Agelasine G, a New Antileukemic Alkaloid from the Okinawan Marine Sponge *Agelas* sp.

Keisuke ISHIDA,[a] Masami ISHIBASHI,[a] Hideyuki SHIGEMORI,[a] Takuma SASAKI,[b] and Jun'ichi KOBAYASHI*,[a]

Faculty of Pharmaceutical Sciences, Hokkaido University,[a] Sapporo 060, Japan and Cancer Research Institute, Kanazawa University,[b] Kanazawa 902, Japan. *Received August 19, 1991*

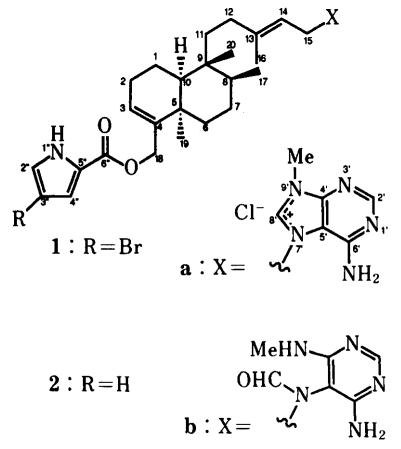
A new antileukemic alkaloid, agelasine G (1a), has been isolated from the Okinawan marine sponge *Agelas* sp. and its structure elucidated to be a bromopyrrole alkaloid containing 9-methyladenine and diterpene moieties on the basis of the spectroscopic data.

Keywords sponge; *Agelas* sp.; Agelasiidae; bromopyrrole; diterpene; 9-methyladenine; cytotoxicity

Marine sponges of the genus *Agelas* have been demonstrated to be a rich source of unique bioactive bromopyrrole alkaloids[1] and diterpene derivatives with polar functionalities.[2] These metabolites exhibit various biological activities such as antimicrobial,[3] antispasmodic,[4] antiserotonergic,[5] Na,K-adenosine triphosphatase (ATPase) inhibitory,[6,7] or actomyosin ATPase-activating activities.[8] During our survey of bioactive substances from Okinawan marine organisms,[9] we further investigated extracts of the sponge *Agelas* sp. to isolate agelasine G (**1a**), a bromopyrrole alkaloid containing 9-methyladenine and diterpene moieties, and having antileukemic activity. Here we describe the isolation and structure elucidation of **1a**.

The sponge *Agelas* sp.[10] was collected off Konbu, Okinawa, and kept frozen until used. The MeOH extract was partitioned between ethyl acetate and water, and the aqueous layer was subsequently extracted with 1-butanol. The 1-butanol-soluble fraction was subjected to C_{18} reversed-phase flash column chromatography (eluent: 80% MeOH containing 0.2 M NaCl), followed by C_{18} reversed-phase high performance liquid chromatography (HPLC) (eluent: 80% MeOH containing 0.2 M NaCl) to give agelasine G (**1a**, 0.01% yield, wet weight).

