A New Bis-γ-pyrone Polypropionate of Onchidiol Family from Marine Pulmonate Mollusk *Onchidium* sp.

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A new bis-γ-pyrone polypropionate, 4,16-di-*epi*-onchidiol (**1**), along with three known related compounds (**2**–**4**) were isolated from the marine pulmonate mollusk *Onchidium* sp. The structure of compound **1** was elucidated by extensive spectroscopic analysis and by comparison the NMR data with its stereoisomers **2**–**4**, whereas its absolute configuration was determined by the combination of X-ray diffraction analysis and TDDFT-ECD calculation. In bioassay, the isolated compounds exhibited broad cytotoxicity against several cancer cell lines with IC_{50} values ranging from 24.6 to 88.5 μM.

Keywords: bis-γ-pyrone polypropionate; X-ray diffraction; absolute configuration; cytotoxicity; marine natural product; marine pulmonated mollusk

1. Introduction

Polypropionates of C_{32} carbon skeleton, with two γ-pyrone rings and several contiguous chiral centers, are a family of marine natural products (MNPs) widely distributed in the marine mollusks (Gavagnin et al. 1997), especially of the genus *Onchidium* (Davies et al. 1998). The characteristic secondary metabolites were reported to play an important role in the chemical defense of the mollusks against their enemies (Guo et al. 2012), which enable them to possess broad biological activities, such as antioxident, antiviral and antitumor (Chakraborty et al. 2018, Carbone et al. 2009, Wang et al. 2012, Carbone et al. 2013, Zhou et al. 2018, Jaime et al. 1992). However, since there are many chiral centers in polypropionates and their structures are highly conformationally flexible, the determination of the absolute configuration is of great challenge (Darias et al. 2006).

In our previous study, the marine pulmonate *Onchidium* sp. has been proven to be a rich source of polypropionates, and many of them showed interesting bioactivities, such as significant activation effects on the XBP1 gene expression to inhibit the growth of tumors (Zhou et al. 2018). In order to accumulate the amount of the bioactive

polyprionates for more-in-depth pharmacological study, and to evaluate the influence of growing period on the variation of second metabolites in the mollusk *Onchidium* sp., we have continued a further chemical study of the same mollusk collected at the intertidal zone along the coast of Hainan situated in the South China Sea, while with a much smaller size of the previous investigated ones. The detailed chemistry investigation of *Onchidium* sp. resulted in the isolation of one new and three known bis-γ-pyrone polypropionates (**1**–**4**) of onchidiol family. Herein, we report the isolation, structure elucidation, and bioactivity evaluation of the above compounds.

2. Results and Discussion

The Et_2O-soluble portion (3.0 g) of the acetone extract was performed by a combination of silica gel column chromatography, Sephadex LH-20 column chromatography, and HPLC to yield the polypropionates **1**–**4**. Among them, the known compounds were rapidly identified by the comparison of their spectroscopic data with those reported in the literature to be onchidiol (**2**) (Carbone et al. 2009, Wang et al. 2012), 4-*epi*-onchidiol (**3**) (Wang et al. 2012) and 16-*epi*-onchidiol (**4**) (Carbone et al. 2009, Wang et al. 2012), respectively (Figure 1).

Compound **1** was isolated as a colorless crystal. Its molecular formula, $C_{32}H_{46}O_8$, was deduced by HR-ESI-MS peak at *m/z* 559.3273 $[M+H]^+$, indicating 10 degrees of unsaturation. The IR absorption at 1723 cm^{-1} suggested the presence of carbonyl; The UV spectrum at λ_{max} 260 nm and IR absorption at 1684 cm^{-1} suggested the presence of the α,β-unsaturated-γ-pyrone moiety. The ^{13}C NMR data of **1**, in combination with the DEPT and HSQC spectra, indicated 32 carbon resonances, including eleven sp^3 methyls, two sp^3 methylenes, seven sp^3 methines, one sp^3 quaternary carbon, and eleven sp^2 quaternary carbons. The diagnostic ^1H and ^{13}C NMR resonances, disclosed the presence of three carbonyl carbons (δ_C 207.5, 179.8, 180.2), four tetra-substituted double bonds (δ_C: 160.9/119.6, C, C-5/C-6; 119.1/165.5, C, C-8/C-9; 163.5/119.9, C, C-17/C-18; 118.2/164.9, C, C-20/C-21). The above unsaturated groups accounted for seven degrees of unsaturation; the remaining three degrees should be ascribed to a tricyclic system. In fact, the spectroscopic data of **1** were reminiscent of the co-occurring known

