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β-CARBOLINE ALKALOIDS FROM THE MARINE BRYOZOAN *COSTATICELLA HASTATA*

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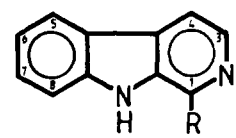
Bryozoans are colonial, sedentary, invertebrate animals, usually of marine origin. The chemistry of their secondary metabolites remains largely unexplored, although it has been the topic of one recent review (1). Most of the compounds isolated to date have been alkaloids (2).

As part of our continuing survey of southern Australian marine invertebrates for alkaloids (3), we report here the results of our chemical investigation of the bryozoan *Costaticella hastata* Busk (order Cheilostomata). This orange foliose bryozoan is common around the coast of Tasmania, occurring mainly on exposed vertical rock faces. A range of 1-substituted β-carboline alkaloids was obtained from two separate collections of *C. hastata* from southern Tasmania. The same major alkaloid was present in both samples, but some variation in the minor alkaloids was observed. Most of the compounds have been previously described, although one has a novel structure.

RESULTS AND DISCUSSION

C. hastata collected from Eaglehawk Neck yielded, after freeze drying and extraction, 0.11% crude alkaloid. Purification of this material mainly by Si gel chromatography gave 1-methyl-β-carboline (harman) [**1**] as the major alkaloid with lesser amounts of 1-ethyl-β-carboline [**2**] and (*S*)-1-(1′-hydroxyethyl)-β-carboline [**3**]. The structures of **1** and **2** were established by comparison of their physical and spectral (^{1}H nmr and ms) characteristics and, in the case of harman, by direct comparison with an authentic synthetic sample.

The third compound, **3**, has a novel structure. Its molecular formula was shown to be $C_{13}H_{12}N_2O$ by high resolution eims. The presence of a 1-substituted β-carboline moiety was revealed by the uv spectrum and the aromatic region of the 300 MHz ^{1}H-nmr spectrum, both of which were very similar to those of **1** and **2**. Ir spectroscopy showed the presence of an alcohol group. This was confirmed by the ^{1}H-nmr spectrum that, furthermore, revealed a quartet at 5.40 ppm and a doublet at 1.67 ppm (J=6.5 Hz), which showed that the remaining part of the molecule consisted of a 1-hydroxyethyl group attached to the β-carboline at the 1-position. The observation of a specific rotation of −11.4° is consistent with the presence of the chiral center and shows that **3** did not form as an artifact, for example by hydration of **4**, during the work-up procedure. Application of Brewster's rules (4) indicates that **3** has *S*- absolute stereochemistry.



1 R=−CH_3
2 R=−CH_2CH_3
3 R=−$CH(OH)CH_3$
4 R=−$CH{=}CH_2$

A second collection of *C. hastata* made in the vicinity of Tinderbox contained 1-vinyl-β-carboline (pavettine) [**4**] in addition to **1**, **2**, and **3**. The identification of **4** followed from comparison of spectral data with published information.

The simple β-carbolines are well-known alkaloids from terrestrial plants and their occurrence and chemistry have been extensively reviewed (5,6). The isolation of β-carbolines from marine

sources is, however, quite rare. This is the first time that **1-4** have been reported from the marine environment (as well as from an animal source), and this is apparently only the third report of marine β-carbolines. The earlier instances were when a series of novel di- and tri-substituted β-carbolines, the eudistomins, were obtained from a Caribbean colonial tunicate *Eudistoma olivaceum* (7), and when manzamine A was isolated from a sponge (8).

Of the β-carbolines that have been isolated from *C. hastata* only (*S*)-1-(1′-hydroxyethyl)-β-carboline [**3**] does not appear to have been described in the literature. Isomers of it are known: 1-(2′-hydroxyethyl)-β-carboline, which occurs, together with pavettine [**4**], in the terrestrial plant *Soulamea fraxinifolia*, Simarubaceae (9), while 3-(1′-hydroxyethyl) β-carboline has been synthesised (10). There are numerous reports of the occurrence of harman [**1**] and pavettine [**4**] from terrestrial plants (5). However, 1-ethyl-β-carboline [**2**] has only been reported once before as a natural product when it was obtained from the roots of *Hannoa klaineana*, Simaroubaceae (11).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Yanagimoto Seisakusho apparatus and are uncorrected. Spectral data were obtained on the following instruments: ^{1}H nmr Bruker AM-300 and JEOL JNH-MH-100 (**4** only); mass spectra, Vacuum General 70/70F; ftir, Digi-Lab FTS:20E; uv, Varian DMS-100. Optical rotation was measured using a Bellingham and Stanley polarimeter. Adsorbents for cc and ptlc, Camag Si gel. Analytical tlc plates, Merck Si gel 60.

