74644-93-2; **13a**, 88668-85-3; **13b**, 74606-97-6; (+)-**15**, 86783-97-3; (−)-**15**, 88668-86-4; (±)-**15**, 74606-93-2; (+)-**15** (*N*-methyl), 86749-47-5; (±) **15** (*N*-methyl), 88668-95-5; (±)-**16**, 88636-12-8; (±)-**19** (isomer 1), 88636-13-9; (±)-**19** (isomer 2), 88668-88-6; (±)-**20** (isomer 1), 82359-56-6; (±)-**20** (isomer), 88668-87-5; (±)-**21**, 82359-57-7; (±)-**22**, 88636-14-0; (±)-**23**, 88636-15-1; (±)-**24**, 82359-60-2; (±)-**25**, 82359-59-9; (+)-**26**, 88668-89-7; (±)-**26**, 74606-94-3; (+)-**27**, 88668-90-0; (±)-**27**, 74606-95-4; (±)-**28**, 88668-91-1; (±)-**29**, 88668-92-2; (±)-**30**, 88668-93-3; (±)-**31**, 88668-94-4; (±)-**38** (isomer 1), 88668-96-6; (±)-**38** (isomer 2), 88668-97-7; (+)-**40**, 86749-46-4; (±)-**40**, 88636-17-3; (+)-**41**, 3623-44-7; (±)-**41**, 88668-98-8; (±)-**42**, 88636-20-8; (±)-**42** (base), 88636-19-5; (±)-**43a**, 62592-63-6; (±)-**43b**, 62592-69-2; (±)-**44b**, 62630-94-8; i, 88636-18-4; CH_2=$C(CH_2Br)CO_2Et$, 17435-72-2; CH_2=$C(CH_3)CH_2Cl$, 563-47-3; L-tryptophan, 73-22-3; DL-tryptophan, 54-12-6.

Antimicrobial Metabolites from a Pacific Sponge, *Agelas* sp.

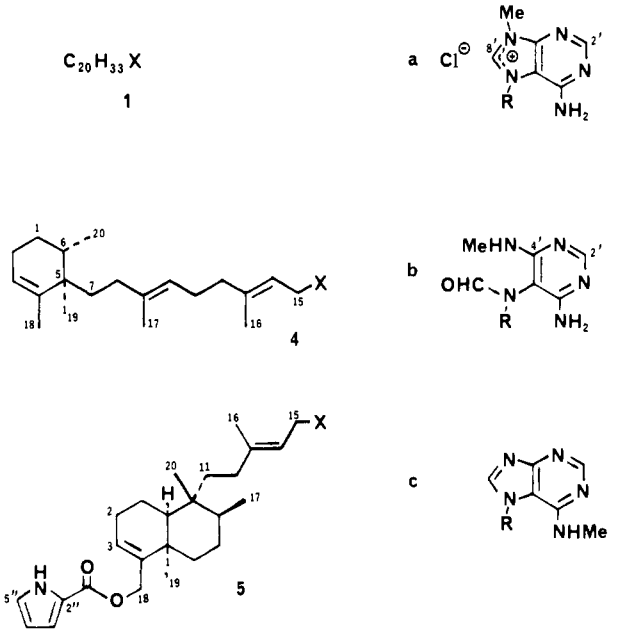
Robert J. Capon and D. John Faulkner*

Contribution from Scripps Institution of Oceanography, A-012F La Jolla, California 92093.
Received October 3, 1983

Abstract: Three novel antimicrobial metabolites have been isolated from the Pacific sponge *Agelas* sp. Two of the metabolites, ageline A (**4a**) and ageline B (**5a**), are quarternary 9-methyladenine salts of diterpenes. The third and minor metabolite was shown to be agelasidine A (**6**), a sesquiterpenoid derivative of taurocyamine. The structures of ageline A and ageline B were elucidated by interpretation of spectral data with particular emphasis on ^{13}C NMR correlations. The agelines are mild ichthyotoxins and show moderate antimicrobial activities.

In 1975, Cullen and Devlin[1] reported that *Agelas dispar* contained agelasine (**1a**), a 9-methyladenine derivative of an unidentified diterpene.[2] This report was in sharp contrast to all other studies[3] of *Agelas* species that described brominated pyrrole derivatives, such as oridin (**2**)[3a] and sceptrin (**3**),[3b] as the typical metabolites of the genus. From an unidentified *Agelas* species collected at Palau, Western Caroline Islands, we have isolated three novel metabolites, two of which were quarternary 9-methyladenine derivatives of diterpenes while the third was agelasidine A (**6**), a sesquiterpene derivative of taurocyamine, recently described by Nakamura et al.[4] In this paper, we report the structural elucidation of ageline A (**4a**) and ageline B (**5a**).

