Leucettamols A and B, Two Antimicrobial Lipids from the Calcareous Sponge *Leucetta microraphis*

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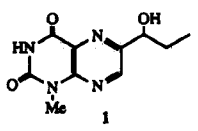
Calcareous sponges of the genus *Leucetta* are best known as sources of imidazole alkaloids.[1] The exception is the pteridine alkaloid leucettidine (1), which was isolated from *Leucetta microraphis* from Bermuda.[2] We recently reported the isolation of the zinc complex of clathridine (2) and (9*E*)-clathridine 9-*N*-(2-sulfoethyl)imine (3) from a specimen of *L. microraphis* from Pohnpei.[3] We have now investigated the chemistry of a maroon-colored calcareous sponge that, although quite different in physical appearance, was also identified as *Leucetta microraphis*. Antimicrobial screening of the crude methanolic extract of this sponge revealed mild activity against *B. subtilis*. A bioassay-guided fractionation using Sephadex LH-20, TSK 40s, and reversed-phase HPLC resulted in the isolation of leucettamols A (4) and B (5) as the active constituents.



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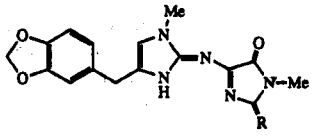
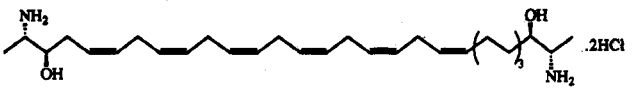


2 R = O 3 R = N-$CH_2CH_2SO_3H$ (*E*)



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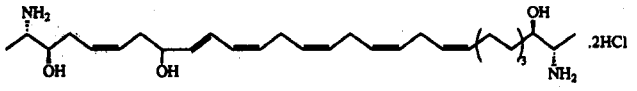

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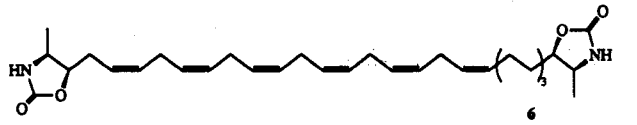

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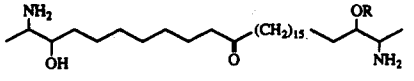

7 R = β-galactosyl 8 R = H

Leucettamol A (4) was isolated as an optically inactive, pale yellow oil. The molecular formula, $C_{30}H_{52}N_2O_2$, required six degrees of unsaturation. The broad signals at δ 5.37 (m, 10 H), 5.45 (m, 1 H, H-5), and 5.51 (m, 1 H, H-6) in the ^1H NMR spectrum (CD_3OD) accompanied by 12 signals in the olefinic region of the ^{13}C NMR spectrum required six disubstituted olefinic bonds that accounted for all of the unsaturation. A broad signal at 2.8–2.9 (m, 10 H) was coupled to the olefinic signals and was assigned to five bis-allylic methylene groups. The presence of ^{13}C NMR signals at δ 26.6 (t, 3 C), 26.8 (t), and 27.0 (t), assigned to the five bis-allylic methylene carbons, indicated the cis geometry for all olefins. The structure of 4, which includes two 2-amino-3-hydroxy end groups, was defined by interpretation of the ^1H NMR spectrum in DMSO-d_6 solution. Both methyl signals at δ 1.06 (d, 3 H, J = 6.5 Hz, H-30) and 1.09 (d, 3 H, J = 6.5 Hz, H-1) were coupled to a signal at 3.09 (m, 2 H, H-2,29) that was assigned to overlapping –C*H*(NH_3^+)– signals. The signal at δ 3.09 was coupled to a very broad signal at 8.01 (6 H, NH),[4] and to signals at 3.69 (m, 1 H, H-3) and 3.60 (m, 1 H, H-28) that were assigned to –C*H*(OH)– groups. The signal at 3.69 was coupled to a signal at 2.15 (m, 2 H, H-4) that was in turn coupled to the olefinic envelope. The signal at 3.60 was coupled to an aliphatic envelope at 1.14–1.44 (m, 10 H). The final signal at δ 2.02 (m, 2 H, H-22) was coupled to both the olefinic and aliphatic envelopes. The ^{13}C NMR spectrum is completely compatible with the structure of leucettamol A (4) defined by the ^1H NMR data.

