Two Auronols from *Pseudolarix amabilis*

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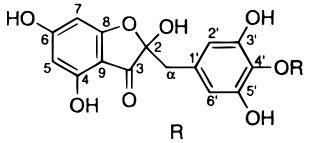
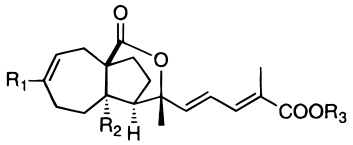
Two new auronols, amaronols A (**1**) and B (**2**), were isolated from the bark of *Pseudolarix amabilis*, along with pseudolaric acid B (**3**), pseudolaric acid C (**4**), demethoxydeacetoxy-pseudolaric acid B (**5**), pseudolaric acid B-β-D-glucoside (**6**), pseudolaric acid A-β-D-glucoside (**7**), and myricetin (**8**). The structures of amaronols A and B were established by spectral data interpretation as 2,4,6-trihydroxy-2-[(3′,4′,5′-trihydroxyphenyl) methyl]-3(*2H*)-benzofuranone and 2,4,6-trihydroxy-2-[(3′,5′-dihydroxy-4′-methoxyphenyl) methyl]-3(*2H*)-benzofuranone, respectively. Antimicrobial testing results of the eight compounds indicated that only pseudolaric acid B was active against *Candida albicans* (MIC, 3.125 μg/mL; MFC, 6.25 μg/mL), while myricetin was marginally active against *Trichophyton mentagrophytes* (MIC, 50 μg/mL).

Pseudolaric acid B (**3**), which was initially isolated from the traditional Chinese herbal medicine, *Pseudolarix kaempferi*,[1,2] was reported to possess significant antifungal[3] and cytotoxic[4] activities. During our antifungal screening program, the ethanol extract of the bark of *Pseudolarix amabilis* L. (Pinaceae) also demonstrated growth inhibitory effects against *Candida albicans*. This extract, when examined by TLC, proved to contain pseudolaric acid B (**3**). Previous phytochemical investigation of this plant indicated the presence of flavonoids.[5] In an effort to search for antifungal compounds from *P. amabilis*, we carried out a bioassay-guided fractionation of the crude ethanol extract. In addition to the isolation of six known compounds which were identified by NMR spectroscopy as pseudolaric acid B (**3**),[1,3] pseudolaric acid C (**4**),[2,3] demethoxydeacetoxy-pseudolaric acid B (**5**),[3] pseudolaric acid B-β-D-glucoside (**6**),[3,6] pseudolaric acid A-β-D-glucoside (**7**),[3,6] and myricetin (**8**),[7] two new auronols (**1** and **2**) were obtained. In the present paper we report the isolation and structure elucidation of these two new compounds and the antimicrobial activity of the eight compounds.

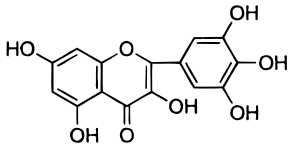
The ethanol extract of the bark of *P. amabilis* was partitioned between hexane and methanol/water (9:1). The methanol/water layer was further partitioned with chloroform. The chloroform-soluble portion was active against *C. albicans* with an MIC of 500 μg/mL. Repeated column chromatography of this portion afforded compounds **1** and **2**, along with the above-mentioned six known compounds (**3**−**8**).



	R
1	H
2	Me



	R_1	R_2	R_3
3	$COOCH_3$	OAc	H
4	$COOCH_3$	OH	H
5	COOH	OH	H
6	$COOCH_3$	OAc	Glc
7	CH_3	OAc	Glc



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Compound **1**, designated as amaronol A, was obtained as a pale yellow powder which on TLC gave a blue color with ferric chloride and a characteristic cherry-red color with acetic anhydride and concentrated sulfuric acid.[8] The UV spectrum showed absorpions at 212, 288, and 333 nm, while the IR spectrum had a characteristic carbonyl absorption at 1685 cm^{-1}. The coloration characteristics together with the above spectral data suggested that compound **1** possessed an auronol skeleton.[8,9] Its molecular formula $C_{15}H_{12}O_8$ was established by high-resolution ESIMS in conjunction with ^{13}C NMR data analysis. The ^1H NMR spectrum displayed two doublets at δ 5.76 (J = 1.2 Hz) and 5.81 (J = 1.2 Hz) integrating for two meta-coupled protons; one singlet at δ 6.10 integrating for two protons; two ABq coupled doublets at δ 2.83 and 2.77 (J = 13.8 Hz), and some broad singlets attributable to phenolic hydroxyl protons. The ^{13}C NMR spectrum exhibited 15 carbon signals (Table 1). The carbonyl signal at δ 193.1 and three relatively downfield hydroxylated carbon signals at δ 172.1, 168.2, and 158.1 indicated that **1** was a 4,6-dihydroxysubstituted auronol derivative.[10] The 3′,4′,5′-trihydroxy substituted pattern on the B-ring was confirmed by the carbon signals at δ 109.5 (CH), 131.7 (C), and 145.4 (C) with an intensity ratio of 2:1:2. Signal assignments were facilitated with the aid of the HMBC experiment (Figure 1). The HMBC correlations of the proton signals at δ 2.83 and 2.77 with the carbon signal at δ 109.5 (C-2′) excluded the possibility of a 2-hydroxy-2,3-dihydroflavone skeleton for the structure of **1**. From the above NMR evidence, the structure of **1** was assigned as 2,4,6-trihy-

