Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Marine Bis-γ-pyrone Polypropionates of Onchidione Family and their effects on the XBP1 gene expression

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ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
pulmonate
polypropionates
absolute configuration
XBP1

ABSTRACT

Two additional new members of the onchidione family, 16-*epi*-onchidione (**1**) and 4-*epi*-onchidione (**2**), co-occurring with six previously reported bis-γ-pyrone polypropionates including onchidione (**3**), were isolated from the marine pulmonate *Onchidium* sp. Their structures were determined by extensive spectroscopic analysis and by comparison with **3** and onchidione-related derivatives. The absolute configuration of **1** was established by X-ray diffraction analysis employing graphite monochromated Cu K_α radiation (λ = 0.71073 Å) with small Flack parameter 0.08. In addition, the absolute stereochemistry of previously reported onchidionol (**6**) was confirmed by the X-ray diffraction analysis. Some of the isolated compounds showed significant activation effects on the splicing of XBP1 mRNA as ER stress modulators to inhibit the growth of tumors.

Marine pulmonate mollusks belonging to the family Onchidiidae are a prolific source of polypropionates possessing a C_{32} carbon skeleton with two γ-pyrone rings and several contiguous stereogenic centers.[1] These polypropionates include either linear members (i.e. ilikonapyrones)[2–4] or compounds exhibiting an additional hemiketal pyrone ring in the middle part of the polypropionate chain (i.e. onchidione and onchidionols).[4–6] Promising *in vitro* growth-inhibitory activity against cancer cell lines have been reported for a series of these polypropionates.[4,7,8]

XBP1 (X-box binding protein-1) is a key transcription factor that regulates ER (endoplasmic reticulum) homeostasis, it is essential for the anti-oxidant defense and cell survival and is required for tumor growth.[9] High expression of XBP1 has been reported for many kinds of cancers, such as breast neoplasms, pancreatic cancers and so on. Due to this, XBP1 is considered a new target for molecular treatment of cancers.[10]

In the framework of our ongoing research on marine organisms focused on the search of new anticancer compounds,[11–15] we recently investigated pulmonate mollusks of the genus *Onchidium* sp.[4–6] Due to the interesting bioactivity showed by *Onchidium* polypropionates, we have examined a further population of the mollusk collected from the intertidal zone along the coast of Hainan situated in the South China Sea, with the aim of isolating new structural analogs to be evaluated for the effects on the splicing of XBP1 gene.

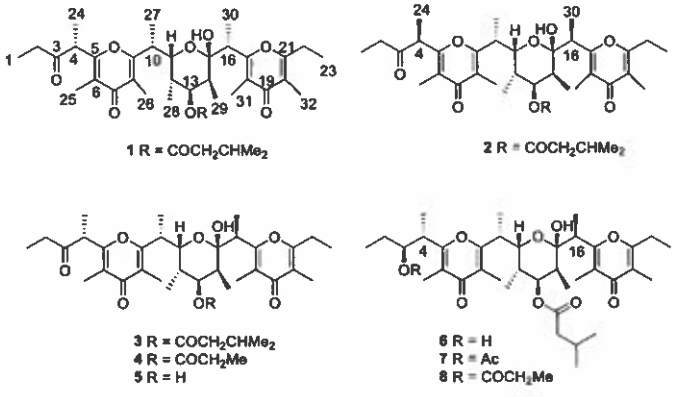

Fig. 1. Structures of compounds **1–8**

The chemical analysis of the lipophilic extract of external parts of the mollusk led to the isolation of two new members of the onchidione family, 16-*epi*-onchidione (**1**) and 4-*epi*-onchidione (**2**), together with previously reported compounds **3–8** (Fig. 1). In this paper, we describe the chemical characterization of compounds **1** and **2**, the assignment of the

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absolute configuration of compound **6**, and the bioactivity evaluation of all isolated metabolites.