The relative stereochemistry of both 2-amino-3-hydroxy end groups was defined by analysis of nuclear Overhauser enhancements in bis-oxazolone 6, that was formed by treatment of 4 with 1,1′-carbonyldiimidazole. Although the ^1H NMR signals for H-2 and H-29 overlap at δ 3.95 (dq, 1 H, J = 7.8, 6.5 Hz, H-2) and 3.90 (dq, 1 H, J = 7.8, 6.5 Hz, H-29) and the H-3 and H-28 signals overlap at 4.61 (ddd, 1 H, J = 9, 7.7, 6 Hz, H-3) and 4.57 (ddd, 1 H, J = 10, 7.7, 4 Hz, H-28), irradiation at 4.59 caused enhancement of only the two overlapping signals at 3.90 and 3.95, indicating the threo stereochemistry at both ends of the molecule. Since the olefinic bonds are not symmetrically situated, it must be assumed that leucettamol A (4) is racemic.

Leucettamol B (5) was isolated as an optically inactive oil of molecular formula $C_{30}H_{52}N_2O_3$. The ^{13}C NMR spectrum contains 12 olefinic carbon signals, which account for the six degrees of unsaturation, and three signals at δ 70.1 (d), 70.2 (d), and 70.8 (d), which indicate that 5 contains one more secondary alcohol than 4. The position of the additional hydroxyl group was deduced by analysis of the ^1H NMR spectra. The COSY spectrum revealed the following sequence: δ 1.09 (d, 3 H, J = 7 Hz, H-1), 3.10 (m, 1 H, H-2), 3.66 (m, 1 H, H-3), 2.12 (q, 2 H, J = 6.3 Hz, H-4), 5.46 (m, 2 H, H-5, H-6), 2.19 (m, 2 H, H-7), 4.05 (q, 1 H, J = 6.3 Hz, H-8), 5.69 (dd, 1 H, J = 15, 6 Hz, H-9), 6.48 (dd, 1 H, J = 15, 11 Hz, H-10), 5.97 (dd, 1 H, J = 11, 10.5 Hz, H-11), 5.39 (m, 7 H, H-12 and others). Since the remaining ^1H NMR data for 5 is almost identical to that of 4, we propose that 5 is an oxidation product of 4 in which the 8,9 double bond has been oxidized to give a new hydroxyl group at C-8 with rearrangement of the double bond to 9,10(*E*).[5]

(1) Carmely, S.; Kashman, Y. *Tetrahedron Lett.* **1987**, *28*, 3003. Carmely, S.; Ilan, M.; Kashman, Y. *Tetrahedron* **1989**, *45*, 2193.
(2) Cardellina, J. H., II; Meinwald, J. *J. Org. Chem.* **1981**, *46*, 4782. Pfleiderer, W. *Tetrahedron Lett.* **1984**, *25*, 1031.
(3) He, H.; Faulkner, D. J.; Lee, A. Y.; Clardy, J. *J. Org. Chem.* **1992**, *57*, 2176.

(4) It was not clear from the spectral data whether leucettamol A (4) existed as a salt or a free amine. The chemical shift of H-2 at δ 3.09 suggested a free amine but the integration of NH signal at 8.10 as six protons implied a salt. However, treatment of 4 with 0.1 M methanolic silver nitrate solution yielded a pale yellow precipitate indicative of a hydrochloride salt. Both amines are therefore drawn as hydrochloride salts.

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The lack of optical activity is an unusual feature of the leucettamols. Their closest analog is rhizochalin (7), which is a 26-galactosyl derivative of 11-keto-2,27-diaminooctacosane-3,26-diol, from the calcareous sponge *Rhizochalina incrustata*.[6] Since both rhizochalin (7) and the corresponding diol (8) have optical rotations of −5° and +11°, respectively, we must conclude that both leucettamols A (4) and B (5) are racemic. Although sufficiently active to be isolated by bioassay-guided fractionation, leucettamols A and B are only mildly active against *B. subtilis* and showed very weak cytotoxicity in the SOS chromotest bioassay.[7]

