Pergamon

Tetrahedron Letters, Vol. 38, No. 43, pp. 7507-7510, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01802-9

Rhapsamine, a Cytotoxin from the Antarctic Sponge *Leucetta leptorhapsis*

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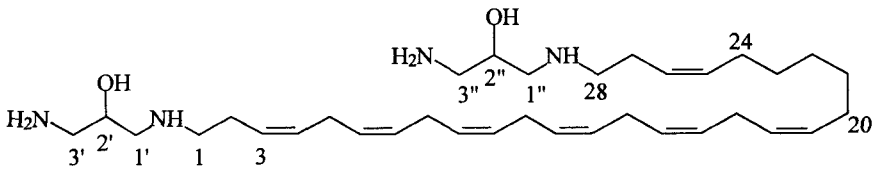
Abstract: Bioassay guided fractionation of the cytotoxic crude extract from the antarctic sponge *Leucetta leptorhapsis* has resulted in the isolation of rhapsamine (**1**), a linear C28 polyene terminally substituted by 1,3-diaminoglycerol groups. © 1997 Elsevier Science Ltd.

Calcareous sponges belonging to the genus *Leucetta* have been the subject of several recent chemical investigations resulting in the isolation of a variety of bioactive alkaloids.[1-10] Among the most bioactive alkaloids reported are a cytotoxic imidazole alkaloid from a Micronesian specimen,[4] a leukotriene B_4 receptor antagonist from a Palauan specimen *of L. microraphis*,[7] and antimicrobial lipids from a Micronesian *L. microraphis*.[8] *Leucetta leptorhapsis* is a common member of the benthic community of Ross Island, Antarctica, and has been reported to possess cytotoxicity[11] and other ecological bioactivity.[12] We have investigated the chemical basis of this bioactivity and report herein a cytotoxic constituent.

Leucetta leptorhapsis, known as the rubber sponge due to its appearance as being stretched, was collected in October, 1996, from several sites in McMurdo Sound.[13] The MeOH/CH_2Cl_2 (1:1) extract of the freeze-dried sponge displayed cytotoxicity in a fertilized sea urchin assay.[14] The extract was fractionated by reversed phase vacuum chromatography, Sephadex® LH-20, and reversed phase high performance liquid chromatography, yielding an amorphous solid, rhapsamine (**1**),[15] as the sole cytotoxic agent in the extract.

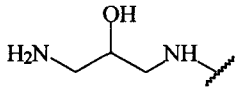


Rhapsamine (**1**)

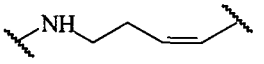
Mass spectral (HRFABMS) analysis indicated a formula of $C_{34}H_{60}N_4O_2$ for rhapsamine (**1**), suggesting seven degrees of unsaturation. The ^{13}C and DEPT NMR data indicated the presence of only methine and methylene carbons. ^1H NMR signals at δ 5.35 (m, 12H) and δ 5.60 (m, 2H), taken with the fourteen ^{13}C NMR signals in the olefinic region (δ 123-135), indicated seven disubstituted double bonds, accounting for the required unsaturations. A broad ^1H NMR signal at δ 2.75-2.85 (m, 10H), which was coupled to the olefinic

envelope, was assigned to five doubly-allylic methylene groups. This suggested the seventh olefinic unit was localized elsewhere in the molecule. The ^{13}C NMR spectrum contained three triplet mono-allylic carbon signals at δ 24.3, 27.3 and 27.4, the former of which accounted for two carbons. These chemical shift values as well as those for doubly-allylic carbons (δ 25.8, t, 5C) indicated the *cis* geometry for all olefins.[16] Heteroatom-bearing carbon signals at δ 64.0 (d), 50.6 (t), 47.0 (t) and 42.9 (t) accounted for two carbons each, suggesting symmetrical structural features for **1**.

