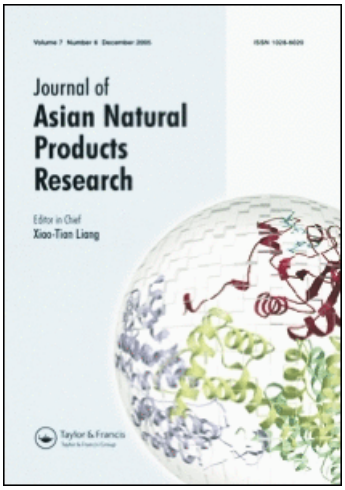

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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Online publication date: 01 February 2010

To cite this Article Wu, Hong-Hua , Tian, Li , Feng, Bao-Min , Li, Zhi-Feng , Zhang, Qi-Hui and Pei, Yue-Hu(2010) 'Three new compounds from the marine fungus *Penicillium* sp.', Journal of Asian Natural Products Research, 12: 1, 15 — 19
To link to this Article: DOI: 10.1080/10286020903442020
URL: http://dx.doi.org/10.1080/10286020903442020

Journal of Asian Natural Products Research
Vol. 12, No. 1, January 2010, 15–19

Taylor & Francis
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ORIGINAL ARTICLE

Three new compounds from the marine fungus *Penicillium* sp.

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(*Received 20 July 2009; final version received 27 October 2009*)

Continuous research on the ethyl acetate extract of the fermentation broth of the marine fungus Y26-02 (*Penicillium* sp.) led to the purification of one known and three new compounds. Their structures were elucidated, respectively, as butyl 2-(4-oxo-5,6-dihydro-2H-pyran-3-yl) acetate (**1**), 4-hydroxyphenethyl 2-(4-oxo-5,6-dihydro-2H-pyran-3-yl) acetate (**2**), 3-hydroxybenzyl 2-(4-oxo-5,6-dihydro-2H-pyran-3-yl) acetate (**3**), and desoxypatulinic acid (**4**) on the basis of their spectroscopic and physicochemical properties.

Keywords: *Clerodendrum inerme*; desoxypatulinic acid; *Penicillium* sp.; marine fungus

1. Introduction

Owing to the source limitation of terrestrial organisms such as plants and microbes, most of which have been well investigated chemically and biologically, scientists have begun to extend their attention to marine or marine-derived organisms since the early 1960s. Also, some marine organisms have been shown to be able to synthesize structurally unique secondary metabolites with the chemical and biological properties unlike those found in terrestrial ones [1,2]. Marine micro-organisms, particularly marine fungi, have recently attracted more and more attention as an important source of structurally diverse, biologically active secondary metabolites [3,4].

The marine fungus Y26-02 (*Penicillium* sp.) was isolated from *Clerodendrum inerme*, a tree from the inter-tidal zone of the South China Sea. Recently, our continuous research on the ethyl acetate extract of the fermentation broth of the fungus afforded four compounds, among which compounds **1**–**3** (Figure 1) were new compounds.

2. Results and discussion

Compound **1** was obtained as a colorless oil. The molecular formula of $C_{11}H_{16}O_4$ was established by HR-FAB-MS at *m/z* 213.1129 $[M+H]^+$. The IR spectrum showed absorption bands due to an α,β-unsaturated ketone group (1675, 1625 cm^{-1}) and a carbonyl group (1730 cm^{-1}). The ^1H NMR signal at δ 7.36 (1H, s) and the ^{13}C NMR signals at δ 190.7, 161.8, 112.7 further affirmed the

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020903442020
http://www.informaworld.com