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Table 1. NMR Data for Compounds **1** and **2** in DMSO-d_6 (ppm)

	1		**2**	
position	δ_C	δ_H	δ_C	δ_H
2	105.7		105.4	
3	193.1		192.9	
4	158.1		158.1	
5	95.9	5.81 (d, J = 1.2 Hz)	95.9	5.83 (d, J = 1.2 Hz)
6	168.2		168.2	
7	89.9	5.76 (d, J = 1.2 Hz)	89.9	5.77 (d, J = 1.2 Hz)
8	172.1		171.9	
9	101.5		101.2	
OH-2		7.39 (br s)		7.43 (br s)
OH-4		10.55 (br s)		10.58 (br s)
1′	124.4		129.5	
2′,6′	109.5	6.10 (s)	109.8	6.15 (s)
3′,5′	145.4		149.9	
4′	131.7		134.2	
α	41.3	2.83, 2.77 (ABq, J = 13.8 Hz)	41.2	2.84, 2.77 (ABq, J = 13.8 Hz)
OMe-4′			59.7	3.61 (s)
OH-3′,5′		8.60 (br s)		8.83 (br s)

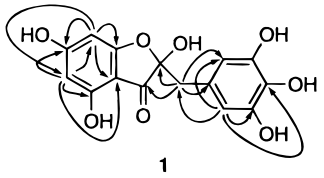

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Figure 1. HMBC correlations of compound **1**.

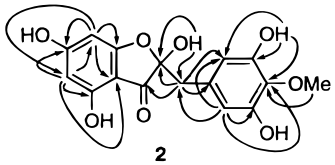

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Figure 2. HMBC correlations of compound **2**.

droxy-2-[(3′,4′,5′-trihydroxyphenyl) methyl]-3(*2H*)-benzofuranone. As already established in the case of 2,4,6-trihydroxy-2-[(4′-trihydroxyphenyl) methyl]-3(*2H*)-benzofuranone (maesopsin),[9] compound **1** is considered to be an enantiomeric pair due to the reversible nature of the hemiketal at C-2.

Compound **2**, designated as amaronol B, gave on TLC a brown color with ferric chloride and a cherry-red color with acetic anhydride and concentrated sulfuric acid.[8] Its UV and IR spectra showed close resemblances with those of **1**. The ^{1}H and ^{13}C NMR spectra of **2** were also similar to those of **1** (Table 1) except for an additional methoxyl group. The ESIMS also suggested the presence of an additional methoxyl group in **2** when compared against **1**. This methoxyl group was placed at C-4′, since the ^{13}C NMR signals of B-ring shifted but remained symmetric (Table 1). In addition, the signal for the methoxyl carbon at δ 59.7, which is in a downfield position when compared to the usual methoxyl group, also suggested an ortho-dihydroxy-substituted environment.[11] Confirmation was made by analysis of the HMBC spectrum (Figure 2). All of the HMBC correlations supported the structure of **2** to be 2,4,6-trihydroxy-2-[(3′,5′-dihydroxy-4′-methoxyphenyl) methyl]-3(*2H*)-benzofuranone. Compound **2** is also an enantiomeric pair due to the reversible nature of the hemiketal at C-2.

Compounds **1**−**8** were examined for antifungal activity against *C. albicans, Cryptococcus neoformans, Aspergillus flavus, A. fumigatus*, and *Trichophyton mentagrophytes* and for antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Pseudomonas aeruginosa*, and *Mycobacterium intracellulare*. Pseudolaric acid B (**3**) selectively inhibited the growth of *C. albicans*; its minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were 3.125 and 6.25 μg/mL, respectively. Myricetin (**8**) was marginally active against *T. mentagrophytes* (MIC, 50 μg/mL). The positive control, amphotericin B, had an MIC and MFC in *C. albicans* of 0.20 and 0.39 μg/mL, respectively. In *T. mentagrophytes*, amphotericin B had an MIC and MFC of 0.39 and 1.56 μg/mL, respectively. The remaining compounds (**1**, **2**, **4**−**7**) were inactive. The testing results indicated that pseudolaric acid B is responsible for the anifungal activity observed in the crude ethanol extract of *P. amabilis*.

