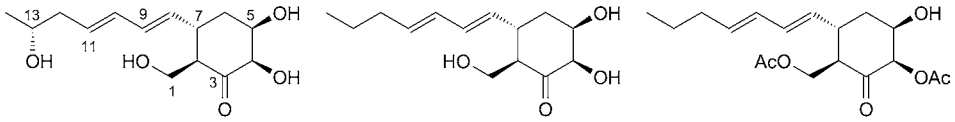
A New Polyketide from the Mangrove Endophytic Fungus *Penicillium* sp. sk14JW2P

by **Xi-Shan Huang**, **Bin Yang**, **Xue-Feng Sun**, **Guo-Ping Xia**, **Ya-Yue Liu**, **Lin Ma**, and **Zhi-Gang She***

School of Chemistry and Chemical Engineering, Sun Yat-sen University, #135, Xingang West Road, Haizhu District, Guangzhou 510275, P. R. China
(phone: +86-20-84113356; cesshzhg@mail.sysu.edu.cn)

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.



1 **2** **2a**

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 1*) 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 (δ(C) 40.4, 56.1, 68.5, 73.8, and 78.8), two CH=CH (δ(C) 131.1, 133.0, 133.3, and 134.5), three CH_2

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Table 1. *^{1}H- 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	δ(H)	δ(C)	HSQC	HMBC
1	3.69–3.76 (*m*, 2 H)	59.3	CH_2	3, 2, 7
2	2.44 (*dddd*, 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 (*dd*, J = 3.4, 1.0, 1 H)	78.8	CH	5, 3, 2
5	4.29 (*dd*, J = 6.2, 3.4, 1 H)	73.8	CH	4, 3, 6
6	1.95–2.03 (*m*, 2 H)	37.9	CH_2	5, 7
7	2.76 (*dtd*, J = 11.6, 9.3, 6.8, 2.9, 1 H)	40.4	CH	2, 6, 8
8	5.50–5.59 (*m*, 1 H)	134.5	CH	7, 9
9	6.13 (*dd*, J = 10.2, 18.4, 1 H)	133.3	CH	8, 10
10	6.10 (*dd*, J = 9.5, 18.4, 1 H)	133.0	CH	9, 11
11	5.69 (*dd*, J = 9.5, 6.8, 1 H)	131.1	CH	10, 12
12	2.16–2.30 (*m*, 2 H)	43.4	CH_2	11, 13
13	3.79 (*dd*, J = 12.4, 6.2, 1 H)	68.5	CH	12, 14
14	1.16 (*d*, J = 6.2, 3 H)	23.0	Me	13, 12

(δ(C) 37.9, 43.4, and 59.3) groups, and one Me (δ(C) 23.0) and one C=O group (δ(C) 210.8); thus, the structure must contain one ring. The ^{1}H,^{1}H-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 ^{1}H,^{1}H 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 ^{1}H,^{1}H-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 ^{1}H,^{1}H-COSY, C(11) was connected to CH_2CH(OH)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 ($[\alpha]_D^{23} = +4.49$ ($c = 0.32$, $CHCl_3$); $[\alpha]_D^{25}$ (**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.

