p-Aminoacetophenonic Acids Produced by a Mangrove Endophyte: *Streptomyces griseus* subsp.

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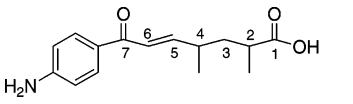
Three new *p*-aminoacetophenonic acids, named 7-(4-aminophenyl)-2,4-dimethyl-7-oxo-hept-5-enoic acid (**1**), 9-(4-aminophenyl)-7-hydroxy-2,4,6-trimethyl-9-oxo-non-2-enoic acid (**2**), and 12-(4-aminophenyl)-10-hydroxy-6-(1-hydroxyethyl)-7,9-dimethyl-12-oxo-dodeca-2,4-dienoic acid (**3**), were isolated from an endophyte of the mangrove plant *Kandelia candel*. The structures of **1**−**3** were elucidated using spectroscopic analyses, primarily NMR and MS.

Mangrove plants are some of the more important ocean resources, and certain mangrove species have been used as medicinal plants in local areas. For example, *Acanthus ilicifolius* extracts have been used to treat rhinopharyngocele and lung cancer, and phytochemical studies of this plant were carried out in recent years.[1,2] However, chemical investigations of most mangrove species have been limited. Endophytes have been rich sources of metabolites with potential for medicinal and agricultural uses.[3] Mangrove-associated fungi have received some attention, and some promising compounds with unique chemical structures and potent activities have been isolated.[4,5] Our aim has been to undertake comprehensive investigations of secondary metabolites of mangrove plants, and their endophytes, in a search for active compounds as leads for drug discovery. A subspecies of *Streptomyces griseus* was isolated from the stems of the mangrove plant *Kandelia candel*, which is one of the primary communities of mangroves in China and has been reported to contain abundant tannins.[6,7]

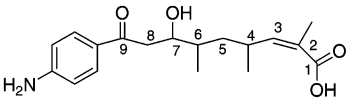
Thirty-three actinomycetes and 21 fungi have been isolated from *K. candel*, from which three of the actinomycetes and several fungal strains were selected by chemical and bioactivity screening to be of interest for further studies. Phytochemical investigation of the culture broth of an isolate identified as a *Streptomyces griseus* subspecies resulted in the isolation of several interesting new compounds (**1**−**3**).

Results and Discussion

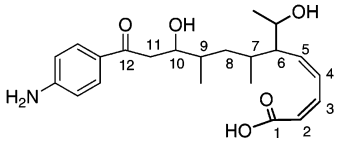
Compound **1** was isolated as a colorless gum; $[\alpha]^{25}_{D}$ −72.7°. Its molecular formula was determined to be $C_{15}H_{19}NO_3$ on the basis of the HRESIMS analysis, which indicated a molecule with seven degrees of unsaturation. Quarternary carbon signals at δ 190.6 and 181.0 implied the presence of carbonyl and carboxylic acid groups. Proton signals at δ 7.77 (dd, 2H, J = 8.8, 2.0 Hz) and 6.64 (dd, 2H, J = 8.8, 2.0 Hz) were readily attributed to a *p*-disubstituted phenyl moiety, the deduction of which was supported by carbon signals at δ 127.0 (C), 132.5 (CH × 2), 114.2 (CH × 2), and 155.0 (C) in the ^{13}C NMR spectrum. Carbon signals at δ 152.9 and 126.1 indicated an olefinic bond, which was supported by coupling of two relevant protons at δ 6.75 (dd, 1 H, J = 8.6, 15.0 Hz, H-5) and 6.97 (d, 1 H, J = 15.0 Hz, H-6) in the ^{1}H−^{1}H COSY spectrum. This olefinic bond was assigned the *E*-configuration on the basis of its large vicinal coupling constant (J = 15.0 Hz).[8] A benzene ring, a carbonyl group, a carboxylic acid group and an unsaturated bond accounted for seven degrees of unsaturation, indicating that the remaining carbon signals at δ 39.5 (CH), 41.8 (CH_2), 36.8 (CH), 18.6 (CH_3), and 20.6 (CH_3) belonged to an aliphatic chain. Thus, compound **1** was characterized as (5*E*)-7-(4-aminophenyl)-2,4-dimethyl-7-oxo-hept-5-enoic acid.



