Botryllamides K and L, new tyrosine derivatives from the Australian ascidian *Aplidium altarium*

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Graphical Abstract

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Abstract— The chemical investigation of the Australian ascidian *Aplidium altarium* led to the isolation of two new tyrosine derivatives, botryllamides K (**1**) and L (**2**), together with six known metabolites, botryllamides A–C (**3–5**), botryllamide G (**6**), and perspicamides A (**7**) and B (**8**). The structures of these compounds were elucidated by spectroscopic analysis. This is the first reported chemistry from this species. Compounds **1–8** were evaluated for their cytotoxicity towards the tumour cell lines, MCF-7 (breast), H460 (lung) and SF268 (central nervous system).

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Ascidians from the genus *Aplidium* have proved to be a rich source of nitrogenous metabolites with a variety of structural types. The first *Aplidium* metabolite to be identified was aplidiasphingosine, which was isolated from an *Aplidium* sp. collected in the Gulf of California in 1978.¹ Since then more than 70 nitrogenous compounds from this genus have been reported, including nucleosides,²⁻⁵ amino acid derivatives,^{6,7} macrocyclic compounds,^{8,9} thiazoles,¹⁰ imidazoles,^{10,11} pyridoacridines,³ indol-pyrimidines,¹² and piperidines.^{13,14} Most of these compounds have been reported to possess cytotoxicity. Among them, dehydridemnin B (DDB or aplidine) from *A. albicans* is one of the most renowned ascidian natural products as it made antitumor phase II clinical trials.¹⁵⁻¹⁷ As a part of our ongoing research into the discovery of new and/or bioactive natural products from Australian ascidians,¹⁸⁻²⁰ two new tyrosine derivatives, botryllamides K (**1**) and L (**2**), together with six known metabolites **3-8**, were isolated from *Aplidium altarium* (Fig. 1). Compounds **1-8** were evaluated for their cytotoxicity towards the tumour cell lines, MCF-7 (breast), H460 (lung) and SF268 (central nervous system). This is the first reported chemistry from *A. altarium*. Herein, we report the isolation, structural elucidation as well as cytotoxicity of compounds **1-8**.

Figure 1. Structures of compounds **1-8**

The ascidian *Aplidium altarium* Sluiter 1909 (Order: Enterogona, Family: Polyclinidae) was collected by scuba diving at a depth of 7.8 m at Double Rock, Elliott Heads, Queensland, Australia, in October 2000. A voucher sample, QMG317322, was lodged at the Queensland Museum, South Brisbane, Queensland, Australia.

The CHCl₃/MeOH extract from the ground and freeze-dried *A. altarium* was initially fractionated by reverse-phase C₁₈ HPLC (MeOH/H₂O/0.1% TFA) to give 60 fractions. Fractions 31-39 were found to possess interesting NMR signals and were further purified by UV-guided isolation using C₁₈ HPLC to yield botryllamides K (**1**, 1.7 mg, 0.043%), L (**2**, 2.6 mg, 0.065%), A (**3**, 8.2 mg, 0.210%), B (**4**, 4.4 mg, 0.110%), C (**5**, 6.4 mg, 0.160%),

and G (**6**, 6.6 mg, 0.170%), and perspicamides A (**7**, 1.6 mg, 0.040%) and B (**8**, 2.4 mg, 0.060%).

