Synthesis and Structure of the Marine Ascidian 8-Oxoadenine Aplidiamine

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Received May 10, 1999; accepted June 14, 1999

Alkylation of 8-oxoadenosine (13) with 4-benzyloxy-3,5-dibromobenzyl bromide (20), followed by Dimroth rearrangement and acid hydrolysis, provided *N*-(3,5-dibromo-4-hydroxybenzyl)-8-oxoadenosine (15). The 2'-deoxy version of this reaction sequence accomplished the first synthesis of *N*-(3,5-dibromo-4-hydroxybenzyl)-8-oxoadenine (16), which is the correct expression for marine ascidian purine apidiamine.

Key words apidiamine synthesis; marine metabolite; 8-oxoadenosine alkylation; Dimroth rearrangement; nucleoside hydrolysis; heteronuclear multiple bond connectivity

Although 8-oxoadenine (type **12**: R=H)¹⁾ and 8-oxoadenosine (**13**)²⁾ have long been known, the natural occurrence of neither of them has been reported. 1,9-Dimethyl-8-oxoadenine (**1**)³⁾ (isolated from the English Channel sponge *Hymeniacidon sanguinea* GRANT only in the form of the *N*⁶-acetyl derivative⁴⁾) was the first instance of naturally occurring 8-oxoadenine derivatives. Later on, biologically active caissarone hydrochloride (**2**) was isolated from the sea anemone *Bunodosoma caissarum* CORREA,⁵⁾ and the zwitterionic structure **3** was assigned to its free base.⁶⁾ Antibiotic nucleotides phosmidosines (**4**–**6**) were then isolated from the fermentation broth of *Streptomyces* sp. strain RK-16.⁷⁾ In 1997, Kang and Fenical reported the isolation of apidiamine from the marine ascidian *Aplidiopsis* sp., and they assigned the unique zwitterionic structure **7** to this newest member of a small family of naturally occurring 8-oxoadenine derivatives rather than the usual undissociated structure **16** on the basis of its heteronuclear multiple bond connectivity (HMBC) spectrum.⁸⁾ The zwitterionic structure **7** seemed to us unlikely because the acidity of the phenol moiety of **16** might be too weak to protonate the weakly basic 2-oxoimidazole moiety. We herein report the first synthesis of apidiamine and propose adopting **16** as its correct structure.⁹⁾

The synthesis of **16** followed the reaction sequence (Chart 1) established in this laboratory for the synthesis of *N*-methyl-8-oxoadenine (**12a**).¹⁰⁾ It has been reported that 1-methyl-8-oxoadenosine (**8a**), which is obtainable by methyla-

tion of 8-oxoadenosine (**13**) in excellent yield, undergoes Dimroth rearrangement to produce *N*-methyl-8-oxoadenosine (**10a**) in good yield.^{10a)} A parallel reaction sequence starting from the reaction of **13** with 4-benzyloxy-3,5-dibromobenzyl bromide (**20**) would produce **10b**, which might be a good precursor for the synthesis of the target purine **16**. The requisite benzyl bromide **20** was prepared from 3,5-dibromo-4-hydroxybenzaldehyde (**17**). The latter compound had been prepared in 93% yield by bromination of 4-hydroxybenzaldehyde with Br₂ in toluene containing BuNH₂ at –78 °C.¹¹⁾ We prepared the same compound **17** more conveniently in 96% yield by treating 4-hydroxybenzaldehyde with an excess of Br₂ in AcOH at room temperature in the presence of AcONa. Benzoylation of **17** with PhCH₂Br in the presence of NaH, followed by sequential treatment with NaBH₄ and PBr₃, afforded the bromide **20** (Chart 2). Thus, 8-oxoadenosine (**13**) was treated with **20** in AcNMe₂ at 50 °C for 111 h, and the crude product was heated in boiling 1 *N* aqueous NaOH for 1 h to afford the rearranged nucleoside **10b** in 58% overall yield. Hydrolysis of *N*-methyl-8-oxoadenosine (**10a**) takes place so slowly in aqueous HCl to yield *N*-methyl-8-oxoadenine (**12a**)^{10b)} that **10b** would produce the purine **16** through the nucleoside **15** under similar conditions. Indeed, **15**·H₂O was obtained in 52% yield by heating **10b** in 1 *N* aqueous HCl for 1 h. More drastic conditions were necessary for removal of the sugar moiety of **15**. However, hydrolysis of the glycosyl bond of **15** was accompanied by *N*-debenzylation to provide mainly 8-oxoadenine (type **12**: R=H), when **10b** was heated in boiling 2 *N* aqueous HCl for 48 h according to the procedure employed for hydrolysis of **10a**.^{10b)}

