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 alkaloids¹⁾ 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 C₁₈ reversed-phase flash column chromatography (eluent: 80% MeOH containing 0.2 M NaCl), followed by C₁₈ 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 C₃₁H₄₂BrN₆O₂ (*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–F²⁾ and attributed to the quaternary 9-methyladenine unit. The presence of this unit in 1a was further confirmed by comparison of the ¹H- and ¹³C-nuclear magnetic resonance (¹H- and ¹³C-NMR) spectral data with those of 7,9-dimethyladeninium perchlorate¹¹⁾ or the 9-methyladenine unit of agelasine A²⁾ [for 1a δ_H: 8.55 (1H, s, H-2'), 11.28 (1H, s, H-8'), 4.12 (3H, s, CH₃-9'), and 6.29 (2H, br s, exchangeable, NH₂-6'); 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, CH₃-9')]. The ¹H- and ¹³C-NMR spectral data also revealed the presence of a 3-bromo-5-carboxypyrrole moiety (C₅H₃BrNO) [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 C₂₀H₃₂O, was inferred to be a diterpenoid from the ¹H- and ¹³C-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 ¹H- and ¹³C-NMR signals were made by application of two-dimensional NMR techniques (¹H–¹H 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 ¹³C-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(CH₃)=CH–CH₂–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 ¹H–¹³C long-range connectivities (H₂-15/C-5' and H₂-15/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 H₂-18/C-3, H₂-18/C-4, H₂-18/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 (H₂-18) 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 1H -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 2N 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** ($[\alpha]_D^{25} -7.1^\circ$ ($c=0.02$, $CHCl_3$)) was comparable to that of **2b** ($[\alpha]_D^{25} -9.2^\circ$ ($c=0.73$, $CHCl_3$)).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 $\mu g/ml$.

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. 1H - 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 $\times$ 2). This extract was partitioned between ethyl acetate (400 ml $\times$ 3) and water (400 ml) and the aqueous layer was subsequently extracted with 1-butanol (400 ml $\times$ 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 $\times$ 330 mm) eluted with 80% MeOH containing 0.2M NaCl, followed by C_{18} reversed-phase HPLC (YMC D-ODS-5, Yamamura Chemical, 20 $\times$ 250 mm; eluent, 80% MeOH containing 0.2M NaCl; flow rate, 7 ml/min) to give agelasine G (**1a**, 0.01% yield, wet weight, t_R 31 min).

Agelasine G (**1a**): $[\alpha]_D^{27} -85^\circ$ ($c=0.02$, $CHCl_3$). UV λ_{max} MeOH nm (ϵ): 212 (24000), 272 (19000). IR (KBr): 3320, 3100, 2900, 1640, 1625, 1610, 1440, 1380, 1290, 1160 cm^{-1} . 1H -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, brt, $J=7.7$ Hz, $H-14$), 5.61 (2H, brd, $J=7.7$ Hz, H_2-15), 5.75 (1H, m, $H-3$), 6.29 (2H, brs, 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'/ $H-8'$, 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 (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 1H -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 2N 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). $[\alpha]_D^{25} -7.1^\circ$ ($c=0.02$, $CHCl_3$). UV λ_{max}^{MeOH} nm (ϵ): 271 (6900). IR (KBr): 3400, 2900, 1660, 1590, 1450, 1380, 1280, 1180, 1120 cm^{-1} . 1H -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, brs, NH_2-6'), 4.76 (2H, m, H_2-18), 5.36 (1H, brt, $J=7.1$ Hz, $H-14$), 5.74 (1H, brs, $H-3$), 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, brs, $NH-1''$). FAB-MS m/z : 629, 627 ($M+H^+$), 611, 609 ($M-H_2O+H^+$). EI-MS m/z : 628, 626 (M^+), 167 (base peak).

Acknowledgements We thank Mr. Z. Nagahama for his help in collecting the marine sponge and Dr. J. Fromont, James Cook University of North Queensland, for identification of the sponge. This study was supported in part by a Grant-in-Aid for Cancer Research (02151037) from the Ministry of Education, Science, and Culture, Japan.