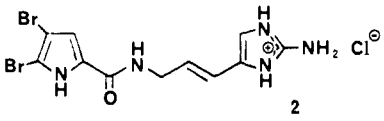
The material from the methanolic extract of the sponge was triturated with dichloromethane and then methanol to obtain two fractions that showed activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, and the marine bacterium B-392. With use of antimicrobial activity to follow the separations, the dichloromethane fraction was repeatedly chromatographed on Sephadex LH-20 first with methanol and then with 1:1 dichloromethane/methanol as eluants and then on silica gel with 6:3:1 chloroform/methanol/ammonia as eluant to obtain agelasidine A (**6**, 0.16% dry weight) and a white solid. The white solid was separated into four fractions by reversed-phase LC, but each of the fractions equilibrated on standing. The four fractions represented pairs of isomers of the two formamides **4b** (1.28% dry weight) and **5b** (0.21% dry weight).



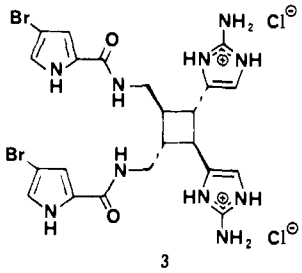
Although the formamides **4b** and **5b** had retained sufficient antimicrobial activity to allow a bioassay-directed fractionation, these metabolites were not present in the crude dichloromethane or methanol extracts. Examination of the ^1H NMR spectra revealed that the formamides were artifacts produced during chromatography on silica gel. The same transformation had been observed by Cullen and Devlin,[1] who obtained the formamide **1b** by hydrolysis of agelasine (**1a**) with aqueous sodium carbonate solution. We were able to isolate a pure sample of ageline A (**4a**, 0.11% dry weight) together with 2:1 mixture of ageline A (**4a**) and ageline B (**5a**) by fractional crystallization of the methanol-soluble fraction from acetonitrile. Since hydrolysis of the 2:1 mixture of **4a** and **5a** gave a 2:1 mixture of **4b** and **5b**, the

(1) Cullen, E.; Devlin, J. P. *Can. J. Chem.* **1975**, *53*, 1690.
(2) The data presented in ref 1 indicated that the diterpene portion of the molecule was probably based on a labdane, kolevane, or related bicyclic skeleton. Devlin (personal communication) has reported that the diterpene portion was not homogeneous.
(3) (a) Minale, L.; Cimino, G.; De Stefano, S.; Sodano, G. *Prog. Chem. Nat. Prod.* **1976**, *33*, 1 and references cited therein. (b) Walker, R. P.; Faulkner D. J.; Van Engen, D.; Clardy, J. *J. Am. Chem. Soc.* **1981**, *103*, 6772. (c) Chevolot, L.; Padua, S.; Ravi, B. N.; Blyth, P. C.; Scheuer, P. J. *Heterocycles* **1977**, *7*, 891.
(4) Nakamura, H.; Wu, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y.; Higashijima, T.; Miyazawa, T. *Tetrahedron Lett.* **1983**, *24*, 4105.

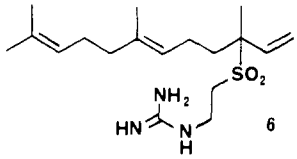
0002-7863/84/1506-1819$01.50/0



2



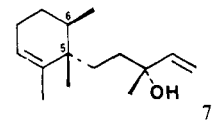
3



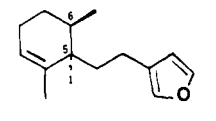
6

structural elucidations were performed on the more stable artifacts.

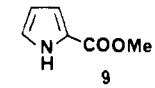
The formamide **4b**, $C_{26}H_{41}N_5O$, was obtained as a white crystalline solid, mp 101–104 °C, from isopropyl ether. The infrared bands at 3380, 3170, 1660, and 1590 cm^{-1} and ultraviolet absorptions at 260 nm (ε 5400) and 224 nm (ε 37 800), shifted to 270 nm (ε 11 200) and 225 nm (ε 24 700) at pH 2, were in excellent agreement with literature values[1] for the 6-amino-5-(formylamino)-4-(methylamino)-1,3-diazine moiety. The ^1H NMR spectrum contained signals at δ 8.14 and 7.96, assigned to the formamide and C-2′ protons, at 2.97 (d, 3 H, J = 4.5 Hz) and 4.90 (br q, 1 H, J = 4.5 Hz) due to the $-NHCH_3$ group, and at 4.84 (br s, 2 H) for the NH_2 group. The substitution pattern about the diazine ring was confirmed by cyclization of the formamide **4b** with sodium hydride in dimethylformamide to obtain the *N*-methyl-7-alkyladenine derivative **4c**. The nitrogenous portion of the molecule was thus well established leaving a $C_{20}H_{33}$ alkyl residue to be identified. The diterpene portion of formamide **4b** contained three olefinic bonds and was not identical with the diterpenoid portion of agelasine (**1a**), reported to be a bicyclic hydrocarbon containing two olefinic bonds.[1] The ^1H NMR spectrum contained five methyl signals at δ 0.85 (d, 3 H, J = 7 Hz), 0.85 (s, 3 H), 1.58 (s, 3 H), 1.59 (d, 3 H, J = 1 Hz), and 1.61 (s, 3 H). The methyl signal at δ 1.59 was allylically coupled to an olefinic proton signal at δ 5.35 (t, 1 H, J = 8 Hz), that was in turn coupled to two methylene proton signals at δ 4.12 (dd, 1 H, J = 12, 8 Hz) and 4.15 (dd, 1 H, J = 12, 8 Hz) that must be attached to nitrogen. The remaining olefinic proton signals at δ 5.41 (br s, 1 H) and 5.03 (br t, 1 H, J = 7 Hz) were assigned to a cycloalkene proton and an olefinic proton in a linear polyisoprenyl chain, respectively. These data were consistent with a structure having the 1-alkyl-1,2,6-trimethyl-2-cyclohexene ring system found in striatol (**7**)[5] and microcionin 2 (**8**),[6] sesquiterpenes that differ in stereochemistry at C-5. The chemical shifts of the methyl signals at C-5 and C-6 (δ 0.85, 0.85) clearly favored the relative stereochemistry of striatol (**7**, δ 0.88, 0.87) over that of microcionin 2 (**8**, δ 1.08, 0.99). A similar comparison of the ^{13}C NMR spectra (Table I) of formamide **4b** with that of striatol (**7**) revealed an excellent correlation for the relevant signals. Comparison of the ^{13}C NMR spectra of the formamide **4b** and ageline A (**4a**) indicated that the diterpenoid moieties were identical. The ^1H NMR spectrum of ageline A (**4a**) contained signals at δ 4.08



