

## Clathculins A and B, Two Novel Nitrogen-Containing Metabolites from the Sponge *Clathrina aff. reticulum*

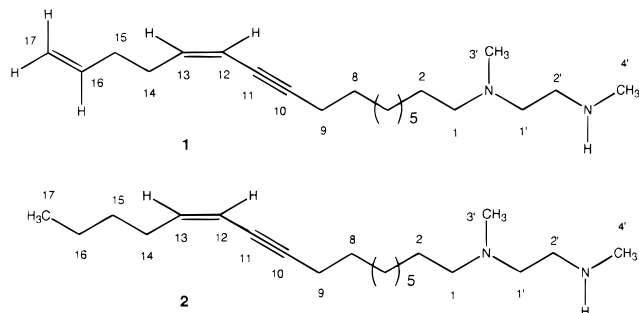
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Two novel acyclic diaza alkenynes, clathculins A and B (**1** and **2**), were isolated from the Indo-Pacific sponge *Clathrina aff. reticulum* collected in Sodwana Bay, South Africa. The structure of the mixture of the two unstable inseparable compounds was established by spectroscopic analysis, mainly 1D and 2D NMR measurements and two chemical transformations. Clathculins A and B are the first reported linear marine metabolites containing a 1,2-diaminoethane moiety.

In connection with our long-standing interest in the chemistry of marine organisms, we investigated the small, brittle, pink Indo-Pacific Ocean sponge *Clathrina aff. reticulum* (Schmidt, 1862) (class Calcarea, subclass Calcinea, order Clathrinida, family Clathrinidae), collected from the five-mile reef in Sodwana Bay, South Africa. In the process of normal chemical examination, we isolated an unstable, inseparable mixture of two new nitrogen-containing metabolites, designated clathculins A and B (compounds **1** and **2**), from the sponge. We hereby report the isolation and structure elucidation of these two compounds, which possess an unprecedented diamine structure.



Nitrogen-containing cyclic and polycyclic marine natural compounds are well-known;<sup>1</sup> less familiar are the acyclic, long-chain, nitrogen-containing compounds. Among the latter compounds are the sinulamides<sup>2</sup> and spermidine derivatives,<sup>3</sup> isolated from soft corals of the genus *Sinu-laria*, and penarmides,<sup>4</sup> rhapsamine,<sup>5</sup> coriacenins,<sup>6</sup> and erylusamines,<sup>7</sup> obtained from sponges. All of the latter acyclic compounds contain one or more  $>N(CH_2)_nN<$  moieties where  $n = 3–5$  or higher. Clathculins A and B are, to the best of our knowledge, the first acyclic long-chain marine metabolites containing a  $>NCH_2CH_2N<$  moiety.

Freshly collected *Clathrina aff. reticulum* was frozen on site and kept frozen until needed. Freeze-dried sponge tissue (6 g dry wt) was extracted three times with a mixture of  $CH_3OH-EtOAc$  (1:1) to give a brown gum (220 mg) after evaporation. The latter extract was subsequently partitioned between aqueous methanol and hexane,  $CCl_4$ , and

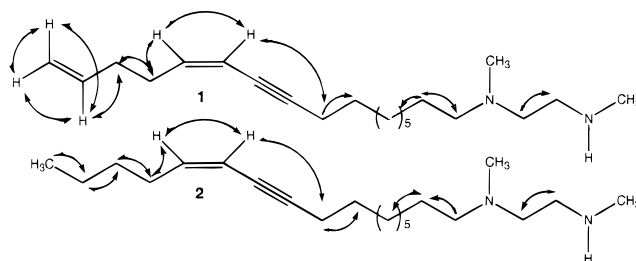


Figure 1. COSY and TOCSY correlations.

$CHCl_3$ , and the  $CHCl_3$  phase was fractionated by chromatography on Sephadex LH-20 [eluting with hexane– $CHCl_3$ – $CH_3OH$  (2:1:1)] to give an unstable 1:1 mixture of compounds **1** and **2** (35 mg). Tedious attempts to separate the mixture, including HPLC, caused a change in the ratio of **1** and **2**, but mostly decomposition of both (new peaks appeared in the  $\delta$  3.0–4.0 ppm region in the  $^1H$  NMR spectrum). Clathculins A and B analyzed by EIMS for  $C_{21}H_{38}N_2$  and  $C_{21}H_{40}N_2$ , respectively; HRMS were obtained for the corresponding monoacetates **3** and **4** (see Experimental Section).