Experimental Section

General Experimental Procedures. Melting points were measured with a Thomas-Hoover capillary melting point apparatus and were uncorrected. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. UV spectra were measured on a Hewlett-Packard 8453 spectrometer. IR spectra were recorded on an ATI Mattson Genesis Series FTIR spectrometer. NMR were recorded in DMSO-d_6 with TMS as an internal standard, using a Bruker Avance DRX-400 NMR spectrometer for the ^{1}H, ^{13}C, and HMBC NMR spectra. ESI−FTMS were measured on a Bruker-Magnex BioAPEX 30es ion cyclotron high-resolution HPLC-FT spectrometer by direct injection into an electrospray interface. Column chromatography was run using silica gel (40 μm, J. T. Baker) and reversed-phase silica gel (RP-18, 40 μm, J. T. Baker). TLC was performed on silica gel sheets (Alugram Sil G/UV$_{254}$, Macherey-Nagel, Germany) and reversed-phase plates (RP-18 F$_{254S}$, Merck, Germany).

Plant Material. The bark of *P. amabilis* L. (Pinaceae) was collected at an altitude of 1000 m, in March, 1996 in Jiujiang, Jiangxi Province, People's Republic of China. A voucher specimen of this plant is deposited at the National Center for the Development of Natural Products (Voucher # 3712).

Extraction and Isolation. Dried bark (4 kg) was ground, and the powdered material was percolated with 95% EtOH (15 L × 3). Removal of the solvent by evaporation under vacuum yielded an EtOH extract (278 g). The EtOH extract was dissolved in MeOH/H_2O (9:1, 4 L) and defatted with hexane (1.2 L × 3). The combined hexane layers were evaporated to dryness to afford a dark residue (26.7 g). To the MeOH/H_2O layer were added 400 mL of H_2O and the resulting solution was further partitioned between $CHCl_3$ (3.8 L). The antifungal activity resided in the $CHCl_3$ fraction (65 g). This $CHCl_3$-soluble material was adsorbed on Celite 545 (160 g) and evaporated to dryness. The resulting Celite-coated material was washed successively with $CHCl_3$ and MeOH to yield a $CHCl_3$-soluble fraction (37 g) and a MeOH-soluble fraction (24 g). The $CHCl_3$-soluble fraction was chromatographed on silica gel ($CHCl_3$/MeOH) followed by reversed-phase column (aqueous MeOH) to afford compounds **3** (0.6 g), **4** (2.0 g), **5** (0.01 g), **6** (3.1 g), and **7** (0.22 g). The MeOH-soluble fraction was chromatographed over a flash silica gel column using a stepwise gradient solvent system consisting of $CHCl_3$/MeOH (5% to 10%) to afford **8** (0.15 g) and a fraction (1.4 g) eluted with 15% MeOH/$CHCl_3$. This fraction was further separated on a RP-18 column using 25% MeOH/H_2O to furnish **1** (0.44 g) and **2** (0.32 g).

Amaronol A (**1**): pale yellow powder, mp 110−112 °C; $[\alpha]^{25}_D$ +2.3° (*c* 1.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 212 (4.23), 230 (sh), 288 (4.09), 333 (sh) nm; IR (KBr) ν_{max} 3350 (br), 1685, 1623, 1456, 1322, 1154, 1032, 830 cm^{-1}; NMR data, see Table 1; ESIMS *m/z* 321.0621 {calcd for [M($C_{15}H_{12}O_8$) + H], 321.0605}.

Amaronol B (**2**): pale yellow powder, mp 94−96 °C; $[\alpha]^{25}_D$ −1.7° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 212 (4.23), 230 (sh), 288 (4.15), 335 (sh) nm; IR (KBr) ν_{max} 3360 (br), 1695, 1628, 1460, 1362, 1154, 1048, 830 cm^{-1}; NMR data, see Table 1; ESIMS *m/z* 335.0769 {calcd for [M($C_{16}H_{14}O_8$) + H], 335.0761}.

Antimicrobial Bioassay. The bioassays used are adaptations of those recommended by the National Committee on Clinical Laboratory Standards.[12] Briefly, inocula of the filamentous fungi, *A. fumigatus*, *A. flavus*, and *T. mentagrophytes*, were prepared by scraping spores from fungus growth into Mycophil nutrient broth. Inocula of the yeasts, *C. albicans* and *C. neoformans*, were prepared by diluting 24 or 72 h cultures in Sabouraud dextrose broth or Mycophil broth, respectively. Inocula of the bacteria, *S. aureus* and *P. aeruginosa*, were prepared by diluting 24 h cultures in Eugon nutrient broth. A 72 h culture of *M. intracellulare* was diluted in Middlebrook nutrient broth supplemented with OADC to prepare the inoculum. Plant extracts and column fractions were added to the wells of a 96-well microtiter plate at final concentrations of 500, 100, and 20 μg/mL and pure compounds at 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, and 0.20 μg/mL. Inocula of the appropriate organism was added such that the final volume in each well was 200 μL. Following appropriate incubation periods at either 30 or 37 °C, the inhibitory activity of the samples were assessed as the Minimum Inhibitory Concentration (MIC), the lowest concentration in which at least 80% growth was inhibited. Amphotericin B was used as a positive control.

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