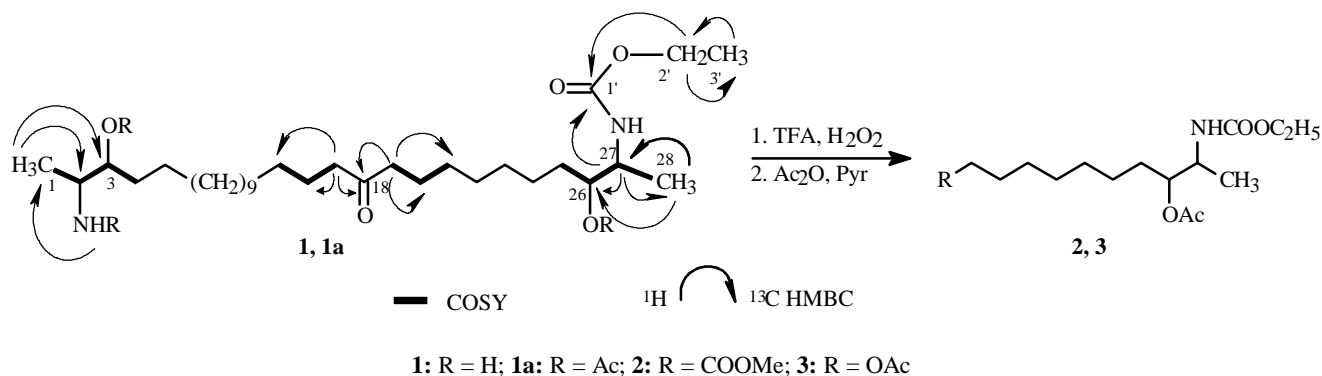


**RHIZOCHALININ A, A NEW ANTILEUKEMIC  
TWO-HEADED SPHINGOLIPID FROM  
THE SPONGE *Rhizochalina incrustata***

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In continuation of research on two-headed sphingolipids from the marine sponge *Rhizochalina incrustata* [1, 2], we isolated a new aglycon, which we called rhizochalinin A (**1**), after acid hydrolysis of total natural galactosylated sphingolipids.



Sponge *R. incrustata* (collected in 1986, Madagascar) was extracted with ethanol. The extract was concentrated in vacuo. The solid was distributed between aqueous ethanol (90%) and hexane. The total substances in the aqueous ethanol were chromatographed over a column of Polychrom using a H<sub>2</sub>O→EtOH gradient. Part of the fraction with two-headed sphingolipids was hydrolyzed by HCl (2 N) in methanol and evaporated. The solid was chromatographed over a silica-gel column using CHCl<sub>3</sub>:MeOH (10:1). The fraction containing sphingolipid aglycons was chromatographed over Sephadex LH-20 using CHCl<sub>3</sub>:EtOH (2:1) to afford rhizochalinin A (**1**, 27.9 mg, 0.05% of sponge dry weight).

The structure of **1** was studied by spectral methods (PMR, <sup>13</sup>C NMR, DEPT, COSY, HSQC, HMBC, MALDI-TOF mass spectrometry) and chemical transformations. The molecular formula of **1** C<sub>31</sub>H<sub>62</sub>N<sub>2</sub>O<sub>5</sub> was calculated from the MALDI-TOF mass spectrum in which peaks for ions at *m/z* 565 [M + Na]<sup>+</sup> for **1** and *m/z* 691 [M + Na]<sup>+</sup> for its triacetate (**1a**) were observed [2]. The PMR and <sup>13</sup>C NMR spectra of **1** exhibited resonances typical of pseudodimeric amino alcohols [1-5], including the corresponding additional resonances for a carbamoyl group [2]. The cross peak H-27[δ<sub>H</sub> 3.65]/C-1'[δ<sub>C</sub> 159.4] in the HMBC spectrum and the production after Baeyer—Villiger degradation [1] of **2** and **3** with pseudomolecular ions at *m/z* 368 [M + Na]<sup>+</sup> indicated unambiguously that the carbamoyl group was bonded to C-27 and the carbonyl was located on C-18.

**Rhizochalinin A**: [α]<sub>D</sub> +9.2° (*c* 0.93, MeOH). MALDI-TOF mass spectrum (*m/z*): 565 [M + Na]<sup>+</sup>.

PMR spectrum (300 MHz, CD<sub>3</sub>OD, δ, ppm, J/Hz): 1.26 (d, J = 6.8, H-1), 3.02 (m, H-2), 3.43 (m, H-3), 1.37 (m, H-4), 1.30 (br.s, H-5-15,21-24), 1.55 (m, H-16), 2.46 (t, J = 7.5, H-17), 2.46 (t, J = 7.5, H-19), 1.55 (m, H-20), 1.42 (m, H-25), 3.47 (m, H-26), 3.65 (ddd, J = 10.0, 6.9, 3.2, H-27), 1.14 (d, J = 6.8, H-28), 4.08 (q, J = 7.0, H-2'), 1.24 (t, J = 7.0, H-3').

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<sup>13</sup>C NMR spectrum (75 MHz, CD<sub>3</sub>OD, δ, ppm): 17.0 (C-1), 54.0 (C-2), 74.2 (C-3), 33.0 (C-4), 28.7-29.0 (C-5-15, 21-24), 25.0 (C-16), 44.1 (C-17), 215.0 (C-18), 44.1 (C-19), 25.4 (C-20), 33.2 (C-25), 75.5 (C-26), 52.5 (C-27), 18.3 (C-28), 159.4 (C-1'), 62.3 (C-2'), 15.6 (C-3').

**Baeyer—Villiger Oxidation of Rhizochalinin A Triacetate (1a).** Rhizochalinin A triacetate (**1a**, 2.0 mg), which was prepared by acetylation of **1** with a mixture of acetic anhydride and pyridine, was treated with methanol (200 μL), conc. CF<sub>3</sub>CO<sub>2</sub>H (150 μL), and H<sub>2</sub>O<sub>2</sub> (30%, 50 μL), heated at 60°C for 2.5 h, left at room temperature for 5 d, evaporated to dryness, acetylated by a mixture of acetic anhydride and pyridine (1:1, 200 μL) for 16 h at room temperature, concentrated in vacuo, purified by HPLC over a column of YMC Pack ODS-A using aqueous ethanol (80%), and analyzed by MALDI-TOF mass spectrometry to afford **2** (*m/z* 368 [M + Na]<sup>+</sup>) and **3** (*m/z* 368 [M + Na]<sup>+</sup>).

Rhizochalinin A exhibited antileukemic activity against human leukemia THP-1 cells (IC<sub>50</sub> = 7.5 μM).

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