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New Nectriapyrones by Salt Water Culture of a Fungus Separated from an Indo-Pacific Sponge

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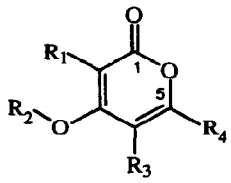
Abstract: The salt water culture of an unidentified fungus separated from an Indo-Pacific marine sponge has yielded new tetraketide natural products, demethyl nectriapyrone A (**1**) and nectriapyrone B (**2**). These α-pyrone containing compounds are analogous to nectriapyrone (**3**), previously reported from a terrestrial fungus.

We have begun to culture marine-derived heterotrophic fungi for new natural products. Recent reviews on this subject have summarized that the entire chemical knowledge-base from these organisms consists of just seventeen structural classes.[1] Our recent description of chlorinated sesquiterpenes from cultures of a fungus separated from the sponge *Jaspis johnstoni* represents an interesting new development.[2] A central strategy in this new program, designed to access relatively unstudied groups of marine microorganisms, is the separation of marine fungal symbionts from atypical sources, especially tropical sponges. Besides the chloriolins, the only other examples of natural products obtained from microorganisms separated from a sponge are alteramide A from *Alteromonas* sp. reported by Kobayashi,[3] urauchimycins from *Streptomyces* sp. Ni-80 reported by Imamura,[4] three diketopiperazines and four benzothiazoles from *Micrococcus* sp. reported by Cardellina,[5] and the trichoharzins from *Trichoderma harzanum* reported by Kitagawa.[6] We now add to this list by describing two new α-pyrone metabolites, demethyl nectriapyrone A (**1**) and nectriapyrone B (**2**).

An unidentified fungus[7] which served as the starting point in this work was obtained as aseptic sections from an encrusting sponge, *Stylotella* sp.,[8] collected by SCUBA in the Somosomo Strait near Taveuni, Fiji. The EtOAc extracts of its liquid broths[9] (filtered from the mycelium) yielded **1** (25 mg) and **2** (3 mg) whose molecular formulae were respectively established as $C_{10}H_{12}O_3$ (HRFABMS [M+1]$^+$ 181.0862; Δ0.3 mmu) and $C_9H_{12}O_3$ (HREIMS [M$^+$] 168.0771; Δ 1.5 mmu). The structure of **1**,[10] the major component of the culture broths, was established first. There were five ^{13}C NMR resonances consisting of δ 171.6, 164.5, 161.3, 97.3, and 88.1 – all characteristic of an α-pyrone moiety substituted by an OCH_3. An additional dimethyl containing trisubstituted double bond (δ 6.78) was also proposed to be attached to this ring. The two pyrone ring proton doublets (J=2.0 Hz) at δ 5.44 and 5.90 had to be in a *meta* orientation and both adjacent to the OCH_3. These structural postulates were further confirmed and pieced together by observing HMBC correlations as follows: $H_3$9 to C5, C6, and C7; $H_3$8 to C7 and C6; H4 to C2, C3 and C5; and H2 to C3 and C4. The *E*-double bond regiochemistry was established based on the characteristic ^{13}C NMR CH_3 shifts. Two compounds have been previously described which are close in structure to that of **1**. These include nectriapyrone (**3**), isolated from the terrestrial fungus *Gyrostroma missouriense* (Seeler),[11] and tetra-acetic acid lactone (**4**), isolated from *Penicillium stipitatum*.[12] As expected, the ^{13}C NMR shifts of the pyrone ring carbons of **1** are analogous to those of **4** and anispyrone A,[13] another α-pyrone containing fungal metabolite.

