# Sculezonones A and B, Two Metabolites Possessing a Phenalenone Skeleton from a Marine-Derived Fungus *Penicillium* Species

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Two new metabolites possessing a phenalenone skeleton, sculezonones A (**1**) and B (**2**), were isolated from cultured broth of the fungus *Penicillium* sp., which was separated from the Okinawan marine bivalve *Mytilus coruscus*, and the structures were elucidated by spectroscopic data.

Marine microorganisms have proven to be a rich source of compounds that might be useful for the development of new pharmaceutical agents.[1] In our search for new metabolites from marine microorganisms,[2] two new compounds having a phenalenone skeleton, sculezonones A (**1**) and B (**2**), were isolated from the broth of the fungus *Penicillium* sp., which was found in the Okinawan marine bivalve *Mytilus coruscus*. In this paper we describe the isolation and structure elucidation of **1** and **2**.

The fungus *Penicillium* sp. was separated from the bivalve *Mytilus coruscus* collected off Seragaki Beach at Okinawa Island, and grown in PYG broth [peptone (1%), yeast extract (0.5%), and glucose (2%) in seawater, pH 7.5] at 25 °C for 14 days. The supernatant of the culture broth (1 L) was extracted with EtOAc, and the EtOAc-soluble portions were subjected to a Si gel column and gel filtration to give sculezonones A (**1**) and B (**2**), together with a known compound, herqueinone (**3**).[3,4]

The molecular formula, $C_{20}H_{20}O_8$, of sculezonone A (**1**) was established by HRFABMS [*m/z* 387.1067 $(M - H)^-$, $\Delta$ −1.3 mmu]. The IR spectrum suggested the presence of hydroxy (3435 $cm^{-1}$) and unsaturated carbonyl (1703 $cm^{-1}$) groups. The backbone structure of **1** was deduced from detailed analyses of $^1H$ and $^{13}C$ NMR data (Table 1) aided with 2D NMR experiments ($^1H-^1H$ COSY, HMQC, and HMBC). The $^{13}C$ NMR data indicated that the molecule possessed three ketone carbonyls, one oxygenated $sp^3$ quaternary carbon, one $sp^3$ quaternary carbon, one methoxy, four methyls, and 10 aromatic carbons, four of which were attached to an oxygen atom. In the $^1H$ NMR spectrum, two downfield singlets ($\delta_H$ 14.24 and 17.97) suggested the presence of two hydrogen-bonded OH protons. The $^1H$ and $^{13}C$ signals due to the following characteristic functional groups were observed for **1**: an acetyl ($\delta_H$ 2.17, $\delta_C$ 28.8, C-1′; $\delta_C$ 209.4, C-2′), a *gem*-dimethyl ($\delta_H$ 0.98, Me-4′ and $\delta_H$ 0.95, Me-5′; $\delta_C$ 55.0, C-3′), a methoxy ($\delta_H$ 3.65 and $\delta_C$ 59.0, MeO-15), and an aromatic proton ($\delta_H$ 6.12, H-12). HMBC correlations of H-12 to C-2, C-10, and C-14 and of $H_3$-14 to C-10, C-11, and C-12 indicated that the methyl group ($\delta_H$ 2.43) was attached to C-11, while correlations of the phenolic hydroxy proton ($\delta_H$ 17.97) to C-2, C-12, and C-13 revealed that the hydroxy group was attached to C-13. The presence of hydroxy and methoxy groups at C-7 and C-8, respectively, was deduced from HMBC correlations of the phenolic hydroxy proton ($\delta_H$ 14.24) to C-6, C-7, and C-8

