

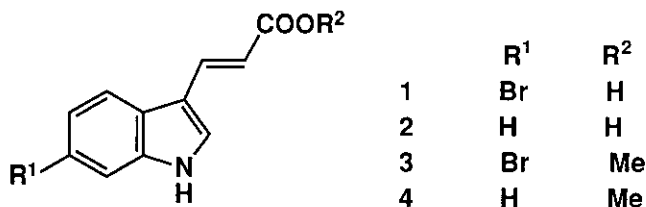
PENARESIN, A NEW SARCOPLASMIC RETICULUM Ca-INDUCER FROM THE OKINAWAN MARINE SPONGE *PENARES* SP.

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Abstract – A new bromoindole alkaloid, penaresin, has been isolated from the Okinawan marine sponge *Penares* sp. as a powerful Ca-inducer in sarcoplasmic reticulum and its structure was assigned by the spectral data.

Among diverse metabolites of marine livings, many indole alkaloids have been isolated from marine algae and sponges.¹ During our continuing research for bioactive compounds from the Okinawan marine organisms,² we have found that the methanol extract of a sponge of the genus *Penares*³ shows a potent Ca-releasing activity in sarcoplasmic reticulum (SR). In this paper, we describe the isolation and structure determination of penaresin (1), a new bromoindole metabolite with a Ca-releasing activity in SR, from the sponge.



The SR of rabbit white muscle was prepared by the method reported previously.⁴ The extravesicular Ca^{2+} concentration was monitored with a Ca^{2+} electrode prepared by the method of Tsien and Rink with modifications.⁴ The sponge *Penares* sp., collected by netting at Unten Bay (-70 m), Okinawa Island, in June 1987, was kept frozen until used. The methanol extract of the sponge was partitioned between toluene and water. The chloroform-soluble portion of the aqueous layer exhibiting Ca-releasing activity was chromatographed on a Sephadex LH-20 column with chloroform-methanol (1:1) to afford an active fraction. This fraction was subjected to a C_{18} reversed-phase hplc column (Capcell Pak C_{18} , 5 μm , 4.6 x 250 mm) with methanol-water (65:35) to give penaresin (**1**, 0.0006% wet weight of the sponge) as a pale yellow solid, mp 189-192 °C (decomp.), and indoleacrylic acid⁵ (**2**, 0.003%) as a colorless solid, mp 183-185 °C (decomp.).

The uv spectrum [λ_{max} (MeOH) 228 (ϵ 27100), 280 (18900) and 307 (18300) nm] of **1** argues the presence of a substituted indole chromophore.⁶ The EIms spectrum exhibited molecular ions ($\text{C}_{11}\text{H}_8\text{NO}_2\text{Br}$) at m/z 265 and 267 (1:1) and intense fragment ions at m/z 221 and 223 (1:1), 195 and 197 (1:1), and 115 by loss of CO_2 , $\text{C}_3\text{H}_2\text{O}_2$ and $\text{C}_3\text{H}_3\text{O}_2\text{Br}$ from M^+ , respectively. The ir spectrum (KBr) showed an NH band at 3350 cm^{-1} and bands assigned to $\text{C}=\text{C}-\text{COOH}$ at $3600\text{--}2800$ and 1640 cm^{-1} . The ^1H nmr spectrum (CDCl_3 , δ in ppm) exhibited two deuterium-exchangeable protons at δ 8.67 (NH) and 11.90 (COOH). The other proton resonances were examined in methanol- d_4 . An AB system due to the olefinic protons of an acrylic acid part appeared at δ 6.43 (H-8) and 7.87 (H-9) with a *trans*-coupling constant of 15.9 Hz. The remaining resonances [δ 7.67 (H-2, s), 7.81 (H-4, d, $J=8.5$ Hz), 7.32 (H-5, dd, $J=1.7$ and 8.5 Hz) and 7.63 (H-7, d, $J=1.7$ Hz)] were similar to the aromatic proton chemical shifts of 6-bromoindole-3-carbaldehyde.⁷ The structure of **1** was thus assigned to be 6-bromoindole-3-acrylic acid. In order to confirm this structure, the acid (**1**) was esterified by diazomethane to afford the methyl ester (**3**). All the spectral data of **3** were identical with those of methyl (*E*)-3-(6-bromoindol-3-yl) prop-2-enoate, which has been isolated from the sponge *Iotrochota* sp.⁸

In SR,⁹ the Ca-releasing activity of penaresin (**1**) was ten times more potent than that of caffeine, a well-known Ca-releaser,⁴ whereas such activity was not observed for indoleacrylic acid (**2**). It is noted that the methyl ester (**3**) of penaresin shows potent

cytotoxicity (IC_{50} 4.5 μ g/ml) against L1210 murine leukemia cells,¹⁰ while **1**, **2**, and the methyl ester (**4**)¹¹ of **2** did not affect the cell growth.

