Trypargine Alkaloids from a Previously Undescribed *Eudistoma* sp. Ascidian

Ryan M. Van Wagoner,[†] Jamaluddin Jompa,[‡] Akbar Tahir,[‡] and Chris M. Ireland*[,†]

Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah 84112 and Faculty of Marine and Fisheries Sciences, University of Hasanuddin, Ujung Pandang, Indonesia

Received December 7, 1998

The MeOH extract of an undescribed *Eudistoma* sp. ascidian was found to contain the known β-carboline trypargine (**3**); the two novel trypargine derivatives trypargimine (**4**) and 1-carboxytrypargine (**5**); and 3′,5′-dibromo-4′-methoxyphenethylamine (**6**). The structures of the novel trypargine derivatives were elucidated through the use of mass spectrometry and NMR. The trypargine isolated in this study was found to be nearly racemic in contrast to the previously described isolate which was chiroptically pure. Other previously described compounds detected in the MeOH extract include 4-hydroxyphenylacetamide, tryptamine, 1,3,7-trimethylguanine, and tetrahydropentoxyline (**7**).

Many novel β-carboline metabolites have been isolated from ascidians of the genus *Eudistoma* since Rinehart and co-workers first reported the structures of eudistomins A−Q.[1,2] These tryptophan-derived compounds often exhibit interesting biological activities.[1−5] Naturally occurring β-carbolines are often formed by the condensation of a tryptophan derivative with a second amino acid to produce a compound bearing an amino acid side chain pendant to the tricyclic β-carboline nucleus. Such compounds were isolated from *Eudistoma glaucus* by Kobayashi and co-workers, including eudistomidins A,[4] B, and C[5] with pendant groups apparently derived from proline, phenylalanine, and methionine, respectively. While the eudistomidins described above contain all of the carbon atoms found in their biosynthetic precursors, other β-carboline products result from biosynthetic pathways in which carbon atoms are lost from the precursor skeleton. Irikawa and co-workers described the isolation of **1**, a compound possibly derived from the condensation of tryptophan with 2-ketoglutaric acid, which spontaneously formed trichotomine (**2**) in neutral MeOH at room temperature.[6] The authors suggested a biosynthetic precursor role of **1** for **2** in the *Clerodendron trichotomum* species they were isolated from. In this paper we describe the isolation of a series of β-carbolines from a new *Eudistoma* sp. ascidian that may be intermediates in a biosynthetic pathway that involves the loss of a carbon atom from one of the precursor carbon skeletons.

The previously undescribed *Eudistoma* sp. ascidian[7] was collected with the aid of SCUBA in October 1996 from Bonne Tambun, Indonesia. The MeOH extract of the ground ascidian was subjected to solvent partitioning and chromatographic separation as described in the Extraction and Isolation section to provide the known tetrahydro-β-carboline trypargine (**3**), its novel derivatives trypargimine (**4**) and 1-carboxytrypargine (**5**), as well as the halogenated tyramine derivative 3′,5′-dibromo-4′-methoxyphenethylamine (**6**) (Chart 1).

