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Polyandrocarpamides A-D, Novel Metabolites from the Marine Ascidian *Polyandrocarpa* sp.

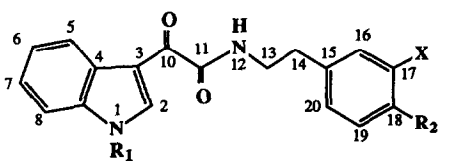
Niels Lindquist and William Fenical*

**Scripps Institution of Oceanography
University of California, San Diego
La Jolla, California 92093-0228**

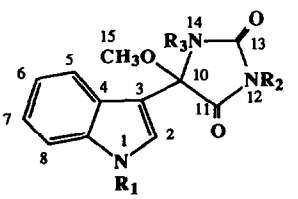
Summary: Four new indole-derived metabolites (**1-4**), including the iodinated polyandrocarpamide B (**2**), have been isolated from the colonial marine ascidian *Polyandrocarpa* sp. collected in the Philippines. The structures of these metabolites were determined through spectral interpretation, chemical conversions and by comparisons with a synthetic phenethylamine analog.

Prior chemical studies have shown marine ascidians to be a rich source of unique and biologically active secondary metabolites that have attracted the interest of both chemists and pharmacologists.[1] Recent chemical[2] and biological[3] investigations have begun to reveal the importance of these metabolites in the survival of many physically vulnerable ascidians in predator-rich habitats and to further illustrate how the presence of chemically defended organisms in the marine environment affects the development of many benthic marine communities. As part of our continuing study of the chemical adaptations of taxonomically diverse ascidians, we began a chemical investigation of the colonial ascidian, *Polyandrocarpa* sp., collected in the Philippines. In this paper, we wish to report the structures of several new natural products that possess α-dicarbonyl functionalities joining an indole moiety with derivatives of 4-hydroxyphenethylamine. *Polyandrocarpa* sp., a rust colored compound ascidian, was collected in the Spring, 1988, at Siquijor Is., the Philippines between -15 and -25 m depth. The acetone extract of the lyophilized ascidian was initially fractionated by vacuum flash chromatography over TLC-grade silica gel. Final purification by reversed-phase HPLC (ODS-silica, 7:3 CH_3OH:H_2O), yielded **1-4** as 0.01, 0.007, 0.0021 and 0.079% dry weight, respectively.

Polyandrocarpamide A (**1**), needles, mp. 178-179°C, analyzed for $C_{18}H_{15}N_2O_3Br$ by HRFABMS in conjunction with ^1H and ^{13}C NMR data (Table). DEPT sequence NMR experiments indicated that 3 protons were bonded to heteroatoms. Of the 12 units of unsaturation in **1**, 4 were accounted for as a 1,2,4 trisubstituted benzene by the



#	R_1	R_2	X
1	H	OH	Br
2	H	OH	I
3	H	OH	H
5	Ac	OAc	Br
6	Me	OMe	Br
7	H	H	H



#	R_1	R_2	R_3
4	H	H	H
8	Ac	Ac	H
9	Me	Me	Me

Table 1. ^1H and ^{13}C NMR Data for Polyandrocarpamides A-D (1-4) and Derivatives 5-9

	1		2		3		4	
#	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H
1								11.32, 1H, bs[b]
2	139.5	8.67, 1H, s	139.6	8.65, 1H, s	139.5	8.67, 1H, s	124.6	7.34, 1H, s
3	114.0		114.0		no[c]		110.6	
4	127.9		127.9		127.8		124.4	
5	122.9	8.29, 1H, m	122.9	8.28, 1H, m	123.0	8.29, 1H, m	120.3	7.74, 1H, dd (7.8, 1.0)
6	123.8	7.25, 1H, m	123.8	7.25, 1H, m	123.8	7.25, 1H, s	119.1	7.03, 1H, ddd (7.8, 7.4, 1.0)
7	124.8	7.25, 1H, m	124.8	7.25, 1H, m	124.8	7.25, 1H, m	121.4	7.13, 1H, ddd (7.8, 7.4, 1.0)
8	113.1	7.47, 1H, m	113.1	7.45, 1H, m	113.1	7.46, 1H, m	111.6	7.36, 1H, dd (7.8, 1.0)
9	138.0		138.0		138.0		136.7	
10	183.3		183.0		182.9		88.6	
11	165.8		166.0		165.7		172.4	
13	41.8	3.51, 2H, t (7.1)	41.8	3.49, 2H, t (7.2)	42.1	3.51, 2H, t (7.7)	156.1	
14	35.2	2.79, 2H, t (7.1)	35.2	2.76, 2H, t (7.2)	35.6	2.80, 2H, t (7.7)		9.08, 1H, bs[b]
15	132.9		131.0		131.0		50.2	3.36, 3H, s
16	134.3	7.38, 1H, d (2.0)	140.5	7.59, 1H, d (2.0)	130.8	7.08, 1H, d (8.5)		
17	110.8		84.9		116.3	6.72, 1H, d (8.5)		
18	154.0		157.1		157.0			
19	117.3	6.82, 1H, dd (8.2)	115.7	7.07, 1H, dd (8.1)	116.3	6.72, 1H, d (8.5)		
20	130.1	7.06, 1H, dd (8.2, 2.0)	133.3	7.07, 1H, dd (8.1, 2.0)	130.8	7.08, 1H, d (8.5)		

