Eudistomin V, a New β-Carboline from the Australian Ascidian *Pseudodistoma aureum*

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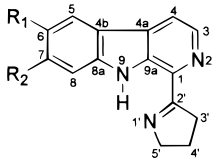
Chemical investigation of the Australian ascidian *Pseudodistoma aureum* has resulted in the isolation of a new β-carboline, eudistomin V (**1**). The known compounds eudistomin H (**2**) and I (**3**) were also isolated, and all compounds had their structures determined by spectroscopic means.

Tryptophan-derived metabolites constitute a large class of nitrogen-containing compounds that have been isolated from ascidians.[1] Examples of this class include the eudistomins,[2−10] the eudistomidins,[11−13] and woodinine,[14] all of which are based on a β-carboline ring system. In this paper we report the isolation and structure elucidation of a new β-carboline, eudistomin V (**1**).

A freeze-dried sample of *Pseudodistoma aureum* Brewin, 1957 (family Pseudodistomidae) was exhaustively extracted with CH_2Cl_2 followed by MeOH. Both extracts were combined and repeatedly chromatographed on Sephadex LH-20 to yield eudistomin V (**1**) (1.86 mg, 0.031% dry wt), eudistomin H (**2**) (8.86 mg, 0.15% dry wt), and eudistomin I (**3**) (0.88 mg, 0.015% dry wt).



R_1= Br R_2=Br (**1**)
R_1= Br R_2=H (**2**)
R_1= H R_2=H (**3**)

Eudistomin V (**1**) was obtained as a yellow gum. Bands in the UV spectrum at λ_{max} 224 and 290 nm indicated the presence of an indole chromophore. The IR spectrum (KBr) established the presence of conjugated double bonds (ν_{max} 1603 cm^{-1}), a hydroxy or amine functionality (ν_{max} 3500−3300 cm^{-1}), and a halogen moiety (ν_{max} 582 cm^{-1}). The HREIMS showed a molecular ion at *m/z* 392.9293, which coupled with a molecular ion cluster at *m/z* 391 [M + H, $C_{15}H_{12}N_3{}^{79}Br_2$], 393 [M + H, $C_{15}H_{12}N_3{}^{79}Br_1{}^{81}Br_1$], and 395 [M + H, $C_{15}H_{12}N_3{}^{81}Br_2$] in the low resolution positive ion electrospray mass spectrum [(+)-LRESMS] were consistent with the molecular formula $C_{15}H_{11}N_3Br_2$ (calcd *m/z* 392.9299, Δ −1.9 ppm).

The ^{1}H NMR spectrum (see Table 1 for NMR data) contained signals for three methylenes, four aromatic protons (two doublets and two singlets), and a downfield exchangeable proton. The COSY spectrum revealed two spin-coupled networks. Protons H-3 and H-4 constituted an isolated vicinal pair, which could be assigned to α and β pyridine protons from their homonuclear coupling constant (J = 5.1 Hz), from their proton chemical shifts [H-3 (δ 8.50) and H-4 (δ 7.95)], and from the chemical shifts of the carbons to which they were attached [C-3 (139.5 ppm) and C-4 (116.5 ppm)]. The second spin system was assigned to three contiguous methylene groups 3′-CH_2, 4′-CH_2, and 5′-CH_2. The ^{1}H−^{13}C chemical shifts for 5′-CH_2 (δ 4.25, 62.8 ppm) were characteristic of a heterosubstituted methylene. All three methylene proton resonances showed HMBC correlations to an imino carbon at 177.3 ppm. Hence, a 2′-substituted pyrroline ring was established.

Table 1. NMR Data for Eudistomin V (**1**)[a]

position	^{13}C (δ)	^{1}H (δ, mult., *J* in Hz)	HMBC (C no.)
1	137.2		
2			
3	139.5	8.50 (d, 5.1)	1, 4a, 4
4	116.5	7.95 (d, 5.1)	9a, 4b, 3
4a	128.3		
4b	122.8		
5	126.7	8.43 (s)	8a, 4a, 7, 6
6	115.4		
7	124.2		
8	117.4	7.93 (s)	7, 4b, 6
8a	140.6		
9			
9-NH		10.97 (br s)	
9a	136.2		
1′			
2′	177.3		
3′	35.3	3.27 (ddt, 9.1, 7.4, 2.1)	2′, 5′, 4′
4′	22.3	2.08 (dddd, 9.1, 7.5, 7.4, 7.3)	2′, 5′, 3′
5′	62.8	4.25 (ddt, 7.5, 7.3, 2.1)	2′, 4′, 3′, 1

[a] In CD_2Cl_2 at 20 °C.

