Natural Product Sciences
21(4) : 248-250 (2015)
http://dx.doi.org/10.20307/nps.2015.21.4.248

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 °C 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.

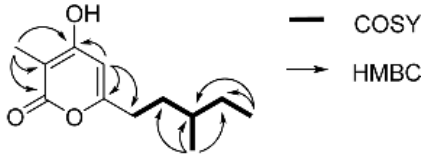

Fig. 2. Key HMBC and COSY correlations of violapyrone J (**1**) in CD_3OD.

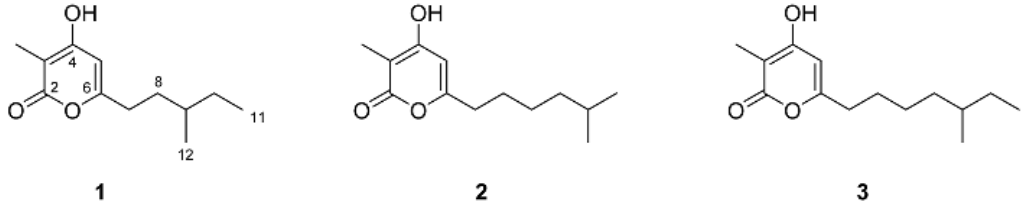

Fig. 1. The structures of violapyrones J (**1**), B (**2**) and C (**3**).

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

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (NRF-2014R1A1A1003492) and by the National Research Foundation of Korea Grants (NRF-2012M1A5A1054307) funded by the Korean Government (Ministry of Science, ICT and Future Planning).

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Received June 30, 2015
Revised August 14, 2015
Accepted August 17, 2015