ANIMAL MATERIAL.—Whole colonies of *C. hastata* were collected at -2 to -8 m. The first collection was made in November 1984, near The Blowhole, Eaglehawk Neck, Tasman Peninsula (147°56′49″ E, 43°2′14″ S), while the second was obtained at Tinderbox, River Derwent Estuary, near Hobart (147°20′ E, 43°3′36″ S), and at several other locations within 7 km during summer 1982. A voucher specimen has been deposited at the Museum of Victoria, Melbourne, Australia (F52864).

EXTRACTION AND PURIFICATION.—*Eaglehawk Neck sample.*—The freeze dried and powdered invertebrate (3450 g) was extracted successively with CH_2Cl_2, MeOH, and finally MeOH containing NH_3. The extracts were separately evaporated and extracted with 10% H_2SO_4. Addition of NH_3 to the aqueous phase followed by extraction with CH_2Cl_2 yielded crude alkaloid fractions that were then combined (3.78 g).

The crude alkaloid material was partially dissolved in CH_2Cl_2. The insoluble material was largely **1**, which was purified by ptlc to give harman, **1** (0.47 g). Si gel flash chromatography of the CH_2Cl_2 extract of the crude alkaloid material with CH_2Cl_2 containing increasing amounts of MeOH followed by purification of the fractions by repetitive ptlc on 4% KOH Si gel with multiple developments with 3% MeOH in CH_2Cl_2 gave, in order of decreasing Rf, **2** (0.20 g), **1** (0.07 g), and **3** (0.50 g).

Harman [**1**].—Combined wt 0.54 g, 1.6×10^{-2}% dry wt. Its mp and mixed mp with an authentic sample, ^{1}H nmr, ms, and hrms were in agreement with those of harman (12,13).

1-Ethyl-β-carboline [**2**].—6×10^{-3}% dry wt. Its ^{1}H nmr, ms, and hrms were in agreement with published data (11,14).

(S)-*1-(1′-hydroxyethyl)-β-carboline* [**3**].—2×10^{-3}% dry wt; mp 163-169° dec. M^+ found 212.0951 $C_{13}H_{12}N_2O$ requires 212.0949, *m/z* 210 (M^+; 33), 210 (49), 197 (23), 194 (70), 193 (100), 182 (20), 168 (86), 162 (31), 140 (30); ir ν max (KBr) 3220 bd, 2980, 2926, 1628, 1570, 1497, 1431, 1238, 743 cm^{-1}; ^{1}H nmr (300 MHz, $CDCl_3$) δ 9.11 (s, 1, OH), 8.32 (d, J=5.1 Hz, 1, H-3), 8.12 (d, J=8 Hz, 1, H-6), 7.86 (d, J=5.1 Hz, 1, H-4), 7.53 (m, 2, H-8 and H-9), 7.29 (m, 1, H-7), 5.40 (q, J=6.5 Hz, 1, H-1′), 1.67 (d, J=6.5 Hz, 3, H-2′); uv (EtOH) ν max 211 (3.78), 234 (3.99), 287 sh (3.79), 337 (3.01) nm; $[\alpha]^{25}D$ (MeOH) −11.4°.

Tinderbox sample.—Wet material (2198 g, equivalent dry wt 620 g) was extracted with MeOH and then MeOH containing NH_3. The extracts were purified as described above to give **1** (5.1×10^{-2}% dry wt), **2** (4.8×10^{-3}% dry wt), **3** (2×10^{-3}% dry wt), and **4** (1.8×10^{-2}% dry wt). The spectral data of **4** were in agreement with those published for pavettine (15).

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