Marine Natural Products. XXXIV.[1] Trisindoline, a New Antibiotic Indole Trimer, Produced by a Bacterium of *Vibrio* sp. Separated from the Marine Sponge *Hyrtios altum*

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Received June 20, 1994; accepted August 4, 1994

A new antibiotic indole trimer named trisindoline (1) was isolated, together with a known dioxopiperazine brevianamide F (2), from the culture of a bacterium of *Vibrio* sp., which was separated from the Okinawan marine sponge *Hyrtios altum*. The structure of trisindoline (1) has been determined on the bases of physicochemical evidence and chemical synthesis.

Keywords trisindoline; *Vibrio* sp.; marine sponge; *Hyrtios altum*; antibiotic; indole trimer

We have isolated extremely cytotoxic macrolides named altohyrtins A, B, and C, and 5-desacetylaltohyrtin A from the Okinawan marine sponge *Hyrtios altum*[2a,b] and have characterized their absolute stereostructures.[2c] Recently, we became aware that several compounds having structures closely similar to altohyrtins have been isolated from different genera of marine sponges, such as *Cinachyra* sp.[3] and *Spongia* sp.[4] The common feature in the isolations of these biologically interesting compounds from marine sponges is that the macrolides were obtainable only in tiny quantities, which limited further pharmacochemical investigation.

Most marine sponges live as a "miniature conglomerate" which usually comprises, besides its own tissue, several kinds of microorganisms such as blue-green algae, fungi, and/or bacteria. In this connection, identification of either symbiotic or parasitic microorganism(s) really responsible for the production of such biologically interesting spongean constituents as altohyrtins, is difficult. During our attempts to find a microorganism(s) producing altohyrtins, we have isolated a bacterium of *Vibrio* sp. from the fresh marine sponge *Hyrtios altum*, and have isolated a new antibiotic indole trimer named trisindoline (**1**) from the culture. This paper deals with the structure elucidation of trisindoline (**1**) and other accompanying constituents produced by this bacterium.

The bacterial strain was separated from the fresh marine sponge *Hyrtios altum* by using an agar plate prepared with a sea-water medium. The separated bacterium, identified as *Vibrio* sp., the extractive of which exhibiting antibiotic activity against *Escherichia coli*, was grown in Zobell 2216E medium. The cultivation was carried out in 5 l round flasks with vigorous shaking at 25 °C for 5 d. The combined culture (80 l) was first homogenized with a biomixer and then partitioned with ethyl acetate (AcOEt). The AcOEt-soluble portion was separated and evaporated under reduced pressure to give 3.2 g of the extractive, which showed antibiotic activities against *E. coli*, *Bacillus subtilis*, and *Staphylococcus aureus*. The extractive was subjected to bioassay-guided separation by means of TLC bioautography and by a paper disk method. Repeated silica gel column chromatography (SiO_2 column) and reversed-phase high-performance liquid chromatography (HPLC) of the extractive provided trisindoline (**1**) in 0.3% yield from the AcOEt-soluble portion together with indole itself and brevianamide F[5] (**2**) (0.6%).

Many years ago, brevianamide F(**2**) was first isolated as a fungal metabolite of *Penicillium brevicompactum* by Birch and his co-workers.[5] The physicochemical properties [ultraviolet (UV), infrared (IR), and mass spectra (MS)] of brevianamide F (**2**) obtained here by us were identical with those reported except for the $[\alpha]_D$ value ($[\alpha]_D$ −64° obtained by us; $[\alpha]_D$ −101° reported[5]). The purity of our brevianamide F was confirmed by the proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) analyses. Next, to examine the optical purity of our brevianamide F, the compound was subjected to complete hydrolysis followed by the amino acid analysis of the hydrolysate. Thus, treatment with 6 N aqueous hydrochloric acid at 110 °C for 20 h liberated proline as a sole constituent amino acid, while the tryptophan moiety was decomposed. The proline portion was further treated with succinimido D(+)-α-phenylethylcarbamate[6] to give the (+)-α-phenylethylcarbamoylated derivative(s). The product was a single compound and was shown to be identical with (+)-α-phenylethylcarbamoyl-L-proline (**3**) by reversed-phase HPLC analysis; (+)-α-phenylethylcarbamoyl-D-proline was not detected. The result demonstrates that brevianamide F (**2**) obtained by us contains L-proline and is optically pure, and consequently is identical with the compound reported by Birch *et al.*[5]