This is the first isolation of indoleacrylic acid (**2**) from marine sources, although various indole alkaloids have been isolated from marine sponges.¹ Since marine microorganisms such as blue-green algae are known to live symbiotically in sponges,¹² the origin of **2** in this sponge might be the symbiotic algae. Penaresin (**1**) is considered to be a metabolite generated by bromination of indoleacrylic acid (**2**). It is interesting from a chemotaxonomic point of view that the methyl ester (**3**) of penaresin has been found in a sponge *Iotrochota* sp.⁸ belonging to the Family (Tedaniidae) which drifts apart from the Family (Stelletidae) of the sponge *Penares* sp.

EXPERIMENTAL

General Methods. The ir spectra were recorded on a Hitachi 260-50 ir spectrophotometer. Uv spectra were obtained on a JASCO 660 UV/VIS spectrophotometer. ¹H and ¹³C NMR spectra were recorded on a Bruker AM-500 spectrometer with an internal TMS standard (0 ppm.). Mass spectra were obtained on a JEOL HX-100 spectrometer operating at 70 eV for EI.

Collection, Extraction, and Separation. The sponge *Penares* sp. was collected by netting at Unten Bay (-70 m), Okinawa island, in June 1987, was kept frozen until used. The methanol (1500 ml x 2) extract was dissolved in methanol-toluene (3:1, 200 ml) and then partitioned between toluene (1000 ml x 2) and 1 M NaCl (1000 ml). The aqueous layer was subsequently extracted with CHCl₃ (1000 ml x 2), EtOAc (1000 ml x 2), and *n*-BuOH (1000 ml x 2). The CHCl₃-soluble fraction was subjected to column chromatography on Sephadex LH-20 (Pharmacia Fine Chemicals) with CHCl₃/MeOH (1:1) followed by hplc (Capcell Pak C18, 5 μ m, 4.6 x 250 mm) with methanol-water (65:35) to give penaresin (**1**, 0.0006% wet weight) and indoleacrylic acid (**2**, 0.003%).

Penaresin (1): a pale yellow solid, mp. 189-192 °C; uv (MeOH) λ_{max} 228 (ϵ 27100), 280 (18900), and 307 (18300) nm; ir (KBr) ν_{max} 3600 - 2800, 3350, and 1640 cm⁻¹; ¹H nmr (CD₃OD) δ 7.87 (1H, d, J =15.9Hz, H-9), 7.81 (1H, d, J =8.5Hz, H-4), 7.67 (1H, s, H-2), 7.63 (1H, d, J =1.7Hz, H-7), 7.32 (1H, dd, J =1.7 and 8.5Hz, H-5), and 6.43 (1H, d, J =15.9 Hz, H-8); Elms m/z 267, 265 (M⁺), 223, 221(M⁺-CO₂), 197, 195 (M⁺-C₃H₂O₂), and 115 (M⁺-C₃H₃O₂Br).

Methyl Ester (3): a pale yellow solid, mp 185 °C; uv (MeOH) λ_{max} 226 (ϵ 34200), 283 (19900) and 324 (26100) nm; ir (KBr) ν_{max} 3300 and 1700 cm⁻¹; ¹H nmr (CDCl₃) δ 8.47 (1H, brs, NH), 7.88 (1H, d, J =16.1 Hz, H-8), 7.77 (1H, d, J =8.6 Hz, H-4), 7.58 (1H, d, J =3.4 Hz, H-2), 7.48 (1H, d, J =1.6 Hz, H-7), 7.36 (1H, dd, J =1.6 and 8.6 Hz, H-5), 6.42 (1H, d, J =16.1 Hz, H-9), and 3.82 (3H, s, COOMe); Elms m/z 279 (M⁺), 281 (M⁺+2), 248 (M⁺-OMe), 250 (M⁺-OMe+2), 221 (M⁺-CO₂Me), 223 (M⁺-CO₂Me+2) and 143 (M⁺-CO₂Me-Br+H).

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