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Extending the Record of Bis-γ-pyrone Polypropionates from Marine Pulmonate Mollusks

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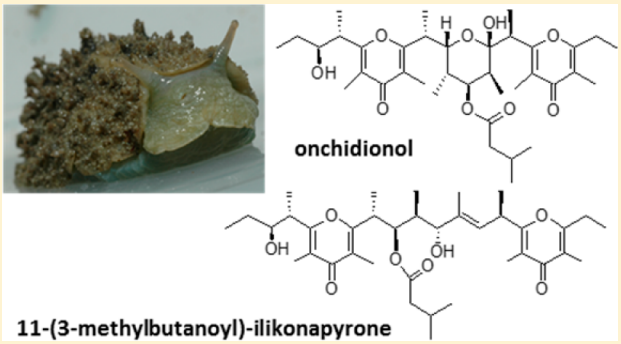
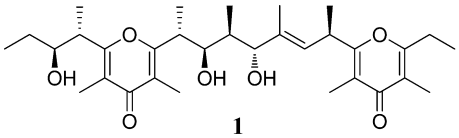
Supporting Information

ABSTRACT: The isolation and structure elucidation of 10 unreported polypropionate metabolites (compounds **6**–**15**), structurally related to either ilikonapyrone (**1**) or onchidione (**3**), from two onchidiid pulmonate mollusk species are discussed. Structure elucidation was achieved by NMR spectroscopy and chemical correlation with model compounds. Evaluation of *in vitro* growth-inhibitory properties in human cancer cells was also carried out on some of the isolated polypropionates including previously reported onchidione metabolites.



Secondary metabolism of marine pulmonate mollusks belonging to the family Onchidiidae is typically characterized by the presence of polypropionates exhibiting a C_{32} carbon skeleton with two γ-pyrone rings and several contiguous stereogenic centers.[1,2] A series of compounds with these structural features have been reported from different onchidiid species including either linear members—ilikonapyrones (e.g., ilikonapyrone, **1**),[3,4] onchitriols (e.g., onchitriol I, **2**),[5−8] and peroniatriols[4,9,10]—or molecules that exhibit an additional hemiketal pyrone ring in the middle part of the polypropionate chain, e.g., onchidione (**3**).[11,12] Interesting cytotoxic properties against several cancer cell lines as well as antiviral activity have been shown for a number of these polypropionates.[5,6]



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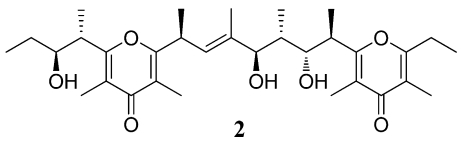

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In our ongoing studies of the chemistry of marine mollusks while searching for new bioactive molecules,[13] we have considered two distinct Onchidiidae species, tentatively called *Onchidium* sp. 1 and *Onchidium* sp. 2, which were collected during August 2010 in the intertidal zone along the coast of Hainan in the South China Sea. A previous study we conducted on the same collection of *Onchidium* sp. 1 had resulted in the isolation of the main secondary metabolites, (−)-onchidione (**3**)[11] and two related alcohol derivatives, (−)-onchidiol (**4**) and (+)-4-*epi*-onchidiol (**5**).[12] Stereochemical aspects in the structure determination of these molecules had been also deeply investigated, and the absolute configurations of compounds **3**−**5** unambiguously assigned.[12] We have now focused our attention on minor polypropionate metabolites of *Onchidium* sp. 1 and, at the same time, examined the related species *Onchidium* sp. 2. Ten new polypropionates, four minor onchidione-related metabolites (**6**−**9**) and six ilikonapyrone ester derivatives (**10**−**15**), that were isolated from *Onchidium* sp. 1 and *Onchidium* sp. 2, respectively, have been chemically characterized. Selected polypropionates have also been submitted to an evaluation of their *in vitro* growth-inhibitory properties against a number of human cancer cell lines.

RESULTS AND DISCUSSION

The polypropionate content of *Onchidium* sp. 1[11,12] was reanalyzed with the aim at characterizing the minor

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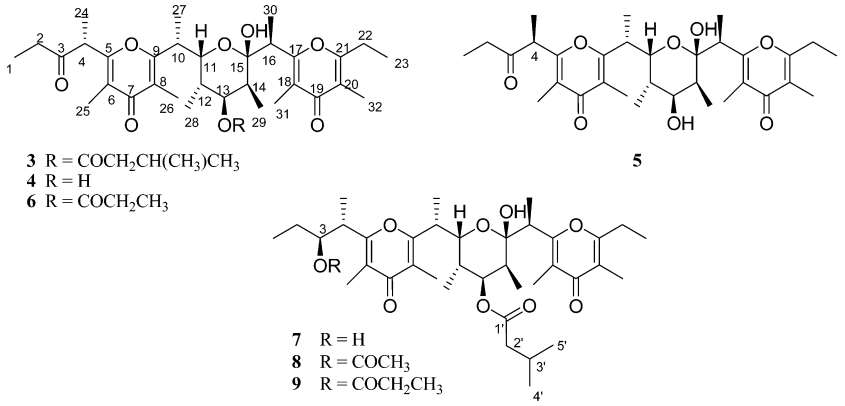

Figure 1. Metabolites of the mollusk *Onchidium* sp. 1.

components. In particular, a further subfraction (50.3 mg), which was previously recovered along with those containing main polypropionates **3**, **4**, and **5** by Si gel purification of a crude polypropionate mixture,[12] was now considered for the chemical analysis. After purification by reversed-phase HPLC, four additional minor onchidione-related metabolites, 13-propanoylonchidiol (**6**), onchidionol (**7**), 3-acetylonchidionol (**8**), and 3-propanoylonchidionol (**9**) (Figure 1), were obtained.

According to the procedure used for *Onchidium* sp. 1,[11,12] the Et_2O-soluble portions of mantle and digestive gland acetone extracts of *Onchidium* sp. 2 were analyzed by TLC, showing different secondary metabolite patterns. In particular, a series of UV−visible bands, which gave a characteristic yellow coloration by spraying with $CeSO_4$, were selectively present in the mantle extract of the mollusk (R_f 0.70−0.45, $CHCl_3$/MeOH, 95:5). A preliminary NMR analysis of this extract indicated the presence in *Onchidium* sp. 2 of polypropionate metabolites exhibiting a bis-γ-pyrone skeleton analogous with *Onchidium* sp. 1. The extract (385.0 mg) was fractionated by Sephadex LH20, yielding a main polypropionate-containing fraction, an aliquot (60.0 mg) of which was purified by reversed-phase HPLC to give six polypropionates, 11-(3-methylbutanoyl)ilikonapyrone (**10**), 13-acetyl-11-(3-methylbutanoyl)ilikonapyrone (**11**), 3,13-diacetyl-11-(3-methylbutanoyl)ilikonapyrone (**12**), 11-(3-methylbutanoyl)-13-propanoylilikonapyrone (**13**), 3-acetyl-11-(3-methylbutanoyl)-13-propanoylilikonapyrone (**14**), and 11-(3-methylbutanoyl)-3,13-dipropanoylilikonapyrone (**15**) (Figure 2).

