Two new α-Methoxy-γ-Pyrone analogs, 2-methoxy-3-methyl-5,6-diethyl-γ-pyrone (2) and 2-methoxy-3,5-dimethyl-6-propyl-γ-pyrone (3), together with 2-methoxy-3,5-dimethyl-6-ethyl-γ-pyrone (1), firstly isolated from natural sources, were obtained from the EtOAc-soluble extract of the mangrove sediment-derived actinomycete strain Streptomyces psammoticus SCSIO NS126, under the optimized fermentation conditions. Their structures were elucidated by detailed spectroscopic analysis and by comparison of their spectroscopic data with those reported in the literature. Those α-methoxy-γ-pyrone compounds were evaluated for their acetylcholinesterase inhibitory activity; however, none of them exhibited obvious activity. Moreover, their biosynthetic relationship with piericidins was also discussed.

Keywords
actinomycete, Streptomyces psammoticus, pyrone, mangrove sediment, bioactivity

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Introduction

Mangrove forest, distributed in the transition between land and sea, possesses extensive microbial diversity and has the potential to discover new bioactive natural products, including those with potential medicinal application. A series of natural products with novel structure and significant activity have been reported from the microbial community, isolated from the sediment, leaves, branches, and roots. Several studies have shown the uniqueness of mangrove sediments with respect to their microbial composition. Soil or sediment samples collected in mangrove forests showed a high diversity of associated microbes due to their unique ecosystem.

During the course of our search for novel lead compounds, the mangrove sediment-derived strain Streptomyces psammoticus SCSIO NS126 was revealed with important potential medicinal value in our previous study. Twenty-seven natural piericidins were obtained in this strain with antirenal cell carcinoma activities. In order to further explore the comprehensive secondary metabolites of this strain, careful chemical separation study was taken to obtain the different types of compounds except the piericidins. Herein, we report the isolation and structural elucidation of those compounds.

Results and Discussion

The strain was fermented and then harvested by extraction with EtOAc. The extract was subjected to repeated silica gel column chromatography (CC) followed by semipreparative high-performance liquid chromatography (HPLC). As a result, a α-methoxy-γ-pyrene compound firstly isolated from natural sources, 2-methoxy-3,5-dimethyl-6-ethyl-γ-pyrene (1), and 2

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new \( \alpha \)-methoxy-\( \gamma \)-pyrone analogs, 2-methoxy-3-methyl-5,6-diethyl-\( \gamma \)-pyrone (2), and 2-methoxy-3,5-dimethyl-6-propyl-\( \gamma \)-pyrone (3) (Figure 1), were obtained and identified.

Compound 1 was obtained as a pale yellow gum. The molecular formula \( \text{C}_{10} \text{H}_{16} \text{O}_{3} \) was determined by HRESIMS at \( m/z \) 183.1015 [M + H]\(^+\). Compared with the literature NMR data (Table 1),\(^{12} \) this \( \alpha \)-methoxy-\( \gamma \)-pyrone analog (1) was the same as the reported synthetic intermediate, which was further proven by the HMBC correlations of H3 to 7 with C-2, H3 to 9 with C-4/C-6, and H2 to 10 with C-5. Hence, the structure of 1 was determined as shown in Figure 1. It is the first time to obtain the compound from natural sources.

Two \( \alpha \)-methoxy-\( \gamma \)-pyrone analogs, 2 and 3, were also isolated as pale yellow gum, which were determined to have the same molecular of \( \text{C}_{10} \text{H}_{16} \text{O}_{3} \) from the HRESIMS data \( [m/z \text{ } 197.1173 \text{ } [M + H]^+] \) for 2 and \( [m/z \text{ } 197.1182 \text{ } [M + H]^+] \) for 3. Comprehensive analysis of the NMR data (Table 1) indicated that 2 and 3 possessed the similar structures as 1. The only change of 2 was the replacement of the methyl (9-CH\(_3\)) in 1 by ethyl (9-CH\(_2\), 12-CH\(_3\)), which was corroborated by the HMBC correlation from H3 to 9 to C-4, as well as the \( ^1\text{H}-^1\text{H} \) COSY correlations of H2 to 10/H2 to 11/H3 to 12 (Figure 2). For 3, the only difference was the replacement of the ethyl (10-CH\(_2\), 11-CH\(_3\)) in 1 by an \( \alpha \)-propyl (10-CH\(_2\), 11-CH\(_3\), 12-CH\(_3\)) (Table 1), which was confirmed by the HMBC correlation from H2 to 10 to C-12, as well as the \( ^1\text{H}-^1\text{H} \) COSY correlations of H2 to 10/H2 to 11/H3 to 12 (Figure 2). Thus, the structures of 2 and 3 were determined as shown in Figure 1.