	5	6	7		8	9
#	^1H	^1H	^{13}C	^1H	^1H	^1H
1	2.79, 3H, s (NAc)	3.90, 3H, s (NMe)			2.51, 3H, s (NAc)	3.79, 3H, s (NMe)
2	9.34, 1H, s	8.54, 1H, s	139.4	8.72, 1H, s	7.84, 1H, s	7.33, 1H, s
3			113.7			
4			127.5			
5	8.48, 1H, m	8.29, 1H, d (7.7)	122.7	8.29, 1H, m	8.42, 1H, d (8.4)	7.22 - 7.31, 1H
6	7.44, 1H, m	7.31, 1H, m	123.6	7.25, 1H, m	7.24, 1H, dd (8.4, 7.3)	7.22 - 7.31, 1H
7	7.44, 1H, m	7.31, 1H, m	124.5	7.25, 1H, m	7.34, 1H, dd (8.5, 7.3)	7.11, 1H, ddd (8.2, 6.8, 0.8)
8	8.37, 1H, m	7.50, 1H, d (7.6)	112.8	7.44, 1H, m	7.31, 1H, d (8.5)	7.22 - 7.31, 1H
9			137.5			
10			182.1			
11			164.9			
12	7.54, 1H, t (6.8)				2.67, 3H, s (NAc)	2.83, 3H, s (NMe)
13	3.66, 1H, dt (7.1, 6.8)	3.54, 1H, t (7.2)	41.4	3.56, 2H, t (7.6)		
14	2.92, 1H, t (7.1)	2.83, 1H, t (7.2)	36.1	2.87, 2H, t (7.6)		3.15, 3H, s (NMe)
15			139.6		3.44, 3H, s	3.39, 3H, s
16	7.52, 1H, d (1.7)	7.45, 1H, d (2.1)	129.2	7.15 - 7.25, 1H		
17			129.4	7.15 - 7.25, 1H		
18	2.35, 3H, s (OAc)	3.84, 1H, s (OMe)	127.1	7.15 - 7.25, 1H		
19	7.09, 1H, d (8.0)	6.95, 1H, d (8.4)	129.4	7.15 - 7.25, 1H		
20	7.21, 1H, dd (8.0, 1.7)	7.21, 1H, dd (8.4, 2.1)	129.2	7.15 - 7.25, 1H		

[a] ^1H and ^{13}C NMR spectra were recorded at 360 and 50 MHz, respectively, and were run in methanol-d_4 for **1-4** and **7-9** and in $CDCl_3$ for **5** and **6**. Coupling constants are reported in Hertz and chemical shifts are given in δ units (downfield of Me_4Si). Assignments were aided by spin decoupling experiments, DEPT sequence experiments and a comparison of spectral data for model compounds and the synthetic derivative **7** and $^1J_{C\text{-}H}$ and $^{2,3}J_{C\text{-}H}$ correlation experiments performed with **4**.

[b] NH protons observed in the ^1H NMR spectra recorded in DMSO-d_6.

[c] resonance not observed due to the small quantity of **3** isolated.

