Pergamon

Tetrahedron Letters, Vol. 37, No. 31, pp. 5555-5556, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01154-9

Stellettadine A: a New Acylated Bisguanidinium Alkaloid Which Induces Larval Metamorphosis in Ascidians from a Marine Sponge *Stelletta* sp.

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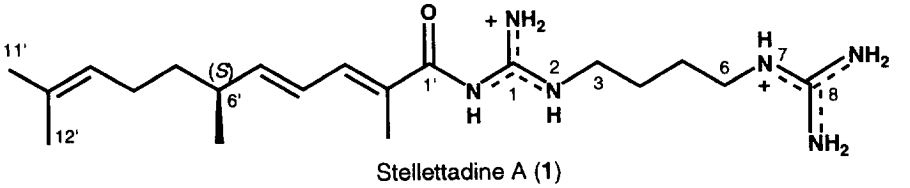
Abstract: A new bisguanidinium alkaloid, stellettadine A (**1**), containing a norsesquiterpene unit has been isolated from a marine sponge *Stelletta* sp. as a metamorphosis-inducing compound. Its structure including the absolute stereochemistry was elucidated on the basis of spectral data and chemical degradation. Compound **1** induced metamorphosis of the ascidian *Halocynthia roretzi* larvae with an ED_{100} value of 50 μM. Copyright © 1996 Elsevier Science Ltd

During our ongoing search for compounds inducing metamorphosis in ascidians, *Halocynthia roretzi* and *Ciona savignyi*, from marine organisms,[1] we found the activity in the MeOH extract of a marine sponge, *Stelletta* sp., collected in the Gulf of Sagami, Japan. Bioassay-guided isolation afforded a new acylated bisguanidinium alkaloid, stellettadine A (**1**).

The water soluble portion of the MeOH extract of the sponge (270 g, wet weight) was partitioned between water and *n*-BuOH; the active *n*-BuOH layer (5.43 g) was repeatedly fractionated by reversed-phase (C_{18}) column chromatography (aq MeOH) to afford a metamorphosis-inducer, stellettadine A (**1**, 0.25 g, 0.093 % wet weight).[2] The positive FAB mass spectrum of **1** showed a pseudomolecular ion peak at *m/z* 377, matching $C_{20}H_{37}N_6O$ by HRFABMS. ^1H and ^{13}C NMR data (DMSO-d_6) revealed the presence of 4 Me, 6 CH_2, 1 CH, 3 double bonds, 2 guanidino, and 1 carbonyl groups. Interpretation of the ^1H-^1H COSY spectrum readily led to two structural units, C2'~C12' and N2~N7. 2'*E*,4'*E*-Geometry was confirmed by a coupling constant ($J_{4',5'}$ = 15.0 Hz), an NOE cross peak (Me-2'/H-4'), and a chemical shift for Me-2' (δ 12.2). HMBC cross peaks[2] indicated that a carbonyl carbon (δ 169.6) was linked to C2' (H_3-2' and H-3'/C1'), while two guanidinium carbons were connected through a $(CH_2)_4$ unit (H_2-3/C1; H_2-6/C8). Thus, the gross structure was constructed. In order to elucidate the stereochemistry of C6', **1** was treated with



Stellettadine A (**1**)

$NaIO_4$ in the presence of $RuCl_3$[3] to afford (*S*)-2-methylglutaric acid[4] ($[\alpha]_D$ +22°), thereby determining 6'*S*-stereochemistry.

Bisguanidino derivatives have been reported from various organisms: saxitoxin and related PSPs from dinoflagellates *Alexandrium* spp.,[5a] zoanthoxanthins, 1,3,7,9-tetrazacyclopent[*e*]azulene derivatives, from the zoanthid *Epizoanthus arenaceus*,[5b] crambines A and B from the marine sponge *Crambe crambe*,[5c] phloeodictine B, a bicyclic amidinium salt, from a marine sponge *Phloeodictyon* sp.,[5d] and aliphatic long-chain muscarinic receptor antagonists from a bacterium *Streptomyces* sp.[5e] The bisguanidino unit C1~C8 of stellettadine A (**1**) corresponds to the long known arcaine from the mollusk *Arca noae*[6a] and the worm *Audouinia tentaculata*.[6b] Interestingly, an indolizidine alkaloid containing a homosesquiterpene unit was reported from another Japanease *Stelletta* sponge.[7] Stellettadine A (**1**) showed metamorphosis-inducing activity with an ED_{100} value of 50 μM on ascidian *H. roretzi* larvae.

