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

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ABSTRACT

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).

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] indolo-pyrimidines[12] and piperidines.[13,14] Most of these compounds have been reported to possess cytotoxicity. Among them, dehydrodidemnin B (DDB or aplidine) from *Aplidium 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 and cytotoxicity of compounds **1**–**8**.

The ascidian *A. 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 $CHCl_3$/MeOH extract from the ground and freeze-dried *A. altarium* was initially fractionated by reverse-phase C_{18} HPLC (MeOH/H_2O/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 C_{18} 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 $C_{18}H_{16}BrNO_4$ to be assigned for **1**. Only 16 resonances were observed in the ^{13}C 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

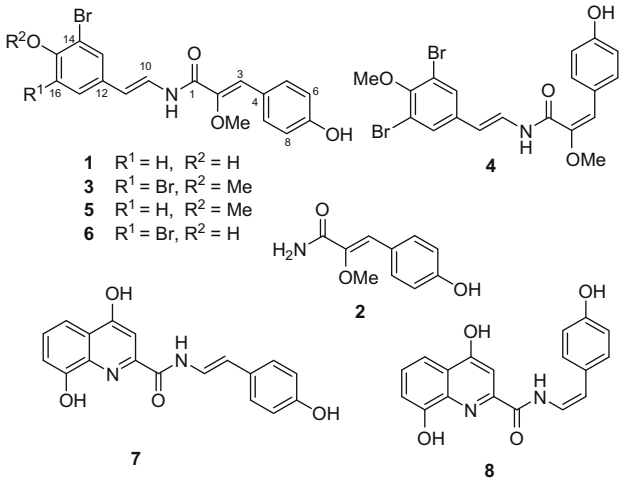

Figure 1. Structures of compounds **1**–**8**.

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

No.	**1**			**2**	
	δ_H (m, *J*, Hz)	δ_C		δ_H (m, *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] ^{1}H and ^{13}C NMR were recorded at 600 and 125 MHz, respectively.

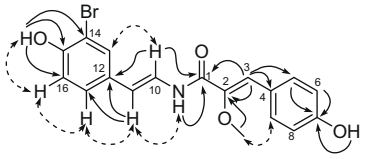

Figure 2. Key HMBC (→) and ROESY (⋯) correlations of **1**.

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 (*N*H) 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 ^{1}H and ^{13}C signals were attributed to an enol methyl ether moiety since both a downfield proton (δ_H 6.79, H-3) and methoxy group protons [δ_H 3.60 (3H, s)] showed HMBC correlations to an sp^2 quaternary carbon at δ_C 146.2 (C-2).[22] The *Z* geometry of Δ^2 was indicated by the characteristic ^{13}C 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**).

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 $[2\,M-H]^-$ and 578 $[3\,M-H]^-$. The ^{1}H 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 eight 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). ^{1}H–^{1}H 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 artefact from the isolation process or a natural product, a small portion of botryllamide C (**5**, 1 mg) was dissolved in MeOH/H_2O/0.1% TFA (400 μL) 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.

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 the 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 μM 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 ± relative standard error of five replicates from a single

Table 2
The cytotoxic activity of compounds **1**–**8**

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

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 μM).[22,26] More recently, botryllamide G has been reported as a potent inhibitor of the membrane-localized human transporter protein ABCG2.[23]

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 *A. 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

Supplementary 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:10.1016/j.tetlet.2010.04.104.

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21. *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 $[C_{18}H_{17}{}^{79}BrNO_4]^+$ (75), 392 $[C_{18}H_{17}{}^{81}BrNO_4]^+$ (100), 412 $[C_{18}H_{16}{}^{79}BrNO_4Na]^+$ (30), 414 $[C_{18}H_{16}{}^{81}BrNO_4Na]^+$ (20), (+)-HRESIMS *m/z* 412.0175 (calcd for $C_{18}H_{16}{}^{79}BrNO_4Na$, 412.0160).
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