Tetrahedron 65 (2009) 4404–4409

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Structure of onchidione, a bis-γ-pyrone polypropionate from a marine pulmonate mollusk

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ARTICLE INFO

Article history:
Received 2 December 2008
Received in revised form 5 March 2009
Accepted 19 March 2009
Available online 27 March 2009

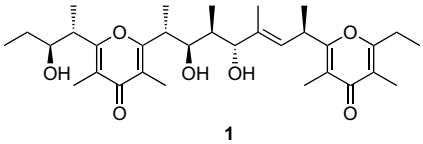
Keywords:
Marine natural products
Polypropionates
Pyrones
Pulmonata
X-ray analysis

ABSTRACT

The chemical investigation of the marine pulmonate *Onchidium* sp. and of its defensive mucous secretion led us to the isolation of a novel polypropionate, onchidione (**4**), containing two γ-pyrone rings. The structure of **4** was elucidated by spectroscopic methods, mainly NMR techniques, and secured by X-ray analysis on a single crystal.

1. Introduction

Marine pulmonates of the family Onchidiidae are shell-less mollusks living on sheltered intertidal shores.[1] Previous studies on distinct species of this family have reported mostly polypropionates exhibiting a carbon skeleton with two γ-pyrone rings and several asymmetric centers.[2] Representative molecules belonging to this group are ilikonapyrone (**1**) from *Onchidium verruculatum*,[3,4] onchitriols (e.g., onchitriol II, **2**) from an *Onchidium* sp.,[5,6] and peronatriols (e.g., peronatriol I, **3**) from *Peronia peronii*.[7,4] The presence of a large number of contiguous stereogenic centers in these molecules has generated many problems with stereochemical assignments but it has also constituted an intriguing target for synthetic studies.[8] Some of them led to the revision of suggested stereochemistries and also to the determination of the absolute configuration.[4,6]



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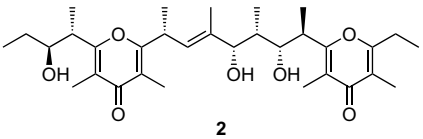

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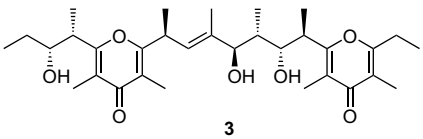

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In the course of our ongoing research on marine gastropod mollusks that has been mainly focused on species of the subclass Opisthobranchia,[9–11] we have analyzed a population of an

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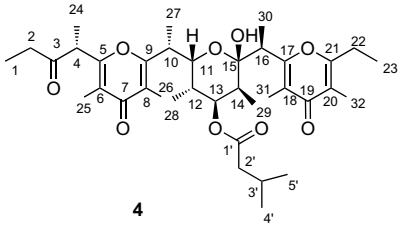
doi:10.1016/j.tet.2009.03.052

Onchidium sp. of the subclass Pulmonata. The animals were collected in the intertidal zone along the coast of Hainan, in the South China Sea, during the Summer of 2004. The chemical investigation of both the mucus and the external part of the mollusk has led to the isolation of a main metabolite, an unprecedented bis-γ-pyrone polypropionate, onchidione (**4**), the structure elucidation of which including the assignment of the relative stereochemistry by X-ray analysis is described here.



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2. Results and discussion

The diethyl ether soluble portion of the acetone extracts from both the external part (by ultrasound) and the internal part of *Onchidium* sp. individuals was analyzed by TLC in comparison with the ether extract from the mucus. A main UV–visible spot (R_f 0.45–0.30, $CHCl_3$/MeOH, 95:5) was detected in both the external part and the mucous secretion whereas it was absent in the internal part. The ether extract of the external part was fractionated and the major component, onchidione (**4**), was isolated as the pure compound. Onchidione (**4**) was also almost the only metabolite present in the mucus extract as revealed by direct ^{1}H NMR analysis.

The molecular formula $C_{37}H_{54}O_9$ was deduced by the sodiated-molecular ion at 665.3665 *m*/*z* in the HRESI mass spectrum. Eight of the 11 degrees of unsaturation required by the formula were assigned to two γ-pyrone rings as it was indicated by the presence in the ^{13}C NMR spectrum of 10 unprotonated carbon signals (C-5/C-9 and C-17/C-21, Table 1). Two intense infrared bands at 1608 and 1654 cm^{-1}, as well as a strong UV absorption at 261 nm (ε 24,977), confirmed this hypothesis.