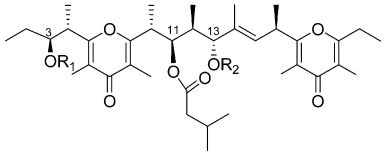

Figure 2. Metabolites of the mollusk *Onchidium* sp. 2.

13-Propanoyl-onchidiol (**6**) had the molecular formula $C_{35}H_{50}O_9$, as deduced by a HRESIMS sodiated molecular ion peak at m/z 637.3339. The ^{1}H NMR spectrum of **6** strongly resembled that of **3**,[11] except for the presence of characteristic signals at δ_H 2.49 (2H, q, J = 8 Hz, H_2-2′) and 1.25 (3H, t, J = 8 Hz, H_3-3′), due to a propanoyl group replacing the 3-methylbutanoic acyl residue in **3**. Accordingly, the ^{13}C NMR spectrum being almost identical to that of **3** only lacks two signals due to the different acyl residue linked to 13-OH. The relative configuration of the stereogenic centers was assumed to be the same on the basis of very close carbon and proton NMR values (Tables 1 and 2) with **3**.[11] Thus, compound **6** was identified as 13-propanoylonchidiol. The ECD profile of compound **6** was similar to that of onchidione (**3**), suggesting the same (4*R*,10*R*,11*R*,12*S*,13*S*,14*S*,15*S*,16*S*) absolute configuration.[12]

Onchidionol (**7**) gave a sodiated molecular ion peak in the HRESIMS spectrum at m/z 667.3812 $[M + Na]^+$, corresponding to a molecular formula with two hydrogen atoms more than onchidione (**3**). The ^{1}H and ^{13}C NMR spectra of **7** showed close similarities to **3**. The only difference was at C-3, where the ketone function in **3** was replaced by the secondary hydroxy group in **7**. Accordingly, in the ^{13}C NMR spectrum of **7** a signal at δ_C 74.4 due to an additional CH linked to oxygen was observed in place of the carbonyl signal at δ_C 208.9 in **3**. This structural hypothesis was confirmed by analysis of 2D NMR experiments including HMBC spectra. The relative configuration of the stereogenic centers was suggested to be the same as in onchidione due to the close similarities in the ^{1}H and ^{13}C NMR values of **3** and **7** (Tables 1 and 2) and confirmed by analysis of ROESY spectra. The *threo* configuration of the C-3/C-4 fragment was suggested by comparison of the coupling constant values of H-3 (ddd, J = 9, 9, and 2 Hz) with those of literature model compounds exhibiting either an *erythro* (i.e., peroniatriol I, Figure 3)[4,9] or *threo* relative configuration (i.e., onchitriol I, Figure 3).[5,8] The stereochemical assignment of onchidionol (**7**) was definitively confirmed by chemical correlation with onchidione (**3**) as described later in this paper.

3-Acetylonchidionol (**8**) had a molecular formula of $C_{39}H_{58}O_{10}$ as determined by HRESIMS. Comparison of the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) with those of **7** showed that **8** differed only in the presence of an acetyl group. In fact, additional signals at δ_H 1.82 (s, 3H), and δ_C 170.7 (C) and 20.6 (CH_3) were observed in the spectra of **8**. In addition, H-3 was shifted downfield to δ_H 4.95 (δ_H 3.55 in **7**), indicating that in **8** the 3-hydroxy group was acetylated.

3-Propanoylonchidionol (**9**) exhibited in the HRESIMS spectrum the sodiated molecular ion at m/z 723.4089 $[M + Na]^+$, with an additional $-CH_2$ unit with respect to **8**. Comparison of the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **9** with those of **8** clearly evidenced the presence of a propanoyl residue in place of the acetyl moiety that esterified the 3-OH group, as depicted in **9**.

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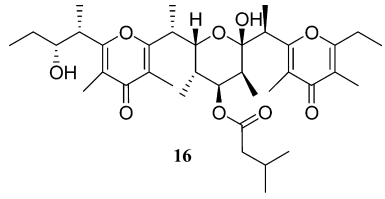
Table 1. ^{1}H NMR Data in $CDCl_3$ (400 MHz) for Compounds 6–9[a]

	6	7	8	9
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	1.00, t (7)	0.99–1.06[b]	0.97, t (7)	0.96, t (7)
2	2.41, m	1.72, m	1.79, m	1.82, m
	2.30, m	1.34, m	1.58, m	1.64, m
3		3.55, ddd (9, 9, 2)	4.95, ddd (10, 9, 3)	4.95, ddd (10, 9, 2)
4	3.92, q (7)	2.82, dq (9, 7)	3.15, dq (10, 7)	3.17, dq (10, 7)
10	3.05, m	3.05, m	3.04, m	3.01, m
11	4.15, dd (10, 2)	4.44, dd (11, 2)	4.46, br d (10)	4.50, dd (11, 2)
12	1.89, m	1.92, m	1.88, m	1.90, m
13	4.89, dd (3, 3)	4.88, dd (3, 3)	4.81, dd (3, 3)	4.81, dd (3, 3)
14	2.10, m	2.15, m	1.98, m	1.94, m
16	3.24, m	3.26, q (7)	3.26, q (7)	3.28, q (7)
22	2.60, m	2.23, m	2.56, m	2.55, m
	2.55, m		2.46, m	
23	1.10, t (8)	1.10, t (7)	1.18, t (7)	1.18, t (6)
24	0.98–1.01[b]	1.00–1.06[b]	1.19, d (7)	1.21, d (7)
25	1.94, s	1.87, s	1.96, s	1.94, s
26	1.94, s	1.97, s	1.97, s	1.96, s
27	0.98–1.01[b]	0.99–1.06[b]	0.98–1.01[b]	0.94–1.01[b]
28	0.98–1.01[b]	0.99–1.06[b]	0.98–1.01[b]	0.86, d (7)
29	1.21, d (7)	0.99–1.06[b]	0.98–1.01[b]	0.94–1.01[b]
30	1.21, d (7)	1.22, d (7)	1.22, d (7)	1.25, d (7)
31	1.98, s	1.95, s	1.92, s	1,94, s
32	1.86, s	1.86, s	1.92, s	1.90, s
2′	2.49, q (8)	2.32, m	2.34, m	2.35, m
		2.22, m	2.21, m	2.24, m
3′	1.25, t (8)	2.20, m	2.20, m	2.20, m
4′		0.99	0.91[c]	1.00[c]
5′		0.99	0.93[c]	1.01[c]
2″			1.82, s	2.16, q (8)
3″				0.94–1.01[b]

[a]Assignments were based on COSY, HSQC, and HMBC (J = 10 Hz) experiments. [b]Not assigned. [c]Assignments in the same column may be interchanged.