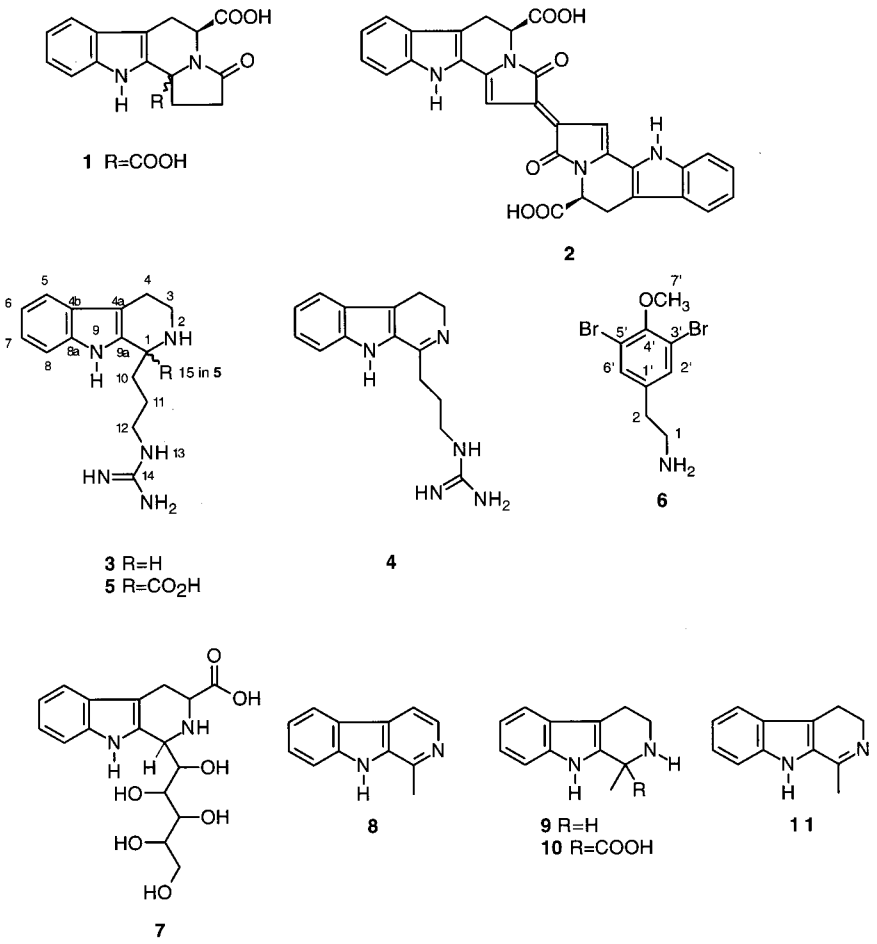
Compound **3** was identified as trypargine, previously isolated from the skin of the African frog *Kassina senegalensis*,[8] through combined use of HRFABMS and ^{1}H, ^{13}C, and two-dimensional heteronuclear correlation NMR spectroscopy. Its identity was confirmed by comparison of the ^{13}C NMR spectrum to previous reports.[9] Compound **4** was recognized as an analogue of **3** due to similarity of the aromatic signals in the ^{1}H NMR spectrum. HRFABMS of **4** indicated a molecular formula of $C_{15}H_{19}N_5$ (Δ −0.7 ppm), differing from that of trypargine only by a deficiency of two protons. The COSY spectrum for **4** in DMSO-d_6 indicated the presence of one aromatic and two aliphatic spin systems. The aromatic spin system composed of four methine protons at δ 7.77, 7.19, 7.45, and 7.55 was consistent with the presence of a 2,3-disubstituted indole ring moiety. One of the two aliphatic spin systems, which spanned three methylene groups, included a COSY correlation between carbon-bound protons at δ 3.25 (H_2-12) and an exchangeable proton at δ 7.95 (H-13). The third spin system was composed of two methylene groups. The upfield ^{13}C NMR chemical shift (δ 18.4) observed for C-4 was consistent with direct attachment to the indole at C-4a. The presence of a dihydro-β-carboline moiety was further supported by the observations of long-range heteronuclear correlations between protons at δ 3.20 (H_2-4) and 3.04 (H_2-10) and the carbon at δ 125.6 (C-9a), and between protons δ 3.92 (H_2-3) and 3.04 (H_2-10) and the carbon at δ 168.9 (C-1). The ^{13}C NMR spectral data for the putative dihydro-β-carboline moiety in **4** was consistent with that reported for isoeudistomin U, whose revised structure[10] contains a 3,4-dihydro-β-carboline moiety. The presence of a long-range heteronuclear correlation between the proton signal at δ 3.25 (H_2-12) and a carbon signal at δ 157.1 (C-14) indicated the presence of a guanidinium moiety at the end of the pendant spin system as is present in **3**, indicating that **4** is the 1,2-dehydro analogue of **3**.