It is well-known that 2-deoxyribofuranosides undergo hydrolysis much more easily than the corresponding ribofuranosides.¹²⁾ Accordingly, selective cleavage of the glycosyl linkage would be possible for the 2'-deoxy analogue **11b**. This compound was obtained from 2'-deoxy-8-oxoadenosine (**14**)¹³⁾ in 74% overall yield by repeating the reaction sequence similar to that described above for the synthesis of **10b**. Hydrolysis of **11b** indeed proceeded selectively at the glycosyl bond, producing *N*-(4-benzyloxy-3,5-dibromobenzyl)-8-oxoadenine (**12b**) as the hemihydrate in 90% yield after treatment of **11b** with boiling 1 *N* aqueous HCl for 10 min. *O*-Debenzylation of **12b** was attained for the duration of the reaction for a further 50 min, providing the target compound **16** as the monohydrate in 78% yield.

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Chart 1

Chart 2

The ^1H - and ^{13}C -NMR spectra of this sample were identical with those⁸⁾ reported for aplidiamine, confirming the correctness of the gross structure assigned to this marine metabolite. However, the ^1H -NMR pattern arising from the purine moiety of this compound (δ 8.05, 9.83, 11.34) presents an almost complete similarity to that of the *O*-benzyl derivative **12b** (δ 8.06, 9.86, 11.37), supporting the correctness of the undissociated structure **16** rather than the zwitterion **7**. The structure **16** was finally identified by our own HMBC experiment using an analytical sample of synthetic aplidiamine: marked ^1H - ^{13}C long-range connectivity was observed between C(3',5') at δ 111.8 and a hydrogen (OH) at δ 9.87; another set observed was correlations between C(8) at δ 152.5 and two hydrogens [N(7)- and N(9)-H] at δ 9.83 and 11.34.

Experimental

General Notes All melting points were determined using a Yamato MP-1 or Büchi model 530 capillary melting point apparatus and values are corrected. Spectra reported herein were recorded on a JEOL JMS-SX102A mass spectrometer, a Hitachi model 320 UV spectrophotometer [for solutions in 95% aqueous EtOH, 0.1 N HCl in 90% (v/v) aqueous EtOH, 90% (v/v) aqueous EtOH, 0.1 N NaOH in 90% (v/v) aqueous EtOH, 0.1 N aqueous HCl (pH 1), 0.005 M phosphate buffer (pH 7), and 0.1 N aqueous NaOH (pH 13)], a Shimadzu FTIR-8100 IR spectrophotometer, a JEOL JNM-EX-270 or a JEOL JNM-GSX-500 NMR spectrometer (measured at 25°C with Me_4Si as an internal standard). Elemental analyses and MS measurements were performed by Dr. M. Takani and her associates at Kanazawa University. Flash chromatography was performed according to the reported procedure.¹⁴⁾

The following abbreviations are used: br=broad, d=doublet, dd=doublet-of-doublets, ddd=doublet-of-doublets-of-doublets, dddd=doublet-of-doublets-of-doublets-of-doublets, m=multiplet, s=singlet, sh=shoulder, t=triplet.

