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# NEUROTROPHIC SESTERTERPENES ISOLATED FROM A MARINE SPONGE, *SPONGIA* SP.

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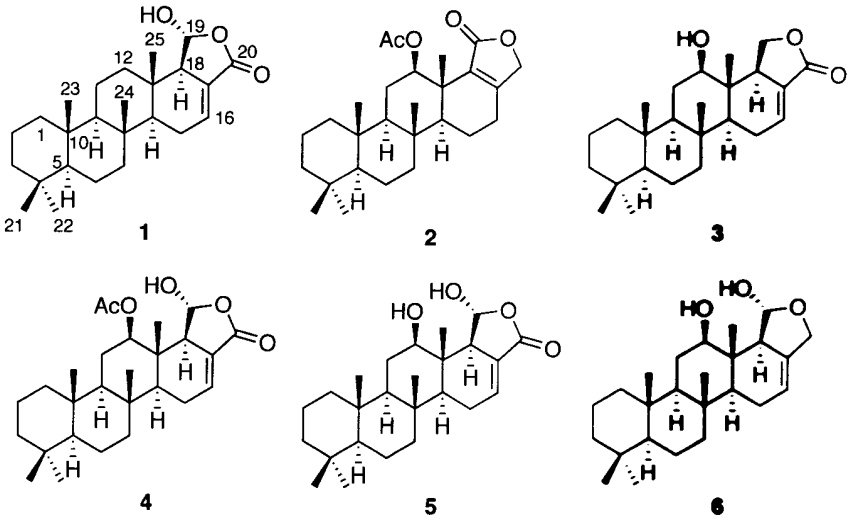
**Abstract** – The MeOH extract of a marine sponge of the genus *Spongia* showed neurotrophic activity in pheochromocytoma (PC-12) cells. Purification of the extract afforded a new sesterterpene, deacetoxyscalarin (**1**), along with five known sesterterpenes (**2-6**). Among them, **2-6** induced neurite outgrowth in PC-12 cells at the concentration of 50 μg/mL but **1** was inactive.

Marine sponges of the genus *Spongia* sp. are well known as rich sources of biologically active metabolites; so far the isolation of cytotoxic macrolides,[1,2] furanoterpenes,[3] cytotoxic sesterterpenes,[4] polyhydroxylated sterols which reverse multidrug resistance,[5,6] and aminoquinone[7] has been reported. In the course of our study on biologically active compounds from marine organisms, we also reported the isolation and structure elucidation of three new cytotoxic sesterterpenes from *Spongia* sp.[8] Recently, we found that the MeOH extract of the same sponge induced neurite outgrowth in pheochromocytoma (PC-12) cells. It is well known that neurons are terminally differentiated cells. Neurotrophic factors are necessary for the functional maintenance and organization of neurons. Among various neurotrophic factors, nerve growth factor (NGF)[9] has been extensively investigated and found to show pleiotrophic effects, such as the induction of neuronal differentiation, neural cell survival, and prevention of apoptosis of neurons, in both central and peripheral nervous systems.[9] PC-12 cells have been used as an *in vitro* model system to study the mechanisms of neuronal differentiation, and NGF induces their differentiation,

leading to the extension of neurites and the development of the characteristics of sympathetic neurons.[10] Then, we recollected the sponge in the Toyama Bay in the Japan Sea and isolated neurotrophic compounds. We report here the isolation, structure elucidation, and biological activity of a new sesterterpene, deacetoxyscalarin (**1**), along with five known compounds, scalarolide acetate (**2**),[11] sesterterpene (**3**),[12] 12-*epi*-scalarin (**4**),[13] 12-*O*-deacetyl-12-*epi*-scalarin (**5**),[8] and sesterterpene (**6**).[14]

The sponge was extracted with MeOH, and the EtOAc-soluble fraction of the extract was partiotioned between hexane and 90% MeOH-$H_2O$. The two fractions were purified on $SiO_2$ and ODS column chromatography followed by ODS HPLC to afford a new sesterterpene, (**1**) (3.1 mg), together with the known sesterterpenes, (**2**) (4.0 mg), (**3**) (3.0 mg), (**4**) (4.4 mg), (**5**) (4.9 mg), and (**6**) (6.3 mg).