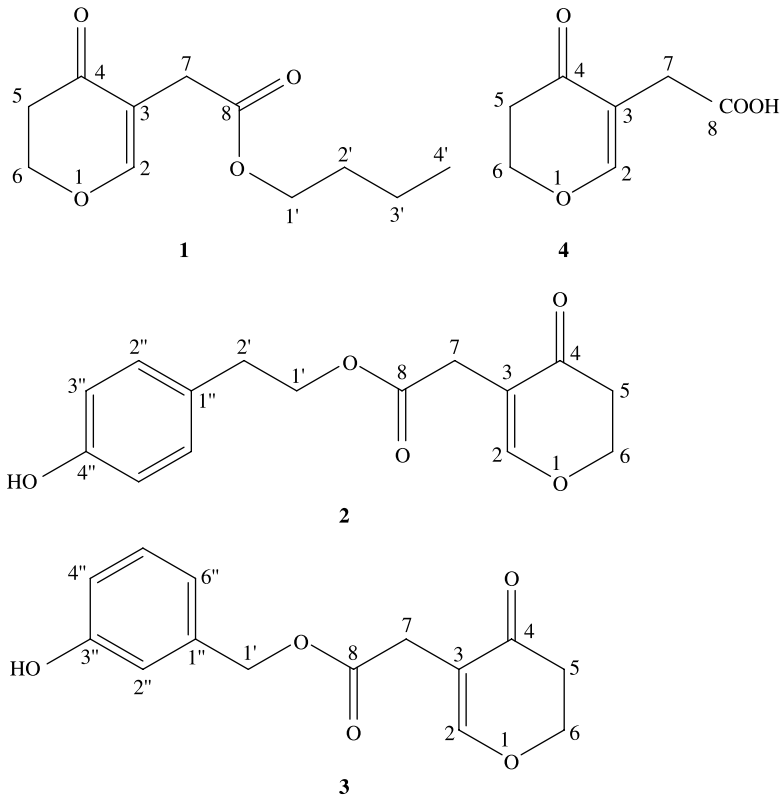

Figure 1. The structures of compounds **1**–**4**.

presence of an α,β-unsaturated ketone group in **1**. The ^{13}C NMR signal at δ 171.4 belongs to a carbonyl group. Therefore, three of the four elements of unsaturation, as indicated by the molecular formula of **1**, could be attributed to an α,β-unsaturated ketone group and a carbonyl group; the molecule thus has one ring. The ^{1}H NMR signals at δ 4.09 (2H, t, $J = 6.6$ Hz), 1.61 (2H, m), 1.37 (2H, m), 0.93 (3H, t, $J = 7.2$ Hz), together with the corresponding carbon signals at δ 64.8, 30.5, 19.1, 13.7, indicated that compound **1** contained a butoxy group. By comparing the 1D NMR spectra of compound **1** with the 1D NMR spectra of compound **4** (desoxypatulinic acid; Figure 1), compound **1** was proved to be a butoxy ester of desoxypatulinic acid. The deduction was also supported by the HMBC experiment (Figure 2). Thus, compound **1** was finally elucidated as butyl 2-(4-oxo-5,6-dihydro-2H-pyran-3-yl) acetate.

Compound **2** was obtained as a colorless oil. The molecular formula was determined to be $C_{15}H_{16}O_5$ by HR-FAB-MS at *m/z* 277.1074 $[M+H]^+$. The IR spectrum of **2** showed absorption bands at 1600, 1510, and 1445 cm^{-1}, indicating the presence of an aromatic ring, which was also supported by the UV absorption maxima at 282 and 230 nm. The ^{1}H NMR signals at δ 9.25 (1H, s), 7.02 (2H, d, $J = 7.2$ Hz), 6.67 (2H, d, $J = 7.2$ Hz), and the ^{13}C NMR signals at δ 155.9, 129.2 × 2, 128.0, 115.2 × 2, indicated the presence of a 4-hydroxybenzene moiety in **2**. By comparing the NMR spectrum of **2** with the corresponding spectrum of **4**, the ^{1}H NMR signals at δ 2.73 (2H, t, 6.6), 4.10 (2H, t, 6.6) and the ^{13}C NMR signals at δ 33.6, 65.1, together with the above

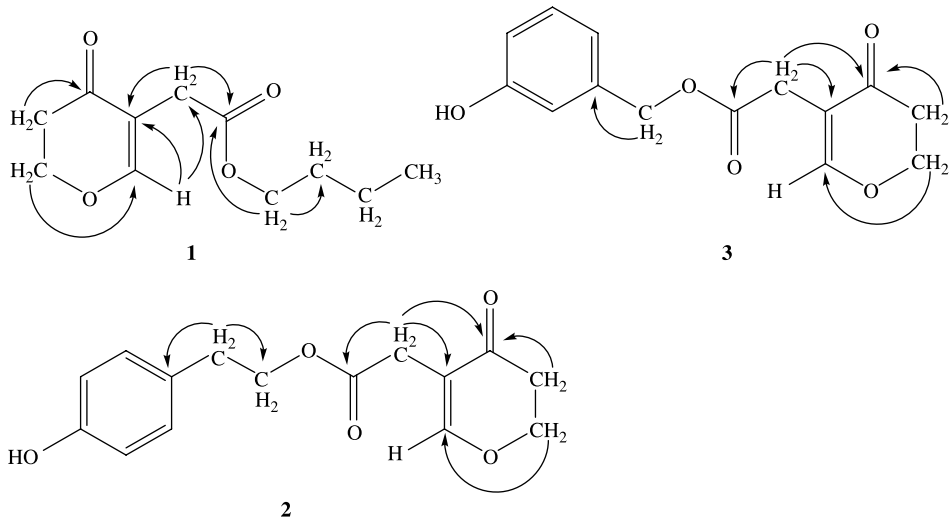

Figure 2. The key HMBC correlations of new compounds **1**–**3**.

deduction, indicated that compound **2** was a 4-hydroxyphenethyl ester of desoxypatulinic acid, which was also supported by the HMBC correlations of H-7 (2H, δ_H 3.02)/C-3 (δ_C 112.2), C-4 (δ_C 190.7) and C-8 (δ_C 170.9), H-5 (2H, δ_H 2.51)/C-4, H-6 (2H, δ_H 4.44)/C-2 (δ_C 162.4) and H-2′ (2H, δ_H 2.73)/C-1′ (δ_C 65.1) and C-1″ (δ_C 128.0). Hence, compound **2** was confirmed as 4-hydroxyphenethyl 2-(4-oxo-5,6-dihydro-2H-pyran-3-yl) acetate.