References and Notes

- 1) J. Kobayashi, M. Tsuda, T. Murayama, H. Nakamura, Y. Ohizumi, M. Ishibashi, M. Iwamura, T. Ohta, and S. Nozoe, *Tetrahedron*, **46**, 5579 (1990) and references cited therein.
- 2) H. Wu, H. Nakamura, J. Kobayashi, M. Kobayashi, Y. Ohizumi, and Y. Hirata, *Bull. Chem. Soc. Jpn.*, **59**, 2495 (1986) and references cited therein.
- 3) R. P. Walker, D. J. Faulkner, D. Van Engen, and J. Clardy, *J. Am. Chem. Soc.*, **101**, 6772 (1981).
- 4) H. Nakamura, H. Wu, J. Kobayashi, Y. Ohizumi, Y. Hirata, T. Higashijima, and T. Miyazawa, *Tetrahedron Lett.*, **24**, 4105 (1983).
- 5) H. Nakamura, Y. Ohizumi, J. Kobayashi, and Y. Hirata, *Tetrahedron Lett.*, **25**, 2475 (1984).
- 6) H. Nakamura, H. Wu, Y. Ohizumi, and Y. Hirata, *Tetrahedron Lett.*, **25**, 2989 (1984).
- 7) H. Wu, H. Nakamura, J. Kobayashi, Y. Ohizumi, and Y. Hirata, *Tetrahedron Lett.*, **25**, 3719 (1984).
- 8) J. Kobayashi, M. Tsuda, and Y. Ohizumi, *Experientia*, **47**, 301 (1991).
- 9) a) J. Kobayashi, T. Murayama, S. Kosuge, F. Kanda, M. Ishibashi, H. Kobayashi, Y. Ohizumi, T. Ohta, and S. Nozoe, *J. Chem. Soc., Perkin Trans. 1*, **1990**, 3301; b) J. Kobayashi, T. Murayama, M. Ishibashi, S. Kosuge, M. Takamatsu, Y. Ohizumi, H. Kobayashi, T. Ohta, S. Nozoe, and T. Sasaki, *Tetrahedron*, **46**, 7699 (1990); c) Y. Kikuchi, M. Ishibashi, T. Sasaki, and J. Kobayashi, *Tetrahedron Lett.*, **32**, 797 (1991); d) M. Tsuda, M. Ishibashi, K. Agemi, T. Sasaki, and J. Kobayashi, *Tetrahedron*, **47**, 2181 (1991); e) J. Kobayashi, J.-F. Cheng, M. Ishibashi, M. R. Wälchli, S. Yamamura, and Y. Ohizumi, *J. Chem. Soc., Perkin Trans. 1*, **1991**, 1135; f) J. Kobayashi, F. Kanda, M. Ishibashi, and H. Shigemori, *J. Org. Chem.*, **56**, 4574 (1991).
- 10) The sponge of *Agelas* sp. used in this study is a carverous sponge with a dermal skin and medium brown color. It is firm and compressible and has a dense internal skeleton. The skeleton is reticulate with echinating verticillate acanthostyles and smooth styles coring the fibres. The acanthostyles are $206 \times 14.5 \mu m$, range $162-252 \times 8-18 \mu m$.
- 11) T. Fujii, F. Tanaka, K. Mohri, T. Itaya, and T. Saito, *Tetrahedron Lett.*, **1973**, 4873.
- 12) J. Kobayashi, Y. Ohizumi, H. Nakamura, and Y. Hirata, *Experientia*, **42**, 1176 (1986).
- 13) A. Bax and S. Subramanian, *J. Magn. Reson.*, **67**, 565 (1986).
- 14) A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, **108**, 2093 (1986).
- 15) Sagittariol acetate (*cis*-fused clerodane): δ_C 34.4 q (C-19) and 23.7 t (C-2); hardwickiilol (*trans*-fused clerodane): δ_C 21.2 q (C-19) and 18.0 t (C-2); S. C. Sharma, J. S. Tandon, B. Porter, M. S. Raju, and E. Wenkert, *Phytochemistry*, **23**, 1194 (1984).
- 16) M. P. Edwards, A. M. Doherty, S. V. Ley, and H. M. Organ, *Tetrahedron*, **42**, 3723 (1986).
- 17) R. J. Capon and D. J. Faulkner, *J. Am. Chem. Soc.*, **106**, 1819 (1984).