Notes

Agelasines J, K, and L from the Solomon Islands Marine Sponge *Agelas* cf. *mauritiana*

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Three new diterpene alkaloids, agelasine J (**3**), agelasine K (**4**), and agelasine L (**5**), were isolated from the marine sponge *Agelas* cf. *mauritiana* collected in the Solomon Islands. The structures of these compounds were elucidated by physical data analyses. They displayed *in vitro* antimalarial activity against *Plasmodium falciparum*.

Agelasidae sponges have been widely investigated, but still represent a good source of new marine metabolites.[1] Several species have been reported to yield new pyrrole-2-aminoimidazole (P-2-AI) alkaloids[2] and the group of purine diterpenes possessing 9-*N*-methyladeninium, named agelasines.[3,4] In the course of our survey of bioactive substances from marine sources, we investigated extracts of several samples of *Agelas* sp. related to *A. mauritiana* collected in the Solomon Islands. The CH_2Cl_2 extract, which displayed antimalarial activity, was found to be rich in agelasines. Agelasines have also been described to exhibit a wide array of related bioactivities such as antibiotic,[5,6] antifouling,[7] and K-ATPase inhibiting effects.[4,8] More recently, antimycobacterial activity was demonstrated for agelasine E,[9] and more modestly for agelasine F,[10] showing the importance of the lipophilic character of the terpenoid side chain to penetrate into the *Mycobacterium* cell.

The antimalarial tests showed that the purine diterpene-rich chromatography fraction was responsible for this unreported property of these compounds. Nevertheless, some guanine base analogues have been described recently as lead compounds for antimalarial chemotherapy;[11] thus we further investigated this bioactive fraction. The isolation and structure determination of novel agelasines J (**3**), K (**4**), and L (**5**) isolated from a complex mixture of related isomers (Figure 1) are described here.

The CH_2Cl_2 extract was purified by chromatography on a Si gel column using a CH_2Cl_2−MeOH gradient. The antimalarial activity was concentrated in the resulting main fraction (19 g). Further HPLC-MS analysis of this fraction using a C_{18} column showed the presence of a complex mixture of isomers of agelasines corresponding to the same molecular mass as the revised agelasine C (**1**)[12] and agelasine B (**2**).[13] This mixture (100 mg) was purified by preparative RPHPLC and further semipreparative HPLC using a graphite column to obtain agelasines J (**3**) (7 mg) and K (**4**) (1.4 mg). Purification of another agelasine-containing fraction (100 mg) directly by semipreparative graphite column using slightly different elution conditions afforded agelasines J (**3**) (15 mg) and L (**5**) (9.3 mg).

The molecular formula of these three metabolites, $C_{26}H_{39}N_5$ (calcd for $C_{26}H_{40}N_5$ 422,3284), was established by HRESIMS as *m/z* 422.3276 $[M + H]^+$ for **3**; *m/z* 422.3311 $[M + H]^+$ for **4**, and *m/z* 422.3284 ($C_{26}H_{39}N_5$) for **5**. The presence of a 9-*N*-methyladeninium moiety was suggested on the basis of UV (λ_{max} 269 nm, MeOH) and ^1H and ^{13}C NMR mass spectral fragmentation (150.1 $[M + H]^+$ for $C_6H_8N_5$) and by comparison with data of various known agelasines. ^{15}N NMR shifts for compounds **3** and **5** (Table 2) were deduced from $^1H-^{15}N$ HMBC experiments.[14] The N-7 of the *N*-methyladeninium part was actually observed to be connected to the C-20 of the terpenoid moiety. The *E*-configuration of the linear olefin (C-17−C-19) was determined by NOESY analysis (Figure 2) for the three new agelasines.

