

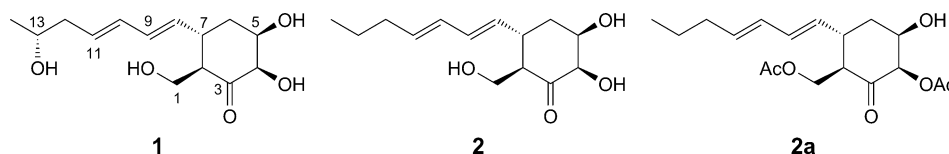
A New Polyketide from the Mangrove Endophytic Fungus *Penicillium* sp. sk14JW2P

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Two polyketides were isolated from the mangrove endophytic fungus, *Penicillium* sp. sk14JW2P, and one derivative with a modified structure. The structures were elucidated by spectroscopic analyses, the structure of the compound **1** was further confirmed by single-crystal X-ray diffraction, and its absolute configuration was determined. Compound **1** and **2** showed acetylcholinesterase (AChE) inhibitory activities with IC_{50} values of 12 ± 0.3 and 79 ± 2 nM, respectively.

Introduction. – Palitantin is a polyketide and cyclitol compound, which was isolated by Birkinshaw and Raistrick from fungus in 1936 [1]. Palitantin, with the ability to mimic the parent saccharides, rendered it a potential candidate as inhibitor of glycosidase [2], and so it has received a high degree of attention regarding its synthesis [3–5]. In the past decade, we have been dedicated to investigating novel bioactive compounds from mangrove endophytic fungi collected from the South China Sea [6–9], and an endophytic fungus, strain sk14JW2P, identified as *Penicillium* sp., was isolated from the mangrove. Study of the extract of this fungus led to a new (+)-palitantin analog, named 13-hydroxypalitantin (**1**), (+)-palitantin (**2**), and 1,4-diacetoxypalitantin (**2a**), a new derivative by structure modification of **2**. Herein, we report the isolation, structure elucidation, and acetylcholinesterase (AChE) inhibitory activities of these compounds.



Results and Discussion. – Compound **1** was isolated as colorless block crystals (MeOH). The molecular formula, $C_{14}H_{22}O_5$, was determined by NMR (Table I) and HR-EI-MS (m/z 270.1459 (M^+)). The formula indicated four degrees of unsaturation. The IR spectrum of **1** displayed absorption bands at 3383, 3018, 2970, 2921, 2882, and 1735 cm^{-1} , which indicating the presence of OH, C=O, and CH=CH groups. The 1D-NMR and HSQC spectra of **1** showed signals corresponding to five CH ($\delta(C)$ 40.4, 56.1, 68.5, 73.8, and 78.8), two CH=CH ($\delta(C)$ 131.1, 133.0, 133.3, and 134.5), three CH_2

Table 1. ^1H - and ^{13}C -NMR Data (400 and 100 MHz, resp., CD_3OD) of Compound **1**. δ in ppm, J in Hz. Arbitrary atom numbering as indicated in the Formulae.

Position	$\delta(\text{H})$	$\delta(\text{C})$	HSQC	HMBC
1	3.69–3.76 (<i>m</i> , 2 H)	59.3	CH_2	3, 2, 7
2	2.44 (<i>dddd</i> , $J = 11.8, 6.1, 2.9, 1.0$, 1 H)	56.1	CH	3, 1, 7
3		210.8	C=O	
4	4.32 (<i>dd</i> , $J = 3.4, 1.0$, 1 H)	78.8	CH	5, 3, 2
5	4.29 (<i>dd</i> , $J = 6.2, 3.4$, 1 H)	73.8	CH	4, 3, 6
6	1.95–2.03 (<i>m</i> , 2 H)	37.9	CH_2	5, 7
7	2.76 (<i>ddd</i> , $J = 11.6, 9.3, 6.8, 2.9$, 1 H)	40.4	CH	2, 6, 8
8	5.50–5.59 (<i>m</i> , 1 H)	134.5	CH	7, 9
9	6.13 (<i>dd</i> , $J = 10.2, 18.4$, 1 H)	133.3	CH	8, 10
10	6.10 (<i>dd</i> , $J = 9.5, 18.4$, 1 H)	133.0	CH	9, 11
11	5.69 (<i>dd</i> , $J = 9.5, 6.8$, 1 H)	131.1	CH	10, 12
12	2.16–2.30 (<i>m</i> , 2 H)	43.4	CH_2	11, 13
13	3.79 (<i>dd</i> , $J = 12.4, 6.2$, 1 H)	68.5	CH	12, 14
14	1.16 (<i>d</i> , $J = 6.2$, 3 H)	23.0	Me	13, 12

