Biosynthetically Diverse Compounds from a Saltwater Culture of Sponge-Derived *Aspergillus niger*

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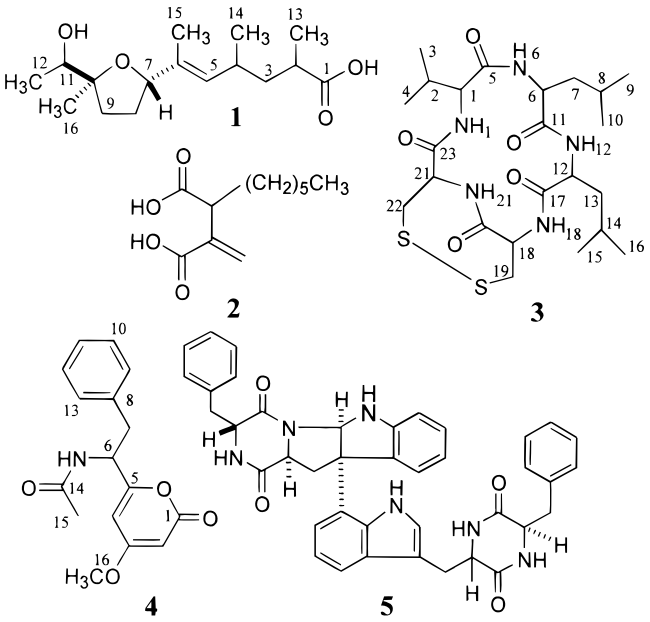
Received June 14, 1999

The new compound, asperic acid (**1**), and the known compounds hexylitaconic acid (**2**), malformin C (**3**), pyrophen (**4**), and asperazine (**5**) were isolated from the saltwater culture of *Aspergillus niger* derived from a Caribbean sponge, *Hyrtios proteus*. The structure elucidation of asperic acid is presented.

There is considerable interest in probing marine sponge-derived fungi as a source of new, bioactive natural products.[1] During the past five years we have developed methods to obtain such cultures from tropical sponges and then carry out moderate-scale saltwater fermentations.[1a,2−5] In fact, these cultures have provided access to a wide variety of novel natural products, including rearranged terpenes,[2] polyketides,[1a,3] pyrones,[4] and unusual amino acids.[5] Although marine-derived fungi may be capable of generating novel secondary metabolites, in many cases these compounds are analogues of those previously discovered from terrestrial fungi.[6] In connection with efforts to explore this subject further, our attention was drawn to a fungal culture separated from a *Hyrtios proteus* sponge because its extracts were toxic to brine shrimp. The chemodiversity of the culture extract was broad; a total of five compounds, belonging to a wide range of biosynthetic classes, was isolated. Reported below are the results of our investigation of this chemically prolific fungus.



Results and Discussion

Employing previously described techniques,[2] a culture of *Aspergillus niger* (culture # 94−1212) was obtained from *H. proteus* collected in the Dry Tortugas National Park, Florida. Several separate fermentations of the fungus were carried out in malt liquid, and the same chemical procedures were performed for each workup. The final methylene chloride partition of the various crude extracts provided the novel compound, asperic acid (**1**), and the known compounds, hexylitaconic acid (**2**),[7] malformin C (**3**),[8] pyrophen (**4**),[9] and asperazine (**5**).[5] The NMR and MS data for hexylitaconic acid (**2**)[7] and pyrophen (**4**)[9] matched the literature values. Complete ^{1}H and ^{13}C NMR assignments for malformin C (**3**) and pyrophen (**4**) were made from ^{1}H−^{1}H COSY, HMQC, and HMBC experiments. The resultant properties are presented here which add to the previous literature data for these compounds.[8,9]

The structure elucidation of asperic acid (**1**) began with the molecular formula of $C_{16}H_{28}O_4$. It was derived from ^{1}H−^{13}C DEPT (135) NMR spectra, along with MS data from LRFAB and HRFAB ([M + H]$^+$ 285.2059, Δ 0.07 mmu of calcd). The ^{13}C NMR signals for the double bond at δ 133.8 (C6) and δ 133.2 (C5), together with the carbonyl signal at δ 180.6, accounted for two of the three unsaturations, indicating the presence of a single ring in the structure. ^{1}H NMR and ^{1}H−^{1}H COSY experiments provided evidence for the subunits I−VI as shown in Figure 1. The placement of Me15 in V was justified by a long-range allylic ^{1}H−^{1}H COSY correlation from it to H5. The ^{13}C−^{1}H HMBC correlations linked these units together to provide the linear structure VII (Figure 1).