Compound **3** was obtained as a colorless powder. The ^1H NMR spectra showed only one vinylic proton (δ 5.54) and one vinylic methyl (δ 1.87) belonging to an isoprenyl moiety linking the 9-*N*-methyladeninium to a bicyclic carbon skeleton bearing four methyl groups as one doublet and three singlets. Furthermore, HMBC correlations (Table 1) showed two *gem*-dimethyl singlets (δ 0.97 and 1.00). The skeleton was thus assigned to a labdane, as for agelasine C (**1**). Furthermore, NMR analysis indicated a tetrasubstituted double bond. These observations led us to propose the structure **3** for agelasine J. This compound was obtained by Nakamura et al. by semisynthesis from agelasine A or B under acidic conditions.[15] Assuming the fact that the double bond could migrate during purification, we confirmed that the isolated agelasine J (**3**) was actually present in the initial mixture by HPLC analysis (column C_{18} Waters SunFire, gradient MeOH/H_2O/$HCOONH_4$, pH = 3.5). Interestingly, the chemical rearrangement of agelasines A and B into agelasine J (**3**) could be a spontaneous biogenetic pathway.

Since the acidic conditions give the rearrangement toward two enantiomers of **3** when starting from agelasine A or B, we confirmed the absolute configuration of the natural product **3** by comparison of its specific rotation $[\alpha]^{25}_D$ +14 (*c* 0.46, MeOH) and the semisynthesis products obtained from agelasine B ($[\alpha]^{25}_D$ +17 (*c* 0.34, MeOH))[15] and from agelasine A ($[\alpha]^{25}_D$ −14 (*c* 0.42,

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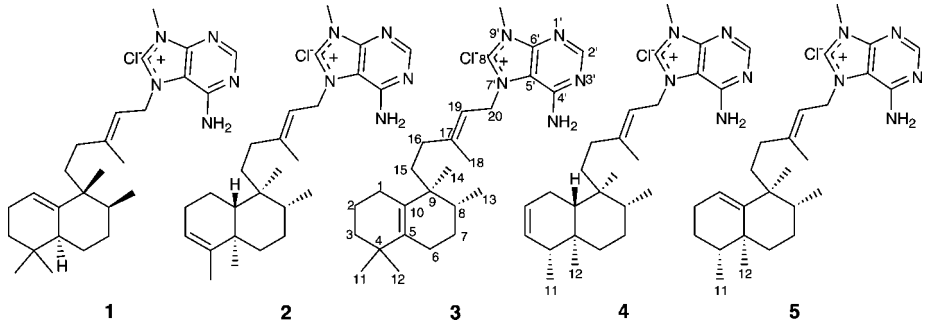

Figure 1

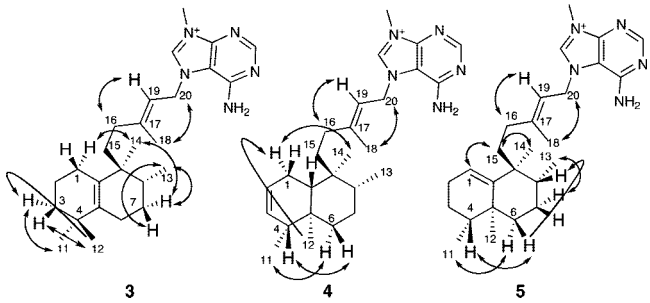

Figure 2. Selected NOE correlations for **3**, **4**, and **5**.

MeOH))[14] since the configuration of both agelasines A and B has been achieved by synthesis.[13,15]

Compound **4** was also obtained as a colorless powder, $[\alpha]^{25}_D$ +60 (*c* 0.11, MeOH). The ^{1}H NMR spectra showed an olefinic proton (δ 5.55) and an olefinic methyl (δ 1.89) belonging to an isoprenyl fragment linking the 9-*N*-methyladeninium moiety to a bicyclic carbon skeleton bearing four methyl groups. The ^{1}H NMR spectrum of **4** (Table 1) showed the presence of an angular methyl (δ 0.77), and the skeleton of this agelasine was thus assigned to a clerodane as in agelasine A or B (**2**), whose structures have been confirmed by synthesis.[13,16] Two coupled olefinic protons (δ 5.23, 5.59) indicated the presence of a disubstituted double bond. The HMBC experiment showed a correlation between the olefinic carbon C-3 (δ 133.0) and a methyl group at C-14 (δ 0.84). These observations led us to propose the structure **4** for this new metabolite named agelasine K. The planar structure of **4** was confirmed by the correlations observed in the HMBC experiment. The *trans* ring fusion mode in agelasine K (**4**) was suggested by a high-field signal for the angular methyl carbon[15] (δ 14.0). The relative configuration of **4** was established from NOE analysis (Figure 2) Assuming again the possibility of the double-bond migration during purification, we confirmed that the isolated agelasine K (**4**) was actually present in the initial mixture by HPLC analysis.

