# Agelasines H and I, 9-Methyladenine-Containing Diterpenoids from an *Agelas* Sponge

Xiong Fu,[†] Francis J. Schmitz,*[,†] Ralph S. Tanner,[‡] and Michelle Kelly-Borges[§]

*Department of Chemistry and Biochemistry and Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma 73019, and Department of Civil and Environmental Engineering, Faculty of Health, Science and Technology, UNITEC Institute of Technology, Private Bag 92025, Auckland, New Zealand*

*Received October 1, 1997*

Two new diterpenes possessing a 9-methyladeninium substituent, agelasines H (**5**) and I (**6**), along with five related known ones, **1**−**4** and **7**, were isolated from a sponge, *Agelas* sp. The structures of the new compounds were determined from spectral data. Compounds **2**−**4**, **6**, and **7** exhibited varying degrees of antimicrobial activity.

A group of diterpenes possessing a 9-methyladeninium substituent has been isolated from marine sponges of the genus *Agelas*.[1−6] Included among these are agelasine,[1] agelasines A−G,[2−6] and agelines A and B.[5] These novel metabolites have shown interesting biological activities including cytotoxicity,[5,6] antimicrobial activity,[5] and inhibition of Na, K-ATPase.[2,3] Two similar compounds, agelasimines A and B, which were isolated from the sponge *Agelas mauritiana*,[7] exhibited cytotoxicity against L-1210 cells, inhibition of adenosine transfer into rabbit erythrocytes, $Ca^{2+}$-channel antagonistic action, and $\alpha_1$ adrenergic blockade. In the course of our survey of bioactive substances from marine sources,[8] we investigated extracts of an *Agelas* sp. that exhibited some antimicrobial activity. This led to the isolation of two new diterpenes, **5** and **6**, containing the 9-methyladeninium moiety together with five known, related compounds: agelasines A (**1**), B (**2**), and F (**3**), ageline B (**4**), and agelasidine A (**7**) (Chart 1). Here we describe the isolation and structure determination of the new compounds.

The sponge was collected at Yap Island, Federated States of Micronesia, and kept frozen until workup. The combined MeOH and MeOH−$CH_2Cl_2$ (1:1) extracts were subjected to solvent partitioning[9] to give hexane, $CH_2Cl_2$, *n*-BuOH, and $H_2O$ solubles. Both $CH_2Cl_2$ and *n*-BuOH solubles showed antifungal activity against *Aspergillus niger* at 1 mg/mL and were therefore fractionated over Si gel and C-18 open columns, respectively. Selective fractions from each column were resolved by reversed-phase HPLC to yield compounds **1**−**7**.

The known compounds, agelasines A (**1**), B (**2**), and F (**3**) and agelasidine A (**7**) were identified by comparison of their FABMS, $^1$H NMR, and $^{13}$C NMR data with literature values.[2] The structure of ageline B (**4**) was determined[5] by interpretation of spectral data of a product obtained from partial hydrolysis because the natural product could not be obtained in pure form. Because we were able to obtain pure ageline B (**4**), its spectral data are reported here.

* To whom correspondence should be addressed. Tel.: (405) 325-5581. Fax: (405) 325-6111. E-mail: fjschmitz@chemdept.chem.ou.edu.
[†] Department of Chemistry and Biochemistry.
[‡] Department of Botany and Microbiology.
[§] UNITEC Institute of Technology.

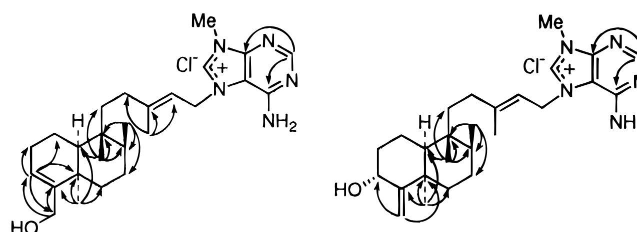


**Figure 1.** Prominent HMBC correlations for agelasines H (**5**) and I (**6**).

Agelasine H (**5**) was obtained as an amorphous solid. Its molecular formula, established by HRFABMS to be $C_{26}H_{39}N_5O$, differs from that of ageline B ($C_{31}H_{42}N_6O_2$) by the elements $C_5H_3NO$. Comparison of $^1$H and $^{13}$C NMR spectra of agelasine H (**5**) with those of ageline B (**4**) revealed that the former lacks signals for the pyrrole-2-carboxylic acid unit but that the rest of the signals for the two compounds are very similar. Therefore, structure **5** was assigned to agelasine H, and this was substantiated by HMBC data (Figure 1).