($\delta(\text{C})$ 37.9, 43.4, and 59.3) groups, and one Me ($\delta(\text{C})$ 23.0) and one C=O group ($\delta(\text{C})$ 210.8); thus, the structure must contain one ring. The ^1H , ^1H -COSY spectrum displayed the cross-peaks H–C(4)/H–C(5)/ CH_2 (6) and CH_2 (6)/H–C(7)/H–C(2), indicating a ^1H , ^1H spin system of CH(4)–CH(5)– CH_2 (6)–CH(7)–CH(2). Combined with the HMBC cross-peaks (Fig. 1) H–C(2)/C(3) and C(7), and cross-peaks H–C(4)/C(3) and C(5), the structure of the six-membered ring was established. The 2D-NMR spectrum revealed that two CH=CH groups were conjugated and attached to CH(7), based on CH(8) showing ^1H , ^1H -COSY and HMBC correlations with CH(7). The H–C(12) showed HMBCs with C(11) and C(13), and, combined with the 1D-NMR and ^1H , ^1H -COSY, C(11) was connected to $\text{CH}_2\text{CH}(\text{OH})\text{Me}$ (C(12)/C(13)/C(14)). Ultimately, the structure of **1** was further confirmed by single-crystal X-ray diffraction, and the absolute configuration was determined using copper radiation. The new compound **1** named 13-hydroxylpalitantin (Fig. 2) with absolute configuration (2*R*,4*R*,5*R*,7*S*,13*S*).

Compound **2** was obtained as colorless block crystals. The NMR spectrum was almost identical to that of **1** and the data were consistent with those (+)-palitantin ($[\alpha]_{\text{D}}^{23} = +4.49$ ($c = 0.32$, CHCl_3); $[\alpha]_{\text{D}}^{25}$ (**2**) = +6.80 ($c = 0.32$, CHCl_3)) [4]. The NOE experiment revealed the same relative configuration as that of **1**, and, together with the biogenenic pathway analysis, we deduced the absolute configuration of **2** as that of **1**. Thus, **2** was determined as (+)-palitantin.

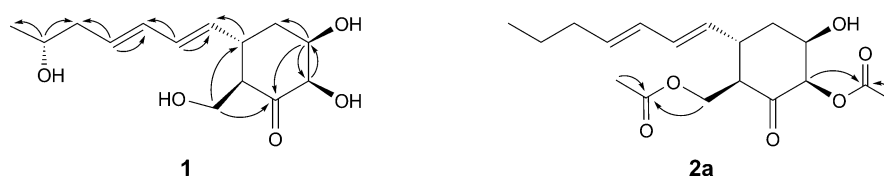
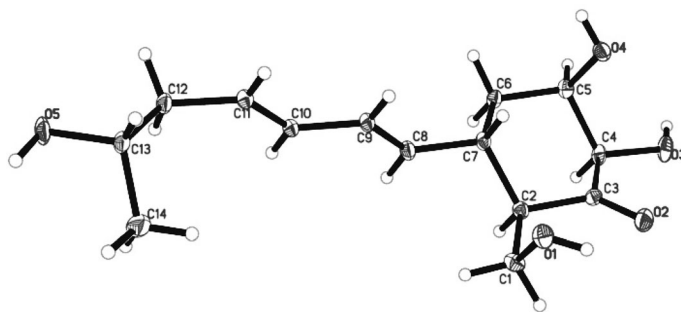


Fig. 1. Main HMBCs (H $\rightarrow$ C) of **1** and **2a**

Fig. 2. X-Ray crystal structure of **1**

Compound **2a** was obtained as colorless powder. It was obtained by structure modification of **2** via the reaction with the AcOH in pyridine. Compared to the NMR data of **2** [4], the spectra of **2a** exhibited two more Me *singlet* signals at $\delta(\text{H})$ 2.21 (4-AcO) and 1.99 (1-AcO), plus four more C-atom signals at $\delta(\text{C})$ 171.0, 169.6, 21.0, and 20.7, attributed to two AcO groups. In the HMBC (Fig. 1), H–C(1) signal showed correlation to that at $\delta(\text{C})$ 171.0, and H–C(4) signal correlated with that at $\delta(\text{C})$ 169.6, suggesting that the OH groups at C(1) and C(4) were acetylated, different from the reported compound 4,5-diacetoxypalitantin [10].

