New Sesterterpenes with Nerve Growth Factor Synthesis-Stimulating Activity from the Okinawan Marine Sponge *Hyrtios* sp.

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Two new pentacyclic sesterterpenes, 12-*O*-desacetylfuroscalarol (1) and 12-*O*-desacetylscalarin (2), which enhance nerve growth factor synthesis, have been isolated from the Okinawan marine sponge *Hyrtios* sp. and their structures elucidated on the basis of spectroscopic data.

Keywords sesterterpene; nerve growth factor; sponge; 12-*O*-desacetylfuroscalarol; 12-*O*-desacetylscalarin; *Hyrtios* sp.

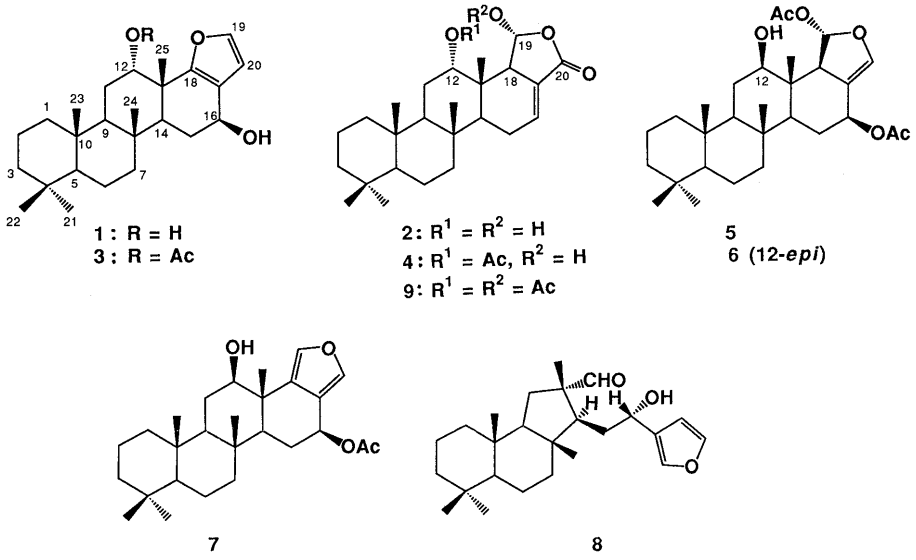
Marine sponges of the order Dictyoceratida have proven to be a rich source of structurally unique sesterterpenes.[1,2] In our continuing studies on bioactive substances from marine organisms,[3] we have investigated extracts of the Okinawan sponge *Hyrtios* sp. and isolated two new scalarane-type sesterterpenes, 12-*O*-desacetylfuroscalarol (**1**) and 12-*O*-desacetylscalarin (**2**), which stimulated nerve growth factor (NGF)[4] synthesis in cultured astroglial cells. In this paper, we describe the isolation and structure elucidation of **1** and **2**.

The sponge *Hyrtios* sp. was collected off Okinawa Island and kept frozen until used. The methanolic extract of the sponge *Hyrtios* sp. was partitioned between EtOAc and H_2O. The EtOAc-soluble material was subjected to silica gel column chromatography followed by silica gel TLC and reversed-phase HPLC to yield 12-*O*-desacetylfuroscalarol (**1**, 0.002%, wet weight) and 12-*O*-desacetylscalarin (**2**, 0.001%) together with known sesterterpenes, furoscalarol[5] (**3**), scalarin[6] (**4**), heteronemin[7] (**5**), 12-*epi*-heteronemin acetate[2] (**6**), scalarafuran[8] (**7**), and hyrtiosal[9] (**8**).

Compound **1**, a colorless solid, was shown to have the molecular formula, $C_{25}H_{38}O_3$, by high resolution electron impact mass spectra (HREIMS) [*m/z* 386.2818 (M^+), $\Delta -0.3$ mmu], indicating seven degrees of unsaturation. The IR absorption at 3400 cm^{-1} indicated the presence of hydroxyl group(s). The UV absorption maximum at 217 nm and the ^{1}H-NMR spectrum (δ 6.72, d, $J=1.8$ Hz; δ 7.43, d, $J=1.8$ Hz) of **1** suggested the presence of a furan ring. The ^{1}H-NMR also indicated the presence of five methyl groups [δ 0.79 (3H, s), 0.81 (6H, s), 0.91 (3H, s), and 1.29 (3H, s, H_3-21)]. The ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of furoscalarol (**3**), suggesting that **1** is the desacetyl derivative of **3**. All spectral data (^{1}H- and ^{13}C-NMR, IR, optical rotation, UV, and EIMS) of **1** were identical with those of 12-*O*-desacetylfuroscalarol derived from furoscalarol (**3**).[10] Therefore the structure of **1** was elucidated to be 12-*O*-desacetylfuroscalarol.