In the ^{13}C NMR spectrum of **4** were also present signals attributed to a carbonyl (δ 208.9, C-3), a carboxyl (δ 172.4, C-1′), and a hemiketal carbon (δ 100.4, C-15). In the high-field region 24 sp^3 carbon signals were observed between δ 77.4 and 7.8 (see Table 1) and assigned to 13 CH_3, three CH_2, and eight CH by DEPT sequence. On the basis of these data the remaining three degrees of unsaturation were thus assigned to a ketone function, an ester group, and a ring. The polypropionate nature of the carbon skeleton of compound **4** was strongly suggested by analysis of the ^{1}H NMR spectrum containing 13 signals due to methyl groups, four singlets at

Table 1
NMR[a,b] data of onchidione (**4**)

C	δ ^{13}C ($CDCl_3$)	m[c]	δ ^{1}H ($CDCl_3$)	m, *J* in Hz	δ ^{1}H (C_6D_6)	m, *J* in Hz	Long-range correlations[d]
1	7.8	q	1.05	t, 7	0.95	t, 7	—
2	35.2	t	2.35–2.50	m	1.98–2.20	m	—
3	208.9	s	—	—	—	—	H_3-1, H_2-2, H-4, H_3-24
4	47.8	d	3.92	q, 7	3.26	q, 7	—
5	159.5	s	—	—	—	—	H_3-24, H_3-25
6	119.2	s	—	—	—	—	—
7	179.2	s	—	—	—	—	—
8	118.6	s	—	—	—	—	—
9	165.3	s	—	—	—	—	H-11, H_3-27
10	36.8	d	3.05	m	2.81	m	—
11	66.9	d	4.32	dd, 2, 11	4.32	dd, 2, 11	H-10, H-13, H_3-27, H_3-28
12	33.1	d	1.91	m	1.88–2.05	m	H-11
13	77.4	d	4.87	dd, 3, 3	4.92	dd, 3, 3	H-12, H_3-28, H_3-29
14	32.3	d	2.10	dq, 3, 7	1.88–2.05	m	—
15	100.4	s	—	—	—	—	H-11, H-13, H-16, H_3-30
16	43.6	d	3.23	q, 7	3.10	q, 7	—
17	161.6	s	—	—	—	—	H-16, H_3-30, H_3-31
18	121.3	s	—	—	—	—	—
19	179.6	s	—	—	—	—	—
20	117.4	s	—	—	—	—	—
21	164.6	s	—	—	—	—	H_2-22, H_3-23, H_3-32
22	24.7	t	2.53 2.10–2.25	dq, 7, 15m	2.42 1.98–2.20	dq, 8, 15m	—
23	11.1	q	1.10	t, 7	0.96	t, 8	—
24	13.5	q	1.35	d, 7	1.02	d, 7	—
25	9.7[e]	q	1.93	s	1.86	s	—
26	9.4[e]	q	1.98	s	2.01	s	—
27	14.6	q	n.a.	—	0.68	d, 7	—
28	13.5	q	n.a.	—	0.71	d, 7	—
29	11.4	q	n.a.	—	0.92	d, 7	—
30	12.6	q	1.21	d, 7	1.05	d, 7	—
31	12.1[e]	q	1.94	s	2.17	s	—
32	9.3[e]	q	1.85	s	1.97	s	—
1′	172.4	s	—	—	—	—	H-13, H_2-2′
2′	43.8	t	2.37, 2.29	dd, 7, 14, dd, 7, 14	1.98–2.20	m	—
3′	26.1	d	2.10–2.25	m	1.98–2.20	m	—
4′	22.4	q	0.95–1.01	—	0.92	d, 7	—
5′	22.4	q	0.95–1.01	—	0.96	d, 7	—

[a] Bruker DPX-300, DPX-600, AVANCE 400 MHz; $CDCl_3$, chemical shifts (ppm) referred to C*H*Cl_3 (δ 7.26) and to CDCl_3 (δ 77.0); C_6D_6, chemical shifts (ppm) referred to C_6H_6 (δ 7.15) and to C_6D_6 (δ 128.0).
[b] Assignment by ^{1}H–^{1}H COSY, HSQC, and HMBC.
[c] By DEPT sequence.
[d] HMBC experiment (*J*=10 Hz).
[e] May be interchanged.