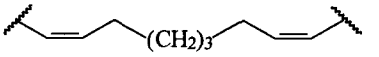
The presence of two end units of partial structure **1a** could be deduced by the interpretation of the ^{1}H and ^{13}C NMR, COSY and HMQC data. Three amino-bearing methylene carbons were observed in the ^{13}C NMR spectrum at δ 42.9, 47.0 and 50.6. The COSY spectrum indicated the protons attached to the δ 42.9 carbon signal, at δ 2.97 (1H, dd) and 3.16 (1H, dd), were correlated to a signal at δ 4.20 (m), a hydroxy-bearing methine proton. The δ 4.20 hydroxymethine was further connected to a doublet of triplets at δ 3.08 (obscured by overlapping H_2-1/H_2-28 signals) and 3.23, which were the methylene protons on the δ 50.56 aminomethylene group. Because these carbon signals each accounted for two carbons, the presence of two, terminal, 1,3-diaminoglycerol (**1a**) groups was suggested.



1a



1b



1c

A third amino-bearing methylene could be further extended to incorporate partial structure **1b**. COSY correlations were observed between the multiplet at δ 3.08 (4H, H_2-1/H_2-28), representing protons on the remaining aminomethylene (δ 47.0), and a 4H multiplet at δ 2.50 (H_2-2 and H_2-27) which was in turn coupled to the olefinic envelope at δ 5.35.

Finally, a six proton multiplet at δ 1.3 (3 X CH_2) showed connectivity only to a mono-allylic methylene signal at δ 2.05 (4H, H_2-20 and H_2-24) which was in turn coupled to the olefin envelope; this indicated the presence of unit **1c** and located the isolated olefin five carbons from the polyolefin chain.

HMBC correlations are compatible with the structure proposed for rhapsamine. In particular, partial structure **1a** could be shown to share the NH of **1b** by observation of a correlation between H_a-1'/H_a-1" at δ 3.08 and the aminomethylene at δ_c 47.0 (C-1/C-28). The reciprocal correlation from C-1'/C-1" (δ 50.6) to H_2-1/H_2-28 (δ 3.09) was observed, as were appropriate correlations among protons and carbons of the 1,3-diaminoglycerol group.

Acylation of rhapsamine (**1**) was carried out.[17] The HRFABMS data secured the molecular formula of $C_{46}H_{72}N_4O_8$, confirming the presence of six reactive sites in **1**. The proton NMR spectrum of the acylation product suffered from extensive signal broadening, due to the multiple conformations that the tetraamide possesses This signal broadening rendered the acylation product of little use for further structural studies.

Lack of optical rotation of the natural product suggests the diaminoglycerol groups each lack chirality.[18] However, the nearly symmetrical features that result in the coincidence of many NMR signals may similarly influence the optical rotation of the two chiral centers, resulting in a very small or nonexistent rotation.

Because of the initial observation of bioactivity, the cytotoxicity of rhapsamine (**1**) was further evaluated. In the KB nasopharyngeal cell line, rhapsamine displayed an LC_{50} of 1.8 μM; in the NCI cell-line panel, rhapsamine displayed a similar level of cytotoxicity with little selectivity. During the course of this work, the coriacenins were reported from the Mediterranean sponge *Clathrina coriacea*;[19] the coriacenins are shorter and symmetrical homologues of rhapsamine with no reported bioactivity.

ACKNOWLEDGMENTS

This work would not have been possible without field and laboratory assistance from Jill Baker, Dr. Mark Hamann, Jim Mastro, and Wesley Yoshida. Dr. David Powell, Director of Spectroscopic Services at the University of Florida, is gratefully acknowledged for mass spectral determinations. Purchase of the NMR instrument was assisted by a grant from NSF. Bioactivity data was kindly provided by Dr. Louis Barrows at the University of Utah and by Dr. Ven Narayanan and colleagues at the National Cancer Institute. The Antarctic Support Associates, Inc., the Antarctic Support Services of the National Science Foundation, and the U.S. Naval Antarctic Support Force provided invaluable logistical support for this project. This research was supported by National Science Foundation grants OPP-9117216, -9118864, -9526610, and -9530735 to BJB and JBM.