HRFABMS for **5** indicated a molecular formula of $C_{16}H_{21}N_5O_2$ (Δ +3 ppm) which differs from **3** by the addition of a carboxyl group. The ^{1}H and ^{13}C NMR spectra of **5** were very similar to **3** with the largest differences being the lack of a proton signal for H-1; that C-1 was a quaternary carbon at δ 64.5; and a new carbon signal appeared at δ 169.7 (C-15). The presence of a carboxyl at C-1 in **5** was confirmed by an HMBC correlation between the proton signal at δ 1.98 (H-10) and the carbon at δ 169.7 (C-15); by the downfield shift of C-1 in **5** compared to **3** (δ 64.5 in **5** compared to δ 51.9 in **3**); as well as by the slight downfield shift of C-10 in **5** compared to **3** (δ 34.1 in **5**

* To whom correspondence should be addressed. Tel.: (801) 581-8305. Fax: (801) 581-6208. E-mail: cireland@deans.pharm.utah.edu.
[†] University of Utah.
[‡] University of Hasanuddin.

10.1021/np9805589 CCC: $18.00
Published on Web 04/15/1999

Chart 1



compared to δ 28.7 in **3**). Thus, **5** has been identified as the 1-carboxyl analogue of **3**.

Chiroptical methods were used to explore the stereochemistry of **3**. Measurement of the optical rotation of the hydrochloride salt of **3** yielded an $[\alpha]^{25}_D$ 0° (*c* 1.0, MeOH). The reported value for natural trypargine is $[\alpha]^{25}_D$ −34.2° (*c* 1.02, MeOH).[8] CD measurements were also undertaken on the hydrochloride salt of **3** in MeOH. Overall the CD curve of **3** resembled that of stereochemically resolved synthetic (−) trypargine[11] but with a much lower intensity. Quantitatively, a value for $\Delta\epsilon^{25}$ @ 215 nm of −1.7° cm^2/dmol (*c* 0.21 mM, MeOH) was measured for the *Eudistoma* isolate of **3** compared to −10.55° cm^2/dmol (*c* 0.01, MeOH) for the synthetic enantiomer.[11] Since the measured ϵ_{272} in MeOH for the *Eudistoma* isolate of **3** was within a factor of 2 of that reported for the synthetic compound, it appears likely that the *Eudistoma* isolate of **3** is nearly fully racemized. The CD spectrum of **5** (*c* 60 μM, MeOH) indicated chirality due to the presence of a positive Cotton effect at 230 nm ($\Delta\epsilon^{25}$ +7° cm^2/dmol) and a negative Cotton effect at 212 nm ($\Delta\epsilon^{25}$ −5° cm^2/dmol). Although it is not possible to derive the absolute stereochemistry of **5** by comparison of the CD spectrum with those of the yohimbine-type β-carbolines which bear a proton at C-1,[12,13] it is worth mentioning that the polarities of the Cotton effects are similar to those observed for the 1-*R*, dimethyl ester derivative of **1** and opposite to those observed for the 1-*S*, dimethyl ester derivative.[14]

HRFABMS for **6** indicated a molecular formula of $C_9H_{11}Br_2NO$ (Δ −2 ppm). The presence of two bromine atoms within the molecule was supported by the isotopic pattern of the peaks at *m*/*z* 308 (relative abundance of 1), 310 (2), and 312 (1). The ^{1}H NMR spectrum in CD_3OD consisted of two singlets and two triplets. Both sets of triplet resonances showed HMBC correlations to the carbon at δ 136.7 (C-1′). The methylene protons at δ 2.82 (H_2-2) also showed HMBC correlations to the protonated carbons at δ 133.1 (C-2′/6′). An integration value higher than one for the aromatic resonance at δ 7.58 (H-2′/6′) and the observance of an HMBC correlation between the same resonance and the carbon atom it was directly bound to suggested a symmetrically substituted phenyl moiety. HMBC correlations from the aromatic protons and the protons of an oxygenated methyl group at δ 3.76 (H_3-7′) into the carbon resonance at δ 152.2 (C-4′) indicated that the aromatic ring was a methylated phenol. Structure **6** was surmised to be the most likely structure consistent with all of the HMBC data and the symmetry requirements of the molecule. The structure was confirmed by reaction of O-methyltyramine with an excess of Br_2 overnight in acetic acid.[15] The synthetic product was identical to the natural product by MS, NMR, and HPLC co-injection.

