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## ISOLATION OF LATRUNCULIN A, 6,7-EPOXYLATRUNCULIN A, FIJIANOLIDE A, AND EURYFURAN FROM A NEW GENUS OF THE FAMILY THORECTIDAE

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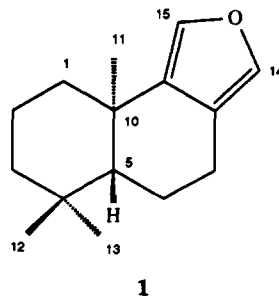
ABSTRACT.—Latrunculin A, 6,7-epoxylatrunculin A, euryfuran [1], and fijianolide A have been isolated from a new genus of a Pacific sponge in the family Thorectidae. Their structures were identified by comparison with the literature data.

Our ongoing studies of marine-derived biologically active metabolites led us to investigate a new genus of the marine sponge family Thorectidae. Here we report the unusual co-occurrence of latrunculin A (1-4), fijianolide A (5,6), euryfuran (7), and 6,7-epoxylatrunculin A (4) in the same sponge. To the best of our knowledge, this is the first report of the isolation of 6,7-epoxylatrunculin A from a sponge other than *Latrunculia magnifica* Keller (4), which contained latrunculins A-D. Latrunculin A has also been reported from nudibranchs: *Chromodoris elizabethina* (8), *Glossodoris quadricolor* (9), *Chromodoris williani* (6), *Chromodoris* sp. (6), and *Chromodoris lochi* (3) associated with *Spongia mycofijiensis*.

Latrunculin A (0.12% wet wt sponge) was isolated as the major metabolite of this sponge. The structure was identified by comparison of nmr data with those previously reported for latrunculin A (1,2,4). In our hands, latrunculin A inhibits the in vitro proliferation of the cultured murine P388 leukemia cells with an IC<sub>50</sub> of 4.1 μg/ml. The growth of *Candida albicans* is also inhibited by the compound with MIC value of 15.6 μg/ml. Latrunculin A inhibited the adherence of PMA-(phorbol-12-myristate-13-acetate) induced EL-4.IL-2 murine lymphoma cells (50% inhibition at 0.125 μg/ml) in an assay which is used to detect agonists/antagonists of protein kinase C (PKC) (10).

Euryfuran [1] (0.07% wet wt sponge) was isolated as the second major compound. We report here the complete as-

signments of the <sup>1</sup>H- and <sup>13</sup>C-nmr data for euryfuran. This is the first report of the complete assignments of the <sup>13</sup>C data for euryfuran, although it has been isolated from *Euryspongia* sp. (7) and has been synthesized (11-15). The <sup>1</sup>H and <sup>13</sup>C assignments were based on the results from COSY (16), HETCORR (17), and HMBC (18) experiments. Euryfuran was unstable in CDCl<sub>3</sub> and decomposed within a week in the freezer. The absolute stereochemistry of euryfuran, [α]<sub>D</sub><sup>23</sup> -21.6° (CHCl<sub>3</sub>, c=0.16), was deduced based on the rotation reported for (+)-euryfuran, [α]<sub>D</sub><sup>19</sup> +19° (CHCl<sub>3</sub>, c=1.0) that was obtained by a total synthesis (15).



Fijianolide A and 6,7-epoxylatrunculin A were isolated as minor compounds. Fijianolide A has been isolated from *Spongia mycofijiensis* (5), *Hyattella* sp. (6) and also from the nudibranch predator *C. lochi* (6). Euryfuran, 6,7-epoxylatrunculin A, and fijianolide A inhibit the in vitro proliferation of the cultured human lung cancer cell line A549 with IC<sub>50</sub> values of 1.5, 0.2, and 1.4 μg/ml, respectively, and the murine P388

leukemia cells with  $IC_{50}$  values of 1.6, 1.8, and 1.0  $\mu\text{g/ml}$ , respectively. 6,7-Epoxyatrunculin A and fijianolide A also inhibit the growth of *Candida albicans* with MIC values of 25 and 6.2  $\mu\text{g/ml}$ , respectively. Both euryfuran and 6,7-epoxyatrunculin A inhibited the PMA-induced adherence of EL-4.IL-2 cells (48% inhibition at 0.025  $\mu\text{g/ml}$  and 100% inhibition at 0.003  $\mu\text{g/ml}$ , respectively). However, fijianolide A induced a significant adherence of EL-4.IL-2 cells in the absence of PMA (0.003–0.025  $\mu\text{g/ml}$ ) indicative PKC agonistic activity.

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—The nmr spectra were recorded on a Bruker instrument operating at 360 MHz for  $^1\text{H}$  and 90.5 MHz for  $^{13}\text{C}$ .

