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Njaoamines G and H, two new cytotoxic polycyclic alkaloids and a tetrahydroquinolone from the marine sponge *Neopetrosia* sp.

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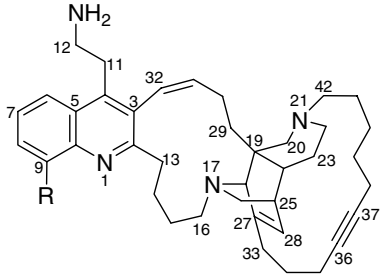
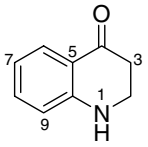
Abstract—Two new polycyclic alkaloids, njaoamines G (**1**) and H (**2**) and 1,2,3,4-tetrahydroquinolin-4-one (**3**) have been isolated from the sponge *Neopetrosia* sp. collected at Pemba Island, Tanzania. Compounds **1** and **2** are close in structure to njaoamines A–F. Compound **3**, known synthetically, is a new natural compound. The structures and relative stereochemistries of compounds **1** and **2** were elucidated on the basis of spectroscopic data. Njaoamines G and H are potent brine shrimp toxins with LD_{50} values of 0.17 μg/mL and 0.08 μg/mL for **1** and **2**, respectively.

In a continuing discovery program for bioactive compounds from marine invertebrates,[1,2] sponge extracts were screened for brine shrimp toxicity. Consequently, we encountered potent activity from a *Neopetrosia* sp. collected off Pemba Island, Tanzania.[3] Bioassay-guided isolation of the active compounds resulted in the isolation of two new polycyclic alkaloids belonging to the 'njaoamines' family, represented by njaoamines A–F, isolated recently from a haplosclerid sponge *Reniera* sp.[4] Hereafter, are described the isolation, structure elucidation and brine shrimp toxicity of njaoamine G (**1**) and H (**2**).



1, R=H
2, R=OH



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Homogenized *Neoptrosia* sp. (7.0 g, dry weight), was extracted with EtOAc–MeOH–H_2O (5:5:1). The extract (370 mg) was then subjected to solvent-partitioned, that is, aq MeOH against hexane and CH_2Cl_2 and the CH_2Cl_2 fraction was chromatographed on Sephadex LH-20, eluting with hexane–MeOH–$CHCl_3$ (2:1:1), to afford a complex cytotoxic mixture, which following RP-HPLC, led to the isolation of njaoamine G (**1**, 10 mg, 0.14% dry weight)[5] and njaoamine H (**2**, 8 mg, 0.11% dry weight).[6]

Njaoamine G (**1**) showed a molecular ion peak at *m*/*z* 577 $(M+H)^+$ in the FABMS. The presence of 39 carbon signals in the ^{13}C NMR spectrum was consistent with the molecular formula of $C_{39}H_{52}N_4$ that was established by HRFABMS (*m*/*z* 577.8956, calcd for $C_{39}H_{53}N_4$, 577.8959), suggesting 16 degrees of unsaturation. The ^{13}C, PND, and DEPT, NMR data (Table 1) revealed 39 carbon signals due to 13 low field signals attributable to olefinic and/or C=N carbons, one sp^3 quaternary carbon, three sp^3 methines, twenty sp^3 methylenes, and two sp carbons (δ_C 79.5 s, 81.4 s) that together with the IR absorption at 2361 cm^{-1} suggested the presence of an acetylene bond. The above functionalities accounted for nine degrees of unsaturation, implying a heptacyclic structure (the numbering is consistent with njaoamines A–F).[4]

The presence of a 2,3,4-trisubstituted quinoline moiety (**a**) in the molecule was inferred from the presence of

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Table 1. NMR data of njaoamine G (**1**)[a,b]