In addition to the novel compounds described in this paper, the *Eudistoma* sp. ascidian was found to produce compounds generally typical of this genus. Among the known compounds observed in the extract were simple amino acid metabolites such as 4-hydroxyphenylacetamide and tryptamine; 1,3,7-trimethylguanine;[16] and the β-carboline tetrahydropentoxyline (**7**), previously isolated from human urine.[17]

Brominated phenolic moieties are often found within marine natural products. A *Verongula* sp. sponge was found to produce the trimethylammonium derivative of **6**.[18] There has been a recent report of a monobrominated tyramine

derivative from the ascidian *Cnemidocarpa bicornuta*.[19] While compound **6** has not been described as a natural product to our knowledge, it was synthesized in the course of testing its analeptic activity in cats.[15]

Neither the physiological relevance nor the biosynthetic interrelationships of the trypargine alkaloids are known. Trypargine has been reported to be toxic to mice (LD_{50} 16.9 mg/kg, intravenous administration)[20] and to block voltage gated sodium currents in squid axon membrane in a potential-dependent manner.[21] We did not observe significant cytotoxicity in fractions containing the trypargine alkaloids against human colon tumor cells.

There are some data that suggest that trypargine is the furthest downstream of the three compounds in the biosynthetic pathway of these alkaloids. The FAB mass spectrum for **5** included a peak at *m/z* 270, more intense than the protonated molecular ion at *m/z* 316, whose high-resolution mass corresponded to the same molecular formula as **4** although trypargimine was not detectable by ^{1}H NMR in the sample. It is possible that protonated **5** has a low energy barrier pathway leading to a loss of an equivalent of formic acid to produce trypargimine. A similar oxidative decarboxylation has been hypothesized to occur in some biosynthetic pathways of the β-carbolines harman (**8**) and eleagnine (**9**). Studies in rats have shown that co-injections of tritium-labeled tryptamine with pyruvic acid into rat brain led to the formation of labeled 1-carboxy-tetrahydroharman (**10**) via an apparent enzymatic reaction.[22] Injection of labeled **10** in mice led to the formation of labeled harmalan (**11**) as the major degradation product and **9** as a minor product in the lung and spleen.[23] Double label studies have also indicated that **10** is a precursor of **8** and **9** in *Passiflora edulis* and *Eleagnus angustifolia*, respectively.[24] The synthesis of **8** and **9** from **10** is believed to occur by way of **11** which has been shown to be a precursor for both compounds.[25,26] Thus, **5**, possibly generated by the enantioselective condensation of tryptamine with the α-keto-acid derivative of arginine, may oxidatively decarboxylate to give **4**. Once generated, **4** may be enzymatically reduced to **3** with low stereoselectivity.

Experimental Section

General Experimental Procedures. The ^{1}H and ^{13}C NMR spectra were obtained at 500 and 125 MHz, respectively, on a Varian Unity 500 spectrometer. Proton chemical shifts are reported in parts per million relative to residual undeuterated solvent. UV spectra were obtained in MeOH on a Hewlett-Packard 8452A diode array spectrophotometer. High- and low-resolution FAB mass spectral measurements were made on a Finnegan MAT 95 high-resolution spectrometer. Circular dichroism spectra were obtained on a Jasco J720A spectropolarograph. HPLC was carried out using the following Rainin Dynamax columns on a Beckman 126 solvent module and a Beckman 168 diode array detector: C_{18} (10 × 250 mm, 8 μm); cyano (4.6 × 250 mm, 5 μm) and (10 × 250 mm, 8 μm); amino (10 × 250 mm, 8 μm). Sephadex LH-20 chromatography gel (25−200 μm bead size) and *O*-methyltyramine were purchased from Sigma.