Trisindoline (**1**) was obtained as a colorless amorphous powder. Its MS showed a molecular ion peak at *m/z* 363 while the molecular formula $C_{24}H_{17}N_3O$ was determined by high-resolution MS. The IR spectrum of **1** showed the presence of an amide carbonyl (1705 cm^{-1}) and the UV spectrum showed characteristic absorption maxima at 290, 280, 274, 254 and 219 nm, which were ascribable to the indole moiety.

The ^{1}H-NMR spectrum of trisindoline (**1**) showed in total fourteen proton signals, among which five signals at δ 6.81, 6.90, 7.03, 7.27, and 7.32 were each observed with two proton intensity. Among the twenty-four carbons of

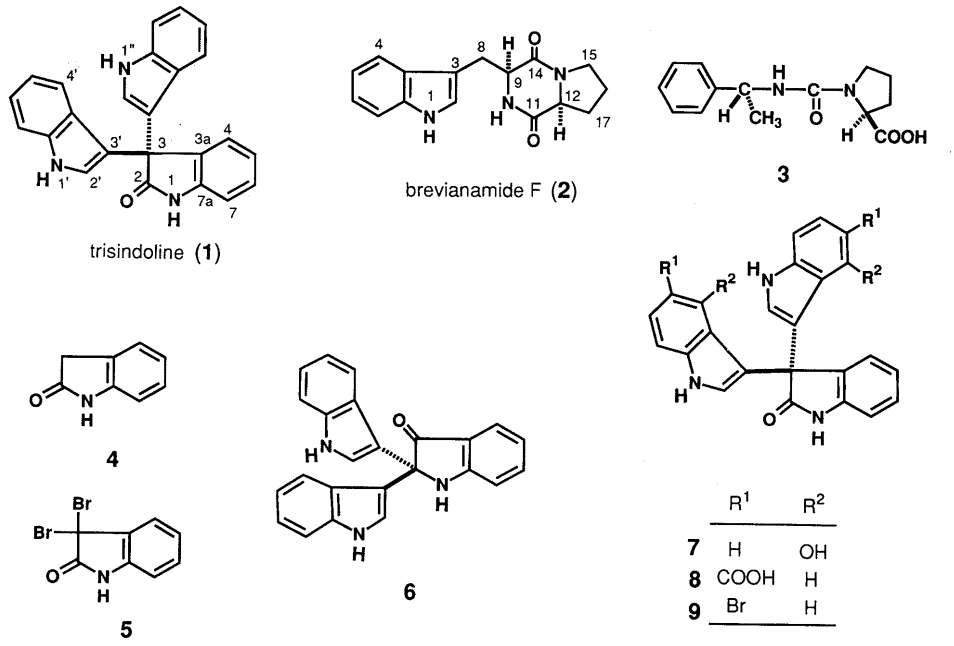

Chart 1

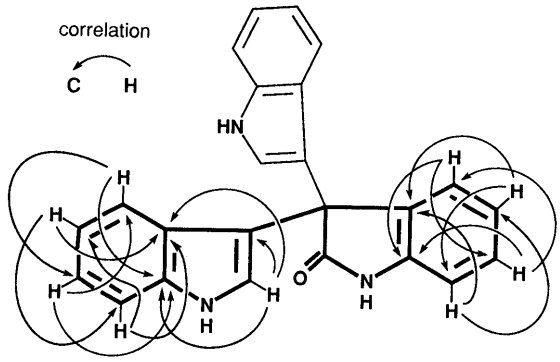

Fig. 1. HMBC of Trisindoline (1)