Compound **2** was obtained as a colorless solid. The HREIMS analysis showed the molecular formula of **2** to be $C_{25}H_{38}O_4$. The IR (3400, 1760 and 1740 cm^{-1}) and the UV [221 nm (ε 7900)] data implied that **2** possesses hydroxyl group(s) and an α,β-unsaturated ester. The ^{1}H-NMR spectrum of **2** was similar to that of scalarin (**4**) and suggested that **2** is the desacetyl derivative of **4**. Acetylation of **2** afforded the diacetyl derivative (**9**), whose spectral data (^{1}H-NMR, EIMS and optical rotation) were identical with those of 19-*O*-acetylscalarin derived from scalarin (**4**). Thus, the structure of **2** was assigned to be 12-*O*-desacetylscalarin.

The new compounds **1** and **2**, and a known compound



8, were found to cause 5- to 6-fold increases of NGF synthesis in cultured astroglial cells at 30—100 μg/ml. Since NGF synthesis-enhancers are candidate drugs for peripheral or central nerve disorders, these sesterterpenes might be useful as lead compounds.[11] Compounds **5**—**8** showed cytotoxicity against human epidermoid carcinoma KB cells *in vitro* with the IC_{50} values of 1.2 (**5**), 2.7 (**6**), 7.2 (**7**), and 2.0 (**8**) μg/ml, respectively, while compounds **1**—**4** were not cytotoxic. Compounds **3** and **8** inhibited rabbit platelet aggregation induced by adenosine diphosphate with the IC_{50} values of 50—100 μg/ml.

Experimental

General Methods Optical rotations were determined on a JASCO DIP-370 polarimeter. UV and IR spectra were obtained on a Shimadzu UV-220 spectrometer and a JASCO IR Report-100 spectrometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL JNM GX-270 and EX-400 spectrometers. EIMS spectra were recorded on a JEOL DX-303 spectrometer. Wako C-300 silica gel (Wako Pure Chemical) was used for glass column chromatography, and TLC was carried out on Merck silica gel GF_{254}.

Sponge Material The sponge *Hyrtios* sp. (order Dictyoceratida, family Thorectidae) was collected off Okinawa Island and kept frozen until used. The sponge is a firm incompressible with a dense interior, spongy. Some fine white fibres are apparent macroscopically. The surface is slightly glossy with fine conules close together. Black-brown exterior and dark brown interior. Primary and secondary fibres are cored with detritus. The primary fibres are fasciculate under the conules, up to 250 μm wide. There is a collagenous skin at the surface, no sand. Some secondary fibres are not cored, secondaries are 50 μm wide. No detrius in mesohyl. Large choanocyte chambers, fibres are pithed. The voucher specimen (SS-305) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University.

Collection, Extraction, and Isolation The sponge (1.0 kg wet weight) was extracted with MeOH (1.5 l × 2). The MeOH extract was partitioned between EtOAc (600 ml × 3) and 1 M NaCl (600 ml). The EtOAc-soluble materials (2.4 g) were subjected to column chromatography on silica gel (2.8 × 43 cm) eluted with $CHCl_3$–MeOH (96 : 4). The fraction from 250 to 450 ml was purified by silica gel column chromatography (2.5 × 40 cm) with hexane–EtOAc (100 : 0→50 : 50) to give 2 fractions (I and II), which were eluted with hexane–EtOAc (65 : 35) and hexane–EtOAc (55 : 45), respectively. Fraction I (347 mg) was then separated on a silica gel column (1.2 × 24 cm, hexane–EtOAc, 95 : 5→70 : 30). The fraction eluting with hexane–EtOAc (85 : 15) was purified on a Sep-Pak ODS cartridge (CH_3CN–EtOAc, 95 : 5) to give 12-*O*-desacetylfuroscalarol (**1**, 17 mg). Fraction II (195 mg) was subjected to silica gel column chromatography (1.1 × 40 cm) with toluene–acetone (100 : 0→75 : 25). The fraction eluted with toluene–acetone (9 : 1) was purified by reversed-phase HPLC [YMC-Pack AM-324 ODS, 10 × 250 mm; flow rate 2.5 ml/min; eluent CH_3CN–H_2O–TFA (80 : 20 : 0.1)] to give 12-*O*-desacetylscalarin (**2**, 9.6 mg, t_R 24.4 min).

