Limaciamine, a New Diacylguanidine Isolated from the North Sea Nudibranch *Limacia clavigera*

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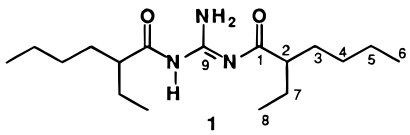
Limaciamine (**1**), a new symmetrical diacylguanidine, has been isolated from skin extracts of the North Sea dorid nudibranch *Limacia clavigera*.

Dorid nudibranchs are shell-less molluscs found in most shallow water habitats of the world's oceans. Skin extracts of dorids have proven to be a rich source of novel secondary metabolites that in some instances apparently play a role in defending the nudibranches from predation.[1,2] The dorid nudibranch *Limacia clavigera* (Muller, 1776) (family Polyceridae) is common in nearshore North Sea habitats along the western coasts of Norway, Great Britain, and France. It has a white body with dramatic yellow or orange tips on its rhinophores and similarly colored ceratal processes on its dorsal edge, making it easy to spot and identify in the field. *L. clavigera* feeds exclusively on a variety of bryozoans. Examination of skin extracts obtained from specimens of *L. clavigera* collected in Norway has resulted in the isolation of limaciamine (**1**), a new diacylguanidine that is related to triophamine (**2**), a known metabolite of the dorid nudibranch *Triopha catalinae*.[3] Details of the structure elucidation of the limaciamine (**1**) are presented below.



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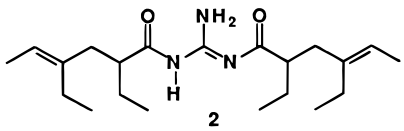

2

Specimens of *L. clavigera* were collected by hand using scuba at −10 m off Tosoy Island, near Bergen, in the North Sea. Freshly collected animals were immediately immersed in methanol and returned to Vancouver for further study. The methanol extract was concentrated in vacuo, and the residue was partitioned between water and ethyl acetate. Fractionation of the ethyl acetate-soluble materials via sequential application of silica gel flash chromatography, and normal-phase HPLC gave pure limaciamine (**1**) as a colorless glass.

Limaciamine (**1**) gave a parent ion in the HREIMS at *m/z* 311.2579 appropriate for a molecular formula of $C_{17}H_{33}N_3O_2$. The ^{13}C NMR spectrum of **1** showed only eight resonances, suggesting a symmetrical molecule. One of the resonances in the ^{13}C NMR spectrum of **1** had a chemical shift of 158.9 ppm, which was identical to the chemical shift of the guanidine carbon resonance in the ^{13}C NMR spectrum of triophamine (**2**).[3] In addition, the IR spectrum of limaciamine (**1**) showed a strong carbonyl stretching band at 1700 cm^{-1}, which was identical to the amide carbonyl stretching frequency (1700 cm^{-1}) observed for triophamine. Taken together, these two pieces of evidence indicated that the three nitrogen and two oxygen atoms in limaciamine (**1**) were present as a diacylguanidine substructure.

Subtracting the atoms present in the amide carbonyl and guanidine fragments ($C_3H_3N_3O_2$) from the molecular formula of limaciamine left $C_{14}H_{30}$. Analysis of the HMQC and ^{13}C NMR data showed that these hydrocarbon atoms belonged to two identical C_7H_{15} aliphatic fragments each containing one methine, four methylene, and two methyl carbons. Resonances at δ 2.17 (H-2) in the ^1H NMR spectrum and at δ 51.5 (C-2) in the ^{13}C NMR spectrum could be assigned from the HMQC data to the methine proton and its attached carbon. Comparison of the methine chemical shifts with the values for the α-methine proton (**2**: H-2 δ 2.39) and its attached carbon (**2**: C-2 δ 50.3) in triophamine (**2**) indicated that the methine carbons in the identical acyl residues of **1** were also attached to the carbonyl functionalities. COSY correlations were observed between the methine proton resonance (δ 2.17: H-2) and a pair of geminal methylene proton resonances at δ 1.66 (H-7) and 1.51 (H-7′), which were in turn both correlated to a methyl triplet at δ 0.89 (Me-8). These COSY correlations identified an ethyl residue as the second substituent on the methine carbon. The third substituent on the methine carbon, which had to account for the remaining methyl and methylene carbons, had to be an *n*-butyl residue. COSY, HMQC, and HMBC correlations confirmed the presence of the *n*-butyl fragment and led to the following ^1H and ^{13}C assignments for this portion of limaciamine (**1**): C-3, δ 31.9; H-3/H-3′, δ 1.41/1.62; C-4, δ 29.6; H-4, δ 1.26; C-5, δ 22.7; H-5, δ 1.31; C-6, δ 13.9; H-6, δ 0.86.