1



2



3

Compound **2**, $[\alpha]^{25}_{D}$ −320.0°, had physicochemical properties similar to those of **1**. The determination of its molecular formula ($C_{18}H_{25}NO_4$) was based on HRESIMS analysis. The extra C_3H_6O moiety and the same degrees of unsaturation as **1** implied that **2** was a homologue of **1**. The quaternary carbon signal at δ 127.8 (C-2) and a tertiary carbon signal at δ 149.4 (C-3) suggested a substituted olefinic bond in the aliphatic chain. It was deduced that the carboxylic acid group was attached to the double bond on the basis of the high-field carbon signal at δ 172.3. The olefinic methyl was consistent with an *E*-configuration of the double bond, having a chemical shift of δ 12.6.[9,10] In the HMBC spectrum, correlations between protons at δ 4.06 (H-7), 2.91, 2.98 (H-8) and a carbonyl carbon at δ 200.2 (C-9) indicated a β-hydroxy-carbonyl in **2** instead of the olefinic ketone in **1**. A carbonyl signal shifted to low field compared with that of **1** (δ 190.6) also supported this deduction. Therefore, **2** was elucidated to be (2*E*)-9-(4-aminophenyl)-7-hydroxy-2,4,6-trimethyl-9-oxo-non-2-enoic acid.

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Compound **3**, $[\alpha]^{25}_D$ −64.1°, was also isolated as a colorless gum. Its molecular formula was determined to be $C_{22}H_{31}NO_5$ on the basis of its HRESIMS data ($[M + H]^+$ ion at *m/z* 390.2199), requiring 8 degrees of unsaturation. Compound **3** contained the same *p*-disubstituted phenyl moiety as **1** and **2** by comparing their NMR data. The appearance of carbonyl and carboxylic acid carbon signals at δ 200.2 and 171.3, respectively, indicated that these two groups existed in the same environments as those of **2**, a conclusion also supported by HMBC data. The ^{13}C NMR spectrum displayed 10 olefinic carbon signals, six of which were consistent with the presence of a benzene ring, and the other four signals appeared at δ 121.4, 146.4, 130.6, and 147.6, suggesting the presence of two olefinic bonds. The configurations of the olefinic bonds were determined as *E* on the basis of their coupling constants ($J_{2,3}$ = 15.0 Hz, $J_{4,5}$ = 15.0 Hz). The HMBC correlations of H-2/C-1, C-3, H-3/C-1, C-5, and H-4/C-2, C-3, C-5, C-6 and $^1H-^1H$ COSY couplings of H-2/H-3, H-3/H-4, H-4/H-5, and H-5/H-6 indicated that these two olefinic bonds were conjugated and attached to a terminal carboxylic acid group. In addition, two oxygenated tertiary carbons at δ 74.1 (C-10) and 80.4 (C_6-CH), observed in the DEPT spectrum, suggested that **3** possessed one more substituted hydroxyl group than **2**. The correlations between a proton at δ 1.05 (3H, d, J = 6.8 Hz) and tertiary carbons at δ 80.4, 41.8 and an olefinic carbon at δ 147.6 indicated a hydroxyethyl substituent at C-6 in the molecule. Thus, compound **3** was (2*E*,4*E*)-12-(4-aminophenyl)-10-hydroxy-6-(1-hydroxyethyl)-7,9-dimethyl-12-oxo-dodeca-2,4-dienoic acid.