1, only sixteen showed distinct signals in the ^{13}C-NMR spectrum. Those signals were assignable to fourteen aromatic carbons, one carbonyl carbon (δ_C 183.4), and one quaternary carbon (δ_C 55.7). The ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) of **1** revealed the presence of two types of *ortho*-disubstituted benzene moieties, one of which showed two-proton signals at δ 7.27 (d-like, J=*ca.* 7.5 Hz), 6.81 (ddd, J=7.5, 7.5, 1.0 Hz), 7.03 (ddd, J=7.5, 7.5, 1.0 Hz), and 7.32 (d-like, J=*ca.* 7.5 Hz). The presence of two identical indole moieties in **1** was figured out from the correlations obtained by ^{1}H–^{13}C COSY and from the heteronuclear multiple bond correlation (HMBC) spectrum of **1** (Fig. 1). Based on these findings, it has been presumed that trisindoline possesses a symmetrical structure, shown as **1**.

In order to verify this presumption, trisindoline was synthesized in the following manner. Oxindole (**4**) was treated with copper(II) bromide in ethyl acetate under reflux for 3 h to give 3,3-dibromoxindole (**5**).[7] 3,3-Dibromoxindole (**5**) was then treated with indole and silver carbonate in tetrahydrofuran at 25 °C for 1.5 h to furnish trisindoline (**1**) in 47% overall yield from **4**. The physical data for the synthesized trisindoline (**1**) were identical with those for natural **1** obtained from the culture. Consequently, the chemical structure of trisindoline (**1**) has been determined as shown. A trinuclear indole derivative (**6**) of different type was reported recently as an oxidation product of indole,[8] but trisindoline (**1**) seems to be the first example among natural microbial products.

Trisindoline (**1**) showed antibiotic activities [16, 17, and 10 mm diameter growth inhibitions for *E. coli*, *B. subtilis*, and *S. aureus* at 10 μg/disk (i.d. = 8 mm)]. In a preliminary examination to shed light on the correlation between chemical structure and antibiotic activity, we have prepared several analogues (**7**, **8**, and **9**) of trisindoline from commercially available indole derivatives *via* the same procedure as for **1**, and examined their antibiotic activities. It was found that a brominated compound **9** showed very weak activity (9 mm) only against *E. coli* at the concentration of 30 μg/disk, while a hydroxylated compound **7** and a carboxylated derivative 8 showed no antibiotic activity.

Experimental

The instruments used to obtain physical data and experimental conditions for chromatography were the same as described in the previous paper.[1]

Cultivation of *Vibrio* sp. The bacterium *Vibrio* sp. was isolated from the marine sponge *Hyrtios altum*, which was collected at Aragusuku Island, Okinawa Prefecture in June 1992, by means of an agar plate method. The bacterium thus isolated was cultured aseptically at 25 °C in an autoclaved Zobell 2216E medium with vigorous shaking, initially in 300 ml flasks for 3 d then in 5 l round flasks for 5 d.

Isolation of Trisindoline (1) and Brevianamide F (2) The combined culture (80 l) was homogenized with a biomixer (BM-4, Nihon Seiki) and the whole was extracted with AcOEt (40 l) twice. Removal of the solvent under reduced pressure from the combined extracts provided the AcOEt extract (3.2 g). The AcOEt extract was subjected to SiO_2 column chromatography (Kieselgel 60, *n*-hexane–AcOEt) to furnish indole (135 mg) and five fractions. The fraction (653 mg) eluted with *n*-hexane–AcOEt (1 : 1) was further separated by SiO_2 column chromatography ($CHCl_3$–MeOH) to furnish seven fractions. The fraction (75 mg)

eluted with $CHCl_3$–MeOH (50 : 1) was subjected to HPLC (Cosmosil $5C_{18}AR$, MeOH–H_2O–CH_2Cl_2 (45 : 54 : 1)) to afford trisindoline (**1**) (10 mg) and brevianamide F (**2**)[5] (18 mg).

