Perspicamides A and B, Quinolinecarboxylic Acid Derivatives from the Australian Ascidian *Botrylloides perspicuum*

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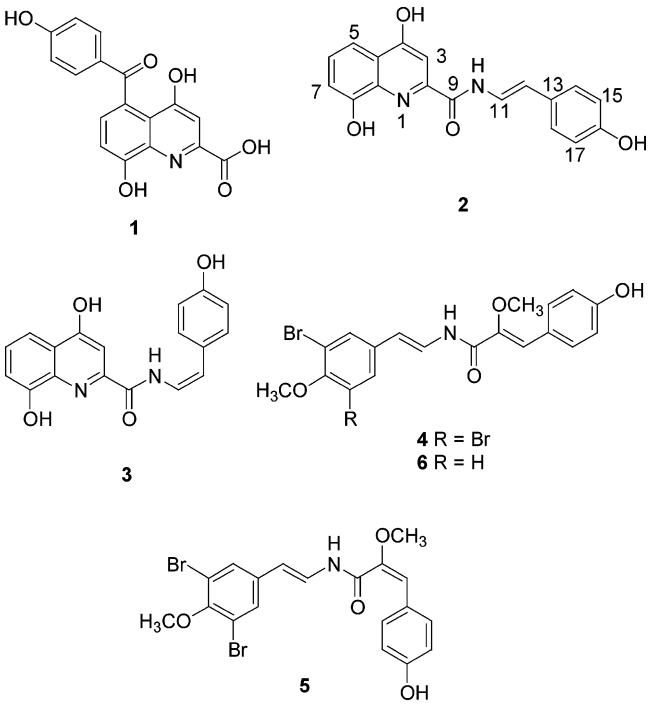
Two novel quinoline-2-carboxylic acid derivatives, perspicamides A (**2**) and B (**3**), were isolated from the Australian ascidian *Botrylloides perspicuum*. Extraction of the freeze-dried ascidian with methanol and purification of the extract by C18 MPLC followed by repeated C18 HPLC yielded the new compounds **2** and **3** as well as the known compounds botryllamides A−C (**4**−**6**). The structures of **2** and **3** were determined from analysis of 2D NMR spectra.

2-Quinolinecarboxylic acid derivatives are relatively uncommon in nature. Simple quinolinecarboxylic acids have been isolated from a variety of insects including cockroaches, flies, and butterflies.[1] Xanthurenic acid, or 4,8-dihydroxy-2-quinolinecarboxylic acid, a metabolite of tryptophan, is present in urine in cases of vitamin B6 deficiency.[2] The 8-methoxy derivative of xanthurenic acid is a possible endogenous carcinogen.[3] Only four quinolinecarboxylic acid deriviatives have been reported from marine organisms: xanthurenic acid and its derivatives, tridemnic acids A (**1**) and B, which have been isolated from the ascidian *Trididemnum* sp. from British Columbia,[4] and 4,5,8-trihydroxyquinolinecarboxylic acid, which has been isolated from the Antarctic sponge *Dendrilla membranosa*.[5] This paper reports the structures of two xanthurenic acid derivatives, perspicamides A (**2**) and B (**3**), which we have isolated from the Australian ascidian *Botrylloides persipicuum* Herdman 1886 (Styelidae). The ascidian also contained the enamide derivatives botrylamides A−C (**4**−**6**), which have been isolated previously from *Botryllus schlosseri* from the Great Barrier Reef and *Botryllus* sp. from the Philippines.[6]

Extraction of the freeze-dried ascidian with methanol and purification of the extract by C18 MPLC followed by repeated C18 HPLC yielded the new compounds **2** and **3** as well as the known compounds botrylamides A−C (**4**−**6**). Perspicamide A (**2**) was isolated as a yellow gum. A pseudomolecular ion in the (+) HRESIMS at *m/z* 323.10357 (Δ 1.2 ppm) allowed a molecular formula of $C_{18}H_{14}N_2O_4$ to be assigned to **2**. Only 16 resonances were observed in the ^{13}C NMR spectrum (Table 1) of **2**, 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 6.74 (2H, J = 8.6 Hz) and 7.25 (2H, J = 8.6 Hz) and two aromatic methine carbon signals at 115.7 (2 carbons) and 126.6 ppm (2 carbons) in the ^{13}C NMR spectrum. gHMBC correlations from both of the aromatic methine doublets and an exchangeable phenolic proton singlet at δ 9.42 to an aromatic oxygenated quaternary carbon at 156.4 ppm indicated that the molecule contained a 4-hydroxyphenyl group. A *trans*-enamide group was supported by the presence of two olefinic protons at δ 6.52 (1H, d, J = 15.0 Hz) and 7.45 (1H, dd, J = 15.0, 10.0 Hz) and by coupling of the olefinic proton at δ 7.45 to an exchangeable amide proton at δ 11.20. The assignment