	R_1	R_2	R_3	R_4
1	H	Me	H	
2	H	Me	Me	Et
3	Me	Me	H	
4	H	H	H	



Only a small amount of the second broth constituent, nectriapyrone B (**2**),[14] was obtained. However, its characterization was efficiently completed using analogies to the spectral and structural features of **1**. The

proton resonances of **2** consisted of three singlets (pyrone ring H at δ 6.0, an OCH_3 at δ 3.9, and a pyrone ring CH_3 at δ 1.9) and an ethyl group spin system. Important information provided by an HMBC correlation from H_37 to the carbon at δ 166.0 established that the ethyl group was attached to C5 of the pyrone ring. At this point, the additional pyrone ring substituents could be assigned as shown in structure **2**.

The set of related α-pyrone containing natural products discussed above (**1** - **4**) collectively represents the simplest examples of such natural products. Alternatively, there are a number of higher molecular weight analogues which have been isolated from several different fungus species.[15] These compounds have an α-pyrone unit as the terminus of $C_{18/20}$ chains of polyacetate or polypropionate biosynthetic origin.[16] Several years ago the suggestion was made that nectriapyrone should be classified as a monoterpene,[11] but the similarities between structures **1** - **4** strongly suggest that they all arise from the common tetraketide core such as in **4** which can then undergo further biogenetic transformations ranging from O-methylation to extensive C-methylation (e.g., the helicascolides),[17] or a combination of C-methylation and demethylation (e.g., structure **2**).

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7. Prof. J. Kohlmeyer (UNC) has kindly provided information about this fungal culture (no. 92948). The agar plate isolate contains two types of conidia which could belong to the same species or are a mixture of two different fungi. The first conidia is small, ellipsoidal, brown and formed in chains. The second is large, fusiform, consists of five cells, the three middle ones are brown, the apical ones hyaline, and the apical cell bears two hyaline projections. The second type of conidia resembles those of the genus *Pleiocheata*.
8. For an example of the chemistry of a *Stylotella* see: Pettit, G.R.; Srirangam, J.K.; Herald, D.L.; Erickson, K.L.; Doubek, D.L.; Schmidt, J.M.; Tackett, L.P.; Bakus, G.J. *J. Org. Chem.* **1992**, *57*, 7217.
9. The initial culture was grown on solid corn meal agar (17g/L; DIFCO) made with filtered Monterey Bay sea water and then transferred to sea water malt media (15g/L; DIFCO). The broth was placed in 1L flasks (8L total volume) and rotary shaken at 120 rpm at 27°C for 21 days. EtOAc extracts of the broth were concentrated and partitioned between hexanes and 10% aq MeOH and then between CH_2Cl_2 and 50% aq MeOH. The CH_2Cl_2 fractions were purified by flash silica column chromatography and HPLC.
10. Demethyl nectriapyrone A (**1**): ^{13}C NMR (62.9 MHz) $CDCl_3$ δ 171.6 s (C3); 164.5 s (C1); 161.3 s (C5); 130.3 d (C7); 126.7 s (C6); 97.3 d (C4); 88.1 d (C2); 56.0 q (OMe); 14.3 q (Me9); 12.1 q (Me8). ^1H NMR (250 MHz) $CDCl_3$ δ 6.78 q, *J*=6.0 Hz (H7); 5.90 d, *J*=2.0 Hz (H4); 5.44 d, *J*=2.0 Hz (H2); 3.81 s (OMe); 1.84 s ($H_3$9); 1.82 d, *J*=6.0 Hz ($H_3$8).
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14. Nectriapyrone B (**2**): ^{13}C NMR (62.9 MHz) $CDCl_3$ δ 166.0 s (C3, C5); 165.5 s (C1); 101.0 s (C4); 93.3 d (C2); 56.2 q (OMe); 27.3 t (C6); 11.3 q (Me7); 8.4 q (Me at C4). ^1H NMR (250 MHz) $CDCl_3$ δ 6.0 s (H2); 3.90 s (OMe); 2.5 q, *J*=7.5 Hz ($H_2$6); 1.9 s (H_3 at C4); 1.2 t, *J*=7.5 Hz ($H_3$7).
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