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A New Polyketide, Secocurvularin, from the Salt Water Culture of a Sponge Derived Fungus

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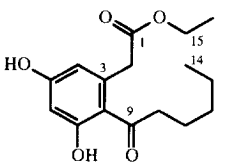
Abstract: The salt water culture of an unidentified fungus separated from the Indo-Pacific sponge *Spirastrella vagabunda* has yielded a new mildly antibiotic polyketide, 14,15-secocurvularin (**1**).

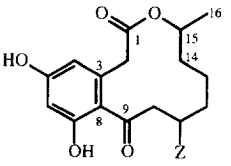
Recent developments in sponge biology suggest this phyla may harbor chemically prolific microorganisms. Interlaced in sponge tissue are the cells and tissue of associants including those of other invertebrates and/or microorganisms such as cyanobacteria (blue-green algae), bacteria, diatoms, and fungi.[1] Sometimes anomolous patterns associated with the occurrence of sponge-derived compounds implicate microorganisms as a source of the chemistry.[2] The best example involves the sesquiterpenes from *Dysidea* sponges which are greatly diminished or even eliminated when large concentrations of the cyanobacteria, *Oscillatoria*, are present.[3,4] It has been shown that the cyanobacterial cells[5] separated from *Dysidea* are rich in halogenated polypeptides.[4] In 1992 we began to explore the potential of tropical sponges as a source of heterotrophic microorganisms worthy of chemical study. We have discovered that sponge-derived fungi are a source of unique natural products including halogenated compounds[6a,b,c] and peptides.[6d] Similarly, between 1992 and 1994 Kitagawa,[7a,b] Oclarit,[7c] and Kobayashi[7d] reported novel alkaloids from the culture of a sponge-derived fungus[7a] and from bacteria isolates[7b,c,d]. We now wish to further contribute to this emerging subject by describing the structure of 14,15-secocurvularin (**1**).

The initial fungus culture[8] (951014) emerged from inside of the encrusting sponge *Spirastrella vagabunda*[9] which itself was known to be a source of a novel cyclopropyl sterol.[10] The culture obtained from this sponge was selected for further study because the ethyl acetate extract of a 125 mL test liquid broth[11] (filtered from the mycelium) showed 23% inhibition against *Bacillus subtilis* in a disc diffusion assay[12]. Bioassay guided fractionation of the EtOAc extract of an 8L broth culture led to the isolation[13] of **1** (6.2 mg) whose molecular formula was established as $C_{16}H_{22}O_5$ (HRFABMS $[M+1]^+$ 295.1547; Δ0.2 mmu).

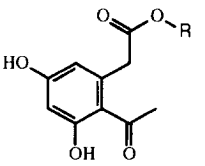
The polyketide nature of **1** was rapidly established. Initial indirect evidence came from the relatively high degree of oxygenation, the six elements of unsaturation, and the partial list of substructures consisting of two methyl groups and a benzene ring. All sixteen carbons in **1** were visible by ^{13}C NMR[14] including two methyls, six aromatic carbons, six methylenes, and two carbonyls (ketone and ester). The ^1H NMR spectrum revealed two OH groups as singlet signals integrating for one proton each with no attached carbons (by HMQC) plus four distinct spin systems: an OEt, an isolated CH_2, two meta-oriented benzene ring protons, and a pentyl group (clarified by ^1H-^1H COSY). There were several possible ways to join these substructures and all of them could be distinguished by the HMBC NMR data and by comparison to ^{13}C NMR data of appropriate models. First, key HMBC correlations allowed the pentyl group to be connected to the ketone C=O (δ 206.8, s). Likewise HMBC correlations required that the isolated CH_2 be flanked by the ester C=O (δ 171.1, s) and a ring carbon (δ 137.0, s). Finally, the choice in favor of attaching the acyl group at C8 versus C3 was justified by the similarity of the C=O shifts of **1** (δ 206.8) and of 2',4'-dihydroxypropriophenone (δ 204.7).[15] The agreement in the experimental and calculated shifts of C4 and C6 of **1** was excellent (calc.: δ 110, C4; δ 102, C6) and was not matched as well by the calculated shifts for the model derived by switching the C3/C8 substituents (calc.: δ 109, C4; δ 108, C6). Finally the large differential chemical shifts observed for OH protons (δ 12.26, C7OH; δ 5.69, C5OH) is only consistent with the arrangement of **1**.[16]



1



2 Z=H 3 Z=OH



4 R=Et 5 R=H

The discovery of 14,15-secocurvularin (**1**) adds a new dimension to the structures of this family of polyketides. The known structural analogs to **1** include curvularin (**2**), β-hydroxy curvularin (**3**), curvulin (**4**), and curvulinic acid (**5**).[17] Interestingly, curvularin (**3**) has been reported from four different fungi (*Curvularia*, *Cochliobolus*, *Penicillium*, and *Alternaria*). Our name for **1** is based on the close structural resemblance of it to **2** but there is an important difference. If the polyketide "starter unit" of curvularin (**2**) actually begins at C16 and continues on through to carbons 15, 14, 13, 12, 11, 10 and 9 as previously shown,[18] then a subsequent carbon-carbon bond break between C14 and C15 to arrive at **1** seems to be an unusual biosynthetic event.