of *trans* geometry for the enamide was indicated by a large, 15 Hz, coupling between the two protons of the double bond. The observation of a gHMBC correlation from the amide proton H-10 to a conjugated carbonyl carbon at 161.4 ppm confirmed the presence of the amide unit. gHMBC correlations from the olefinic proton at δ 6.52 to the aromatic methine carbon at 126.6 ppm and a quaternary aromatic carbon resonance at 127.2 ppm provided evidence that the *trans*-enamide unit was directly attached to the 4-hydroxyphenyl group. The coupling pattern observed for three downfield aromatic methine protons at δ 7.58 (1H, dd, J = 7.2, 1.0 Hz), 7.43 (1H, dd, J = 7.2, 7.2 Hz), and 7.13 (1H, dd, J = 7.2, 1.0 Hz) indicated that a 1,2,3-trisubstituted benzene ring was present in the molecule. This observation was consistent with the correlation pattern observed in the gCOSY spectrum of **2**. The aromatic methine proton at δ 7.13 showed a gHMBC correlation to the aromatic methine carbon at 111.8 ppm and two quaternary aromatic carbon resonances at 153.5 and 138.2 ppm. An exchangeable phenolic proton at δ 10.10 also showed gHMBC correlations to each of the quaternary carbon resonances at 153.5 and 138.2 ppm, and to the aromatic methine carbon at 111.7 ppm, indicating that a

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Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Data for Perspicamides A (**2**) and B (**3**) in DMSO-d_6

	perspicamide A (**2**)			perspicamide B (**3**)		
position	δ_C	δ_H (mult, *J*, Hz)	HMBC	δ_C	δ_H (mult, *J*, Hz)	HMBC
2	147.8 (qC)			147.8 (s)		
3	102.0 (CH)	7.57 (s, 1H)	C-4a, C-8a,[a] C-9	102.0 (d)	7.56 (s, 1H)	C-4a, C-8a,[a] C-9
4	162.6 (qC)			162.6 (s)		
4-OH		11.87 (s, 1H)	C-3, C-4, C-4a		11.84 (s, 1H)	C-3, C-4, C-4a
4a	122.1 (qC)			122.0 (s)		
5	111.8 (CH)	7.58 (dd, 7.2, 1.0, 1H)	C-4, C-8a, C-7	111.7 (d)	7.58 (dd, 7.6, 1.1, 1H)	C-4, C-8a, C-7
6	127.7 (CH)	7.43 (dd, 7.2, 7.2, 1H)	C-4a, C-8, C-8a[a]	127.6 (d)	7.44 (dd, 7.6, 7.6, 1H)	C-4a, C-8, C-8a[a]
7	111.7 (CH)	7.13 (dd, 7.2, 1.0, 1H)	C-5, C-8, C-8a	112.1 (d)	7.11 (dd, 7.6, 1.1, 1H)	C-5, C-8, C-8a
8	153.5 (qC)			153.7 (s)		
8-OH		10.10 (s, 1H)	C-7, C-8, C-8a		10.10 (s, 1H)	C-7, C-8, C-8a
8a	138.2 (qC)			138.5 (s)		
9	161.4 (qC)			161.4 (s)		
10-NH		11.22 (d, 10.0, 1H)	C-9, C-12		10.95 (d, 9.6, 1H)	C-9, C-12
11	120.6 (CH)	7.45 (dd, 15.0, 10.0, 1H)	C-9, C-12, C-13	119.7 (d)	6.73 (dd, 9.6, 9.4, 1H)	C-9, C-12, C-13
12	114.1 (CH)	6.52 (d, 15.0, 1H)	C-11, C-13, C-14	114.1 (d)	5.85 (d, 9.4, 1H)	C-11, C-13, C-14
13	127.2 (qC)			126.5 (s)		
14/18	126.6 (CH)	7.25 (d, 8.6, 2H)	C-12, C-14, C-16	129.9 (d)	7.34 (d, 8.6, 2H)	C-12, C-14, C-16
15/17	115.7 (CH)	6.74 (d, 8.6, 2H)	C-13, C-15, C-16	115.4 (d)	6.77 (d, 8.6, 2H)	C-13, C-15, C-16
16	156.4 (qC)			156.3 (s)		
16-OH		9.42 (s, 1H)	C-15, C-16		9.53 (s, 1H)	C-15, C-16

[a] Weak, $^4J_{C-H}$.

2,3-disubstituted phenol ring was present. Further evidence for a 2,3-disubstituted phenol ring included the observation of gHMBC correlations from the aromatic methine proton H-6 to the quaternary carbon C-8 and a quaternary carbon C-4a and gHMBC correlations from the downfield aromatic methine H-5 to the methine carbon C-7 and the quaternary aromatic carbon C-8a. The relatively downfield shift of C-8a suggested that it was α to an electronegative atom, most likely a nitrogen substituent. A downfield phenolic proton at δ 11.87 showed gHMBC correlations to C-4a, to an oxygenated quaternary carbon at 162.6 ppm, and to an aromatic methine carbon C-3, at 102.0 ppm, indicating that the previously assigned 2,3-disubstituted phenol ring was likely to be part of a trisubstituted quinoline system. The aromatic methine singlet at δ 7.57, H-3, showed a gHMBC correlation to the quaternary carbon, C-4a, providing further evidence for a trisubstituted quinoline system. No gHMBC correlations were observed to a quaternary carbon at 147.8 ppm; however the relatively downfield shift of this carbon was consistent with it being α to a nitrogen atom, allowing it to be assigned to C-2. A strong gHMBC correlation from H-3 to the amide carbonyl carbon, C-9, indicated that **2** contained a 4,8-dihydroxyquinoline-2-carboxamide, which was directly attached to the aminovinylphenol.

