Pergamon

*Tetrahedron* Vol. 51, No. 19, pp. 5523-5528, 1995

0040-4020/95 $9.50+0.00

0040-4020(95)00241-3

# Isolation and Structure of Convolutamydines B ~ D from Marine Bryozoan *Amathia convoluta*

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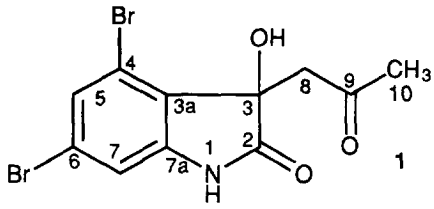
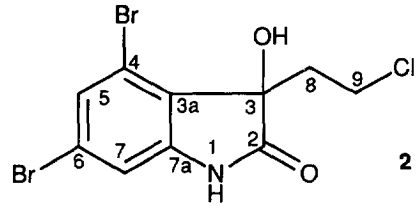
***Abstract:*** Convolutamydines B ~ D (**2** ~ **4**), three new alkaloids containing a dibromohydroxyoxindole moiety, were isolated from the Floridian bryozoan *Amathia convoluta* and the structures were elucidated on the basis of spectroscopic data. Convolutamydine B (**2**) has been found to exhibit a biological activity in the differention of HL-60 cells.

Marine bryozoans have proved to be a rich source of intriguing structures and interesting biological activities,[1,2] e. g. antineoplastic macrolide bryostatins.[3,4] During our studies on bioactive substances from the Floridian marine bryozoan *Amathia convoluta*,[5,6] we recently obtained a novel dibromohydroxyoxindole alkaloid with a 2-oxopropyl group, named convolutamydine A (**1**), which exhibited a potent activity in the differentiation of HL-60 human promyelocytic leukemia cells.[7] We further continued investigation on extracts of this bryozoan which resulted in the isolation of three new dibromohydroxyoxindole derivatives, convolutamydines B ~ D (**2** ~ **4**), bearing a variety of 3-substituents. This paper describes the isolation and structure elucidation of compounds **2** ~ **4** in detail.

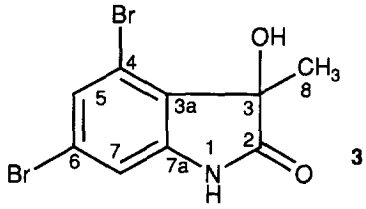
The bryozoan *Amathia convoluta* (100 kg), collected off Northeastern Gulf of Mexico in Florida, was extracted with EtOH. The extract was partitioned between aqueous MeOH and hexane, and the aqueous MeOH phase was further extracted with EtOAc. The EtOAc-soluble material was subjected successively to silica gel (hexane/acetone, 1:1), Sephadex LH-20 (hexane/$CH_2Cl_2$/MeOH, 10:10:1), Sep-Pak cartridge® (first: $C_{18}$, 75% MeOH; second: $SiO_2$, $CHCl_3$/MeOH, 94:6), and short column ($SiO_2$, hexane/acetone, 7:3) chromatographies to yield convolutamydine A (**1**, 8.6 x $10^{-6}$% wet weight). On the other hand, two side fractions , one of which was eluted from the next fraction of **1** on the Sephadex LH-20 column and the other was prior to the fraction of **1** by the short column chromatography, were noted to be more less polar than **1** on the TLC and similar to **1** in NMR spectral properties. Consequentially, the former was isolated by a short column ($SiO_2$, benzene/EtOAc, 1:1) chromatography to yield convolutamydine B (**2**, 10.7x$10^{-6}$%), and the latter was isolated carefully by reverse phase high-performance liquid chromatography (65% MeOH) to afford convolutamydines C (**3**, 6.0x$10^{-7}$%) and D (**4**, 3x$10^{-7}$%), respectively.



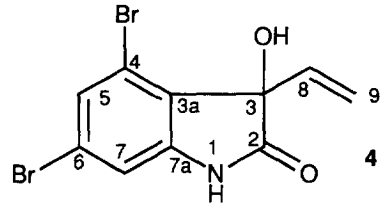

1




2




3




4

We have communicated the structural elucidation of convolutamydine A (**1**) by extensive spectroscopic techniques.[7] The structure of convolutamydine A was determined to be **1**, 4, 6-dibromo-3-hydroxy-3-(2-oxopropyl)-2-indolinone, which contained a dibromohydroxyoxindole moiety and a 2-oxopropyl group, as shown above. The $^{1}H$ and $^{13}C$ NMR chemical shifts were summarized in Table 1, which were assigned by analysis of $^{1}H$-$^{1}H$ COSY, HMQC,[8] and HMBC[9] spectral data. In comparison with the $^{1}H$ NMR and EIMS spectral data of **1**, convolutamydines B ~ D (**2~4**) were inferred to be related analogs of **1**. Furthermore, detection of compounds **1** ~ **4** could be made by the colors , which appeared ultramarine, vermilion, light yellow and yellow, respectively, on TLC (silica gel) with hexane/acetone (7:3) by spraying anisaldehyde reagent.