Compound **1**²¹ was isolated as a yellow powder. The (+)-LRESIMS showed an isotopic cluster of [M+H]⁺ ions in the ratio of 1:1 at *m/z* 390 and 392, indicating the presence of one bromine atom. The (+)-HRESIMS at *m/z* 412.0175 [M+Na]⁺ allowed the molecular formula C₁₈H₁₆BrNO₄ to be assigned for **1**. Only 16 resonances were observed in the ¹³C NMR spectrum (Table 1) of **1**, indicating the presence of symmetry in some portion of the molecule. The presence of a *para*-substituted phenyl ring (accounting for the element of symmetry) was indicated by the observation of two coupled aromatic doublets at δ_H 6.80 (2H, *J* = 8.6 Hz) and 7.57 (2H, *J* = 8.6 Hz) and two aromatic methine carbon signals at δ_C 115.6 (2C) and 131.4 (2C). HMBC correlations (Fig. 2) from both of the aromatic methine doublets and an exchangeable phenolic proton singlet at δ_H 9.81 to an aromatic oxygenated quaternary carbon at δ_C 158.0 indicated that this benzene ring was a 4-hydroxyphenyl group. A 1,2,4-trisubstituted benzene ring was suggested by the coupling pattern observed for three downfield aromatic methine protons at δ_H 7.43 (1H, d, *J* = 1.6 Hz), 6.88 (1H, d, *J* = 8.4 Hz), and 7.20 (1H, dd, *J* = 8.4, 1.6 Hz). A *trans*-enamide group was supported by the presence of two olefinic protons at δ_H 7.33 (1H, dd, *J* = 14.6, 10.1 Hz) and 6.39 (1H, d, *J* = 14.6 Hz) and by coupling of the former proton to an exchangeable amide proton at δ_H 10.22. The *trans* geometry of the enamide was indicated by a large coupling constant (*J* = 14.6 Hz) between the two olefinic protons. The HMBC correlation from the amide proton (NH) to a conjugated carbonyl carbon at δ_C 161.3 confirmed the presence of the amide unit. The *trans*-enamide unit was directly attached to the 1,2,4-trisubstituted benzene ring since HMBC correlations were observed from the olefinic proton at δ_H 6.39 to two aromatic methine carbons at δ_C 129.4 and 125.4, and a quaternary aromatic carbon at δ_C 129.5. ROESY correlations from an exchangeable phenolic proton (δ_H 10.16) to H-16, from H-16 to H-17, and from H-11 to H-17 located a hydroxy group at C-15 of the 1,2,4-trisubstituted benzene ring. This left the only position, C-14, to place the bromine atom that was implied by the molecular formula. The remaining ¹H and ¹³C signals were attributed to an enol methyl ether moiety since a downfield proton (δ_H 6.79, H-3) and methoxy group protons [δ_H 3.60 (3H, s)] both showed HMBC correlations to an sp² quaternary carbon at δ_C 146.2 (C-2).²² The *Z* geometry of Δ² was indicated by the characteristic ¹³C chemical shift of C-3 at δ_C 119.8, as C-3 of botryllamides with *Z* geometry is known to resonate at *ca.* δ_C 120 while C-3 of botryllamides with *E* geometry resonates δ_C <110.^{22,23} This was further supported by the ROESY correlations between H-9/H-5 and 2-OMe (Fig. 2). The HMBC correlations from H-3 to C-1 and C-4 incorporated the enol methyl ether group between the amide unit and the *para*-substituted ring and finally led to the proposed structure for botryllamide K (**1**).