Agelasine I (**6**), obtained as an amorphous solid, has a molecular formula of $C_{26}H_{39}N_5O$ on the basis of a FABMS ion at $m/z$ 438 $[M]^+$ and $^{13}$C NMR data (Table 1). This formula is the same as found for agelasine H, suggesting that they are isomers. Comparison of the NMR data for **6** with those of **5** indicated that significant changes were apparent only in the substituted decalin portion of the molecule. The presence of an exocyclic methylene group and an oxymethine in **6** was indicated by NMR data [$\delta_H$ 4.86 (br s, H-18), 4.93 (br s, H-18), and 4.20 (br s, $W_{1/2}$ = 9.6 Hz, H-3) ppm; $\delta_C$ 110.0 (t, C-18), 154.9 (s, C-4), and 71.2 (d, C-3) ppm]. HMBC data (Figure 1) confirmed most of the connections predicted by spectral comparisons, and hence agelasine I was assigned structure **6**. The relative configuration of the 3-OH was deduced from the solvent-induced shift[10] of the Me-19 signal in pyridine-$d_5$ ($\delta$ 1.60) vs DMSO-$d_6$ ($\delta$ 1.27). This large downfield shift ($\Delta\delta$ −0.33 ppm) indicated that the 3-OH must be 1,3-diaxial to Me-19. Thus, the 3$\alpha$-OH configuration was assigned.

The relative stereochemistry of agelasines H (**5**) and I (**6**) is presumed to be the same as in ageline B (**4**) based on comparison of NMR data. The absolute configurations of all the compounds are assumed to be the same because all exhibit negative optical rotations.

S0163-3864(97)00454-0 CCC: $15.00 © 1998 American Chemical Society and American Society of Pharmacognosy
Published on Web 04/03/1998

