# (3*R*,4a*R*,5*S*,6*R*)-6-Hydroxy-5-methylramulosin: a New Ramulosin Derivative from a Marine-Derived Sterile Mycelium

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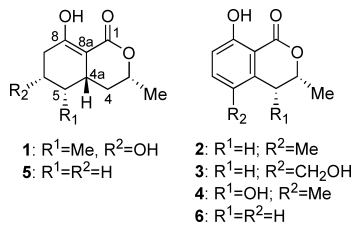
**(3*R*,4a*R*,5*S*,6*R*)-6-Hydroxy-5-methylramulosin (1) was isolated from a culture of a sterile mycelium, which was derived from the green alga, *Codium fragile*, along with (−)-5-methylmellein (2), (−)-5-hydroxymethylmellein (3), and (−)-(3*R*,4*R*)-*cis*-4-hydroxy-5-methylmellein (4). The absolute configuration of 1 was determined by the NMR data along with the lactone sector rule by circular dichroism (CD). Compound 1 exhibited moderate cytotoxic activity against HeLa cells.**

**Key words** 6-hydroxy-5-methylramulosin; sterile mycelium; marine-derived fungus

Marine microorganisms have attracted considerable attention as important sources of structurally diverse secondary metabolites and as potential leads for drug discovery.[1—3] In the course of our search for new biologically active compounds from marine-derived organisms,[4—6] we report here the isolation and structure elucidation of (3*R*,4a*R*,5*S*,6*R*)-6-hydroxy-5-methylramulosin (**1**), along with three known compounds, (−)-5-methylmellein (**2**), (−)-5-hydroxymethylmellein (**3**), and (−)-(3*R*,4*R*)-*cis*-4-hydroxy-5-methylmellein (**4**), which were isolated from *Valsa ceratosperma*, the pathogenic fungus of apple canker.[7]

The marine-derived fungus was isolated from the green alga, *Codium fragile* (SURINGAR) HARIOT, collected in Toyama Bay in the Japan Sea. The fungus was grown on agar plates composed of 50% seawater with nutrients. The culture plates were extracted with EtOH. The extract was partitioned between EtOAc and $H_2O$. The EtOAc-soluble fraction (4.5 g) was subjected to ordinary and reverse-phase silica gel column chromatography and HPLC to furnish **1** (16.0 mg), **2** (12.0 mg), **3** (26.0 mg), and **4** (3.6 mg).

Compound **1** has a molecular formula of $C_{11}H_{16}O_4$ as established by HR-FAB-MS, and the molecular formula requires four degrees of unsaturation. The $^1$H-NMR spectrum in $CDCl_3$ (Table 1) revealed two doublet methyl groups [$\delta$ 0.81 (3H, d, $J$=7.0 Hz, 5-Me) and 1.38 (3H, d, $J$=6.8 Hz, 3-Me)], two methines bearing an oxygen functional group [$\delta$ 4.10 (ddd, $J$=10.8, 6.5, 3.2 Hz, H-6) and 4.76 (ddq, $J$=1.5, 5.4, 6.8 Hz, H-3)], two hydrogen signals [$\delta$ 1.63 (br s, 6-OH) and 13.27 (s, 8-OH)], and six methylene and methine signals ($\delta$ 1.5—2.9). The $^1$H–$^1$H correlation spectroscopy ($^1$H–$^1$H COSY) experiment of **1** indicated the presence of a partial structure in bold lines (Fig. 1). The heteronuclear multiple-bond correlation (HMBC) experiment of **1** showed the correlations between $H_2$-7 and two quaternary carbons C-8 ($\delta$ 173.1) and C-8a ($\delta$ 93.1) and between three hydrogens H-4$\beta$, H-4a, and H-5 and C-8a (Fig. 1). HMBCs were observed from H-3 to a carbonyl carbon C-1 ($\delta$ 171.7) and from the downfield hydroxy resonance 8-OH to C-8 and C-8a, which clarified the connectivity of the quaternary carbons C-1, C-8, and C-8a. Thus, the planar structure of **1** was shown (Fig. 1). The relative stereochemistry of **1** was elucidated by its NOE experiment. NOE correlations between H-4a and two signals H-3 and H-6 indicated that they were oriented on the same side (Fig. 1) at axial positions; hence the 3-Me and 6-OH groups were in equatorial orientation. While the correlations between 5-Me and two hydrogens H-4$\alpha$ ($\delta$ 1.90) and H-7$\alpha$ ($\delta$ 2.31) revealed that they were the opposite side of H-3, H-4a, and H-6 and that they were in axial orientaion. The absolute stereochemistry of **1** was substantiated by applying the lactone sector rule by CD measurement, in which the signs, used in the ketone octant rule, are reversed for lactone sectors.[8] The sector projection, in which **1** is viewed in the plane of the lactone group along the line of the carboxyl group and its attached carbon atom, is shown in Fig. 2. The CD spectrum of **1** showed the positive Cotton effect observed at 260 nm ($\Delta\varepsilon$=+126) due to the lactone group with the exocyclic $\alpha,\beta$-double bond and with the $\beta$-hydroxy group bearing hydrogen bonding to the oxygen at C-1. As shown in Fig. 2, the positive contribution was exhibited for **1**. Therefore, the structure of **1** was established as (3*R*,4a*R*,5*S*,6*R*)-6-hy-