Considering positions of the oxygenated carbons and the carbonyl functionality, six alternatives could be drawn for the ring. The presence of a lactone or an epoxide was discounted because the ^{13}C or ^{1}H NMR shift values for C1, C10, C11, C7, and H7 could not support either possibility. Two other ring structures were good candidates, and these included an oxane with the oxygen between C7 and C11 or an oxolane with C7 and C10 joined by the oxygen atom. To utilize the resonance of the hydroxyl proton, ^{1}H, HMBC, and NOESY NMR experiments were performed in dioxane-d_8. The HMBC correlation observed from OH11 to C12 was only possible in a structure with an oxolane because the oxane would require the OH10 to C12 correlation to arise through four bonds. Further confirmation of the oxolane was provided by a NOESY correlation from OH11 to H7 across one face of the ring. In the case of a six-membered ring this would be a 1−4 transannular correlation, which would be beyond the range of an NOE coupling. In addition to confirming the oxolane ring, the OH11−H7 NOE signal indicated that Me16 and the C1−C6 chain are located on the same face of the ring. Due to sample limitations, the absolute stereochemistry of asperic acid (**1**) was not pursued.

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10.1021/np9902892 CCC: $19.00
Published on Web 12/23/1999

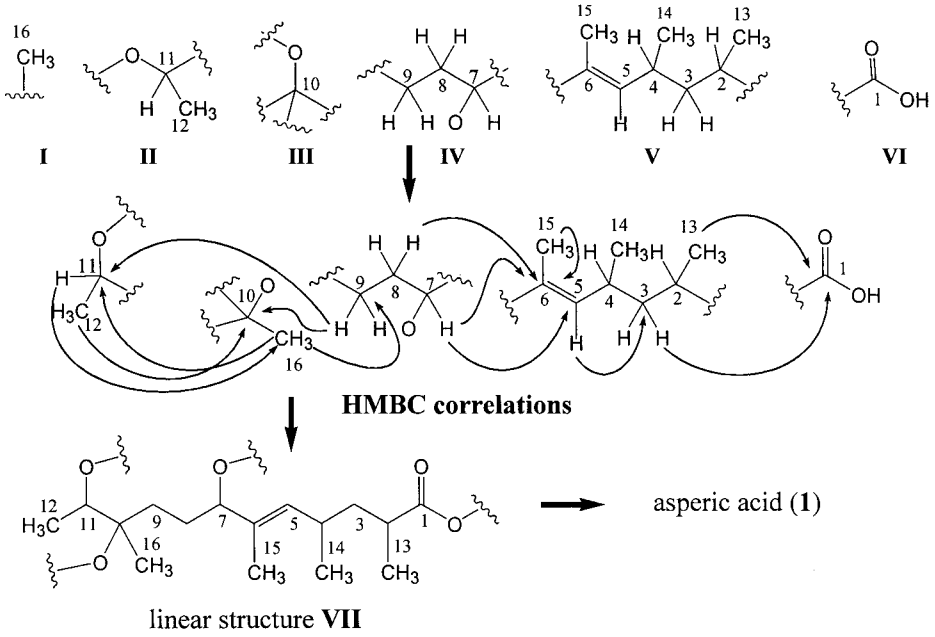

Figure 1. Substructures and the HMBC correlations used to determine linear structure **VII**.

Eventually, asperazine (**5**)[5] was discovered to be responsible for the bioactivity that initiated this project. Asperic acid (**1**) was evaluated in a variety of cancer cell lines,[10] but it was inactive, as was pyrophen (**4**). Malformin C (**3**) and asperazine (**5**) displayed tumor- and leukemia-selective bioactivity, respectively.

Overall, the new compound, asperic acid (**1**), and the four known compounds (**2**−**5**) were isolated during this study. Asperic acid does not have any close structural counterparts, whereas **2**−**4** are analogues of, or are identical to, metabolites reported from terrestrial fungi. It has been previously recognized that the members of the genus *Aspergillus*, obtained from terrestrial sources, are prolific producers[11] of diverse natural products.[12] The interesting assemblage of compounds reported above illustrates that a marine-derived *Aspergillus* cultured in seawater can mirror this behavior. Most importantly, our results provide encouragement that the study of known fungi from new habitats[13] will lead to the discovery of additional novel natural products.

Experimental Section

General Experimental Procedures. The NMR spectra were recorded at 250 or 500 MHz for ^{1}H and 62.9 and 125.7 MHz for ^{13}C. UV/vis measurements were performed with a diode array detector. High performance liquid chromatography (HPLC) was performed with columns of 10 μm ODS. All solvents were HPLC grade for HPLC and spectral grade for NMR.

Taxonomic Identification. Taxonomic identification was performed by Analytical Services, Inc., using both growth characteristics and fatty acid analysis. The strain was plated out on malt extract agar, potato dextrose agar, and two varieties of cellulose yeast agar. The organism was keyed to the description of *Aspergillus niger* var. *awamori*; however, other similar varieties of *A. niger* are *A. niger* var. *niger* and *Aspergillus foetidus*. By fatty acid analysis the strain was identified as *Aspergillus niger*. The culture is preserved as UCSC specimen #94−1212, and specimens are available from P.C.

Culture Conditions. The growth conditions consisted of 500 mL of malt extract broth (Difco) in filtered Monterey Bay seawater in 1 L shake flasks grown at 29 °C for 21 days.