Agelasine G (**1a**) showed intense molecular ions at *m/z* 609 and 611 (M^+) in the positive ion fast atom bombardment mass spectrum (FAB-MS). The intensity ratio of these peaks (*ca.* 1 : 1) suggested that agelasine G (**1a**) contains one bromine atom in the molecule. High-resolution FAB-MS (HRFAB-MS) of the M^+ ion revealed the molecular formula $C_{31}H_{42}BrN_6O_2$ (*m/z* 609.2575, Δ +2.3 mmu). The ultraviolet (UV) spectrum showed an absorption maximum at 272 nm (ε 19000), which was analogous to those of agelasines A—F,[2] having a quaternary 9-methyladenine moiety. The electron impact mass spectrum (EI-MS) of **1a** exhibited a characteristic fragment ion at *m/z* 149 that is commonly observed in the EI-MS of agelasines A—F[2] and attributed to the quaternary 9-methyladenine unit. The presence of this unit in **1a** was further confirmed by comparison of the ^{1}H- and ^{13}C-nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectral data with those of 7,9-dimethyladeninium perchlorate[11] or the 9-methyladenine unit of agelasine A[2] [for **1a** δ_H: 8.55 (1H, s, H-2′), 11.28 (1H, s, H-8′), 4.12 (3H, s, CH_3-9′), and 6.29 (2H, br s, exchangeable, NH_2-6′); δ_C: 156.2 (d, C-2′), 149.7 (s, C-4′), 110.0 (s, C-5′), 152.5 (s, C-6′), 142.1 (d, C-8′), and 32.0 (q, CH_3-9′)]. The ^{1}H- and ^{13}C-NMR spectral data also revealed the presence of a 3-bromo-5-carboxypyrrole moiety (C_5H_3BrNO) [δ_H: 9.14 (1H, br s, exchangeable, NH-1″), 6.95 (1H, dd, H-2″), and 6.86 (1H, dd, H-4″); δ_C: 122.8 (d, C-2″), 97.8 (s, C-3″), 116.7 (s, C-4″), 123.4 (s, C-5″), and 160.2 (s, C-6″)] on the basis of comparison with the NMR data of hymenidin,[12] sceptrin,[3] or ageliferin.[1] The rest of the molecule, consisting of $C_{20}H_{32}O$, was inferred to be a diterpenoid from the ^{1}H- and ^{13}C-NMR spectral data, including the signals due to two olefins, one oxymethylene, one vinyl methyl, one secondary methyl, and two tertiary methyl groups. Assignments of the ^{1}H- and ^{13}C-NMR signals were made by application of two-dimensional NMR techniques (^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum connectivity (HMQC),[13] and heteronuclear multiple bond connectivity (HMBC)[14] experiments) and it was revealed that these NMR data correlated well to those of a bicyclic clerodane diterpenoid agelasine A.[2] The ^{13}C-NMR chemical shifts of C-19 (methyl on C-5, δ_C 34.7 q) and C-2 (δ_C 23.9 t) of **1a** implied that **1a** possesses a *cis*-fused clerodane skeleton.[15,16] The terminal grouping [–C(CH_3)=CH–CH_2–X] is the same as that commonly contained in agelasines A—F,[2] whereby the diterpenoid portion was connected to the N-7′ atom of the 9-methyladenine unit. This finding was verified by the ^{1}H–^{13}C long-range connectivities (H_2-15/C-5′ and H_2-15/C-8′) obtained from the HMBC spectrum. The high-field resonance of the vinylmethyl (C-16, δ_C 17.6 q) indicated the *E*-geometry of the C-13 double bond. The oxymethylene carbon (δ_C 66.4 t) was assigned to C-18, *viz.* attached to C-4, since the HMBC correlations for H_2-18/C-3, H_2-18/C-4, H_2-18/C-5, and H-3/C-18 were observed. The bromopyrrole unit was shown to be linked to C-18 through an ester bond based on the HMBC connectivity observed between the oxymethylene protons (H_2-18) and the ester carbonyl (C-6″). The presence of the 3-bromo-5-carboxypyrrole moiety was confirmed by the fact that hydrogenation



of agelasine G (**1a**) with H_2/Pd–C followed by acid hydrolysis (HCl/MeOH) afforded methyl pyrrole-2-carboxylate, whose ^{1}H-NMR data corresponded well to those in the literature.[16] From these observations the structure of agelasine G was deduced to be **1a**, which is closely related to ageline B (**2a**) previously isolated from the Palauan sponge *Agelas* sp.[17] as a mixture with ageline A. Since ageline B (**2a**) has been characterized as its formamide derivative (**2b**, a hydrolysis product) and no spectral data of the natural form of ageline B (**2a**) have been described, agelasine G (**1a**) was treated with 2 N sodium hydrogen carbonate solution at room temperature for 5 min to obtain the formamide (**1b**). The spectral data of **1b** corresponded well to those of the formamide (**2b**) derived from ageline B (**2a**).[17] The optical rotation of **1b** ($[\alpha]_D^{25}$ $-7.1°$ ($c=0.02$, $CHCl_3$)) was comparable to that of **2b** ($[\alpha]_D$ $-9.2°$ ($c=0.73$, $CHCl_3$)).[17] Thus, the absolute configuration of agelasine G (**1a**) was assigned to be the same as that of ageline B (**2a**), which was proposed to be antipodal to the terrestrial plant metabolite sagittariol.[15]

