Journal of Natural Products
Vol. 55, No. 4, pp. 525-527, April 1992

BROMINATED β-CARBOLINES FROM THE MARINE TUNICATE *EUDISTOMA ALBUM*

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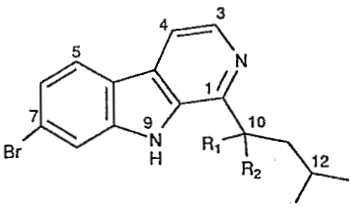
ABSTRACT.—Chemical investigation of the cytotoxic EtOH extract of the marine tunicate *Eudistoma album* led to the isolation of three brominated β-carbolines which were characterized by their spectral data. Two of them, eudistomin E, which is already known for its potent antiviral activity, and the novel compound eudistalbin A [**1**], were shown to possess cytotoxic activity (ED_{50}<5.0 ng and 3.2 μg/ml respectively) in vitro against the growth of KB human buccal carinoma cells. The third compound, eudistalbin B [**2**], is a new inactive natural product.

Recent investigations into the constituents of marine tunicates have led to the characterization of a new class of metabolites, based on the β-carboline ring system, with antiviral, antibiotic, and other biological activities (1,2). The majority of the compounds found were named eudistomins A–T. These were isolated from *Eudistoma olivaceum* collected from the Caribbean Sea (3–6), except eudistomin K, which together with debromoeudistomin was obtained from the ascidian *Ritterella sigillinoides* from the sea around New Zealand (7). Eudistomidins A–F were isolated from *Eudistoma glaucus* found in the sea around Japan (8–10), while 5-bromo-*N,N*-dimethylaminoethyltryptamine and woodinine are constituents of *Eudistoma fragum* located in the lagoon of New Caledonia (11).

In continuation of our investigation of New Caledonian marine organisms for bioactive compounds (11), we have analyzed the EtOH extract of *Eudistoma album* F. Monniot (Polycitorodae), found to be cytotoxic in vitro against the growth of KB human buccal carinoma cells.

Partitioning of the acidified aqueous solution of the EtOH extract with Et_2O gave an inactive Et_2O fraction. Basification and extraction of the alkaloids into CH_2Cl_2 led to an active alkaloidal fraction. Chromatographic analysis of the alkaloidal extract gave eudistomin E and **1**, while the Et_2O fraction afforded **2**. Eudistomin E was characterized by comparison of its physical and spectral data with literature values (3,6).



1 R_1=H, R_2=NH_2
2 R_1, R_2=O

The presence of the indole chromophore in **1** was deduced from its uv, λ max 240 (ε 45,368), 295 (ε 23,157) nm. The hrfabms had one of its doublet $[MH]^+$ peaks at *m/z* 334.0754 ($C_{16}H_{19}{}^{81}BrN_3$), indicating the presence of bromine. The eims shows a fragment peak at *m/z* 248 attributed to the brominated β-carboline moiety (5,11).

The aromatic region of the ^{1}H-nmr spectrum of **1** had one-proton signals at δ 7.31 (dd, *J*=8.4, 1.3 Hz), 7.70 (d, *J*=1.3 Hz), and 7.98 (d, *J*=8.4 Hz), representing a trisubstituted benzene ring compo-

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nent of the brominated β-carboline system. Comparison of these signals with similar ones reported for eudistomin N, eudistomin O (4), and eudistomidin B (9) located the bromine on C-7. In addition, the ^{1}H-nmr spectrum was almost identical with that of eudistomin O except for the lack of the H-1 signal (4). Thus, the coupled signals at δ 7.79 and 7.98 (d, J=5.0 Hz) were assigned to H-4 and H-3, respectively, leaving the location of the aliphatic chain at C-1 of a fully aromatic system. The ^{1}H/^{13}C HETCOR showed the presence of two methyl, one methylene, and two methine groups in the nonaromatic region. The couplings of these protons in the ^{1}H-nmr spectrum indicated a $(Me)_2CHCH_2CH(NH_2)$-linkage. The relative downfield chemical shift of the last methine group (δ 4.53 for its proton and δ 55 for its carbon) confirmed the presence of NH_2. These data support the proposed structure **1** for eudistalbin A.