A β-carboline moiety was assigned from HMBC correlations observed from the four aromatic protons to the remaining 11 carbons. All possible $^3J_{CH}$ and some $^2J_{CH}$ correlations were observed. The singlets assigned to aromatic protons H-5 and H-8 (δ 8.43 and 7.93) showed HMBC correlations to quaternary carbons C-6 and C-7 (115.4 and 124.2 ppm). H-5 also showed two $^3J_{CH}$ correlations to C-8a and C-4a (140.6 and 128.3 ppm). The ^{13}C chemical shift of C-8a was assigned to a carbon α to a nitrogen, while C-4a was part of the trisubtituted pyridine system. H-8 showed a $^3J_{CH}$ correlation to C-4b (122.8 ppm), which was assigned to a carbon β to a nitrogen. Hence, H-5 and H-8 were positioned para to

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each other on the benzenoid ring, with the two bromine atoms attached to C-6 and C-7. A C-1/C-2′ linkage of the 2′-pyrroline ring to the β-carboline was determined by means of a weak four-bond HMBC correlation from H-5′ to C-1. It followed that eudistomin V was assigned structure **1**.

The chemical shift values observed for eudistomin V (**1**) were in close agreement with those reported for β-carboline compounds that have been previously isolated from ascidians.[2−14] The known compounds eudistomin H (**2**) and eudistomin I (**3**) were identified by comparison of their ^{1}H NMR, UV, and IR spectra and (+)-LRESMS analysis.[3]

It is interesting to note that the isolation of eudistomins has not been restricted to a particular genus or even family. *Eudistoma olivaceum* (family Polycitoridae), *Ritteralla sigillinoides* (family Ritterellidae), *Lissoclinum fragile* (family Didemnidae), and now *Pseudodistoma aureum* (family Pseudodistomidae) are all species known to produce eudistomins.

Experimental Section

General Experimental Procedures. NMR spectra were recorded at 20 °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 solvent peak (CD_2Cl_2) δ 5.32 and 54.00 ppm, respectively. HREIMS was recorded on a KRATOS mass spectrometer, and (+)-LRESMS was recorded on a FISONS VG platform mass spectrometer. FTIR and UV spectra were recorded on a Perkin−Elmer 1725X spectrophotometer and a GBC UV/vis 916 spectrophotometer, respectively. Size exclusion chromatography was performed on Pharmacia Biotech Sephadex LH-20 (40-mm D × 520-mm H) connected to a Waters 486 tunable UV detector and Waters fraction collector. Standard parameters were used for the 2D NMR spectra obtained, which included gradient COSY, HMQC, and HMBC.

Animal Material. A specimen of *Pseudodistoma aureum* was collected by scuba diving (−18m) at Heron Reef, Capricorn-Bunker Group, Great Barrier Reef, and kept frozen prior to freeze-drying and extraction. Voucher specimen QMG307359 has been deposited at the Queensland Museum, South Brisbane, Queensland, Australia.

Extraction and Isolation. The freeze-dried ascidian (6.0 g dry wt) was extracted with CH_2Cl_2 (3 × 100 mL) followed by MeOH (3 × 100 mL), and both these extracts were concentrated under vacuum and combined to yield a dark green gum (1.87 g). This gum was dissolved in MeOH (6 mL) and chromatographed on Sephadex LH-20 using MeOH as the eluent and a flowrate of 3 mL/min. The fractions were monitored at 262 nm. The last eluting fraction (>3 h) contained a mixture of compounds **1**, **2** and **3**. This fraction was concentrated under vacuum, then rechromatographed on Sephadex LH-20 using the same conditions yielding eudistomin I (**3**) (0.88 mg, 0.015%), eudistomin H (**2**) (8.86 mg, 0.15%), and eudistomin V(**1**) (1.86 mg, 0.031%), respectively.

Eudistomin V (1): isolated as a yellow gum; UV (MeOH) λ_{max} (ϵ) 224 (30 000), 290 (16 000), 311 (11 900), 371 (6300); IR ν_{max} (KBr disk) 3500−3300, 2920, 1603, 1470, 1535, 1260, 1282, 1085, 582 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; (+)-LRESMS *m/z* 391(50) [M+H, $C_{15}H_{12}N_3{}^{79}Br_2$], 393(100) [M + H, $C_{15}H_{12}N_3{}^{81}Br_1{}^{79}Br_1$], 395(100) [M + H, $C_{15}H_{12}N_3{}^{81}Br_2$]; HREIMS *m/z* 392.9293 (calcd for $C_{15}H_{11}N_3{}^{79}Br_1{}^{81}Br_1$ 392.9299, Δ −1.9 ppm)