**Chart 1**

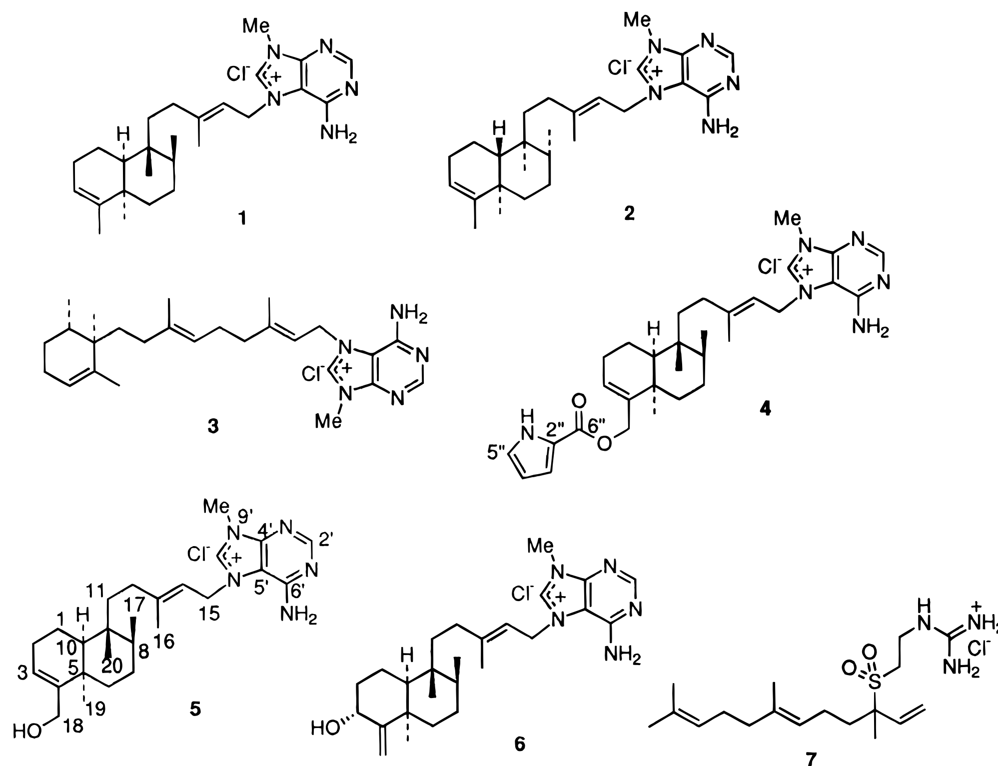

**Table 1.** $^{13}$C NMR Data for Ageline B (**4**) and Agelasines H (**5**) and I (**6**)[a,b]

| position (mult) | ageline B (4) | agelasine H (5) | agelasine I (6) |
|---|---|---|---|
| 1 (t) | 16.8 | 17.0 | 16.8 |
| 2 (t) | 23.3 | 23.1 | 30.5 |
| 3 (d) | 127.7 | 121.8 | 71.2 |
| 4 (s) | 138.3 | 143.0 | 154.9 |
| 5 (s) | 35.7 | 35.7 | 38.1 |
| 6 (t) | 36.6 | 36.2 | 38.5 |
| 7 (t) | 28.4 | 28.6 | 26.9 |
| 8 (d) | 36.8 | 36.8 | 37.7 |
| 9 (s) | 39.2[c] | 39.2[c] | 39.5[c] |
| 10 (d) | 44.5 | 44.6 | 45.9 |
| 11 (t) | 35.5 | 35.5 | 35.7 |
| 12 (t) | 32.3 | 32.3 | 32.1 |
| 13 (s) | 146.0 | 146.3 | 146.0 |
| 14 (d) | 115.2 | 114.9 | 114.8 |
| 15 (t) | 47.1 | 47.0 | 46.9 |
| 16 (q) | 16.9 | 16.9 | 16.6 |
| 17 (q) | 15.8 | 15.9 | 15.6 |
| 18 (t) | 64.8 | 61.9 | 110.0 |
| 19 (q) | 34.2 | 34.3 | 34.4 |
| 20 (q) | 17.1 | 17.1 | 18.7 |
| 2′ (d) | 155.4 | 155.4 | 155.2 |
| 4′ (s) | 149.0 | 148.9 | 148.8 |
| 5′ (s) | 109.2 | 109.2 | 109.1 |
| 6′ (s) | 152.4 | 152.4 | 152.2 |
| 8′ (d) | 141.3 | 141.0 | 140.9 |
| 9′-N-Me (q) | 31.4 | 31.4 | 31.3 |
| 2″ (s) | 121.9 | | |
| 3″ (d) | 114.8 | | |
| 4″ (d) | 109.4 | | |
| 5″ (d) | 124.2 | | |
| 6″ (s) | 160.1 | | |

[a] Spectra recorded at 125 MHz in DMSO-$d_6$, referenced to the central solvent peak at $\delta$ 39.5 ppm. [b] Multiplicities were determined by DEPT experiments and assignments made by HMQC and HMBC experiments. [c] Overlapping with solvent peak, however, identified by long-range coupling between this carbon and H-17 and H-20.

Purified compounds were tested against *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, *Asperigillus niger* ATCC 9642, and *Saccharomyces cerevisiae* ATCC 18824 at concentrations of 200, 100, 10, and 1 μg/mL in a minimum inhibitory concentration (MIC) assay conducted in triplicate.[11] Agelasine A (**1**) and agelasine H (**5**) were inactive in all the assays. Agelasine B (**2**) and ageline B (**4**) inhibited *S. cerevisiae* at 10 μg/mL, but did not inhibit the other test organisms at 200 μg/mL. Agelasine F (**3**) inhibited *A. niger* at 100 μg/mL and *S. cerevisiae* at 10 μg/mL. Agelasine I (**6**) inhibited *S. cerevisiae* at 200 μg/mL but was otherwise inactive. Agelasidine A (**7**) was tested in a mixture with ageline B (**4**), and this mixture showed inhibitory activity against *A. niger* at about 40 μg/mL.

## Experimental Section

**General Experimental Procedures.** All solvents were redistilled. Merck Si gel 60 (230–240 mesh) and Whatman LRP-2 C-18 material were used for vacuum flash chromatography. HPLC was conducted using a UV detector and a Spherex 5 C-18 column. IR spectra were obtained on a Bio-Rad 3240-SPC FT instrument; UV spectra, on a Hewlett-Packard spectrophotometer. NMR experiments were conducted with a Varian VXR-500 instrument; signals are reported in parts per million ($\delta$), referenced to the solvent used. FABMS were measured on VG ZAB-E mass spectrometer; and optical rotations, on a Rudolph Autopol III Automatic Polarimeter.

**Animal Material.** The sponge was collected in August 1995, at Yap Island, Federated States of Micronesia, and frozen shortly after collection. The sponge forms an elongate branch, 4–8 cm long and 3–5 cm thick, attached to the substrate at various points. Oscules are 2–3 mm in diameter and are scattered irregularly, each within a wrinkled cavity on a smooth surface. Color in life is reddish-orange, the texture

tough and elastic. The sample was collected from a depth of 15−20 m. The specimen is an undescribed species of *Agelas* (order Agelasida, family Agelasidae), and a voucher specimen has been deposited at The Natural History Museum, London (BMNH 1997.6.20.7) and The University of Oklahoma (38-YA-95).