Trisindoline (**1**), an amorphous solid; IR ν_{max}^{KBr} cm^{-1}: 3200, 1705, 1472. UV λ_{max}^{MeOH} nm (ε): 290 (10000), 280 (12000), 274 (12000), 254 (12000), 219 (62000). ^{1}H-NMR (500 MHz, CD_3OD) δ: 6.81 (2H, ddd, J=7.5, 7.5, 1.0 Hz, 5′,5″-H), 6.90 (2H, s, 2′,2″-H), 6.97 (1H, ddd, J=7.5, 7.5, 1.0 Hz, 5-H), 7.03 (2H, ddd, J=7.5, 7.5, 1.0 Hz, 6′,6″-H), 7.05 (1H, d-like, J=*ca.* 7.5 Hz, 7-H), 7.25 (1H, ddd, J=7.5, 7.5, 1.0 Hz, 6-H), 7.27 (3H, d-like, J=*ca.* 7.5 Hz, 4,4′,4″-H), 7.32 (2H, d-like, J=*ca.* 7.5 Hz, 7′,7″-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 183.4 (s, C-2), 143.1 (s, C-7a), 139.6 (2C, s, C-7′a,7″a), 137.5 (s, C-3a), 129.8 (d, C-6), 128.1 (2C, s, C-3′a,3″a), 127.3 (d, C-4), 126.4 (2C, d, C-2′,2″), 124.1 (d, C-5), 123.2 (2C, d, C-4′,4″), 122.9 (2C, d, C-6′,6″), 120.3 (2C, d, C-5′,5″), 116.5 (2C, s, C-3′,3″), 113.1 (2C, d, C-7′,7″), 111.8 (d, C-7), 55.7 (s, C-3). FAB-MS *m/z*: 363 (M^+). High-resolution FAB-MS *m/z*: Calcd for $C_{24}H_{17}N_3O$: 363.137. Found: 363.140. Brevianamide F (**2**), $[\alpha]_D$ −64° (*c*=0.69, MeOH, 25 °C). IR ν_{max}^{KBr} cm^{-1}: 3264, 1694, 1265. UV λ_{max}^{MeOH} nm (ε): 290 (1700), 281 (2000), 276 (1900), 220 (11000). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 2.0—2.3 (4H, m, 16,17-H), 2.97 (1H, dd, J=11, 15 Hz, 8-H_a), 3.64 (2H, m, 15-H), 3.76 (1H, dd, J=3.5, 15 Hz, 8-H_b), 4.08 (1H, t-like, J=*ca.* 7.5 Hz, 12-H), 4.38 (1H, dd, J=3.5, 11 Hz, 9-H), 5.74 (1H, s, 10-NH), 7.1—7.2 (3H, m, 2,5,6-H), 7.39, 7.59 (both 1H, d-like, J=*ca.* 8 Hz, 4,7-H), 8.30 (1H, s, 1-NH). ^{13}C-NMR (67.5 MHz, CD_3OD) δ_C: 169.3, 165.4 (both s, C-11, 14), 136.6 (s, C-7a), 126.7 (s, C-3a), 123.3 (d, C-2), 122.7 (s, C-5), 120.0 (d, C-6), 118.5 (s, C-4), 111.5 (d, C-7), 109.9 (s, C-3), 59.2 (d, C-12), 54.5 (d, C-9), 45.4 (t, C-15), 28.3 (t, C-17), 26.8 (t, C-8), 22.6 (t, C-16). MS *m/z*: 283 (M^+).

Complete Acidic Hydrolysis of Brevianamide F (2) Followed by HPLC Analysis A solution of **2** (5 mg) in 6 N aqueous HCl (0.5 ml) was heated at 110 °C for 20 h in a sealed tube. After cooling, the reaction mixture was neutralized with saturated aqueous $NaHCO_3$ and then treated with 0.1 M succinimido D-(+)-α-phenylethylcarbamate in CH_3CN (0.5 ml) at 25 °C for 1 h. The whole mixture was filtered and the filtrate was analyzed by HPLC [YMC-Pack ODS-AM, MeOH–H_2O–TFA (35 : 64 : 1)]. D,L-Proline and L-proline were converted to the respective (+)-α-phenylethylcarbamates in the same manner as the authentic samples.