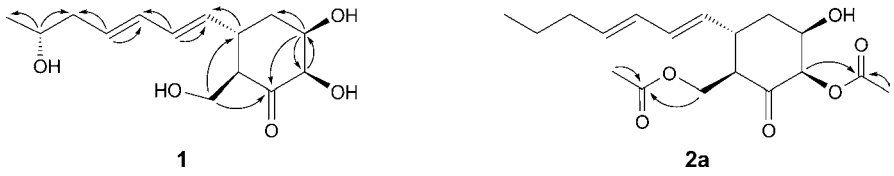

Fig. 1. *Main HMBCs* (H → C) *of* **1** *and* **2a**

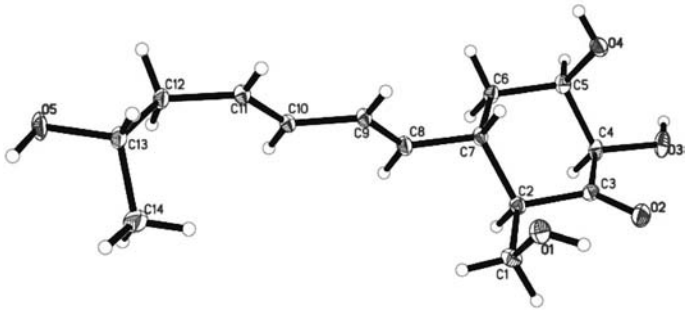

Fig. 2. *X-Ray crystal structure of* **1**

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 δ(H) 2.21 (4-AcO) and 1.99 (1-AcO), plus four more C-atom signals at δ(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 δ(C) 171.0, and H–C(4) signal correlated with that at δ(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. *^{1}H- 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	δ(H)	δ(C)	HSQC	HMBC
1	4.22–4.27 (*m*, 2 H)	60.4	CH_2	3, 2, 7, 1-Me*C*O
2	2.55–2.60 (*m*, 1 H)	52.7	CH	3, 1, 7, 8
3		200.3	C=O	
4	5.25 (*dd*, *J* = 3.0, 1 H)	78.8	CH	5, 3, 4-Me*C*O
5	4.42 (*dd*, *J* = 7.1, 3.0, 1 H)	71.0	CH	4, 3, 6
6	2.17 (*dt*, *J* = 14.5, 4.1, 1 H),	36.9	CH_2	5, 4, 2, 7
	1.87–1.94 (*m*, 1 H)			3, 2, 1, 6, 8
7	2.78–2.87 (*m*, 1 H)	39.5	CH	
8	5.41 (*dd*, *J* = 14.7, 9.1, 1 H)	130.7	CH	2, 6, 7, 9
9	6.08 (*dd*, *J* = 9.1, 18.4, 1 H)	132.9	CH	7, 10
10	6.00 (*dd*, *J* = 14.7, 18.4, 1 H)	129.5	CH	9, 12
11	5.65 (*dd*, *J* = 14.7, 6.9, 1 H)	135.5	CH	10, 12, 13
12	2.02–2.07 (*m*, 2 H)	34.8	CH_2	10, 11, 13, 14
13	1.36–1.45 (*m*, 2 H)	22.5	CH_2	11, 12, 14
14	0.90 (*t*, *J* = 7.4, 3 H)	13.9	Me	12, 13
4-AcO	2.21 (*s*, 3 H)	20.7, 169.6	Me, C	
1-AcO	1.99 (*s*, 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])
IC_{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 Me_4Si 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; $CHCl_3$/MeOH from 1:0 to 1:3) to give four fractions, *Frs. 1–4*. *Fr. 4* (320 mg) with $CHCl_3$/MeOH from 1:0 to 1:3 afforded compound **2** (50 mg). The *Fr. 3* was subjected to CC (*Sephadex LH-20*; petroleum ether/$CHCl_3$/MeOH 2:1:1) to furnish compound **1** (4 mg).

(+)-*13-Hydroxypalitantin* (=*(2*R,*3*R,*5*S,*6*R*)-2,3-Dihydroxy-5-[(1*E*,*3*E,*6*R)-6-hydroxyhepta-1,3-dien-1-yl]-6-(hydroxymethyl)cyclohexanone*; **1**). Colorless crystals. M.p. 144–146°. $[\alpha]_D^{20} = +46.0$ (*c* = 0.10, MeOH). IR (KBr): 3383, 3018, 2970, 2921, 2909, 2882, 1735, 1404, 988. ^{1}H- and ^{13}C-, and 2D-NMR: *Table 1*. HR-EI-MS: 270.1459 (M^+, $C_{14}H_{22}O_5^+$; calc. 270.1467).

(+)-*1,4-Diacetoxypalitantin* (=*{(1*R,*3*R,*4*R,*6*S*)-3-(Acetyloxy)-6-[(1*E,*3*E)-hepta-1,3-dien-1-yl]-4-hydroxy-2-oxocyclohexyl}methyl Acetate*; **2a**). Colorless powder. $[\alpha]_D^{25} = +40.0$ (*c* = 0.10, MeOH). ^{1}H- and ^{13}C-NMR: *Table 2*. HR-EI-MS: 338.1729 (M^+, $C_{18}H_{26}O_6^+$; calc. 338.1729).

X-Ray Crystallography of **1**. *Crystal data:* $C_{14}H_{22}O_5$, M_r 270.32, crystal size 0.32 × 0.26 × 0.25 mm, monoclinic, space group: $P2_1$, unit cell dimensions *a* = 8.6284(2) Å, *b* = 5.0503(2) Å, *c* = 16.0474(4) Å,

$\alpha = 90^\circ$, $\beta = 103.753(3)^\circ$, $\gamma = 90^\circ$, $V = 679.23(4)$ Å^3, $Z = 2$, $D_x = 1.322$ g/cm^3, $F(000) = 292$. The reflection data were collected on an *Oxford Diffraction XcaliburNova* single-crystal diffractometer using CuK_α radiation at 150(2) K, $\lambda = 1.54178$ Å. A total of 12262 reflections were collected in the range $2.83^\circ \leq \theta \leq 67.09^\circ$, of which 2,397 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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