Compound **3** was obtained as a colorless oil. The molecular formula was determined to be $C_{14}H_{14}O_5$ by HR-FAB-MS at *m/z* 263.0918 $[M+H]^+$. The IR spectrum of **3** showed absorption bands at 1610, 1500, and 1450 cm^{-1} and indicated the presence of an aromatic ring in **3**, which was also supported by the UV absorption maxima at 280 and 231 nm. The ^1H NMR signals at δ 9.48 (1H, s), 7.15 (1H, t, 7.8), 6.74 (1H, dd, 7.8, 2.4), 6.70 (1H, dd, 7.8, 2.4), 6.72 (1H, t, 2.4), 4.97 (2H, s) and the ^{13}C NMR signals at δ 157.4, 137.6, 129.5, 118.3, 115.0, 114.6, 65.6, together with the above inference, indicated the presence of a 3-hydroxybenzyl group in **3**. Therefore, compound **3** was proved to be a 3-hydroxybenzyl ester of desoxypatulinic acid by comparing the NMR spectra of **3** with the corresponding spectra of **4**. The deduction was also supported by the HMBC correlations of H-7 (2H, δ_H 3.11)/C-3 (δ_C 112.2), C-4 (δ_C 190.7) and C-8 (δ_C 170.8), H-5 (2H, δ_H 2.56)/C-4, H-6 (2H, δ_H 4.46)/C-2 (δ_C 162.5) and H-1′ (2H, δ_H 4.97)/C-1″ (δ_C 137.6). Therefore, compound **3** was finally determined as 3-hydroxybenzyl 2-(4-oxo-5,6-dihydro-2H-pyran-3-yl) acetate.

The structure of the known compound desoxypatulinic acid (**4**) was established by comparing its spectral data with those reported in the literature [5,6].

3. Experimental

3.1 General experimental procedures

UV spectra were measured on a Shimadzu UV-1601. IR spectra were taken on a Bruker IFS-55 infrared spectrophotometer. The NMR spectral data were recorded on Bruker AV-600 (600 MHz for ^1H and 150 MHz for ^{13}C) with TMS as the internal standard. The HR-FAB-MS data were obtained on the Micross Mass Autospec-UltimaE TOF mass spectrophotometer. Chromatography was performed on silica

gel (200–300 mesh; Qingdao Haiyang Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia, Piscataway, NJ, USA), and reversed-phase HPLC (Shimadzu LC-8A vp, Kyoto, Japan).

3.2 Fungal material

The strain Y26-02 was isolated from *C. inerme* collected in the inter-tidal zone of the South China Sea in December 2006. A voucher specimen (No. HTTA-Z06001) was identified as *Penicillium* sp. by Prof. Li Tian, and has been deposited in the Marine Microbial Medicinal Resource Library of the First Institute of Oceanography SOA funded by the Ministry of Science and Technology.

3.3 Extraction and isolation

The supernatant of the fermentation broth of the strain Y26-02 (50 liters) was concentrated to 5 liters *in vacuo* and extracted with ethyl acetate and *n*-butanol, successively. The EtOAc crude extract (20.7 g) was subjected to silica gel column, eluted with $CHCl_3$–CH_3OH (100:1–0:1), yielding 12 fractions. Fraction 2 (2.5 g) was then subjected to silica gel column again, eluted with petroleum ether–EtOAc (100:1–0:1), yielding 14 fractions. Then, fraction 1 (155 mg) of these 14 fractions was subjected to Sephadex LH-20 eluted with $CHCl_3$–CH_3OH (1:1), silica gel column eluted with petroleum ether–acetone (4:1), preparative-TLC eluted with $CHCl_3$–CH_3OH (20:1, $R_f = 0.47$) successively to get compound **1** (7.5 mg), while fraction 8 (210 mg) of these 14 fractions was subjected to Sephadex LH-20 eluted with $CHCl_3$–CH_3OH (1:1), silica gel column eluted with $CHCl_3$–CH_3OH (10:1), and preparative-HPLC eluted with CH_3OH–H_2O (35%) successively to yield compounds **2** (3 mg, 49.2 min) and **3** (4 mg, 40.9 min). Fraction 3 (300 mg) was subjected to Sephadex LH-20 eluted with CH_3OH and preparative-TLC eluted with $CHCl_3$–CH_3OH–HCOOH (10:1:0.1) successively to get compound **4** (15 mg, $R_f = 0.32$).