Those \( \alpha \)-methoxy-\( \gamma \)-pyrones (1-3) were tested for their acetylcholinesterase (AChE) inhibitory activity,\(^{11} \) and tacrine was used as a positive control. However, none of them exhibited obvious AChE inhibitory activity, with the concentration 1 mg/ml.

Because this strain produces a rich variety of natural piericidin compounds,\(^{11} \) the biosynthetic relationship between those \( \alpha \)-methoxy-\( \gamma \)-pyrone analogs and piericidins is now discussed. The formation of the pyridone ring in piericidins is dependent on the amidation and cyclization of the linear \( \beta \)-diketo carboxylic acid, and ATP-dependent amidotransferase PieD plays a key role in introducing the nitrogen into the pyridone ring in piericidin.\(^{14} \) The only difference of pyrone ring formation in the biosynthetic pathway is the absence of amidation.\(^{15} \) Actinopyrones with \( \alpha \)-methoxy-\( \gamma \)-pyrone ring, such as actinopyrones A-D,\(^{16,17} \) M050511, PM050463, PM060054, and PM060431,\(^{18} \) were also discovered from Streptomyces strains. Not only the chemical structural similarity, actinopyrones showed resembling antibiotic/antitumor activities and related biosynthetic pathway with piericidins.\(^{18,19} \) It’s interesting that 3 \( \alpha \)-methoxy-\( \gamma \)-pyrone analogs have been discovered in this piericidins productive strain, without actinopyrones metabolites.

### Experimental

#### General Experimental Procedures

UV spectra were recorded on a Shimadzu UV-2600 PC spectrometer (Shimadzu). IR spectra were measured on an IR Affinity-1spectrometer (Shimadzu). The NMR spectra were obtained on a Bruker Avance spectrometer (Bruker) operating at 700 MHz for \( ^1\text{H} \) NMR and 175 MHz for \( ^13\text{C} \) NMR, using tetramethylsilane as an internal standard. HRESIMS spectra were collected on a Bruker mix is TOF-QII mass spectrometer (Bruker). Thin layer chromatography (TLC) and CC were performed on plates precoated with silica gel GF254 (10-40 μm) and over silica gel (200-300 mesh) (Qingdao Marine Chemical Factory) and Sephadex LH-20 (Amersham Biosciences), respectively. All solvents employed were of analytical grade (Tianjin Fuyu Chemical and Industry Factory). The semipreparative HPLC was performed on an HPLC (Hitachi-L2130, diode