Finally, compound **2a** was identified as a new compound and named 1,4-diacetoxypalitantin.

All compounds were evaluated for their inhibitory activities against AChE *in vitro* according to the modified *Ellman* method [11]. Compound **2** showed significant inhibitory activity against AchE with an IC_{50} value of 79 nM (Table 3); however, the

Table 2. ^1H - and ^{13}C -NMR Data (400 and 100 MHz, resp., CDCl_3) of Compound **2a**. δ in ppm, J in Hz. Arbitrary atom numbering as indicated in the Formulae.

Position	$\delta(\text{H})$	$\delta(\text{C})$	HSQC	HMBC
1	4.22–4.27 (<i>m</i> , 2 H)	60.4	CH_2	3, 2, 7, 1-MeCO
2	2.55–2.60 (<i>m</i> , 1 H)	52.7	CH	3, 1, 7, 8
3		200.3	$\text{C}=\text{O}$	
4	5.25 (<i>dd</i> , $J=3.0$, 1 H)	78.8	CH	5, 3, 4-MeCO
5	4.42 (<i>dd</i> , $J=7.1$, 3.0, 1 H)	71.0	CH	4, 3, 6
6	2.17 (<i>dt</i> , $J=14.5$, 4.1, 1 H), 1.87–1.94 (<i>m</i> , 1 H)	36.9	CH_2	5, 4, 2, 7 3, 2, 1, 6, 8
7	2.78–2.87 (<i>m</i> , 1 H)	39.5	CH	
8	5.41 (<i>dd</i> , $J=14.7$, 9.1, 1 H)	130.7	CH	2, 6, 7, 9
9	6.08 (<i>dd</i> , $J=9.1$, 18.4, 1 H)	132.9	CH	7, 10
10	6.00 (<i>dd</i> , $J=14.7$, 18.4, 1 H)	129.5	CH	9, 12
11	5.65 (<i>dd</i> , $J=14.7$, 6.9, 1 H)	135.5	CH	10, 12, 13
12	2.02–2.07 (<i>m</i> , 2 H)	34.8	CH_2	10, 11, 13, 14
13	1.36–1.45 (<i>m</i> , 2 H)	22.5	CH_2	11, 12, 14
14	0.90 (<i>t</i> , $J=7.4$, 3 H)	13.9	Me	12, 13
4-AcO	2.21 (<i>s</i> , 3 H)	20.7, 169.6	Me, C	
1-AcO	1.99 (<i>s</i> , 3 H)	21.0, 171.0	Me, C	

Table 3. *Acetylcholinesterase (AChE) Inhibitory Activity of Compounds 1, 2, and 2a^a*

Compound	1	2	2a	HUP ^b)
<i>IC</i> ₅₀ [μM]	12	0.079	> 200	0.060

^a) Expressed in *IC*₅₀ values. ^b) Huperzine A used as positive control.

corresponding value for **1** showed only 12 μM. Since **1** possesses an additional OH group at C(13) as the only difference to **2**, the results of the biological assay indicated that the Pr group was essential for inhibitory activity. Similarly, **2a** showed no activity, its *IC*₅₀ was > 200 μM, indicating that the OH groups are indispensable for the AChE inhibitory activity.

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Experimental Part

General. Column chromatography (CC): silica gel (200–300 mesh; *Qingdao Marine Chemicals*, P. R. China). M.p.: *Fisher–Johns* hot-stage apparatus. Optical rotation: *WZ-1* polarimeter at 25°. IR Spectra: *Nicolet 5DX-FT-IR*, in KBr discs; $\tilde{\nu}$ in cm^{−1}. NMR: *Varian Inova-400 NB* spectrometer, δ in ppm rel. to Me₄Si as internal standard, *J* in Hz. HR-EI-MS: *VG-ZAB* mass spectrometer; in *m/z*.

Fungal Material. The fungal strain *Penicillium* sp. sk14JW2P (GenBank accession No. KC545799) was isolated as endophytic fungus from a mangrove, root of the *Kandelia candel* (L.) DRUCE, collected from Shankou, Guangxi Province, P. R. China, in September 2008. The fungus was deposited with the School of Chemistry and Chemical Engineering, Sun Yat-sen University.