3.3.1 Butyl 2-(4-oxo-5,6-dihydro-2H-pyran-3-yl) acetate (*1*)

Colorless oil, UV(MeOH) λ_{max}: 269 nm; IR (KBr) ν_{max} (cm^{-1}): 1730, 1675, 1625; ^1H and ^{13}C NMR spectral data, see Table 1; HR-FAB-MS *m/z*: 213.1129 $[M+H]^+$ (calcd for $C_{11}H_{17}O_4$, 213.1127).

Table 1. ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectral data of compounds **1** (in $CDCl_3$) and **4** (in DMSO-d_6).

1			**4**		
Position	δ_C	δ_H (*J*, Hz)	Position	δ_C	δ_H (*J*, Hz)
2	161.8	7.36 (1H, s)	2	162.2	7.56 (1H, s)
3	112.7	–	3	112.7	–
4	190.7	–	4	190.7	–
5	30.7	2.65 (2H, t, 7.2)	5	30.4	2.52 (2H, t, 6.9)
6	68.4	4.51 (2H, t, 7.2)	6	68.2	4.44 (2H, t, 6.9)
7	36.1	3.11 (2H, s)	7	35.7	2.94 (2H, s)
8	171.4	–	8	172.4	11.9 (1H, br s, —COOH)
1′	64.8	4.09 (2H, t, 6.6)	1′	–	–
2′	30.5	1.61 (2H, m)	2′	–	–
3′	19.1	1.37 (2H, m)	3′	–	–
4′	13.7	0.93 (3H, t, 7.2)	4′	–	–

Table 2. ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz) spectral data of compounds **2** and **3**.

2			**3**		
Position	δ_C	δ_H (J, Hz)	Position	δ_C	δ_H (J, Hz)
2	162.4	7.59 (1H, s)	2	162.5	7.64 (1H, s)
3	112.2	–	3	112.2	–
4	190.7	–	4	190.7	–
5	30.4	2.51 (2H, t, 6.6)	5	30.4	2.56 (2H, t, 7.2)
6	68.2	4.44 (2H, t, 6.6)	6	68.2	4.46 (2H, t, 7.2)
7	35.6	3.02 (2H, s)	7	35.6	3.11 (2H, s)
8	170.9	–	8	170.8	–
1′	65.1	4.10 (2H, t, 6.6)	1′	65.6	4.97 (2H, s)
2′	33.6	2.73 (2H, t, 6.6)	2′	–	–
1″	128.0	–	1″	137.6	–
2″	129.9	7.02 (d, 7.2)	2″	114.6	6.72 (1H, t, 2.4)
3″	115.2	6.67 (d, 7.2)	3″	157.4	–
4″	155.9	–	4″	115.0	6.70 (1H, dd, 7.8, 2.4)
5″	115.2	6.67 (d, 7.2)	5″	129.5	7.15 (1H, t, 7.8)
6″	129.9	7.02 (d, 7.2)	6″	118.3	6.74 (1H, dd, 7.8, 2.4)
—OH	–	9.25 (1H, s)	—OH	–	9.48 (1H, s)

*3.3.2 4-Hydroxyphenethyl 2-(4-oxo-5,6-dihydro-2H-pyran-3-yl) acetate (**2**)*

Colorless oil, UV(MeOH) λ_{max}: 230, 265, 282 nm; IR (KBr) ν_{max} (cm^{-1}): 3500, 1730, 1680, 1620, 1600, 1510, 1445, 845; ^{1}H and ^{13}C NMR spectral data, see Table 2; HR-FAB-MS *m/z*: 277.1074 $[M+H]^+$ (calcd for $C_{15}H_{17}O_5$, 277.1076).

*3.3.3 3-Hydroxybenzyl 2-(4-oxo-5,6-dihydro-2H-pyran-3-yl) acetate (**3**)*

Colorless oil; UV(MeOH) λ_{max}: 231, 269, 280 nm; IR (KBr) ν_{max} (cm^{-1}): 3450, 1730, 1675, 1625, 1610, 1500, 1450, 780, 690; ^{1}H and ^{13}C NMR spectral data, see Table 2; HR-FAB-MS *m/z*: 263.0918 $[M+H]^+$ (calcd for $C_{14}H_{15}O_5$, 263.0919).

*3.3.4 Desoxypatulinic acid (**4**)*

White amorphous powder; ^{1}H and ^{13}C NMR spectral data, see Table 1.

Acknowledgements

This work was supported by the Ministry of Science and Technology '863' project of China under contract Nos 2007AA09Z413, 2007AA09Z435 and the National Natural Science Foundation of China under contract No. 40776098.

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