**Extraction and Isolation.** Thawed specimens (300 g wet wt) were minced and extracted twice with MeOH overnight, and then twice with MeOH−$CH_2Cl_2$ (1:1) overnight. All extracts were combined after removal of solvents and subjected to solvent partitioning as described previously.[9] This led to three organic-soluble fractions: hexane (0.83 g), $CH_2Cl_2$ (2.78 g), and *n*-BuOH (1.01 g). Both $CH_2Cl_2$ and *n*-BuOH solubles showed antifungal activity against *A. niger* (MIC 1 mg/mL). Fractionation of the $CH_2Cl_2$ fraction by flash vacuum chromatography over $SiO_2$ using a MeOH−$CH_2Cl_2$ step gradient (5% to 50%) as eluent yielded several fractions. A portion (1/3) of the fractions (10% to 20% MeOH in $CH_2Cl_2$), which contained compounds **1**−**4** was resolved by C-18 reversed-phase HPLC using MeOH−$H_2O$ (8:2) containing 0.08 M $HCOONH_4$ (pH adjusted to approximately 4 by HCOOH), with the elution order being ageline B (**4**) (29 mg, 0.029% of the wet wt of sponge), agelasine A (**1**) (6.8 mg, 0.0068%), agelasine B (**2**) (43 mg, 0.043%), and ageline A (**3**) (42 mg, 0.042%). The compounds were recovered by concentration under reduced pressure to remove MeOH, followed by repeated lyophilization to remove the $H_2O$ and buffer salts.

The *n*-BuOH fraction was chromatographed on a C-18 open column and eluted with 30% to 20% $H_2O$ in MeOH containing 0.08 M $HCOONH_4$ to furnish five fractions. The third and fourth fraction therefrom contained crude agelasines H (**5**) (4.2 mg, 0.0014%) and I (**6**) (3.6 mg, 0.0012%), respectively, and each fraction was ultimately purified by reversed-phase HPLC using 35% $H_2O$−MeOH containing 0.08 M $HCOONH_4$ as eluent. The fifth fraction from the C-18 open column contained agelasidine A (**7**) and ageline B (**4**), in a ratio of 2:3, but was not further purified. Agelasidine A also appeared in the less polar chromatographic fractions of $CH_2Cl_2$ solubles.

**Agelasine A (1)**: amorphous solid; $[\alpha]_D$ −29.8° (*c* 0.22, MeOH) (lit.[2] $[\alpha]_D$ −31.3°); FABMS *m/z* $[M]^+$ 422.

**Agelasine B (2)**: amorphous solid; $[\alpha]_D$ −23.5° (*c* 1.53, MeOH) (lit.[2] $[\alpha]_D$ −21.5°); FABMS *m/z* $[M]^+$ 422.

**Ageline A (=Agelasine F) (3)**: amorphous solid; $[\alpha]_D$ −6.8° (*c* 0.86, MeOH) (lit.[2] $[\alpha]_D$ −5.5°); FABMS *m/z* $[M]^+$ 422.

**Ageline B (4)**: amorphous solid; $[\alpha]_D$ −11.6° (*c* 2.5, MeOH); IR (neat) $\nu_{max}$ 3340, 3170, 1690, 1650, 1610, 1590, 1450, 1405, 1310 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ 268 ($\epsilon$ 8320) nm; $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 0.75 (3H, d, *J* = 7.5 Hz), 0.80 (3H, s), 1.15 (3H, s), 1.88 (3H, s), 4.08 (3H, s), 4.74 (1H, d, *J* = 15.5 Hz), 4.79 (1H, d, *J* = 15.5 Hz), 5.40 (2H, br d, *J* = 8.5 Hz), 5.46 (1H, m), 5.75 (1H, br s), 6.26 (1H, m), 6.90 (1H, m), 6.96 (1H, m), 8.36 (2H, br s), 8.52 (1H, s), 9.16 (1H, br), 10.48 (1H, br) ppm; $^1H$ NMR (DMSO-$d_6$, 500 MHz), $\delta$ 0.74 (3H, d, *J* = 6.5 Hz, H-17), 0.75 (3H, s, H-20), 1.09 (3H, s, H-19), 1.79 (3H, s, H-16), 3.87 (3H, s, N-9′-Me), 4.71 (2H, br s, H-18), 5.18 (2H, m, H-15), 5.45 (1H, m, H-14), 5.67 (1H, br s, H-3), 6.73 (1H, m, H-3″), 6.14 (1H, m, H-4″), 7.00 (1H, m, H-5″), 7.99 (2H, br, $NH_2$), 8.41 (1H, s, H-2′), 8.51 (1H, br, NH), 9.62 (1H, br, H-8′) ppm; $^{13}C$ NMR, see Table 1; HRFABMS *m/z* $[M]^+$ 531.3447 (calcd for $C_{31}H_{43}N_6O_2$ 531.3448).