| Table 1. \( ^1\text{H} \) (700 MHz) and \( ^13\text{C} \) NMR (175 MHz) NMR Data of 1 to 3 in CD\(_3\)OD (δ in ppm). |
|---|---|---|---|---|
| Pos. | \( \delta_\text{C} \), type | \( \delta_\text{H} \) (in Hz) | \( \delta_\text{C} \), type | \( \delta_\text{H} \) (in Hz) | \( \delta_\text{C} \), type | \( \delta_\text{H} \) (in Hz) |
| 1 | | | | | | |
| 2 | 164.6, C | 4.04 (s) | 164.6, C | 4.04 (s) | 164.7, C | 4.03 (s) |
| 3 | 100.0, C | 7.0, CH\(_3\) | 2.12 (t, 7.6) | 12.1, CH\(_3\) | 1.29 (t, 7.6) | 21.5, CH\(_2\) | 1.74 (sextet, 7.5) |
| 4 | 183.2, C | 1.81 (s) | 18.7, CH\(_2\) | 2.44 (q, 7.5) | 10.1, CH\(_3\) | 1.93 (s) |
| 5 | 118.4, C | 1.93 (s) | 24.7, CH\(_2\) | 2.71 (q, 7.6) | 33.3, CH\(_2\) | 2.68 (t, 7.5) |
| 6 | 162.1, C | 2.71 (q, 7.6) | 24.7, CH\(_2\) | 2.71 (q, 7.6) | 33.3, CH\(_2\) | 2.68 (t, 7.5) |
| 7 | 56.3, CH\(_3\) | 4.04 (s) | 56.3, CH\(_3\) | 4.04 (s) | 56.3, CH\(_3\) | 4.03 (s) |
| 8 | 7.0, CH\(_3\) | 1.81 (s) | 7.0, CH\(_3\) | 1.81 (s) | 7.0, CH\(_3\) | 1.81 (s) |
| 9 | 11.5, CH\(_3\) | 1.93 (s) | 18.7, CH\(_2\) | 2.44 (q, 7.5) | 10.1, CH\(_3\) | 1.93 (s) |
| 10 | 25.0, CH\(_2\) | 2.71 (q, 7.6) | 24.7, CH\(_2\) | 2.71 (q, 7.6) | 33.3, CH\(_2\) | 2.68 (t, 7.5) |
| 11 | 9.8, CH\(_3\) | 1.29 (t, 7.6) | 12.1, CH\(_3\) | 1.29 (t, 7.6) | 21.5, CH\(_2\) | 1.74 (sextet, 7.5) |
| 12 | 14.0, CH\(_3\) | 1.05 (t, 7.5) | 13.8, CH\(_3\) | 1.01 (t, 7.5) |  |  |
array detector, Hitachi L-2455, Tokyo, Japan) using a Phenomenex Octadeckylsil (ODS) column (250 mm × 10.0 mm i.d., 5 μm; Phenomenex). The artificial sea salt was a commercial product (Guangzhou Haili Aquarium Technology Company).

**Bacteria Material**

The strain information and the fermentation have been reported in literature.11

**Extraction and Isolation**

The culture broth of this strain was extracted with an equal volume of EtOAc 3 times. The organic extract was then concentrated under vacuum to afford the EtOAc extract (38.2 g). The extract was subjected to silica gel vacuum liquid chromatography using step gradient elution of petroleum ether (PE)–CH2Cl2 (1:0, 2:1, 0:1), CH2Cl2–MeOH (200:1, 100:1, 50:1, 30:1, 0:1) to yield 8 fractions according to TLC profiles (Frs.B1–B8). Frs.B4 (205 mg) was separated into 4 subfractions (Frs.B4–1–B4–4) by ODS silica gel chromatography eluting with MeCN/H2O (5%-100%). Frs.B4 to 3 (20 mg) was directly separated by semipreparative HPLC (30% MeCN/H2O, 2 ml/min, 280 nm) to provide 1 (2.78 mg, tR = 14 min), 2 (2.16 mg, tR = 16 min), and 3 (2.10 mg, tR = 18 min).

2-Methoxy-3,5-dimethyl-6-propyl-pyrole (1): pale yellow gum; IR (film) \( \nu_{\text{max}} \) 1666, 1581, 1464, 1416, 1379, 1338, 1311, 1172 cm\(^{-1}\); UV (MeOH) \( \lambda_{\text{max}} \) (log ε) 203 (3.84), 253 (3.42) nm; \(^1\)H and \(^{13}\)C NMR see Table 1; (+)-HR-ESIMS \( m/z \) 183.1015 [M + H]\(^+\) (calcd for C10H15O3 183.1021).

2-Methoxy-3,5-dimethyl-6-ethyl-pyrole (2): pale yellow gum; IR (film) \( \nu_{\text{max}} \) 1662, 1321, 1142, 1257, 1203, 1172, 1134, 1032, 800 cm\(^{-1}\); UV (MeOH) \( \lambda_{\text{max}} \) (log ε) 205 (3.88), 254 (3.68) nm; \(^1\)H and \(^{13}\)C NMR see Table 1; (+)-HR-ESIMS \( m/z \) 197.1173 [M + H]\(^+\) (calcd for C11H17O3 197.1178).

AChE Inhibitory Bioassay

AChE inhibitory bioassay was assayed using Ellman method and the enzyme was from *Saccharomyces cerevisiae*, Sigma Aldrich by a spectrophotometric method. Tacrine was used as a positive control.13

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