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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A new antibiotic indole trimer named trisindoline (1) was isolated, together with a known dioxopiperazine breviamamide 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 altolythins A, B, and C, and 5-desacetylandaltolythrin A from the Okinawan marine sponge Hyrtios altum and have characterized their absolute stereochemistries. 2) Recently, we became aware that several compounds having structures closely similar to altolythins have been isolated from different genera of marine sponges, such as Cinachyra sp. 3) and Spongia sp. 4) The common feature in the isolation of these biologically interesting compounds from marine sponges is that the macrolides were obtainable only in tiny quantities, which limited further pharmacological 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 sponge constituents as altolythins, is difficult. During our attempts to find a microorganism(s) producing altolythins, 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 51 round flasks with vigorous shaking at 25°C for 5d. The combined culture (801) 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 3.2g 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 (SiO2 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 breviamamide F (2) (0.6%).

Many years ago, breviamamide 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 breviamamide F (2) obtained here by us were identical with those reported except for the [x]D value ([x]D = 64° obtained by us; [x]D = 101° reported5). The purity of our breviamamide F was confirmed by the proton nuclear magnetic resonance (1H-NMR) and carbon-13 nuclear magnetic resonance (13C-NMR) analyses. Next, to examine the optical purity of our breviamamide F, the compound was subjected to complete hydrolysis followed by the amino acid analysis of the hydrolysate. Thus, treatment with 6N aqueous hydrochloric acid at 110°C for 20h 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 breviamamide 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 C24H17N2O was determined by high-resolution MS. The IR spectrum of I 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 1H-NMR spectrum of trisindoline (1) showed in total fourteen proton signals, among which five signals at 6 8.61, 6.90, 7.93, 7.27, and 7.32 were each observed with two proton intensity. Among the twenty-four carbons of

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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 ($\delta_c$ 183.4), and one quaternary carbon ($\delta_c$ 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 $\delta$ 7.27 (d-like, $J = 6.5$ 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, $J = 6.5$ 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). 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, 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 $\mu$g/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 $\mu$g/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 albus, 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$_3$–EtOH) to furnish seven fractions. The fraction (75 mg)
Tritrinolide (I) is an amorphous solid. IR ν\text{max} cm⁻¹: 3200, 1705, 1472. UV λ\text{max} nm (e): 290 (10000), 278 (10000), 220 (11000). 1H-NMR (500 MHz, CDOD) 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, dd, J = 7.5, 7.5, 1.0 Hz, 4-H), 7.03 (2H, dd, J = 7.5, 7.5, 1.0 Hz, 6,6'-H), 7.05 (1H, d, J = 7.5 Hz, 7-H), 7.25 (1H, dd, J = 7.5, 7.5, 1.0 Hz, 6,6'-H), 7.27 (3H, d, J = 7.5 Hz, 4,4'-H), 7.32 (2H, d, J = 7.5 Hz, 7,7'-H). 13C-NMR (125 MHz, CDOD) δ: 83.4 (C-2, 143.1 (C-3), 139.6 (2C, s, C-7a,7'Ha), 137.5 (s, C-3a), 129.8 (d, C-6), 128.1 (2C, s, C-3a,3'a), 127.3 (d, C-4), 126.4 (2C, d, C-2',2'-H), 124.1 (d, C-5), 122.2 (2C, d, C-4',4'-H), 122.9 (2C, d, C-6,6'-H), 120.3 (2C, d, C-5,5'-H), 116.5 (2C, s, C-3,3'), 111.1 (2C, d, C-7,7'-H), 111.8 (d, C-7), 55.7 (s, C-3). FAB-MS m/z: 363 (M⁺). High-resolution FAB-MS m/z: Caled for C₂₆H₂₅NO₂ 363.170. Found: 363.140. Brevinamide F (2), [x]D ° -64 (c = 0.69, MeOH, 25°C). IR νmax cm⁻¹: 3164, 1694, 1265. UV λmax nm (e): 290 (1700), 261 (2000), 220 (1000). 1H-NMR (270 MHz, CDCl₃) δ: 2.0-2.3 (4H, m, 16.17-H), 2.97 (1H, dd, J = 11, 15 Hz, 8-H), 3.64 (2H, m, 5-H), 3.76 (1H, dd, J = 3.5, 15 Hz, 8-H), 4.08 (1H, t-like, J = 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). 13C-NMR (67.5 MHz, CDCl₃) δ: 66.9, 165.4 (both s, C-11, 14), 136.6 (s, C-7a), 126.7 (d, C-3a), 123.3 (d, C-2), 122.7 (s, C-5), 120.0 (d, C-6), 118.3 (s, C-4), 111.5 (d, C-7), 109.9 (s, C-3), 55.7 (s, C-3'), 42.7 (s, C-15), 28.5 (t, C-17), 26.8 (t, C-8), 22.6 (t, C-16). MS m/z: 283 (M⁺).

Complete Acidic Hydrolysis of Brevinamide 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 20h in a sealed tube. After cooling, the reaction mixture was neutralized with saturated aqueous NaHCO₃ and then treated with 0.1 M succinimido (+) -α-phenylethylcarbamate in CH₂CN (0.5 ml) for 25°C for 1h. The whole mixture was filtered and the filtrate was then evaporated under reduced pressure to furnish a product. The product was subjected to SiO₂ column chromatography [CHCl₃-MeOH (30:1)] to give tritrinolide (I) (80 mg, 65%). This product was identical with authentic tritrinolide in terms of 1H-NMR, 13C-NMR, and IR data. Compounds 7, 8, and 9 were synthesized from commercially available 4-hydroxyindole, indole-5-carboxylic acid, and 5-bromonitriole in a similar manner.

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References and Notes

9) Compound 6 is unstable and decomposes gradually.