6-Bromotryptamine Derivatives from the Gulf of California Tunicate Didemnum candidum

Eoin Fahy, Barbara C. M. Potts, D. John Faulkner, and Keith Smith

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6-BROMOTRYPTAMINE DERIVATIVES FROM THE GULF OF CALIFORNIA TUNICATE *DIDEMNUM CANDIDUM*

EOIN FAHY, BARBARA C.M. POTTS, D. JOHN FAULKNER,*

Scripps Institution of Oceanography (A-012F), University of California, San Diego, La Jolla, California 92093-0212

and KEITH SMITH

Department of Chemistry, University College of Swansea, Swansea, SA2 8PP, UK

ABSTRACT.—Two slightly different specimens of the encrusting grey tunicate *Didemnum candidum* were collected in the southern Gulf of California and were examined separately. 6-Bromotryptamine [**1**] was isolated from the first specimen (87-045). The second specimen (87-061) contained 2,2-bis(6′-bromo-3′-indolyl)ethylamine [**2**] and 2,5-bis(6′-bromo-3′-indolyl)piperazine [**3**].

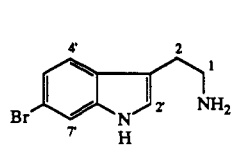
6-Bromotryptamine derivatives have been encountered in several marine phyla including sponges (1–11), coelenterates (12,13), tunicates (14,15), a bryozoan (16), and a mollusc (17). This broad distribution among the phyla may indicate that the compounds are produced by symbionts, and in the case of the mollusc *Babylonia japonica*, a food-chain origin has been demonstrated (18). Brominated indole derivatives are most closely associated with the bryozoans, particularly *Flustra foliacea* (19). As part of a study of the metabolites of *F. foliacea*, 6-bromotryptamine [**1**] was synthesized in order to provide material for pharmacological screening but was not found to be a natural product (20). In this paper we report the first occurrence of 6-bromotryptamine [**1**] as a natural product and the isolation and characterization of 2,2-bis(6′-bromo-3′-indolyl)ethylamine [**2**] and 2,5-bis(6′-bromo-3′-indolyl)piperazine [**3**] from the tunicate *Didemnum candidum* (Savigny, 1816) (Didemnidae).

RESULTS AND DISCUSSION

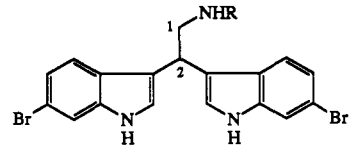
Two samples of *D. candidum* were collected from different environments in the Gulf of California. Specimen 87-045, which is a thin off-white encrusting tunicate, was collected by hand from submerged mangrove roots (−1 m) at Isla San Jose. Specimen 87-061, a thin blue-gray encrusting tunicate, was collected by hand using scuba (−3 m) from rock faces at Isla Carmen. Both specimens were subsequently identified as *D. candidum*. The apparent difference in the surface colors may well be due to different symbionts associated with the two forms. The samples were frozen and later extracted with MeOH. The combined hexane, CH_2Cl_2, and EtOAc extracts from the MeOH-soluble material from 87-045 were chromatographed on Sephadex LH-20, and an antimicrobial fraction from that separation was further purified by chromatography on Si gel to obtain 6-bromotryptamine [**1**] (0.015% dry wt). A similar treatment of the MeOH extracts of 87-061 resulted in the isolation of 2,2-bis(6′-bromo-3′-indolyl)ethylamine [**2**] (0.023% dry wt) and 2,5-bis(6′-bromo-3′-indolyl)piperazine [**3**] (0.01% dry wt).

6-Bromotryptamine [**1**] was obtained as a pale yellow oil. The molecular formula, $C_{10}H_{11}BrN_2$, was established by high resolution mass measurement of the molecular ion. The structure was elucidated by interpretation of spectral data followed by comparison of the spectral data, particularly the ^1H- and ^{13}C-nmr spectra recorded in DMSO-d_6 solution (Tables 1 and 2), with those reported for a sample of synthetic 6-bromotryptamine [**1**] (20).