Purification of Metabolites. The culture broth was extracted three times with equal volumes of ethyl acetate to obtain an extract that was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2-soluble fraction was partitioned between 10% aqueous CH_3OH and hexanes, the polarity of the CH_3OH layer was adjusted to 40% aqueous CH_3OH and further partitioned with CH_2Cl_2 to yield 0.15 g of the examined CH_2Cl_2-soluble fraction. This fraction was chromatographed with a linear gradient of 30% aqueous CH_3OH to 100% CH_3OH over the period of 1 h on a C_{18} HPLC column with UV detection at 254 nm to yield asperic acid (**1**) (4.0 mg), hexylitaconic acid (**2**) (13 mg), malformin C (**3**) (5.4 mg), pyrophen (**4**) (9.8 mg), and asperazine (**5**) (2.1 mg).

Asperic acid (1): yellow wax, $[\alpha]_D = 35°$ (*c*, 0.5, CH_2Cl_2); IR (film) 3301, 2967, 2874, 1672, 1533, 1454, 1370, 1064, 1018 cm^{-1}; λ_{max} 280, 225 nm; ^{1}H NMR ($CDCl_3$, 500 MHz,) δ 0.94 (d, J = 6.5 Hz, Me14), 1.15 (d, J = 7 Hz, Me12), 1.18 (d, J = 6.5 Hz, Me13), 1.19 (s, Me16), 1.39 (m, H3′), 1.55 (m, H9′), 1.62 (s, Me15), 1.69 (m, H3), 1.89 (m, H8), 2.22 (dt, J = 6.5, 12 Hz, H9), 2.40 (m, H2), 2.49 (m, H4), 3.79 (br q, J = 6.5, 12.5 Hz, H11), 4.35 (dd, J = 10, 6 Hz, H7), 5.16 (d, J = 9.5 Hz, H5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.2 (q, C15), 16.7 (q, C13), 17.7 (q, C12), 21.5 (q, C14), 24.7 (q, C16), 29.9 (d, C4), 30.3 (t, C9), 30.7 (t, C8), 36.9 (d, C2), 40.7 (t, C3), 72.1 (d, C11), 87.2 (d, C7), 87.7 (s, C10), 133.2 (d, C5), 133.8 (s, C6), 180.6 (s, C1).

Malformin C (3): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.93−0.98 (overlapped doublets, Me3, Me4, Me9, Me10, Me15, Me16), 1.56 (t, J = 6.5 Hz, H7), 1.61 (m, H8, H14), 1.66 (m, H13), 2.15 (m, H2), 3.13 (dd, J = 1, 10 Hz, H19′), 3.36 (m, H22), 3.83 (dd, J = 2, 10 Hz, H19), 4.04 (m, H1), 4.10 (m, H12), 4.36 (m, H18), 4.41 (m, H6), 4.94 (dt, J = 2, 8 Hz, H21), 6.18 (d, J = 9 Hz, NH6), 6.58 (d, J = 10 Hz, NH1), 6.71 (d, J = 6 Hz, NH18), 7.15 (d, J = 12 Hz, NH21); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.6 (q, C4), 19.8 (q, C3), 22.2 (q, C10), 22.3 (q, C9), 22.5 (q, C16), 22.8 (q, C15), 24.8 (d, C14), 25.1 (d, C8), 27.3 (d, C2), 39.2 (t, C13), 40.5 (t, C7), 46.2 (t, C22), 47.5 (t, C19), 52.0 (d, C6), 52.7 (d, C18), 53.6 (d, C12), 54.6 (d, C21), 59.5 (d, C1), 171.4 (s, C23), 171.8 (s, C5), 172.8 (s, C17), 174.9 (s, C11, C20).

Pyrophen (4): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.96 (s, Me15), 3.08 (d, J = 8.0 Hz, H7, H7′), 3.75 (s, Me16), 5.00 (t, J = 8.0 Hz, H6), 5.42 (d, J = 2.2 Hz, H2), 5.75 (d, J = 2.2 Hz, H4), 6.06 (d, J = 8.0 Hz, NH), 7.15 (m, H9, H13), 7.22 (m, H11), 7.30 (m, H10, H12); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 23.2 (q, C15), 38.9 (t, C7), 52.4 (d, C6), 56.0 (q, C16), 88.5 (d, C2), 101.2 (d,

C4), 127.2 (d, C11), 128.7 (d, C10, C12), 129.1 (d, C9, C13), 135.8 (s, C8), 161.3 (s, C5), 164.4 (s, C1), 169.6 (s, C14), 170.5 (s, C3).

Acknowledgment. Financial support was provided by the California Sea Grant College (NA36R60537) project R-MP/63, NMR equipment grants from NSF BIR-94-19409 and the Elsa U. Pardee Foundation. We warmly thank Ms. Bethel Borgeson for carrying out fungal cultures, Dr. Leif M. Abrell and Mr. Roy Appulguise for collection of the fungal sample, and Dr. M. C. Diaz (UCSC) for sponge taxonomy. We also thank the Dry Tortugas National Park, Florida, for collection permission.

Supporting Information Available: Spectra of **1** including 1D and 2D NMR, IR, and UV. This material is available free of charge via the Internet at http://pubs.acs.org.

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NP9902892