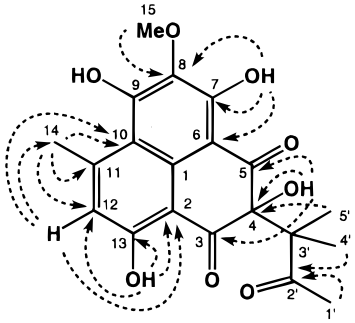


**Figure 1.** HMBC correlations of sculezonone A (**1**).

**Table 1.** $^1H$ and $^{13}C$ NMR Data of Sculezonones A (**1**) and B (**2**) in DMSO-$d_6$

| | **1** | | **2** | |
|---|---|---|---|---|
| position | $^1H^a$ | $^{13}C^a$ | $^1H^a$ | $^{13}C^a$ |
| 1 | | 136.6 s | | 129.9 s |
| 2 | | 110.1 s | | 109.1 s |
| 3 | | 192.8[b] s | | 192.8[c] s |
| 4 | | 84.5 s | | 84.5 s |
| 4-OH | 5.25 s | | 5.19 s | |
| 5 | | 196.9[b] s | | 196.7[c] s |
| 6 | | 98.7 s | | 98.9 s |
| 7 | | 161.2 s | | 161.0 s |
| 7-OH | 14.24 s | | 14.30 s | |
| 8 | | 129.2 s | | 128.0 s |
| 9 | | 168.5 s | | 169.5 s |
| 9-OH | 3.15 s | | 3.15 s | |
| 10 | | 114.1 s | | 112.1 s |
| 11 | | 144.2 s | | 128.3 s |
| 12 | 6.12 s | 111.6 d | | 137.7 s |
| 12-OH | | | 7.91 s | |
| 13 | | 172.3 s | | 158.2 s |
| 13-OH | 17.97 s | | 18.40 s | |
| 14 | 2.43 s | 22.5 q | 2.37 s | 13.9 q |
| 15 | 3.65 s | 59.0 q | 3.66 s | 59.1 q |
| 1′ | 2.17 s | 28.8 q | 2.15 s | 28.8 q |
| 2′ | | 209.4 s | | 209.2 s |
| 3′ | | 55.0 s | | 55.0 s |
| 4′ | 0.98 s | 20.2 q | 0.97 s | 20.3 q |
| 5′ | 0.95 s | 20.2 q | 0.95 s | 20.3 q |

[a] $\delta$, in ppm. [b,c] Interchangeable.

and of the methoxy protons ($\delta_H$ 3.65) to C-8. HMBC correlations of $H_3$-4′ and $H_3$-5′ to C-2′ and C-3′ and of $H_3$-1′ to C-2′ indicated the presence of a 3-methyl-2-butanone moiety (C-1′−C-5′). HMBC correlations of a hydroxy proton ($\delta_H$ 5.25) to C-3, C-4, C-5, and C-3′ and of $H_3$-4′ and $H_3$-5′ to C-4 revealed the presence of the hydroxy group at C-4 and the connection between C-3′ and C-4, between C-3 and C-4, and between C-4 and C-5. The presence of a hydroxy group ($\delta_H$ 3.15) at C-9 was implied by the $^{13}C$ chemical shift

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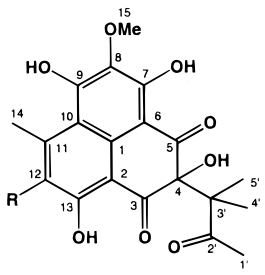
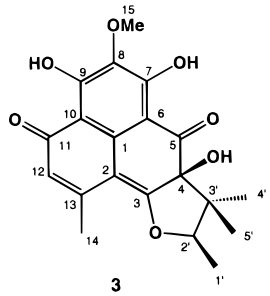
of C-9 ($\delta_C$ 168.5). The phenalenone−dione skeleton of **1** was deduced from comparison of $^1$H and $^{13}$C NMR data of **1** and aurantionone (**4**).[5] Thus, the gross structure of sculezonone A was elucidated to be **1**.

The molecular formula, $C_{20}H_{20}O_9$, of sculezonone B (**2**), was established by HRFABMS [*m*/*z* 403.1034 $(M - H)^-$, $\Delta$ +0.5 mmu], indicating that **2** was an oxygenated form of **1**. The $^1$H NMR spectrum of **2** was very close to that of **1** except for observation that an aromatic proton ($\delta_H$ 6.12, H-12) in **1** was replaced by a hydroxy proton ($\delta_H$ 7.91, OH-12) in **2**. HMBC correlations of $H_3$-14 ($\delta_H$ 2.37) and OH-13 ($\delta_H$ 18.40) to C-12 ($\delta_C$ 137.7) indicated that the hydroxy group was attached to C-12. Thus, the gross structure of sculezonone B was assigned as **2**. The CD spectra of **1** and **2** were similar to that of herqueinone (**3**), of which the absolute configuration has been established as 2′*R* and 4*S*,[4] indicating that the absolute configuration at C-4 for **1** and **2** may be *S*.

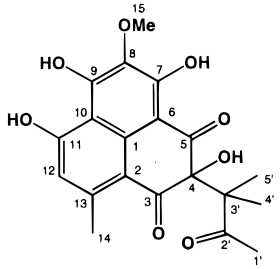
Sculezonones A (**1**) and B (**2**) are new metabolites containing a phenalene−dione nucleus from the broth of *Penicillium* sp., which was isolated from a marine bivalve, although similar phenalene−dione compounds such as aurantionone (**4**) and FR-901235 (**5**) have been obtained from the terrestrial fungi *Penicillium aurantio-virens*[5] and *Paecilomyces carneus*.[6] Among them, compounds **1** and **2** possess a 3-methyl-2-butanone moiety at C-4 as in **4**, while compound **5** has an acetonyl group at C-4. Sculezonones A (**1**) and B (**2**) may be biosynthetically derived from heptaketide (C-1−C-14) and the isoprene-like $C_5$ unit (C-1′−C-5′) as in known phenalenone compounds.[3−5] It is noted that the methyl group (C-14) of **1** and **2** is attached to C-11 as in **5**, whereas most of phenalenone compounds, including herqueinone (**3**) and aurantionone (**4**), possess the methyl group at C-13.