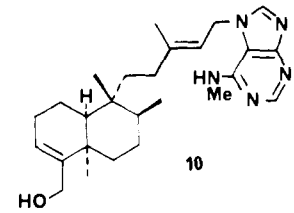
7



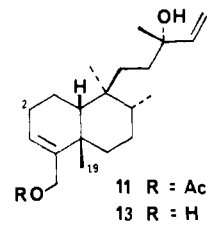
8



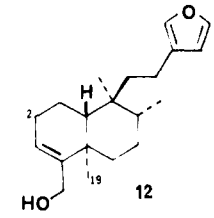
9



10



11 R = Ac
13 R = H



12

(s, 3 H) due to the methyl group on the quarternary nitrogen, at δ 8.47/8.48 (s, 1 H)[7] due to the C-2′ proton, and at δ 10.78/10.88 (br s, 1 H)[7] assigned to the C-8′ proton. These data, together with the infrared bands at 3400, 1640, and 1600 cm^{-1} and ultraviolet absorptions at 272 nm (ε 10 000) and 212 nm (ε 18 100), were in good agreement with the spectral data of agelasine (**1a**).[1] The absolute configuration of ageline A (**4a**) is *probably* 5*R*,6*S* since the sign of the molecular rotation, M_D −35°, is opposite that of striatol, M_D +100°, and both molecules possess "isolated" chiral units[8,9] with the same relative stereostructure.

The formamide **5b**, $C_{31}H_{44}N_6O_3$, possessed all the spectral characteristics of a 6-amino-5-(formylamino)-4-(methylamino)-1,3-diazine moiety and was converted into the corresponding *N*-methyl-7-alkyladenine derivative **5c** on treatment with sodium hydride in dimethylformamide. The presence of a base peak in the mass spectrum at m/z 438 ($M^+ - 110$) indicated the facile loss of a $C_5H_4NO_2$ fragment, identified from spectral data and chemical degradation as pyrrole-2-carboxylic acid. The ^1H NMR spectrum contained signals at δ 6.26 (m, 1 H), 6.90 (m, 1 H), and 6.97 (m, 1 H) assigned to the pyrrole protons while signals in the ^{13}C NMR spectrum at δ 161.2 (s), 123.0 (d), 122.9 (s), 115.2 (d), and 110.3 (d) were all within 1 ppm of the signals for 2-carbomethoxypyrrole (**9**).[10] Hydrolysis of derivative **5c** with use of a 1 N potassium hydroxide solution in methanol gave the alcohol **10** and pyrrole-2-carboxylic acid, identical in all respects with authentic samples.

The diterpene portion of the molecule was defined as a *cis*-clerodane by comparison of the NMR spectral data with those of an array of known clerodane diterpenes.[11] The ^1H NMR spectrum contained signals at δ 4.14 (d, 2 H, J = 7.6 Hz), 5.35 (t, 1 H, J = 7.6 Hz), and 1.62 (s, 3 H) due to a 3-methyl-2-butenyl entity attached to the *N*-formyl nitrogen of the 6-amino-5-(formylamino)-4-(methylamino)-1,3-diazine moiety, and signals at δ 0.74 (d, 3 H, J = 6 Hz), 0.78 (s, 3 H), 1.15 (s, 3 H), and 5.74 (br s, 1 H, $W_{1/2}$ = 9 Hz) were compatible with a clerodane ring

(5) Takeda, R.; Naoki, H.; Iwashita, T.; Mizukawa, K.; Hirose, Y.; Isida, T.; Inoue, M. *Bull. Chem. Soc. Jpn.* **1983**, *56* 1125.