δ 1.85 (H_3-32), 1.93 (H_3-25), 1.94 (H_3-31), and 1.98 (H_3-26), which were attributed to the methyls in β position of γ-pyrone rings, two triplets due to terminal methyls at δ 1.05 (J=7 Hz, H_3-1) and 1.10 (J=7 Hz, H_3-23), and the remaining seven doublets resonating between δ 0.95 and δ 1.35, assigned to the methyls of the polypropionate chain. The ^{1}H NMR spectrum of **4** recorded in C_6D_6 (Table 1) was better resolved than in $CDCl_3$, in particular with regards to the methyl region, thus the spectroscopic 2D NMR analysis was carried out also in this solvent.

Six distinct spin systems including two ethyl groups (H_3-1/H_2-2 and H_2-22/H_3-23), two CH–CH_3 moieties (H-4/H_3-24 and H-16/H_3-30), the eight-membered fragment H-10 (H_3-27)/H-14 (H_3-29), and a 3-methylbutyric residue (H-1′/H-5′) were defined by examining ^{1}H–^{1}H COSY and HSQC spectra recorded in both $CDCl_3$ and C_6D_6 (Table 1). Analysis of HMBC experiments (see relevant correlations in Table 1) allowed the connection of all partial moieties leading to the polypropionate structure **4**.

The relative stereochemistry of the substituents at the hemiketal oxane ring was suggested by analysis of proton coupling constants as well as of NOE effects. 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 and 3 Hz), which was equatorial. Significant steric effects were observed between the methyl at C-12 and H-14, and between the hydroxyl group at C-15 and H-11 thus suggesting for all the axial orientations.

With the aim at confirming the structure and also at establishing the stereochemistry of the chiral centers C-4, C-10, and C-16, a suitable single crystal, obtained by careful crystallization from *n*-hexane/water, was used for an X-ray diffraction study. The final X-ray model of **4** is shown in Figure 1.

The molecule adopts a folded conformation with the two γ-pyrone rings facing each other, stabilized by means of an intramolecular hydrogen bond between the hydroxyl group at C15 and the carbonyl group at C3 [O6···O1=2.789(6) Å, O6–H···O1=160.6°] [Fig. 1(b)]. A water molecule of crystallization acts as a bridge between two molecules, forming a hydrogen bond with the carbonyl group at C7 [OW···O3=2.885(7) Å, OW–H···O3=167.3°]. The tetrahydropyran ring (C11, C12, C13, C14, C15, O4) approximates to an ideal chair with puckering parameters[12] Q=0.575(3) Å, θ=7.4(4)° and φ_2=14.6(5)°. The atomic displacements are 0.707(9) Å for C11, and 0.636(9) Å for C14. The γ-pyrone rings are planar, with rms deviations of 0.014 Å (C5, C6, C7, C8, C9, O2) and 0.006 Å (C17, C18, C19, C20, C21, O7), while the two carbonyl oxygens O3 and O8 are 0.083(9) and 0.079(9) Å out of plane, respectively. The two γ-pyrone rings are almost parallel to each other, with an angle of about 30°. The orientation of these rings can be described by the torsion angles O4–C11–C10–C9 [−58.2(7)°] and C11–C10–C9–O2 [−50.3(7)°] for the first ring, and O4–C15–C16–C17 [73.1(8)°] and C15–C16–C17–O7 [−88.6(8)°] for the second. The geometry of the molecule was in good accord with those reported in the literature for similar compounds.[3,13–16]