Compound **5** was isolated as a colorless powder. Comparison of the ^{1}H and ^{13}C NMR spectroscopic data (Table 1) of compounds **4** and **5** suggested a clerodane skeleton for **5**. The only difference between these two compounds was the position of the ring double bond at C-1 in **5** instead of C-3 in **4**. These results were confirmed by the HMBC long-range correlations of Me-14 (δ 1.02) with C-8, C-9, C-10, and C-15 and the correlations of Me-12 (δ 0.99) with C-4, C-5, C-6, and C-10. The relative configuration of **5** was established from NOE analysis (Figure 2). The structure of the new compound was thus assigned as a clerodane stereoisomer of agelasine J and named agelasine L.

Agelasines J, K, and L displayed mild activity on *P. falciparum* (FcB1-Columbia strain), with respective IC_{50} values of 6.6, 8.3, and 18 μM, and a low cytotoxicity on MCF7 cells, with IC_{50} values of 33, 30, and 80 μM, respectively. The cytotoxicity was consistent with what has been recently described for agelasine D, which was more active on different leukemia tumor cell cultures than solid tumor cells.[6] Furthermore, it would be interesting to test these compounds on *Mycobacterium tuberculosis*, as well as agelasines E and F on *P. falciparum*, to correlate the structure of the terpenoid side chain to the activity of those agelasines.

In summary, we isolated three new diterpene alkaloids, agelasines J (**3**), K (**4**) and L (**5**), that differ from agelasines C (**1**) and B (**2**) by the position of the double bond on the diterpenoid carbon skeleton. From the natural chemodiversity point of view, the presence of these agelasines in a complex cocktail mixture of isomers suggests again the ability of the sponges to synthesize an array of ionic molecules of biological significance.

Experimental Section

General Experimental Procedures. Optical rotation was determined in methanol with a Jasco P1010 polarimeter. The IR spectrum was measured (neat) on a Perkin-Elmer BX-FT-IR spectrometer. The UV spectrum was recorded on a Waters 996 photodiode array detector in MeOH. NMR experiments were carried out with Bruker Avance 600 MHz and DRX 500 MHz spectrometers using CD_3OD and CD_3OH. HRMS were obtained with an electrospray source (Lockspray) coupled with a time-of-flight analyzer (LCT, Micromass). Samples were prepared in MeOH and injected in the MS system using a Waters 2795 system. The mobile phase was MeOH/H_2O (50/50, v/v) with a 0.2 mL/min flow. Preparative HPLC was performed on an autoprep system (Waters 600 controller and Waters 600 pump with a Waters 996 photodiode array detector). Samples were injected with the Waters 2700 sample manager.

Collection and Identification of the Sponge. *Agelas* cf. *mauritiana* samples were collected off the northern Guadalcanal coast in the Solomon Islands on June 30, 2004, using scuba at 18−30 m. A voucher specimen is deposited in the Queensland Museum under the accession number G324813.

Extraction and Isolation. The sponge was frozen after collection and later ground, freeze-dried, and stored at −20 °C until workup. The dry, powdered sponge (600 g) was extracted by maceration with CH_2Cl_2 (3 × 3 L) at room temperature. After removal of the solvent under reduced pressure, the resulting crude extract (30 g) was chromatographed on Si gel (500 g, 70−200 μm Chromagel) using a CH_2Cl_2/MeOH gradient as eluent and monitored by TLC. The main fraction (19 g) containing the mixture of agelasines was eluated with CH_2Cl_2/MeOH (90/10).

After LC-MS analysis, 100 mg of the mixture was purified by preparative HPLC using a C_{18} column (Symmetryshield, RP_{18}, 7 μm, 19 × 150 mm column, 22 mL/min) with an isocratic elution with CH_3CN/H_2O/HCOOH (35/65/0.5). Further purification on a semi-preparative graphite column (Hypercarb, Thermo Hypersil, 5 μm, 10 × 150 mm column, 5 mL/min) using a gradient of CH_3CN/H_2O/TFA (92/8/0.5 to 100/0/0.5 in 30 min) afforded agelasines J (**3**) (7 mg, 0.0012%) and K (**4**) (1.4 mg, 0.0002% of the freeze-dried sponge).