(6) Cimino, G.; De Stefano, S.; Guerriero, A.; Minale, L. *Tetrahedron Lett.* **1975**, 3723.

(7) The relative intensities of these signals varied but always totalled one proton. The reason for the "double" signals is not known, but the presence of hydrates or other complexes would provide a reasonable explanation. there is no relationship between the intensities of these signals and the **4a**:**5a** ratio.

(8) According to the methodology used by Carman[9] for labdane diterpenes, the molecular rotation (M_D) of a molecule equals the sum of the molecular rotations of "isolated" chiral units in the molecule.

(9) Carman, R. M. *Aust. J. Chem.* **1966**, *19*, 629.

(10) Shamma, M.; Hindenlang, D. M. "Carbon-13 NMR Shift Assignments of Amines and Alkaloids"; Plenum Press: New York, 1979; p 2.

(11) (a) Berti, G.; Livi, O.; Segnini, D. *Tetrahedron Lett.* **1970**, 1401. (b) Henderson, M. S.; McCrindle, R.; McMaster, D.; *Can. J. Chem.* **1973**, *51*, 1346. (c) Jefferies, P. R.; Knox, J. R.; Scaf, B. *Aust. J. Chem.* **1973**, *26*, 2199. (d) McCrindle, R.; Nakamura, E.; Anderson, A. B. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1590. (e) Sharma, S. C.; Tandon, J. S.; Dhar, M. M. *Phytochemistry* **1975**, *14*, 1055 (see ref 12).

system. Comparison of the ^{13}C NMR spectral data of formamide **5b** with those of sagittariol acetate (**11**)[12] confirmed the location of a trisubstituted olefin at C-3 and the relative stereochemistry of the bicyclic ring system. The signals at δ 23.9 (t, C-2) and 34.7 (q, C-19) are characteristic of a *cis*-clerodane [cf. sagittariol acetate (**11**), δ 23.7 (t, C-2), 34.4 (q, C-19)] rather than a *trans*-clerodane [cf. hardwickiiol (**12**),[12] δ 18.0 (t, C-2), 21.2 (q, C-16)] ring system. Although ageline B (**5a**) was not obtained in a pure form, its presence in an ~1:2 mixture with ageline A (**4a**) was characterized by a peak in the mass spectrum at m/z 515 ($M^+ - 1$) and pyrrole proton signals at δ 6.23 (br s), 6.80 (br s), 6.98 (br s), and 9.65 (br s) in the ^1H NMR spectrum. The absolute configuration of ageline B (**5a**) is probably antipodal to the terrestrial metabolite sagittariol (**13**), since the molecular rotation of the alcohol **10** (M_D −41.1°) is opposite in sign to that attributed to the bicyclic chiral unit in **13** (M_D +105°).

While this manuscript was in preparation, the structural elucidation of agelasidine A (**6**) was published by Nakamura et al.[4] Our structural studies on agelasidine A (**6**) were essentially the same as those of Nakamura et al. and resulted in an identical structure being proposed.

The agelines and their derivatives have been screened for antimicrobial activity, cytotoxicity, and ichthyotoxicity. With use of the standard disk assay, ageline A (**4a**) inhibited the growth of *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans* at 5 μg/disk. The corresponding formamide (**4b**) showed only slight inhibitory activity against *B. subtilis* at 100 μg/disk while the purine **4c** inhibited *S. aureus* and *B. subtilis* at 10 μg/disk. Agelasidine A (**6**) inhibited *C. albicans* at 5 μg/disk, *B. subtilis* at 10 μg/disk, and *S. aureus* at 25 μg/disk. The corresponding 3,5-dimethylpyrimidine derivative was inactive. The crude dichloromethane extract inhibited cell division in the fertilized sea urchin egg at 10 μg/mL, but the pure compounds **4a**, **4b**, **5b**, and **6** showed no activity at 20 μg/mL. Ageline A (**4a**) and agelasidine A (**6**) but not formamide **4b** were lethal to goldfish (*Carassius auratus*) at 25 μg/mL, the only concentration tested.

Experimental Section

Collection, Extraction, and Separation. *Agelas* sp. (81-047), a massive orange sponge, was collected by had using SCUBA (−20 m) at Argulpelu Reef, Palau, and frozen for transport and storage. The diced sponge was divided into two portions. The first portion (91 g dry weight) was extracted with methanol and the solvent evaporated to obtain a gum that was triturated with first dichloromethane (5 × 250 mL) then methanol (5 × 250 mL) to obtain fractions of 3.3 g and 7.2 g, respectively. Both fractions inhibited growth of *B. subtilis*, *S. aureus*, *C. albicans*, and B-392. The second portion (100 g dry weight) was extracted with 1:1 methanol/dichloromethane and on addition of water (~10% of volume) partitioned into dichloromethane-soluble (3.7 g) and aqueous-methanol-soluble (10 g) fractions. Again, both fractions contained antimicrobial material.