Animal Material. The ascidian *Eudistoma* sp., identified as a previously undescribed species by Dr. Françoise Monniot (Muséum National d'Histoire Naturelle, Paris, France) (Sample No. CMI-96-29-3; Registry No. A3 Eud 198), was collected by SCUBA at Bonne Tambun (5° 1.923′ S × 119° 16.745′ E at −3 to −15 m), Indonesia, in October 1996. It is characterized by a cushion-like colony without sedimentary inclusions and zooids grouped in irregular systems. The zooids have an incubatory pouch neatly protruding out of the thorax but without peduncle. The larva is large with three oval wide adhesive papillae in a line, not separated by epidermic ampullae. Thin threadlike extensions lie anteriorly on each side of the larval papillae. A voucher specimen was deposited at the Muséum National d'Histoire Naturelle, Paris, France.

Extraction and Isolation. The freeze-dried *Eudistoma* sp. ascidians (∼30−40 g dry weight) were extracted repeatedly with MeOH followed by filtration. The combined extracts were concentrated in vacuo and redissolved in a 90% aqueous MeOH solution. This solution was extracted with hexanes prior to dilution with water to 70% MeOH and extraction with $CHCl_3$. The $CHCl_3$ soluble material was fractionated by LH-20 size exclusion chromatography using MeOH as an eluent. Fractions containing **3** and **4** were combined and purified by flash C_{18} chromatography using a step gradient from 10 to 100% aqueous MeOH. The loading solution contained 0.1% TFA to enhance solubility. Both compounds eluted during the late 10% MeOH/early 30% MeOH steps of the gradient. Compound **3** was further purified by HPLC on a cyano column using an isocratic mobile phase composition of 30% aqueous MeOH (0.1% TFA). Compound **3** (trifluoroacetate salt) was obtained as a light yellow solid (3.7 mg, 0.01%). Compound **4** was further purified by HPLC (cyano column) using an isocratic mobile phase composition of 5% aqueous TFA (0.1%) in MeOH followed by HPLC (amino column; 95% aqueous MeCN for 15 min; gradient from 95 to 50% aqueous MeCN over 20 min) to provide **4** as a light yellow solid (0.4 mg, 0.001%).

The aqueous MeOH soluble material was dried and repeatedly triturated with MeOH to remove insoluble salts. The MeOH soluble material was dissolved in water and purified by C_{18} vacuum liquid chromatography (VLC) using a step gradient from 0 to 100% aqueous MeOH followed by a MeOH (0.1% TFA) rinse of the column. The aqueous fraction was partitioned between water and *n*-butanol. The *n*-butanol soluble material was further fractionated by flash C_{18} chromatography using a step gradient from 10 to 100% aqueous MeOH followed by a MeOH (0.1% TFA) rinse. Compound **3** was obtained as a slightly impure, light yellow solid (11 mg, 0.03%), which eluted during the late 10% MeOH/early 30% MeOH steps of the purification.

The fractions eluting from 5 to 50% aqueous MeOH by the C_{18} VLC purification described in the previous paragraph were combined and fractionated by reversed-phase C_{18} flash chromatography. A step gradient was used with the mobile phase composition varied from 10 to 100% aqueous MeOH and a MeOH (0.1% TFA) rinse. Fractions containing **5** eluted with 30% aqueous MeOH. Compound **5** was then purified by HPLC (cyano column; 30% aqueous MeOH (0.05% TFA)) followed by C_{18} HPLC (10% aqueous MeOH (0.05% TFA)). Compound **5** was obtained as a light yellow solid (0.2 mg, 6 ppm).