**CLASSIFICATION OF THE SPONGE.**—The sponge was collected by scuba at a depth of 20 m in Larsen Bay, Tutuila Island, American Samoa. It grew as coalescent branches and was rare. Its color is dark brown externally, both alive and in EtOH. The surface of the sponge is finely tuberculate to rugose. The oscules are contractile and tend to be raised on low mounds. Its fibers are laminated, clear of inclusions, and form a regular, rectangular reticulation. The laminated fibers are diagnostic for the family Thorectidae (Class Demospongiae, Order Dictyoceratida), but there are no described genera with characteristics which match those of our sponge. Thus, we believe that our sponge belongs to a new genus of the family Thorectidae. A taxonomic voucher specimen is deposited in the Harbor Branch Oceanographic Museum (catalog number 003:00062). The sponge is the most similar in morphology, surface texture, and architecture to *S. mycofijiensis* (3), which also has laminated fibers and may, therefore, have been incorrectly assigned to the genus *Spongia* (family Spongiidae) (3). An examination of the type of *S. mycofijiensis* indicates that there are notable differences between the two sponges, sufficient to distinguish the two as different species. The fibers of *S. mycofijiensis* are not clear as in our sponge, but are filled with foreign spicules. Moreover, the fibers do not form a regular mesh as in our sponge. The consistency of *S. mycofijiensis* is compressible and easily torn, whereas our sponge is firm, not compressible, and not as easily torn. A revision of *S. mycofijiensis* and a description of the new genus of our sponge are desirable, but are not within the scope of this study. We have established that the two sponges are different species, and most likely

different genera within the family Thorectidae (see note following Acknowledgments).

**EXTRACTION AND ISOLATION.**—The wet sponge (311 g) was extracted three times with MeOH-toluene (3:1). The concentrated extract was partitioned between EtOAc and  $\text{H}_2\text{O}$ . The EtOAc-soluble fraction was chromatographed on Si gel using  $\text{CH}_2\text{Cl}_2$  and  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  step gradient to give eleven fractions. Rechromatography of a portion of the combined fractions 1 and 2 on Si gel followed by hplc ( $\text{SiO}_2$ ,  $5\mu$ ,  $250\times 10$  mm) with heptane gave euryfuran (27 mg, 0.07% wet wt sponge):  $^1\text{H}$  nmr ( $\text{CDCl}_3$ , 360 MHz)  $\delta$  7.07 (d,  $J=1.4$  Hz, H-15), 7.04 (q,  $J=1.5$  Hz, H-14), 2.76 (dddd,  $J=1.0$ , 1.0, 6.5, 16.5, H-7), 2.48 (dddd,  $J=1.7$ , 7.4, 11.8, 16.5, H-7), 1.96 (m, H-1), 1.80 (ddd,  $J=7.4$ , 13.4, 1.0, H-6), 1.68 (m, H-2), 1.61 (m, H-6), 1.51 (m, H-2), 1.48 (m, H-1), 1.47 (m, H-3), 1.24 (m, H-3), 1.26 (m, H-5), 1.20 (s, H-11), 0.93 (s, H-12), 0.90 (s, H-13) (the chemical shifts assignments were based on H-H COSY experiment);  $^{13}\text{C}$  nmr ( $\text{CDCl}_3$ , 90 MHz)  $\delta$  137.4 (C-9), 136.9 (C-14), 134.9 (C-15), 119.9 (C-8), 51.5 (C-5), 42.1 (C-3), 39.5 (C-1), 33.9 (C-10), 33.5 (C-12), 33.1 (C-4), 25.0 (C-11), 21.6 (C-13), 20.5 (C-7), 19.2 (C-2), 19.1 (C-6). Similarly, the fraction 3 on Si gel cc furnished latrunculin A (378 mg, 0.12% wet wt sponge), fraction 4 produced impure 6,7-epoxyatrunculin A, and fraction 6 produced impure fijianolide A. Fraction 4 on hplc ( $\text{SiO}_2$ ,  $5\mu$ ) with heptane-EtOAc (1:1) yielded 6,7-epoxyatrunculin A (0.8 mg, 0.0003%). Chromatography of fraction 6 on a column of Sephadex LH-20 with MeOH followed by hplc ( $\text{SiO}_2$ ,  $5\mu$ ) with heptane-EtOAc (2:3) gave fijianolide A (3.2 mg, 0.001% wet wt sponge). Latrunculin A, 6,7-epoxyatrunculin A, and fijianolide A were identified by comparison of spectral data (uv, ir, nmr) with those reported in the literature.

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Institution Contribution No. 891.

NOTE ADDED IN PROOF: Professor Bergquist recently re-examined our sponge as well as *Spongia mycofijiensis* (5) and *Hyattella* sp. (6) and has revised her earlier diagnosis. She believes that all three sponges are the same species, exhibiting extreme variation in growth form, skeletal density, fiber structure, and arrangement. Based on the presence of a fine surface fiber network characteristic of *Hyattella* (but only weakly developed in our sponge), she suggests assigning all three sponges to the genus *Hyattella*, which necessitates amending the diagnosis of this genus to include forms with clear, laminated fibers.

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