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Violapyrone J, α-Pyrone Derivative from a Marine-derived Actinomycetes, *Streptomyces* sp.

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Abstract – A new α-pyrone derivative, violapyrone J (**1**), and along with the two known violapyrones B (**2**) and C (**3**) were isolated from the fermentation broth of a marine actinomycete *Streptomyces* sp. SC0718. The structure of violapyrone J (**1**) was elucidated from 1D and 2D NMR spectroscopic analyses.
Keywords – α-Pyrone, Violapyrone, Marine actinomycetes, *Streptomyces* sp.

Introduction

Microbial natural products are responsible for more than 50% of the anticancer medications and antibiotics available commercially today.[1] However, the frequent re-discovery of the secondary metabolites from soil-derived microorganisms has decreased the attention for natural products in drug discovery programs. Recently, microorganisms isolated from marine environments have been recognized as a prolific source for discovering structurally unique natural products with diverse biological activities.[2-7] In addition, several researches suggested that the actual producers of the half of drugs in the market developed from marine natural products could be microorganisms.[8] Microorganisms isolated from unexplored marine environments could be a great niche to provide new biologically active secondary metabolites.[9]

As part of the program for discovering marine microorganisms from Korean marine environments, a *Streptomyces* sp. SC0718 was isolated from the marine sediment in Sunchon Bay. Intensive study for chemical components of this strain has yielded a new α-pyrone derivative, violapyrone J (**1**), with the two known violapyrone derivatives, violapyrones B (**2**) and C (**3**).

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Experimental

General experimental procedures – The optical rotation was measured on a Autopol III polarimeter (Rudolph Research) with a 5 cm cell. IR spectrum was recorded on a Varian Scimitar Series spectrometer. NMR spectra were recorded on Varian Inova NMR spectrometer (700 and 175 MHz for ^{1}H and ^{13}C NMR, respectively), using the signals of the residual solvent protons and the solvent carbons as internal references (δ_H 3.33 and δ_C 49.3 ppm for MeOD). Low resolution LC-MS data were measured using an Agilent Technology 6120 quadrupole LC/MS system with a reversed phase column (Phenomenex luna C18(2) 100 Å, 50 mm × 4.6 mm, 5 μm) at a flow rate of 1.0 mL/min.

Strain and cultivation – *Streptomyces* sp. SC0718 was isolated from a marine sediment collected from the mudflat of Suncheon bay, South Sea of Korea. It was classified according to 16S rRNA analysis with 99.7% identity with *Streptomyces* sp. zx-10-19 (Gene bank accession no. HQ611066.1).

Strain SC0718 was cultured in 20 of 2.5-L Ultra Yield Flasks each containing 1 L of the medium (10 g/L of soluble starch, 2 g/L of yeast, 4 g/L of peptone, dissolved in 750 mL natural seawater and 250 mL of distilled water) at 25 ºC with shaking at 150 rpm. After 7 days, the whole culture (20 L) was extracted with EtOAc, and was concentrated *in vauo* to yield 1.3 g of an extract.

Table 1. NMR data of violapyrone J (**1**) in CD_3OD. (δ in ppm)[a]

No.	1			
	δ_C, mult.[b]	δ_H (*J* in Hz)	COSY	HMBC
2	181.1, C			
3	96.6, C			
4	171.4, C			
5	108.2, CH	5.77, s		3, 6, 7
6	163.4, C			
7	32.3, CH_2	2.35-2.45, m	8	5
8	35.3, CH_2	1.70, 1.20, m	7, 9	
9	35.4, CH	1.45, m	8, 10,12	
10	30.6 CH_2	1.40, 1.20, m	9, 11	11, 12
11	12.0, CH_3	0.91, t (7.0)	10	
12	19.6, CH_3	0.93 d (6.3)	9	8
3-Me	9.0, CH_3	1.81, s		2, 3, 4

[a]700 MHz for ^1H NMR and 175 MHz for ^{13}C NMR. [b]Numbers of attached protons were determined by analysis of 2D spectroscopic data.

Isolation of violapyrones J, B and C (1 - 3) – The crude extract (1.3 g) was fractionated by C18 vacuum column chromatography eluting with a step gradient from 10 to 100% MeOH in H_2O. The 60% MeOH/H_2O fraction (63.3 mg) was subjected to reversed-phase HPLC with 35% aqueous acetonitrile (Phenomenex Luna C-18 (2), 250 × 100 mm, 2.5 mL/min, 5 μm, 100 Å, UV = 254 nm) to afford violapyrones J (**1**, 1.0 mg) and B (**2**, 4.0 mg), with retention times of 22 and 33 min, respectively. The 70% MeOH/H_2O fraction (72.7 mg) from C18 vacuum column chromatography was also subjected to reversed-phase HPLC with 55% aqueous acetonitrile (Phenomenex Luna C-18 (2), 250 × 100 mm, 2.5 mL/min, 5 μm, 100 Å, UV =254 nm) to afford violapyrones C (**3**, 4.0 mg), with a retention time of 24 min.

