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Aplicyanins A–F, new cytotoxic bromoindole derivatives from the marine tunicate *Aplidium cyaneum*

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ABSTRACT

Six new bromoindole derivatives, aplicyanins A–F (**1**–**6**), have been isolated from the CH_2Cl_2/MeOH extract of the tunicate *Aplidium cyaneum* collected in Antarctica. Their structures were determined by extensive analysis of their spectroscopic features, particularly 1D and 2D NMR spectra, and comparison with related compounds. Cytotoxic and antimitotic activities were found for compounds **2** and **4**–**6**.

1. Introduction

Marine tunicates of the genus *Aplidium* have been the source of numerous nitrogen-containing metabolites belonging to unprecedented structural families of natural products.[1] Some of these compounds possess interesting biological properties, with the cyclodepsipeptide aplidin isolated from *Aplidium albicans* being perhaps the most renowned due to its interesting antitumor activity.[2] Other cytotoxic nitrogenous metabolites isolated from ascidians of the genus *Aplidium* include the lobatamides, macrocycles with an uncommon methylated oxime moiety isolated from *Aplidium lobatum*,[3] a group of iodotyrosine-derived compounds obtained from an unidentified species of an Australian tunicate of this genus,[4] a 1,2,3-trithiane derivative isolated from a New Zealand species of *Aplidium*,[5] pantherinine, a pyridoacridine from *Aplidium pantherinum*,[6] the haouamines from *Aplidium haouarianum*,[7] and the conicaquinones[8] and thiaplidiaquinones[9] obtained from *Aplidium conicum*. The presence in this genus of indole alkaloids is restricted to the isolation of the meridianins, a family of compounds with a brominated and/or hydroxylated indole nucleus and a 2-aminopyrimidine substituent at C-3 from *Aplidium meridianum*,[10,11] and the histamine antagonist conicamycin from the Mediterranean *A. conicum*.[12] Moderate cytotoxicity toward murine mamarian adenocarcinoma cells as well as protein kinase inhibitory properties have been described for the meridianins, making these compounds promising scaffolds for the development of new protein kinase inhibitors.[10,13]

In the course of our ongoing program for the search of new antitumor agents from marine organisms, the CH_2Cl_2/MeOH extracts of the hitherto uninvestigated ascidian *Aplidium cyaneum* were found to display cytotoxicity against the human tumor cell lines A-549, HT-29, and MDA-MB-231 as well as antimitotic properties. Bioassay-guided fractionation of these extracts led to the isolation of aplicyanins A–F (**1**–**6**), a group of cytotoxic and antimitotic alkaloids containing a bromoindole nucleus and a 6-tetrahydropyrimidine substituent at C-3. Herein, we report the isolation, structural characterization, and cytotoxic and antimitotic properties of this new family of marine metabolites.

2. Results and discussion

Samples of *A. cyaneum* were collected by bottom trawling at the Weddell sea (Antarctica) and kept frozen until used. The crude CH_2Cl_2/MeOH extract of the frozen tunicate was subjected to reversed phase C_{18} chromatography. Semipreparative reversed phase HPLC of selected active fractions from this chromatography led to the isolation of compounds **1**–**6** as their TFA salts.

The most polar compound of the extract, aplicyanin A (**1**), was isolated as an optically active pale yellow oil. A pseudomolecular

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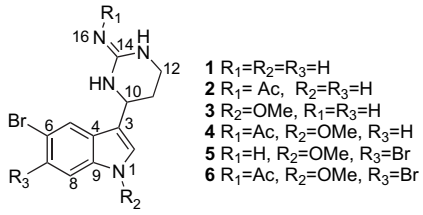

1 $R_1=R_2=R_3=H$
2 R_1= Ac, $R_2=R_3=H$
3 R_2=OMe, $R_1=R_3=H$
4 R_1=Ac, R_2=OMe, $R_3=H$
5 $R_1=H$, R_2=OMe, R_3=Br
6 R_1=Ac, R_2=OMe, R_3=Br

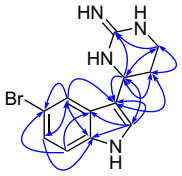

Figure 1. HMBC (H–C) correlations for aplicyanin A (**1**).

ion in the (+)-HRESIMS at *m*/*z* 293.0399, with an isotopic cluster for one bromine atom, and the presence of 12 signals in the ^{13}C NMR spectrum (Table 1) were consistent with a molecular formula of $C_{12}H_{13}BrN_4$. The ^{13}C NMR spectrum contained nine low field signals, eight of them attributable to olefinic carbons. The remaining low field signal was assigned to the presence of a guanidine group in the molecule on the basis of its chemical shift (δ_C 155.7 ppm). Signals in the UV, ^{13}C NMR, and the low field region of the ^1H NMR spectra (Table 1) accounted for the presence of a 3-substituted indole in the molecule. In addition, the proton coupling pattern [7.75 (d, 2.0), 7.26 (dd, 9.0, 2.0), and 7.34 (d, 9.0)] indicated a substitution either at C-6 or C-7. A bromine atom was placed at C-6 based on the correlations observed in the HMBC spectrum (Fig. 1), the downfield shift of proton H-5, which appeared as a *m*-coupled doublet, and comparison with reference data for similar compounds.[11] The nature of the substituent at C-3 was deduced from the analysis of the high field region of the ^1H NMR spectrum and correlations observed in the HSQC and HMBC spectra. Signals for a nitrogenated methine and two methylenes, one of them also nitrogenated, were observed in the ^1H NMR spectrum. Correlations observed in the COSY spectrum established the sequence from C-10 to C-12, and HMBC correlations from H-10 and both H-12 protons to the guanidine carbon C-13 confirmed the presence of a 6-substituted tetrahydropyrimidin-2(1*H*)-imine in the molecule. Finally, HMBC cross-peaks between H-10 and C-2 and C-3, between both H-11 protons and C-3, and between H-2 and C-10 confirmed the attachment of C-3 to C-10 establishing the structure of the compound as depicted in **1**.

