

## A $\beta$ -CARBOLINE DIMER FROM AN ASCIDIAN, *DIDEMNUM* SP.

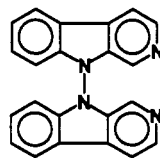
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ABSTRACT.—A  $\beta$ -carboline dimer (**1**) has been isolated from a *Didemnum* sp. of ascidian collected from the Great Barrier Reef in Australia.

Ascidians of the family Didemnidae are generally highly colored, encrusting organisms that characteristically contain compounds derived from amino acids (1). A specimen of a green encrusting *Didemnum* sp. was collected from Sykes Reef in the Capricorn Bunker Group of the southern Great Barrier Reef. Extraction of the freeze-dried ascidian with hexane followed by EtOAc gave fractions containing one major uv absorbing compound, as indicated by tlc. This compound was purified by cc on Si gel, using EtOAc as the solvent. Low-resolution ms of the compound showed a molecular ion at  $m/z$  334, with a base peak at  $m/z$  of 167, and with no intervening fragment ions, thereby suggesting a dimeric structure for the compound. The  $^1\text{H}$ -nmr spectrum contained only seven signals for aromatic hydrogens, arranged in two isolated spin-systems consistent with 1,2-disubstituted and 1,2,4-trisubstituted aromatic ring systems. The  $^{13}\text{C}$ -nmr spectrum revealed eleven signals, all in the aromatic region, of which four were derived from quaternary carbons. The spectral evidence thus supports a symmetrical dimer with a probable molecular formula of  $\text{C}_{22}\text{H}_{14}\text{N}_4$ . The structure was further defined by the values of the ortho coupling constants in the two spin-systems. These were smaller in the 1,2,4-trisubstituted aromatic ring system, suggesting a fused pyridine ring, with the nitrogen at the  $\beta$  position. All spectroscopic data thus pointed to a  $\beta$ -carboline (9H-pyrido[3,4-b]indole) dimer with  $N,N$ -dimerization, as shown in **1**.

Compound **1** is a known synthetic compound derived from photochemical dimerization of  $\beta$ -carboline (norharmane)



**1**

(2). To the best of our knowledge, however, neither **1** itself, nor  $\beta$ -carboline dimers in general, have been reported previously as natural products. Our spectroscopic data are in agreement with those reported for **1** (2,3).

The ability of **1** to form complexes with transition metal ions was briefly investigated. However, no change was observed in the uv spectrum of MeOH solutions of **1** on addition of  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ , or  $\text{Ni}^{2+}$ .

Further aromatic compounds are also present in the extracts of the *Didemnum* sp. Spectroscopic evidence suggests that they are also  $\beta$ -carboline dimers, but with  $N,C$ -dimerization<sup>1</sup>. The present collection has not yet yielded sufficient quantities of these compounds to enable complete structural elucidation.

### EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.— $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra were recorded in  $\text{DMSO}-d_6$  on a Bruker AMX300 spectrometer. Low-resolution ms were recorded on a Shimadzu QP2000 quadrupole mass spectrometer using the direct insertion probe. Cc was performed on 230–400 mesh Si gel. Ir spectra were recorded on a Perkin Elmer 1600 Ft-ir, and uv spectra on a Varian Cary 4E.

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**ANIMAL MATERIAL.**—The ascidian *Didemnum* sp. was collected by scuba from Sykes Reef, Great Barrier Reef, and kept frozen until used. The ascidian occurs as a thin, hard and brittle investing sheet-like colony, crowded with spicules throughout. Some minute pigment cells occur in the surface, mixed with the upper layer of spicules. Spicules are large, to 0.1-mm diameter, with 9 to 11 long conical rays in the optical section. The colony is in an active vegetative state, with gonads not detected. The cloacal cavity is shallow, at thorax level. Spicules and zooids (with 8 or 9 stigmata per row and a forked alrial tongue) resemble those of *D. caesium* Sluiter, 1909. *D. caesium* has spicules present only in the surface test and not throughout the colony. A voucher specimen (QM G308286) has been deposited at the Queensland Museum, Brisbane, Queensland, Australia.

**EXTRACTION AND ISOLATION.**—The freeze-dried ascidian (42 g) was extracted with hexane (200 ml $\times$ 3) and EtOAc (200 ml $\times$ 3). The hexane and EtOAc extracts were separately chromatographed on Si gel with EtOAc as solvent. The major uv-absorbing fraction from each column was combined and rechromatographed on Si gel with EtOAc-HOAc (99:1). Recovery of the major fraction and recrystallization from EtOAc/hexane yielded the dimer **1**. Colorless needles (33 mg), mp 209–210° (dec); ir (KBr disk)  $\nu$  max 3043, 2928, 1617, 1566, 1452, 1426, 1318, 1229, 821, 745, 720  $\text{cm}^{-1}$ ; uv (MeOH)  $\lambda$  max 211 (log  $\epsilon$  4.71), 230

(5.01), 278 (4.37), 283 (4.50), 328 (3.79); 338 (3.89) nm; eims  $m/z$  [M]<sup>+</sup> 334 (35), 167 (100), 140 (36); <sup>1</sup>H nmr  $\delta$  6.95 (1H, dd,  $J=8$  and 1 Hz, H-8), 7.48 (1H, dt,  $J=8$  and 1 Hz, H-6), 7.54 (1H, dt,  $J=8$  and 1 Hz, H-7), 8.38 (1H, d,  $J=5$  Hz, H-4), 8.40 (1H, s, H-1), 8.50 (1H, dd,  $J=8$  and 1 Hz, H-5), 8.60 (1H, d,  $J=5$  Hz, H-3); <sup>13</sup>C nmr  $\delta$  109 (C-8), 116 (C-4), 120 (C-4a), 122 (C-6), 123 (C-5), 128 (C-4b), 129 (C-7), 132 (C-1), 136 (C-8a), 140 (C-9a), 142 (C-3).

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