A marine *Micrococcus* produces metabolites ascribed to the sponge *Tedania ignis*

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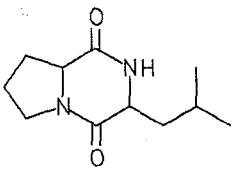
Summary. Extracts of the sponge *Tedania ignis* have been reported to contain several diketopiperazines. As part of an investigation of the commensal and symbiotic microflora of sponges, we have consistently isolated, from specimens of *T. ignis*, a *Micrococcus* sp. which produces diketopiperazines in laboratory cultural media. This is the first demonstration that a bacterium associated with a sponge produces secondary metabolites ascribed to the sponge host.
Key words. Secondary metabolites; sponge; *Tedania ignis;* marine bacterium; *Micrococcus* sp.

In the last few years, several macrolides and polyethers with antitumor or antiobiotic properties have been isolated from marine invertebrates, notably sponges[1-3]. In all cases, these unique compounds were very minor constituents of the extracts and this fact, together with the structural characteristics of the compounds, has provided a basis for the hypothesis that such metabolites might actually be produced by microorganisms living in or on the invertebrates. Support for this idea is provided in reports that surugatoxins[4-6] are produced by a bacterium associated with the Japanese ivory shell[7], tetrodotoxin is produced by bacteria from puffer fish[8], and okadaic acid, found in extracts of the sponge *Haliclona okadai*[9], is produced by a dinoflagellate[10].

In 1983, we initiated a study of microorganisms growing on or in sponges from Bermudian waters. As part of this investigation, we collected specimens of the sponge *Tedania ignis* from two different locations during consecutive summer visits (1986, 1987). All bacterial isolates were grown in liquid culture (Marine Broth 2216, DIFCO). Each sponge specimen yielded several distinctly different strains of marine bacteria, among which were bright orange-pigmented, gram-positive species that have been identified as members of the genus *Micrococcus*.

The *Micrococcus* sp. was grown in 20-l batch cultures (Marine Broth 2216, DIFCO) for 21 days. The cultures were lyophilized and extracted with CH_3OH-CH_3CN (4:1) and CH_2Cl_2; the CH_3OH-CH_3CN solubles were partitioned between CH_2Cl_2 and water and the two CH_2Cl_2 extracts were combined and chromatographed on Sephadex LH-20, then Bio-Beads S-X8. Fractions 5–7 from the latter chromatography were pooled and subjected to centrifugal countercurrent chromatography (CCC) with $CHCl_3-CH_3OH-H_2O$ (25:34:20) to give the diketopiperazines **1–3**. The structures were secured by analysis of the MS, IR and ^{1}H-NMR data, which were identical to those previously reported for these compounds[11].



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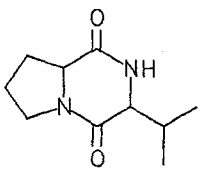

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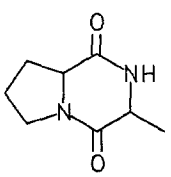

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The significance of isolating these diketopiperazines from a marine bacterium associated with *Tedania ignis* resides in Schmitz's report of these same diketopiperazines from extracts of the sponge[11]. Given the propensity of microorganisms to produce diketopiperazines[12], the low yield of **1–3** observed by Schmitz[11] and the consistent association of this *Micrococcus* with *T. ignis*, there is substantial cause to believe that these compounds are actually produced by the bacterium living in association with the sponge.

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1 Faulkner, D. J., Nat. Prod. Rep. *4* (1987) 539.
2 Faulkner, D. J., Nat. Prod. Rep. *3* (1986) 1.
3 Faulkner, D. J., Nat. Prod. Rep. *1* (1984) 551.
4 Kosuge, T., Zenda, H., Ochiai, A., Masaki, N., Noguchi, M., Kimura, S., and Narita, H., Tetrahedron Lett. (1972) 2545.
5 Kosuge, T., Tsuji, K., Hirai, K., Yamaguchi, K., Okamoto, T., and Iitaka, Y., Tetrahedron Lett. *22* (1981) 3417.
6 Kosuge, T., Tsuji, K., and Hirai, K., Chem. pharm. Bull. *30* (1982) 3255.
7 Kosuge, T., Tsuji, K., Hirai, K., and Fukuyama, T., Chem. pharm. Bull. *33* (1985) 3059.
8 Yotsu, M., Yamazaki, T., Meguro, Y., Endo, A., Murata, M., Naoki, H., and Yasumoto, T., Toxicon *25* (1987) 225.
9 Tachibana, K., Scheuer, P. J., Tsukitani, Y., Kikuchi, H., Van Engen, D., Clardy, J., Gopichand, Y., and Schmitz, F. J., J. Am. chem. Soc. *103* (1981) 2469.
10 Yasumoto, T., Seino, N., Murakami, Y., and Murata, M., Biol. Bull. *172* (1987) 128.
11 Schmitz, F. J., Vanderah, D. J., Hollenbeak, K. H., Enwall, C. E. L., Gopichand, Y., Sengupta, P. K., Hossain, M. B., and van der Helm, C., J. org. Chem. *48* (1983) 3941.
12 Sammes, P. G., Fortschr. Chem. org. Naturstoffe *32* (1975) 51.

0014-4754/88/11-12/1021-01$1.50 + 0.20/0