The  $^1H$  and  $^{13}C$  NMR data (Table 1) exhibited great similarity between compounds **1** and **2**. From the NMR data, it was evident that both are acyclic compounds possessing, on one side, the same *N,N*-dimethyl-1,2-diaminoethane moiety as one terminus of the molecule and a different one on the other end (see below). In the common part, two separate  $N-CH_3$  groups ( $CH_3-3'$  and  $CH_3-4'$ ) and a 1,2-diaminoethane segment [ $>NCH_2(1')CH_2(2')N<$ ] were deduced from the NMR chemical shifts<sup>8</sup> as well as from COSY, TOCSY (Figure 1), HMBC correlations (C to H correlations between C-1' and 2H-1 and  $CH_3-3'$  and between C-2' and H-4', and vice versa, together with H–H correlations between 2H-1',  $\delta$  2.70, and 2H-2',  $\delta$  2.95, see Table 1, established unequivocally the diaminoethylene moiety), and the strong MS fragmentation between C(1') and C(2')– $\alpha$ -cleavage to both nitrogen atoms.

The end  $-NHCH_3$  group was further confirmed by acetylation of the mixture of **1** and **2** with a mixture of  $Ac_2O$ /methanol (known to acetylate only amino groups),<sup>9</sup> at room temperature overnight, to afford the corresponding mono acetamides **3** and **4**, respectively (see Experimental Section). The mixture of **3** and **4** was more stable than that of **1** and **2**; however, still no good separation of the two was achieved. Attempts to use silver nitrate-impregnated alu-

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**Table 1.** NMR Data of Clathculins A and B (**1** and **2**) (125 and 500 MHz in CDCl<sub>3</sub>)

no.	<b>1</b>		HMBC (C>H)	<b>2</b>	
	$\delta_C$ (m) <sup>c</sup>	$\delta_H$ (m) (J in Hz)		$\delta_C$ (m)	$\delta_H$ (m) (J in Hz) <sup>b</sup>
1	57.7 t	2.45 q (7.1)	3'	57.7 t	
2	26.2 t	1.41 m	1	26.2 t	
3	27.2 t	1.17 m		27.2 t	
4	29.5 t	1.15 m		29.5 t	
5 <sup>a</sup>	29.4 t	1.20 m		29.4 t	
6 <sup>a</sup>	29.4 t	1.20 m		29.4 t	
7 <sup>a</sup>	29.3 t	1.20 m		29.3 t	
8	28.8 t	1.43 m		28.8 t	
9	19.5 t	2.25 m	8	19.5 t	
10	94.6 s		8, 9, 12	94.3 s	
11	77.2 s		8, 9, 12, 13, 14	77.0 s	
12	109.7 d	5.44 d (10.8)	13, 14	109.1 d	5.43 d (9.8)
13	142.6 d	5.81 dt (10.8, 7.0)	14, 15	141.4 d	5.80 dt (9.8, 7.0)
14	29.1 t	2.30 m		29.6 t	2.20 m
15	32.9 t	2.05 q (7.1)	17	22.5 t	1.25 m
16	137.0 d	5.84 ddt (17.1, 10.2, 7.1)	14, 15, 17	31.2 t	2.25 m
17	114.8 t	5.05 d (17.1)	15, 16	13.9 q	0.80 t (7.5)
		4.95 d (10.2)			
1'	52.9 t	2.70 t (6.8)	3', 1	52.9 t	
2'	46.0 t	2.95 t (6.8)	4'	46.0 t	
3'	41.4 q	2.25 s	1'	41.4 q	
4'	33.5 q	2.60 s	2'	33.5 q	

<sup>a</sup> Overlapping signals. <sup>b</sup> H<sub>2</sub>-1-9 and H<sub>2</sub>-1'-4' are the same in **1** and **2**. <sup>c</sup> Multiplicity inferred from DEPT experiment.

mina for the separation of **1** and **2**, or **3** and **4** were unsuccessful.

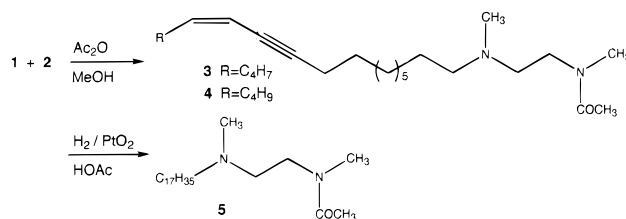
A second functionality in both **1** and **2** was an enyne group (-CH=CH-C≡C-), suggested by the carbon chemical shifts (94.6 s, 77.2 s, 109.7 d, 142.6 d and 94.3 s, 77.0 s, 109.1 d, 141.4 d ppm for **1** and **2**, respectively),<sup>10</sup> as well as by the UV absorptions (see Experimental Section), which agree well with such a system.<sup>11</sup> Differentiation between the two enyne groups of **1** and **2**, and the attached allylic and propargylic groups was achieved by a selective-SPT (INAPT) experiment.<sup>12</sup>

The difference between **1** and **2** was in the second terminus of the molecules, that is, the existence of a terminal methylene in **1** ( $\delta$  4.95 and 5.05 d and  $\delta_C$  114.8 t and 137.0 d) and an end methyl group in **2** ( $\delta$  0.80 t and  $\delta_C$  13.9 q). Furthermore, from the NMR data (Table 1), and especially from the H-H COSY and TOCSY correlations (Figure 1), it was deduced that the end =CH<sub>2</sub>(17) group of **1** and the end CH<sub>3</sub>(17) group of **2** are separated from the enyne group by two methylenes in **1** and three methylenes in **2**. This suggestion was strongly supported by an HMBC experiment (Table 1).