Compound **2** also had doublet $[M]^+$ peaks at *m/z* 332 ($C_{16}H_{14}{}^{81}BrN_2$) and *m/z* 330 together with a fragment at *m/z* 248 in its eims, indicating a β-carboline system similar to that of **1** and differing only in the composition of the aliphatic chain. The uv spectrum with λ max at 224 (ε 28,400) and 285 nm (ε 14,697) supports this suggestion. The chemical shifts and coupling pattern of the aromatic protons in the ^{1}H-nmr spectrum also indicated a similar 7-bromo-β-carboline structure with an aliphatic chain at C-1. The ir spectrum had a peak at 1670 cm^{-1}, indicating the presence of a keto group on the chain. The ^{1}H-nmr spectrum had two equivalent methyls giving a signal at δ 1.06 (d, J=7.0 Hz) coupled to a methine with a multiplet at δ 2.40, which is also coupled to the equivalent methylene protons at δ 3.30 (d, J=7.0 Hz). Thus the aliphatic chain is $(Me)_2CHCH_2CO$-, consistent with the proposed structure **2** for eudistalbin B.

Only the eudistomidins (in vitro) and eudistomin K (in vitro and in vivo) have so far been shown to possess strong cytotoxic activities (7,9). Eudistomin E, known to be strongly antiviral (6), has now also been shown to be cytotoxic to KB human nasopharyngeal carcinoma cells (100% cytotoxicity at 5.0 ng/ml). Eudistalbin A [**1**] was found to be less cytotoxic (ED_{50} 3.2 μg/ml), while eudistalbin B [**2**] was inactive. Eudistalbins A [**1**] and [**2**] may be derived biosynthetically from tryptophan and leucine. This suggestion, when considered with similar biosynthetic proposals for other β-carbolines from various tunicates, indicates a basic ability of tunicates to join various amino acids with tryptophan to form this growing class of compounds (1–9,12).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (uncorrected) were determined on a micro hot-stage apparatus. Optical rotations at 20° were taken on a Perkin-Elmer 241 polarimeter. Uv spectra were recorded on a Shimidzu UV-161 uv-visible spectrophotometer; ir spectra on a Nicolet 205 FT-IR spectrometer; eims (70 eV) on a Kratos MS50; hrfabms on a Kratos MS 80; and nmr on a Bruker AC 250 (normal ^{1}H and ^{13}C spectra), AC 400 (^{1}H/^{13}C HETCOR spectrum). All nmr spectra were recorded in CD_3CN with TMS as internal standard. Cc was performed using Si gel Merck H60, and tlc with Si gel 60 F_{254}. Visualization was by viewing under uv light and spraying with Dragendorff's reagent followed by 50% H_2SO_4.

ANIMAL MATERIAL.—The tunicate *E. album* was collected in February 1989 in the intertidal area at Cap Noua, New Caledonia, in the frame of the CRNS-ORSTOM program Substances Marines d'Intérêt Biologique (SMIB). Samples (ref. UA 323) were identified by Mrs. F. Monniot of the Museum d'Histoire Naturelle de Paris, France and conserved at ORSTOM, Nouméa, New Caledonia.

EXTRACTION AND PURIFICATION.—The freeze-dried animal material (160 g) was extracted with 80% EtOH (×5) at room temperature, and the pooled extract was concentrated to dryness in vacuo. The concentrate was dissolved in H_2O, acidified with 10% HCl, and partitioned with Et_2O. Removal of Et_2O from the pooled organic fraction gave 10 g of inactive extract. The acidic medium was basified with NH_4OH and partitioned with CH_2Cl_2. Concentration of the pooled CH_2Cl_2 fractions gave 795 mg of active extract which was fractionated by cc eluted with CH_2Cl_2,

followed by increasing amounts of MeOH in CH_2Cl_2. Tlc monitoring of fractions in CH_2Cl_2-MeOH (9:1) led to eleven bulked Dragendorff-positive fractions in order of their increasing polarity. Fraction 8 gave pure **1** (30 mg) on removal of solvent. Fraction 9 (60 mg) was further purified by cc eluted with CH_2Cl_2-MeOH (98:2) to give **1** (19 mg) and eudistomin E (20 mg). The inactive Et_2O fraction was also subjected to cc eluted with CH_2Cl_2 (20-ml fractions collected). Combined fractions 21–70 were purified by another cc and preparative tlc in heptane-EtOAc (95:5) to give pure **2** (2 mg).

