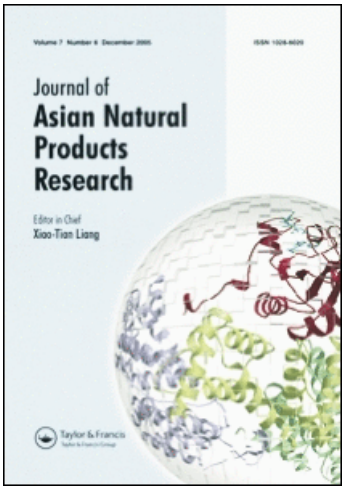

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Xuan Lu[a]; Nan Xu[b]; Hao-Fu Dai[c]; Wen-Li Mei[c]; Zi-Xin Yang[a]; Yue-Hu Pei[a]
[a] School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, China [b] College of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian, China [c] The Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Hainan, China

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Three new compounds from endophytic fungus L10 of *Cephalotaxus hainanensis*

Xuan Lu[a], Nan Xu[b], Hao-Fu Dai[c], Wen-Li Mei[c], Zi-Xin Yang[a] and Yue-Hu Pei[a]*

[a]*School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China;* [b]*College of Pharmacy, Liaoning University of Traditional Chinese Medicine, Dalian 116600, China;* [c]*The Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Hainan 571101, China*

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Three new compounds, 4-hydroxyphenethyl-2′-hydroxypropanoate (**1**), 6-(1′,2′-dimethyloxiran-1′-yl)-4-methoxy-3-methyl-2*H*-pyran-2-one (**2**), 6-(1-hydroxyethyl)-4-methoxy-3-methyl-2*H*-pyran-2-one (**3**), along with the two known compounds, nectriapyrone (**4**) and wermopyrone (**5**), have been isolated from the endophytic fungus of *Cephalotaxus hainanensis*. Their structures were determined on the basis of chemical and spectroscopic methods.

Keywords: *Cephalotaxus hainanensis* Li; endophytic fungus; α-pyrone

1. Introduction

Cephalotaxus hainanensis Li is a Chinese medicine with anticancer activity. The biological active constituents are alkaloids [1]. Some research studies indicated that the endophytic fungus generated some compounds like the plants [2]. So we investigated the fungus in order to isolate some alkaloids with anticancer function. In this paper, we report the isolation and structural elucidation of three new compounds and two known compounds (Figure 1) from the endophytic fungus of *C. hainanensis*.

2. Results and discussion

Compound **1** was obtained as a colorless oil with $[\alpha]_D^{20} + 3.6$ ($c = 5.5$, MeOH). The molecular formula was determined to be $C_{11}H_{14}O_4$ by HR-FAB-MS at *m/z* 211.0971 $[M+H]^+$. The IR spectrum of **1** showed absorption bands at 1614, 1516, and 1450 cm^{-1} indicating the presence of an aromatic ring, which was supported by the observation of the UV spectral data ($\lambda_{max} = 276$ and 229 nm). The ^1H NMR spectrum of compound **1** showed aromatic proton signals at δ 7.02 (2H, d, $J = 8.4$ Hz) and 6.67 (2H, d, $J = 8.4$ Hz). The ^{13}C NMR spectrum of **1** showed six aromatic carbon signals at δ 156.0, 129.9 × 2, 127.9, and 115.3 × 2. Hence, compound **1** was considered to contain a *para*-substituted benzene moiety. Moreover, the ^1H NMR spectrum showed a phenolic hydroxyl proton signal at δ 9.22 and two methylene signals at δ 4.13 (2H, ddd, $J = 10.8$, 7.2, 7.2 Hz) and δ 2.76 (2H, t, $J = 7.2$ Hz), and the ^{13}C NMR spectrum of **1** showed the corresponding carbons of the two methylenes at δ 65.0 and 33.7. On the basis of the above evidence, compound **1** was considered to contain a 4-hydroxyphenethyl. The ^1H NMR spectrum showed

