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Short Note

5-(6-Hydroxy-2,5,7,8-tetramethylchroman-2-yl)-2-methyl-pentanoic Acid Methyl Ester

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Abstract: A natural diastereomeric mixture of 5-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-2-methyl-pentanoic acid methyl ester (**1**), was isolated from the soft coral *Sinularia arborea*. The structure of **1** was elucidated by spectroscopic methods and **1** displayed a significantly inhibitory effect on the generation of superoxide anion by human neutrophils.

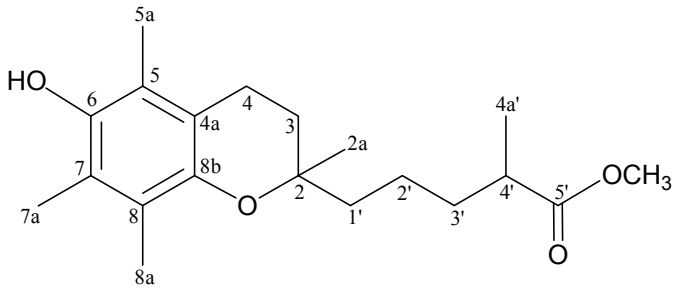
Keywords: *Sinularia arborea*; tocopherol; superoxide anion

Introduction

Octocorals belonging to the genus *Sinularia* have been proved to be rich sources of interesting secondary metabolites. The octocoral *Sinularia arborea* (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonacea, family Alcyoniidae) was studied, as their organic extract was found to

show meaning signals in NMR studies. In previous studies on the chemical constituents of the soft coral *Sinularia arborea* [1], six new diterpenoids, arbolides A–C, (+)-sarcophytol T, sinularbols A and B, along with a trihydroxysteroid, crassarosterol A, were isolated [2–4]. In the continuing studies on this interesting species, a mixture of diastereoisomers, 5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)-2-methyl-pentanoic acid methyl ester (**1**), was isolated (Scheme 1). In this paper, we describe the isolation, structure determination and bioactivity of compound **1**.

Scheme 1. The structure of 5-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-2-methyl-pentanoic acid methyl ester (**1**).



Results and Discussion

5-(6-Hydroxy-2,5,7,8-tetramethylchroman-2-yl)-2-methyl-pentanoic acid methyl ester (**1**) was isolated as a colorless oil. Its molecular formula was established as $C_{20}H_{30}O_4$ from a sodium salt ion at *m/z* 357 in the ESIMS and further supported by the HRESIMS at *m/z* 357.20365 (calcd. for $C_{20}H_{30}O_4Na$, 357.20363), thus implying six degrees of unsaturation. The presence of hydroxy and ester groups in **1** were evidenced by IR absorptions at υ_{max} 3,450 and 1,727 cm^{-1}. From the ^{13}C and DEPT spectral data, six tetrasubstituted olefins were deduced from the signals at δ_C 145.40, 144.59, 122.58, 121.04, 118.48 and 117.25. A methyl esterified carboxyl group was supported by the ^{13}C-NMR signals at δ_C 177.28 (C), 51.45 (CH_3) and the ^1H-NMR signal (Table 1) at δ_H 3.67 (3H, s, -OMe). Comparison of the ^{13}C-NMR and DEPT spectra with the molecular formula indicated that there must be an exchangeable proton, requiring the presence of a hydroxy group. From the above data, four degrees of unsaturation were accounted for and **1** must be a compound with two rings.

From the ^1H–^1H COSY spectrum of **1** (Table 1 and Figure 1), the separate spin systems of H_2-3/H_2-4 and H_2-1'/H_2-2'/H_2-3'/H-4'/H_3-4a' were differentiated. These data, together with the key HMBC correlations between protons and quaternary carbons (Table 1 and Figure 1), such as H_3-2a, H_2-4/C-2; H_2-4, H_3-5a/C-4a; H_2-4, H_3-5a/C-5; H_3-5a/C-6; H_3-7a, H_3-8a/C-7; H_3-7a, H_3-8a/C-8; H_2-4/C-8b; and H_3-4a'/C-5', established the main carbon skeleton of **1**. The vinyl methyls at C-5, C-7 and C-8 were confirmed by the HMBC correlations noted, thus, the remaining hydroxy group is positioned at C-6, an oxygen-bearing olefin carbon at δ_C 144.59. Based on the above findings, the structure of **1** is similar in structure to α-tocopherol. A set of additional carbon signals observed in the ^{13}C-NMR spectrum of **1** (Table 1) supported the presence diastereoisomers. The stereochemistry of chiral carbons C-2 and C-4' was not determined at this stage.