This conformation of the molecule exhibits a pseudo-twofold symmetry, which does not differentiate the two planar parts of the molecule, relatively to the intermolecular contacts. Indeed, the obtained crystals are assignable to either the monoclinic or the trigonal crystallographic system, indicating that the package is not governed by the typical intermolecular forces. Moreover, the 3-methylbutyric acid chain linked to the tetrahydropyran ring is characterized by several isoenergetic conformers, that, in the monoclinic form [a=18.956(5) Å, b=11.554(4) Å, c=20.115(6) Å, β=103.06(1)°], displays disorder even at low temperatures (−130 °C). This conformational disorder did not allow us to define with the needed precision the crystalline structure in the monoclinic phase. The peculiarities of the trigonal form can be related to pseudo-twofold symmetry of the molecule. Indeed, the crystals present a single axis of more than 50 Å, as found in crystal structures of macromolecules, that considerably affects data collection at the expense of significance, especially for the presence of a large number of weak reflections. Additionally, the tendency to form molecular layers resulted in germination of most crystals.

Having established the structure and the relative stereochemistry (4*R**, 10*R**, 11*R**, 12*R**, 13*S**, 14*S**, 15*S**, 16*S**) of **4** we tried to determine the absolute configuration by applying the Mosher method on a suitable derivative obtained by methanolysis of **4**. The reaction gave a mixture of alcohol derivatives **5a**–**5b** that were observed to undergo to an interconversion on TLC and also in $CHCl_3$ solution. This transformation led us to hypothesize that an inversion of the configuration at C-15 occurred in the conditions used by opening followed by cyclization of the hemiketal ring. But a careful NMR analysis conducted on the two isolated molecules and in particular some diagnostic NOE difference experiments indicated that the relative stereochemistry of the hemiketal ring was

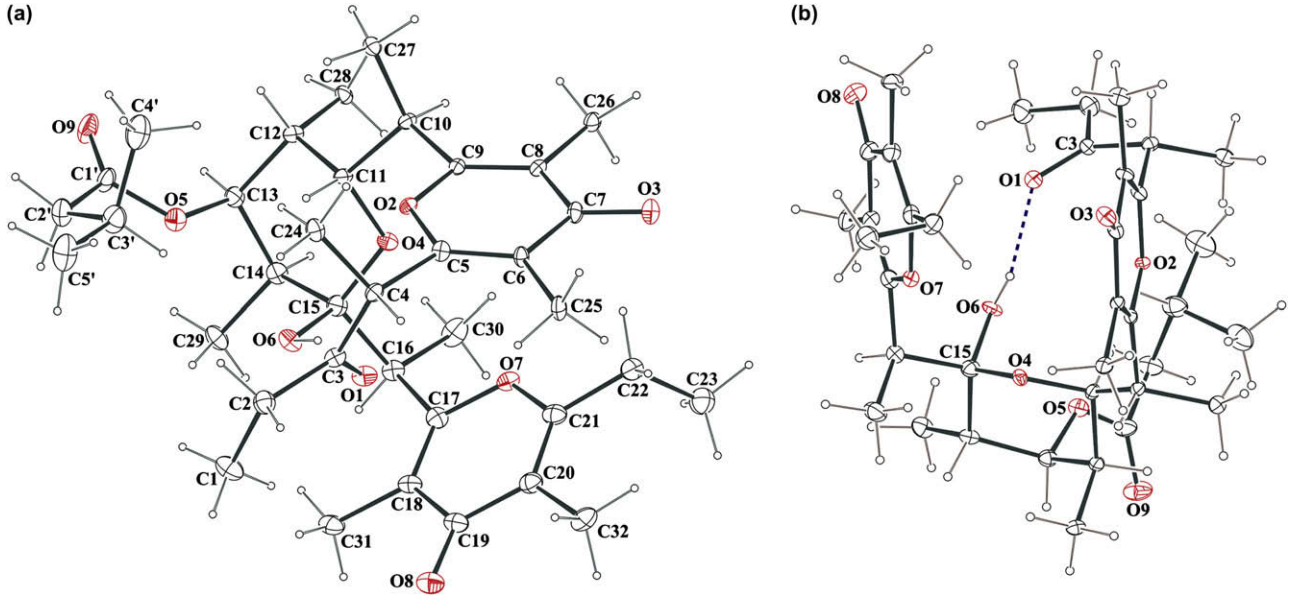
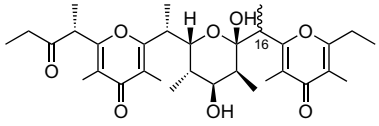