The structural relationship among compounds **7**, **8**, and **9** was confirmed by chemical transformation (Scheme 1). The esters obtained by treatment of **7** with $(CH_3CO)_2O$ and $(CH_3CH_2CO)_2O$ were identical in all respects with natural compounds **8** and **9**, respectively. With the aim of confirming the stereochemical assignment including the absolute configurations of onchidionols **7**–**9**, which exhibited similar ECD profiles, it was decided to convert the remaining aliquot of **7** to onchidione (**3**) by Jones oxidation. Unfortunately, the sample degraded before carrying out the reaction. Thus the reduction of **3** to **7** was pursued. A sample of onchidione was treated with $NaBH_4$/MeOH to give a mixture of two expected reaction products (ratio ca. 5:2), which were purified by HPLC and characterized by NMR. The most abundant product showed spectroscopic data identical with those of natural onchidionol (**7**), whereas the other compound was identified as the C-3 epimer **16** (see Experimental Section). In addition, the CD profile of onchidionol obtained from **3** was identical to that of the naturally occurring alcohol (Figure 4), thus inferring that the absolute configuration of **7**, and consequently of the ester derivatives **8** and **9**, was the same as that of onchidione (**3**). Finally, the absolute configuration of C-3 was confirmed to be *S* by applying the modified Mosher's method.[14,15] Two aliquots of compound **7** were treated with (*R*)- and (*S*)-MTPA chlorides to obtain the (*S*)- and (*R*)-esters, **7a** and **7b**, respectively. Both Mosher's esters were characterized by 2D-NMR experiments (see Experimental Section). The $\Delta\delta$ values (δS ester − δR ester) observed for the signals of the protons close to 3-OH (Figure 5) indicated the *S* configuration at this carbon.



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Proton and carbon NMR analysis of compounds **10**–**15** immediately showed that all molecules isolated from *Onchidium* sp. 2 were structurally related (Tables 3 and 4).

11-(3-Methylbutanoyl)ilikonapyrone (**10**) gave a sodiated molecular ion peak at m/z 651.3871 $[M + Na]^+$ in the HRESIMS spectrum, corresponding to the molecular formula $C_{37}H_{56}O_8$. The ^{13}C NMR spectrum showed 37 resonances including 12 C, 9 CH, 3 CH_2, and 13 CH_3 (Table 4). The ^{1}H NMR spectrum contained signals attributable to four methyls on two γ-pyrone rings, four secondary methyl groups, a proton on a trisubstituted double bond, a vinyl methyl, two terminal ethyl moieties, three carbinolic methines, and a 3-methylbutanoic acyl residue (Table 3). These structural features were consistent with an acyclic C_{32} polypropionate architecture, such

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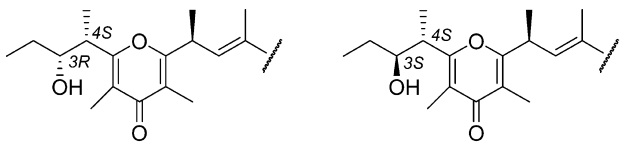
Table 2. ^{13}C NMR Data in $CDCl_3$ (100 MHz) for Compounds 6–9[a]

	6	7	8	9
position	δ_C, type	δ_C, type	δ_C, type	δ_C, type
1	7.8, CH_3	9.3, CH_3	8.9, CH_3	8.8, CH_3
2	35.3, CH_2	27.5, CH_2	24.7, CH_2	24.7, CH_2
3	209.2, C	74.4, CH	77.2, CH	76.6, CH
4	47.8, CH	42.8, CH	39.0, CH	39.0, CH
5	159.5, C	161.6, C	162.0, C	162.0, C
6	119.1, C	118.4, C	119.4, C	119.5, C
7	179.3, C	179.4, C	179.6, C	179.5, C
8	118.5, C	117.6, C	118.8, C	118.8, C
9	165.3, C	164.7, C	165.0, C	165.0, C
10	36.8, CH	36.7, CH	37.0, CH	37.0, CH
11	66.9, CH	67.1, CH	67.4, CH	67.3, CH
12	33.1, CH	33.1, CH	33.4, CH	33.3, CH
13	77.2, CH	77.2, CH	77.4, CH	77.2, CH
14	32.3, CH	31.7, CH	33.1, CH	33.2, CH
15	100.3, C	100.5, C	100.0, C	100.3, C
16	43.5, CH	42.4, CH	45.7, CH	46.4, CH
17	161.6, C	164.6, C	162.8, C	163.1, C
18	121.3, C	121.6,C	120.7, C	120.6, C
19	179.7, C	180.2, C	179.9, C	179.9, C
20	117.3, C	117.5, C	117.7, C	117.8, C
21	164.7, C	165.8, C	164.6, C	164.4, C
22	24.7, CH_2	24.8, CH_2	24.7, CH_2	24.7, CH_2
23	10.6, CH_3	12.3, CH_3	11.5, CH_3	11.5, CH_3
24	13.6, CH_3	14.4, CH_3	13.6, CH_3	13.6, CH_3
25	9.8, CH_3	9.6, CH_3	9.6, CH_3	9.7, CH_3
26	9.5, CH_3	9.3, CH_3	9.5, CH_3	9.6, CH_3
27	14.4, CH_3	15.6, CH_3	14.2, CH_3	14.3, CH_3
28	13.0, CH_3	10.9, CH_3	13.1, CH_3	13.3, CH_3
29	11.4, CH_3	12.3, CH_3	10.3, CH_3	10.3, CH_3
30	12.6, CH_3	11.2, CH_3	13.1, CH	13.3, CH_3
31	12.5, CH_3	10.0, CH_3	9.9, CH_3	9.9, CH_3
32	9.3, CH_3	9.3, CH_3	9.3, CH_3	9.3, CH_3
1′	173.3, C	172.4, C	172.9, C	172.9, C
2′	28.5, CH_2	44.1, CH_2	44.0, CH_2	44.0, CH_2
3′	9.4, CH_3	26.1, CH	26.0, CH	26.0, CH
4′		22.5, CH_3	22.5, CH_3	22.5, CH_3
5′		22.4, CH_3	22.5, CH_3	22.5, CH_3
1″			170.7, C	174.3, C
2″			20.6, CH_3	27.4, CH_2
3″				9.0, CH_3

[a]The assignments were based on DEPT, HSQC, and HMBC (J = 10 Hz) experiments.



peroniatriol I $\delta_{H\text{-}3}$: 3.75 (ddd, J = 7, 7, 7 Hz)

onchitriol I $\delta_{H\text{-}3}$: 3.55 (ddd, J = 7, 8, 3 Hz)

Figure 3. Coupling constant values of H-3 in 3,4- *erythro* and 3,4-*threo* literature model compounds.

as ilikonapyrone (**1**) and onchitriol I (**2**), with a secondary −OH esterified by a 3-methylbutanoic acid unit. Analysis of COSY and HSQC experiments led us to identify five spin systems in the carbon skeleton, whereas HMBC correlations allowed the positioning of tertiary methyls on γ-pyrone rings and the partial connection of the defined spin systems (Supporting Information). However, two possible molecular arrangements, ilikonapyrone-like and onchitriol-like, could be proposed on the basis of spectroscopic data. In order to discriminate between the two alternatives and confirm the structure, compound **10** was submitted to methanolysis to give the corresponding triol derivative, which showed spectroscopic and optical data identical with those reported for ilikonapyrone (**1**).[3] Thus the structure of compound **10** was assigned as 11-(3-methylbutanoyl)ilikonapyrone with the same absolute configuration of **1**.