Compound (**1**) had a molecular formula of $C_{25}H_{38}O_3$ as established by **HREIMS and** $^{13}C$ NMR data. In the IR spectrum the presence of hydroxyl and carbonyl groups was suggested by a broad band at 3500 $cm^{-1}$ and a sharp band at 1742 $cm^{-1}$, respectively. The $^{13}C$ NMR spectrum (Table 1) displayed a carbonyl signal at δ 164.5 (C-20), two olefin signals at δ 136.7 (C-16) and δ 127.6 (C-17), an oxygen-derived carbon at δ 98.4 (C-19), and five singlet methyl carbons at δ 15.2 (C-25), 16.4 (C-23), 16.4 (C-24), 21.3 (C-22), and 33.3 (C-21). The $^1H$ NMR spectrum (Table 1) showed two signals in the low-field region at δ 6.85 (1H, br d, $J$ = 3.1 Hz, H-16) and 5.62 (1H, br d, $J$ = 5.5 Hz, H-19) and five singlet methyls at δ 0.78 ($H_3$-25), 0.79 ($H_3$-22), 0.83 ($H_3$-21), 0.83 ($H_3$-23), and 0.91 ($H_3$-24), which was similar to that of **5** except for the absence of 12-OH in **1**. The COSY spectrum showed that a signal at δ 5.62 (H-19) was coupled with a methine signal at δ 2.44 (br s, H-18) (Figure 1), and that H-18 was further long-range coupled with an olefin hydrogen at δ 6.85 (br d, $J$ = 3.1 Hz, H-16). The carbon signals in the low-field region at δ 98.4

(CH, C-19), 127.6 (C, C-17), 136.7 (CH, C-16), and 164.5 (C, C-20) were reminiscent of those of **5**.[8] The HMBC correlations (Figure 1), H-14/C-18, $H_2$-15/C-17, H-16/C-18 and C-20, H-19/C-13 and C-20, $H_3$-25/C-13, C-14, and C-18, suggested that **1** was a derivative of scalarin.[11] The NOE correlation between H-19 and $H_3$-25 revealed the β-orientation of H-19, and the configuration of the pentacyclic sesterterpene ring system of **1** was also established as shown in Figure 2.

**Table 1.** $^1$H and $^{13}$C NMR Data of **1** ($CDCl_3$)

| No. | $\delta_H$ mult. *J* (Hz) | $\delta_C$ mult | COSY | HMBC |
|---|---|---|---|---|
| 1 | 0.78 m | 39.9 $CH_2$ | H-1', H-2' | |
| | 1.69 m | | H-1 | C-2 |
| 2 | 1.40 m | 18.6 $CH_2$ | H-2' | C-3, C-4, C-10 |
| | 1.58 m | | H-1, H-2, H-3' | C-1, C-3, C-10 |
| 3 | 1.12 dt 3.5, 13.0 | 42.1 $CH_2$ | H-3' | C-2, C-4, C-21, C-22 |
| | 1.35 m | | H-2', H-3 | C-5 |
| 4 | | 33.3 C | | |
| 5 | 0.79 m | 56.4 CH | H-6, H-6' | C-6 |
| 6 | 1.33 m | 18.0 $CH_2$ | H-5, H-6', H-7, H-7' | |
| | 1.51 br d 13.5 | | H-5, H-6, H-7, H-7' | |
| 7 | 0.97 br d 12.0 | 41.7 $CH_2$ | H-6, H-6', H-7' | |
| | 1.69 m | | H-6, H-6', H-7 | |
| 8 | | 37.6 C | | |
| 9 | 0.83 m | 61.2 CH | H-11, H-11' | |
| 10 | | 37.6 C | | |
| 11 | 1.35 m | 17.1 $CH_2$ | H-9 | C-8, C-10 |
| | 1.54 m | | H-9, H-12' | |
| 12 | 1.38 m | 40.6 $CH_2$ | H-12' | |
| | 1.87 br d 7.0 | | H-11', H-12 | C-9 |
| 13 | | 33.3 C | | |
| 14 | 1.30 m | 54.5 CH | H-15 | C-7, C-8, C-15, C-18, C-24, C-25 |
| 15 | 2.08 m | 24.0 $CH_2$ | H-14, H-15', H-16 | C-16, C-17 |
| | 2.29 m | | H-15, H-16 | C-17 |
| 16 | 6.85 br d 3.1 | 136.7 CH | H-15, H-15', H-18 | C-15, C-18, C-20 |
| 17 | | 127.6 C | | |
| 18 | 2.44 br s | 59.4 CH | H-16, H-19 | C-16, C-19 |
| 19 | 5.62 br d 5.5 | 98.4 CH | H-18 | C-13, C-20 |
| 20 | | 164.5 C | | |
| 21 | 0.83 (3H) s | 33.3 $CH_3$ | | C-3, C-4, C-5, C-22 |
| 22 | 0.79 (3H) s | 21.3 $CH_3$ | | C-3, C-4, C-5, C-21 |
| 23 | 0.83 (3H) s | 16.4 $CH_3$ | | C-1, C-5, C-9, C-10 |
| 24 | 0.91 (3H) s | 16.4 $CH_3$ | | C-7, C-8, C-9, C-14 |
| 25 | 0.78 (3H) s | 15.2 $CH_3$ | | C-12, C-13, C-14, C-18 |