Experimental Section

Isolation of Leucettamol A (4) and Leucettamol B (5). The specimen of *Leucetta microraphis* (41.2 g dry wt., collection no. 89-027) was collected by hand (−15 m) at Ahnd Atoll, Pohnpei, Micronesia, and was stored frozen until extracted. The sponge was extracted with MeOH (2 × 200 mL), and the combined extracts were evaporated to obtain an aqueous suspension (ca. 100 mL) that was extracted with EtOAc (3 × 100 mL). The aqueous solution was freeze-dried to obtain a residue that was redissolved in MeOH and chromatographed on Sephadex LH-20 using MeOH as eluant. The fractions were assayed for antimicrobial activity against *B. subtilis*, using the standard disk assay procedure. The active fractions were combined to obtain a brown oil (610 mg), half of which was chromatographed on TSK 40s using MeOH–H_2O–NaCl (8:2:0.01) to obtain an active fraction (130 mg). The final purification was achieved by reversed-phase HPLC (ODS, MeOH–H_2O–NH_4Cl, 75:25:0.1) which gave two active compounds, leucettamol A (4, 5.3 mg, 0.026% dry wt) and leucettamol B (5, 7.5 mg, 0.032% dry wt).

Leucettamol A (4): pale yellow oil; $[\alpha]_D = 0$ (*c* 1.26, MeOH); IR (film) 3360, 3310, 2930, 2850, 1610, 1505, 1390, 1045 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.01 (br, 6 H, NH), 5.36 (m, 12 H), 5.36 (1 H, OH), 5.13 (br s, 1 H, OH), 3.69 (m, 1 H), 3.60 (br s, 1 H), 3.09 (m, 2 H), 2.82 (m, 10 H), 2.15 (m, 2 H), 2.02 (q, 2 H, *J* = 7 Hz), 1.15–1.40 (br m, 10 H), 1.09 (d, 3 H, *J* = 7 Hz), 1.06 (d, 3 H, *J* = 7 Hz); (CD_3OD) δ 5.52 (m, 1 H, H-6), 5.45 (m, 1 H, H-5), 5.37 (m, 10 H), 3.78 (m, 1 H), 3.44 (m, 1 H), ca. 3.25 (m, 2 H), 2.86 (m, 8 H), 2.81 (m, 2 H), 2.29 (m, 2 H), 2.07 (m, 2 H, *J* = 7 Hz), 1.36 (br m, 10 H), 1.24 (d, 3 H, *J* = 7 Hz), 1.20 (d, 3 H, *J* = 7 Hz); ^{13}C NMR (CD_3OD) δ 11.9 (q), 12.1 (q), 26.6 (t, 3 C), 26.8 (t), 27.0 (t), 28.2 (t), 30.3 (t), 30.5 (t), 30.7 (t, 2 C), 32.4 (t), 34.0 (t), 51.9 (d), 52.6 (d), 71.5 (d), 71.6 (d), 125.9 (d), 128.8 (d), 128.9 (d, 2 C), 129.1 (d, 2 C), 129.2 (d, 2 C), 129.4 (d, 2 C), 131.1 (d), 131.7 (d); HRFABMS obsd *m/z* 495.3901 (M + Na), $C_{30}H_{52}N_2O_2Na$ requires *m/z* 495.3926.