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13. The sponge *Leucetta leptorhapsis* was collected at the depth of 40 to 45 m in McMurdo Sound, Antarctica. The freeze dried sponge (10 g) was extracted with 1:1 MeOH/CH_2Cl_2 (2 x 200 mL) and the combined extracts evaporated in vacuo to give 1.0 g of residue which was active in a sea urchin fertilization assay at 500 ppm. The residue in 25% aqueous methanol was filtered through C-18 silica (30 g) in a small funnel and washed successively with 100 mL each 25, 50, 75% aqueous methanol, followed by 100% methanol. The residue (11.3 mg) from combined 75 and 100% methanol elutes (active at 100 ppm) was dissolved in MeOH (2 mL) and chromatographed on Sephadex LH-20 (2 X 50 cm) using MeOH as eluant. The active fractions were combined to obtain rhapsamine (**1**) as an amorphous white solid (6.3 mg). The final purification was achieved by reversed phase HPLC (YMC-Aq column, 9:1 MeOH/H_2O) to give pure rhapsamine (5.4 mg).

14. Fusetani, N. In *Bioorganic Marine Chemistry*; Scheuer, P.J., ed.; Springer-Verlag: Berlin, 1987; Vol. 1; pp. 61-92.

15. Rhapsamine (**1**): white amorphous solid; $[\alpha]_D = 0$ (*c* 0.006 , MeOH); IR (KBr) 3000-3400, 2732, 1608, 1500, 1465, 1066 cm^{-1}; UV (MeOH) nm (ε) 232 (6341); ^1H NMR (CD_3OD) δ 1.3 (m, 6H, H_2-21, -22, -23), 2.05 (m, 4H, H_2-20, -24), 2.5 (m, 4H, H-2, -27), 2.82 (m, 10H, H_2-5, -8, -11,-14, -17), 2.97 (dd, 2H, J = 13.75, 8.75 Hz, H_a-3', 3"), 3.16 (dd, 2H, J = 13.75, 3.75 Hz, H_b-3', -3"), 3.07 (overlapping m, 2H, H_a-1', -1"), 3.23 (dt, 2H, J = 3.75, H_b-1', -1"), 3.09 (m, 4H, H_2-1, -28), 4.21 (m, 2H, H-2', -2") and 5.35-5.60 (m, 14H, unresolved olefinic H's); ^{13}C NMR (CD_3OD) δ 24.3 (t, C-2, -27), 25.8 (t, C-5, -8, -11, -14, -17), 27.3 (t, C-20 or C-24), 27.4 (t, C-20 or C-24), 29.1, 29.6, 29.7 (t, C-21, -22, -23), 42.9(t, C-3', -3"), 47.0 (t, C-1, -28), 50.6 (t, C-1', 1"), 64.0 (d, C-2', -2"), 123.3 (d, C-26), 123.8 (d, C-3), 128.1 (d, C-18 or C-19), 127.9, 128.2, 128.4 (2C), 128.6, 128.7, 128.8, 129.1 (d, C-6, -7, -9, -10, -12, -13, -15, -16), 130.6 (d, C-18 or C-19), 132.6 (d, C-4), 134.7 (d, C-25); HRFABMS observed *m/z* 557.4794 [M^++H], $C_{34}H_{61}N_4O_2$ requires *m/z* 557.4794.

16. Wenkert, E.; Buckwater, B. L.; Burfitt, I. R.; Gasic, M. J.; Gottlieb, H. E.; Hagman, E. W.; Schell, F. M.; Wovkulich, P. M. In *Topics in Carbon-13 NMR Spectroscopy*; Levy, G. G., ed.; Academic Press: New York, 1976; Vol. 2; pp. 81-87.

17. Acetylation of **1**: A mixture of 1 mg of **1**, Ac_2O (0.1 mL) and pyridine (0.1 mL) was allowed to stand overnight at RT and then was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract dried and evaporated. HRFABMS observed *m/z* 809.5353 [M^++H], $C_{46}H_{72}N_4O_8$ requires *m/z* 809.5428.

18. Very small observed rotations were recorded with two different polarimeters; however, these rotations were of opposite sign. Therefore we believe rhapsamine to be optically inactive.

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(*Received in USA* 21 *July* 1997; *accepted* 21 *August* 1997)