The extraction of *Onchidium* sp. specimens was performed according to our previous work.[4–6] Silica gel column and HPLC purification of selected fractions from the Et_2O-soluble portion of the acetone extract yielded the polypropionate derivatives **1–8**. Among them, the known compounds were readily identified by spectroscopic data as onchidione (**3**),[5,6] 13-propanoyl-onchidiol (**4**),[4] onchidiol (**5**),[6] onchidionol (**6**),[4] 3-acetylonchidionol (**7**),[4] and 3-propanoylonchidionol (**8**).[4]

16-*Epi*-onchidione (**1**) was isolated as a colorless crystal. The molecular formula $C_{37}H_{54}O_9$, the same as onchidione (**3**), was deduced by HRESIMS data. Analysis of NMR spectra (Table 1) clearly indicated the same polypropionate nature as the co-occurring known metabolites. In particular, the ^1H NMR spectrum contained thirteen signals due to methyl groups, four singlets at δ 1.96 (H_3-25), 1.98 (H_3-32), 1.99 (H_3-31), and 2.15 (H_3-26), which were attributed to the methyls in β position of γ-pyrone rings, two triplets due to terminal methyls at δ 1.00 (J=7.2 Hz, H_3-1) and 0.92 (J=7.2 Hz, H_3-23), and the remaining seven doublets resonating between δ 0.61 and δ 1.16, assigned to five methyls of the polypropionate chain and to the isopropyl methyls of the acyl residue at C-13 (Table 1). The ^{13}C NMR spectrum displayed carbon resonances consistent with the C_{32} polypropionate onchidione-like skeleton[4–6] containing two substituted γ-pyrone rings, a ketone, a hemiketal oxane ring and a 3-methyl butanoyl residue esterifying 13-OH (Table 1). Detailed analysis of 2D NMR experiments, which were recorded in C_6D_6 (Table 1) and $CDCl_3$ (Suppl. Material) revealed that compound **1** had the same planar structure as onchidione[5,6] and differed with the latter one only in their stereochemical aspects. Analysis of the proton coupling constants and NOE effects of **1** indicated that the relative configuration of the substituents at the hemiketal oxane ring was the same as **3**[5,6] and related derivatives.[4] In particular, the esterified hydroxyl group at C-13 was deduced to be axial by the coupling constants of the geminal proton H-13 (dd, J=3.0 and 3.0 Hz) which was equatorial. Significant steric effects were observed between the methyl at C-12 and H-14, and between 15-OH and H-11 thus suggesting for all the axial orientation. Thus, differences were assumed to be in the configuration of one or more stereogenic centers in the chain.