Compound **2** had a molecular formula of $C_{14}H_{15}BrN_4O$, according to its (+)-HRESIMS (*m*/*z* $[M+H]^+$ 335.0508, calcd for $C_{14}H_{16}{}^{79}BrN_4O$, 335.0501) and ^{13}C NMR spectra (Table 1). The two additional carbon atoms with respect to **1** were assigned to an acetyl group based on the observation of signals for a carbonyl at δ_C 173.9 ppm and a methyl group at δ_H/δ_C 2.21/24.1 ppm. ^{15}N HMBC studies carried out on aplicyanin F (see below) located this acetyl group at N-16. To the best of our knowledge, this is the first report of the presence of *N*-acetylguanidines in marine natural products.

The third compound in the series, aplicyanin C (**3**), was isolated as an optically active yellowish oil of molecular formula $C_{13}H_{15}BrN_4O$, according to the (+)-HRESIMS and ^{13}C NMR spectra. A direct comparison of the ^1H and ^{13}C NMR spectra (Table 1) of this compound with those of aplicyanin A revealed the presence of a methoxyl group in **3** as the only noticeable difference between both compounds. That function was placed at the indole nitrogen based on correlations observed in the ROESY spectrum between the methoxyl singlet at δ_H 4.10 ppm and protons H-2 and H-8. *N*-Methoxyindoles are fairly uncommon in marine natural products. Only two compounds containing that functionality have been previously reported: pibocin B, an ergoline alkaloid isolated from an *Eudistoma* species ascidian,[14] and the nematocide convolutindole A, obtained from dichloromethane extracts of the bryozoan *Amathia convoluta*.[15] The ^1H and ^{13}C NMR chemical shifts reported for the *N*-methoxy functionality of these compounds are well in agreement with those observed for aplicyanin C.

The major differences found in the ^1H and ^{13}C NMR spectra of aplicyanin D (**4**) with respect to **3** were the presence of signals attributable to the presence of an acetyl group in the molecule. It was placed at N-16 based on ^{15}N HMBC studies carried out on the structurally related aplicyanin F (see below). A parent ion at *m*/*z* 365.0611 ($[M+H]^+$) in the (+)-HRESIMS with an isotopic cluster for one bromine, accounting for a molecular formula of $C_{15}H_{17}BrN_4O_2$, corroborated the structural proposal.

Compound **5** had a molecular formula of $C_{13}H_{14}Br_2N_4O$ according to the (+)-HRESIMS (*m*/*z* $[M+H]^+$ 400.9609 (calcd for $C_{13}H_{15}{}^{79}Br_2N_4O$, 400.9607)) and ^{13}C NMR spectra (Table 2). The major differences in the NMR spectra of this compound compared to **3** were found in the aromatic region of the proton spectrum. Three singlet signals accounted for the presence of a 3,6,7-trisubstituted indole in the molecule. Substituents at C-6 and C-7

Table 1
^1H and ^{13}C NMR data (CD_3OD, 500/125 MHz) for aplicyanins A–C (**1**–**3**)[a]

No	**1**		**2**		**3**	
	^{13}C	^1H (multiplicity, *J*)	^{13}C	^1H (multiplicity, *J*)	^{13}C	^1H (multiplicity, *J*)
2	125.3 d	7.31 (s)	125.6 d	7.37 (s)	123.8 d	7.56 (s)
3	113.6 s		113.8 s		112.0 s	
4	124.1 s		127.8 s		124.3 s	
5	121.9 d	7.75 (d, 2.0)	121.8 d	7.80 (d, 2.0)	122.5 d	7.80 (d, 2.0)
6	115.2 s		114.3 s		114.4 s	
7	125.9 d	7.26 (dd, 9.0, 2.0)	126.1 d	7.29 (dd, 8.5, 2.0)	126.9 d	7.37 (dd, 8.5, 2.0)
8	114.5 d	7.34 (d, 9.0)	114.6 d	7.36 (d, 8.5)	111.4 d	7.42 (d, 8.5)
9	137.2 s		137.2 s		132.7 s	
10	48.1 d	4.95 (dd, 6.5, 6.0)	48.2 d	5.14 (dd, 6.5, 6.0)	47.6 d	4.94 (dd, 8.0, 4.5)
11	28.3 t	2.25 (m) 2H	26.9 t	2.34 (m), 2H	28.3 t	2.26 (dddd, 13.5, 8.5, 8.0, 5.5); 2.18 (dddd, 13.5, 5.5, 4.5, 4.5)
12	38.6 t	3.46 (ddd, 12.5, 6.5, 6.5); 3.41 (ddd, 12.5, 5.0, 5.0)	38.6 t	3.60 (ddd, 13.0, 7.5, 6.5); 3.53 (ddd, 13.0, 5.5, 5.0)	38.4 t	3.45 (ddd, 12.5, 8.5, 4.5); 3.40 (ddd, 12.5, 5.5, 5.0)
14	155.7 s		152.3 s		155.7 s	
O*Me*					66.8 q	4.10 (s)
CH_3CO			173.9 s			
CH_3CO			24.1 q	2.21 (s)		

[a] Assignments were made with the help of edited HSQC and HMBC experiments.