The two termini of clathculins A and B, established above, left a nine methylene chain to connect the diaza end with the enyne moiety. Of these nine methylenes, four, CH<sub>2</sub>(1), CH<sub>2</sub>(2), CH<sub>2</sub>(8), and CH<sub>2</sub>(9) were readily assigned on the basis of their chemical shifts, COSY, TOCSY (Figure 1), and HMBC (Table 1) correlations. The above information may be represented by structures **1** and **2**.

Hydrogenation of **1** and **2** under a variety of neutral conditions failed to give a defined saturated compound, most likely due to complexation between the catalyst and the diaminoethane moiety.<sup>13</sup> Proof of the structures of **1** and **2** was finally obtained upon hydrogenation of the mixture of compounds **3** and **4** over PtO<sub>2</sub> in acetic acid to give a single saturated derivative **5** (Scheme 1). Compound **5** is a new compound, however, lower synthetic diamino homologues with 9-14 C-atoms in the long chain are known.<sup>13</sup> As with clathculins A and B, a strong peak in the mass spectrum at *m/z* 282 was observed, due to  $\alpha$ -cleavage to both nitrogen atoms [M - CH<sub>2</sub>=N<sup>+</sup>(CH<sub>3</sub>)-COCH<sub>3</sub>, 100%].

### Scheme 1. Chemical Transformation of **1** and **2**



Sponges of the genus *Clathrina* are a well-known source of the imidazolyl alkaloid clathridine.<sup>14,15</sup> *C. coriacea*,<sup>6</sup> on the other hand, does not contain the latter heterocyclic compound but rather the coriacenins—a family of long-chain polyenes bearing, at both ends, a polar 1,3-diamino-propan-2-ol moiety.

*C. aff. reticulum* is another example of this genus containing long-chain nitrogen-containing compounds, although the clathculins (**1** and **2**) differ from the coriacenins. They possess a single unprecedented diaminoethane terminus and an enyne moiety in a C<sub>17</sub> long chain.<sup>16</sup> Thus, compounds **1** and **2** represent a new class of marine natural products.

### Experimental Section

**General Experimental Procedures.** IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. The UV spectrum was obtained on a Unikom 931 spectrophotometer. EIMS and FABMS were recorded on a Fisons Autospec Q instrument. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on Bruker AMX-360 and ARX-500 spectrometers. All chemical shifts are reported with respect to TMS ( $\delta_H = 0$ ) and CDCl<sub>3</sub> ( $\delta_C = 77.0$ ).

**Animal Material.** The small, brittle, pink sponge (TASA 53) was collected several times during the years 1995-1999 from the five-mile reef in Sodwana Bay, South Africa, using scuba at a depth of 39 m. The sponge agrees best with *Clathrina reticulum* (Schmidt, 1862) (class Calcarea, subclass Calcinea, order Clathrinida, family Clathrinidae). As the latter sponge was reported only from the Mediterranean, it is suggested that the investigated sponge is *Clathrina aff. reticulum*. A voucher sample ZMA POR 15734 is deposited at O. R. I., Durban.

**Extraction and Isolation.** The freeze-dried sponge (6 g) was extracted 3 times with a mixture of MeOH-EtOAc (1:1)

to give, after evaporation, a brown gum (220 mg) that was partitioned between aqueous methanol and hexane,  $\text{CCl}_4$ , and  $\text{CHCl}_3$ . The  $\text{CHCl}_3$  fraction (80 mg) was chromatographed twice on Sephadex LH-20 eluted with a mixture of hexane–MeOH– $\text{CHCl}_3$  (2:1:1) to give clathculins A and B (**1** and **2**) (35 mg). Additional chromatographies changed the ratio of **1/2** but did not give a single pure compound. The ratio between **1** and **2** was determined according to the  $^1\text{H}$  NMR ratio of end-methyl to vinyl protons.