The EIMS data of convolutamydine B (**2**) displayed a molecular ions $(M)^+$ at *m/z* 367, 369, 371, and 373 in the ratio of 3:7:5:1, suggesting the presence of two bromines and one chlorine. The molecular formula of **2** was determined as $C_{10}H_8O_2NBr_2Cl$ by HREIMS [*m/z* 366.8610 $(M)^+$ for $C_{10}H_8O_2N^{79}Br_2{}^{35}Cl$; $\Delta$ +0.0 mmu], indicating six unsaturation degrees. The $^{13}C$ NMR spectrum (Table 1) of convolutamydine B (**2**), assisted with DEPT experiment, showed ten carbon signals attributable to two aromatic methines, two aliphatic methylenes, one oxygenated quaternary carbon, one carbonyl carbon, and four aromatic quaternary carbons. The assignment of the protonated carbons was made by the HMQC data (Table 1). The $^{13}C$ chemical shifts of **2** revealed that **2** lacked one carbon due to methyl (C-10) of **1**. And a new signal due to methylene ($\delta_C$ 39.6 t) was observed instead of the resonace due to oxocarbon ($\delta_C$ 206.0, C-9) of **1**. Interpretation of the $^{1}H$ and $^{13}C$ NMR data of **2** facilitated by application of 2D NMR spectra suggested that **2** consist of partial structures of a dibromohydroxyoxindole moiety and a 2-chloroethyl group.

The presence of a 1, 2, 3, 5-tetrasubstituted benzene ring of **2** was proved by means of the similar methods of **1** as follows. First, $^{1}H$ and $^{13}C$ NMR spectra of **2** (Table 1) revealed the presence of two aromatic methines (positions 5 and 7) and four aromatic quaternary carbons. The methines were deduced from the $^{1}H$ long range coupling constants for H-5/H-7 ($J$=1.5 Hz) to be in the meta positions. The $^{13}C$ NMR chemical shifts for quaternary aromatic carbons of C-4 ($\delta_C$ 124.1 s), C-6 ($\delta_C$ 120.9 s) and C-7a ($\delta_C$ 146.2 s) implied that bromine atoms were substituted at C-4 and C-6, and that an NH-group was attached at C-7a. Consecu-

Table 1. $^1H$ and $^{13}C$ NMR Data of Convolutamydines A (**1**) and B (**2**)

| | Compound **1**[a] | | | | Compound **2**[a] | | | |
|---|---|---|---|---|---|---|---|---|
| position | $\delta_H$ | (*J*/*Hz*) | $\delta_C$ | HMBC ($^1H$) | $\delta_H$ | (*J*/*Hz*) | $\delta_C$ | HMBC ($^1H$) |
| 1-NH | 10.60 s[b] | | | | 10.65 s[b] | | | |
| 2 | | | 177.7 s | H-8 | | | 177.8s | H-8 |
| 3 | | | 75.0 s | H-8 | | | 77.0 s | H-8, H-9 |
| 3-OH | 6.19 s[b] | | | | 6.27 s[b] | | | |
| 3a | | | 126.7 s | H-5, H-7, H-8 | | | 128.8 s | H-5, H-7, H-8 |
| 4 | | | 124.2 s | H-5 | | | 124.1 s | H-5 |
| 5 | 7.32 bd | (1.5) | 129.2 d | H-7 | 7.35 bd | (1.5) | 128.9 d | H-7 |
| 6 | | | 119.6 s | H-5 | | | 120.9 s | H-5, H-7 |
| 7 | 6.93 bd | (1.5) | 113.2 d | H-5 | 7.10 bd | (1.5) | 113.4 d | H-5 |
| 7a | | | 144.2 s | | | | 146.2 s | H-7 |
| 8 | 3.22 d | (17.7) | 47.6 t | H-10 | 2.67 ddd | (15.1, 8.6, 7.6) | 38.8 t | |
| | 3.68 d | (17.7) | | | | | | |
| 9 | | | 206.0 s | H-8, H-10 | 3.53 m[c] | | 39.6 t | |
| 10 | 2.09 s | | 30.6 q | | | | | |