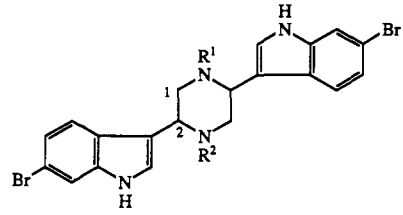
2,2-Bis(6′-bromo-3′-indolyl)ethylamine [**2**] was obtained as an optically inactive yellow oil. The high resolution fab mass spectrum contained a cluster of peaks at *m/z* 431.9701, 433.9711, and 435.9668 that correspond to $[M + H]^+$ ions for a molecular



1



2 R=H **4** R=Ac



3 $R^1=R^2=H$*
5 $R^1=R^2=Ac$*
6 $R^1=Me$, $R^2=H$*

*The numbering scheme for compounds **3**, **5**, and **6** allows comparison of the nmr signals in Tables 1, 2, and 3 and differs from the usual numbering scheme for this class of compounds.

formula of $C_{18}H_{15}Br_2N_3$. The ^{13}C-nmr spectrum contained only ten signals, and inspection of the relative intensities of the signals indicated that the eight downfield signals assigned to indole carbons were due to two carbons each. Comparison of the chemical shifts of the downfield signals with those of the corresponding signals in 6-bromotryptamine [**1**] led to the conclusion that **2** contained two symmetrically located 6-bromo-3-indolyl rings. The position of the bromine atom was confirmed by an nOeds experiment in which irradiation of the NH signal at δ 11.35 (br s, 2H) caused enhancements of the H-2′ signal at 7.38 (d, 2H, $J=2.1$ Hz) and the H-7′ signal at 7.54 (d, 2H, $J=1.6$ Hz). The two upfield ^{13}C-nmr signals at δ 32.7 (d) and 43.2 (t) were assigned to a 2,2-disubstituted ethylamine residue. The ^1H nmr signals at δ 3.46 (t, 2H, $J=7$ Hz) and 4.80 (m, 1H) were at appropriate chemical shifts for 2,2-bis(6′-bromo-3′-indolyl)ethylamine [**2**]. Peaks at *m/z* 401, 403, 405 (1:2:1) and 317, 319 (1:1) in

TABLE 1. ^1H-nmr Data (200 MHz, chemical shift, integral, multiplicity) for Indoles **1**–**5**.

Proton	Compound					
	1[a]	**2**[a]	**4**[b]	**3**[a]	**5**[c]	*J* (Hz)
H-1	2.80 2H, m	3.46 2H, m	3.98 2H, t	3.03 4H, m	see text	7[d]
H-2	2.80 2H, m	4.80 1H, m	4.64 1H, t	4.13 2H, t	see text	7[d]
H-1′	11.09 1H, s	11.35 2H, s	8.15 2H, s	11.00 2H, s	9.02 2H, s	
H-2′	7.18 1H, d	7.38 2H, d	7.00 2H, d	7.48 2H, d	6.95 2H, d	2.1
H-4′	7.48 1H, d	7.40 2H, d	7.38 2H, d	7.59 2H, d	7.47 2H, d	8.5
H-5′	7.09 1H, dd	7.00 2H, dd	7.13 2H, dd	7.05 2H, dd	7.28 2H, dd	8.5, 1.6
H-7′	7.53 1H, d	7.54 2H, d	7.52 2H, d	7.51 2H, d	7.52 2H, d	1.6
Ac			1.79 3H, s		2.00 3H, s	
NH			5.55 1H, br t			7

[a]Solvent = DMSO-d_6.
[b]Solvent = $CDCl_3$.
[c]Solvent = 5% CD_3OD in $CDCl_3$.
[d]Coupling constant for acyclic compounds **1**, **2**, and **4**. For **3** and **5**, see text.

TABLE 2. ^{13}C-nmr Data (50 MHz, chemical shift, multiplicity) for Indoles **1–5**.