Perspicamide B (**3**) was also isolated as a yellow gum. The pseudomolecular ion in the (+) HRESIMS was the same as for perspicamide A (**2**), indicating that the two compounds were isomeric. The ^{1}H and ^{13}C NMR data (Table 1) for **3** were also very similar to that of **2**. The only major differences between the spectra for the two compounds was for signals associated with the enamide group. H-10, H-11, and H-12 were all significantly upfield shifted in **3** relative to **2**, and the magnitude of the coupling between H-11 and H-12 was significantly smaller (J = 9.4 Hz). These data indicated that perspicamide B (**3**) was the *cis*-enamide derivative of **2**. Two-dimensional NMR analysis confirmed this assignment. Perspicamide B (**3**) isomerized to **2** on exposure to light.

Experimental Section

General Experimental Procedures. UV and FTIR spectra were recorded on a Camspec MS01 single beam scanning UV/vis spectrophotometer and a Bruker Tensor 27 IR spectrophotometer, respectively. NMR spectra were recorded at 30 °C on a Varian 600 MHz Unity INOVA at 599.926 MHz for ^{1}H and 149.98 MHz for ^{13}C. The ^{1}H and ^{13}C chemical shifts were referenced to the proto-deutero solvent peak (DMSO-d_6) at δ 2.49 and 39.5 ppm, respectively. HRESIMS was recorded on a Mariner Biospectrometry TOF workstation using positive electrospray ionization, mobile phase 1:1 MeOH/H_2O containing 0.1% formic acid. A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler were used for HPLC separations. Alltech Davisil 30−40 μm 60 A C18 bonded silica was used for MPLC work. A Hypersil BSD 5 μm 120 Å C18 silica HPLC column (10 mm × 250 mm) was used for HPLC semipreparative separations. All solvents used for HPLC, UV, and MS were Merck Omnisolv grade, and the H_2O used was Millipore Milli-Q PF filtered.

Animal Material. The ascidian *Botrylloides perspicuum* Herdman 1886 was collected by scuba from Flinders Reef in Morton Bay SE Queensland in December 1997. A voucher specimen, G313625, was deposited at the Queensland Museum.

Extraction and Purification. The freeze-dried, ground, ascidian (11.4 g) was extracted with a continuous flow gradient of H_2O to MeOH. The eluent from the extraction was immediately fractionated by C18 in a continuous flow process. Ninety fractions were collected and analyzed by (+) ESIMS. Mass spectrometry results indicated that a series of alkaloids were present in fractions 17−23. Fractions 17−23 were combined, yielding an intensively red gum. HPLC on C18 using a gradient from 84.5% water/14.5% ACN/1% TFA to 99% ACN/1% TFA resulted in the isolation of a mixture of perspicamides A (**1**) and B (**2**), botryllamide C (**6**) (3.3 mg), and a mixture of botryllamides A (**4**) and B (**5**). The mixture of perspicamides A and B was purified by HPLC on C18 elution with 54.5% water/44.5% ACN/1% TFA to yield perspicamide A (**2**) (4.3 mg) and perspicamide B (**3**) (2.7 mg). The mixture of botryllamides A and B was purified by HPLC on C18 elution with 34.5% water/64.5% ACN/1% TFA to yield botryllamide A (**4**) (9.3 mg) and botryllamide B (**5**) (2.1 mg).

Perspicamide A (2): pale yellow solid; UV (MeOH) λ_{max} 230 nm (ϵ 15 830), 246 (16 000), 314 (6620), 349 (7460); IR (film) ν_{max} 3365, 1712, 1678, 1661, 1636, 1609 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); (+)-LRESIMS *m/z* (rel int) 323 (100%) $[MH^+, C_{18}H_{15}N_2O_4]^+$; (+)-HRESIMS *m/z* 323.10357 (calcd for $C_{18}H_{15}N_2O_4$ $[MH]^+$, 323.10318).

Perspicamide B (3): pale yellow solid; UV (MeOH) λ_{max} 221 nm (ϵ 17 725), 246 (16 645), 315 (6800), 347 (7680); IR (film) ν_{max} 3360, 1723, 1680, 1662, 1608, 1589 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); (+)-LRESIMS *m/z* (rel int): 323 (100%) $[MH^+, C_{18}H_{15}N_2O_4]^+$; (+)-HRESIMS *m/z* 323.10330 (calcd for $C_{18}H_{15}N_2O_4$ $[MH]^+$, 323.10318).

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