**Agelasine H (5)**: amorphous solid; $[\alpha]_D$ −63.9° (*c* 0.36, MeOH); IR (neat) $\nu_{max}$ 3400, 3300, 3140, 1640, 1610, 1590, 1460, 1355 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ 272 ($\epsilon$ 9015) nm; $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$ 0.72 (3H, d, *J* = 6.7 Hz, H-17), 0.74 (3H, s, H-20), 1.03 (3H, s, H-19), 1.78 (3H, s, H-16), 2.02 (1H, m, H-2), 2.08 (1H, m, H-2), 3.86 (3H, s, N-9′-Me), 3.88 (1H, d, *J* = 13.7 Hz, H-18), 3.97 (1H, d, *J* = 13.7 Hz, H-18), 5.16 (2H, m, H-15), 5.44 (1H, m, H-14), 5.49 (1H, br s, H-3), 7.97 (2H, br, $NH_2$), 8.44 (1H, s, H-2′), 9.58 (1H, br, H-8′) ppm; $^{13}C$ NMR, see Table 1; HRFABMS *m/z* $[M]^+$; 438.3252 (calcd for $C_{26}H_{40}N_5O$ 438.3233).

**Agelasine I (6)**: amorphous solid; $[\alpha]_D$ −2.5° (*c* 0.20, MeOH); IR (neat) $\nu_{max}$ 3400, 3300, 3180, 1640, 1606, 1590, 1460, 1357; UV (MeOH) $\lambda_{max}$ 272 ($\epsilon$ 8230) nm; $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$ 0.74 (3H, d, *J* = 7.5 Hz, H-17), 0.73 (3H, s, H-20), 1.27 (3H, s, H-19), 1.58 (1H, m, H-2), 1.76 (3H, s, H-16), 1.93 (1H, m, H-2), 3.88 (3H, s, N-9′-Me), 4.20 (1H, br s, H-3), 4.86 (1H, br s, H-18), 4.93 (1H, br s, H-18), 5.18 (2H, m, H-15), 5.45 (1H, m, H-14), 7.87 (2H, br, $NH_2$), 8.45 (1H, s, H-2′), 9.62 (1H, br, H-8′) ppm; $^{13}C$ NMR, see Table 1; FABMS *m/z* $[M]^+$ 438.

**Acknowledgment.** This work was supported by NIH Grant No. CA 52955. We thank Shelli Stammann for her assistance with antimicrobial assays. We gratefully acknowledge NSF Grant CHE 8113507 and the University of Oklahoma Research Associates Fund for funds to purchase a highfield NMR spectrometer. We thank the Government of Yap, Federated States of Micronesia, for permission to collect specimens.

## References and Notes

(1) Cullen, E.; Devlin, J. P. *Can. J. Chem.* **1975**, *53*, 1690−1691.
(2) Wu, H.; Nakamura, H.; Kobayashi, J.; Kobayashi, M.; Ohizumi, Y.; Hirata Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2495−2504.
(3) Nakamura, H.; Wu, H.; Ohizumi, Y.; Hirata, Y. *Tetrahedron Lett.* **1984**, *25*, 2989−2992.
(4) Wu, H.; Nakamura, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *Tetrahedron. Lett.* **1984**, *25*, 3719−3722.
(5) Capon, R. J.; Faulkner, D. J. *J. Am. Chem. Soc.* **1984**, *106*, 1819−1822.
(6) Ishida, K.; Ishibashi, M.; Shigemori, H.; Sasaki, T.; Kobayashi, J. *Chem. Pharm. Bull.* **1992**, *40*, 766−767.
(7) Fathi-Afshar, R.; Allen, T. M. *Can. J. Chem.* **1988**, *66*, 45−50.
(8) Fu, X.; Ng, P.-L.; Schmitz, F. J.; Hossain, M. B.; van der Helm, D.; Kelly-Borges, M. *J. Nat. Prod.* **1996**, *59*, 1104−1106.
(9) Fu, X.; Schmitz, F. J.; Lee, R. H.; Papkoff, J. S.; Slate, D. L. *J. Nat. Prod.* **1994**, *57*, 1591−1594.
(10) Demarco, P. V.; Farkas, E.; Doddrell, D.; Mylari, B. L.; Wenkert, E. *J. Am. Chem. Soc.* **1968**, *90*, 5480−5486.
(11) Tanner, R. S. *J. Indust. Microbiol.* **1989**, *4*, 145−154.

NP970454I