Carbon	Compound				
	1[a]	**2**[a]	**4**[b]	**3**[a]	**5**[c]
C-1	41.1 t	43.2 t	43.7 t	49.1 t	42.9 t
C-2	29.2 t	32.7 d	34.1 d	50.8 d	53.6 d
C-2′	123.9 d	124.0 d	122.9 d	124.1 d	123.5 d
C-3′	112.2[d] s	114.5[d] s	116.8[d] s	116.2[d] s	116.3[d] s
C-3a′	126.3 s	125.3 s	125.6 s	125.6 s	123.8 s
C-4′	120.1[e] d	120.2[e] d	120.6[e] d	120.9[e] d	120.1[e] d
C-5′	121.0[e] d	121.1[e] d	122.5[e] d	120.9[e] d	121.5[e] d
C-6′	113.6[d] s	113.8[d] s	115.1[d] s	113.5[d] s	114.5[d] s
C-7′	113.8 d	114.1 d	114.2 d	113.8 d	114.6 d
C-7a′	137.1 s	137.5 s	137.4 s	137.0 s	137.5 s
MeCO			23.4 q		21.4 q
H_3CO			170.3 s		171.6 s

[a]Solvent = DMSO-d_6.
[b]Solvent = $CDCl_3$.
[c]Solvent = 5% CD_3OD in $CDCl_3$.
[d,e]Assignments may be reversed.

the fabms were attributed to losses of methylamine and 6-bromoindole respectively. In order to confirm the proposed structure, the amine was treated with Ac_2O in pyridine to prepare a monoacetate **4**. In the ^{1}H-nmr spectrum, the signal for the methylene group bearing the acetamide at δ 3.98 (t, 2H, J = 7 Hz) was coupled to the methine signal at 4.64 (t, 2H, J = 7 Hz) and to the amide proton signal at 5.55 (br t, 1H, J = 7 Hz). All other spectral data were in accord with those expected for a monoacetamide derived from 2,2-bis(6′-bromo-3′-indolyl)ethylamine.

2,5-Bis(6′-bromo-3′-indolyl)piperazine [**3**] was obtained as an optically inactive opaque glass that resisted all attempts at crystallization. The molecular formula, $C_{20}H_{18}Br_2N_4$, was established from the fabms peaks at *m/z* 473, 475, 477 $[M+H]^+$ coupled with high-resolution data for the diacetamide **5**. Both the ^{1}H- and ^{13}C-nmr data contained only half the number of signals expected from the molecular formula, indicating that **3** was a symmetrical dimer. The ^{1}H-nmr spectrum in DMSO-d_6 solution contained the usual signals assigned to the indole ring system together with signals at δ 4.13 (br m, 2H) and 3.03 (br m, 4H). In the ^{1}H-nmr spectrum in Me_2CO-d_6 solution, the aliphatic signals appeared at δ 3.16 (dd, 2H, J = 12, 3 Hz), 3.26 (dd, 2H, J = 12, 6 Hz), and 4.30 (dd, 2H, J = 6, 3 Hz). The lack of an optical rotation requires that **3** have C_i symmetry and that both substitutents at C-2 and C-5 be equatorial. The ^{13}C-nmr signals at δ 49.1 (t) and 50.8 (d) are at reasonable chemical shifts for the 2,5-disubstituted piperazine carbons. After comparing these data with those of dragmacidon A [**6**] (11), which is a mono-*N*-methyl derivative of **3** that has ^{1}H-nmr coupling constants of 11, 10.5, and 3 Hz for $J_{1,1}$, $J_{1ax,2ax}$, and $J_{1eq,2ax}$, it was concluded that the ^{1}H-nmr signals were at appropriate chemical shift values for the hydrogens on a 2,5-disubstituted piperazine ring but that the differences in coupling constants, possibly due to conformational differences, required further explanation. We therefore decided to examine the spectral data of the corresponding *N,N*′-diacetylpiperazine [**5**]. The mass spectral peaks at *m/z* 557, 559.0167, 561 $[M+H]^+$ confirmed the expected molecular formula $C_{24}H_{22}Br_2N_4O_2$. When recorded at room temperature in various solvents, the signals in the ^{1}H-nmr spectra of the diacetamide **5** were broadened due to interconversion of the amide diastereomers. At −40° in CD_2Cl_2 solution or −55° in $CDCl_3$ sol-