[a]The NMR spectral data of **1** were determined in $CDCl_3$ (400MHz), while those of **2** were determined in acetone-$d_6$ (500MHz). [b]The signals were recorded in DMSO-$d_6$ and disappeared after $D_2O$ exchange. [c]This coupling belong to a AA'XX'.

tivelly, the coupling pattern of aromatic portion (positions 3a ~ 7a) was verfied by the HMBC cross-peaks for H-5/C-3a, H-5/C-6, H-7/C-6, H-5/C-7, and H-7/C-3a. The assignments of C-2, C-3, C-3a, and C-7a were determined by the HMBC correlations from $H_2$-8/C-2, $H_2$-8/C-3, $H_2$-9/C-3, $H_2$-8/C-3a, and H-7/C-7a. The HMBC cross-peak H-7/C-7a of **2** provided the evidence of connectivity between C-7 and C-7a, albeit this cross-peak was not obtained from the HMBC spectrum of **1**. The $^1H$ NMR signals due to the hydroxyl proton on C-3 and the NH proton were observed at $\delta_H$ 6.27 s and 10.65 s in DMSO-$d_6$, respectively, and disappeared by addition of $D_2O$. The assignment of 3-OH was also clarified by the $^{13}C$ chemical shift ($\delta_C$ 77.0 s) due to C-3. These results indicated the presence of a 4, 6-dibromo-3-hydroxyoxindole ring to be the same carbon skeleton in **1**. The presence of oxindole ring was also supported by UV absorption [$\lambda_{max}$ (MeOH) 225.0 (ε 33222), 262.5 (ε 5196), and 294.0 nm (ε 3180)].[10] On the other hand, the presence of the 2-chloroethyl group was confirmed as follows. The $^1H$-$^1H$ COSY spectrum of **2** revealed the correlation between $H_2$-8 ($\delta_H$ 2.67td) and $H_2$-9 ($\delta_H$ 3.53m), suggesting the presence of a vicinal methylene. A chlorine in the molecule of **2** was connected to C-9, which was supported by the HMBC correlations due to $H_2$-8/C-3, $H_2$-8/C-3a, and $H_2$-9/C-3, but no cross-peak due to $H_2$-9/C-3a. The $^1H$ and $^{13}C$ chemical shifts ($\delta_{H\text{-}9}$ 3.53m and $\delta_{C\text{-}9}$ 39.6t) were in good agreement with those of chloroalkyl system.[11] The EIMS fragmentation [(M-$CH_2CH_2Cl$)$^+$, *m*/*z* 304, 306, 308 (1:2:1)] also supported the presence of the chloroethyl unit. The 2-chloroethyl unit was indicated to be attached at C-3 mainly by the HMBC cross-peak for $H_2$-8/C-3. In conclusion, the structure of convolutamydine B was determined to be **2**, 4, 6-dibromo-3-hydroxy-3-(2-chloroethyl)-2-indolinone.

The molecular formula, $C_9H_7O_2NBr_2$, of convolutamydine C (**3**) was established by HREIMS [*m*/*z* 318.8815 (M)$^+$, Δ -2.8 mmu], indicating six unsaturation degrees. The EIMS of **3** revealed two fragmentations, one was due to (M-$CH_3$)$^+$ and the other was due to (M-$CH_3$-CO)$^+$, at *m*/*z* 304, 306, 308 and

Table 2. The NMR Spectral Data of Convolutamydine C (**3**)[a]

| position | $\delta_H$ (*J*/*Hz*) | $\delta_C$ | HMBC ($^1$H) | position | $\delta_H$ (*J*/*Hz*) | $\delta_C$ | HMBC ($^1$H) |
|---|---|---|---|---|---|---|---|
| 1-NH | 9.55 brs[b] | | | 5 | 7.31 bd (1.5) | 129.5 d | |
| 2 | | 177.5 s | H-8 | 6 | | 123.1 s[d] | H-7 |
| 3 | | 74.5 s | H-8 | 7 | 7.08 bd (1.5) | 112.8 d | |
| 3-OH | 5.03 s[b] | | | 7a | | 132.5 s[d] | H-7 |
| 3a | | 127.3 s | H-7, H-8 | 8 | 1.76 s | 22.5 q | |
| 4 | | 124.2 s[c] | | | | | |

[a]The $^1$H and $^{13}$C NMR spectra were detected in acetone-$d_6$ and $CDCl_3$, respectively, and the HMBC spectrum was recorded in $CDCl_3$, operating 400 MHz and 500 MHz NMR spectrometer. [b]The signals disappeared after $D_2O$ exchange. [c]The signal was not observed, and was assigned in comparison with that of **1**. [d]The signals were deduced only from 125.72 MHz NMR spectral data.