**1**: $R^1$=Me, $R^2$=OH
**5**: $R^1$=$R^2$=H
**2**: $R^1$=H; $R^2$=Me
**3**: $R^1$=H; $R^2$=$CH_2OH$
**4**: $R^1$=OH; $R^2$=Me
**6**: $R^1$=$R^2$=H

Chart 1

Table 1. NMR Data ($CDCl_3$) of **1**

| | $\delta_H$ | $J$ (Hz) | $\delta_C$ | HMBC |
|---|---|---|---|---|
| 1 | | | 171.7 C | |
| 3 | 4.76 ddq | 5.4, 1.5, 6.8 | 74.2 CH | C-1, C-4a |
| 4 | $\alpha$ 1.90 dt | 5.4, 13.8 | 30.1 $CH_2$ | C-3, C-4a, 3-Me |
| | $\beta$ 1.59 ddd | 1.5, 4.6, 13.8 | | C-4a, C-8a |
| 4a | 2.86 br d | 13.8 | 29.6 CH | C-4, C-5, C-8a, 5-Me |
| 5 | 2.06 m | | 37.2 CH | C-4, C-6, C-7, C-8a |
| 6 | 4.10 ddd | 10.8, 6.5, 3.2 | 68.5 CH | 5-Me |
| 7 | $\alpha$ 2.31 dd | 18.6, 10.8 | 34.2 $CH_2$ | C-6, C-8, C-8a |
| | $\beta$ 2.63 dd | 18.6, 6.5 | | C-5, C-6, C-8, C-8a |
| 8 | | | 173.1 C | |
| 8a | | | 93.1 C | |
| 3-Me | 1.38 d | 6.8 | 20.2 $CH_3$ | C-3, C-4 |
| 5-Me | 0.81 d | 7.0 | 5.3 $CH_3$ | C-5, C-6, C-4a |
| 6-OH | 1.63 br s | | | |
| 8-OH | 13.27 s | | | C-7, C-8, C-8a |

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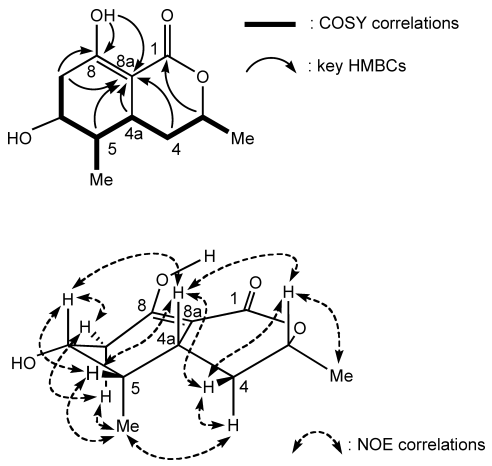