Having established the structure of **10**, co-occurring metabolites **11**–**15** were characterized by referring to this compound and NMR resonances were fully assigned (Tables 3 and 4). All molecules exhibited the 3-methylbutanoic acyl residue esterifying the 11-OH functionality the same as **10** and were different from each other in the nature of one or two additional acyl moieties linking the other two hydroxy groups at C-3 and C-13 of the carbon framework.

13-Acetyl-11-(3-methylbutanoyl)ilikonapyrone (**11**) had the molecular formula $C_{39}H_{58}O_9$. The comparison of ^1H and ^{13}C NMR spectra (Tables 3 and 4) showed that **11** differed from **10** only in the presence of an acetyl group. Accordingly, signals at δ_H 1.96 (s, 3H) and δ_C 169.7 (C) and 20.9 (CH_3) were observed in the spectra of **11**. In addition, H-13 was shifted downfield to δ_H 4.82 (δ_H 3.53 in **10**), locating the acetyl residue at 13-OH.

3,13-Diacetyl-11-(3-methylbutanoyl)ilikonapyrone (**12**) was the most abundant component of a polypropionate mixture from *Onchidium* sp. 2. The molecular formula $C_{41}H_{60}O_{10}$ was deduced by the HRESIMS sodiated molecular peak at m/z 735.4055 with 84 amu more than **10**. The ^1H NMR spectrum contained two 3H singlet signals at δ_H 1.98 and 2.04, whereas in the ^{13}C NMR spectrum additional signals at δ_C 169.8 (C), 170.5 (C), 21.1 (CH_3), and 21.0 (CH_3) were observed. This was consistent with the presence of two acetyl groups esterifying both the 3-OH ($\delta_{H\text{-}3}$ 4.99) and 13-OH ($\delta_{H\text{-}13}$ 4.77) groups.

The molecular formula $C_{40}H_{60}O_9$ of 11-(3-methylbutanoyl)-13-propanoylilikonapyrone (**13**) as deduced by HRESIMS contained an additional C_3H_4O fragment with respect to compound **10**. Analysis of the ^1H NMR spectrum of **13** evidenced a spin system corresponding to an ethyl moiety [δ_H 1.02 (3H, app. t, J = 7 Hz, H_3-3‴), 2.29 (1H, dq, J = 17 and 7 Hz, H_2-2a‴), 2.23 (1H, dq, J = 17 and 7 Hz, H_2-2b‴)] as well as the low-field shift of H-13 (δ_H 4.86) according for the presence of a propanoic acid residue linked to 13-OH.

Compound **14** was characterized as 3-acetyl-11-(3-methylbutanoyl)-13-propanoylilikonapyrone. The molecular formula $C_{42}H_{62}O_{10}$ was indicated by the HRESIMS sodiated molecular peak at m/z 749.4259. Comparison of the ^1H NMR spectrum of **14** with that of compound **10** showed the presence of additional signals attributable to an acetyl (δ_H 2.04, s, 3H) and to a propanoyl residue [δ_H 1.02 (3H, app. t, J = 7 Hz, H_3-3‴), 2.39 (1H, dq, J = 15 and 7 Hz, H_2-2a‴), 2.19 (1H, dq, J = 15 and 7 Hz, H_2-2b‴)]. Analysis of HMBC spectra of **14** led us to link the acetyl group to 3-OH and the propanoyl residue to 13-OH.

Finally, compound **15** was the 3,13-dipropanoyl ester derivative of **10**. The sodiated molecular peak in the HRESIMS spectrum at m/z 763.4402 corresponding to the molecular formula $C_{43}H_{64}O_{10}$ implied the presence of an additional 122

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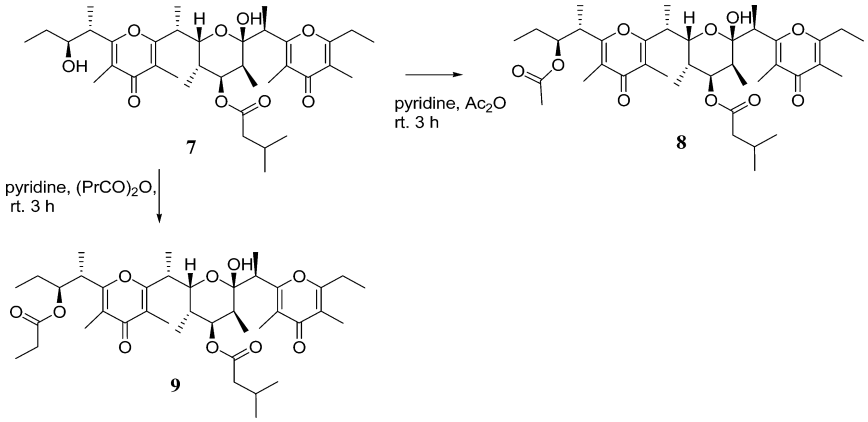
Scheme 1. Chemical Correlation of 7−9



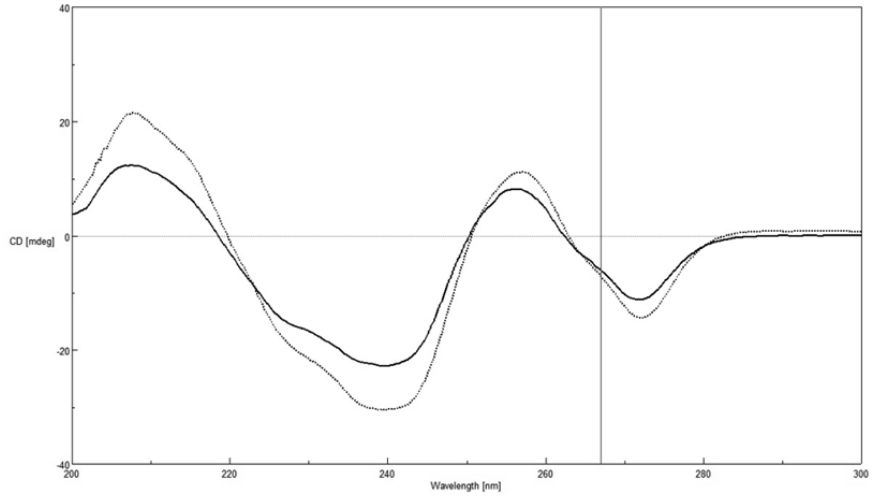


Figure 4. ECD profiles of natural **7** (dotted line) and the major diastereomer obtained from reduction of **3** (solid line).

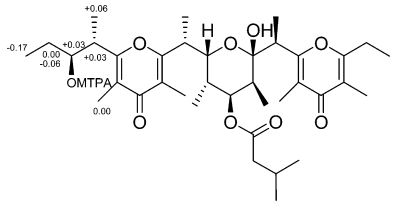


Figure 5. $\Delta\delta$ ($\delta S - \delta R$) values (in ppm) for the MTPA esters of compound **7**.

amu ($C_6H_8O_2$) with respect to compound **10** that was assigned to two propanoyl residues [δ_H 1.11 (3H, t, J = 7 Hz, H_3-3′) and 2.32 (2H, m, H_2-2′); δ_H 1.02 (3H, app. t, J = 7 Hz, H_3-3‴) 2.34 (1H, dq, J = 15 and 7 Hz, H_2-2a‴), 2.21 (1H, dq, J = 15 and 7 Hz, H_2-2b‴)] esterifying both 3-OH (δ 4.96) and 13-OH (δ 4.79).