3,5-Dibromo-4-hydroxybenzaldehyde (17) A solution of Br_2 (30.2 g, 190 mmol) in AcOH (50 ml) was added dropwise to a stirred mixture of 4-hydroxybenzaldehyde (11.0 g, 90 mmol), AcONa (22.9 g, 279 mmol), and AcOH (150 ml) at room temperature over a period of 15 min. The mixture was stirred at room temperature for a further 1 h and concentrated *in vacuo*. The residue was triturated with H_2O (200 ml), and the insoluble solid was collected by filtration, washed with H_2O (3×30 ml), and dried to give **17** (24.3 g, 96%), mp 181–183°C. Recrystallization of this product from EtOH– H_2O (7:3, v/v) afforded an analytical sample of **17** as colorless needles, mp 182–184°C. MS m/z : 277, 279, 281 (M^+). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm^{-1} : 3153 (OH), 1673 (C=O). ^1H -NMR (CDCl_3) δ : 6.43 (1H, brs, OH), 8.00 (2H, s, $\text{Br}_2\text{C}_6\text{H}_2$), 9.80 (1H, s, CHO). Anal. Calcd for $\text{C}_7\text{H}_4\text{Br}_2\text{O}_2$: C, 30.04; H, 1.44. Found: C, 30.12; H, 1.32.

4-Benzyloxy-3,5-dibromobenzaldehyde (18) NaH (of 60% purity) (1.00 g, 25.1 mmol) was washed with hexane (2×50 ml) and suspended in HCONMe_2 (100 ml). Compound **17** (6.38 g, 22.8 mmol) was added to this suspension to give a slightly yellow solution. A solution of PhCH_2Br (4.30 g, 25.1 mmol) in HCONMe_2 (30 ml) was then added to the solution, and the mixture was stirred at room temperature for 24 h. The resulting solution was concentrated *in vacuo*, and the residue was partitioned between 10% aqueous Na_2CO_3 (80 ml) and CHCl_3 (80 ml). The aqueous layer was extracted with CHCl_3 (80 ml). The CHCl_3 layers were combined, washed successively with 10% aqueous Na_2CO_3 (5×40 ml), 10% aqueous NaOH (2×40 ml), and saturated aqueous NaCl (40 ml), dried (MgSO_4), and concentrated *in vacuo*. The residual solid was recrystallized from EtOH– H_2O (7:3, v/v) to give **18** (2.92 g), mp 73–75°C. The mother liquor was concentrated *in vacuo*, and the residue was recrystallized from EtOH– H_2O (7:3, v/v) to afford a second crop of **18** (1.99 g), mp 73–75°C. A third crop of **18** (0.89 g, the total yield

was 69%) (mp 73–75 °C) was obtained by treatment of the mother liquor of the second recrystallization in a manner similar to that described for obtaining the second crop of **18**. Further recrystallization of crude **18** from EtOH–H₂O (7:3, v/v) provided an analytical sample of **18** as colorless needles, mp 78–79 °C. MS *m/z*: 368, 370, 372 (*M*⁺). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 1694 (C=O). ¹H-NMR (CDCl₃) δ : 5.12 (2H, s, PhCH₂), 7.35–7.70 (5H, m, PhCH₂), 8.06 (2H, s, Br₂C₆H₂), 9.88 (1H, s, CHO). *Anal.* Calcd for C₁₄H₁₀Br₂O₂: C, 45.44; H, 2.72. Found: C, 45.57; H, 2.81.

4-Benzoyloxy-3,5-dibromobenzyl Alcohol (19) NaBH₄ (115 mg, 3.04 mmol) was added to a solution of **18** (746 mg, 2.02 mmol) in MeOH (10 ml), and the mixture was stirred at room temperature for 30 min. It was concentrated *in vacuo* after addition of acetone (1 ml). The residue was mixed with H₂O (20 ml), and the mixture was neutralized with 10% aqueous H₃PO₄ and extracted with CHCl₃ (4×20 ml). The CHCl₃ layers were combined, dried (MgSO₄), and concentrated *in vacuo* to leave **19** (736 mg, 98%), mp 95.5–96 °C. Recrystallization of this product from hexane afforded an analytical sample of **19** as colorless needles, mp 96–97 °C. MS *m/z*: 370, 372, 374 (*M*⁺). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 3293 (OH). ¹H-NMR (CDCl₃) δ : 1.76 (1H, brt, *J*=5 Hz, CH₂OH), 4.65 (2H, d, *J*=5 Hz, CH₂OH), 5.03 (2H, s, PhCH₂), 7.35–7.63 (5H, m, PhCH₂), 7.56 (2H, s, Br₂C₆H₂). *Anal.* Calcd for C₁₄H₁₂Br₂O₂: C, 45.20; H, 3.25. Found: C, 45.07; H, 3.27.