ution, the signals due to a major diastereomer sharpened and those due to minor diastereomers intensified. The low temperature CD_2Cl_2 spectrum contained a signal at δ 3.18 (dd, 1H, J = 13, 10 Hz) that was assigned to H-1_{ax} of the major diastereomer, but the H-1_{eq} and H-2 signals were obscured by the solvent peak. The low temperature $CDCl_3$ spectrum contained an AA'X system for the major diastereomer with the H-1_{ax} signal at δ 3.14 and the H-1_{eq} and H-2 signals at 5.33 and 5.36 (observed by irradiation at δ 3.14). A low temperature (−75°) Me_2CO-d_6 spectrum contained four sets of signals (Table 3) of approximately equal intensity that were assigned to the three possible diastereomers of the diacetamide **5**. An analysis of the low temperature COSY experiment allowed the coupling patterns of the four sets of signals to be assigned, and all signals clearly showed the expected coupling constants for a six-membered ring in the chair conformation.

TABLE 3. Selected ^{1}H-nmr Data (500 MHz, Me_2CO-d_6, −75°) for the Geometrical Isomers of Diacetamide **5**.[a]

Proton		
H-1_{ax}	H-1_{eq}	H-2
3.73 J = 15, 11 Hz	4.30 J = 15, 7 Hz	6.04 J = 11, 7 Hz
3.70 J = 15, 12 Hz	4.43 J = 15, 7 Hz	5.76 J = 12, 7 Hz
3.45 J = 13.5, 12 Hz	5.11 J = 13.5, 7 Hz	5.72 J = 12, 7 Hz
3.37 J = 13.5, 11 Hz	5.11 J = 13.5, 7 Hz	5.40 J = 11, 7 Hz

[a]Four sets of signals were assigned by interpretation of the COSY spectrum.

EXPERIMENTAL

COLLECTION, EXTRACTION AND PURIFICATION OF *D. CANDIDUM*.—Specimens of *D. candidum* (collection #87-045, SIO Benthic Invertebrate Collection # AS138), a thin off-white encrusting tunicate, were collected from submerged mangrove roots at Isla San Jose, Gulf of California, Mexico, in May 1987. The sample (197 g dry wt) was immediately frozen and later extracted with MeOH (2 × 2 liters) at room temperature to give a green solution. The combined MeOH extracts were evaporated in vacuo to obtain an aqueous suspension (200 ml) that was successively extracted with hexane (2 × 200 ml), CH_2Cl_2 (2 × 200 ml), and EtOAc (2 × 200 ml). All three organic phases were dried over anhydrous Na_2SO_4, the solvents were evaporated, and the combined extracts were chromatographed on a Sephadex LH-20 column with MeOH-CH_2Cl_2 (1:1) as eluent. One of the fractions (150 mg), which exhibited in vitro antibacterial and antifungal activity, was further purified by flash silica chromatography, using a gradient of 5% NH_4OH/MeOH in CH_2Cl_2, to yield 6-bromotryptamine [**1**] (35 mg, 0.015% dry wt).

Specimens of *D. candidum* (collection #87-061, SIO Benthic Invertebrate Collection # AS139), a thin blue-gray encrusting tunicate, were collected by hand using scuba from rock faces (−3 m) near Isla Carmen in the Gulf of California, Mexico, in May 1987. The sample (266 g dry wt) was immediately frozen and later extracted with MeOH (2 × 2 liters) to obtain a bluish-green solution. The combined MeOH extracts were evaporated in vacuo, and the resulting aqueous suspension (200 ml) was successively extracted with hexane (2 × 200ml), Et_2O (2 × 200 ml), and EtOAc (2 × 200 ml). The hexane and Et_2O extracts were combined (400 mg), dried over anhydrous Na_2SO_4, and chromatographed on a Sephadex LH-20 column with MeOH-CH_2Cl_2 (1:1) as eluent. A late-eluting fraction contained pure 2,2-bis(6'-bromo-3'-indolyl)ethylamine [**2**] (60 mg, 0.023% dry wt). A second LH-20 fraction was subjected to flash chromatography on a Si gel column using an MeOH in CH_2Cl_2 gradient to yield 2,5-bis(6'-bromo-3'-indolyl)piperazine [**3**] (35 mg, 0.01% dry wt).