Position	δ_C	δ_H (J in Hz)	COSY	HMBC (H to C)	NOESY
*N*1					
2	160.3 s				
3	131.0 s				
4	141.4 s				
5	126.4 s				
6	124.3 d	8.23 d (7.9)	7, 8	4, 8, 10	7, 11, 12
7	126.5 d	7.43 t (7.9)	6, 8, 9	5, 9	6, 8
8	128.9 d	7.61 t (7.9)	6, 7, 9	6, 10	7, 9
9	130.0 d	8.30 d (7.9)	7, 8	5, 7	8
10	147.5 s				
11	28.1 t	3.85 dt (12.1, 7.1) 3.62 m	12	3, 4, 5	6, 12, 32
12	39.3 t	3.51 m, 3.40 m	11	4, 11	6, 11
13	38.8 t	3.22 m, 3.14 m	14	2, 3, 15	32
14	26.0 t	2.42 m, 1.57 m	13, 15	2, 16	
15	26.1 t	1.30 m	14, 16	13	
16	56.3 t	2.45 br d (12.1), 1.95 m	15	18	18
*N*17					
18	57.3 d	2.78 s		16, 19, 20, 24, 27, 28	16
19	43.5 s				
20	48.4 t	3.38 d (13.4), 2.25 d (13.4)		18, 22, 24, 29, 42	23, 24
*N*21					
22	48.6 t	3.52 m, 3.02 m	23	20, 24, 42	23
23	24.5 t	1.58 m, 1.10 m	24, 22	19, 25	20, 22, 24, 25
24	41.2 d	1.11 m	23	18, 20, 22, 28	20, 23, 25
25	36.9 d	2.04 m	24, 26, 28	19, 23, 27	23, 24, 28
26	56.6 t	3.03 m, 1.74 m	25	16, 18, 24, 28	
27	144.0 s				
28	123.4 d	5.78 br d (6.3)	25	24, 26, 27, 33	25, 33
29	36.3 t	2.25 m, 1.96 m	30	18, 20, 24	31
30	23.8 t	2.86 m, 1.90 m	29, 31	19, 32	31
31	136.7 d	6.02 t (10.7)	30, 32	3, 29	29, 30, 32
32	124.6 d	6.39 d (10.7)	31	2, 3, 4, 30	11, 13, 31
33	34.7 t	1.73 m, 1.34 m	34	18, 28, 35	28, 34
34	27.0 t	1.31 m, 0.88 m	33, 35	36	33, 35
35	17.4 t	1.90 m, 1.76 m	34	36, 37	34
36	81.4 s				
37	79.5 s				
38	17.5 t	2.15 m	39	35, 36, 37	
39	27.0 t	1.45 m	38, 40	37	
40	24.5 t	1.32 m	39, 41	38	
41	20.2 t	2.05 m, 1.80 m	40, 42	39	
42	57.0 t	3.11 m	41	20, 22, 40	

[a] Data recorded in d_5-pyridine on a Bruker Avance-500 instrument, except ^{13}C NMR (100 MHz), which was recorded on a Bruker Avance-400.
[b] The numbering is according to the njaoamines A–F.[4]

signals for four adjacent aromatic protons in the ^1H NMR spectrum at δ_H 8.23 (d), 7.43 (t), 7.61 (t), and 8.30 (d) ppm (H-6 to H-9, respectively) (Table 1) and from the HMBC correlations observed for these protons with the non-protonated C-atoms C-4, C-5, and C-6 (Fig. 1). The substituent at C-4 was identified as a 2-aminoethyl chain on the basis of ^1H–^1H couplings observed between H-11 and H-12, the chemical shifts of both carbons[4] and HMBC correlations are shown in Figure 1.

Through analysis of the COSY, TOCSY, and HMBC experimental data, we identified a second substructure (**b**), containing a tricyclic core with two nitrogen atoms similar to that recently described for the njaoamine family[4] and earlier for the ingenamine family,[7,8] and keramaphidin B.[9] The stereochemistry of **b** was deduced from NOESY correlations, and confirmed to be comparable to the 'njaoamines' and 'ingenamines' families (Fig. 2).

The connection between the two substructures, the 2,3,4-trisubstituted quinoline (**a**) and the tricyclic core (**b**), was established by COSY and HMBC experiments, see Table 1, and determined to be the same as reported for njaoamines A and C–F. The stereochemistry of the Δ^{31} double bond was determined to be Z on the basis of the coupling constant of 10.7 Hz observed between the two protons.[4] The remaining signals, present in both the ^1H and ^{13}C NMR spectra, were eight methylenes and two acetylene carbons. Interpretation of the COSY, TOCSY, and HMBC experiments led to a 4-decenyne unit and closure of the seventh ring (**c**) as shown in Figure 1. The 4-decenyne unit encompasses an unprecedented acetylene comprising 17-membered ring system, representing a structurally unique portion of njaoamine G (**1**).

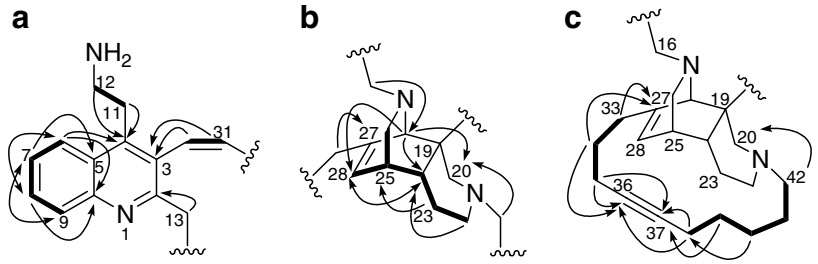

Figure 1. Key COSY, TOCSY (▬) and HMBC (⌒) correlations of the three functionalized moieties of njaoamine G (**1**).

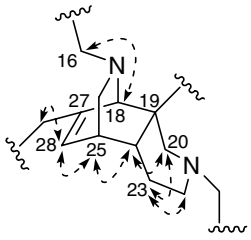

Figure 2. Key NOESY (⌒) correlations of njaoamine G (**1**).