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REFERENCES AND NOTES

1. van Soest, R.W.M.; van Kempen, T.M.G.; Braekman, J.C. *Sponges in Time and Space*; A.A. Balkema: Netherlands, 1994.
2. Kobayashi, J.; Ishibashi, M. *Chem. Rev.* **1993**, *93*, 1753.
3. Clark, W.D.; Crews, P. *Tetrahedron Lett.* **1995**, *36*, 1185.
4. (a) Unson, M.D.; Faulkner, D.J. *Experientia* **1993**, *2*, 713. (b) Unson, M.D.; Faulkner, D.J. *Pure Appl. Chem.* **1994**, *66*, 1983.
5. Hinder, R.; Pironet, F.; Borowitzka, M.A. *Mar. Biol.* **1994**, *119*, 99.
6. (a) Abrell, L.M.; Borgeson, B.; Crews, P. *Tetrahedron Lett.* **1996**, *37*, 2331. (b) Abrell, L.M.; Cheng, X-C.; Crews, P. *Tetrahedron Lett.* **1994**, *35*, 9159. (c) Cheng, X-C.; Varoglu, M.; Abrell, L.; Crews, P.; Lobkovsky, E.; Clardy, J. *J. Org. Chem.* **1994**, *59*, 6344. (d) Varoglu, M.; Crews, P. 1996, submitted.
7. (a) Kobayashi, M.; Uehara, H.; Matsunami, K.; Aoki, S.; Kitagawa, I. *Tetrahedron Lett.* **1993**, *34*, 7925. (b) Kobayashi, M; Aoki, S.; Gato, K.; Matsunami, K.; Kurosu, M.; Kitagawa, I. *Chem. Pharm. Bull.* **1994**, *42*, 2449. (c) Oclarit, J.M.; Okada, H.; Ohta, S.; Kaminura, K.; Yamaoka, Y.; Iizuka, T.; Miyashiro, S; Ikegami, S. *Microbios*, **1994**, *78*, 7. (d) Shigemori, H.; Bae, M-A.; Yazawa, K.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1992**, *57*, 4317.
8. Isolated on: 15g malt extract agar, 100mg penicillin G, 100mg streptomycin, 1L filtered Monterey Bay sea water.
9. This sponge was identified by Dr. M. C. Diaz (UCSC, IMS) and it was collected by SCUBA from the Togian Islands in central Sulawesi, Indonesia.
10. Catalan, C.A.N.; Lakshmi, V.; Schmitz, F.J.; Djerassi, C. *Steroids* **1982**, *40*, 455.
11. Sea water malt media: 15 g/L of malt extract in 0.2 μm filtered Monterey Bay sea water. The broth (125ml) was grown on a rotary shaker (125 RPM, 27°C, 21 days) and then harvested.
12. All samples are tested at 200μg/disc and compared to tetracycline (30μg/disc) control as 100% inhibition (**1** = 20% inhib.).
13. EtOAc extracts 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 sephadex column chromatography and reverse phase gradient hplc (50% aq. MeOH - 100% H_2O).
14. 14,15-Secocurvularin (**1**): ^{13}C NMR (125 MHz) $CDCl_3$ δ 206.8 s (C9), 171.1 s (C1); 164.8 s (C5); 160.2 s (C7); 137.0 s (C3), 116.4 s (C8), 112.5 d (C4), 103.3 d (C6), 61.6 t (C15); 43.4 t (C10); 41.9 t (C2); 31.5 t (C12); 24.8 t (C11), 22.6 t (C13), 14.2 q (Me16); 14.0 q (Me14). ^1H NMR (500 MHz) $CDCl_3$ δ 12.26 s, (OH5); 6.33 d, *J*=2.5, (H6); 6.29 d, *J*=2.5, (H4); 5.69 s (OH7); 4.20 q, *J*=7.3 ($H_2$15); 3.86 s ($H_2$2); 2.84 t, *J*=7.3 ($H_2$10); 1.71 pent., *J*=7.3 ($H_2$11); 1.36 m ($H_2$13), 1.32 m ($H_2$12), 1.28 t, *J*=7.3 ($H_3$16), 0.91 t, *J*=7.0 ($H_3$14). HMBC (J=9) NMR correlations (500 MHz) $CDCl_3$ H6 to C5,7,8,4; H4 to C8,6,2; $H_2$2 to C1,3,8,4; $H_2$10 to C12,11; $H_2$13 to C12; $H_2$12 to C13; $H_3$16 to C15; $H_3$14 to C12,13.
15. Model compound data from Pouchert, C. J.; Behnke, J. *The Aldrich Library of ^{13}C and ^1H FT NMR Spectra*; Aldrich Chemical Co.: Vol. 2, 1993 has ^{13}C ketone shift as follows: 2',4'-dihydroxypropriophenone (#858A) δ 204.7. Calculated carbonyl δ's using commerically available packages did not afford acceptable data for the ketone C=O.
16. Masento, M.S.; Morris, H.R.; Taylor, G.W.; Johnson, S.J.; Skapski, A.C.; Kay, R.R. *Biochem. J.* **1988**, *256*, 23.
17. Turner, W.B.; Aldridge, D.C. *Fungal Metabolites II*; Academic Press: New York, N.Y., 1983; pp. 96, 167, 516.
18. Yoshizawa, Y.; Li, Z.; Reese, P.B.; Vederas, J.C. *J. Am. Chem. Soc.* **1990**, *112*, 3212.

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