Compounds **1**−**3** appear to be precursors of aminoacetophenone heptaene antibiotics such as levorin[11,12] and trichomycin,[13] which are fungicidal compounds known to be produced by *Streptomyces* species and which have a wide spectrum of therapeutic activities.[13,14] These antibiotic compounds could be considered as macrolides esterified at the seventh hydroxyl-substituted carbon (counting from the carbonyl carbon in the side chain). Our HPLC-MS analyses of the crude extract of the *S. griseus* title strain could not detect this class of macrolide compounds. However, three aliphatic-chain aminoacetophenonic acids (**1**−**3**) were obtained, none of which demonstrated any activity in bioassays against macrophages, hydroxysteroid dehydrogenase, peroxidase, or bacteria or fungi, and no cytotoxic activity against L-929, K-562, or HeLa cells.

Experimental Section

General Experimental Procedures. NMR spectra were measured on Bruker Avance dpx 300 (1D) and Brucker Avance drx 500 (2D) instruments. Optical rotations were measured on a Dr. Kernchen digital automatic polarimeter. ESIMS was recorded on an LCQ Thermo Finnigan mass spectrometer. EIMS was recorded using a MAT 95 XL Thermo Finnigan mass spectrometer. HPLC-MS was recorded on a Finnigan Surveyor PDA Detector/Finnigan TSQ Quantum instrument. IR spectra were recorded on a Bruker IFS 55 spectrometer. Reversed-phase chromatography was carried out on Lichrospher RP-18 (12 μm).

Plant Material. Stems of *Kandelia candel* (L.) (Rhizophoraceae) were collected near Xiamen City of Fujian Province, People's Republic of China, in August 2002, and identified by Prof. Peng Lin of Xiamen University. Samples were deposited in the School of Pharmaceutical Sciences, Peking University (200208082). Microbial materials were isolated from the stems of the plant. The *S. griesus* strain (HKI0412), subject of this study, was deposited in Hans-Knöll-Institute, Jena, Germany.

Strain Description and Fermentation. The medium was supplemented with nalidixic acid (20 mg/L) and cycloheximide (30 mg/L). The *K. candel* stem surface was rinsed with sterile H_2O, sterilized by soaking in 70% EtOH for 1 min, and rinsed again with sterile H_2O. The stem was cut into tiny pieces using a sterile knife. The pieces were placed on the surface of agar plates and incubated at 28 °C until growth appeared. Microbial colonies were transferred to fresh agar plates for further growth. The title strain *Streptomyces griseus* subsp. was isolated on ISP 2 medium[15] prepared with artificial sea salt solution (800 mL/L). On the basis of morphological and chemotaxonomic characteristics [the presence of LL-diaminopimelic acid in the peptidoglycan and their characteristic menaquinones MK-9(H_6), MK-9(H_8)] and comparison of 16S rDNA sequence, the title strain was tentatively assigned as *S. griseus* subsp. with the similarity of 99.7%. General laboratory cultivation was performed on oatmeal agar (ISP medium 3[15]) or in liquid organic medium 79[16] at an incubation temperature of 28 °C. For long-term preservation, the culture strain in liquid organic medium 79 was supplemented with 5% DMSO and maintained in the vapor phase of liquid nitrogen.

Liquid organic medium 79 (2 × 100 mL/flask) was inoculated with a suspension of mycelium and spores (about 1 × 1 cm) of the title strain grown on agar slants or agar plates (ISP medium 3). After incubation for 48 h on a rotary shaker at 28 °C, the culture was transferred to 3200 mL of medium 1 (eight 1000 mL-scale Erlenmeyer flasks with 400 mL of medium 1 each) and incubated at 28 °C under shaking conditions for 48 h to yield prefermentation culture, which was poured into a 300 L-scale fermenter filled with 200 L of medium 1 and fermented for 5 days.

