6.84 (1H, d, J = 2.4 Hz, H-7), 4.02−3.99 (6H, s, H-16, H-17), 2.77 (3H, s, H-15); ^{13}C NMR ($CDCl_3$) δ 183.5 (s, C-10), 180.5 (s, C-9), 164.9 (s, C-6), 164.1 (s, C-3), 162.7 (s, C-8), 149.2 (d, C-1), 137.5 (s, C-13), 136.9 (s, C-11), 125.4 (s, C-14), 117.9 (d, C-4), 115.6 (s, C-12), 105.4 (d, C-7), 103.6 (d, C-5), 56.6 (q, C-17), 56.1 (q, C-16), 24.7 (q, C-15); EIMS *m*/*z* 283 (M+; 100%), 266 (16%), 254 (47%), 237, 210; HRMS *m*/*z* 283.0846 (calcd for $C_{16}H_{13}NO_4$: 283.0845), *m*/*z* 266.0811 (calcd for $C_{16}H_{12}NO_3$: 266.0817) and *m*/*z* 254.0820 (calcd for $C_{15}H_{12}NO_3$: 254.0817).

Crystal Data for 1·0.5H₂O: $C_{16}H_{13}NO_4 \cdot 0.5H_2O$; M = 292.28, monoclinic, space group $C2/c$ (no. 15), a = 43.613(6) Å, b = 3.857(1) Å, c = 37.363(5) Å, β = 121.89(1)°, U = 5337-(2) Å^3, Z = 16, D_c = 1.455 g cm^{-3}, μ(Cu Kα) = 0.896 mm^{-1}, $F(000)$ = 2448, T = 293 K; colorless needle, 0.659 × 0.023 × 0.007 mm, 2915 independent measured reflections, refinement based on F^2 to give R_1 = 0.075, wR_2 = 0.163 for 1113 observed reflections $|F_o| > 4\sigma(|F_o|)$, $2\theta < 105°$and 403 parameters. Computations were carried out using the SHELXTL program package version 5.03. CCDC reference number is 165464.

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(14) Crystallographic data for compound **1** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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