

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

Author

Yin, Sheng, Cullinane, Carleen, Carroll, Anthony R, Quinn, Ronald J, Davis, Rohan A

Published

2010

Journal Title

Tetrahedron Letters

Version

Accepted Manuscript (AM)

DOI

[10.1016/j.tetlet.2010.04.104](https://doi.org/10.1016/j.tetlet.2010.04.104)

Rights statement

© 2010 Elsevier. Licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International Licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, providing that the work is properly cited.

Downloaded from

<http://hdl.handle.net/10072/36763>

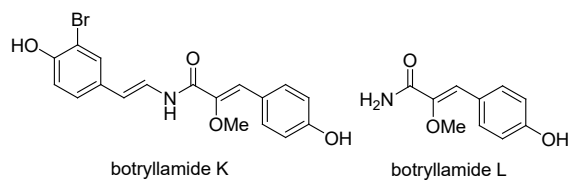
Griffith Research Online

<https://research-repository.griffith.edu.au>

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

Leave this area blank for abstract info.

Sheng Yin, Carleen Cullinane, Anthony R. Carroll, Ronald J. Quinn, Rohan A. Davis



Graphical Abstract

Sheng Yin,^a Carleen Cullinane,^b Anthony R. Carroll,^a Ronald J. Quinn,^a and Rohan A. Davis^{a*}

^a*Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia*

^b*Peter MacCallum Cancer Centre, St. Andrew's Place, East Melbourne, VIC 3002, Australia*

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).

© 2021 Elsevier Science. All rights reserved

* Corresponding author. Tel.: +61-7-3735-6043; fax: +61-7-3735-6001; e-mail: r.davis@griffith.edu.au.

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, dehydrididemnin 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**.

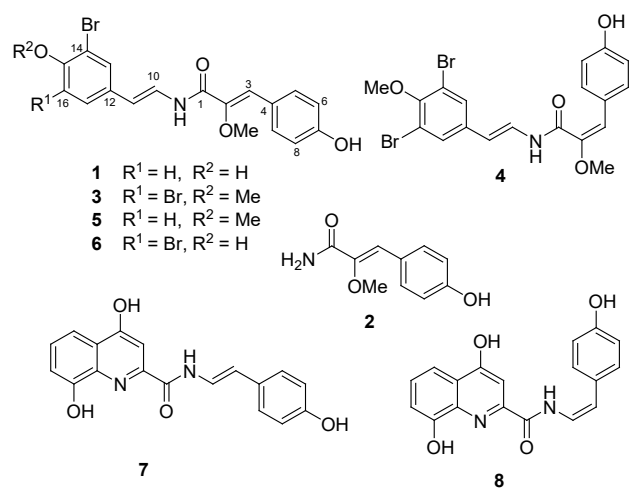


Figure 1. Structures of compounds **1-8**

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**).

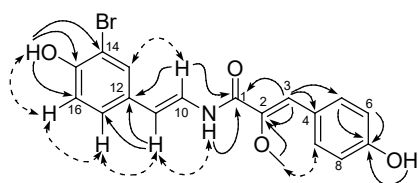


Figure 2. Key HMBC (\rightarrow) and ROESY (\curvearrowright) correlations of **1**

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 (IC₅₀ 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).

Acknowledgements

We thank Hoan The Vu from Griffith University for acquiring the HRESIMS measurements. Alison Slater is acknowledged for technical assistance in performing the cell culture assays. We would also like to thank John N. A. Hooper from the Queensland Museum for ascidian collection and identification.

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.