6-BROMOTRYPTAMINE [**1**].—Pale yellow oil: ir ($CHCl_3$) 3470, 2940, 2860, 1455, 1090, 805 cm^{-1}; uv (MeOH) 225 nm (ε 15,800), 286 nm (ε 3,100), 294 nm (ε 2,700); ^{1}H nmr (200 MHz, DMSO-d_6) see Table 1; ^{13}C nmr (50 MHz, DMSO-d_6) see Table 2 (cf. reference 20).

2,2-BIS(6'-BROMO-3'-INDOLYL)ETHYLAMINE [**2**].—Pale yellow oil: ir ($CHCl_3$) 3630, 3440 (br), 3010, 1615, 1455, 1330, 890, 805 cm^{-1}; uv (MeOH) 227 nm (ε 28700), 286 nm (ε 5300); ^{1}H nmr (200 MHz, DMSO-d_6) see Table 1; ^{13}C nmr (50 MHz, DMSO-d_6) see Table 2; fabms *m/z* (rel. int.) 432/434/

436 (3), 401/403/405 (7), 237/239 (12); hrfabms *m/z* 431.9701, 433.9711, 435.9668 ($C_{18}H_{16}Br_2N_3$ $[M+H]^+$ requires 431.9711, 433.9691, 435.9671).

2,5-BIS(6'-BROMO-3'-INDOLYL)PIPERAZINE [**3**].—Opaque glass: ir (KBr disc) 3300 (br), 1615, 1540, 1455, 1335, 1225, 1100, 1050, 895, 800 cm^{-1}; uv (MeOH) 226 nm (ε 27400), 286 nm (ε 4600); ^1H nmr (200 MHz, DMSO-d_6) see Table 1, (500 MHz, Me_2CO-d_6) δ 3.16 (dd, 2H, $J=12$, 3 Hz), 3.26 (dd, 2H, $J=12$, 6 Hz), 4.30 (dd, 2H, $J=6$, 3 Hz), 7.09 (d, 2H, $J=8$ Hz), 7.58 (s, 2H), 7.59 (s, 2H), 7.68 (d, 2H, $J=8$ Hz), 10.26 (br s, 2H); ^{13}C nmr (50 MHz, DMSO-d_6) see Table 2; fabms *m/z* (rel. int.) 473/475/477 (5), 237/239 (14).

PREPARATION OF ACETAMIDE **4**.—2,2-Bis(6'-bromo-3'-indolyl)ethylamine [**2**] (10 mg) was treated with Ac_2O (0.25 ml) and pyridine (0.5 ml) at room temperature for 16 h. Evaporation of the solvents under high vacuum yielded the acetamide **4** (9 mg) as a pale yellow oil: ir ($CHCl_3$) 3630, 3470, 3010, 1710, 1660 (s), 1615 cm^{-1}; uv (MeOH) 228, 287 nm; ^1H nmr (200 MHz, $CDCl_3$) see Table 1; ^{13}C nmr (50 MHz, $CDCl_3$) see Table 2; fabms *m/z* (rel. int.) 401/403/405 (6), 279/281 (7), $[M]^+$ not observed.