The structures of polypropionates **11**−**15** were confirmed by chemical correlation with ilikonapyrone (**1**). Each compound was submitted to methanolysis to give the corresponding triol derivative, the spectroscopic data of which were identical with those reported for ilikonapyrone (**1**).[3,4] Analogous with **10**, the absolute configurations of **11**−**15** were suggested to be the same as ilikonapyrone (**1**) by biogenetic considerations and by comparing the $[\alpha]_D$ values.

The *in vitro* growth-inhibitory activities of selected polypropionates (**3**−**5**, **10**−**14**) isolated from the two *Onchidium* species were investigated on a panel of six human cancer cell lines as detailed in Table 5. The most active compound was 3-acetyl-11-(3-methylbutanoyl)-13-propanoyl-ilikonapyrone (**14**) with IC_{50} growth-inhibitory activity of <10 μM in all six cell lines analyzed, whereas onchidione (**3**), onchidiol (**4**), 4-*epi*-onchidiol (**5**), and ilikonapyrone (**10**) were inactive (IC_{50} >10 μM against all of the cell lines) (Table 5). The remaining three compounds (**11**−**13**) displayed *in vitro* growth-inhibitory activity against the PC-3 cell line (Table 5). When comparing these results to those of two reference compounds, i.e., etoposide and camptothecin used as a positive control, compound **14** appeared to be as active as etoposide on A549, MCF7, Hs683, and SKMEL28 cells, while being more potent on PC3 and U373 cells but less active than camptothecin.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO DIP 370 digital polarimeter. The UV spectra and CD curves were recorded on an Agilent 8453 spectrophotometer and a JASCO F815 spectropolarimeter, respectively. ^1H and ^{13}C NMR spectra were recorded on DRX 600, Avance 400, DRX 400, and DPX 300 MHz Bruker spectrometers in $CDCl_3$, with chemical shifts reported in ppm referred to $CHCl_3$ (δ 7.26 for proton and δ 77.0 for carbon) and to C_6D_6 (δ 7.15 for proton) as internal standards. ESIMS and HRESIMS spectra were measured on a Micromass Q-TOF Micro spectrometer coupled with an HPLC Waters Alliance 2695. The

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Table 3. ^{1}H NMR Data (600 MHz, $CDCl_3$) for Compounds 10−15[a]

	10[b]	11	12	13	14	15
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	1.03, t (7)	1.03, t (7)	0.90, t (7)	1.04, t (7)	0.89, t (7)	0.89, t (7)
2	1.72, m	1.72, m	1.80, m	1.71, m	1.84, m	1.83, m
3	3.78, m	3.73, m	4.99, m	3.71, m	4.98, m	4.96, m
4	3.00, m	2.99, m	3.31, m	2.98, m	3.30, m	3.33, m
10	3.28, m	3.23, m	3.23, m	3.23, m	3.23, m	3.23, m
11	5.59, br d (10)	5.30, br d (10)	5.28, br d (10)	5.27, br d (11)	5.27, br d (10)	5.28, br d (10)
12	1.99, m	2.20, m	2.15, m	2.20, m	2.15, m	2.14, m
13	3.53, dd (3, 10)	4.82, d (11)	4.77, d (11)	4.86, d (11)	4.80, d (11)	4.79, d (11)
15	5.44, d (10)	5.67, d (9)	5.65, d (9)	5.66, d (9)	5.64, d (9)	5.64, d (9)
16	3.88, m	3.82, m	3.81, m	3.82, m	3.81, m	3.81, m
22	2.61, m	2.59, app. q (7)	2.60, app. q (7)	2.59, app. q (7)	2.60, app. q (8)	2.60, app. q (8)
23	1.20, t (7)	1.20, t (7)	1.21, t (7)	1.20, t (7)	1.21, t (8)	1.21, t (8)
24	1.17, d (7)	1.21, d (7)	1.20, d (7)	1.20, d (7)	1.20, d (8)	1.20, d (8)
25	1.95, s	1.96,[c] s	1.98, s	1.95,[c]s	1.98, s	1.99, s
26	1.96, s	1.93,[c] s	1.93,[c] s	1.930,[c]s	1.93,[c]s	1.93,[c]s
27	1.25, d (7)	1.25, d (7)	1.23, d (7)	1.24, d (7)	1.24, d (8)	1.23, d (7)
28	0.83, d (7)	0.92, d (7)	0.89, d (7)	0.92, d (7)	0.90, d (7)	0.90, d (7)
29	1.70, br s	1.70, br s	1.62, br s	1.64, br s	1.62, br s	1.62, br s
30	1.29, d (7)	1.29, d (7)	1.28, d (7)	1.29, d (7)	1.28, d (7)	1.28, d (7)
31	1.99, s	1.93,[c] s	1.94,[c] s	1.926,[c] s	1.94,[c] s	1.94,[c] s
32	1.94, s	1.93,[c]s	1.95,[c]s	1.926,[c]s	1.95,[c]s	1.95,[c]s
2′			2.04, s		2.04, s	2.32, m
3′						1.11, t (7)
2″	2.07, m	2.07, m	2.07, m	2.08, m	2.06, m	2.06, m
3″	1.91, m	1.80, m	1.80, m	1.76, m	1.81, m	1.81, m
4″	0.89, d (6)	0.80, d (6)	0.882, d (6)	0.80, d (6)	0.81, d (6)	0.82, d (6)
5″	0.83, d (6)	0.72, d (6)	0.75, d (6)	0.71, d (6)	0.74 d (6)	0.75 d (6)
2‴		1.96, s	1.98, s	2.29, dq (17, 7)	2.39, dq (15, 7)	2.34, dq (15, 7)
				2.23, dq (17, 7)	2.19, dq (15, 7)	2.21, dq (15, 7)
3‴				1.02, app. T (7)	1.02, aap. t (7)	1.02, app. t (7)

[a]Assignments were based on COSY, HSQC, and HMBC (J = 10 and 7 Hz) experiments. [b]^{1}H spectrum recorded at 400 MHz. [c]Assignments in the same column may be interchanged.

instrument was calibrated by using a PEG mixture from 200 to 1000 MW. Silica gel chromatography was performed using precoated Merck F254 plates and Merck Kieselgel 60 powder. HPLC purification was carried out on a Shimadzu LC-10AD liquid chromatograph equipped with a UV SPD-10A wavelength detector.