polypropionates **2**–**4**, which all comprised the same C_{32} carbon skeleton, indicating **1** of the same onchidiol skeleton. Detailed comparison of the ^{13}C NMR data of **1** and 4-*epi*-onchidiol (**3**) showed that the only differences between them occurred in chemical shifts of C-14, C-15, C-16, C-17, C-18, C-30 ($\delta_{C\text{-}14}$: 33.8 in **1** vs. 32.4 in **3**; $\delta_{C\text{-}15}$: 100.6 in **1** vs. 102.7 in **3**; $\delta_{C\text{-}16}$: 43.6 in **1** vs. 42.5 in **3**; $\delta_{C\text{-}17}$: 163.5 in **1** vs. 161.0 in **3**; $\delta_{C\text{-}18}$: 119.9 in **1** vs. 121.5 in **3**; $\delta_{C\text{-}30}$: 11.2 in **1** vs. 12.3 in **3**) (Table S1), suggesting that compound **1** should be an epimer of **3** with possibly different configurations at C-14, C-15 or C-16 positions. Further detailed analysis of 2D NMR experiments, including ^{1}H-^{1}H COSY, and HMBC (Figure S1), confirmed that compound **1** shared the same planar structure as **3**.

The relative configuration around the saturated oxygen-containing six-membered ring were deduced based on the NOESY cross-peaks indicated in Figure S1. In particular, the clear correlations between H-11 and H-12, H-12 and H_3-29, indicated the same orientation of H-11, H-12 and CH_3-29, arbitrarily assigned as β. Whereas the NOE cross-peaks between H-13 and H-14, H-13 and H_3-28, suggested the α-orientation of H-13, H-14 and CH_3-28. However, due to the conformational flexibility of the molecule, the relative configuration of the chiral centers C-4, C-10 and C-16 could not be addressed by NOESY experiment.

Fortunately, after several attempts, we successfully obtained the single crystal of **1**, The crystal of **1** was submitted to diffraction analysis using graphite-monochromated Ga K_α radiation (λ = 1.34139). As shown in Figure S2, the X-ray structure confirm the deduced planar structure of **1**. Further, since the Flack parameter of 0.34 (15) of the X-ray structure is not small enough to certainly determine the absolute configuration of **1**, the X-ray diffraction (XRD) analysis can only assign its relative configuration as 4*S**, 10*R**, 11*R**, 12*S**, 13*S**, 14*S**, 15*S**, 16*R**.

The absolute configuration of compound **1** was finally determined by the time-dependent density functional theory electronic circular dichroism (TDDFT-ECD) calculation, since this method was proved to be a reliable and powerful tool for the determination of the Absolute configurations of chiral natural products (Ye et al. 2018). As shown in Figure S3, the experimental ECD spectrum (MeCN) of compound **1**

displayed a negative absorption effect at 234 nm and a positive absorption effect at 285 nm, which highly matched to the calculated ECD of (4*S*, 10*R*, 11*R*, 12*S*, 13*S*, 14*S*, 15*S*, 16R)-**1.** Consequently, the absolute configuration of all chiral carbons of **1** was determined to be 4*S*, 10*R*, 11*R*, 12*S*, 13*S*, 14*S*, 15*S*,16*R*, and its structure was drawn as shown in Figure 1.

All the isolated compounds were evaluated for their cytotoxicity by the sulforhodamine B (SRB) method in 96-well plates, using four human cell lines: human lung cancer cell line A549, human colon cancer cell line HT-29, human pancreatic cancer cell line Capan-1 and human hepatocellular carcinoma cell line SNU-398, with vincristine (VCR) as positive control. As shown in Table S2, the three known compounds displayed moderate to weak cytotoxicity against all the four human cell lines, while the new compound **1** showed even less activity against all tested cell lines (IC_{50} > 100 μM). Interestingly, compounds **3** (25.3 μM) and **4** (24.7 μM) displayed the strongest cytotoxicity against A549 compared with SNU-398, Capan-1 and HT-29, indicated a selectivity of these two compounds on A549 cells. In addition, the different activity of all the four stereoisomers further proved the impact of stereochemistry on the biological activities. In addition, the other biological studies, such as immunosuppressive activity and PTP 1B inhibitory activity of these compounds are still in progress.

4,16-di-*epi*-onchidiol: Colorless crystals; m.p. 190–191 ℃; $[\alpha]^{20}_{,D}$ + 1.3 (*c* 0.4, $CHCl_3$); UV (MeOH): λ_{max} (log ε) 260 (4.4) nm; IR (KBr): ν_{max} 3390, 3357, 2962, 2921, 2851, 1723, 1648, 1592, 1456, 1379, 1260, 1093, 799 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ_H : 1.03 (3H, t, *J* = 7.2 Hz, H-1), 2.43 (2H, m, H-2), 3.91 (1H, q, *J* = 7.2 Hz, H-4), 3.17 (1H, m, H-10), 4.40 (1H, dd, *J* = 10.4, 2.0 Hz, H-11), 1.96 (1H, m, H-12), 3.74 (1H, br d, *J* = 3.2 Hz, H-13), 1.58 (1H, m, H-14), 3.16 (1H, m, H-16), 2.63 (1H, m, H-22), 2.47 (1H, m, H-22), 1.19 (3H, t, *J* = 7.6 Hz, H-23), 1.47 (3H, d, *J* = 6.8 Hz, H-24), 2.04 (3H, s, H-25), 2.01 (3H, s, H-26), 1.05 (3H, d, *J* = 7.2 Hz, H-27), 0.90 (3H, d, *J* = 7.2 Hz, H-28), 1.00 (3H, d, *J* = 6.8 Hz, H-29), 0.91 (3H, d, *J* = 7.2 Hz, H-30), 1.95 (3H, s, H-31), 1.92 (3H, s, H-32); and ^{13}C NMR (100 MHz, $CDCl_3$) δ_C : 8.0 (C-1), 34.5 (C-2), 207.5