The dichloromethane-soluble material from the first extraction was chromatographed on Sephadex LH-20 with methanol, and the antimicrobial fractions were then rechromatographed with use of 1:1 methanol/dichloromethane as eluant to obtain an antimicrobial oil that showed two spots on silica gel thin-layer chromatography (9:4:1 CH_2Cl_2:MeOH:NH_4OH). Column chromatography of the active fraction on silica gel with use of 6:3:1 dichloromethane/methanol/ammonia as eluant caused separation of a yellow oil, agelasidine A (**6**), 145 mg, 0.16% dry weight) from a white solid (1.7 g, 1.9% dry weight). A sample of the white solid (300 mg) was chromatographed on a Partisil M9 10/50 ODS column with use of 10% 0.05 M Na_2HPO_4/MeOH (pH 5) as eluant and gave pure samples of formamide **4b** (206 mg) and formamide **5b** (34 mg). Each of these materials appeared as a "double" peak on LC due to separation of geometrical isomers.

The methanol-soluble material from the first extraction was separated into water-soluble (1.2 g) and water-insoluble (6.0 g) fractions. A portion of the water-insoluble material was repeatedly chromatographed on Sephadex LH-20 with use of methanol of 1:1 methanoldichloromethane as eluants until a further portion of agelasidine A (**6**, 3.2 mg, 0.08% dry weight) was separated from a white solid (148 mg) that showed antimicrobial activity. The white solid was triturated with hot acetonitrile and filtered to obtain insoluble material (51 mg) and a solution from which ageline A (**4a**, 30 mg, 0.06% dry weight) separated on cooling. The mother liquor contained a mixture of ageline A and ageline B. Since it was apparent that there had been some decomposition of agelines A and B, an alternative purification is presented.

The methanol-soluble material from the second portion of sponge was triturated with acetonitrile. The acetonitrile-soluble material was chromatographed on Sephadex LH-20 with use of 1:1 dichloromethane/methanol as eluant. The fractions containing ageline C were hydrolyzed to obtain agelasidine A (**6**, 58 mg) and a mixture of formamides **4b** and **5b** (258 mg). The remaining active fractions were evaporated, and the solid residue was crystallized from acetonitrile to obtain ageline A (**4a**, 125 mg). The mother liquors contained a 2:1 mixture of ageline A (**4a**) and ageline B (**5a**) (520 mg). It is estimated that the sponge originally contained 1.7% dry weight of ageline A (**4a**), 0.6% of ageline B (**5a**), and 0.25 dry weight of agelasidine A (**6**).

Ageline A (4a): White plates (CH_3CN); mp 175–176 °C $[\alpha]_D$ − 8.4° (*c* 3.0, $CHCl_3$); UV (EtOH) 212 (ε 18 100), 272 (10 000), (EtOH + HCl), 212 (18 100), 272 nm (10 000); IR ($CHCl_3$) 3400, 1640, 1600 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.84 (s, 3 H), 0.84 (d, 3 H, J = 6.5 Hz), 1.56 (s, 3 H), 1.57 (s, 3 H), 1.85 (s, 3 H), 4.08 (s, 3 H), 5.00 (br s, 1 H), 5.40 (br s, C-3), 5.45 (br t, 1 H, J = 4 Hz), 5.72 (br d, 2 H, J = 4 Hz), 6.86 (br s, 2 H), 8.47/8.48 (s, 1 H),[7] 10.78/10.88 (br s, 1 H),[7] ^1H NNR (CD_3OD, 360 MHz) δ 0.86 (s, 3 H), 0.87 (d, 3 H, J = 7.2 Hz), 1.60 (s, 3 H), 1.62 (s, 3 H), 1.88 (s, 3 H), 3.97 (s, 3 H), 5.12 (br m, 1 H), 5.21 (d, 2 H, J = 6.8 Hz), 5.42 (br m, 1 H, C-3), 5.56 (br t, 1 H, J = 7 Hz), 8.46 (s, 1 H); ^{13}C NMR ($CDCl_3$, 50 MHz) see Table I; EI mass spectrum, m/z 421, 406, 404, 298, 272, 257, 230, 216, 189, 149 (100%), 122 (100%); high resolution mass spectrum, obsd 421.3196, $C_{26}H_{39}N_5$ requires 421.3205. Scanning electron microscope analysis (SEM) indicated Br, 8%; Cl, 92%.