Fig. 1. COSY, Key HMBC, and NOE Correlations Observed for **1**

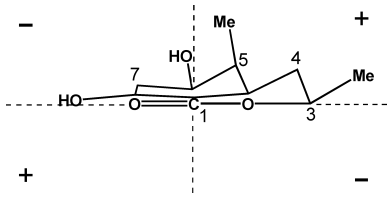

Fig. 2. Lactone Sector Projection of **1**

droxy-5-methylramulosin. Metabolites belonging to a family of ramulosin (**5**)[9—13] isolated so far contain commonly 3*R*,4a*R* configuration, and also mellein (**6**)[7,14] derivatives bear 3*R* configuration. Among them, 6-hydroxyramulosin, which was isolated from several fungi,[9—11] possesses a $\beta$-oriented hydroxy group at C-6, while the hydroxy group at C-6 in **1** was found to be $\alpha$-oriented.

Since the metabolites in the group of ramulosin (**5**) or mellein (**6**) exhibited a variety of biological activities, a number of the synthetic studies have been carried out to date. Recently, (+)-**6**, (−)-**5**, and their related natural products were synthesized as optically active form.[15,16] Compound **1** showed 65% growth inhibition against HeLa cells at a concentration of 50 $\mu$g/ml, while compounds **2**—**4** were inactive.

## Experimental

**General Experimental Procedures** Optical rotation was determined with a Horiba SEPA-300 high sensitive polarimeter. UV spectrum was measured on a Shimadzu UV-1600 UV–visible spectrometer. CD spectrum was measured on a JASCO J-820 spectropolarimeter. IR spectrum was recorded on a Shimadzu IR-460 infrared spectrophotometer. NMR spectra were recorded on a JEOL GSX500 in $CDCl_3$. Mass spectra were measured on a JEOL SX-102 mass spectrometer.

**Fungal Strain** The marine fungus was isolated from the green alga, *Codium fragile* (Suringar) Hariot, collected in Toyama Bay in the Japan Sea. The identification of the fungus was evaluated by TechonoSuruga Co., Ltd. (Shizuoka, Japan), and it was just clarified to be a sterile mycelium. A voucher specimen is deposited at Kanazawa University with the code MF593.

**Extraction and Isolation** The fungus was cultivated on agar medium (20 ml×500 plates) composed of 2.0% malt extract and 5.0% peptone in 50% natural seawater for 10 d at 25 °C. The culture was extracted with EtOH three times. The extract was evaporated under reduced pressure and partitioned between EtOAc and $H_2O$. The EtOAc fraction (4.5 g) was subjected to silica gel column chromatography using a step-wise gradient from hexane to EtOAc. The hexane/EtOAc (4 : 1, 2 : 1, and 1 : 1) fractions were combined and further purified by silica gel HPLC (hexane/EtOAc) and ODS HPLC (MeOH/$H_2O$) to afford **1** (16.0 mg), **2** (12.0 mg), **3** (26.0 mg), and **4** (3.6 mg).

**1**: Colorless oil, $[\alpha]_D^{25}$ +30° ($c$=0.24, EtOH); UV $\lambda_{max}$ (MeOH) nm (log $\varepsilon$): 263 (3.8); CD (MeOH) $\Delta\varepsilon_{260}$ +126, $\Delta\varepsilon_{300}$ −35; IR (film) $cm^{-1}$: 3370, 1647; $^1$H- and $^{13}$C-NMR, see Table 1; FAB-MS (positive) *m*/*z*: 213 $[M+H]^+$; HR-FAB-MS (positive) *m*/*z*: 213.1124 (Calcd for $C_{11}H_{17}O_4$ 213.1127).

**Cytotoxicity Test** Cytotoxicity test was carried out with HeLa cells. Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin (50 units/ml), and streptomycin (50 $\mu$g/ml) under a humidified atmosphere of 5% $CO_2$ at 37 °C. The cells were seeded into 96-well microplates ($3\times10^3$ cells/well) and pre-cultured for a day. The medium was replaced with that containing test compounds at various concentrations and the cells were further cultured at 37 °C for 3 d. The medium was then replaced with 50 $\mu$l of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (0.2 mg/ml in medium) and the cells were incubated under the same conditions for 4 h. After the addition of 200 $\mu$l of DMSO, the optical density at 570 nm was measured with a microplate reader.

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