N-(4-Benzoyloxy-3,5-dibromobenzyl)-8-oxoadenosine (10b) A solution of PBr₃ (870 mg, 3.21 mmol) in anhydrous Et₂O (3 ml) was added dropwise to a solution of **19** (2.00 g, 5.38 mmol) in Et₂O (25 ml) at 0 °C over a period of 5 min, and the mixture was stirred at 0 °C for a further 1 h. The resulting solution was diluted with Et₂O (25 ml), washed successively with saturated aqueous NaHCO₃ (2×30 ml), dried (MgSO₄) with stirring at room temperature for 1 h, and concentrated *in vacuo* to leave crude **20** (1.75 g) as a colorless oil.

A mixture of **13**^(10a) (205 mg, 0.724 mmol), **20** (410 mg, 0.943 mmol), and AcNMe₂ (6 ml) was stirred at 50 °C for 111 h and concentrated *in vacuo*. The residue was washed with Et₂O (30 ml) and heated in 1 N aqueous NaOH (5 ml) under reflux for 1 h. The solution was neutralized with 10% aqueous H₃PO₄, extracted with AcOEt (20 ml and then 3×10 ml). The organic layers were combined, dried (MgSO₄), and concentrated *in vacuo* to leave a colorless glass (368 mg). This was subjected to flash chromatography [AcOEt–EtOH (10:1, v/v)] to afford **10b** (267 mg, 58%) as a colorless glass. ¹H-NMR [(CD₃)₂SO] δ : 3.46 (m), 3.61 (ddd, *J*=12, 4, 4 Hz) [1H each, C(5')–H₂], 3.87 [1H, m, C(4')–H], 4.14 [1H, m, C(3')–H], 4.67 (2H, d, *J*=5.6 Hz, NHCH₂), 4.88 [1H, ddd, *J*=6 Hz each, C(2')–H], 4.96 (2H, s, PhCH₂), 5.05 [1H, d, *J*=5 Hz, C(3')–OH], 5.09 [1H, dd, *J*=4, 8 Hz, C(5')–OH], 5.23 [1H, d, *J*=6 Hz, C(2')–OH], 5.69 [1H, d, *J*=6.5 Hz, C(1')–H], 7.12 (1H, brt, *J*=5.6 Hz, NHCH₂), 7.24–7.51 (5H, m, PhCH₂), 7.69 (2H, s, Br₂C₆H₂), 8.14 [1H, s, C(2)–H], 10.32 [1H, brs, N(7)–H].

N-(3,5-Dibromo-4-hydroxybenzyl)-8-oxoadenosine Monohydrate (15·H₂O) A suspension of **10b** (100 mg, 0.157 mmol) in 1 N aqueous HCl (100 ml) was heated under reflux for 1 h. The resulting solution was neutralized with 8 N aqueous NaOH (12.5 ml), brought to pH 5 with 10% aqueous H₃PO₄, and kept at 4 °C overnight. The precipitate that resulted was collected by filtration, washed with H₂O (3 ml), and dried to give crude **15**·H₂O (46 mg, 52%), mp 164–168 °C (dec.). This was recrystallized from H₂O after purification by preparative TLC [silica gel, CHCl₃–MeOH (6:1, v/v)], dried (P₂O₅) at 2 mmHg and 50 °C for 15 h, and exposed to air until a constant weight was reached to afford an analytical sample of **15**·H₂O as colorless needles, mp 167.5–169 °C (dec.). [α]_D²⁵ –34° (*c*=0.248, MeOH). UV $\lambda_{\text{max}}^{95\% \text{ EtOH}}$ 279 nm (ϵ 23000); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 1) 284 (18200); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 7) 211 (49900), 277 (22400), 304 (sh) (4400); $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ (pH 13) 289 (24100). IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 1734, 1717 (C=O). ¹H-NMR [(CD₃)₂SO] δ : 3.47 (ddd, *J*=12.2, 4.9, 8.3 Hz), 3.61 (ddd, *J*=12.2, 3.9, 3.9 Hz) [1H each, C(5')–H₂], 3.86 [1H, ddd, *J*=4.9, 3.9, 2.9 Hz, C(4')–H], 4.14 [1H, ddd, *J*=2.9, 5.4, 4.9 Hz, C(3')–H], 4.58 (2H, d, *J*=5.9 Hz, CH₂NH), 4.87 [1H, ddd, *J*=5.4, 6.4, 6.4 Hz, C(2')–H], 5.06 [1H, d, *J*=4.9 Hz, C(3')–OH], 5.11 [1H, dd, *J*=8.3, 3.9 Hz, C(5')–OH], 5.23 [1H, d, *J*=6.4 Hz, C(2')–OH], 5.69 [1H, d, *J*=6.4 Hz, C(1')–H], 7.03 (1H, t, *J*=5.9 Hz, CH₂NH), 7.54 (2H, s, Br₂C₆H₂), 8.13 [1H, s, C(2)–H], 9.88 (1H, brs, Br₂C₆H₂OH), 10.31 [1H, brs, N(7)–H]. *Anal.* Calcd for C₁₇H₁₇Br₂N₅O₆·H₂O: C, 36.13; H, 3.39; N, 12.39. Found: C, 36.31; H, 3.17; N, 12.28.