The isolation of **5** was somewhat optimized for better yields on a different specimen of the *Eudistoma* sp. ascidian. The organism (700 g) was extracted with EtOH, and the resulting extract was partitioned between hexanes, $CHCl_3$ and aqueous MeOH as described above for the original specimen. The aqueous MeOH fraction was partitioned between *n*-butanol and water. The *n*-butanol fraction was resolved by flash C_{18} chromatography using a multistep gradient (0−100% aqueous MeOH). Compound **5** eluted with 30% aqueous MeOH. Fractions containing compound **5** were then iteratively purified by three successive steps of LH-20 chromatography (100% MeOH) followed by HPLC (amino column; 85% aqueous MeCN for 20 min; gradient from 85 to 50% aqueous MeCN over 20 min). Compound **5** was obtained as a colorless solid (0.8 mg, 0.0001%).

The MeOH (0.1% TFA) rinses of several flash C_{18} chromatography purifications of the $CHCl_3$ soluble material were combined and partitioned between hexanes and 90% MeOH in water. The aqueous MeOH soluble material was then triturated with 10% aqueous MeOH and loaded onto a flash C_{18} chromatography column. A step gradient from 10% aqueous MeOH (0% TFA) to 100% MeOH (0.1% TFA) was used for the purification. At each step the MeOH soluble material was again triturated with mobile phase and the supernatant loaded onto the column. Compound **6** was finally purified by C_{18} HPLC (20% MeCN in aqueous TFA (0.1%) for 20 min; gradient

Table 1. NMR Assignments for Trypargimine (**4**) in DMSO-d_6

atom no.	δ ^{13}C	δ ^{1}H	integration, mult, *J* (Hz)	HMBC
1	168.9			
2				
3	42.4	3.92	2H, t, 8.9	1, 4, 4a
4	18.4	3.20	2H, m	3, 4a, 9a
4a	123.6			
4b	124.2			
5	121.7	7.77	1H, d, 8.0	7, 8a
6	121.2	7.19	1H, t, 7.4	4b, 8
7	128.2	7.45	1H, t, 7.5	5, 8a
8	113.1	7.55	1H, d, 8.3	4b, 6
8a	140.6			
9		10.91	1H, s	
9a	125.6			
10	29.6	3.04	2H, t, 7.7	1, 9a, 11, 12
11	26.0	1.90	2H, p, 7.3	10, 12
12	40.0	3.25	2H, m	10, 11, 14
13		7.95	1H, t, 5.3	
14	157.1			

Table 2. NMR Assignments for 1-Carboxytrypargine (**5**) in DMSO-d_6

atom no.	δ ^{13}C	δ ^{1}H	integration, mult, *J* (Hz)	HMBC
1	64.5			
2				
3	39.9	3.38	2H, m	1, 4, 4a
4	18.1	2.83	2H, t, 5.7	3, 4a, 4b, 9a
4a	104.7			
4b	125.6			
5	117.2	7.38	1H, d, 7.8	4a, 7, 8a
6	118.0	6.94	1H, t, 7.0	4b, 8
7	120.6	7.01	1H, t, 7.1	5, 8a
8	111.1	7.30	1H, d, 8.0	4b, 6
8a	136.2			
9		10.86	1H, s	4a, 8a, 9a
9a	132.5			
10	34.1	2.30	1H, m	1, 9a, 11, 12
		1.98	1H, m	1, 11, 12, 15
11	23.1	1.60	2H, m	1, 10, 12
12	40.1	3.10	2H, m	
13		8.51	1H, m	
14	156.7			
15	169.7			

from 20 to 90% MeCN in aqueous TFA (0.1%) over 30 min) to yield **6** (trifluoroacetate salt) as a colorless solid (0.2 mg, 0.0006%).