*Corresponding author. Email: peiyueh@vip.163.com

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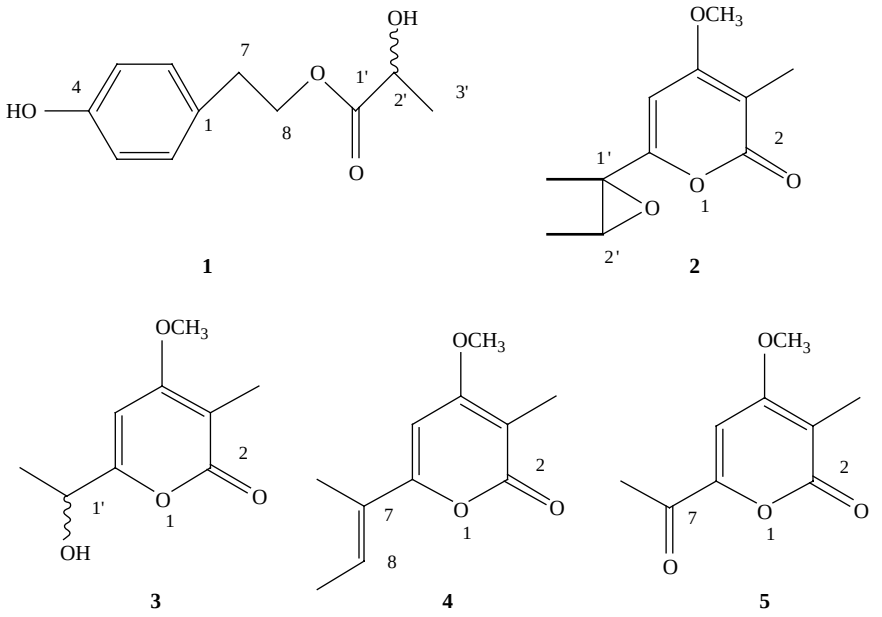

Figure 1. The structures of compounds **1**–**5**.

a methyl signal at δ 1.18 (3H, d, $J = 6.6$ Hz) and a hydroxyl signal at δ 5.32 (1H, d, $J = 3.6$ Hz), and the ^{13}C NMR spectrum of **1** showed the presence of a carbonyl and a methine at δ 174.7 and 66.0, respectively. Hence, compound **1** was considered to contain a 2′-hydroxypanone. In the HMBC experiment of **1** (Figure 2), the correlations of H-7 (δ 2.76) with C-2, 6 (δ 129.9), C-1 (δ 127.9), and H-8 (δ 4.16) with C-1′ (δ 174.8), C-7 (δ 33.7) were shown. On the basis of the above evidence, compound **1** was identified as 4-hydroxyphenethyl-2′-hydroxypropanoate.

Compound **2** was obtained as a yellow oil with $[\alpha]_D^{20} - 3.4$ ($c = 0.33$, MeOH).

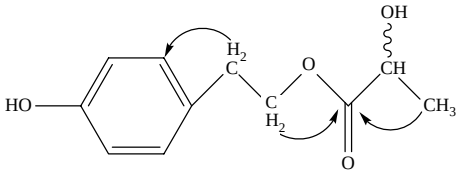

Figure 2. The key HMBC correlations of compound **1**.

The molecular formula was determined to be $C_{11}H_{14}O_4$ by HR-FAB-MS at *m/z* 211.0968 $[M+H]^+$. The ^{1}H NMR spectrum of **2** showed three methyl signals at δ 1.77 (3H, s, 3-CH_3), 1.53 (3H, s, 1′-CH_3), and 1.34 (3H, d, $J = 6.0$ Hz, 2′-CH_3), and a methoxyl signal at δ 3.90 (3H, s, 4-OCH_3). The presence of an α-pyrone ring in **2** was indicated by the ^{13}C NMR signals at δ 166.0 (C-4), 163.7 (C-2), 162.5 (C-6), 100.2 (C-3), 93.3 (C-5), which was supported by the IR absorptions (1680, 1620, and 1565 cm^{-1}) and UV maximum ($\lambda_{max} = 300$ nm). The presence of an oxirane in **2** was indicated by the ^{13}C NMR signals at δ 60.5 (C-1′) and 57.6 (C-2′). In the HMBC experiment of **2**, the correlations between H-5 (δ 6.47) and C-1′ (60.5) were shown. In the NOESY experiment of **2** (Figure 3), the correlations of methyl signal at δ 1.53 with H-5 (δ 6.47) and 2′-CH_3 (δ 1.34), the correlations of methyl signal at δ 1.34 with 1′-CH_3 (δ 1.53), the correlations of methyl signal at δ 1.77 with 4-OCH_3 (δ 3.90), the correlations of methoxyl signal at δ 3.90