Agelasine G (**1a**) exhibited cytotoxic activity against murine lymphoma L1210 cells *in vitro* with the IC_{50} value of 3.1 μg/ml.

Experimental

General Methods Optical rotations were measured on a JASCO DIP-370 polarimeter. Infrared (IR) and UV spectra were recorded on a JASCO IR-A102 and a Shimadzu UV-220 spectrometer, respectively. ^{1}H- and ^{13}C-NMR spectra were obtained on a JEOL JNM GX-270 spectrometer. EI-MS and FAB-MS were taken on a JEOL DX-303 and an HX-110 spectrometer, respectively.

Collection, Extraction, and Separation The sponge *Agelas* sp. was collected off Konbu, Okinawa Island, in 1988 by SCUBA and was kept frozen until used. The sponge (0.5 kg, wet weight) was extracted with methanol (1 l × 2). This extract was partitioned between ethyl acetate (400 ml × 3) and water (400 ml) and the aqueous layer was subsequently extracted with 1-butanol (400 ml × 3). A part of the 1-butanol-soluble fraction (1.2 g) was subjected to C_{18} reversed-phase flash column chromatography (YMC-GEL ODS-A60-350/250, Yamamura Chemical, 25 × 330 mm) eluted with 80% MeOH containing 0.2 M NaCl, followed by C_{18} reversed-phase HPLC (YMC D-ODS-5, Yamamura Chemical, 20 × 250 mm; eluent, 80% MeOH containing 0.2 M NaCl; flow rate, 7 ml/min) to give agelasine G (**1a**, 0.01% yield, wet weight, t_R 31 min).

Agelasine G (**1a**): $[\alpha]_D^{27}$ $-85°$ ($c=0.02$, $CHCl_3$). UV λ_{max} MeOH nm (ε): 212 (24000), 272 (19000). IR (KBr): 3320, 3100, 2900, 1640, 1625, 1610, 1440, 1380, 1290, 1160 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ_H: 0.76 (3H, d, $J=6.6$ Hz, H_3-17), 0.79 (3H, s, H_3-19), 1.14 (3H, s, H_3-20), 1.91 (3H, s, H_3-16), 4.12 (3H, s, H_3C-N9′), 4.77 (2H, m, H_2-18), 5.48 (1H, br t, $J=7.7$ Hz, H-14), 5.61 (2H, br d, $J=7.7$ Hz, H_2-15), 5.75 (1H, m, H-3), 6.29 (2H, br s, exchangeable, NH_2-6′), 6.86 (1H, dd, $J=2.8$, 1.7 Hz, H-4″), 6.95 (1H, dd, $J=3.3$, 1.7 Hz, H-2″), 8.55 (1H, s, H-2′), 9.14 (1H, m, exchangeable, NH-1″), 11.29 (1H, s, H-8′). ^{13}C-NMR ($CDCl_3$) δ_C: 17.4 (t, C-1), 23.9 (t, C-2), 129.7 (d, C-3), 138.2 (s, C-4), 36.3 (s, C-5), 36.1 (t, C-6), 28.8 (t, C-7), 37.5 (d, C-8), 40.2 (s, C-9), 45.2 (d, C-10), 37.1 (t, C-11), 32.9 (t, C-12), 147.6 (s, C-13), 115.9 (d, C-14), 48.8 (t, C-15), 17.6 (q, C-16), 16.0 (q, C-17), 66.8 (t, C-18), 34.7 (q, C-19), 17.3 (q, C-20), 156.2 (d, C-2′), 149.7 (s, C-4′), 110.0 (s, C-5′), 152.5 (s, C-6′), 142.1 (d, C-8′), 32.0 (q, CH_3-N9′), 122.8 (d, C-2″), 97.8 (s, C-3″), 116.7 (s, C-4″), 123.4 (s, C-5″), 160.2 (s, C-6″). HMBC correlations (C/H): C-3/H_2-18, C-4/H_2-18, C-4/H_3-19, C-5/H-3, C-5/H_2-18, C-5/H_3-19, C-7/H_3-17, C-9/H_3-17, C-9/H_3-20, C-10/H_3-19, C-10/H_3-20, C-12/H-14, C-12/H_3-16, C-13/H_2-15, C-13/H_3-16, C-14/H_2-15, C-14/H_3-16, C-16/H-14, C-18/H-3, C-4′/H-2′, C-4′/H-8′, C-4′/H_3C-N9′, C-5′/H-8′, C-5′/H-15, C-6′/H-2′, C-8′/H-15, C-8′/H_3C-N9′, C-2″/H-4″, C-3″/H-2″, C-4″/H-2″, C-5″/H-2″, C-6″/H_2-18. FAB-MS (positive ion, 3-nitrobenzyl alcohol matrix) *m/z*: 611, 609 (M^+), 436, 422. EI-MS *m/z*: 461, 459 ($M^+ -$9-methyladenine), 446, 444, 380, 378, 364, 362, 306, 189, 149 (base peak). HRFAB-MS found *m/z*: 609.2527, Calcd for $C_{31}H_{42}{}^{79}BrN_6O_2$ (M): 609.2552.