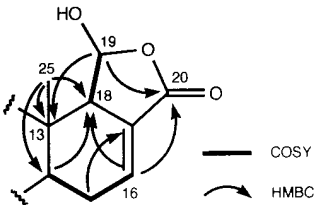



Figure 1. COSY and key HMBC correlations for **1**

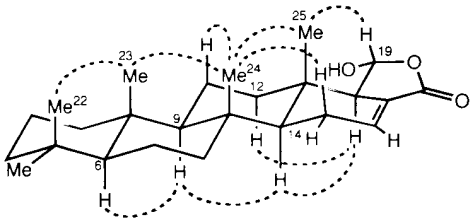



Figure 2. Key NOE correlations for **1**

Neurotrophic activity was tested using PC-12 cells (Table 2). At the concentration of 50 μg/mL, **2-6** induced neurite outgrowth in PC-12 cells but **1** was inactive; 68, 65, 58, 50, and 24% of the cells underwent neurite outgrowth by treatment with **2-6**, respectively.

**Table 2.** Neurite outgrowth activity of **1-6**.

| Compounds | Neurite outgrowth (%) |
|---|---|
| | 50 μg/mL |
| Control | 1.0 |
| NGF (50 ng/mL) | 21 |
| **1** | - |
| **2** | 68 |
| **3** | 65 |
| **4** | 58 |
| **5** | 50 |
| **6** | 24 |

## EXPERIMENTAL

### GENERAL

Optical rotation was determined with a HORIBA SEPA-300 high sensitive polarimeter. UV spectrum was measured on a SHIMADZU UV-1600 uv-visible spectrophotometer. IR spectrum was recorded on a SHIMADZU IR-460 infrared spectrophotometer. NMR spectra were recorded on a JEOL GSX500 and a Bruker Avance500 NMR spectrometers in $CDCl_3$. All chemical shifts were reported with respect to $CDCl_3$ ($\delta_H$ 7.26, $\delta_C$ 77.0). Mass spectra (MS) were measured on a JEOL SX-102 mass spectrometer.

## ANIMAL MATERIAL

The marine sponge was collected at a depth of 5 m in Toyama Bay in the Japan Sea, frozen immediately, and kept frozen until processed. The sponge was previously identified as *Spongia* sp. (class Demospongiae, order Dictyoceratida, family Spongiidae).[8]

## EXTRACTION AND ISOLATION

The frozen sponge (1.4 kg, wet wt) was extracted with MeOH. The extract was concentrated under reduced pressure. The EtOAc-soluble fraction of the MeOH extract (3.6 g) was partitioned between hexane and 90% MeOH-$H_2O$. The hexane fraction (1.9 g) and 90% MeOH-$H_2O$ fraction (1.5 g) were purified on $SiO_2$ column chromatography with hexane/EtOAc. The fractions which contained sesterterpenes were combined and purified by ODS column chromatography with MeOH/$H_2O$ followed by ODS HPLC with MeOH/$H_2O$ to afford **1** (3.1 mg, $2.2 \times 10^{-4}$%, wet weight), **2** (4.0 mg, $2.9 \times 10^{-4}$%), **3** (3.0 mg, $2.1 \times 10^{-4}$%), **4** (4.4 mg, $3.1 \times 10^{-4}$%), **5** (4.9 mg, $3.5 \times 10^{-4}$%), and **6** (6.3 mg, $4.6 \times 10^{-4}$%).

**Deacetoxyscalarin (1).** $[\alpha]_D^{26}$ –20° (*c* 0.095, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log ε) 220.5 nm (3.7); IR (film) $\nu_{max}$ 3610, 3500, 3025, 2925, 2390, 1742, 1719, 1511, 1464, 1415, 1218, 1037 $cm^{-1}$; NMR data, see Table 1; EIMS *m/z* 386 $[M]^+$; HREIMS *m/z* 386.28571 (calcd for $C_{25}H_{38}O_3$, 386.28210).

## NEURITE OUTGROWTH ASSAY

Neurite outgrowth assay was carried out with rat pheochromocytoma (PC-12) cells. PC-12 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 5% horse serum, penicillin (50 units/mL), and streptomycin (50 μg/mL) in an incubator containing 5% $CO_2$ at 37 °C. PC-12 cells were seeded onto 24-well multiplates ($1 \times 10^5$ cells/mL) and cultivated for a day. The medium was replaced with that containing 50 μg/mL samples or 50 ng/mL NGF as positive control, and then PC-12 cells were cultivated for 2 days and observed under a phase-contrast microscope. The percentage of the cells with neurites was determined by counting 200 cells.

## ACKNOWLEDGEMENTS

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