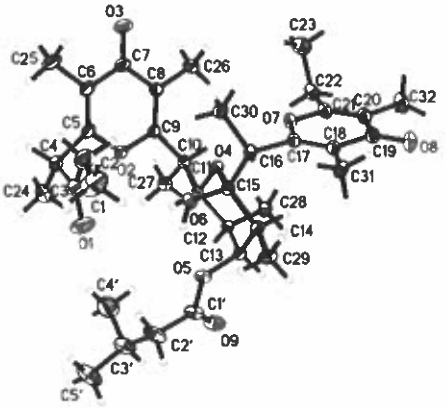

Figure 2. X-ray crystallographic structure for compound **1**

Table 1
^{13}C and ^1H NMR data of compound **1** in C_6D_6

Position	$\delta^{13}C$	δ^1H	m (J in Hz)	Long range correlations	Position	$\delta^{13}C$	δ^1H	m (J in Hz)	Long range correlations
1	7.8	1.00	t, 7.2	—	20	118.3	—	—	—
2a	34.7	2.34	m	—	21	163.9	—	—	H-22, H-23, H-32
2b		2.03	m	—	22a	25.0	2.24	m	—
3	207.7	—	—	H_3-1, H_3-2, H_3-24, H_4-4	22b		2.11	m	—
4	48.1	3.33	q, 7.2		23	11.8	0.92	t, 7.2	—
5	159.4	—	—	H_3-24, H_3-25	24	13.1	1.16	d, 7.2	—
6	119.6	—	—	—	25	10.8,	1.96	s	—
7	178.7	—	—	—	26	9.8	2.15	s	—
8	119.4	—	—	—	27	13.4	0.75	d, 6.6	—
9	164.1	—	—	H-11, H_3-27	28	9.3	0.61	d, 7.2	—
10	37.1	2.89	dq, 7.2, 10.8		29	12.4	0.80	d, 7.2	—
11	68.2	4.22	dd, 2.4, 10.8	H-10, H-13, H_3-27, H_3-28	30	11.2	1.07	d, 6.6	—
12	33.5	1.87	m	H-11	31	9.9	1.99	s	—
13	77.5	4.82	dd, 3.0, 3.0	H-10, H_3-28, H_3-29	32	10.2	1.98	s	—
14	33.4	1.61	dq, 3.0, 7.2		1′	171.5	—	—	H-13, H-2′
15	99.6	—	—	H-11, H-13, H-16, H-29, H-30	2′a	43.7	2.25	m	—
16	43.7	3.10	q, 6.6		2′b		3.10	m	—
17	162.5	—	—	H-16, H-30, H-31	3′	26.4	2.24	m	—
18	120.2	—	—	—	4′	22.4	1.02	d, 6.6	—
19	179.1	—	—	—	5′	22.4	0.98	d, 6.6	—
15-OH		4.09	s						

Bruker-DRX-600 spectrometer (600 MHz for ^1H and 150 MHz for ^{13}C NMR) in C_6D_6, chemical shifts (ppm) referred to C_6H_6 (δ_H 7.16) and to C_6D_6 (δ_C 128.1).
The assignments were based on ^1H-^1H COSY, HSQC, HMBC experiments.

In order to clarify these stereochemical aspects and determine the absolute configuration of **1**, a suitable crystal of **1**, which was obtained by careful crystallization from *n*-hexane/H_2O, was submitted to X-ray diffraction analysis. On the basis of the eight oxygen atoms, the final refinement on the Cu K_α data resulted in a Flack parameter of 0.08, allowing unambiguous assignment of the absolute configuration of **1** as shown in Fig. 2. Thus, compound **1** was determined to be the C-16 epimer of onchidione (**3**).

Compound **2** was isolated as a colorless gum. The molecular formula, $C_{37}H_{54}O_9$, the same as **1** and **3**, was established by HRESIMS. The ^1H and ^{13}C NMR spectra (C_6D_6 data in Table 2, $CDCl_3$ data in Suppl. Material) showed great similarities with those of co-occurring **1** and **3**, indicating they shared the same planar structure. Again, the relative orientation of the substituents in the hemiketal ring was suggested to be the same as onchidione (**3**)[5,6] and all previously reported related derivatives[4] by inspection of the proton coupling constants and NOESY experiments recorded in both solvents. In particular, H-13 resonating as a double doublet (J = 3.0, 3.0 Hz) was assumed to be equatorial whereas the methyl at C-12 and H-14 were suggested to be axial by the steric effects observed in the NOESY experiment. NOE effects were also showed by H-11 and the hydroxyl at C-15 according to their axial orientation. Also in this case, the structural differences of **2** with respect to **3** were thus ascribed to the relative configuration of one or more stereogenic centers in the chain. Detailed analysis of proton and carbon resonances of **2** in comparison with those of **3** revealed that the NMR assignment of the part of the molecule including C-10 and C-16 centers substantially resembled onchidione (**3**) whereas significant differences were observed in the chemical shift values of C-1/C-4 fragment. In particular, this set of signals were very similar with those of 4-*epi*-onchidiol.[5,6] Thus, compound **2** was proposed to be 4-*epi*-onchidione. Further, the similar CD profiles of **2** with those of 4-*epi*-onchidiol[6] suggested they have the same absolute configuration (Fig. 3).