N-(4-Benzoyloxy-3,5-dibromobenzyl)-2'-deoxy-8-oxoadenosine (11b) A mixture of 2'-deoxy-8-oxoadenosine (**14**)⁽¹³⁾ (338 mg, 1.26 mmol), **20** (704 mg, 1.62 mmol), and AcNMe₂ (8 ml) was stirred at 50 °C for 141 h. The resulting slightly yellow solution was concentrated *in vacuo*, and the residue was washed with Et₂O (2×15 ml). Crude **9b** thus obtained was heated under reflux in 1 N aqueous NaOH (10 ml) for 1 h. The solution was neutralized with 10% aqueous H₃PO₄ and extracted with AcOEt (20 ml). The organic

layer was dried (MgSO₄) and concentrated *in vacuo* to leave a slightly yellow foam (734 mg). This was subjected to flash chromatography [AcOEt–EtOH (10:1, v/v)] to afford **11b** (584 mg, 74%) as a colorless foam. ¹H-NMR [(CD₃)₂SO] δ : 2.01 (ddd, *J*=13, 2.4, 6.8 Hz), 2.99 (ddd, *J*=13, 5.8, 8.3 Hz) [1H each, C(2')–H₂], 3.46 (ddd, *J*=12, 4.4, 7.8 Hz), 3.61 (ddd, *J*=12, 4.4, 4.4 Hz) [1H each, C(5')–H₂], 3.81 [1H, ddd, *J*=2.4, 4.4, 4.4 Hz, C(4')–H], 4.39 [1H, dddd, *J*=2.4, 5.8, 2.4, 4.4 Hz, C(3')–H], 4.66 (2H, d, *J*=5.9 Hz, NHCH₂), 4.96 (2H, s, PhCH₂), 5.07 [1H, dd, *J*=7.8, 4.4 Hz, C(5')–OH], 5.20 [1H, d, *J*=4.4 Hz, C(3')–OH], 6.16 [1H, dd, *J*=6.8, 8.3 Hz, C(1')–H], 7.10 (1H, brt, *J*=5.9 Hz, NHCH₂), 7.37–7.46 (3H), 7.53–7.59 (2H) (m each, PhCH₂), 7.69 (2H, s, Br₂C₆H₂), 8.13 [1H, s, C(2)–H], 10.29 [1H, brs, N(7)–H].