Acknowledgment: We thank Prof. P. J. Scheuer of the University of Hawaii for reading this manuscript. Thanks are also due to Prof. I. Kitagawa of Kinki University for valuable discussions, to Dr. T. Numakunai of the Asamushi Marine Biological Laboratory of Tohoku University for his valuable advice on screening, and to Dr. R. van Soest of the University of Amsterdam for identification of the sponge.

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2. $[\alpha]_D^{24}$ -32.8 ° (*c* 1.00, MeOH). IR (film) ν_{max} 3250, 3150, 1660, 1650, and 1620 cm^{-1}. UV (MeOH) λ_{max} 285 nm (ε 11100). ^1H NMR (DMSO-d_6) δ 1.00 (3H, d, *J* = 6.7 Hz, Me-6'), 1.35 (2H, q, *J* = 7 Hz, H_2-7'), 1.54 (3H, s, H_3-12'), 1.55 (2H, m, H_2-4), 1.56 (2H, m, H_2-5), 1.63 (3H, s, H_3-11'), 1.92 (3H, s, Me-2'), 1.93 (2H, m, H_2-8'), 2.31 (1H, quint, *J* = 6.7 Hz, H-6'), 3.13 (2H, m, H_2-3), 3.30 (2H, m, H_2-6), 5.08 (1H, t, *J* = 7 Hz, H-9'), 6.13 (1H, dd, *J* = 15.0 and 8.0 Hz, H-5'), 6.41 (1H, dd, *J* = 15.0 and 11.0 Hz, H-4'), 7.23 (4H, br.s, 2 x NH_2-8), 7.47 (1H, d, *J* = 11.0 Hz, H-3'), 7.84 (1H, s, H-2), 8.86 and 9.13 (each 1H, br.s, NH_2-1), 9.61 (1H, s, H-7), and 11.57 (1H, br.s, NH-1). ^{13}C NMR (DMSO-d_6) δ 12.2 (q, Me-2'), 17.5 (q, C12'), 19.7 (q, Me-6'), 24.9 (t, C5), 25.2 (t, C8'), 25.4 (q, C11'), 25.6 (t, C4), 36.1 (t, C7'), 36.3 (d, C6'), 40.1 (t, C3), 40.4 (t, C6), 124.1 (d, C9'), 124.3 (d, C4'), 126.3 (s, C2'), 130.8 (s, C10'), 138.9 (d, C3'), 150.5 (d, C5'), 154.1 (s, C8), 156.9 (s, C1), and 169.6 (s, C1'). HMBC coss peaks: H_2-3/C1, C4, and C5; H_2-4/C3 and C5; H_2-5/C4 and C6; H_2-6/C4, C5, and C8; Me-2'/C1', C2', and C3'; H-3'/C1', Me-2', and C5'; H-4'/C3' and C6'; H-5'/C3', C6', and Me-6'; H-6'/C4', C5', Me-6', and C8'; H_3-6'/C5', C6', and C7'; H_2-7'/C5', C6', Me-6', C8', and C9'; H_2-8'/C7', C9', and C10'; H-9'/C7', C8', and C-11'; H_3-11'/C9', C10', and C12'; H_3-12'/C9', C10', and C11'. FABMS (positive, glycerol matrix) *m/z* 377 $(M+H)^+$. HRFABMS (positive, PEG matrix) *m/z* 377.3037 (Δ +0.9 mmu, calcd for $C_{20}H_{37}N_6O$).
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   (*S*)-2-Methylglutaric acid from **1**: $[\alpha]_D^{24}$ +22° (*c* 0.033, MeOH). ^1H NMR (D_2O) δ 1.22 (3H, d, *J* = 7.0 Hz, H_3-2), 1.83 (1H, dq, *J* = 7.5 and 15.0 Hz, H-3), 1.96 (1H, dq, *J* = 7.5 and 15.0 Hz, H-3), 2.49 (2H, t, *J* = 7.5 Hz, H_2-4), and 2.60 (1H, sext, *J* = 7.0 Hz, H-2). FABMS (negative, glycerol matrix) *m/z* 145 $(M-H)^-$.
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(*Received in Japan* 17 *May* 1996; *accepted* 10 *June* 1996)