Agelasidine A (6): an unstable yellow oil; $[\alpha]_D$ +12.2° (*c* 3.4, $CHCl_3$); UV (EtOH) 227 (ε 5400), 265 (1800), (EtOH + HCl) 226 (4000), 270 nm (2000); IR ($CHCl_3$) 3450 (br), 1650, 1290 (br), 1140 cm^{-1}; IR (Nujol) 3450 (br), 1650, 1210 (br), 1040 cm^{-1} (br); ^1H NMR (CD_3OD, 360 MHz) δ 1.52 (s, 3 H), 1.59 (s, 3 H), 1.59 (s, 3 H), 1.66 (s, 3 H), 3.28 (t, 2 H, J = 6 Hz), 3.71 (t, 2 H, J = 6 Hz), 5.07 (br t, 1 H, J = 6.5 Hz), 5.13 (br t, 1 H, J = 6.5 Hz), 5.49 (d, 1 H, J = 17.5 Hz), 5.59 (d, 1 H, J = 11 Hz), 6.0 (dd, 1 H, J = 17.5, 11 Hz); ^1H NMR ($CDCl_3$, 360 MHz) δ 1.50 (s, 3 H), 1.57 (s, 3 H), 1.59 (s, 3 H), 1.68 (s, 3 H), 2.56 (br s, 1 H, −NH), 3.31 (br s, 2 H), 3.78 (br s, 2 H), 5.09 (br s, 2 H), 5.44 (d, 1 H, J = 17.6 Hz), 5.56 (d, 1 H, J = 10.8 Hz), 5.94 (dd, 1 H, J = 17.6, 10.8 Hz), 7.15 (br s, 2 H, $-NH_2$), 7.74 (br s, 1 H, =NH); ^{13} NMR ($CDCl_3$, 50 MHz) 15.9 (q), 16.1 (q), 17.7 (q), 22.0 (t), 25.7 (q), 26.6 (t), 31.4 (t), 34.9 (t), 39.7 (t), 45.8 (t), 68.2 (s), 121.7 (t), 122.5 (d), 124.1 (d), 132.2 (s), 136.4 (s), 134.6 (d), 157.4 (s); EI mass spectrum, m/z 355 (M^+), 286, 253, 205, 189, 175, 152, 135, 119, 107, 93, 87, 81, 69 (100%); FD mass spectrum, 356 (M + 1); high resolution mass spectrum, obsd 286.1584 ($M^+ - C_5H_9$), $C_{13}H_{24}N_3O_2S$ requires 286.1589.

Formamide 4b: white crystalline solid (isopropyl ether); mp 101–104 °C; $[\alpha]_D$ −8.2° (*c* 2.4, $CHCl_3$); UV (EtOH) 206 (ε 28 700), 224 (37 800), 260 (5400), (EtOH + HCl) 204 (27 000), 225 (24 700), 270 nm (11 200); IR (Nujol) 3380, 3170, 1660, 1590 cm^{-1}; IR ($CHCl_3$) 3500, 1660, 1600 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.85 (s, 3 H), 0.85 (d, 3 H, J = 7 Hz), 1.58 (s, 3 H), 1.59 (d, 3 H, J = 1 Hz), 1.61 (s, 3 H), 2.97 (d, 3 H, J = 4.5 Hz), 4.12 (dd, 1 H, J = 12, 8 Hz), 4.15 (dd, 1 H, J = 12, 8 Hz), 4.84 (br s, 2 H, $-NH_2$), 4.90 (br q, 1 H, J = 4.5 Hz, −NH), 5.03 (br s, 1 H), 5.35 (br t, 1 H, J = 7.8 Hz), 5.41 (br s, 1 H), 7.96 (s, 1 H), 8.14 (s, 1 H); ^{13}C NMR ($CDCl_3$, 50 MHz) see Table I; EI mass spectrum, m/z 439 (M^+) 424, 410, 316, 234, 167, 150, 139, 123, 105, 95, 81 (100%); FD mass spectrum, 439 (M^+); high resolution mass spectrum, obsd m/z 439.3312, $C_{26}H_{41}N_5O$ requires 439.3311.

Formamide 5b: $[\alpha]_D$ −9.2° (*c* 0.73, $CHCl_3$); UV (EtOH) 224 (ε 41 800), 263 (10 800), (EtOH + HCl) 227 (31 500), 266 (28 600); IR ($CHCl_3$) 3700, 3650, 3450, 1660, 1610 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.74 (d, 1 H, J = 6 Hz), 0.78 (s, 3 H), 1.15 (s, 3 H), 1.62 (s, 3 H), 2.98 (d, 1 H, J = 4.5 Hz), 4.14 (d, 2 H, J = 7.5 Hz), 4.72 (d, 1 H, J = 12 Hz), 4.79 (d, 1 H, J = 12 Hz), 5.12 (br s, 1 H, NH_2), 5.35 (br t, 1 H, J = 7.5 Hz), 5.40 (br g, 1 H, J = 4.5 Hz, NH), 5.74 (br s, 1 H, $w_{1/2}$ = 9 Hz), 6.26 (m, 1 H, J (D_2O)[13] = 3.5, 2.5 Hz), 6.90 (m, 1 H, J (D_2O) = 3.5, 1.5 Hz), 6.97 (m, 1 H, J (D_2O) = 2.5, 1.5 Hz), 7.97 (s, 1 H), 8.14 (s, 1 H), 9.71 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) see Table I; FAB mass spectrum, m/z 548 (M^+), 504, 454, 438 (100%), 422, 248, 234, 192, 189, 167, 150, 139, 119, 105, 94 (100%), 81, 69; FD mass spectrum, 549 (M^+ 1); high resolution mass spectrum, obsd 548.3478, $C_{31}H_{44}N_6O_3$ requires 548.3475, obsd 167.0806, $C_6H_9N_5O$ requires 167.0807.