characteristic coupling constants for the C16, C19 and C20 protons. The relative upfield shift of the C19 proton suggested the presence of a phenol, and the extreme downfield shift of the C2 singlet in the ^{1}H NMR spectrum of **1** established the presence of a carbonyl substituent at C3 of the indole. The chemical shifts and coupling patterns of the 4 remaining aromatic protons characterized the unsubstituted nature at C5 through C8 of the indole nucleus. The 2 remaining unsaturations were accounted for as carbonyls, based on quaternary carbon resonances in the ^{13}C NMR spectrum at δ 183.3 and 165.8, and IR absorptions at 1680 and 1637 cm^{-1}. The λ max at 324 nm in the UV spectrum of **1** supported the assignment of a carbonyl substituent at the C3 position of the indole and indicated further conjugation when compared to model compounds.[5] The N12 D_2O exchangeable amide proton in the ^{1}H NMR spectrum of **1** (DMSO-d_6) was coupled to the C13 methylene protons, which in turn were coupled to the C14 methylene protons, suggesting a 3,4-disubstituted phenethylamine amide. Acetylation of **1** with excess Ac_2O yielded the diacetylated product **5**. An acetamide and phenolic acetate were suggested by the chemical shift of the methyl resonances and IR absorptions at 1727 and 1768 cm^{-1}, respectively. Methylation of **1** with excess CH_3I and K_2CO_3 in acetone afforded the dimethylated product **6**. New methyl resonances in the ^{1}H NMR spectrum of **6** were observed at δ 3.90 (s) and 3.84 (s). In NOEDS experiments with **6**, irradiation of the δ 3.90 methyl group enhanced the C2 and C8 protons of the indole by 8.0 and 18.2%, respectively, allowing assignment of this methyl group at N1. Irradiation of the δ 3.84 methoxyl group produced a 27.4% enhancement in the C19 proton, while irradiation of this proton resulted in a 16.0% enhancement of the C18 methoxyl protons. In addition, irradiation of the C14 methylene protons enhanced the aromatic protons at C16 and C20. The NOEs within the phenethylamide subunit unambiguously placed the hydroxyl group at C18 and the bromine at C17, thus completing the structural elucidation of **1**. Spectral features (Table, ref. 5) of the synthetic unsubstituted phenethylamine analog **7**, prepared by the condensation of oxalyl chloride with indole[6] and subsequent condensation with phenethylamine, provided a firm basis for positioning the C10-C11 dicarbonyl substituent at C3 of the indole moiety.

Polyandrocarpamide B (**2**), analyzed for $C_{18}H_{15}N_2O_3I$, and exhibited highly analogous ^{1}H and ^{13}C NMR spectra. The UV and IR spectra of **2** indicated the identical chromophore as in **1**. Placement of iodine at C17 was required on the basis of the significant upfield shift (δ 84.9) observed for the C17 carbon.

Polyandrocarpamide C (**3**), a white solid, analyzed for $C_{18}H_{16}N_2O_3$. Comparisons of key spectral features of **1**, **2** and **7**, with those of **3** revealed that the indole and dicarbonyl subunits were also present. Two sets of degenerate aromatic carbons (C16,20 and C17,19), and 2 doublet proton resonances (each 2H, J = 8.5 Hz) were observed for **3**. These data characterized polyandrocarpamide C as the unhalogenated 4-hydroxyphenethylamine analog of **1** and **2**.

Polyandrocarpamide D (**4**), a yellow solid, produced a diacetamide, **8**, which confidently analyzed by HRMS for $C_{16}H_{15}N_3O_5$, thus establishing the molecular formula of **4** as $C_{12}H_{11}N_3O_3$ (9 units of unsaturation). Proton and ^{13}C NMR spectra revealed the presence of the 3-substituted indole, however, the shift of the C-2 proton (δ 7.34) and the simple indole UV absorptions for **4** precluded further conjugation. The indole moiety accounted for 6 units of unsaturation leaving 3 unsaturations for the remaining $C_4H_5N_2O_3$ subunit. Two carbonyls were evident (^{13}C NMR: δ 172.4 and 156.1; IR: 1780 and 1735 cm^{-1}), hence one ring was present in this subunit. In the COLOC spectrum of **4**, the C15 methyl protons (δ 3.36) correlated through three bonds to C10, thus a ketal, bearing methoxyl as one substituent, was assigned at C10. Based on the spectral features of **4** and its derivatives, and conserving a basic structural component of **1-3**, the structure of polyandrocapamide D was suggested as **4**. Polyandrocarpamide D shows $[\alpha]_D = 0.00^o$, indicating that C10 is a racemic center. The possibility thus exists that **4** may be produced by methanol addition to a carbonyl-containing precursor during the initial reverse-phase HPLC purification.