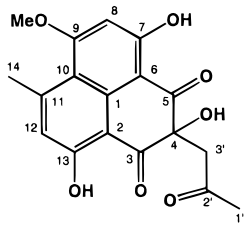

**1**: R = H
**2**: R = OH




**3**




**4**




**5**

## Experimental Section

**General Experimental Procedures.** The 2.49 ppm resonance of residual DMSO and 39.5 ppm of DMSO-$d_6$ were used as internal references for $^1$H and $^{13}$C NMR spectra, respectively. FABMS were obtained in the negative mode using glycerol as a matrix.

**Collection and Cultivation.** The fungus *Penicillium* sp. (K036) was separated from the marine bivalve *Mytilus coruscus*, which was collected off Seragaki Beach at Okinawa Island. Subcultures of the organism are deposited at Graduate School of Pharmaceutical Sciences, Hokkaido University. The fungus was grown in PYG broth at 28 °C for 14 days. The cultured broth (1 L) was filtered.

**Extraction and Separation.** The aqueous supernatant of the cultured broth (1 L) was extracted with EtOAc (500 mL × 2). The EtOAc-soluble portions (380 mg) were subjected to a Si gel column ($CHCl_3$−MeOH, 1:1) to afford herqueinone (**3**, 15.7 mg) and a fraction (63 mg), which was separated on a Sephadex LH-20 column (MeOH) to give sculezonones A (**1**, 7.2 mg) and B (**2**, 6.4 mg).

**Sculezonone A (1):** yellow amorphous solid; $[\alpha]^{23}_D$ +45° (*c* 0.2, MeOH); UV (EtOH) $\lambda_{max}$ 393 ($\epsilon$ 15 100), 274 (34 700), 217 (24 200) nm; IR (KBr) $\nu_{max}$ 3435, 1703, 1624, 1576 cm$^{-1}$; CD (EtOH) 431 ($\Delta\epsilon$ +0.6) 394 (0), 336 (−1.5), 306 (0), 290 (1.0), 263 (−0.6) nm; $^1$H and $^{13}$C NMR, see Table 1; FABMS *m*/*z* 387 $[M - H]^-$; HRFABMS *m*/*z* 387.1067 $[M - H]^-$ (calcd for $C_{20}H_{19}O_8$, 387.1080); HMBC correlations (DMSO-$d_6$) H-12/C-2, OH-13/C-2, OH-4/C-3, OH-4/C-4, $H_3$-4′/C-4, $H_3$-5′/C-4, OH-4/C-5, OH-7/C-6, OH-7/C-7, OH-7/C-8, MeO-8/C-8, H-12/C-10, $H_3$-14/C-10, $H_3$-14/C-11, $H_3$-14/C-12, OH-13/C-12, H-12/C-13, OH-13/C-13, H-12/C-14, $H_3$-1′/C-2′, $H_3$-4′/C-2′, $H_3$-5′/C-2′, $H_3$-4′/C-3′, $H_3$-5′/C-3′, $H_3$-4′/C-4, $H_3$-5′/C-4.

**Sculezonone B (2):** yellow amorphous solid; $[\alpha]^{23}_D$ +130° (*c* 0.2, MeOH); UV (EtOH) $\lambda_{max}$ 412 ($\epsilon$ 25 500), 276 (57 800), 214 (39 300) nm; IR (KBr) $\nu_{max}$ 3434, 1701, 1578 cm$^{-1}$; CD (EtOH) 448 ($\Delta\epsilon$ +5.4) 394 (0), 338 (−4.7), 301 (0), 290 (4.1), 265 (−4.8) nm; $^1$H and $^{13}$C NMR, see Table 1; FABMS *m*/*z* 403 $[M - H]^-$; HRFABMS *m*/*z* 403.1034 $[M - H]^-$ (calcd for $C_{20}H_{19}O_9$, 403.1029); HMBC correlations (DMSO-$d_6$) OH-13/C-2, OH-4/C-3, $H_3$-4′/C-4, $H_3$-5′/C-4, OH-7/C-6, OH-7/C-7, OH-7/C-8, MeO-8/C-8, $H_3$-14/C-10, $H_3$-14/C-11, $H_3$-14/C-12, OH-13/C-12, OH-13/C-13, $H_3$-1′/C-2′, $H_3$-5′/C-2′, $H_3$-4′/C-3′, $H_3$-5′/C-3′, $H_3$-4′/C-4, $H_3$-5′/C-4.

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