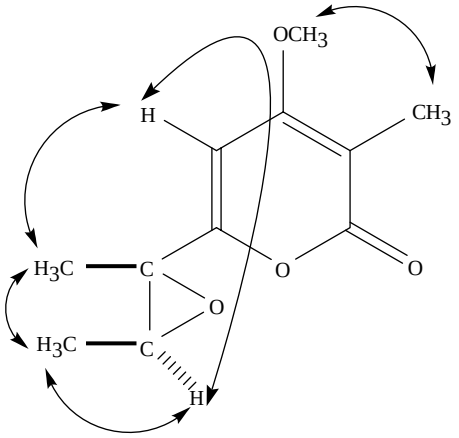

Figure 3. The key NOESY correlations of compound **2**.

with H-5 (δ 6.47) and 3-CH_3 (δ 1.77), the correlations of H-5 (δ 6.47) and H-1′ (δ 3.26) were shown. On the basis of the above evidence, compound **2** was identified as 6-(1′,2′-dimethyloxiran-1′-yl)-4-methoxy-3-methyl-2H-pyran-2-one.

Compound **3** was obtained as a yellow oil with $[\alpha]_D^{20} - 5.9$ ($c = 0.17$, MeOH). The molecular formula was determined to be $C_9H_{12}O_4$ by HR-FAB-MS at *m/z* 185.0812 $[M+H]^+$. The ^{1}H NMR spectrum of compound **3** showed two methyl signals at δ 1.76 (3H, s, 3-CH_3), 1.21 (3H, d, $J = 6.0$ Hz, 1′-CH_3), and a methoxyl signal at δ 3.89 (3H, s, 4-OCH_3). The presence of an α-pyrone ring in **2** was indicated by the ^{13}C NMR signals at δ 167.0 (C-4), 166.2 (C-2), 164.1 (C-6), 99.2 (C-3), and 92.3 (C-5), which was supported by the IR spectral data (1675, 1619, and 1550 cm^{-1}) and UV spectral data ($\lambda_{max} = 287$ nm). The ^{1}H NMR spectrum showed a hydroxyl signal at δ 5.68 (1H, d, $J = 3.6$ Hz). In the HMBC experiment of **3**, the correlations of H-5 (δ 6.52) and C-1′ (65.4) were shown. On the basis of the above evidence, furthermore, by the comparison of its ^{1}H and ^{13}C NMR spectra with those of **2**, compound **3** was established to be 6-(1-hydroxyethyl)-4-methoxy-3-methyl-2H-pyran-2-one.

Two known compounds, nectriapyrone (**4**) and wermopyrone (**5**), were identified by comparison of their spectral data (^{1}H and ^{13}C NMR) with those reported in the literature [3].

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. The UV spectra were measured on a Shimadzu UV-1601. IR spectra were taken on a Bruker IFS-55 infrared spectrophotometer. The NMR data were recorded on Bruker AV-600 (600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR) in DMSO-d_6 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, Uppsala, Sweden), and reversed-phase HPLC (Shimadzu LC-8A vp, Kyoto, Japan).

3.2 Fungus material

C. hainanensis Li was collected in June 2005 in Hainan province of China. A voucher specimen (No. CE20050612) was identified by Associate Researcher Zengfu Dai and has been deposited in the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences. The endophytic fungus was isolated from the phloem of the plant. The hyphostroma was brownish-black.

3.3 Extraction and isolation

The fermentation broth of the endophytic fungus L10 (70 l) was concentrated to 1200 ml, which was extracted with ethyl acetate and *n*-butanol, successively. The ethyl acetate soluble fraction (17.3 g) was subjected to silica gel column, eluted with

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

Position	**2**	**3**
2	163.7	166.2
3	100.2	99.2
4	166.0	167.0
5	93.3	92.3
6	162.5	164.1
1′	60.5	65.4
2′	57.6	
3-CH_3	8.6	8.5
4-OCH_3	56.9	56.8
1′-CH_3	13.9	21.6
2′-CH_3	13.7	

$CHCl_3$—CH_3OH (100:1 → 0:1), yielding 10 fractions. Fraction 3 (2 g) was purified by Sephadex LH-20 column chromatography (CH_3OH) and preparative HPLC (CH_3OH–H_2O: 40:100, flow rate 1 ml/min, wavelength 210 nm) to obtain compound **1** (15 mg, retention time 32 min). Fraction 4 (5 g) was purified by Sephadex LH-20 column chromatography (CH_3OH) and preparative HPLC (CH_3OH—H_2O: 58.5:100, flow rate 1 ml/min, wavelength 210 nm) to obtain **2** (5 mg, retention time 124 min), **3** (3 mg, retention time 56 min), **4** (10 mg, retention time 67 min), and **5** (12 mg, retention time 120 min).