Fermentation and Extraction. Spores of *Penicillium* sp. sk14JW2P were directly spread into 4 × 500-ml *Erlenmeyer* flasks, each containing 200 ml of potato-liquid media composed of glucose (20 g/l) and NaCl (30 g/l), and the final pH was adjusted to 6.9. Four *Erlenmeyer* flasks of the inoculated media were incubated at 25° on a rotary shaker at 180 rpm for 72 h to yield the seed liquid. The rice solid-substrate medium was carried out in 50 *Erlenmeyer* flasks (1000 ml) each containing 100 g of rice and 20 ml of 3% sea salt liquid, all were prepared by autoclaving at 120° ca. 30 min and cooling to r.t. The seed liquid was transferred into the rice solid-substrate medium under aseptic conditions and incubated at 25° for 28 d. The mycelia and rice medium were extracted with MeOH. The MeOH layer was concentrated under reduced pressure to give a dark brown gum (26.6 g).

Purification. The extract was separated by CC (silica gel; CHCl₃/MeOH from 1:0 to 1:3) to give four fractions, *Frs. 1–4*. *Fr. 4* (320 mg) with CHCl₃/MeOH from 1:0 to 1:3 afforded compound **2** (50 mg). The *Fr. 3* was subjected to CC (*Sephadex LH-20*; petroleum ether/CHCl₃/MeOH 2:1:1) to furnish compound **1** (4 mg).

(+)-13-Hydroxypalitantin (= (2R,3R,5S,6R)-2,3-Dihydroxy-5-[(1E,3E,6R)-6-hydroxyhepta-1,3-dien-1-yl]-6-(hydroxymethyl)cyclohexanone; **1**). Colorless crystals. M.p. 144–146°. [α]_D²⁰ = +46.0 (*c* = 0.10, MeOH). IR (KBr): 3383, 3018, 2970, 2921, 2909, 2882, 1735, 1404, 988. ¹H- and ¹³C-, and 2D-NMR: *Table 1*. HR-EI-MS: 270.1459 (*M*⁺, C₁₄H₂₂O₅⁺; calc. 270.1467).

(+)-1,4-Diacetoxypalitantin (= [(1R,3R,4R,6S)-3-(Acetyloxy)-6-[(1E,3E)-hepta-1,3-dien-1-yl]-4-hydroxy-2-oxocyclohexyl)methyl Acetate; **2a**). Colorless powder. [α]_D²⁵ = +40.0 (*c* = 0.10, MeOH). ¹H- and ¹³C-NMR: *Table 2*. HR-EI-MS: 338.1729 (*M*⁺, C₁₈H₂₆O₆⁺; calc. 338.1729).

X-Ray Crystallography of 1. Crystal data: C₁₄H₂₂O₅, *M*_r 270.32, crystal size 0.32 × 0.26 × 0.25 mm, monoclinic, space group: *P*2₁, unit cell dimensions *a* = 8.6284(2) Å, *b* = 5.0503(2) Å, *c* = 16.0474(4) Å,

$\alpha = 90^\circ$, $\beta = 103.753(3)^\circ$, $\gamma = 90^\circ$, $V = 679.23(4) \text{ \AA}^3$, $Z = 2$, $D_s = 1.322 \text{ g/cm}^3$, $F(000) = 292$. The reflection data were collected on an *Oxford Diffraction XcaliburNova* single-crystal diffractometer using $\text{CuK}\alpha$ radiation at $150(2) \text{ K}$, $\lambda = 1.54178 \text{ \AA}$. A total of 12262 reflections were collected in the range $2.83^\circ \leq \theta \leq 67.09^\circ$, of which 2397 unique reflections with $I > 2\sigma(I)$ were collected for the analysis. The structure was solved by direct methods and refined by full-matrix least-squares based on F^2 using the SHELXL-97. The final R and wR_2 factors were 0.0294 and 0.0764, resp. The absolute-structure *Flack* parameter was $-0.05(16)$. Crystallographic data for the structure reported in this article has been deposited with the *Cambridge Crystallographic Data Centre* (accession No. CCDC 944995).

Biological Assay. Acetylcholinesterase (AChE) inhibitory activity was evaluated *in vitro* according to the modified *Ellman* method [11]. The IC_{50} value represents the concentration of a compound required to inhibit the activity by 50%.

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