**Clathculins A and B (1 and 2):** amorphous solid; IR (neat)  $\nu_{\text{max}}$  3400, 2950, 2900, 2275, 2125, 1500  $\text{cm}^{-1}$ ; UV (EtOH)  $\lambda_{\text{max}}$  234 (6300), 246 (7600), 285 (2800) nm; for  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; CIMS  $m/z$  (%) 321 ( $\text{MH}^+$  of **2**, 100), 305 ( $\text{M} - \text{CH}_3$ , 25), 276 ( $\text{C}_{17}\text{H}_{29}\overset{+}{\text{N}}(\text{CH}_3)=\text{CH}_2$ , 15), 220 ( $\text{C}_{16}\text{H}_{28}^+$ , 15). The peaks assigned to **2** are accompanied by the corresponding peaks of **1**, which are 2 mu lower than those of **2**.

**Acetylation of 1 and 2 to Acetates 3 and 4.** A mixture of **1** and **2** (10 mg) in a mixture of  $\text{Ac}_2\text{O}/\text{MeOH}$  (1:20, 5 mL) was kept at room temperature overnight. After evaporation, the residue was chromatographed on a Sephadex LH-20 column eluted with hexane–MeOH– $\text{CHCl}_3$  (2:1:1) to afford compounds **3** and **4** (10 mg): an oil; IR  $\nu_{\text{max}}$  (neat) 2950, 2910, 2275, 2120, 1700, 1400  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{C}_6\text{D}_6$ , 500 MHz) 12.0 (1H, s, NH),<sup>17</sup> 5.85 (1H, ddt,  $J = 17.1, 10.2, 6.5$ , H-16 of **3**), 3.75 (2H, m, H-2'), 3.65 (2H, m, H-1'), 3.14 (3H, s, H-4'), 2.80 and 2.78 (3H, two s, for two isomeric acetamide groups), 0.90 (3H, t,  $J = 6.5$ ,  $\text{H}_3$ -17 of **4**);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz) 171.9 s, 142.6 d, 141.5 d, 138.0 d, 114.8 t, 109.8 q, 109.3 d, 94.8 s, 94.3 s, 77.2 s, 77.0 s, 56.2 t, 52.1 t, 42.8 t, 39.9 q, 31.0 q, 21.8 q, 13.9 q; HREIMS  $m/z$  (calcd) 362.3299 ( $\text{M}^+$  of **2**,  $\text{C}_{23}\text{H}_{42}\text{N}_2\text{O}$ , 362.3297), 288.2693 ( $\text{M} - \text{C}_3\text{H}_8\text{NO}$ , 288.2691), 276.2694 ( $\text{C}_{19}\text{H}_{34}\text{N}^+$ ,  $\text{R}-\overset{+}{\text{N}}(\text{CH}_3)=\text{CH}_2$ , 276.2691). As in the natural compounds, so too are the peaks of **4** here accompanied by peaks of **3** at –2 mu.

**Hydrogenation of 3 and 4 to 5.** A mixture of **3** and **4** (5 mg) in  $\text{CH}_3\text{CO}_2\text{H}$  (1 mL) was hydrogenated over  $\text{PtO}_2$  at 3 atm at room temperature for 2 h. Filtration of the catalyst and evaporation of the solvent afforded compound **5** as an oil: IR (neat)  $\nu_{\text{max}}$  2950, 2910, 1700, 1400  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  7.95 (1H, s), 3.75 (2H, m), 3.30 (2H, m), 3.15 (3H, s), 2.95 (3H, s), 2.15 (3H, s), 1.25 (m,  $\text{CH}_2$ 's), 0.85 (3H, t);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 125 MHz)  $\delta$  176.2 s, 56.1 t, 52.8 t, 42.5 t, 39.7 q, 36.3

q, 29.2 t ( $\times 3$ ), 29.1 t ( $\times 2$ ), 29.0 t ( $\times 3$ ), 28.9 t, 28.8 t, 28.7 t, 26.1 t, 23.5 t, 22.2 t, 21.3 q, 20.9 t, 13.5 q; EIMS  $m/z$  (%) (296,  $\text{M} - \text{C}_3\text{H}_6\text{NO}$ , 20), 282 ( $\text{M} - \text{C}_4\text{H}_8\text{NO}$ , 100), 268 ( $\text{M} - \text{C}_5\text{H}_{10}\text{NO}$ , 20); HREIMS  $m/z$  296.3324 [ $\text{M}^+ - \text{C}_3\text{H}_6\text{NO}$ ] ( $\text{C}_{20}\text{H}_{42}\text{N}$  requires 296.3320).

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## References and Notes

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- (16) Enyne moieties are well-known as a terminus of many marine natural products,<sup>1</sup> but they are also found in long-chain fatty acids.<sup>9,10</sup>
- (17) A similar low-field NH chemical shift ( $\delta$  12.0) was observed for the corresponding proton of the acetamide of *N,N*-diethyl-*N*-methylethyleneamine prepared for this comparison in the same manner as **3** and **4**. This low-field peak may suggest a hydrogen bond with the second amine group.

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