A New Bromopyrrole Alkaloid and the Optical Resolution of the Racemate from the Marine Sponge *Homaxinella* sp.

Akemi Umeyama,*,† Seiichi Ito,† Eri Yuasa,† Shigenobu Arihara,† and Takeshi Yamada‡

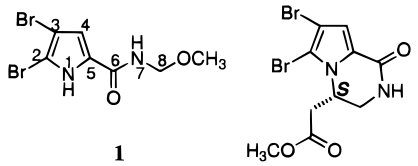
Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770, Japan, and Osaka University of Pharmaceutical Sciences 4-20-1, Nasahara, Takatsuki, Osaka 569-1041, Japan

Received May 18, 1998

A new bromopyrrole alkaloid (**1**) along with (±)-**2** and (±)-longamide (**3**) has been isolated from the Japanese marine sponge *Homaxinella* sp. The structures of the compounds were proposed on the basis of spectroscopic data. The optical resolution of (±)-**2** by chiral HPLC was successful and afforded the two enantiomers, (+)-**2** and (−)-**2**.

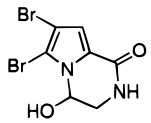
Many bromopyrrole derivatives have been reported from the marine sponge *Agelas* genus since 1971. A series of similar alkaloids such as oroidin[1,2] and hymenidin[3] is the hypothesized precursor of alkaloids such as longamide,[4] cyclized between the α position and NH of a pyrrole ring, and alkaloids such as hymenialdisine,[5] cyclized between the α position and the *β* position of a pyrrole ring. Our chemical interest in marine organisms led to the isolation of the bromopyrrole alkaloid (**1**), along with (±)-**2** and (±)-longamide (**3**) from the marine sponge *Homaxinella* sp.[4,6,7] We now report the isolation and structure elucidation of **1** and the optical resolution of (+)- and (−)-**2**.[8]

A frozen sample (250 g) of the sponge was exhaustively extracted with MeOH−CH_2Cl_2 (3:1) at room temperature for 1 day. The extract was divided into EtOAc- and H_2O-soluble portions. The EtOAc-soluble portion (5.9 g) was subjected to Si gel column chromatography, followed by a Sephadex LH-20 column chromatography. Final purification by reversed-phase HPLC afforded **1**, (±)-**2**, and (±)-longamide (**3**).

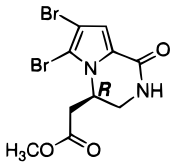


1

(+) - 2



(±) - 3



(−) - 2

The EIMS of the new amide (**1**) showed molecular ion peaks at *m*/*z* 310, 312, and 314 in the ratio 1:2:1, indicating the presence of two bromine atoms. The molecular formula of **1**, $C_7H_8Br_2N_2O_2$, was determined by HREIMS. A peak at 1650 cm^{-1} in the IR spectrum of **1** indicated the presence of an amide carbonyl function, and this was corroborated by a ^{13}C NMR chemical shift at *δ* 160.9. The ^1H NMR spectrum contained peaks at *δ* 14.85 (br s) (pyrrole NH), 9.73 (exchangeable, t, 1H, *J* = 6.5 Hz), 7.29 (s) (aromatic proton), 5.02 (d, 2H, *J* = 6.5 Hz), and 3.39 (s) (methoxy). The ^{13}C NMR spectrum had peaks that indicated the presence of a carbon bearing an oxygen function [*δ* 71.7 (t)] and a methoxy carbon [*δ* 55.6 (q)]. The ^{13}C NMR signals at *δ* 129.3 (s), 113.9 (d), 106.2 (s), and 99.2 (s) implied that there was a 2-carbonyl-4,5-dibromopyrrole ring. The HMBC experiment revealed that H-4 was coupled to C-2, C-3, C-5, and C-6. Furthermore, the HMBC spectrum showed correlations among H-8/C-6, H-8/O*C*H_3, and O*C*H_3/C-8. Thus, the structure of **1** was determined.

Compound (±)-**2** was obtained as a colorless solid. The EIMS showed molecular ion peaks at *m*/*z* 364, 366, and 368 in the ratio 1:2:1, indicating the presence of two bromine atoms. The molecular formula of (±)-**2**, $C_{10}H_{10}Br_2N_2O_3$, determined by HREIMS, was in accordance with six degrees of unsaturation. The IR spectrum of (±)-**2** exhibited absorption bands at 1730 and 1670 cm^{-1}, suggesting the presence of ester and amide carbonyl functions, respectively. A detailed analysis of the ^1H and ^{13}C NMR spectrum revealed that (±)-**2** was the methyl ester analogue of hanishin.