*m/z* 276, 278, 280, respectively. These fragmentations were confirmed by HREIMS data, namely, *m/z* 303.8595 $(M\text{-}CH_3)^+$, calc. 303.8581 for $C_8H_4O_2N^{79}Br_2$; and *m/z* 275.8665 $(M\text{-}CH_3\text{-}CO)^+$, calc. 275.8670 for $C_7H_4ON^{79}Br_2$. The $^1$H NMR spectrum of **3** in $CDCl_3$ showed three signals at $\delta_H$ 7.38 bd (*J* = 1.5Hz), 6.99bd (*J* = 1.5 Hz), and 1.76s, respectively. The structure of **3** was therefore considered to be similar to **1**. The $^{13}$C NMR spectral data revealed only protoned carbon signals at $\delta_C$ 129.5 d, 112.8 d, and 22.5 q by reason of the limited sample quantity (no more than 0.6 mg). The assignment of these carbons was made by the HMQC data. Althouhg any one of quaternary carbons was not observed on the $^{13}$C spectrum, the assignment was clarified by the detailed elucidation of HMBC data (Table 2). It was provided that a methyl group was located at position 3 by the HMBC correlations H-8/C-2, H-8/C-3, and H-8/3a. The $^1$H NMR spectral data (Table 2) of **3** in acetone-$d_6$ also revealed the presence of two exchangeable proton due to 1-NH and 3-OH by addition of $D_2O$. These results led to structure of convolutamydine C assigned to be **3**, namely, 4, 6-dibromo-3-hydroxy- 3-methyl-2-indolinone.

The EIMS spectrum of convolutamydine D (**4**) showed molecular ions at *m/z* 331, 333, and 335 in the ratio of 1:2:1, suggesting the presence of two bromines. The molecular formula of **2** was determined as $C_{10}H_7O_2NBr_2$ by HREIMS [*m/z* 330.8870, $(M)^+$ for $C_{10}H_7O_2N^{79}Br_2$; $\Delta$ 2.7mmu], indicating seven unsaturation degrees. Although the available sample amount of convolutamydine D (**4**) was no more than 0.3 mg, the structure of **4** was unequivocally elucidated by detailed analyses of the spectral data of **4**. The $^1$H NMR spectrum (500 MHz) showed four signals ( δppm) at $\delta_H$ 7.20bd (1H, *J* = 1.5 Hz), 7.00 bd (1H, *J* = 1.5 Hz), 6.03 dd (1H, *J* = 17.1, 10.7 Hz) , 5.51 d (1H, *J* = 17.1Hz) and 5.45 d (1H, *J* = 10.7 Hz). In comparison with those of compounds **1** ~ **3** as represented above, the signals at $\delta_H$ 7.20 and 7.00 were assignable to H-5 and H-7, respectively. An olefinic proton at $\delta_H$ 6.05 was assigned to position 8 and exo-methylene protons at $\delta_H$ 5.51 and $\delta_H$ 5.45 were assigned to positon 9. The presence of this vinyl group was supported by the EIMS fragmentation $(M\text{-}CHCH_2)^+$. From these results, convolutamydine D was established to be **4**, 4, 6-dibromo-3-hydroxy-3-($\Delta^{1,2}$-vinyl)-2-indolinone.

Convolutamydines A ~ D (**1** ~ **4**) belong to a new class of alkaloids, which a dibromohydroxyoxindole with a variety of 3-substituents, from marine natural origin. The compound with oxindole was the first example from the marine Bryozoa.[7] These compounds were considered to be a series of related biogenetic analogs. It is interest in attempting to study the biosythesis of convolutamydines A ~ D. On the other hand,

any of convolutamydines A ~ D showed a negative Cotton effect in their CD spectra. The stereochemistries of them should be investigated in combination with synthesis or X-ray crystal structure analysis in the future.