Synthesis of 6. *O*-Methyltyramine (105 mg) was dissolved in 5 mL of AcOH. To this was added 2 mL of a 1:1 mixture of Br_2 and AcOH. The deep red solution was stirred overnight and the AcOH and excess Br_2 were removed by evaporation. The resulting product was partitioned between 10% aqueous Na_2CO_3 and $CHCl_3$. The $CHCl_3$ soluble material was further purified by flash C_{18} chromatography using a single step gradient from 30% aqueous MeOH (0% TFA) to 30% aqueous TFA (0.1%) in MeOH to yield **6** (trifluoroacetate salt) as an off-white solid (168.9 mg, 78% yield): UV (MeOH) λ_{max} (log ϵ) 208 (4.53), 224 (3.91), 284 (3.08); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.80 (3H, br, NH_3^+), 7.58 (2H, s, H-2′/6′, HMBC C-2, C-2′/6′, C-3′/5′, C-4′), 3.76 (3H, s, H_3-7′, HMBC C-4′), 3.06 (2H, t, 7.4 Hz, H_2-1, HMBC C-2, C-1′), 2.82 (2H, t, 7.4 Hz, H_2-2, HMBC C-1, C-1′, C-2′/6′); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 152.1 (C-4′), 136.8 (C-1′), 133.1 (C-2′/6′), 117.4 (C-3′/5′), 60.3 (C-7′), 39.4 (C-1), 31.5 (C-2); HRFABMS *m/z* 307.9279 (calcd for $C_9H_{12}Br_2NO$, 307.9286).

Trypargimine (4): light yellow solid; UV (MeOH) λ_{max} (log ϵ) 206 (4.5), 236 (4.3), 254 (4.0), 316 (4.3), 348 (3.9); ^{1}H, ^{13}C, and HMBC data: Table 1; HRFABMS *m/z* 270.1717 (calcd for $C_{15}H_{20}N_5$, 270.1719).

1-Carboxytrypargine (5): colorless solid; UV (MeOH) λ_{max} (log ϵ) 204 (4.0), 226 (4.3), 274 (3.7), 282 (3.7), 290 (3.6); ^{1}H, ^{13}C, and HMBC data: Table 2; CD (*c* 60 μM, MeOH) 212 ($\Delta\epsilon$ −5), 222 ($\Delta\epsilon$ 0), 230 ($\Delta\epsilon$ +7), 252 ($\Delta\epsilon$ 0), 270 nm ($\Delta\epsilon$ −0.4); HRFABMS *m/z* 316.1783 (calcd for $C_{16}H_{22}N_5O_2$, 316.1774).

Table 3. NMR Assignments for 3′,5′-Dibromo-4′-methoxyphenethylamine (**6**) in DMSO-d_6

atom no.	δ ^{13}C	δ ^{1}H	integration, mult, *J* (Hz)	HMBC
1	39.4	3.06	2H, t, 7.4	1′
2	31.4	2.82	2H, t, 7.4	1, 1′, 2′/6′
1′	136.7			
2′/6′	133.1	7.58	2H, s	2, 2′/6′, 3′/5′, 4′
3′/5′	117.6			
4′	152.2			
7′	60.3	3.76	3H, s	4′
NH		7.78	3H, br	

3′,5′-Dibromo-4′-methoxyphenethylamine (6): trifluoroacetate salt, colorless solid; UV (MeOH) λ_{max} (log ϵ) 208 (4.9), 224 (4.3), 284 (3.0); ^{1}H, ^{13}C, and HMBC data: Table 3; HRFABMS *m/z* 307.9280 (calcd for $C_9H_{12}Br_2NO$, 307.9286).

Acknowledgment. This work was supported by NIH Grant CA 36622 (C.M.I.) and by NIH Fellowship GM 08573 and an American Foundation for Pharmaceutical Education pre-doctoral fellowship (R.M.V.). Funding for the Varian Unity 500 MHz NMR spectrometer was provided through NCI Grant No. 5 P30 CA 42014 and NIH Grant No. 1 S10 RR 06262. Mass spectrometry was performed by Elliot M. Rachlin on a Finnigan Mat 95 funded by NSF Grant CHE-9002690 and the University of Utah Institutional Funds Committee. We thank Derek M. Schmehl for the performance of cytotoxicity assays.