The equatorial orientation of the proton at the C-9 position was determined by the coupling pattern of NH, H-8, and H-9; the bulky substitution at the C-9 position is considered to be axial-like because of the influence of bromine at the C-2 position. The specific rotation of (±)-**2**, $[\alpha]^{25}_D$ 0° (*c* 0.95, MeOH), the absence of a CD maximum, and the appearance of the well-separated two peaks by chiral HPLC indicated that (±)-**2** was a racemate of 9*S* and 9*R* enantiomers. The optical resolution of (±)-**2** by chiral HPLC was successful and afforded the two enantiomers, (+)-**2** and (−)-**2**.

The absolute stereochemistry at the sole asymmetric carbon C-9 of (+)-**2** was established as *S* on the basis of negative Cotton effects at 226 nm (Δϵ −2.64) by the CD spectrum. On the other hand, the absolute stereochemistry at C-9 of (−)-**2** was established as *R* on the basis of positive Cotton effects at 226 nm (Δϵ +1.96) by the CD spectrum. These spectroscopic data were almost identical to those reported by Sharma et al. for dibromophakellin.[9]

Compound **3** was identified as the racemic form of (+)-longamide ($[\alpha]^{25}_D$ +86°), previously reported by Cafieri et al.,[4] by comparison of spectroscopic data (^1H NMR, ^{13}C NMR) with those reported. The specific rotation of **3**, $[\alpha]^{25}_D$ 0° (*c* 1.04, MeOH), and the absence of a CD maximum indicated that **3** was a racemate. Resolution of **3** by the method used for **2** was unsuccessful, presumably because of the readily epimerizable chiral center in **3**.

* To whom correspondence should be addressed. Tel: 0886-22-9611. Fax: 0886-55-3051. E-mail: umeyama@ph-bunri.ac.jp.
† Tokushima Bunri University.
‡ Osaka University of Pharmaceutical Sciences.

10.1021/np980207u CCC: $15.00
Published on Web 09/26/1998

Compounds **1** and (±)-**2** exhibited weak cytotoxic activity against P-388 lymphocytic leukemia cells in vitro, while compound **3** was inactive, with ED_{50} values of 21.5 μg/mL, 30 μg/mL, and >100 μg/mL, respectively.

Experimental Section

General Experimental Procedures. The following instruments were used: a JASCO FT/IR-5300 (IR), a JASCO DIP-360 polarimeter (optical rotation), a JASCO J-500C (CD), a JEOL JMS−HX-100 mass spectrometer (HRMS), and a Varian UNITY 600 NMR spectrometer (^{1}H and ^{13}C NMR).

Sponge Material. The marine sponge *Homaxinella* sp. (250 g, wet wt) was collected by netting at a depth of 70 m off the coast of Tokushima prefecture and was kept frozen (−20 °C) until used. The sponge was identified by Professor P. R. Bergquist of Auckland University. The voucher sample (TS023) of the organism under consideration is deposited in the herbarium of the Department of Pharmacognosy, Tokushima Bunri University. The sponge body is an undulating lamella, 0.4 cm thick and 6.5 cm across, disposed approximately at right angles to a short cylindrical stalk, 2.5 cm high by 1.0 cm thick. The spicules have styles range in form from slightly curved apically, to wavy or, most frequently, rhabdostyles with marked apical flexure; dimensions, 209−286 μm × 10−14 μm. The skeleton is a typical axinellid arrangement with vertically oriented spicule fiber columns in the stalk and central lamella with an extra-axial skeleton diverging at right angles toward the surface, where tracts terminate in spicule tufts. The species is distinct within *Homaxinella* in having a lamellate form and rhabdostyles as the dominant form for the skeletal styles. The specimen has been dehydrated, and no soft tissue information is available. A full description must await collection of better specimens.

Extraction and Isolation of Metabolites. The frozen sample (250 g) was exhaustively extracted with MeOH−CH_2Cl_2 (3:1) (1 L × 4) at room temperature for 1 day. The extract was concentrated, and the resulting residue was extracted with EtOAc (300 mL × 3). The EtOAc-soluble portion (5.9 g) was repeatedly subjected to Si gel flash column chromatography (using increasing concentrations of MeOH in CH_2Cl_2 as eluent), followed by reversed-phase HPLC (30−50% MeOH) to give **1** (13.5 mg, 0.0054% wet wt), (±)-**2** (19.3 mg, 0.0077%), and (±)-longamide (**3**) (23.0 mg, 0.0092%).