It was found that the ^1H and ^{13}C-NMR data of **1** are almost identical to those of a known synthetic compound, 5-(6-hydroxy-2,5,7,8-tetramethyl-chroman-2-yl)-2-methyl-pentanoic acid methyl ester,

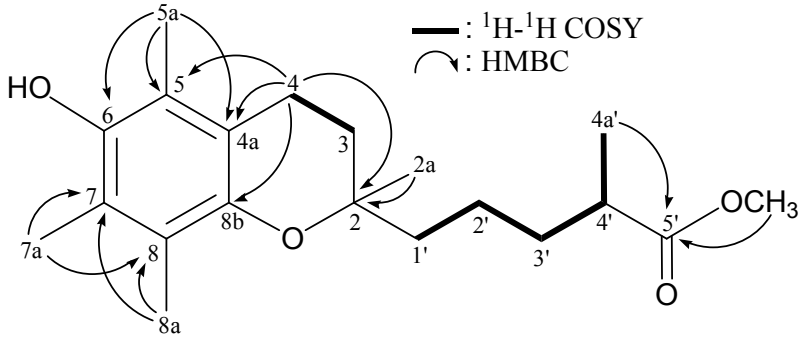
that was shown to exist as a set of diastereoisomers [5]. However, compound **1** has not been isolated previously from any natural sources.

Table 1. ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data, ^{1}H–^{1}H COSY and HMBC correlations for **1**.

Position	δ_H (*J* in Hz)	δ_C, Multiple			^{1}H–^{1}H COSY	HMBC (H→C)
2		74.25		C		
2a	1.22 s	23.67	23.65	CH_3		C-2, -3, -1',
3	1.78 m	31.55		CH_2	H_2-4	C-4
4	2.60 t (6.8)	20.69		CH_2	H_2-3	C-2, -3, -4a, -5, -8b
4a		118.48		C		
5		117.25		C		
5a	2.11 s	11.26		CH_3		C-4a, -5, -6
6		144.59		C		
7		121.04		C		
7a	2.10 s	11.75		CH_3		C-7, -8
8		122.58		C		
8a	2.16 s	12.19		CH_3		C-7, -8
8b		145.40		C		
1'	1.59 m, 1.51 m	39.35	39.31	CH_2	H_2-2'	n.o. [a]
2'	1.44 m	21.30	21.26	CH_2	H_2-1', H_2-3'	n.o.
3'	1.66 m, 1.42 m	34.20		CH_2	H_2-2', H-4'	n.o.
4'	2.46 sext (5.6)	39.40	39.36	CH	H_2-3', H_3-4a'	n.o.
4a'	1.15 d (6.8)	17.06	17.02	CH_3	H-4'	C-3', -4', -5'
5'		177.28	177.24	C		
5'-OCH_3	3.67 s	51.45	51.44	CH_3		C-5'
6-OH	4.20 br s					

[a] n.o. = not observed.

Figure 1. ^{1}H–^{1}H COSY and selective key HMBC correlations (protons→quaternary carbons) for **1**.



In a previous study, the cembranoid, arbolide C, which was also isolated from *S. arborea*, showed an inhibitory effect on the release of elastase (IC_{50} = 16.99 μM) by human neutrophils [4]. The *in vitro* anti-inflammatory effects of **1** was examined; **1** displayed an inhibitory effect on the generation of

superoxide anion (IC_{50} = 7.42 μM), but not active in inhibition of elastase release by human neutrophils, respectively.

