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

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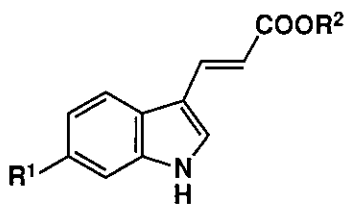
Abstract – A new bromoindole alkaloid, penaresin, has been isolated from the Okinawan marine sponge *Penares* sp. as a powerful Ca-inducer in sarcoplasmic reticulum and its structure was assigned by the spectral data.

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.



	R^1	R^2
1	Br	H
2	H	H
3	Br	Me
4	H	Me

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 acid[5] (**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 ($C_{11}H_8NO_2Br$) 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, $C_3H_2O_2$ and $C_3H_3O_2Br$ from M^+, respectively. The ir spectrum (KBr) showed an NH band at 3350 cm^{-1} and bands assigned to C=C-COOH at 3600-2800 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 μg/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 (Stellettidae) 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 ^{13}C 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 El.

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 subseqently extracted with $CHCl_3$ (1000 ml x 2), EtOAc (1000 ml x 2), and *n*-BuOH (1000 ml x 2). The $CHCl_3$-soluble fraction was subjected to column chromatography on Sephadex LH-20 (Pharmacia Fine Chemicals) with $CHCl_3$/MeOH (1:1) followed by hplc (Capcell Pak C_{18}, 5 μm, 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 (CD_3OD) 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^+-CO_2), 197, 195 (M^+-$C_3H_2O_2$), and 115 (M^+-$C_3H_3O_2Br$).

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 ($CDCl_3$) 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^+-CO_2Me), 223 (M^+-CO_2Me+2) and 143 (M^+-CO_2Me-Br+H).

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