Methyl Pyrrole-2-carboxylate Agelasine G (**1a**, 1.0 mg) in methanol was reduced catalytically with 10% Pd–C under a hydrogen atmosphere at room temperature for 3 h. After filtration of the reaction mixture to remove the catalyst the filtrate was treated with 1% HCl in MeOH (2 ml) at room temperature for 3 h to afford, by usual work-up, methyl pyrrole-2-carboxylate.[16] ^{1}H-NMR ($CDCl_3$) δ: 3.90 (3H, s, $-$OMe), 6.18 (1H, m, H-3), 6.82 (1H, m, H-4), 6.95 (1H, m, H-2).

Formamide (1b) Agelasine G (**1a**, 12.6 mg) was treated with 2 N sodium hydrogen carbonate aqueous solution at room temperature for 5 min. The reaction mixture was extracted with dichloromethane, and evaporation of the solvent afforded the formamide (**1b**, 4.7 mg), $[\alpha]_D^{25}$ $-7.1°$ ($c=0.02$, $CHCl_3$). UV λ_{max}^{MeOH} nm (ε): 271 (6900). IR (KBr): 3400, 2900, 1660, 1590, 1450, 1380, 1280, 1180, 1120 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ_H: 0.75 (3H, d, $J=7.1$ Hz, H_3-17), 0.77 (3H, s, H_3-20), 1.14 (3H, s, H_3-19), 1.63 (3H, s, H_3-16), 2.98 (3H, d, $J=5.0$ Hz, H_3C-N9′), 4.15 (2H, d, $J=7.1$ Hz, H_2-15), 4.74 (2H, br s, NH_2-6′), 4.76 (2H, m, H_2-18), 5.36 (1H, br t, $J=7.1$ Hz, H-14), 5.74 (1H, br s, H-3), 6.86 (1H, d, $J=1.1$ Hz, H-4″), 6.94 (1H, d, $J=1.1$ Hz, H-2″), 7.98 (1H, s, H-8′), 8.16 (1H, s, H-2′), 9.35 (1H, br s, NH-1″). FAB-MS *m/z*: 629, 627 $(M+H)^+$, 611, 609 $(M-H_2O+H)^+$. EI-MS *m/z*: 628, 626 (M^+), 167 (base peak).

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