Human cancer cell lines were obtained from the American Type Culture Collection (ATCC), the European Collection of Cell Culture (ECACC), and the Deutsche Sammlung von Mikroorganismen and Zellkulturen (DSMZ). The code number and histological type of each of the cell lines used in the current study are detailed in Table 5. The cell lines were cultured as detailed previously.[16−18]

Biological Material. For *Onchidium* sp. 1 see ref 12. *Onchidium* sp. 2 (138 individuals, average size 4 cm) was collected in the intertidal zone along the coast of Lingshui Bay, Hainan Province, China, during August 2010. The mollusks were frozen immediately after collection. A brief description of the two species is reported in the Supporting Information. Voucher specimens of *Onchidium* sp. 1 (LS 350G) and of *Onchidium* sp. 2 (LS 350 V) are available for inspection at ICB.

Extraction and Isolation of Compounds 6−9 from *Onchidium* sp. 1. A selected fraction (50.3 mg) containing minor polypropionate components was recovered from Si gel chromatography of the polypropionate mixture obtained from the external part acetone extract of *Onchidium* sp. 1, as previously described.[12] The fraction was purified by reversed-phase HPLC [Supelco-Discovery 5 μm C_{18}, 25 cm × 10 mm, 20 min 50% CH_3CN in H_2O, 5 min gradient from 50% to 100% CH_3CN, 20 min 100% CH_3CN, flow 2 mL/min, UV detector 254 nm] to give pure compounds **6** (2.3 mg), **7** (11.5 mg), **8** (4.2 mg), and **9** (3.5 mg).

*13-Propanoylonchidiol (**6**):* $[\alpha]^{20}_D$ +14 (c 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 260 (4.5) nm; ECD (MeOH, c 0.85 × 10^{-3}) λ_{max} ($\Delta\varepsilon$) 273 (−7.07), 255 (+6.10), 239 (−12.35), 213 (−0.04), 204 (−5.31) nm; ^{1}H and ^{13}C NMR (see Tables 1 and 2); HRESIMS m/z 637.3339 $[M + Na]^+$ (calcd for $C_{35}H_{50}O_9Na$, 637.3353).

*Onchidionol (**7**):* $[\alpha]^{20}_D$ +26 (c 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 260 (4.5) nm; ECD (MeOH, c 1.26 × 10^{-3}) λ_{max} ($\Delta\varepsilon$) 272 (−11.31), 257 (+8.08), 239 (−22.86), 208 (+12.24), 200 (−3.66) nm; ^{1}H and ^{13}C NMR in $CDCl_3$ (see Tables 1 and 2); HRESIMS m/z 667.3812 $[M + Na]^+$ (calcd for $C_{37}H_{56}O_9Na$, 667.3822).

*3-Acetylonchidionol (**8**):* $[\alpha]^{20}_D$ +24 (c 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 260 (4.5) nm; ECD (MeOH, c 1.09 × 10^{-3}) λ_{max} ($\Delta\varepsilon$) 273 (−5.62), 256 (+7.50), 240 (−15.73), 208 (+6.26), 200 (−5.09) nm; ^{1}H and ^{13}C NMR (see Tables 1 and 2); HRESIMS m/z 709.3940 $[M + Na]^+$ (calcd for $C_{39}H_{58}O_{10}Na$, 709.3928).

*3-Propanoylonchidionol (**9**):* $[\alpha]^{20}_D$ +15 (c 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 260 (4.5) nm; ECD (MeOH, c 1.05 × 10^{-3}) λ_{max} ($\Delta\varepsilon$) 271 (−8.02), 257 (+5.09), 242 (−15.03), 207 (+13.78), 198 (−11.37) nm; ^{1}H and ^{13}C NMR (see Tables 1 and 2); HRESIMS m/z 723.4089 $[M + Na]^+$ (calcd for $C_{40}H_{60}O_{10}Na$, 723.4084).

Extraction and Isolation of Compounds 10−15 from *Onchidium* sp. 2. According to the reported procedure,[11] specimens of *Onchidium* sp. 2 (138 individuals) were first immersed in acetone (250 mL × 3) and sonicated for 1 min. Then, after the original solvent was decanted, the whole animal residue was extracted once more with fresh acetone by crumbling in a mortar and ultrasound. Both extracts were concentrated under reduced pressure, and the resulting aqueous suspensions were extracted with diethyl ether, affording, after

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Table 4. ^{13}C NMR Data (150 MHz, $CDCl_3$) for Compounds 10−15[a]

	10	11	12	13	14	15
position	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type
1	9.1, CH_3	9.7, CH_3	8.9, CH_3	10.1, CH_3	10.0, CH_3	9.1, CH_3
2	27.2, CH_2	27.3, CH_2	24.7, CH_2	27.2, CH_2	23.1, CH_2	22.9, CH_2
3	74.6, CH	74.9, CH	76.0, CH	74.9, CH	76.1, CH	76.0, CH
4	42.0, CH	42.3, CH	38.1, CH	42.4, CH	38.2, CH	38.5, CH
5	165.2, C	164.8, C	163.1, C	164.8, C	162.5, C	162.9, C
6	119.7, C	119.9, C	119.5, C	119.9, C	119.6, C	119.7, C
7	179.9,[b] C	179.9,[b] C	179.5,[b] C	179.9,[b] C	179.5,[b] C	179.5,[b] C
8	119.4, C	119.6, C	119.8, C	119.6, C	119.8, C	119.7, C
9	162.2, C	161.7, C	162.4, C	161.7, C	163.0, C	162.5, C
10	37.3, CH	37.2, CH	37.4, CH	37.1, CH	37.4, CH	37.5, CH
11	74.4, CH	72.0, CH	71.9, CH	72.9, CH	71.9, CH	71.9, CH
12	36.9, CH	34.6, CH	35.1, CH	34.7, CH	35.2, CH	35.2, CH
13	78.5, CH	78.5, CH	78.6, CH	78.2, CH	78.4, CH	78.5, CH
14	136.6, C	132.5, C	132.8, C	132.7, C	133.0, C	133.0, C
15	129.2CH	132.2, CH	131.8, CH	132.0, CH	131.6, CH	131.6, CH
16	34.3, CH	34.3, CH	34.3, CH	34.3, CH	34.3, CH	34.3, CH
17	164.4, C	164.2, C	164.3, C	164.2, C	163.9, C	164.3, C
18	117.3, C	117.3, C	117.2, C	117.3, C	117.3, C	117.3, C
19	179.5,[b] C	179.4,[b] C	179.9,[b] C	179.4,[b] C	179.9,[b] C	179.9,[b] C
20	117.9, C	117.9, C	117.8, C	117.9, C	117.8, C	117.8, C
21	164.2, C	164.4, C	164.5, C	164.4, C	164.5, C	164.4, C
22	24.7, CH_2	24.7, CH_2	24.8, CH_2	24.8, CH_2	24.8, CH_2	24.8, CH_2
23	11.3, CH_3	11.2, CH_3	11.2, CH_3	11.2, CH_3	11.2, CH_3	11.2, CH_3
24	15.2, CH_3	15.3, CH_3	13.2, CH_3	15.3, CH_3	13.1, CH_3	12.7, CH_3
25	9.1,[c] CH_3	9.6,[c] CH_3	10.0,[c] CH_3	9.6,[c] CH_3	9.6,[c] CH_3	9.6,[c] CH_3
26	9.7,[c] CH_3	9.5,[c] CH_3	9.49,[c] CH_3	9.5,[c] CH_3	9.5,[c] CH_3	9.5,[c] CH_3
27	13.9, CH_3	13.5, CH_3	13.5, CH_3	13.5, CH_3	13.5, CH_3	13.6, CH_3
28	9.7, CH_3	8.8, CH_3	8.9,CH_3	9.0, CH_3	9.0, CH_3	9.0, CH_3
29	10.9, CH_3	11.6, CH_3	11.7, CH_3	11.6, CH_3	11.8, CH_3	11.8, CH_3
30	18.7, CH_3	18.7, CH_3	18.7, CH_3	18.6, CH_3	18.6, CH_3	18.6, CH_3
31	9.7,[c] CH_3	9.3,[c] CH_3	9.53,[c] CH_3	9.3,[c] CH_3	9.3,[c] CH_3	9.3,[c] CH_3
32	9.7,[c] CH_3	9.9,[c] CH_3	9.3,[c] CH_3	9.9,[c] CH_3	9.0,[c] CH_3	9.9,[c] CH_3
1′			170.5, C		170.5, C	173.9, C
2′			21.1,[d] CH_3		21.1, CH_3	27.6, CH_2
3′						9.0, CH_3
1″	173.7, C	172.9, C	170.8, C	172.8, C	170.8, C	170.8, C
2″	43.1, CH_2	42.4, CH_2	42.4, CH_2	42.4, CH_2	42.4, CH_2	42.4, CH_2
3″	25.5, CH	24.8, CH	23.1, CH	24.7, CH	24.7, CH	24.7, CH
4″	22.2, CH_3	22.2, CH_3	22.2, CH_3	22.2, CH_3	22.2, CH_3	22.2, CH_3
5″	22.1, CH_3	21.9, CH_3	22.0, CH_3	21.9, CH_3	22.0, CH_3	22.0, CH_3
1‴		169.7, C	169.8, C	173.0, C	173.0, C	173.0, C
2‴		20.9, CH_3	21.0,[d] CH_3	27.4, CH_2	27.3, CH_2	27.3, CH_2
3‴				8.7, CH_3	8.7, CH_3	8.7, CH_3