Eudistomin H (2): isolated as a yellow gum; UV (MeOH) λ_{max} (ϵ) 221 (18 300), 286 (8300), 371 (3000); IR ν_{max} (KBr disk) 3500−3300, 2925, 1606, 1474, 1272, 1054, 602 cm^{-1}; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 8.47 (1H, d, J = 5.1 Hz, H-3), 7.96 (1H, d, J = 5.1 Hz, H-4), 8.28 (1H, d, J = 2.0 Hz, H-5), 7.64 (1H, dd, J = 8.7, 2.0 Hz, H-7), 7.49 (1H, d, J = 8.7 Hz, H-8), 10.93 (1H, br s, NH-9), 3.27 (2H, ddt, J = 9.1, 7.5, 2.0 Hz, H-3′), 2.07 (2H, dddd, J = 9.1, 7.5, 7.2, 7.5 Hz, H-4′), 4.25 (2H, ddt, J = 7.2, 7.5, 2.0 Hz, H-5′); (+)-LRESMS *m/z* 314(100) [M + H, $C_{15}H_{13}N_3{}^{79}Br_1$], 316(100) [M + H, $C_{15}H_{13}N_3{}^{81}Br_1$]

Eudistomin I (3): isolated as a yellow gum; UV (MeOH) λ_{max} (ϵ) 215 (13 800), 238 (8500), 280 (7000), 368 (2800); IR ν_{max} (KBr disk) 3500−3300, 2924, 1631, 1384, 1215, 746 cm^{-1}; ^{1}H NMR (CD_2Cl_2, 600 MHz) δ 8.47 (1H, d, J = 5.1 Hz, H-3), 8.03 (1H, d, J = 5.1 Hz, H-4), 8.18 (1H, br d, J = 7.5 Hz, H-5), 7.31 (1H, ddd, J = 7.5, 7.5, 1.1 Hz, H-6), 7.57 (1H, ddd, J = 7.5, 7.8, 1.1 Hz, H-7), 7.61 (1H, br d, J = 7.8 Hz, H-8), 10.89 (1H, br s, NH-9), 3.29 (2H, ddt, J = 9.1, 7.4, 2.1 Hz, H-3′), 2.08 (2H, dddd, J = 9.1, 7.4, 7.4, 7.3 Hz, H-4′), 4.27 (2H, ddt, J = 7.4, 7.3, 2.1 Hz, H-5′); (+)-LRESMS *m/z* 236 (100) [M + H, $C_{15}H_{14}N_3$].

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References and Notes

(1) Davidson, B. S. *Chem. Rev.* **1993**, *93*, 1771−1791.
(2) Rinehart, K. L., Jr.; Kobayashi, J.; Harbour, G. C.; Hughes, R. G., Jr.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1984**, *106*, 1524−1526.
(3) Kobayashi, J.; Harbour, G. C.; Gilmore, J.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1984**, *106*, 1526−1528.
(4) Rinehart, K. L., Jr.; Kobayashi, J.; Harbour, G. C.; Gilmore, J.; Mascal, M.; Holt, T. G.; Shield, L. S.; Lafargue, F. *J. Am. Chem. Soc.* **1987**, *109*, 3378−3387.
(5) Kinzer, K. F.; Cardellina, J. H., II. *Tetrahedron Lett.* **1987**, *28*, 925−926.
(6) Lake, R. J.; Brennan, M. M.; Blunt, J. W.; Munro, M. H. G. *Tetrahedron Lett.* **1988**, *29*, 2255−2256.
(7) Blunt, J. W.; Lake, R. J.; Munro, M. H. G. *Tetrahedron Lett.* **1987**, *28*, 1825−1826.
(8) Lake, R. J.; Blunt, J. W.; Munro, M. H. G. *Aust. J. Chem.* **1989**, *42*, 1201−1206.
(9) Badre, A.; Boulanger, A.; Abou-Mansour, E.; Banaigs, B.; Combaut, G.; Francisco, C. *J. Nat. Prod.* **1994**, *57*, 528−533.
(10) Massiot, G.; Nazabadioko, S.; Bliard, C. *J. Nat. Prod.* **1995**, *58*, 1636−1639.
(11) Kobayashi, J.; Nakamura, H.; Ohizumi, Y.; Hirata, Y. *Tetrahedron Lett.* **1986**, *27*, 1191−1194.
(12) Kobayashi, J.; Cheng, J.; Ohta, T.; Nozoe, S.; Ohizumi, Y.; Sasaki, T. *J. Org. Chem.* **1990**, *55*, 3666−3670.
(13) Marata, O.; Shigemori, H.; Ishibashi, M.; Sugama, K.; Hayashi, K.; Kobayashi, J. *Tetrahedron Lett.* **1991**, *32*, 3539−3542.
(14) Pais, M.; Debitus, C.; Laurent, D. *J. Nat. Prod.* **1988**, *51*, 799−801.

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