Leucettamol B (5): pale yellow oil; $[\alpha]_D = 0$ (*c* 0.27, MeOH); IR (film) 3360, 3310, 2855, 1610, 1505, 1400, 1040 cm^{-1}; UV (MeOH) 235 nm (ε 5400); ^1H NMR (DMSO-d_6) δ 7.88 (br, 6 H, NH), 6.48 (dd, 1 H, *J* = 15, 11 Hz, H-10), 5.97 (t, 1 H, *J* = 11 Hz, H-11), 5.69 (dd, 1 H, *J* = 15, 6 Hz, H-9), 5.46 (m, 2 H, H-5,6), 5.39 (m, 7 H), 5.30 (d, 1 H, *J* = 5.5 Hz, OH), 5.12 (d, 1 H, *J* = 5.5 Hz, OH), 4.90 (br s, 1 H, OH), 4.05 (q, 1 H, *J* = 6 Hz, H-8), 3.66 (m, 1 H, H-3), 3.58 (m, 1 H, H-28), 3.10 (m, 2 H, H-2,29), 2.92 (br t, 2 H, *J* = 7 Hz, H-13), 2.82 (br t, 2 H, *J* = 7 Hz, H-16), 2.78 (br t, 2 H, *J* = 7 Hz, H-19), 2.19 (m, 2 H, H-7), 2.12 (q, 2 H, *J* = 6.5 Hz, H-4), 2.02 (q, 2 H, *J* = 6.5 Hz, H-22), 1.40 (m, 1 H, H-27a), 1.18–1.35 (br m, 9 H), 1.09 (d, 3 H, *J* = 7 Hz, H-1), 1.06 (d, 3 H, *J* = 7 Hz, H-30); ^{13}C NMR (DMSO-d_6) δ 12.2 (q), 12.3 (q), 25.7 (t, 2 C), 25.8 (t), 26.0 (t), 27.1 (t), 29.0 (t), 29.3 (t), 29.5 (t), 31.6 (t), 32.9 (t), 35.9 (t), 50.6 (d), 51.1 (d), 70.1 (d), 70.2 (d), 70.8 (d), 124.3 (d), 127.1 (d), 128.0 (d), 128.1 (d, 2 C), 128.4 (d), 128.6 (d, 2 C), 128.8 (d), 129.2 (d), 130.4 (d), 138.3 (d); HRFABMS obsd *m/z* 621.3041 (M + Cs), $C_{30}H_{52}N_2O_3Cs$ requires *m/z* 621.3032.

Conversion of Leucettamol A (4) into Bis-oxazolone 6. 1,1′-Carbonyldiimidazole (20 mg) was added to a solution of leucettamol A (9.6 mg) in 1:1 CH_2Cl_2–DMF (1 mL), and the solution was stirred under an atmosphere of argon for 18 h. The solvents were evaporated under high vacuum, and the residue was chromatographed by reversed-phase HPLC (ODS, 9:1 MeOH–H_2O) to obtain the bis-oxazolone 6 (8 mg): IR (film) 1760 cm^{-1}; ^1H NMR (CD_3OD) δ 5.52 (dtt, 1 H, *J* = 10.5, 7, 1.5 Hz, H-6), 5.43 (dtt, 1 H, *J* = 10.5, 7, 1.5 Hz, H-5), 5.37 (m, 10 H), 4.61 (ddd, 1 H, *J* = 9, 7.7, 6 Hz, H-3), 4.57 (ddd, 1 H, *J* = 10, 7.7, 4 Hz, H-28), 3.95 (dq, 1 H, *J* = 7.8, 6.5 Hz, H-2), 3.90 (dq, 1 H, *J* = 7.8, 6.5 Hz, H-29), 2.87 (br m, 8 H), 2.82 (t, 2 H, *J* = 5.5 Hz), 2.51 (dddd, 1 H, *J* = 15, 8.5, 7, 1.5 Hz, H-4a), 2.43 (dddd, 1 H, *J* = 15, 7, 6, 1.5 Hz, H-4b), 2.08 (q, 2 H, *J* = 7 Hz, H-22), 1.66 (m, 1 H, H-27a), 1.55 (m, 1 H, H-27b), 1.37 (br m, 8 H), 1.16 (d, 3 H, *J* = 6.5 Hz, H-1), 1.11 (d, 3 H, *J* = 6.5 Hz, H-30); ^{13}C NMR (CD_3OD) δ 15.9 (q), 15.9 (q), 26.6 (t, 3 C), 26.8 (t), 27.1 (t), 28.2 (t), 28.4 (t), 30.2 (t), 30.4 (t), 30.6 (t), 52.3 (d), 52.4 (d), 81.2 (d), 81.9 (d), 125.3 (d), 128.8 (d), 128.9 (d, 2 C), 129.1 (d), 129.1 (d), 129.2 (d, 2 C), 129.4 (d, 2 C), 131.1 (d), 132.0 (d), 161.6 (s, 2 C); HRFABMS obsd *m/z* 657.2669 (M + Cs), $C_{32}H_{48}N_2O_4Cs$ requires *m/z* 657.2668.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra of compounds 4–6 (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(5) This transformation is analogous to the oxidation of the 11,12 double bond of arachidonic acid to form 12-HETE.

(6) Makarieva, T. N.; Denisenko, V. A.; Stonik, V. A.; Milgrom, Y. M.; Rashkes, Y. V. *Tetrahedron Lett.* **1989**, *30*, 6581.

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