Another 100 mg of the mixture was purified directly by a semi-preparative graphite column (Hypercarb, Thermo Hypersil, 5 μm, 10 × 150 mm column, 5 mL/min) using a gradient of CH_3CN/H_2O/TFA (96/4/0.5 in 5 min to 100/0/0.5 in 30 min), affording agelasines J (**3**) (15 mg, 0.0025%) and L (**5**) (9.3 mg, 0.0016% of the freeze-dried sponge).

Agelasine J (3): colorless powder; $[\alpha]^{25}_D$ +14 (*c* 0.46, MeOH); UV (MeOH) λ_{max} 269 nm; IR (neat) ν_{max} 3091, 2959, 2922, 1667, 1649,

Table 1. NMR Data Including HMBC Correlations (600 MHz for ^{1}H data, 125 MHz for ^{13}C NMR data, CD_3OD) for Agelasines J, K, and L

	agelasine J (**3**)			agelasine K (**4**)			agelasine L (**5**)		
position	δ_H (J in Hz)	δ_C	HMBC H to C	δ_H	δ_C	HMBC H to C	δ_H (J in Hz)	δ_C	HMBC H to C
1	1.83 m	27.2	3, 5, 10	1.97 m	24.8	2	5.45 m	121.3	2, 5, 9, 10
	2.06 m			2.03 m					
2	1.58 m	21.2		5.59 m	126.5		2.08 m, 2.21 m	27.0	
3	1.37 m	41.3	1, 4, 5	5.23 m	133.0	1, 5	1.40 m	27.6	1, 2, 5, 11
	1.48 m						1.52 m		
4		35.6		1.93 m	47.4	2	1.41 m	43.0	
5		139.1			36.7[a]			38.0	
6	1.98 m	26.6		1.08 m	40.7	5, 8, 10, 12	1.08 m	39.6	5
	2.06 m			1.79 m			1.74 m		
7	1.37 m	28.5	5	1.35 m	28.4		1.39 m	28.1	
	1.50 m			1.48 m			1.61 m		
8	1.67 m	35.2		1.49 m	38.3		1.52 m	37.2	7
9		42.0			40.0[a]			43.5	
10		133.8		1.48 m	46.5	1, 4, 5, 9, 14		149.3	
11	1.00 s	28.2	3, 4, 5, 12	0.84 d (J = 6,3)	14.9	3,4,5	0.85 d (J = 6.5)	16.0	3, 4, 5
12	0.97 s	29.8	3, 4, 5, 11	0.77 s	14.0	4, 5, 6, 10	0.99 s	21.2	4, 5, 6, 11
13	0.87 d (J = 6.8)	16.7	7, 8, 9	0.85 d (J = 6,3)	16.4	7,8,9	0.89 d (J = 6.5)	16.9	7, 8, 9
14	0.86 s	21.7	8, 9, 10, 15	0.84 s	18.4	8, 9, 10, 15	1.02 s	26.8	8, 9, 10, 15
15	1.59 m	35.6	9, 10, 17	1.43 m	37.3		1.63 m	37.7	9
				1.52 m			1.81 m		
16	1.87 m	35.7	15, 17, 18, 19	2.03 m	34.1	15, 17, 19	2.07 m	35.2	15, 17, 19
	2.14 m			2.11 m			2.15 m		
17		150.3			150.0			150.2	
18	1.87 s	17.3	16, 17, 19	1.89 s	17.3	16, 17, 19	1.89 s	17.3	16, 17, 19
19	5.54 t (J = 7)	115.4	10, 16	5.55 t (J = 7.3)	115.5	16, 18, 20	5.55 t (J = 6.9)	115.2	16, 18, 20
20	5.20 d (J = 7)	49.0[a]	17, 19, 5′, 8′	5.20 d (J = 7.3)	48.8[a]	17, 19, 5′, 8′	5.22 d (J = 6,9)	48.7	17, 19, 5′, 8′
2′	8.46 brs	157.3	4′, 6′	8.47 brs	157.2	4′, 6′	8.48 brs	157.2	4′, 6′
4′		151.1			151.3[a]			154.3	
5′		111.3			111.2[a]			111.3	
6′		154.3			154.3			151.0	
8′		141.8[a]			141.8[a]		9.32 s	142.1	5′, 6′
Me 10′	3.97 s	32.1	4′, 8′	3.98 s	32.1	4′, 8′	3.98 s	32.1	