The spectral data of the second isolated compound, njaoamine H (**2**), pointed to a very similar structure to compound **1**. The FABMS for the $(M+H)^+$ peak at *m/z* 593 indicated the presence of an additional oxygen atom in the molecule (HRFABMS *m/z* 593.4221 calcd for $C_{39}H_{53}N_4O$, 593.4219). The major difference in the NMR spectra of **2** was the disappearance of the CH-9 group of **1**, and the appearance, in the ^{13}C NMR, of a singlet carbon resonating at δ_C 154.4 ppm suggesting a hydroxyl group. The 2D NMR experiments confirmed the suggested 2,3,4-trisubstituted hydroxyquinoline moiety as in njaoamines A–F.[4]

Compound **3** was shown, by HREIMS, to have the molecular formula C_9H_9NO (*m/z* 147.0681 calcd 147.0684).[10] The ^1H NMR spectrum exhibited four aromatic protons at δ_H 7.69 (d), 7.28 (t), 6.79 (d), and 6.58 (t) ppm (H-6 to H-9, respectively), two vicinal aliphatic protons at δ_H 3.28 (t) and 3.12 (t) ppm and one NH group at δ_H 7.25 (br s) ppm. In the ^{13}C NMR spectrum, nine carbon signals including three quaternary carbons, four CH carbons, and two CH_2 carbons were observed.[10] On the basis of 2D NMR spectral data, compound **3** was characterized as 1,2,3,4-tetrahydroquinolin-4-one. Though this compound is commercially available, this is the first report of this compound from a natural source.

Njaoamines G and H were both tested for toxicity to brine shrimp (*Artemia salina*) and were found to be highly active. Njaoamine H (**2**) showed greater potency with an LD_{50} value of 0.08 μg/mL; njaoamine G had an LD_{50} value of 0.17 μg/mL.[11] It is worth noting that njaoamines A–F showed cytotoxic activity against three human tumor cell lines.[4] Although the present metabolites (**1** and **2**) are structurally close to the early reported alkaloids from the sponge *Reniera* sp. (njaoamines A–F),[4] they give an additional insight into the unique metabolic processes in their construction. The highly potent and exceptional brine shrimp toxic activity of njaoamine G (**1**) and H (**2**) will encourage us to continue the current investigation of their biological activity.

Supplementary data

General experimental procedures and NMR data (^1H NMR, ^{13}C NMR, COSY, and HMBC) for njaoamines G and H. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.08.079.

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2. Sorek, H.; Rudi, A.; Aknin, M.; Gaydou, E.; Kashman, Y. *Tetrahedron Lett.* **2006**, *47*, 7237–7239.
3. The sponge *Neopetrosia* sp. was collected from Pemba Island, Tanzania, 4°57.743′ S; 39°39.843′ E (30 November, 2004). Voucher specimens are deposited at the Zoological Museum, Tel Aviv University, Israel (ZMTAU PO 25469) and at the Zoological Museum, University of Amsterdam (ZMAPOR 19909). This sponge was collected from a shallow reef at a depth of 3–5 m, inhabited by a large variety of other sponges, octocorals and tunicates.
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5. Njaoamine G (**1**): pale yellow oil, $[\alpha]_D^{21}$ +22 (*c* 0.8, MeOH), ^1H and ^{13}C NMR data, see Table 1; IR (KBr) ν_{max} 3404, 2933, 2361, 1678, 1436, 1384, 1204, 1135 cm^{-1}, FABMS *m/z* 577 $(M+H)^+$, HRFABMS *m/z* 577.8956 (calcd for $C_{39}H_{53}N_4$, 577.8959).
6. Njaoamine H (**2**): pale yellow oil, $[\alpha]_D^{21}$ +20 (*c* 0.8, MeOH), ^1H and ^{13}C NMR data, see Supporting Information; IR (KBr) ν_{max} 3428, 2934, 2361, 1679, 1432, 1204, 1134 cm^{-1}, FABMS *m/z* 593 $(M+H)^+$, HRFABMS *m/z* 593.4221 (calcd for $C_{39}H_{53}N_4O$, 593.4219).
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10. 1,2,3,4-Tetrahydroquinolin-4-one (**3**): pale yellow oil, IR (KBr) ν_{max} 3428, 3050, 1690, 1432 cm^{-1}; ^1H NMR (DMSO) δ 7.69 (1H, d, $J = 8.0$ Hz, H-6), 7.28 (1H, t, $J = 8.0$ Hz, H-8), 7.25 (1H, br s, H-1), 6.79 (1H, d, $J = 8.0$ Hz, H-9), 6.58 (1H, t, $J = 8.0$ Hz, H-7), 3.28 (2H, t, $J = 6.2$ Hz, H-2), 3.12 (2H, t, $J = 6.2$ Hz, H-3); ^{13}C NMR (DMSO) δ 199.0 (C-4), 151. 7 (C-10), 135.0 (C-8), 131.4 (C-6), 117.5 (C-9), 116.3 (C-5), 115.0 (C-7), 36.8 (C-2), 34.8 (C-3); EIMS m/z 147 M^+, HREIMS m/z 147.0681 (calcd for C_9H_9NO, 147.0684).
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