The optical resolution of (±)-**2** by chiral HPLC (CHIRALCEL OJ-R; 15% CH_3CN) afforded optical isomers, (+)-**2** (7.7 mg, 0.0031%) and (−)-**2** (9.7 mg, 0.0039%).

Compound 1: FT-IR (film) 1650 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 9.73 (t, 1H, J = 6.5 Hz, NH), 7.29 (s, 1H, H-4), 5.02 (d, 2H, J = 6.5 Hz, H-8), 3.39 (s, 3H, OCH_3); ^{13}C NMR (C_5D_5N) δ 160.9 (s, C-6), 129.3 (s, C-5), 113.9 (d, C-4), 106.2 (s, C-2), 99.2 (s, C-3), 71.7 (t, C-8), 55.6 (q, OCH_3); EIMS m/z 310, 312, 314; HREIMS m/z 309.8950 (calcd for $C_7H_8Br_2N_2O_2$, 309.8953).

Compound (±)-2: $[\alpha]^{25}_D$ 0° (c 0.95, MeOH); UV λ_{max} (MeOH) 236 nm (log ε 4.00); FT-IR (film) 1730, 1670 cm^{-1}; ^{1}H NMR (C_5D_5N) δ 8.96 (d, 1H, J = 5.0 Hz, NH), 7.33 (s, 1H, H-4), 4.92 (ddd, 1H, J = 10.5, 3.5, 3.5 Hz, H-9), 3.95 (dd, 1H, J = 13.0, 3.5 Hz, H-8), 3.87 (dd, 1H, J = 13.0, 5.0 Hz, H-8), 3.56 (s, 3H, OCH_3), 3.80 (dd, 1H, J = 16.0, 10.5 Hz, H-10), 2.69 (dd, 1H, J = 16.0, 3.5 Hz, H-10); ^{13}C NMR (C_5D_5N) δ 170.5 (s, C-11), 158.9 (s, C-6), 126.8 (s, C-5), 115.3 (d, C-4), 106.2 (s, C-2), 100.9 (s, C-3), 51.9 (q, OCH_3), 51.1 (d, C-9), 43.2 (t, C-8), 36.0 (t, C-10); EIMS m/z 368, 366, 364; HREIMS m/z 363.9033 (calcd for $C_{10}H_{10}Br_2N_2O_3$, 363.9059); **(+)-2:** $[\alpha]^{25}_D$ +2.6° (c 0.39, MeOH); CD Δε −2.64 (226 nm) (c 0.011, MeOH). **(−)-2:** $[\alpha]^{25}_D$ −2.1° (c 0.49, MeOH); CD Δε +1.96 (226 nm) (c 0.013, MeOH).

Acknowledgment. We are grateful to Professor P. R. Bergquist, Auckland University, for her kind identification and the taxonomic description of the sponge. We thank Mr. K. Murakami and Mr. K. Iwasaki for help with collections. We are indebted to Dr. M. Tanaka for measurements of NMR spectra and Ms. I. Okamoto for measurements of mass spectra.

References and Notes

(1) Forenza, S.; Minale, L.; Riccio, R.; Fattorusso, E. *J. Chem. Soc. Chem. Commun.* **1971**, 1129−1130.
(2) Garcia, E. E.; Benjamin, L. E.; Fryer, R. I.*J. Chem. Soc. Chem. Commun.* **1973**, 78−79.
(3) Kobayashi, J.; Ohizumi, Y.; Nakamura, H.; Hirata, Y. *Experientia* **1986**, *42*, 1176−1177.
(4) Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O. *Tetrahedron Lett.* **1995**, *36*, 7893−7896.
(5) Cimino, G.; De Rosa, S.; De Stefano, S.; Mazzarella, L.; Puliti, R.; Sodano, G. *Tetrahedron Lett.* **1982**, *23*, 767−768.
(6) Mancini, I.; Guella, G.; Amade, P.; Roussakis, C.; Pietra, F. *Tetrahedron Lett.* **1997**, *38*, 6271−6274.
(7) Cafieri, F.; Fattorusso, E.; Mangoni, A.; Taglialatela-Scafati, O. *J. Nat. Prod.* **1998**, *61*, 122−125.
(8) The structures of **1**, (+)-**2**, and (−)-**2** were presented by us at the 117th Annual Meeting of the Japanese Society of Pharmacy on March 27, 1997, in Japan. Umeyama, A.; Ito, S.; Yuasa, E.; Arihara, S. Symposium Papers, no. 2; 1977, p 158.
(9) Sharma, G.; Magdoff-Fairchild, B. *J. Org. Chem.* **1977**, *42*, 4118−4124.

NP980207U