[a]The assignments were based on DEPT, HSQC, and HMBC (J = 10 Hz) experiments. [b,c,d]Assignments in the same column with the same superscript may be interchanged.

evaporation of the organic phases, 385.0 mg and 2.0 g of external and internal part extracts, respectively.

The diethyl ether portion (385.0 mg) of the external part extract was fractionated on Sephadex LH20, yielding a main polypropionate-containing fraction (130.5 mg). An aliquot (60.0 mg) of this fraction was purified by reversed-phase HPLC [Supelco-Discovery 5 μm C_{18}, 25 cm × 10 mm, 60 min gradient from 80% to 100% CH_3CN in H_2O, flow 2 mL/min, UV detector 254 nm], affording pure compounds **10** (2.2 mg), **11** (3.2 mg), **12** (14.4 mg), **13** (13.4 mg), **14** (12.6 mg), and **15** (2.0 mg).

*11-(3-Methylbutanoyl)ilikonapyrone (**10**):* $[\alpha]^{20}_D$ −1.9 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 216 (3.4), 260 (3.5) nm; ECD (MeOH, *c* 2.40 × 10^{-5}) λ_{max} ($\Delta\varepsilon$) 269 (4.32), 240 (−23.59) nm; ^1H and ^{13}C NMR (see Tables 3 and 4); HRESIMS *m*/*z* 651.3871 [M + Na]$^+$ (calcd for $C_{37}H_{56}O_8Na$, 651.3873).

*13-Acetyl-11-(3-methylbutanoyl)ilikonapyrone (**11**):* $[\alpha]^{20}_D$ −1.7 (*c* 0.32, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 259 (4.3), 216 (4.2) nm; ECD (MeOH, *c* 4.03 × 10^{-5}) λ_{max} ($\Delta\varepsilon$) 267 (5.45), 240 (−30.22) nm; ^1H and ^{13}C NMR (see Tables 3 and 4); HRESIMS *m*/*z* 693.3981 [M + Na]$^+$ (calcd for $C_{39}H_{58}O_9Na$, 693.3979).

*3,13-Diacetyl-11-(3-methylbutanoyl)ilikonapyrone (**12**):* $[\alpha]^{20}_D$ −5.7 (*c* 0.24, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 260 (4.3), 216 (4.2) nm; ECD (MeOH, *c* 3.93 × 10^{-5}) λ_{max} ($\Delta\varepsilon$) 269 (4.87), 240 (−27.6) nm; ^1H and ^{13}C NMR (see Tables 3 and 4); HRESIMS *m*/*z* 735.4055 [M + Na]$^+$ (calcd for $C_{41}H_{60}O_{10}Na$, 735.4084).

*11-(3-Methylbutanoyl)-13-propanoylilikonapyrone (**13**):* $[\alpha]^{20}_D$ −1.9 (*c* 0.19, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 259 (4.3), 216

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Table 5. Determination of the IC_{50} *in Vitro* Growth-Inhibitory Concentrations in Six Human Cancer Cell Lines after 72 h of Cell Culture with the Compound of Interest

	IC_{50} in vitro growth-inhibitory concentration (μM)						
	carcinoma			glioma		melanoma	
compound	A549[a]	MCF-7[a]	PC-3[a]	Hs683[a]	U373[a]	SKMEL28[a]	mean ± SEM
camptothecin	<0.01	<0.01	0.010	<0.01	0.015	<0.01	<0.011
etoposide	2	4	>100	2	25	3	>23
3	26	21	16	23	25	23	22 ± 2
4	>100	>100	>100	>100	>100	>100	>100
5	>100	87	82	>100	>100	>100	>95
10	>100	>100	>100	>100	>100	>100	>100
11	15	15	3	30	21	25	18 ± 4
12	28	25	7	24	32	33	25 ± 4
13	12	12	3	13	21	25	14 ± 3
14	3	4	2	3	9	7	5 ± 1

[a]The origin and histological type of each human cell line analyzed are as follows. Carcinoma models included the A549 NSCLC (DSMZ code ACC107), the MCF-7 breast (DSMZ code ACC115), and the PC-3 prostate (DSMZ code ACC465) cancer cell lines. Glioma models included the Hs683 oligodendroglioma (ATCC code HTB-138) and the U373 (ECACC code 08061901) cell lines. One melanoma model included the SKMEL-28 (ATCC code HTB-72) cell line.

(4.2) nm; ECD (MeOH, *c* 4.26 × 10^{-5}) λ_{max} ($\Delta\varepsilon$) 268 (5.30), 240 (−26.72) nm; ^{1}H and ^{13}C NMR (see Tables 3 and 4); HRESIMS *m/z* 723.4095 [M + Na]$^+$ (calcd for $C_{40}H_{60}O_9Na$, 707.4135).