Conversion of Agelines A (4a) and B (5a) to Formamides 4b and 5b. A suspension of a 2:1 mixture (10 mg) of agelines A and B (**4a**, **5a**) in a 2 N sodium bicarbonate solution (5 mL) was stirred at room temper-

(12) Sharma, S. C.; Tandon, J. S.; Porter, B.; Raju, M. S.; Wenkert, E. *Phytochemistry*, in press.

(13) $J(D_2O)$ is an abbreviation for the coupling constants observed after equilibration of the sample with D_2O to remove NH–CH couplings.

Table I. The ^{13}C NMR Spectral Data for Ageline A (4a) and Formamides 4b and 5b Compared with the Relevant Spectral Data for Striatol (7),[5] 2-Carbomethoxypyrrole (9),[10] and Sagittariol Acetate (11)[11]

carbons	7	4a	4b	5b	11	9
1	25.4	25.5 (t)	25.5 (t)	17.3 (t)	17.0 (t)	122.0 (s)
2	27.0	27.0 (t)	27.0 (t)	23.9 (t)	23.7 (t)	115.1 (d)
3	124.0	122.4 (d)	122.6 (d)	129.4 (d)	128.9 (d)	109.8 (d)
4	139.3	139.5 (s)	139.5 (s)	138.3 (s)	137.9 (s)	122.9 (d)
5	40.0	40.3 (s)	40.3 (s)	36.2 (s)	36.0 (s)	161.4 (s)
6	33.2	33.1 (d)	33.1 (d)	36.4 (t)	37.1 (t)	
7		34.1[a] (t)	34.1[a] (t)	28.7 (t)	28.5 (t)	
8		35.1[a] (t)	35.1[a] (t)	37.4 (d)	37.1 (d)	
9		136.9 (s)	136.7 (s)	40.0 (s)	39.5 (s)	
10		124.0 (d)	124.0 (d)	45.1 (d)	44.7 (d)	
11		26.2 (t)	26.3 (t)	32.7[a] (t)	31.3 (t)	
12		39.5 (t)	39.6 (t)	37.1[a] (t)	34.8 (t)	
13		146.8 (s)	142.8 (s)	144.4 (s)	73.1 (s)	
14		115.6 (d)	117.5 (d)	117.0 (d)	114.8 (d)	
15		48.6 (t)	41.5 (t)	41.5 (t)	111.5 (t)	
16		17.3 (q)	16.3[b] (q)	16.5 (q)	27.5 (q)	
17		16.2 (q)	16.2[b] (q)	15.9 (q)	15.7 (q)	
18	15.7	15.8 (q)	15.8 (q)	66.4 (t)	66.4 (q)	
19	19.0	19.2 (q)	19.2 (q)	34.7 (q)	34.4 (q)	
20	21.0	21.0 (q)	21.0 (q)	17.3 (q)	17.2 (q)	
2'		155.9 (d)	164.6[c] (d)	164.6[b] (d)		
4'		149.3[b] (s)	160.4[d] (s)	160.4[c] (s)		
5'		109.7 (s)	99.3 (s)	99.2 (s)		
6'		152.3[b] (s)	159.8[d] (s)	159.6[c] (s)		
8'		141.4 (d)				
NCHO			157.6[c] (d)	157.1[b] (d)		
NCH_3		36.2 (q)	27.9 (q)	28.0 (q)		
2''				122.9 (s)		
3''				115.2 (d)		
4''				110.3 (d)		
5''				123.0 (d)		
OC=O				161.2 (s)		

[a-d] Values with identical superscript within a column may be interchanged.

ature for 2 min and the product then extracted with dichloromethane (3 × 20 mL). Examination of the product by thin-layer chromatography and ^1H NMR spectroscopy revealed >90% conversion to a mixture of the formamides **4b** and **5b**.