PREPARATION OF DIACETAMIDE **5**.—2,5-Bis(6'-bromo-3'-indolyl)piperazine [**3**] (8 mg) was allowed to react overnight in a stirred solution of Ac_2O (0.25 ml) and pyridine (0.5 ml) to obtain the diacetamide **5** (6 mg) as the major reaction product: ir ($CHCl_3$) 3630, 3470, 3010, 1640 (s) cm^{-1}; uv (MeOH) 226 nm, 286 nm; ^1H nmr (200 MHz, $CD_3OD/CDCl_3$) see Table 1; ^{13}C nmr (50 MHz, $CD_3OD/CDCl_3$) see Table 2; cims *m/z* (rel. int.) 557/559/561 $[M-H]^+$ (3), 479/481 (2), 401 (3), 364/366 (2), 296/298 (7), 279/281 (100); hr cims (NH_3) *m/z* 559.0167 ($C_{24}H_{23}{}^{79}Br^{81}BrN_4O_2$ $[M+H]^+$ requires 559.0168).

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LITERATURE CITED

1. W.D. Raverty, R.H. Thomson, and T.J. King, *J. Chem. Soc., Perkin Trans. 1*, 1204 (1977).
2. R.J. Andersen and R.J. Stonard, *Can. J. Chem.*, **57**, 2325 (1979).
3. P. Djura and D.J. Faulkner, *J. Org. Chem.*, **45**, 735 (1980).
4. P. Djura, D.B. Stierle, B.J. Sullivan, D.J. Faulkner, E. Arnold, and J. Clardy, *J. Org. Chem.*, **45**, 1435 (1980).
5. A.A. Tymiak, K.L. Rinehart Jr., and G.J. Bakus, *Tetrahedron*, **41**, 1039 (1985).
6. G. Lindgren, L. Bohlin, and J. Bergman, *Tetrahedron Lett.*, **27**, 3283 (1986).
7. K. Bartik, J.-C. Braekman, D. Daloze, C. Stoller, J. Huysecom, G. Vandevyver, and R. Ottinger, *Can. J. Chem.*, **65**, 2118 (1987).
8. S. Kohmoto, Y. Kashman, O.J. McConnell, K.L. Rinehart Jr., A. Wright, and F. Koehn, *J. Org. Chem.*, **53**, 3116 (1988).
9. S. Tsujii, K.L. Rinehart Jr., S.P. Gunasekera, Y. Kashman, S.S. Cross, M.S. Lui, S.A. Pomponi, and M.C. Diaz, *J. Org. Chem.*, **53**, 5446 (1988).
10. S.A. Morris and R.J. Andersen, *Can. J. Chem.*, **67**, 677 (1989).
11. S.A. Morris and R.J. Andersen, *Tetrahedron*, **46**, 715 (1990).
12. E. Fattorusso, V. Lanzotti, S. Magno, and E. Novelino, *J. Nat. Prod.*, **48**, 924 (1985).
13. G. Guella, I. Mancini, H. Zibrowius, and F. Pietra, *Helv. Chim. Acta*, **72**, 1444 (1989).
14. K.L. Rinehart Jr., J. Kobayashi, G.C. Harbour, R.G. Hughes, Jr., S.A. Mizsak, and T.A. Scahill, *J. Am. Chem. Soc.*, **106**, 1524 (1984).
15. J.W. Blunt, R.J. Lake, M.H.G. Munro, and T. Toyokuni, *Tetrahedron Lett.*, **28**, 1825 (1987).
16. P. Wulff, J.S. Carle, and C. Christophersen, *Comp. Biochem. Physiol.*, **71B**, 523 (1982).
17. T. Kosuge, H. Zenda, A. Ochiai, N. Masaki, M. Noguchi, S. Kimura, and H. Narita, *Tetrahedron Lett.*, 2545 (1972).
18. T. Kosuge, K. Tsuji, K. Harai, and T. Fukuyama, *Chem. Pharm. Bull.*, **33**, 3059 (1985).

19. U. Anthoni, P.H. Nielsen, M. Pereira, and C. Christophersen, *Comp. Biochem. Physiol.*, **96B**, 431 (1990).
20. C. Grøn and C. Christophersen, *Acta Chem. Scand.*, **B38**, 709 (1984).

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