Eudistalbin A [**1**].—Brown amorphous; $[\alpha]_D$ −10° (MeOH, c=0.1); uv λ max (MeOH) 220 sh, 240 (ε 45,368), 290 sh, 295 (ε 23,157), 338 (ε 5789), 350 (ε 5789) nm; ir ν film cm^{-1} 1565, 1616, 2961, 3400; hr fabms *m/z* $[MH]^+$ 334.0754 ($C_{16}H_{19}{}^{81}BrN_3$ requires 334.07423); eims *m/z* (% rel. int.) $[M-H]^+$ 332 (3), 330 (4), 277 (98), 276 (99), 275 (100), 274 (80), 248 (12), 246 (15), 195 (15), 168 (40), 140 (20); ^1H nmr δ 0.88 (3H, d, J=7.0 Hz, Me), 0.94 (3H, d, J=7.0 Hz, Me), 1.63 (1H, m, H-12), 1.72 (2H, m, H-11), 4.53 (1H, t, J=7.0 Hz, H-10), 7.31 (1H, dd, J=8.4, 1.3 Hz, H-6), 7.70 (1H, d, J=1.3 Hz, H-8), 7.79 (1H, d, J=5.0 Hz, H-4), 7.98 (1H, d, J=8.4 Hz, H-5), 8.25 (1H, d, J=5.0 Hz, H-3); ^{13}C nmr δ 22.4 (Me), 23.4 (Me), 25.5 (C-12), 47.0 (C-11), 55.1 (C-10), 113.8 (C-4), 115.6 (C-8), 118.1 (C-4a), 121.2 (C-5a), 123.2 (C-6), 123.5 (C-5), 128.9 (C-8a), 134.8 (C-9a), 138.9 (C-3), 141.9 (C-1), 150.1 (C-7).

Eudistalbin B [**2**].—Light brown, amorphous; uv λ max (MeOH) 210, 224 (ε 28,402), 245 (ε 11,744), 285 (ε 14,697), 310 (ε 8123), 376 (ε 4732) nm; ir ν film cm^{-1} 1670; eims *m/z* (% rel. int.) $[M]^+$ 332 (70) ($C_{16}H_{14}{}^{81}BrN_2O$), 330 (80), 317 (50), 315 (50), 304 (30), 302 (35), 262 (30), 260 (30), 248 (100), 246 (98); ^1H nmr δ 1.06 (6H, d, J=7.0 Hz, 2×Me), 2.40 (1H, m, H-12), 3.30 (2H, d, J=7.0 Hz, H-11), 7.43 (1H, dd, J=8.4, 1.4 Hz, H-6), 7.75 (1H, d, J=1.5 Hz, H-8), 8.00 (1H, d, J=8.4 Hz, H-5), 8.10 (1H, d, J=5.0 Hz, H-4), 8.53 (1H, d, J=5.0 Hz, H-3).

BIOASSAY PROCEDURES.—The products were evaluated for cytotoxicity basically by the procedure established at the National Cancer Institute using the human nasopharyngeal carcinoma KB cell line (13). Results: Eudistomin E 100% cytotoxicity at seven concentrations ranging from 10 to 0.005 μg/ml; ED_{50}<5.0 ng/ml. Eudistalbin A [**1**] 100% cytotoxicity at 10, 92% at 5, and 0% at 1 μg/ml; ED_{50}. 3.2 μg/ml. Eudistalbin B [**2**] 0% cytotoxicity at 10 and 1 μg/ml.

ACKNOWLEDGMENTS

S.A.A. acknowledges fellowship support from the OAU/STRC, Lagos, Nigeria, and CNRS, France. The authors thank C. Tempête (CNRS, Gif-sur-Yvette) for the cytotoxicity tests.

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Received 19 August 1991