Figure 2. Key HMBC (→) and ROESY (↔) correlations of **1**

Compound **2**²⁴ was isolated as a pale white powder. The (+)-HRESIMS displayed a pseudo-molecular ion at m/z 216.0621 $[M+Na]^+$ (calcd, 216.0637) consistent with a molecular formula of $C_{10}H_{11}NO_3$. This was supported by the (-)-LRESIMS at m/z 192 $[M-H]^-$, 385 $[2M-H]^-$, and 578 $[3M-H]^-$. The 1H NMR spectrum of **2** exhibited signals for a *para*-substituted phenyl ring [δ_H 7.51 (2H, d, J = 8.6 Hz) and 6.77 (2H, d, J = 8.6 Hz)], a methoxy group [δ_H 3.56 (3H, s)], a downfield olefinic proton [δ_H 6.66 (1H, s)], and three exchangeable protons [δ_H 9.71 (s), 7.51 (s), and 7.24 (br s)]. The ^{13}C NMR spectrum of **2** revealed 8 unique carbon resonances, which were assigned to a *para*-substituted phenyl ring [δ_C 124.4, 131.0 (2C), 115.5 (2C), and 157.6], an enol methyl ether group with a *Z* geometry [δ_C 147.3, 118.3, 58.4], and an amide carbonyl (δ_C 165.4). The aforementioned NMR data were very similar to those arising from the C-1 to C-9 part of **1**, indicating that **2** contained one of the modified tyrosine residues in **1**. The presence of the enol methyl ether group in **2** was further supported by an HMBC correlation from the methoxy group (δ_H 3.56) to the quaternary sp^2 carbon at δ_C 147.3 (C-2). 1H - 1H COSY correlations between the two exchangeable protons at δ_H 7.51 and 7.24 revealed that **2** contained a primary amide moiety. This was further supported by the strong IR absorptions at 1667 and 1614 cm^{-1} . Thus, the structure of **2** was assigned as botryllamide L. Since enamides are known to undergo hydrolysis to amides and carbonyl compounds under acidic conditions,²⁵ we speculated that compound **2** might be a botryllamide degradation product. In order to determine whether **2** was an artifact from the isolation process or a natural product, a small portion of botryllamide C (**5**, 1 mg) was dissolved in MeOH/H₂O/0.1% TFA (400 μ L) and heated at 50 °C for 2 h. Subsequent analysis of the reaction solution by TLC and LC-MS showed that no reaction had occurred. Thus, botryllamides appear to be stable under mildly acidic isolation conditions and botryllamide L (**2**) is a genuine natural product.

Table 1. NMR data for botryllamides K (**1**) and L (**2**) in DMSO- d_6 ^a

no	1		2	
	δ_H (mult, J , Hz)	δ_C	δ_H (mult, J , Hz)	δ_C
1		161.3		165.4
2		146.2		147.3
3	6.79 (s)	119.8	6.66 (s)	118.3
4		124.1		124.4
5	7.57 (d, 8.6)	131.4	7.51 (d, 8.6)	131.0
6	6.80 (d, 8.6)	115.6	6.77 (d, 8.6)	115.5
7		158.0		157.6
8	6.80 (d, 8.6)	115.6	6.77 (d, 8.6)	115.5
9	7.57 (d, 8.6)	131.4	7.51 (d, 8.6)	131.0
10	7.33 (dd, 14.6, 10.1)	122.4		
11	6.39 (d, 14.6)	112.0		
12		129.5		
13	7.43 (d, 1.6)	129.4		
14		109.6		
15		152.5		
16	6.88 (d, 8.4)	116.7		
17	7.20 (dd, 8.4, 1.6)	125.4		
2-OMe	3.60 (s)	58.9	3.56 (s)	58.4
7-OH	9.81 (s)		9.71 (s)	
15-OH	10.16 (s)			
NH	10.22 (d, 10.1)		NH ₂ 7.24 (br s)	
			7.51 (s)	

^a 1H and ^{13}C NMR were recorded at 600 and 125 MHz, respectively.

Six other compounds were purified during these chemical investigations and were identified as botryllamides A–C (**3**–**5**)²² and G (**6**),²⁶ and perspicamides A (**7**) and B (**8**)¹⁸ following comparison of NMR and MS data with literature values.

Compounds **1**–**8** were tested for their cytotoxicity against the tumour cell lines, MCF-7 (breast), H460 (lung) and SF268 (central nervous system).^{27,28} Initial dosing at 10 μ M for 72 h for all compounds showed no significant growth inhibition towards any of the cancer cell lines. The results are presented as the mean percent cell growth $\pm$ relative standard error of five replicates from a single experiment (Table 2). No biological activity has previously been reported for **7** and **8**. Some of the previously reported botryllamides have exhibited weak cytotoxicity against the human colon cancer cell line HCT-116 (IC_{50} values ranging from 17 to 110 μ M).^{22,26} More recently, botryllamide G has been reported as a potent inhibitor of the membrane-localized human transporter protein ABCG2.²³