Figure 1. Perspective views of the final X-ray model of **4**—(a) atomic labeling scheme for non-H atoms; (b) view of the intramolecular hydrogen bond O6–H···O1.

the same as onchidione (**4**) for both alcohol derivatives. Thus, according to the isomerization mechanism already reported for denticulatins A and B,[17,18] derivatives **5a** and **5b** were suggested to undergo a racemization at C-16 through a retrohemiketalization via the α,β-unsaturated enolate at C-15. We were not able to assign the relative stereochemistry at C-16 of the two epimers but because this center did not influence the assignment by Mosher method of the absolute configuration at C-13 we decided however to carry out the reaction on both compounds. Unfortunately, most likely due to a strong hydrogen bond involving the 1,3 hydroxyl groups at C-13 and C-15 axially oriented in both **5a** and **5b**, no reaction was observed to occur by using different esterification conditions. Therefore, the absolute stereochemistry remained unassigned. The enantiomer represented in formula **4** has been chosen arbitrarily.



5a-5b

The presence of onchidione (**4**) exclusively in the external part and in the mucus of *Onchidium* sp. led us to suppose its involvement in the chemical defense of the mollusk. This was subsequently demonstrated by feeding deterrence assays. In fact, food treated with pure onchidione (**4**) turned out to be significantly unpalatable to marine generalist shrimps,[19] with a minimum effective dose at 1.0 mg/mL. This finding suggested that the effectiveness of the viscous fluid secretion in repelling predators, previously documented in other onchidiids,[20] would be ascribed to lipophilic metabolites.

Even though biological activities have been reported for bis-γ-pyrones,[3,5] onchidione (**4**) was found to be inactive in the antifungal and antibacterial assays as well as in the antiproliferative tests on PTP1B, P-388, and A-549 cell lines conducted in our laboratory.

3. Experimental section

3.1. General procedures

TLC plates (Merck Silica Gel 60 F254) were used for analytical TLC and Merck Kieselgel 60 was used for preparative column chromatography. HPLC purification was carried out on a Shimadzu apparatus equipped with an LC-10ADVP pump and an UV SPD-10AVP detector by using reverse-phase semi-preparative column (250×10 mm, Phenomenex, Kromasil C_{18}). 1D and 2D NMR spectra were acquired in $CDCl_3$ and C_6D_6 (shifts are referenced to the solvent signal) on a Bruker Avance-400 operating at 400 MHz, using an inverse probe fitted with a gradient along the *Z*-axis and a Bruker DRX-600 operating at 600 MHz, using an inverse TCI CryoProbe fitted with a gradient along the *Z*-axis. ^{13}C NMR spectra were recorded in $CDCl_3$ and C_6D_6 (δ values are reported to the solvent signal) on a Bruker DPX-300 operating at 300 MHz, using a dual probe. Optical rotations were measured on a Jasco DIP 370 digital polarimeter. IR spectra were measured on a Biorad FTS 155 FTIR spectrophotometer. Both ESIMS and HRESIMS spectra were recorded on a Micromass Q-TOF microinstrument.

3.2. Biological material

Onchidium sp. (82 individuals, average size 4 cm) were collected in the intertidal zone along the coast of Lingshui County (Hainan province, China), during August, 2004. A large quantity of mucus (ca. 70 mL) secreted by the animals was recovered *in field* and frozen. The mollusks were separately frozen and both samples were transferred to ICB where they were stored at −20 °C until the extraction. A voucher pulmonate specimen is stored for inspection at ICB (LS-230).

3.3. Isolation procedure

Onchidium specimens were first extracted with acetone by using ultrasound vibration (each 150 mL×3) to get metabolites present in the external part of the mollusk. The organic fraction was evaporated under vacuum, and the resulting aqueous suspension was partitioned between diethyl ether and water. The organic phase was concentrated affording 384.6 mg of crude extract (external part extract). The whole residue of animal was homogenized with a pestle and extracted with acetone (150 mL×3). After removing the organic solvent the aqueous suspension was extracted with diethyl ether. The organic portion was evaporated affording 1.7 g of crude residue (internal part extract). The mucous secretion was directly extracted by diethyl ether (150 mL×4) to give 11.2 mg of crude extract. All extracts were analyzed by TLC chromatography. An UV–visible band (R_f 0.45–0.30, $CHCl_3$/MeOH, 95:5) was selectively detected in the external part and mucus.