N-(4-Benzoyloxy-3,5-dibromobenzyl)-8-oxoadenosine Hemihydrate (12b·1/2H₂O) A suspension of **11b** (228 mg, 0.367 mmol) in 1 N aqueous HCl (30 ml) was heated under reflux for 10 min. The resulting suspension was cooled and neutralized with 8 N aqueous NaOH (3.75 ml). The insoluble solid was collected by filtration, washed with H₂O (5 ml), and dried to give crude **12b**·1/2H₂O (170 mg, 90%), mp 234–235 °C (dec.). This was recrystallized from EtOH–H₂O (9:1, v/v) and dried (P₂O₅) at 2 mmHg and 80 °C for 20 h to afford an analytical sample of **12b**·1/2H₂O as colorless needles, mp 250–251 °C (dec.). MS *m/z*: 503, 505, 507 (*M*⁺). UV $\lambda_{\text{max}}^{90\% \text{ EtOH}}$ (0.1 N HCl) 282 nm (ϵ 17100); $\lambda_{\text{max}}^{90\% \text{ EtOH}}$ 277 (22000); $\lambda_{\text{max}}^{90\% \text{ EtOH}}$ (0.1 N NaOH) 286 (ca. 21000). ¹⁵N IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 1732, 1717 (C=O). ¹H-NMR [(CD₃)₂SO] δ : 4.64 (2H, d, *J*=5.9 Hz, CH₂NH), 4.96 (2H, s, PhCH₂), 6.94 (1H, t, *J*=5.9 Hz, CH₂NH), 7.36–7.59 (5H, m, PhCH₂), 7.69 (2H, s, Br₂C₆H₂), 8.06 [1H, s, C(2)–H], 9.86 [1H, brs, N(7)–H], 11.37 [1H, brs, N(9)–H]. *Anal.* Calcd for C₁₉H₁₃Br₂N₅O₂·1/2H₂O: C, 44.38; H, 3.14; N, 13.62. Found: C, 44.59; H, 3.06; N, 13.56.

N-(3,5-Dibromo-4-hydroxybenzyl)-8-oxoadenosine (Aplidiamine) Monohydrate (16·H₂O) A solution of **11b** (240 mg, 0.386 mmol) in 1 N aqueous HCl (600 ml) was heated under reflux for 1 h and cooled to 40 °C. A small amount of **12b**·1/2H₂O (5 mg), which remained undissolved, was filtered off at this temperature. The filtrate was neutralized by addition of 8 N aqueous NaOH (75 ml), and the mixture was adjusted to pH 5 with 10% aqueous H₃PO₄. After cooling the mixture at 0 °C overnight, the precipitate that deposited was collected by filtration, washed with H₂O (20 ml), and dried to give **16**·H₂O (130 mg, 78%), mp 231–232 °C (dec.). Recrystallization of this sample from EtOH–H₂O (7:3, v/v), followed by drying (P₂O₅) at 2 mmHg and 50 °C for 24 h and exposure to air at room temperature until a constant weight was reached, afforded an analytical sample of **16**·H₂O, mp 239–239.5 °C (dec.). MS *m/z*: 413, 415, 417 (*M*⁺). UV $\lambda_{\text{max}}^{90\% \text{ EtOH}}$ (0.1 N HCl) 284 nm (ϵ 19000); $\lambda_{\text{max}}^{90\% \text{ EtOH}}$ 277 (22400); $\lambda_{\text{max}}^{90\% \text{ EtOH}}$ (0.1 N NaOH) 249 (sh) (ca. 13000), ¹⁵N 288 (ca. 27000), ¹⁵N 310 (sh) (ca. 7000). ¹⁵N IR $\nu_{\text{max}}^{\text{Nujol}}$ cm⁻¹: 1717 (C=O). ¹H-NMR [(CD₃)₂SO] δ : 4.55 (2H, d, *J*=5.9 Hz, CH₂NH), 6.84 (1H, t, *J*=5.9 Hz, CH₂NH), 7.53 (2H, s, Br₂C₆H₂), 8.05 [1H, s, C(2)–H], 9.83 [1H, brs, N(7)–H], 9.87 [1H, brs, Br₂C₆H₂OH], 11.34 [1H, brs, N(9)–H]. ¹³C-NMR [(CD₃)₂SO] δ : 41.7 (CH₂NH), 104.5 [C(5)], 111.8 [C(3',5')], 131.2 [C(2',6')], 134.0 [C(1')], 145.1 [C(6)], 147.4 [C(4)], 149.5 [C(4')], 150.7 [C(2)], 152.5 [C(8)]. *Anal.* Calcd for C₁₅H₉Br₂N₅O₂·H₂O: C, 33.28; H, 2.56; N, 16.17. Found: C, 33.36; H, 2.55; N, 16.29.

Acknowledgment This work was supported by the Ministry of Education, Science, Sports and Culture, Japan, under a Grant-in-Aid for Encouragement of Young Scientists (No. 10771244 to T. K.). We thank Professor W. Fenical (University of California, San Diego) for providing us with spectral copies of natural aplidiamine.

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