Dehydration of Formamide 4b. Excess sodium hydride (10 mg) was added to a solution of formamide **4b** (35 mg, 0.08 mmol) in dry dimethylformamide (2 mL) and the resulting suspension was stirred at room temperature for 2 h. The reaction was quenched with water (5 mL), the solvent evaporated under high vacuum, and the product extracted with dichloromethane (2 × 20 mL). The extract was dried over sodium sulfate and the solvent evaporated to obtain the *N*-methyl-7-alkyladenine derivative **4c** (32 mg, 96% theoretical) that crystallized from acetonitrile as white plates: mp 147–8 °C; UV (EtOH) 218 (ε 20 200), 277 (15 200), (EtOH + HCl) 210 (17 300), 283 nm (18 700); ^1H NMR ($CDCl_3$, 360 MHz) δ 0.84 (s, 3 H), 0.85 (d, 3 H, *J* = 6.8 Hz), 1.58 (s, 3 H), 1.59 (s, 3 H), 1.86 (s, 3 H), 3.08 (d, 3 H, *J* = 4 Hz), 4.88 (d, 2 H, *J* = 4 Hz), 5.05 (br s, 1 H), 5.30 (br s, 1 H, NH), 5.41 (br s, 1 H), 5.42 (br s, 1 H), 7.88 (s, 1 H), 8.54 (s, 1 H); partial ^{13}C NMR ($CDCl_3$, 50 MHz) 159.0 (s), 153.1 (d), 151.4 (s), 143.9 (d), 112.1 (s); mass spectrum, *m/z* 421 (M^+), 402, 376, 298, 283, 265; high resolution mass spectrum, obsd 421.3197, $C_{26}H_{39}N_5$ requires 421.3205.

Dehydration of Formamide 5b. Sodium hydride (10 mg) was added to a solution of formamide **5b** (21 mg, 0.04 mmol) and the suspension was stirred at room temperature for 2 h. The reaction was extracted as above to obtain the *N*-methyl-7-alkyladenine derivative **5c** (17 mg, 54% theoretical) as a white solid: $[\alpha]_D$ −8.4° (*c*, $CHCl_3$); UV (EtOH) 268 (ε 17 500), (EtOH + HCl) 217 nm (16 000); ^1H NMR ($CDCl_3$, 360 MHz) δ 0.78 (d, 3 H, *J* = 6.5 Hz), 0.82 (s, 3 H), 1.16 (s, 3 H), 1.89 (br s, 3 H), 3.10 (d, 3 H, *J* = 4.7 Hz), 4.73 (d, 1 H, *J* = 12 Hz), 4.80 (d, 1 H, *J* = 12 Hz), 4.88 (d, 2 H, *J* = 6 Hz), 5.25 (br g, 1 H, *J* = 5 Hz, NH), 5.44 (br t, 1 H, *J* = 6 Hz), 5.75 (br s, 1 H), 6.25 (m, 1 H, $J(D_2O)$[12] = 3, 2.5 Hz), 6.90 (m, 1 H, $J(D_2O)$ = 3, 1 Hz), 6.97 (m, 1 H), 7.86 (s, 1 H), 8.54 (s, 1 H), 9.29 (br s, 1 H); mass spectrum, *m/z* 530 (M^+), 486, 437, 419, 404, 338, 270, 255, 230, 217, 216, 189, 150, 149, 104; high resolution mass spectrum, obsd 530.3340, $C_{31}H_{42}O_2N_6$ requires 530.3369.

Hydrolysis of 5c. A solution of the adenine derivative **5c** (13 mg, 0.02 mmol) in 1:1 aqueous potassium hydroxide/methanol (2 mL) was stirred at reflux temperature under an atmosphere of dry nitrogen for 3 h. The solution was neutralized by addition of dry ice (~0.5 g), and the solvent was evaporated under vacuum to obtain a solid residue that was partitioned into dichloromethane-soluble and methanol-soluble fractions. The methanol-soluble material (2 mg, 73% theoretical) was identical in all respects with pyrrole-2-carboxylic acid. The dichloromethane-soluble material was the primary alcohol **11** (6 mg, 56% theoretical): $[\alpha]_D$ −9.4° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$, 360 MHz) δ 0.76 (d, 3 H, *J* = 6 Hz), 0.79 (s, 3 H), 1.12 (s, 3 H), 1.88 (br s, 3 H), 3.10 (d, 3 H, *J* = 4.5 Hz), 4.12 (d, 1 H, *J* = 13 Hz), 4.22 (d, 1 H, *J* = 13 Hz), 4.88 (d, 2 H, *J* = 6 Hz), 5.27 (br q, 1 H, *J* = 4.5 Hz), 5.44 (br t, 1 H, *J* = 6 Hz), 5.63 (br s, 1 H), 7.85 (s, 1 H), 8.53 (s, 1 H); mass spectrum *m/z* 437 (M^+), 420, 404, 314, 298, 286, 230, 217, 216, 203, 202, 189, 150, 104, 94, 92, 91; high resolution mass spectrum, obsd 437.3149, $C_{26}H_{39}N_5O$ requires 437.3155.

Acknowledgment. We thank Janice E. Thompson for identifying the sponge and Mary Kay Harper for performing bioassays. Mass spectra were provided by Dr. J. Occolwitz, Lilly Research Laboratories, and Dr. A. L. Burlingame, U.C. San Francisco (PHS Grant No. RR00719). Research grants from the National Institutes of Health (AI-11969) and the California Sea Grant Program (NA80AA-D00120-Project R/MP 23) are gratefully acknowledged.