References and Notes

(1) Rinehart, K. L., Jr.; Kobayashi, J.; Harbour, G. C.; Hughes, R. G., Jr.; Mizsak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1984**, *106*, 1524−1526.
(2) Kobayashi, J.; Harbour, G. C.; Gilmore, J.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1984**, *106*, 1526−1528.
(3) Murata, O.; Shigemori, H.; Ishibashi, M.; Sugama, K.; Hayashi, K.; Kobayashi, J. *Tetrahedron Lett.* **1991**, *32*, 3539−3542.
(4) Kobayashi, J.; Nakamura, H.; Ohizumi, Y. *Tetrahedron Lett.* **1986**, *27*, 1191−1194.
(5) Kobayashi, J.; Cheng, J.; Ohta, T.; Nozoe, S.; Ohizumi, Y.; Sasaki, T. *J. Org. Chem.* **1990**, *55*, 3666−3670.
(6) Toyoda, Y.; Kumagai, H.; Irikawa, H.; Okumura, Y. *Chem. Lett.* **1982**, 903−906.
(7) Monniot, F. Personal communication.
(8) Akizawa, T.; Yamazaki, K.; Yasuhara, T.; Nakajima, T.; Roseghini, M.; Erspamer, G. F.; Erspamer, V. *Biomed. Res.* **1982**, *3*, 232−234.
(9) Shimizu, M.; Ishikawa, M.; Komoda, Y.; Matsubara, Y.; Nakajima, T. *Chem. Pharm. Bull.* **1982**, *30*, 4529−4533.
(10) Massiot, G.; Nazabadioko, S.; Bliard, C. *J. Nat. Prod.* **1995**, *58*, 1636−1639.
(11) Shimizu, M.; Ishikawa, M.; Komoda, Y.; Nakajima, T. *Chem. Pharm. Bull.* **1982**, *30*, 909−914.
(12) Bláha, K.; Koblicová, Z.; Trojánek, J. *Collect. Czech. Chem. Commun.* **1974**, *39*, 3168−3176.
(13) Lee, C. M.; Trager, W. F.; Beckett, A. H. *Tetrahedron* **1967**, *23*, 375−385.
(14) Irikawa, H.; Toyoda, Y.; Kumagai, H.; Okumura, Y. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 880−887.
(15) Benington, F.; Morin, R. D.; Clark, L. C., Jr.; Fox, R. P. *J. Org. Chem.* **1958**, *23*, 1979−1984.
(16) Perry, N. B.; Blunt, J. W.; Munro, M. H. G. *J. Nat. Prod.* **1987**, *50*, 307−308.
(17) Horiuchi, K.; Yonekawa, O.; Iwahara, K.; Kanno, T.; Kurihara, T.; Fujise, Y. *J. Biochem.* **1994**, *115*, 362−366.
(18) Ciminello, P.; Fattorusso, E.; Magno, S. *J. Nat. Prod.* **1994**, *57*, 1564−1569.
(19) Lindsay, B. S.; Battershill, C. N.; Copp, B. R. *J. Nat. Prod.* **1998**, *61*, 857−858.
(20) Shimizu, M.; Ishikawa, M.; Komoda, Y.; Nakajima, T.; Yamaguchi, K.; Sakai, S. *Chem. Pharm. Bull.* **1984**, *32*, 1313−1325.
(21) Seyama, I.; Yakehiro, M.; Nakajima, T. *Japan. J. Physiol.* **1985**, *35*, 367−373.
(22) Susilo, R.; Rommelspacher, H. *Naunyn-Schmeideberg's Arch. Pharmacol.* **1987**, *335*, 70−76.
(23) Susilo, R.; Rommelspacher, H. *Naunyn-Schmeideberg's Arch. Pharmacol.* **1988**, *337*, 566−571.
(24) Herbert, R. B.; Mann, J. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1523−1525.
(25) Slaytor, M.; McFarlane, I. J. *Phytochemistry* **1968**, *7*, 605−611.
(26) McFarlane, I. J.; Slaytor, M. *Phytochemistry* **1972**, *11*, 229−234.

NP9805589