Extraction and Isolation. Fermentation supernatant of *S. griseus* subsp. was filtered by centrifugation and subjected to a XAD-161M resin column (20 × 20 cm) eluted with MeOH/H_2O (gradient from 40:60 to 90:10 in 38 min). Seven fractions were collected and lyophilized. Fractions 5, 6, and 7 (total 52 g) were combined on the basis of similar appearance by TLC and extracted with MeOH to obtain 36 g of extract. Nineteen grams of the extract was separated on a silica gel column (5.5 × 40 cm) eluted with $CHCl_3$/MeOH (9:1, 8.8 L) to yield eight fractions. Fraction 3 (120 mg) was further chromatographed on Sephadex LH-20 eluted with MeOH (900 mL) to give five subfractions. Subfraction 4 (12 mg) was purified on a silica gel column (1.4 × 35 cm) eluted with CH_3Cl/MeOH (30:1, 250 mL) to yield compound **2** (6 mg). Subfraction 5 (10 mg) was purified on a RP-18 silica gel column (2 × 40 cm) eluted with MeOH/H_2O (1:1, 500 mL) to yield compound **1** (2 mg). Fraction 8 (160 mg) was further chromatographed on a RP-18 silica gel column (2 × 40 cm) eluted with MeOH/H_2O (3:2, 800 mL) to give five subfractions; subfraction 1 (18 mg) was purified on a silica gel column (1.4 × 35 cm) eluted with CH_3Cl/MeOH (9:1, 150 mL) to yield compound **3** (4.2 mg).

(5*E*)-7-(4-Aminophenyl)-2,4-dimethyl-7-oxo-hept-5-enoic acid (1): colorless gum; $[\alpha]^{25}_D$ −72.7° (*c* 0.22, MeOH); UV λ_{max} (MeOH) 330 nm (ϵ 10 641), 237 nm (ϵ 6967), 202 nm (ϵ 14 676); IR ν_{max} 3358, 2966, 2932, 1705, 1592, 1176, 832 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 2.42 (1H, m, H-2), 1.14 (3H, d, J = 6.6, Hz, CH_3-2), 1.41, 1.80 (2H, m, H-3), 2.48 (1H, m, H-4), 1.05 (3H, d, J = 6.7, Hz, CH_3-4), 6.75 (1H, dd, J = 15.0, 8.6, Hz, H-5), 6.97 (1H, d, J = 15.0, Hz, H-6), 7.77 (2H, dd, J = 8.8, 2.0, Hz, aromatic-ring H-2 × 2), 6.64 (2H, dd, J = 8.8, 2.0, Hz, aromatic-ring H-3 × 2); ^{13}C NMR data, see Table 1; ESIMS *m/z* positive 262.2 $(M + H)^+$, 284.3 $(M + Na)^+$, 545.1 $(2M + Na)^+$, 567.2 $[(2M - H + Na) + Na]^+$; negative 260.3 $(M - H)^-$, 543.4 $[(2M - H + Na) - H]^-$; HRESIMS *m/z* 262.1359 $[(M + H)^+$, calcd for $C_{15}H_{20}NO_3$, *m/z* 262.1367].

(2*E*)-9-(4-Aminophenyl)-7-hydroxy-2,4,6-trimethyl-9-oxo-non-2-enoic acid (2): colorless gum; $[\alpha]^{25}_D$ −320.0° (*c* 0.05, MeOH); UV λ_{max} (MeOH) 330 nm (ϵ 2876), 237 nm (ϵ 1883), 202 nm (ϵ 3967); IR ν_{max} 3363, 3232, 2961, 2929, 1716, 1698, 1684, 1652, 1592, 1174, 832 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 1.82 (3H, d, J = 1.3, Hz, CH_3-2), 6.57 (2H, dd, J = 10.0, 1.3, Hz, H-3), 2.63 (1H, m, H-4), 0.97 (3H, d, J = 6.6, Hz, CH_3-4), 1.18, 1.55 (2H, m, H-5), 1.65 (1H, m, H-6), 0.99 (3H, d, J = 6.6, Hz, CH_3-6), 4.06 (1H, m, H-7), 2.91 (1H, dd, J = 15.6, 4.1, Hz, H-8), 2.98 (1H, dd, J = 15.6, 8.2, Hz, H-8), 7.77 (2H, dd, J = 8.8, 2.0, Hz, aromatic-ring H-2 × 2), 6.64 (2H,