[a] Carbon chemical shifts are deduced from HMBC correlations.

Table 2. ^{15}N NMR Data Deduced from ^{1}H−^{15}N HMBC Correlations (60.77 MHz, CD_3OD at 300 K and CD_3OH at 323 K) for Agelasines J and L

	agelasine J (**3**)		agelasine L (**5**)	
position	δ_H	δ_N	δ_H	δ_N
1′		241.1		242.0[a,c]
3′		223.4		222.7[a,c]
7′		165.4		165.3[a]
9′		156.1		156.1[a]
NH_2	7.47[b]	83.7[b]	7.37/7.52[b]	83.7[b]

[a] CD_3OD. [b] CD_3OH. [c] Nitrogen chemical shifts are deduced from ref 11 and converted to the CD_3OH scale following IUPAC recommendations and ref 14.

1591, 1455, 1302, 1200, 1176, 1129, 825, 800, 718 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 422.3276 [M + H]$^+$, calcd for $C_{26}H_{40}N_5$ 422.3284.

Agelasine K (4): colorless powder; $[\alpha]^{25}_D$ +60 (*c* 0.11, MeOH); UV (MeOH) λ_{max} 269 nm; IR (neat) ν_{max} 2954, 2946, 1672, 1651, 1591, 1460, 1376, 1303, 1202, 1177, 1119, 825, 801, 718 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRESIMS *m/z* 422.3311 [M + H]$^+$, calcd for $C_{26}H_{40}N_5$ 422.3284.

Agelasine L (5): colorless powder; $[\alpha]^{25}_D$ −3.2 (*c* 1, MeOH); UV (MeOH) λ_{max} 269 nm; IR (neat) ν_{max} 3331, 3148, 2922, 1647, 1613, 1589, 1456, 1369, 1297, 1199, 1175, 1128, 828, 800, 719 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m/z* 422.3284 [M + H]$^+$, calcd for $C_{26}H_{40}N_5$ 422.3284.

Biological Assays. Antiplasmodial activity of the compounds was evaluated *in vitro*, using *P. falciparum,* the causative agent of lethal malaria. Parasites (FcB1-Columbia strain) were cultured on human erythrocytes maintained in RPMI 1640 supplemented with 5% human serum, according to the method described by Trager and Jensen[17] with modifications described by Benoit et al.[18] The cultures were synchronized every 48 h by 5% D-sorbitol lysis (Merck, Darmstadt, Germany) in order to discard every old stage parasite. The FcB1-Columbia was considered to be a chloroquine-resistant strain (chloroquine IC_{50} = 145 nM). *In vitro* antimalarial activity testing was performed by [^{3}H]-hypoxanthine (Perkin-Elmer, France) incorporation. Fifty percent inhibitory concentrations (IC_{50}) were graphically determined on inhibition versus concentration curves.

Cytotoxicity was estimated on human breast cancer cells (MCF7). Cells were cultured under the same conditions as those used for *P. falciparum*, except for the replacement of the 5% human serum by 5% fetal calf serum (Cambrex, Verviers, Belgium). For the determination of cytotoxicity, cells were distributed in 96-well plates at 2×10^4 cells/100 μL in each well, and then 100 μL of culture medium containing investigated compounds at various concentrations was added. Cell growth was estimated by [^{3}H]-hypoxanthine incorporation after 48 h incubation exactly as for the *P. falciparum* assay. The [^{3}H]-hypoxanthine incorporation in the presence of extracts or pure compounds was compared with that of control cultures without extract (positive control being doxorubicin (Sigma)). IC_{50} values were graphically determined on inhibition versus concentration curves.

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