*3-Acetyl-11-(3-methylbutanoyl)-13-propanoylilikonapyrone (**14**):* $[\alpha]^{20}_D$ −1.4 (*c* 0.87, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 260 (4.3), 217 (4.2) nm; ECD (MeOH, *c* 3.33 × 10^{-5}) λ_{max} ($\Delta\varepsilon$) 267 (5.30), 240 (−29.19) nm; ^{1}H and ^{13}C NMR (see Tables 3 and 4); HRESIMS *m/z* 749.4259 [M + Na]$^+$ (calcd for $C_{42}H_{62}O_{10}Na$, 749.4241).

*11-(3-Methylbutanoyl)-3,13-dipropanoylilikonapyrone (**15**):* $[\alpha]^{20}_D$ −2.3 (*c* 0.05, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 260 (4.2), 217 (4.1) nm; ECD (MeOH, *c* 5.40 × 10^{-5}) λ_{max} ($\Delta\varepsilon$) 268 (5.45), 239 (−27.3) nm; ^{1}H and ^{13}C NMR (see Tables 3 and 4); HRESIMS *m/z* 763.4402 [M + Na]$^+$ (calcd for $C_{43}H_{64}O_{10}Na$, 763.4397).

Reduction of Compound 3. Pure onchidione (**3**, 5.0 mg) was dissolved in 2.0 mL of dry MeOH and treated at 0 °C with $NaBH_4$. After stirring for 1 h at 0 °C, the reaction mixture was allowed to warm to room temperature (rt), quenched with 6 N HCl, and extracted with $CHCl_3$ (3 × 20 mL). The subsequent purification by reversed-phase HPLC (Supelco-Discovery 5 μm C18, 25 cm × 10 mm, 20 min 50% of CH_3CN in H_2O, flow 2 mL/min, UV detector 254 nm) of the crude reaction mixture afforded the two expected alcohols at C-3. NMR, MS, and ECD analysis of the main product revealed that it was identical in all aspects with natural compound **7**, whereas the minor derivative was identified as 3-*epi*-onchidionol (**16**).

*3-epi-Onchidionol (**16**):* ^{1}H NMR data (C_6D_6, 400 MHz) δ 4.93 (1H, app. t, *J* = 3.3 Hz, H-13), 4.46 (1H, dd, *J* = 2, 11 Hz, H-11), 3.44 (1H, m, H-3), 3.05 (1H, q, *J* = 7 Hz, H-16), 2.85 (1H, m, H-10), 2.60 (1H, m, H-4), 2.50 (1H, m, H-22a), 2.21 (1H, m, H-22b), 2.15 (1H, m, H-3′), 2.13 (3H, s, H_3-31 or H_3-32), 2.09 (2H, m, H_2-2′), 2.02 (3H, s, H_3-31 or H_3-32) 1.99 (3H, s, H_3-24 or H_3-25), 1.95 (3H, s, H_3-24 or H_3-25), 1.89 (1H, m, H-12), 1.87 (1H, m, H-14), 1.36 (1H, m, H-2a), 1.20 (1H, m, H-2b), 0.994 (3H, d, *J* = 7 Hz, H_3-24), 0.985 (3H, d, *J* = 7 Hz, H_3-30), 0.975 (3H, t, *J* = 7 Hz, H_3-1), 0.92 (3H, t, *J* = 8 Hz, H_3-23), 0.87 (3H, d, *J* = 7 Hz, H_3-29), 0.86 (6H, d, *J* = 7 Hz, H_3-5′ and H_3-4′), 0.75 (3H, d, *J* = 7 Hz, H_3-28), 0.70 (3H, d, *J* = 7 Hz, H_3-27); HRESIMS *m/z* 667.3825 [M + Na]$^+$ (calcd for $C_{37}H_{56}O_9Na$, 667.3822).

Preparation of MTPA Esters of Compound 7. (*R*)- and (*S*)-MTPA-Cl (10 μL) and a catalytic amount of DMAP were separately added to two different aliquots of onchidionol (**7**) (1.0 mg each) in dry CH_2Cl_2 (0.5 mL). The resulting mixtures were allowed to stand at rt for 12 h. After the evaporation of the solvent, the mixtures were purified on a SiO_2 Pasteur pipet (CH_2Cl_2/MeOH, 99:1), affording pure (*S*)- and (*R*)-MTPA esters of **7**, respectively.

*(S)-MTPA ester of **7**:* selected ^{1}H NMR values ($CDCl_3$, 600 MHz) δ 5.34 (1H, m, H-3), 4.81 (1H, app. t, *J* = 3 Hz, H-13), 4.46 (1H, dd, *J* = 10 and 2 Hz, H-11), 3.33 (1H, m, H-4), 3.02 (1H, m, H-10), 1.85 (3H, s, H_3-25), 1.83 (1H, m, H-2a), 1.71 (1H, m, H-2b), 1.22 (3H, d, *J* = 7 Hz, H_3-24), 0.85 (3H, d, *J* = 7 Hz, H_3-1); ESIMS *m/z* 883 [M + Na]$^+$.

*(R)-MTPA ester of **7**:* selected ^{1}H NMR values ($CDCl_3$, 600 MHz) δ 5.31 (1H, m, H-3), 4.80 (1H, app. t, *J* = 3 Hz, H-13), 4.51 (1H, dd, *J* = 10 and 2 Hz, H-11), 3.30 (1H, m, H-4), 3.06 (1H, m, H-10), 1.85 (3H, s, H_3-25), 1.83 (1H, m, H-2a), 1.77 (1H, m, H-2b), 1.16 (3H, d, *J* = 7 Hz, H_3-24), 1.02 (3H, t, *J* = 7 Hz, H_3-1); ESIMS *m/z* 883 [M + Na]$^+$.

Methanolysis Reactions of Compounds 10−15. Each of the ilikonapyrone esters **10**−**15** was dissolved in dry MeOH (1.0 mg in 1.5 mL), and an excess of Na_2CO_3 was added. The solution was stirred at rt for 4 h. Then the reaction mixture was filtered, and the solvent was evaporated. Crude products were purified by reversed-phase HPLC (Supelco-Discovery 5 μm C_{18}, 25 cm × 10 mm, 70% CH_3CN in H_2O, flow 2 mL/min, UV detector 254 nm) to afford ∼0.5 mg of pure ilikonapyrone (**1**) from each reaction. Ilikonapyrone from methanolysis of compound **10**:[19] $[\alpha]_D$ −7 (*c* 0.1, CH_2C1_2) *vs* lit.[3] $[\alpha]_D$ −16 (*c* 1.5, CH_2C1_2).

Determination of the IC_{50} Growth-Inhibitory Concentrations *in Vitro*. The MTT colorimetric assay was used as detailed previously.[16−18] Briefly, this test measures the number of metabolically active (thus living) cells that are able to transform the yellow substrate 3-(4,5-dimethylthazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into the blue formazan dye via a mitochondrial reduction involving succinate dehydrogenase. The amount of formazan obtained at the end of the experiment (measured by spectrophotometry) is directly proportional to the number of living cells. The determination of the optical density in the control compared to the treated cells helps measure the effects of compounds on the growth of cancer cells *in vitro*. Each experimental condition was assessed in six replicates.

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR spectra of compounds **6**−**9** and **10**−**15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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