The mucus extract was submitted to ^1H NMR spectroscopy without further purification.

The extract of the external part (384.6 mg) was fractionated on an LH-20 Sephadex column ($CHCl_3$/MeOH, 1:1). Selected fractions were combined (217.5 mg) and purified by a SiO_2 gel column ($CHCl_3$/MeOH gradient) to give 43.2 mg of pure onchidione (**4**): R_f ($CHCl_3$/CH_3OH, 95:0.5) 0.45. $[\alpha]_D^{25}=-15.5^\circ$ (c=0.80, $CHCl_3$); IR (liquid film, cm^{-1}) 1608, 1654, 1723; UV (MeOH): λ_{max} 261 nm (ε=24,977); ^1H and ^{13}C NMR: see Table 1; HRESIMS (*m*/*z*): $(M+Na)^+$: found 665.3665 (665.3666 calculated for $C_{37}H_{54}O_9Na$).

3.4. Methanolysis of 4

A sample of onchidione (**4**, 10.0 mg) was dissolved in anhydrous methanol (1.0 mL) and Na_2CO_3 was added to the resulting solution. The reaction mixture was stirred overnight at room temperature to afford, after the usual work up, 12 mg of a residue, which was purified by reverse-phase HPLC purification using PHENOMENEX-Kromasil C_{18} column (250×10 mm, particle size=5 μm) eluted with CH_3CN/H_2O, 1:1 (flow rate=2 mL/min) to give pure alcohols **5a** (2.9 mg) and **5b** (1.6 mg).

3.4.1. Alcohol *5a*

R_f ($CHCl_3$/CH_3OH, 95:0.5) 0.40. $[\alpha]_D^{25}=+15.5^\circ$ (c=0.17, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6): δ 4.69 (br s, 1H, –OH), 4.27 (dd, J=2 and 11 Hz, 1H, H-11), 3.27 (q, J=7 Hz, 1H, H-4), 3.18–3.13 (m, 1H, H-13, $w_{1/2}$=9 Hz), 3.04 (q, J=7 Hz, 1H, H-16), 2.81 (dq, J=7 and 14 Hz, 1H, H-10), 2.50 (dq, J=7 and 15 Hz, 1H, H-22a), 2.19–2.01 (m, 1H, H-22b), 2.18 (s, 3H, H_3-31), 2.05 (s, 3H, H_3-26), 2.01 (s, 3H, H_3-32), 1.97 (s, 3H, H_3-25), 1.96–1.72 (m, 2H, H_2-2), 1.65–1.59 (m, 1H, H-14), 1.44–1.38 (m, 1H, H-12), 1.26 (d, J=7 Hz, 3H, H_3-24), 0.97 (d, J=7 Hz, 3H, H_3-30), 0.92 (t, J=8 Hz, 3H, H_3-23), 0.84 (d, J=7 Hz, 3H, H_3-29), 0.83 (t, J=8 Hz, 3H, H_3-1), 0.69 (d, J=7 Hz, 3H, H_3-27), 0.59 (d, J=7 Hz, 3H, H_3-28). ^{13}C NMR (300 MHz, C_6D_6, quaternary carbons were not detected): δ 76.8 (C-13), 66.2 (C-11), 48.2 (C-4), 42.1 (C-16), 36.9 (C-10), 36.2 (C-12), 34.2 (C-2), 32.3 (C-14), 25.0 (C-22), 14.4 (C-27), 13.2 (C-24), 12.7 (C-29), 11.8 (C-30), 11.6 (C-31), 11.2 (C-23), 10.8 (C-28), 9.8 (C-25 or C-26 or C-32), 9.7 (C-25 or C-26 or C-32), 9.4 (C-26 or C-25 or C-32), 7.8 (C-1). HRESIMS (*m*/*z*): $(M+Na)^+$: found 581.3069 (581.3090 calculated for $C_{32}H_{46}O_8Na$).