(C-3), 48.7 (C-4), 160.9 (C-5), 119.6 (C-6), 179.8 (C-7), 119.1 (C-8), 165.5 (C-9), 37.1 (C-10), 67.4 (C-11), 36.3 (C-12), 77.0 (C-13), 33.8 (C-14), 100.6 (C-15), 43.6 (C-16), 163.5 (C-17), 119.9 (C-18), 180.2 (C-19), 118.2 (C-20), 164.9 (C-21), 25.3 (C-22), 12.0 (C-23), 13.7 (C-24), 9.7 (C-25), 9.7 (C-26), 14.1 (C-27), 10.7 (C-28), 12.8 (C-29), 11.2 (C-30), 10.0 (C-31), 10.1 (C-32); HR-ESI-MS *m/z* 559.3273 $[M + H]^+$ (calcd for $C_{32}H_{47}O_8$, 559.3265).

3. Conclusions

In summary, further chemical investigation of the marine pulmonate *Onchidium* sp. resulted in the discovery of four bis-γ-pyrone polypropionate of onchidiol family, including a new one (**1**). The absolute configuration of **1** was determined by the combination of XRD analysis and TDDFT-ECD calculation. Further *in vitro* study revealed that compounds **2**, **3** and **4** exhibited weak cytotoxicity against several cancer cell lines with different inhibition rate varied by different compounds and different cells, which not only indicated the selectivity of the compounds on different cells, but also proved the impact of stereochemistry on the biological activity. Intriguingly, as shown in Figure S4, over comparison of our *Onchidium* sp. with the previous investigated ones, we could obviously find that the main difference on the chemical components of the two animals of different size was that the polypropionates from the big size *Onchidium* sp. were mainly esterified at C-13 position (Zhou et al. 2018), which inspired us to make the proposal that the polypropionates could be esterified during the growth of *Onchidium* sp. to possibly strengthen their chemically defensive weapons, leading to the chemical diversity and broader biological activity of these metabolites. Further biological activity comparison of these different polypropionates should be conducted to verify our hypothesis.

Supplementary materials

Supplementary data associated with this article is available online, alongside Experimental, Tables S1-S3, Figures S1-S16.

Disclosure statement

The authors declare no potential conflicts of interest.

Funding

This work was supported by the Natural Science Foundation of China [grant number 81520108028]; the NSFC/CNRS joint project [grant number 81811530284]; NSFC-Shandong Joint Fund for Marine Science Research Centers [grant number U1606403]; the SKLDR/SIMM Project [grant number SIMM1705ZZ-01].

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Graphical Abstract

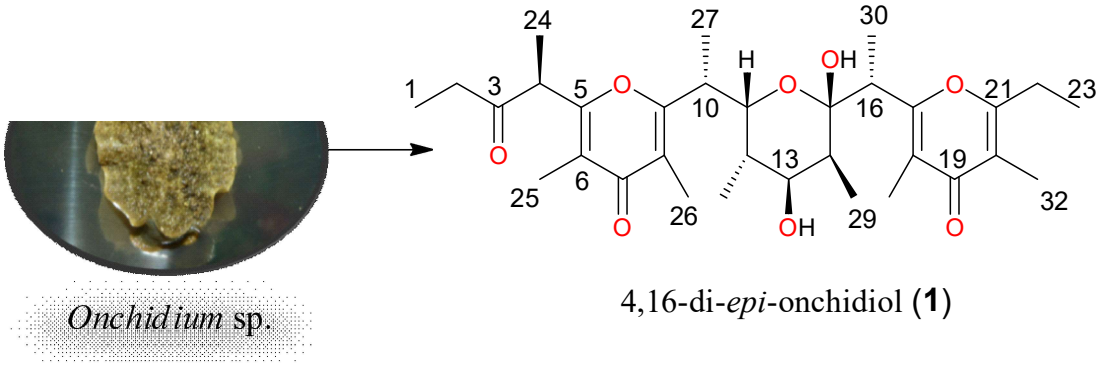

Figure 1. Structures of compounds **1**–**4**.