The HREIMS data recorded for limaciamine were in complete agreement with the assigned structure **1**. Thus, fragment ions were observed at *m/z* 282.2184 ($C_{15}H_{28}N_3O_2$, $M^+ - C_2H_5$ (ethyl)), 255.1944 ($C_{13}H_{25}N_3O_2$, $M^+ - C_4H_8$ (*n*-butene) via a McLafferty rearrangement), 212.1403 ($C_{10}H_{18}N_3O_2$, $M^+ - C_7H_{13}$ (3-heptyl)), 127.1118

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($C_8H_{15}O$, 2-ethylhexanoyl), 99.1178 (C_7H_{13}, 3-heptyl), 86.03502 ($C_2H_4N_3O$, $(NH_2)_2C{=}N{-}C{=}O$), and 57.0705 (C_4H_9, *n*-butyl). One interesting feature of the NMR data recorded for limaciamine (**1**) was the absence of a signal that could be confidently assigned to the carbonyl carbons. The corresponding carbonyl resonance in the ^{13}C NMR spectrum of triophamine (**2**) was a very weak and very broad signal at δ 185.6 that was only detectable in the spectra of relatively concentrated samples. A broad hump was also present at $\sim\delta$ 185 in the ^{13}C NMR spectrum of limaciamine (**1**), but the poor signal-to-noise present in the spectrum as a consequence of the limited sample size that was available for NMR made it impossible to be completely confident that this hump was a real NMR resonance.

It has recently been demonstrated that triophamine (**2**) is biosynthesized *de novo* by *T. catalinae*.[4] The two identical 10-carbon acyl residues in **2** are formed from one acetate and two butyrate units in a manner consistent with a processive polyketide pathway.[4,5] In light of the structural similarities between **1** and **2**, it seems reasonable to conclude that limaciamine (**1**) is also synthesized *de novo* by *L. clavigera*. The acyl residues found in limaciamine (**1**) are missing one of the ethyl branches present in the acyl residues of triophamine (**2**), suggesting that the limaciamine acyl groups are triketides formed from one butyrate and two acetate units.

Experimental Section

Collection and Isolation. *L. clavigera* (50 animals) was collected at depths of −10 m off Tosoy Island in the North Sea. A voucher sample is available in the Zoology Department at UBC. Freshly collected specimens were extracted whole in MeOH (250 mL). After 1 week, the original MeOH was decanted, and the animals were subsequently extracted with two additional aliquots of MeOH (250 mL each). The combined MeOH extracts were filtered and concentrated in vacuo to give an aqueous suspension that was diluted with distilled H_2O (500 mL) and extracted with 4 × 500 mL portions of EtOAc. Fractionation of the EtOAc-soluble materials by silica gel flash chromatography using step-gradient elution proceeding from 100% hexanes to 100% EtOAc gave partially purified limaciamine (**1**) eluting at 80% hexane/20% EtOAc. Further fractionation on normal-phase HPLC (eluent: 15% EtOAc/85% hexane) gave 4.3 mg of pure limaciamine (**1**).

Limaciamine (1): isolated as a colorless glass; ^1H NMR ($CDCl_3$) δ 0.86 (t, J = 7 Hz, Me-6), 0.89 (t, J = 7 Hz, Me-8), 1.26 (m, H-4), 1.31 (m, H-5), 1.41 (m, H-3), 1.51 (m, H-7), 1.62 (m, H-3′), 1.66 (m, H-7′), 2.17 (m, H-2); ^{13}C NMR ($CDCl_3$) δ 11.9 (q, C-8), 13.9 (q, C-6), 22.7 (t, C-5), 25.2 (t, C-7), 29.6 (t, C-4), 31.9 (t, C-3), 51.5 (d, C-2), 158.9 (s, C-9); HREIMS *m/z* 311.2579 (calcd for $C_{17}H_{33}N_3O_2$, 311.2585).

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References and Notes

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