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5. Additional spectral data: For **1**: white needles; mp 178-179°C; UV (MeOH) 324 nm (ε 8400), 290 (5000), 274 (9600), 267 (9900), 255 (10300), 205 (41400); UV (MeOH + NaOH) 309 nm (ε 9800), 272 (8600), 266 (9900), 247 (1600), 207 (58500); IR ($CHCl_3$) 3010, 1680, 1635, 1500, 1420, 1215, 1120 cm^{-1}; HRFABMS obsd. (M^++H) *m/z* 387.0313, $C_{18}H_{15}N_2O_3{}^{79}Br$ requires 387.0345, Δ 8.2; ^1H and ^{13}C NMR (Table). For **2**: white amorphous solid; UV (MeOH) 325 nm (ε 6600), 274 (8200), 267 (sh), 254 (9100), 230 (sh), 205 (50000); UV (MeOH + NaOH) 311 nm (ε 8200), 274 (7600), 267 (sh), 247 (13200), 203 (79100); IR (neat, NaCl) 2930, 1680, 1640, 1505, 1420, cm^{-1}; HREIMS obsd. (M^+) *m/z* 434.0146, $C_{18}H_{15}N_2O_3I$ requires 434.0129, Δ -3.9; ^1H and^{13}C NMR (Table). For **3**: white amorphous solid; UV (MeOH) 324 nm (ε 4600), 285 (sh), 272 (5700), 266 (6100), 254 (6400), 205 (19200); UV (MeOH + NaOH) 218 nm (ε 4600), 272 (5300), 266 (6100), 246 (8300), 205 (21400); HREIMS obsd. (M^+) *m/z* 308.1159, $C_{18}H_{16}N_2O_3$ requires 308.1162, Δ 1.0); ^1H and ^{13}C NMR (Table). For **4**: yellow amorphous solid; $[\alpha]_D$ 0.00 (MeOH, c 5.8) UV (MeOH) 287 nm (ε 3800), 276 (400), 268 (5000), 212 (27500); UV (MeOH + NaOH) 289 nm (ε 3700), 277 (4600), 268 (4600), 217 (sh), 204 (44800); IR (neat, NaCl) 3600-2900, 1780, 1735, 1390, 1250, 1025, 100 cm^{-1}; Positive LRCI obsd. 214; Negative LRCI obsd. 212; HREIMS obsd (M^+-OMe-H) *m/z* 213.0504, $C_{11}H_7N_3O_2$ requires 213.0591, Δ 40.8; ^1H and ^{13}C NMR (Table). For **5**: UV (MeOH) 318 nm (ε 8400), 274 (sh), 255 (10600), 221 (1900), 203 (37400); UV (MeOH + NaOH) 307 nm (ε 9700), 273 (sh), 267 (sh), 245 (14800), 205 (54000); IR ($CHCl_3$) 3020, 1768, 1727, 1680, 1650, 1530, 1445, 1420, 1215, 1045, 930 cm^{-1}; HREIMS obsd. (M^+) *m/z* 470.0478, $C_{22}H_{19}N_2O_5{}^{79}Br$ requires 470.0477, Δ 0.1; ^1H NMR (Table). For **6**: UV (MeOH) 328 nm (ε 3600), 287 (1800), 273 (3400), 259 (4400), 203 (1700); UV (MeOH + NaOH) 328 nm (ε 3500), 287 (1800), 273 (3400), 259 (4200), 203 (22700); IR (neat, NaCl) 2930, 1680, 1626, 1505, 1370, 1255, 1205, 1125, 1095, 1020 cm^{-1}; HREIMS obsd. (M^+) *m/z* 414.0591, $C_{20}H_{19}N_2O_3{}^{79}Br$ requires 414.0580, Δ -2.7; ^1H NMR (Table). For **7**: UV (MeOH) 324 nm (ε 4800), 284 (sh), 273 (6000), 266 (6500), 254 (6600), 204 (2500); IR ($CHCl_3$) 3010, 1680, 1640, 1420, 1215, 1110 cm^{-1}; HREIMS obsd. (M^+) *m/z* 292.1219, $C_{18}H_{16}N_2O_2$ requires 292.1213, Δ 2.1; ^1H and ^{13}C NMR (Table). For **8**: UV (MeOH) 297 nm (ε 2000), 288 (2000), 232 (4600), 204 (18700); UV (MeOH + NaOH) 296 nm (ε 1200), 287 (1900), 232 (sh), 206 (23600); IR (neat, NaCl) 3400-2900, 2940, 1800, 1760, 1720, 1600, 1455, 1380, 1350, 1300, 1215, 1150, 1080, 1040, 930 cm^{-1}; HREIMS obsd. (M^+) *m/z* 329.1013, $C_{16}H_{15}N_3O_5$ requires 329.1013, Δ 0.0; ^1H NMR (Table). For **9**: IR (neat, NaCl) 2930, 1780, 1720, 1460, 1085 cm^{-1}; HREIMS obsd. (M^+) *m/z* 287.1270 (27% bp), $C_{15}H_{17}N_3O_3$ requires 287.1271, Δ 0.3, obsd. (M^+-OMe) *m/z* 256.1070 (bp), $C_{14}H_{14}N_3O_2$ requires 256.1087, Δ 6.6; ^1H NMR (Table).

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