Bromination of Oxindole (4) A solution of **4** (100 mg) in AcOEt (3 ml) was treated with copper(II) bromide (670 mg) and the reaction mixture was heated under reflux with stirring for 3 h. After cooling, the reaction mixture was partitioned into an AcOEt–H_2O mixture. The AcOEt phase was taken, washed with brine, and evaporated under reduced pressure to furnish a product. The product was subjected to SiO_2 column chromatography (benzene : acetone = 30 : 1) to give 3,3-dibromoxindole (**5**) (159 mg, 72%). **5**, IR ν_{max}^{KBr} cm^{-1}: 3096, 1719, 1474. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 6.96 (1H, d, J=7.5 Hz, 7-H), 7.17 (1H, dd, J=7.5, 7.5 Hz, 5-H), 7.31 (1H, dd, J=7.5, 7.5 Hz, 6-H), 7.61 (1H, d, J=7.5 Hz, 4-H). FAB-MS *m/z*: 290, 292, 294 ($M+H)^+$.

Treatment of 3,3-Dibromoxindole (5) with Indole or Indole Derivatives Giving Trisindoline (1) or 7, 8, and 9 A solution of **5** (100 mg) in tetrahydrofuran (4 ml) was treated with indole (160 mg) and silver carbonate (380 mg) and the reaction mixture was stirred at 25 °C for 1.5 h. The whole mixture was filtered and the filtrate was then evaporated under reduced pressure to furnish a product. The product was subjected to SiO_2 column chromatography [$CHCl_3$–MeOH (30 : 1)] to give trisindoline (**1**) (80 mg, 65%). This product was identical with authentic trisindoline in terms of ^{1}H-NMR, ^{13}C-NMR, and IR data. Compounds **7**, **8**, and **9** were synthesized from commercially available 4-hydroxyindole, indole-5-carboxylic acid, and 5-bromoindole in a similar manner.

7,[9] IR ν_{max}^{KBr} cm^{-1}: 3390, 1688, 1471. FAB-MS *m/z*: 396 $(M+H)^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{24}H_{18}N_3O_3$: 396.135. Found: 396.133. **8**, IR ν_{max}^{KBr} cm^{-1}: 3270, 1687, 1472. ^{1}H-NMR (270 MHz, CD_3OD) δ: 6.96 (1H, dd, J=7.5, 7.5 Hz, 5-H), 6.98 (2H, s, 2′,2″-H), 7.10 (1H, d, J=7.5 Hz, 7-H), 7.25 (1H, dd, J=7.5, 7.5 Hz, 6-H), 7.29 (1H, d, J=7.5 Hz, 4-H), 7.38 (2H, d, J=8.5 Hz, 7′,7″-H), 7.71 (2H, d, J=8.5 Hz, 6′,6″-H), 8.03 (2H, s, 4′,4″-H). FAB-MS *m/z*: 474 $(M+Na)^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{26}H_{17}N_3NaO_5$: 474.107. Found: 474.108. **9**, IR ν_{max}^{KBr} cm^{-1}: 3410, 1711, 1470. ^{1}H-NMR (270 MHz, CD_3OD) δ: 6.94 (2H, s, 2′-H), 7.03 (1H, dd, J=7.5, 7.5 Hz, 5-H), 7.08 (1H, d, J=7.5 Hz, 7-H), 7.13 (2H, d, J=8.5 Hz, 7′,7″-H), 7.21 (1H, dd, J=7.5, 7.5 Hz, 6-H), 7.28 (1H, d, J=7.5 Hz, 4-H), 7.42 (2H, d, J=8.5 Hz, 6′,6″-H), 7.87 (2H, s, 4′,4″-H). FAB-MS *m/z*: 519, 521, 523 (M^+). High-resolution FAB-MS *m/z*: Calcd for $C_{24}H_{15}{}^{79}Br_2N_3O$: 518.954; $C_{24}H_{15}{}^{79}Br^{81}BrN_3O$: 520.956; $C_{24}H_{15}{}^{81}Br_2N_3O$: 522.954. Found: 518.954, 520.956, 522.951.

Acknowledgements The authors are grateful to Dr. Akira Yokota, Institute for Fermentation, Osaka for identification of the bacterium. Thanks are due to the Ministry of Education, Science, and Culture of Japan for financial support.

References and Notes

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