Table 2. The cytotoxic activity of compounds **1-8**

Compound	Percent cell growth at 10 μ M $\pm$ relative standard error		
	H460	MCF-7	SF268
1	87 $\pm$ 7	74 $\pm$ 3	78 $\pm$ 5
2	89 $\pm$ 8	91 $\pm$ 3	93 $\pm$ 6
3	73 $\pm$ 11	85 $\pm$ 3	84 $\pm$ 4
4	71 $\pm$ 8	80 $\pm$ 5	78 $\pm$ 3
5	75 $\pm$ 10	88 $\pm$ 3	75 $\pm$ 6
6	86 $\pm$ 6	89 $\pm$ 4	81 $\pm$ 5
7	94 $\pm$ 7	86 $\pm$ 3	88 $\pm$ 4
8	81 $\pm$ 8	93 $\pm$ 4	66 $\pm$ 3

In conclusion, two new tyrosine derivatives, botryllamides **K (1)** and **L (2)**, together with six known metabolites **3-8**, were isolated from the Australian ascidian *Aplidium altarium*. This is the first reported chemistry from this species. Compounds **1-8** were evaluated for their cytotoxicity towards the tumour cell lines, MCF-7 (breast), H460 (lung) and SF268 (central nervous system).

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Supplementary data

Supplemental data (^1H and ^{13}C NMR spectra for botryllamides **K (1)** and **L (2)**, general experimental details, ascidian collection details, extraction and isolation procedure for **1-8**, cytotoxicity assay) associated with this article can be found in the online version, at doi:

References and notes

- Carter, G. T.; Rinehart, K. L. *J. Am. Chem. Soc.* **1978**, *100*, 7441-7442.
- Dematte, N.; Guerriero, A.; Lafargue, F.; Pietra, F. *Comp. Biochem. Physiol., B: Biochem. Mol. Biol.* **1986**, *84*, 11-13.
- Kim, J. W.; Pordesimo, E. O.; Toth, S. I.; Schmitz, F. J.; Vanaltena, I. *J. Nat. Prod.* **1993**, *56*, 1813-1816.
- Kobayashi, J.; Doi, Y.; Ishibashi, M. *J. Org. Chem.* **1994**, *59*, 255-257.
- Doi, Y.; Ishibashi, M.; Kobayashi, J. *Tetrahedron* **1994**, *50*, 8651-8656.
- Schmitz, F. J.; Yasumoto, T. *J. Nat. Prod.* **1991**, *54*, 1469-1490.
- Carroll, A. R.; Bowden, B. F.; Coll, J. C. *Aust. J. Chem.* **1993**, *46*, 825-832.
- Galini, D. L.; McKee, T. C.; Pannell, L. K.; Cardellina, J. H.; Boyd, M. R. *J. Org. Chem.* **1997**, *62*, 8968-8969.
- McKee, T. C.; Galini, D. L.; Pannell, L. K.; Cardellina, J. H.; Laakso, J.; Ireland, C. M.; Murray, L.; Capon, R. J.; Boyd, M. R. *J. Org. Chem.* **1998**, *63*, 7805-7810.
- Arabshahi, L.; Schmitz, F. J. *Tetrahedron Lett.* **1988**, *29*, 1099-1102.
- Copp, B. R.; Blunt, J. W.; Munro, M. H. G.; Pannell, L. K. *Tetrahedron Lett.* **1989**, *30*, 3703-3706.
- Franco, L. H.; Joffe, E. B. K.; Puricelli, L.; Tatian, M.; Seldes, A. M.; Palermo, J. A. *J. Nat. Prod.* **1998**, *61*, 1130-1132.
- McCoy, M. C.; Faulkner, D. J. *J. Nat. Prod.* **2001**, *64*, 1087-1089.
- Garrido, L.; Zubia, E.; Ortega, M. J.; Salva, J. *J. Org. Chem.* **2003**, *68*, 293-299.
- Depenbrock, H.; Peter, R.; Faircloth, G. T.; Manzanera, I.; Jimeno, J.; Hanauske, A. R. *Br. J. Cancer* **1998**, *78*, 739-744.
- Urdiales, J. L.; Morata, P.; DeCastro, I. N.; Sanchez-Jimenez, F. *Cancer Lett.* **1996**, *102*, 31-37.
- Cardenas, F.; Thormann, M.; Feliz, M.; Caba, J. M.; Lloyd-Williams, P.; Giralt, E. *J. Org. Chem.* **2001**, *66*, 4580-4584.
- McKay, M. J.; Carroll, A. R.; Quinn, R. J. *J. Nat. Prod.* **2005**, *68*, 1776-1778.
- Davis, R. A.; Carroll, A. R.; Quinn, R. J. *J. Nat. Prod.* **2002**, *65*, 454-457.
- Davis, R. A.; Carroll, A. R.; Quinn, R. J. *J. Nat. Prod.* **1999**, *62*, 158-160.
- Botryllamide **K (1)**: yellow powder (1.7 mg, 0.043%); UV (MeOH) λ_{max} (log ϵ) 336 (4.31), 225 (4.11) nm; IR (KBr) ν_{max} 3294, 1700, 1647, 1604, 1509, 1407 cm^{-1} ; ^1H (600 MHz) and ^{13}C (150 MHz) NMR, see Table 1; (+)-LRESIMS m/z (35 eV) (rel. int.) 390 [$\text{C}_{18}\text{H}_{17}^{79}\text{BrNO}_4$] $^+$ (75), 392 [$\text{C}_{18}\text{H}_{17}^{81}\text{BrNO}_4$] $^+$ (100), 412 [$\text{C}_{18}\text{H}_{16}^{79}\text{BrNO}_4\text{Na}$] $^+$ (30), 414 [$\text{C}_{18}\text{H}_{16}^{81}\text{BrNO}_4\text{Na}$] $^+$ (20), (+)-HRESIMS m/z 412.0175 (calcd for $\text{C}_{18}\text{H}_{16}^{79}\text{BrNO}_4\text{Na}$, 412.0160).
- McDonald, L. A.; Swersey, J. C.; Ireland, C. M.; Carroll, A. R.; Coll, J. C.; Bowden, B. F.; Fairchild, C. R.; Cornell, L. *Tetrahedron* **1995**, *51*, 5237-5244.
- Henrich, C. J.; Robey, R. W.; Takada, K.; Bokesch, H. R.; Bates, S. E.; Shukla, S.; Ambudkar, S. V.; McMahon, J. B.; Gustafson, K. R. *ACS Chem. Biol.* **2009**, *4*, 637-647.
- Botryllamide **L (2)**: pale white powder (2.6 mg, 0.065%); UV (MeOH) λ_{max} (log ϵ) 297 (4.12), 220 (3.88) nm; IR (KBr) ν_{max} 3323, 3175, 1667, 1614, 1508, 1416 cm^{-1} ; ^1H (600 MHz) and ^{13}C (150 MHz) NMR, see Table 1; (-)-LRESIMS m/z (35 eV) (rel. int.) 177 (100), 192 [M-H] $^-$ (32), 385 [2M-H] $^-$ (10), 578 [3M-H] $^-$ (3); (+)-HRESIMS m/z 216.0621 (calcd for $\text{C}_{10}\text{H}_{11}\text{NO}_3\text{Na}$, 216.0637).
- Csizmadia, V. M.; Koshy, K. M.; Lau, K. C. M.; McClelland, R. A.; Nowlan, V. J.; Tidwell, Thomas T. *J. Am. Chem. Soc.* **1979**, *101*, 974-979.
- Rao, M. R.; Faulkner, D. J. *J. Nat. Prod.* **2004**, *67*, 1064-1066.
- Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107-1112.
- Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigrowolf, A.; Graygoodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757-766.