3.4.2. Alcohol *5b*

R_f ($CHCl_3$/CH_3OH, 95:0.5) 0.45. $[\alpha]_D^{25}=-32.5^\circ$ (c=0.20, $CHCl_3$); ^1H NMR (400 MHz, C_6D_6): δ 4.95 (d, J=1.3 Hz, 1H, –OH), 4.58 (dd, J=2 and 11 Hz, 1H, H-11), 4.12 (dd, J=2 and 9 Hz, 1H, –OH), 3.57–3.50 (m, 1H, H-13), 3.24 (q, J=7 Hz, 1H, H-4), 3.20 (q, J=7 Hz, 1H, H-16),

2.90 (dq, J=7 and 14 Hz, 1H, H-10), 2.45 (dq, J=7 and 15 Hz, 1H, H-22a), 2.25 (s, 3H, H_3-31), 2.18 (dq, J=7 and 15 Hz, 1H, H-22b), 2.02 (s, 3H, H_3-26), 2.00–1.95 (m, 2H, H_2-2), 1.98–1.82 (m, 2H, H-12 and H-14), 1.91 (s, 3H, H_3-32), 1.80 (s, 3H, H_3-25), 1.13 (d, J=7 Hz, 3H, H_3-29), 1.05 (d, J=7 Hz, 3H, H_3-30), 0.99 (t, J=7 Hz, 3H, H_3-1), 0.92 (t, J=8 Hz, 3H, H_3-23), 0.82 (d, J=7 Hz, 3H, H_3-24), 0.77 (d, J=7 Hz, 3H, H_3-27), 0.70 (d, J=7 Hz, 3H, H_3-28). ^{13}C NMR (300 MHz, C_6D_6, quaternary carbons were not detected): δ 76.2 (C-13), 67.1 (C-11), 46.8 (C-4), 42.3 (C-16), 36.7 (C-10), 36.2 (C-12), 35.5 (C-2), 31.9 (C-14), 24.7 (C-22), 13.9 (2C, C-24 and C-27), 13.1 (C-29), 12.7 (C-30), 11.7 (C-31), 11.0 (C-23), 9.8 (C-28), 9.6 (C-25 or C-26 or C-32), 9.4 (C-26 or C-25 or C-32), 9.3 (C-32 or C-25 or C-26), 7.8 (C-1). HRESIMS (m/z): $(M+Na)^+$: found 581.3080 (581.3090 calculated for $C_{32}H_{46}O_8Na$).

3.5. X-ray analysis

A selected crystal was mounted on the glass fiber and the diffraction intensity data were collected at −120 °C by a Nonius KappaCCD diffractometer with graphite monochromatized Mo Kα radiation (λ=0.71069 Å). Accurate cell parameters were obtained by least-squares refined of the setting angles of 216 reflections at medium θ using DIRAX software.[21] Data collection was carried out with φ and ω scans, using COLLECT software.[22] Data reduction and absorption correction were performed using SADABS.[23] Structure solution was solved using direct method (SIR97)[24] and refinement was performed using the SHELXL97 software package.[25] ORTEP-3 software was employed for molecular graphics. [26] All H atoms were found in difference Fourier maps and were included in the final refinement assuming idealized geometry, with C–H distances=1.00, 0.99, and 0.98 Å for tertiary CH, secondary CH_2, and methyl CH_3, respectively, and with O–H distance of 0.84 Å. They were refined with U_{iso} values equal to $1.2U_{eq}$ parent atoms.

Crystal data: $C_{37}H_{54}O_9$, $^1/_2$ H_2O (651.81 g/mol), colorless prism crystal with size 0.46×0.35×0.27 mm^3, trigonal, space group $P3_121$, T=153(2) K, a=b=11.003(4) Å, c=56.35(2) Å, α=β=90°, γ=120°, V=5908(4) Å^3, D_c=1.099 Mg/m^3, Z=6, $F_{(000)}$=2118, $\mu_{Mo\ K\alpha}$=0.078 mm^{-1}. A total of 36,049 reflections ($-10\leq h\leq 11$, $-11\leq k\leq 11$, $-60\leq l\leq 60$) were collected in the range of $2.14°<\theta<22.49°$, with 2962 independent reflections [R(int)=0.079], completeness to θ max was 97.3%. The structure was refined by full-matrix least-square on F^2, with a final discrepancy index R of 0.0547 based on 1964 observed reflections [$I>2\sigma(I)$] and 424 variable parameters. The overall Rw value was 0.1991 with $w=1/[\sigma^2(F_o^2)+(0.11268P)^2]$ where $P=(F_o^2+2F_c^2)/3$; goodness-of-fit=1.088; $(\Delta/\sigma)_{max}<0.001$. No residual electron density was outside the range −0.294 to 0.565 e Å^{-3}. The anomalous dispersion effect is small and no reliable evidence of the absolute configuration could be obtained; indeed the final Flack parameter=0(3), using 2021 Friedel opposite reflections, is not significant.