Convolutamydine A (**1**) induced the change of characteristics of HL-60 cells such as growth arrest, adhesiveness to culture plate, and phagocytosis of latex paricles at concentrations 0.1 ~ 25 μg/mL, while convolutamydine B (**2**) exhibit the bioactivity in the differentiation of HL-60 cells at 12.5 ~ 25 μg/mL. It is of interest to examine the differentiation of tumor cells such as HL-60 cells, THP-1 monocytic leukemia cells by convolutamydines A and B in view of these results. Unfortunately, the biological evaluation of convolutamydines C and D could not be achieved, becouse both were only a few quantity of yielded sample. Detailed discussion of biological activities about **1** and **2** will be reported elsewhere.

## Experimental Section

**General Methods.** Optical rotations were recorded on a HORIBA SEPA-300 digital polarimeter. UV and IR spectra were taken on a JASCO Ubest V-520 spectrometer and a Nicolet 510-FT IR infrared spectrometer, respectively. CD spectra were performed on a JASCO J-720 spectrometer. The NMR spectra were measured on JEOL JNM EX-400, or ALPHA-500 spectrometers. EI mass spectra were obtained on a JEOL JMS-AX 505H spectrometer. TLC plates silica gel 60 $F_{254}$ were supplied from Merck Inc.. The TLC plate results were interpreted by UV light and/or developed by a anisaldehyde reagent spray by heating at approximately 150°C for 2 ~ 5 min.

**Collection, Extraction and Isolation.** The bryozoan *Amathia convoluta* (Bryozoan phylum) was collected off the Northeastern Gulf of Mexico, Florida, in 1982. The *A. convoluta* (100 kg, wet weight) was extracted with EtOH. The EtOH extract (639 g) was partitioned between 10% aqueous MeOH and hexane. The MeOH phase was diluted with water to give 70% MeOH solution, and this was partitioned with EtOAc. The EtOAc-soluble materials (150 g) were subjected to a silica gel column (Wakogel C-200, Wako Pure Chemical, Φ107 x 300 mm) with hexane/acetone (2:1 → 1:1). The fraction eluted with hexane/acetone (1:1) was chromatographed on Sephadex LH-20 (Pharmacia, Φ30 x 350 mm) with hexane/$CH_2Cl_2$/MeOH (10:10:1) to give a fraction A (66 ~ 93 mL) and a fraction B (94 ~ 200 mL). The fraction A was passed through a Sep-Pak $C_{18}$ cartridge (Waters, Φ10 x 10 mm) and then a Sep-Pak $SiO_2$ cartridge (Waters, Φ10 x 10 mm). Finally, the eluted fraction was purified by a short column chromatography on silica gel (Wakogel C-300, Wako Pure Chemical, Φ5 x 90 mm) with hexane/acetone (7:3) to afford convolutamydine A (**1**, 8.6mg, wet weight, yield 8.6 x $10^{-6}$%), accompanied by a fraction C less polarized than convolutamydine A. The fraction C was further purified by reversed-phase HPLC (Develosil ODS-HG-5, 10 x 250 mm, 65% MeOH; flow rate, 2.0 mL/min) to give convolutamydines C (**3**, $t_R$ 22.58 min, 0.6 mg, 6 x $10^{-7}$%), and D (**4**, $t_R$ 25.10 min, 0.3 mg, 3 x $10^{-7}$%). Separation of the fraction B was achieved on a short column as represented above with benzene/EtOAc (1:1) to give convolutamydine B (**2**, 10.7 mg, 10.7 x $10^{-6}$%).

**Convolutamydine A:** a colorless amorphous solid, m.p. 190-195°C from MeOH; $[\alpha]_D^{26}$ +27.4° (*c* 0.06, MeOH); CD (MeOH) $\lambda_{ext}$ 228.20 nm (Δε -2.86). UV (MeOH) $\lambda_{max}$ 220.5 (ε 21625), 224.0 (ε 21600), 262.5 (ε 5200, sh), 265.8 (ε 4050, sh), and 291.5 nm (ε 3100); IR ($CHCl_3$) $\nu_{max}$ 3300 ~3400, 1740, 1720, 1716, and 1615 $cm^{-1}$; TLC (silica gel) $R_f$ 0.22 (7:3 hexane/acetone); EIMS *m/z* 361, 363, and 365 $(M)^+$, in ratio of 1:2:1; HREIMS *m/z* 360.8956 $(M)^+$, calc. 360.8963 for $C_{11}H_9O_3N^{79}Br_2$. EIMS fragmentations, *m/z* 317.8782 $(M\text{-}COCH_3)^+$, calc. 317.8799 for $C_9H_6O_2N^{79}Br_2$, and *m/z* 303.8684 $(M\text{-}CH_2COCH_3)^+$, calc.