12-*O*-Desacetylfuroscalarol (**1**): Colorless solid (MeOH), mp 238—242 °C, $[\alpha]_D^{20}$ +62.0° (c=0.4, $CHCl_3$). UV λ_{max}^{MeOH} 217 nm (ε 6600). IR ν_{max}^{KBr} 3400 cm^{-1}. ^{1}H-NMR (C_5D_5N) δ: 7.43 (1H, d, J=1.8 Hz, H-19), 6.72 (1H, d, J=1.8 Hz, H-20), 5.07 (1H, dd, J=2.5, 9.2 Hz, H-16), 4.62 (1H, br t, H-12), 1.29 (3H, s), 0.91 (3H, s), 0.81 (6H, s), 0.79 (3H, s). ^{13}C-NMR (C_5D_5N) δ 158.9 (s, C-18), 141.4 (d, C-19), 123.1 (s, C-17), 110.0 (d, C-20), 71.4 (d, C-12), 67.2 (d, C-16), 57.1 (d, C-5), 52.4 (d, C-9), 48.2 (d, C-14), 43.2 (s, C-13), 42.6 (t, C-7), 42.2 (t, C-3), 40.1 (t, C-1), 38.2 (s, C-10), 37.7 (s, C-8), 33.8 (q, C-25), 33.7 (s, C-4), 30.8 (t, C-15), 25.7 (t, C-11), 23.3 (q, C-21), 21.7 (q, C-24), 19.1 (t, C-2), 18.9 (t, C-6), 18.0 (q, C-22), 16.8 (q, C-23). EIMS m/z: 386 (98, M^+), 371 (10, M^+-CH_3), 368 (6, M^+-H_2O), 353 (17, $M^+-CH_3-H_2O$), 335 (10, $M^+-CH_3-2H_2O$), 205 (16), and 191 (100). HREIMS m/z: 386.2818. Calcd for $C_{25}H_{38}O_3$: 386.2821.

12-*O*-Desacetylscalarin (**2**): Colorless solid (MeOH), mp 194°C, $[\alpha]_D^{16}$ +15.4° (c=0.35, $CHCl_3$). UV λ_{max}^{MeOH} 221 nm (ε 7900). IR ν_{max}^{KBr} 3400, 1760, 1740, 1220, 1200 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.83 (1H, m, H-16), 5.67 (1H, br d, J=5.0 Hz, H-19), 3.83 (1H, m, H-12), 3.20 (1H, m, H-18), 2.31 (1H, m, H-15), 0.93 (3H, s), 0.85 (6H, s), 0.81 (6H, s). ^{13}C-NMR ($CDCl_3$) δ: 77.2 (d, C-12), 56.3 (d, C-5), 51.2 (d, C-9, C-18), 49.1 (d, C-14), 42.0 (t, C-7), 41.6 (t, C-3), 39.6 (t, C-1), 37.7 (s, C-8), 36.9 (s, C-10, C-13), 33.3 (s, C-4), 33.3 (q, C-21), 24.5 (t, C-15), 24.3 (t, C-11), 21.4 (q, C-22), 18.5 (t, C-6), 18.1 (t, C-2), 16.3 (q, C-24), 16.0 (q, C-23), 15.2 (q, C-25). EIMS m/z: 402 (2, M^+), 384 (13, M^+-H_2O), 368 (27), 256 (17), 236 (23), 205 (13), 191 (21). HREIMS m/z: 402.2812. Calcd for $C_{25}H_{38}O_4$: 402.2770.

Acetylation of 12-*O*-Desacetylscalarin (2) and Scalarin (4) 12-*O*-Desacetylscalarin (0.7 mg) was dissolved in pyridine (0.2 ml) and acetic anhydride (0.1 ml). After standing at room temperature for 14 h, the mixture was evaporated under reduced pressure to give 19-*O*-acetylscalarin (**9**, 0.9 mg), colorless oil, $[\alpha]_D^{17}$ +40.0° (c=0.18, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 6.90 (1H, dd, J=3.7, 7.0 Hz, H-16), 6.39 (1H, d, J=6.2 Hz, H-19), 4.81 (1H, t, J=2.7 Hz, H-12), 3.28 (1H, dd, J=4.1, 5.9 Hz, H-18), 2.13 (3H, s, AcO-19), 2.08 (3H, s, AcO-12), 0.95 (3H, s), 0.91 (3H, s), 0.86 (3H, s), 0.82 (3H, s), 0.81 (3H, s). EIMS m/z: 426 (35, M^+), 384 (35), 366 (39, M^+-CH_3COOH), 351 (20, $M^+-CH_3COOH-CH_3$), 275 (13), 258 (29), 242 (18), 228 (18), 215 (18), 205 (14), 191 (50).

According to essentially the same procedure as described above, **4** (1.0 mg) afforded 19-*O*-acetylscalarin (**9**, 1.1 mg), $[\alpha]_D^{17}$ +47.6° (c=0.20, $CHCl_3$). ^{1}H-NMR and EIMS spectral data were identical with those of the acetate (**9**) derived from **2**.

Biological Assay The cell culture of mouse astroglial cells and determination of NGF synthesized and secreted by the cells were carried out according to Furukawa *et al.*[12] The inhibitory activities against rabbit platelet aggregation were measured by a nephrometric technique.[13]

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