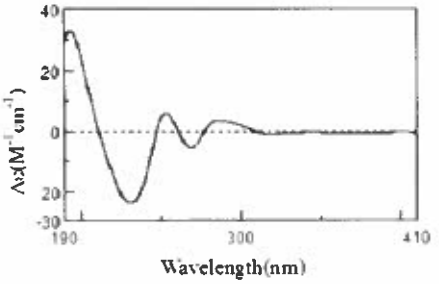

Figure 3. CD spectrum of compound **2**

Table 2
^{13}C and ^1H NMR data of compound **2** in C_6D_6

Position	$\delta^{13}C$	δ^1H	m (J in Hz)	Long range correlations[d]	Position	$\delta^{13}C$	δ^1H	m (J in Hz)	Long range correlations[d]
1	7.9	0.78	t, 7.2	—	20	117.9	—	—	—
2a	34.4	1.63	m	—	21	163.9	—	—	H-22, H-23, H-32
2b		1.78	m	—	22a	25.0	2.49	m	—
3	205.9	—	—	H_3-1, H_3-2, H_3-24, H-4	22b		2.14	m	—
4	48.1	3.22	q, 6.6		23	11.3	0.92	t, 7.2	—
5	160.8	—	—	H_3-24, H_3-25	24	13.0	1.26	d, 7.2	—
6	119.5	—	—	—	25	9.7	1.97	s	—
7	178.7,	—	—	—	26	9.4	1.97	s	—
8	118.1,	—	—	—	27	14.3	0.56	d, 7.2	—
9	164.5,	—	—	H-11, H_3-27	28	9.3	0.68	d, 7.2	—
10	36.8	2.74	dq, 6.6,11.2		29	12.2	0.81	d, 7.2	—
11	66.6	4.17	dd, 1.8, 11.2	H-10, H-13, H_3-27, H_3-28	30	11.7	0.98	d, 7.0	—
12	33.3,	1.81	m	H-11	31	12.0	2.00	s	—
13	78.0	4.90	dd, 3.0, 3.0	H-10, H_3-28, H_3-29	32	9.7	2.07	s	—
14	31.5	1.87	m		1'	171.4	—	—	H-2', H-4', H-5'
15	101.9	—	—	H-11, H-13, H-16, H-29, H-30	2'a	43.4	2.25	m	—
16	41.9	3.08	q, 7.2		2'b		2.19	m	—
17	160.6	—	—	H-16, H-30, H-31	3'	26.3	2.20	m	—
18	121.7	—	—	—	4'	22.3	0.98	d, 7.0	—
19	179.1	—	—	—	5'	22.3	0.96	d, 7.0	—
15-OH		4.15	s						

Bruker-DRX-600 spectrometer (600 MHz for ^1H and 150 MHz for ^{13}C NMR) in C_6D_6, chemical shifts (ppm) referred to C_6H_6 (δ_H 7.16) and to C_6D_6 (δ_C 128.1).
The assignments were based on ^1H-^1H COSY, HSQC, HMBC experiments

Finally, the absolute configuration of co-occurring onchidionol (**6**), that was previously suggested by chemical correlation with onchidione (**3**)[4] has been further confirmed in this work by an X-ray diffraction study. A suitable crystal of **6** was obtained by careful crystallization from petroleum ether/methanol and submitted to diffraction analysis employing graphite monochromated Cu K_α radiation (λ = 0.71073 Å) with small Flack parameter 0.0(2). The X-ray analysis indicated the absolute configuration as reported in structure **6**. This was in

agreement with the previous assignment, which was made by comparison of the CD profile of the alcohol **6** obtained by reduction of onchidione (**3**) with that of the naturally occurring onchidionol.[4]

All isolated compounds were evaluated for the effects on the splicing of XBP1 mRNA, XBP1 is an important regulator of a subset of genes related to the tumor growth. Compounds **2**, **3**, and **7** showed significant activation on the splicing of the XBP1 mRNA, with activity (%) of 217.8%, 210.2%, 233.6%, respectively (two folds than the blank control DMSO), at the concentration of 10 μg/mL, and the activity (%) of positive control Tunicamycin is 178.4% at the concentration of 1 μg/mL.