*3.3.1 4-Hydroxyphenethyl-2′-hydroxypropanoate (**1**)*

Colorless oil; $[\alpha]_D^{20}$ + 3.6 (c = 5.5, MeOH), UV (MeOH) λ_{max}: 276, 229 nm; IR (KBr) ν_{max} (cm^{-1}): 1614, 1516, 1450; ^1H NMR (DMSO-d_6) δ: 1.18 (3H, d, J = 6.6 Hz, H-3′), 2.76 (2H, t, J = 6.6 Hz, H-7), 4.10 (1H, q, J = 6.6 Hz, H-2′), 4.16 (2H, ddd, J = 10.8, 7.2, 6.6 Hz, H-8), 6.67 (2H, d, J = 8.4 Hz, H-3, 5), 7.02 (2H, d, J = 8.4 Hz, H-2, 6), 9.22 (1H, s, 4-OH). ^{13}C NMR (DMSO-d_6) δ: 20.5 (C-3′), 33.7 (C-7), 65.0 (C-8), 66.0 (C-2′), 115.3 (C-3, 5), 127.9 (C-1), 129.9 (C-2, 6), 156.0 (C-4), 174.7 (C-1′). HR-FAB-MS *m/z*: 211.0971 $[M+H]^+$ (calcd for $C_{11}H_{15}O_4$, 211.0970).

*3.3.2 6-(1′,2′-Dimethyloxiran-1′-yl)-4-methoxy-3-methyl-2H-pyran-2-one (**2**)*

Yellow oil; $[\alpha]_D^{20}$ − 3.4 (c = 0.33, MeOH), UV (MeOH) λ_{max}: 300 nm; IR (KBr) ν_{max} (cm^{-1}): 1680, 1620, 1565; ^1H NMR (DMSO-d_6) δ: 1.34 (3H, d, J = 6.0 Hz, 2′-CH_3), 1.53 (3H, s, 1′-CH_3), 1.77 (3H, s, 3-CH_3), 3.26 (1H, q, J = 6.0 Hz, 2′-H), 3.90 (3H, s, 4-OCH_3), 6.56 (1H, s, H-5). ^{13}C NMR spectral data, see Table 1. HR-FAB-MS *m/z*: 211.0968 $[M+H]^+$ (calcd for $C_{11}H_{15}O_4$, 211.0970).

*3.3.3 6-(1-Hydroxyethyl)-4-methoxy-3-methyl-2H-pyran-2-one (**3**)*

Yellow oil; $[\alpha]_D^{20}$ − 5.9 (c = 0.17, MeOH), UV (MeOH) λ_{max}: 287 nm; IR (KBr) ν_{max} (cm^{-1}): 1675, 1619, 1550; ^1H NMR (DMSO-d_6) δ: 1.21 (3H, d, J = 6.0 Hz, 1′-CH_3), 1.76 (3H, s, 3-CH_3), 3.89 (3H, s, 4-OCH_3), 4.43 (1H, m, H-1′), 5.68 (1H, d, J = 3.6 Hz, 1′-OH), 6.52 (1H, s, H-5). ^{13}C NMR spectral data, see Table 1. HR-FAB-MS *m/z*: 185.0812 $[M+H]^+$ (calcd for $C_9H_{13}O_4$, 185.0814).

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References

[1] Jiangsu New Medical College, *Dictionary of Traditional Chinese Medicines* (Shanghai People's Publishing House, Shanghai, 1986), Vol. 2, p. 338.
[2] P. Chen, J. Wu, H.F. Dai, X.C. Xie, and W.L. Mei, *Chin. J. Med. Chem.* **18**, 279 (2008).
[3] A.G. Avent, J.R. Hanson, and A. Truneh, *Phytochemistry* **31**, 1065 (1992).