Table 1. ^{13}C NMR Data of Compounds **1**, **2**, and **3**[a]

position	1	2	3
1	181.0	172.3	171.3
2	39.5	127.8	121.4
2-CH_3	18.6	12.6	
3	41.8	149.4	146.4
4	36.8	32.3	130.6
4-CH_3	20.6	16.0	
5	152.9	40.4	147.6
6	126.1	37.9	41.8
6-CH_3		19.8	
6-(1-hydroxyethyl)			
CH			80.4
CH_3			18.0
7	190.6	73.3	34.6
7-CH_3			14.0
8		42.1	36.9
9		200.2	37.3
9-CH_3			15.6
10			74.1
11			42.4
12			200.2
aromatic ring			
1	127.0	127.2	127.3
2 (×2)	132.5	132.1	132.1
3 (×2)	114.2	114.2	114.2
4	155.0	155.3	155.3

[a] Recorded in CD_3OD at 75.5 MHz. Chemical shifts (δ) in ppm.

dd, J = 8.8, 2.0, Hz, aromatic-ring H-3 × 2); ^{13}C NMR data, see Table; ESIMS *m/z* positive 320.2 $(M + H)^+$, 342.4 $(M + Na)^+$, 364.3 $[(M - H + Na) + Na]^+$, 661.3 $(2M + Na)^+$, 683.4 $[(2M - H + Na) + Na]^+$, 705.5 $[(2M - 2H + 2Na) + Na]^+$, 1024.1 $[(3M - 2H + 2Na) + Na]^+$; negative 318.4 $(M - H)^-$, 659.6 $[(2M - H + Na) - H]^-$, 1000.6 $[(3M - 2H + 2Na) - H]^-$; HRESIMS *m/z* 320.1854 $[(M + H)^+$, calcd. for $C_{18}H_{26}NO_4$, *m/z* 320.1861].

(2*E*,4*E*)-12-(4-Aminophenyl)-10-hydroxy-6-(1-hydroxyethyl)-7,9-dimethyl-12-oxo-dodeca-2,4-dienoic acid (3): colorless gum; $[\alpha]^{25}_D$ −64.1° (*c* 1.03, MeOH); UV λ_{max} (MeOH) 317 nm (ϵ 16 273), 259 nm (ϵ 19 234); 202 nm (ϵ 24 010); IR ν_{max} 3351, 3321, 2963, 2930, 1684, 1636, 1592, 1174, 1000.4 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) δ 5.80 (1H, d, J = 15.0, Hz, H-2), 7.23 (1H, dd, J = 10.0, 15.0, Hz, H-3), 6.25 (1H, dd, J = 10.0, 15.0, Hz, H-4), 6.15 (1H, dd, J = 15.0, 7.8, Hz, H-5), 2.52 (1H, m, H-6), 3.17 (1H, m, CH-6), 1.05 (3H, d, J = 6.8, Hz, CH_3-6), 1.63 (1H, m, H-7), 0.88 (3H, d, J = 6.8, Hz, CH_3-7), 1.22, 1.35 (2H, m, H-8), 1.71 (1H, m, H-9), 0.91 (3H, d, J = 6.8, Hz, CH_3-9), 4.05 (1H, m, H-10), 2.93 (1H, dd, J = 15.4, 4.3, Hz, H-11), 2.99 (1H, dd, J = 15.4, 8.1, Hz, H-11), 7.77 (2H, dd, J = 8.8, 2.0, Hz, aromatic-ring H-2 × 2), 6.64 (2H, dd, J = 8.8, 2.0, Hz, aromatic-ring H-3 × 2); ^{13}C NMR data, see Table; ESIMS *m/z* positive 390.5 $(M + H)^+$, 412.6 $(M + Na)^+$, 434.6 $[(M - H + Na) + Na]^+$, 801.4 $(2M + Na)^+$, 823.5 $[(2M - H + Na) + Na]^+$, 845.6 $[(2M - 2H + 2Na) + Na]^+$; negative 388.8 $(M - H)^-$, 799.9 $[(2M - H + Na) - H]^-$; HRESIMS *m/z* 390.2199 $[(M + H)^+$, calcd for $C_{22}H_{32}NO_5$, *m/z* 390.2280].

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