All the crystallographic data have been deposited with the Cambridge Crystallographic Data Centre (Accession No. CCDC 708802).

3.6. Biological assays

3.6.1. Feeding deterrence assay

Feeding deterrence test was performed according to the previous reported procedure.[19] Marine shrimps (*Palaemon elegans*) significantly rejected food treated with pure onchidione (**4**) at the volumetric concentration of 3.2, 1.6 and 1 mg/mL (two-tailed Fisher's exact test, P<0.05 vs control), while the rejection rate of food treated with **4** at 0.8 mg/mL turned out to be not significantly different with respect to the control (P=0.07).

3.6.2. Antifungal and antibacterial assays

Antifungal assay was performed by the broth macrodilution method following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) document M27-P.[27] The medium used to prepare the drug dilutions and the inoculum suspension was liquid RPMI 1640 with L-glutamine (Sigma Aldrich), 0.165 M morpholinopropanesulfonic acid (MOPS), and 2% glucose (pH 7.0).[28,29] The yeast suspension was adjusted with the aid of a spectrophotometer to a cell density of 0.5McFarland (2×10^8 CFU/mL) standard at 530 nm and diluted to 1:4000 (50,000 CFU/mL) in RPMI 1640 broth medium. The yeast inoculums (0.9 mL) were added to each test tube that contained 0.1 mL of 10 twofold dilutions (256–0.05 mg/mL final) of compound **4**. Broth macrodilution MICs were determined after 48 h of incubation at 35 °C. MIC was defined as the lowest concentration of the compound that completely inhibited the growth of the test organism. The antibacterial assay was performed by using the same method as the antifungal test, only differing in the assay medium (Luria–Bertani medium: 10 g/L bactotryptone, 5 g/L bactoyeast, and 10 g/L NaCl, pH 7.5) and in the incubation temperature (37 °C for 24 h).

3.6.3. Antiproliferative assays

Cells (6000–10,000) in 100 μl culture medium per well were seeded into 96-well microtest III plates. Cells were treated in triplicate with gradient concentrations of tested compound and incubated at 37 °C for 72 h. The microculture tetrazolium [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, MTT; Sigma Chemical Co., St. Louis, MO] assay was performed to measure the antiproliferative effects.[30,31] The optical density (OD) was read using a multiwell spectrophotometer (VERSAmax, Molecular Devices, USA) at a wavelength of 570 nm. The mean percentage of growth inhibition at each concentration of tested compound was calculated as [1−(the mean OD value of the treated wells)/(the mean OD value of the control wells)]×100%.

Acknowledgements

We are grateful to Dr. R. Puliti (ICB) for stimulating discussion in the X-ray analysis and to Prof. M. Ghiselin (California Academy of Sciences) for a critical reading of the manuscript. Thanks are also due to Mr. R. Turco for the drawings, to Mr. C. Iodice for spectrophotometric measurements, and to Mrs. D. Melck for antimicrobial assays. The NMR spectra were recorded at the ICB NMR Facility, the staff of which is gratefully acknowledged. The X-ray experiments were performed at the 'Centro di Metodologie Chimico-Fisiche' of University Federico II of Naples, using the equipment of the 'Centro Regionale di Competenza Nuove Tecnologie per le Attività Produttive' of Campania Region. This work was partially supported by the Italian-Chinese bilateral project CNR-CAS 'Chemical studies on bioactive molecules with biotechnological interest from Chinese marine invertebrates'. Y.W. Guo thanks the grants from National Marine 863 Project (No. 2006AA09Z412) and Natural Science Foundation of China (Nos. 30730108, 20721003).

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