Violapyrones J (1) – white amorphous powder; $[\alpha]_D^{24}$ +72 (c 0.038, MeOH); UV/vis (MeOH) λ_{max} (log ε) 196 (3.27), 292 (2.47) nm; IR (KBr) ν_{max} 3387, 1960, 1644 cm^{-1}; ^1H and ^{13}C NMR data, See Table 1; HRESQTOF *m/z* 211.1335 $[M+H]^+$ (calculated for $C_{12}H_{19}O_3$, 211.1334).

Result and Discussion

Violapyrone J (**1**) was isolated as a white amorphous powder. The molecular formula $C_{12}H_{18}O_3$ was deduced from the $[M+H]^+$ peak at *m/z* 211.1335 (calcd for 211.1334) in the HRESQTOF, which required four degrees of unsaturation. Its IR absorptions at 3419, 1638, and 1577 cm^{-1} and the UV maximum peak at 292 nm indicated the typical α-pyrone chromophore.[10,11]

The ^1H NMR spectrum of **1** displayed an olefinic proton H-5 [δ_H 5.77 (s)], a methyl triplet H-11 [(δ_H 0.91, t, *J* = 7.0)], a methyl doublet H-12 [(δ_H 0.93, d, *J* = 6.3)], and a methyl singlet 3-Me [(δ_H 1.81, s)]. The ^{13}C and HSQC spectroscopic data revealed three methyl, three methylene, two methine, and four fully-substituted carbons. The ^1H and ^{13}C spectra of **1** had very similar features to those of previously reported natural product, violapyrone A,[12] except for the differences of the carbon chemical shifts at C-11 and C-12.

Two substructures, a linear aliphatic chain and a α-pyrone moiety, were assigned by analyses of ^1H, ^{13}C, COSY, HSQC, and HMBC NMR spectroscopic data (Fig. 2). A linear aliphatic chain with a six carbons unit was determined by the interpretation of ^1H–^1H COSY and HMBC spectroscopic data. The COSY cross-peaks [H-7/H-8/H-9/H-10/H-11, H-9/H12] and HMBC correlations from H-12 to carbons C-8, C-9, C-10, and from H-11 to carbons C-9, and C-10 permitted the construction of a linear aliphatic chain. A α-pyrone moiety was established by HMBC correlations from 3-Me to carbons C-2, C-3, and C-4, and from H-5 to a carbon C-4. Lastly, HMBC correlations from H-5 to carbons C-6, and C-7 provided the attachment of C-6/C-7, completing the assignment of **1** as shown in Fig. 2.

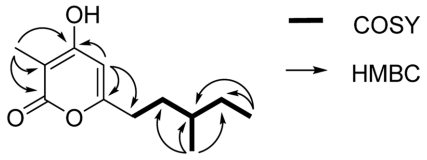

Fig. 2. Key HMBC and COSY correlations of violapyrone J (**1**) in CD_3OD.

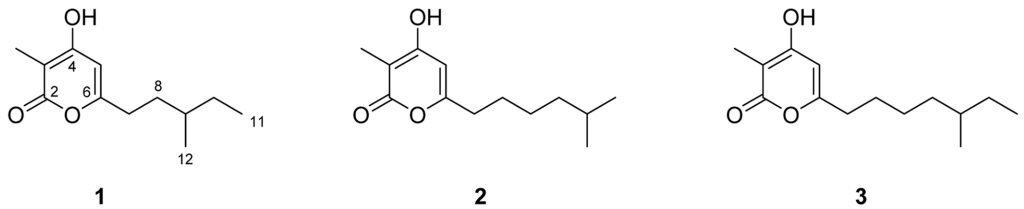

Fig. 1. The structures of violapyrones J (**1**), B (**2**) and C (**3**).

Together with **1**, the two previously reported violapyrones B (**2**) and C (**3**) were isolated.[12] The chemical structures of **2** and **3** were confirmed by the comparison of ^{1}H and ^{13}C NMR data to those of previously reported ones.

Violapyrone J (**1**) possesses a 4-hydroxy-6-alkyl-α-pyrone moiety in the molecules. 4-Hydroxy-6-alkyl-α-pyrones have been reported mainly from fungi, but they are also found in marine animals, plants, and bacteria.[13,14] However, there are few reports of the isolation of these compounds from streptomycetes.[15] Violapyrones A-G were first produced by the culture broth of *Streptomyces violascens* isolated from *Hylobates hoolock* (the east Asian primate) faces, and violapyrones B (**2**) and C (**3**) were found to exhibit antibacterial activities on *Staphylococcus aureus* and *Bacillus subtilis* within the range of MIC values of 4~16 μg/mL.[12] This study also indicated that the shorter alkyl chains within the molecules possessed stronger antibacterial activities on *S. aureus* and *B. subtilis*. The biological activity of violapyrone J (**1**) on bacterial pathogens is under the investigation.

Acknowledgments

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