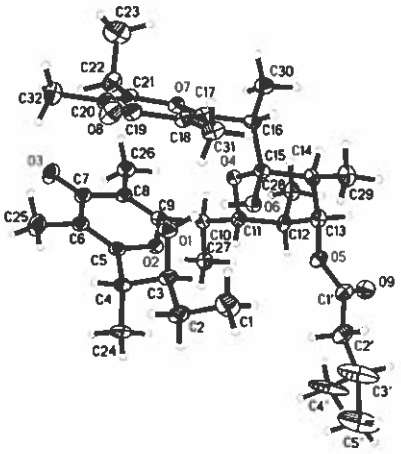

Figure 4. X-ray crystallographic structure for compound **6**

Based on the test of all these compounds, a preliminary structure-activity relationships were discussed. For example, all the bioactive compounds **2**, **3** and **7** share specific structural features including a 3-methylbutanoyl group esterifying 13-OH of the hemiketal oxane ring as well as the 16*S* configuration suggesting that the presence of both features should be relevant for the activity. On the other side, they differ in the C-1/C-4 fragment displaying either opposite configuration at C-4 or reduced C-3 carbonyl group. Onchidione (**3**) and 4-*epi*-onchidione (**2**) show comparable inhibition properties thus the activity seems to be not affected by the configuration of C-4. 3-Acetyl-onchidionol (**7**) is the most active compound suggesting that the reduction of the carbonyl at C-3 has a positive effect on the inhibitory activity. However, the closely related onchidionol (**6**) and 3-propanoyl-onchidionol (**8**) also having the –CHOR at C-3 are inactive. This could indicate that the functionalization at C-3 is important for the pharmacophore interaction because loss of the activity is observed in the presence of the free hydroxyl as well as in the presence of an acyl group with a longer chain with respect to the acetyl. However, it should be stressed that tested compounds represent a small collection of naturally occurring products. A larger number of natural and/or synthetic analogs should be considered for an exhaustive SAR study.

In conclusion, this work extends the record of bis-γ-pyrone polypropionates from marine pulmonate mollusks and gives further support to their structure elucidation. Besides, the activation effects of the active natural products on the expression of the XBP1 gene has provided a clue for the further chemical and pharmacological studies to have more evidence on their potential on anti-tumor drug discovery.

Acknowledgments

This research work was financially supported by the NSFC key international collaboration project (No. 81520108028), other NSFC projects (Nos. 21672230, 41506187, 41476063, 41676073, 81603022, 81473244), SCTSM Project (Nos. 14431901100, 15431901000), the SKLDR/SIMM Projects (SIMM 1705ZZ-01), and by a grant from MIUR-ITALY PRIN2015 (Project No. 2015MSCKCE_003).

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Supplementary Material

Supplementary data related to this article can be found, in the online version, at doi:#.

Graphical Abstract.

Marine Bis-γ-pyrone Polypropionates of Onchidione Family and their effects on the XBP1 Gene Expression

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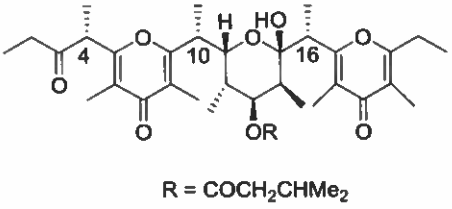

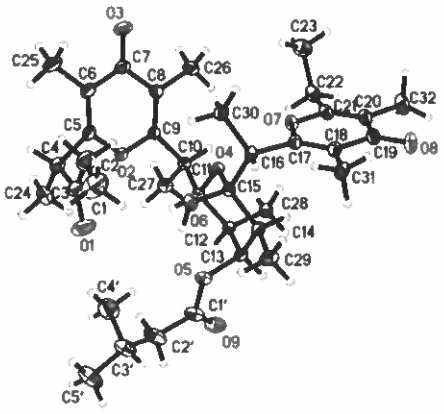

Structure and X-ray crystallographic structure for 16-*epi*-onchidione (compound **1**).

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