Experimental

General

Infrared spectra were recorded on a Varian Diglab FTS 1000 FT-IR spectrometer (Varian Inc., Palo Alto, CA, USA); peaks are reported in cm^{-1}. NMR spectra were recorded on a Varian Mercury Plus 400 NMR spectrometer (Varian Inc.) using the residual $CHCl_3$ signal (δ_H 7.26 ppm) as the internal standard for ^{1}H-NMR and $CDCl_3$ (δ_C 77.1 ppm) for ^{13}C-NMR. Coupling constants (*J*) are given in Hz. ESIMS and HRESIMS were recorded using a Bruker 7 Tesla solariX FTMS system (Bruker, Bremen, Germany). Column chromatography was performed on silica gel (230–400 mesh, Merck, Darmstadt, Germany). TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck); spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. The normal phase HPLC (NP-HPLC) was performed using a system comprised of a Hitachi L-7110 pump (Hitachi Ltd. Tokyo, Japan), a Hitachi L-2455 photodiode array detector (Hitachi Ltd.) and a Rheodyne 7725 injection port (Rheodyne LLC. Rohnert Park, CA, USA). A normal phase column (Supelco Ascentis® Si Cat #:581515-U, 25 cm × 21.2 mm, 5 μm, Sigma-Aldrich, St. Louis, MO, USA) was used for NP-HPLC. The reverse phase HPLC (RP-HPLC) was performed using a system comprised of a Hitachi L-7100 pump (Hitachi Ltd.), a Hitachi L-2455 photodiode array detector (Hitachi Ltd.), a Rheodyne 7725 injection port (Rheodyne LLC.) and a Varian Polaris 5 C-18-A column (25 cm × 10 mm, 5 μm).

Animal Material

Specimens of the octocoral *Sinularia arborea* [1] were collected by hand using SCUBA equipment off the coast of southern Taiwan on October, 2012, and stored at −20 °C until extraction. A voucher specimen (NMMBA-TWSC-1200X) was deposited in the National Museum of Marine Biology and Aquarium, Taiwan.

Extraction and Isolation

The freeze-dried and minced material of *Sinularia arborea* (wet weight 1.6 kg, dry weight 576 g) were minced and extracted with ethyl acetate (EtOAc). The extract (12.5 g) was separated by silica gel and eluted using a mixture of *n*-hexane/EtOAc in a stepwise fashion from 100:1 to pure EtOAc to yield 11 fractions A–K. Fraction E was purified by NP-HPLC, using a mixture of dichloromethane and methanol (90:1, flow rate: 2.0 mL/min) to yield 26 subfractions E1–E26. Fraction E20 was repurified by NP-HPLC, using a mixture of *n*-hexane and acetone (10:1, flow rate: 2.0 mL/min) to yield 9 subfractions E20A–E20I. Fraction E20G was separated by RP-HPLC, using a mixture of methanol and water (83:17, flow rate: 1.0 mL/min) to yield 5-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)-2-methyl-pentanoic acid methyl ester (**1**, 6.2 mg, t_R = 27 min) as a colorless oil; IR (neat) ν_{max} 3450, 1727 cm^{-1}; ^{1}H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data, see Table 1; ESIMS: *m/z* 357 $(M+Na)^+$; HRESIMS: *m/z* 357.20365 (calcd for $C_{20}H_{30}O_4Na$, 357.20363).

Generation of Superoxide Anions by Human Neutrophils

Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Measurements of superoxide anion generation were carried to previously described procedures [6,7]. Briefly, superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*. LY294002 (2-morpholin-4-yl-8-phenylchromen-4-one), a phosphatidylinositol-3-kinase inhibitor, was used as a positive control (IC_{50} = 4.00 μM) for superoxide anion generation.

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Author Contributions

Ping-Jyun Sung designed the whole experiment and contributed to manuscript preparation. Kuan-Hua Chen researched data and wrote the mansucript. Chang-Feng Dai and Tsong-Long Hwang analyzed the data and performed data acquisition. All authors read and approved the finnal manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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