303.8720 for $C_8H_4O_2N^{79}Br_2$.

**Convolutamydine B:** a colorless amorphous solid, m.p. 225-227°C from acetone; $[\alpha]_D^{25}$ +18.1° (*c* 0.42, MeOH); CD (MeOH) $\lambda_{ext}$ 225.0 nm ($\Delta\varepsilon$ -2.79); UV (MeOH) $\lambda_{max}$ 220.0 ($\varepsilon$ 32995), 225.0 ($\varepsilon$ 33222), 262.5 ($\varepsilon$ 6563, sh), 265.5 ($\varepsilon$ 5196, sh), 287.0 ($\varepsilon$ 3205), and 294.0 nm ($\varepsilon$ 3183); IR ($CHCl_3$) $\nu_{max}$ 3300 ~ 3400, 1723, and 1615 $cm^{-1}$; TLC (silica gel) $R_f$ 0.42 (7:3 hexane/acetone); EIMS *m/z* fragmentations *m/z* 349, 351, 353, and 355 $(M\text{-}H_2O)^+$, in ratio of 3:7:5:1, *m/z* 304, 306, and 308 $(M\text{-}CH_2CH_2Cl)^+$, in ratio of 1:2:1. $^1H$ NMR ($CDCl_3$:$CD_3OD$, 10:1) $\delta_H$ 2.69 (2H, m, H-8), 3.40 (2H, m, H-9), 7.00 (1H, bd, 1.5 Hz, H-7), 7.34 (1H, bd, 1.5 Hz, H-5); $^{13}C$ NMR ($CDCl_3$:$CD_3OD$, 10:1) $\delta_C$ 37.8 (t, C-8), 38.8 (t, C-9), 76.7 (s, C-3), 113.1 (d, C-7), 120.2 (s, C-6), 123.9 (s, C-4), 127.9 (s, C-3a), 129.1 (d, C-5), 144.5 (s, C-7a), 179.1 (s, C-2).

**Convolutamydine C:** a colorless amorphous solid, m.p. 175-180°C from acetone; $[\alpha]_D^{25}$ +32.4° (*c* 0.03, MeOH); CD (MeOH) $\lambda_{ext}$ 216.2 ($\Delta\varepsilon$ -1.68) and 243.60 nm ($\Delta\varepsilon$ -1.52). UV (MeOH) $\lambda_{max}$ 220.0 ($\varepsilon$ 8339), 223.0 ($\varepsilon$ 8232), 262.5 ($\varepsilon$ 2306, sh), 267.5 ($\varepsilon$ 1919, sh), and 292.5 nm ($\varepsilon$ 909); IR ($CHCl_3$) $\nu_{max}$ 3300 ~ 3400, 1739, and 1612 $cm^{-1}$; TLC (silica gel) $R_f$ 0.32 (7:3 hexane/acetone); HREIMS fragmentations *m/z* 303.8595 $(M\text{-}CH_3)^+$, calc. 303.8581 for $C_8H_4O_2N^{79}Br_2$; *m/z* 275.8665 $(M\text{-}CH_3\text{-}CO)^+$, calc. 275.8670 for $C_7H_4ON^{79}Br_2$.

**Convolutamydine D**: a colorless amorphous solid; $[\alpha]_D^{26}$ +14.0° (*c* 0.04 MeOH); CD (MeOH) $\lambda_{ext}$ 241.00 ($\Delta\varepsilon$ -0.52) and 252.80 nm ($\Delta\varepsilon$ -0.52). UV (MeOH) $\lambda_{max}$ 220.5 ($\varepsilon$ 7488), 260.0 ($\varepsilon$ 3266, sh), and 290.0 nm ($\varepsilon$ 1375); IR ($CHCl_3$) $\nu_{max}$ 3300 ~ 3400, 2923, 1731, and 1605 $cm^{-1}$. TLC (silica gel) $R_f$ 0.36 (7:3 hexane/acetone); EIMS *m/z* 304, 306, and 308, in ratio of 1:2:1, HREIMS *m/z* 305.8616 $(M\text{-}CHCH_2)^+$, calc. 305.8643 for $C_8H_4O_2N^{79}Br^{81}Br$.

**Acknowledgment**: We are very pleased to acknowledge assistance with the MS operation by Mr. Shinpei Kidokoro, Tobishi Pharmaceutical Co. Ltd. and with the 400 NMR operation by Ms. Haruka Yamada, Faculty of Science, Kanagawa University.

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(*Received in Japan* 9 *February* 1995; *accepted* 20 *March* 1995)