Cytotoxic and Antioxidative Phenolic Compounds from the Traditional Chinese Medicinal Plant, *Myristica fragrans*

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Key words
- *Myristica fragrans*
- Myristicaceae
- cytotoxicity
- antioxidative activity
- phenylpropanoids

Abstract

Two new phenolic compounds were isolated from the fruits of *Myristica fragrans* and their structures were elucidated as (−)-1-(2,6-dihydroxyphenyl)-9-[4-hydroxy-3-(*p*-menth-1-en-8-oxy)-phenyl]-1-nonanone (**1**) and (7*R*,8*R*)-7,8-dihydro-7-(3,4-dihydroxyphenyl)-3′-methoxy-8-methyl-1′-(*E*-propenyl)benzofuran (**2**). In addition, the absolute configuration of (+)-Δ8′-7-acetoxy-3,4,3′,5′-tetramethoxy-8-*O*-4′-neolignan (**3**) was determined for the first time through spectroscopic and chemical methods. Their antioxidative activities against 2,2-diphenyl-1-picrylhydrazyl radical and cytotoxicity against K-562 cells were tested, and (7*S*,8*S*,7′*R*,8′*S*)-4,5′-dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan (**4**) showed the corresponding activities with IC_{50} values of 39.4 and 2.11 μM, respectively.

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Introduction

Reactive oxygen radicals are known to be one of the inducers for cancer [1], as demonstrated in the antitumor effect of lipid peroxidation and the antioxidant defense system [2]. Many antioxidants also exhibit antitumor activities [3, 4]. There is an increasing interest in natural antioxidants due to their safety rather than synthetic antioxidants with their potential toxicity [5]. *Myristica fragrans* Houtt (Myristicaceae) is a traditional Chinese medicinal plant, and its fruits, nutmeg, are used as an aromatic stomachic, analgesic, and anti-inflammatory agent [6]. Up to now, more than 70 compounds have been identified from this plant, which showed various bioactivities, such as antioxidative [7], antitumor [8], antibacterial [9], and hepatoprotective effects [10]. This work is part of our efforts for identifying new anticancer agents and antioxidants from the traditional Chinese medicinal herb. The ethanol extract of the fruits of *M. fragrans* exhibited significant cytotoxicity against the human leukemia cell line (K562), as well as radical-scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) (see **Tables 1** and **2**). The present chemical investigation resulted in the isolation and identification of two new phenolic compounds **1** and **2**, as well as 10 known analogues: (+)-*erythro*-(7*S*,8*R*)-Δ$^{8'}$-7-acetoxy-3,4,3′,5′-tetramethoxy-8-*O*-4′-neolignan (**3**) [11], (7*S*,8*S*,7′*R*,8′*S*)-4,5′-dihydroxy-3,3′-dimethoxy-7,7′-epoxylignan (**4**) [12], (+)-*erythro*-(7*S*,8*R*)-Δ$^{8'}$-4,7-dihydroxy-3,3′,5′-trimethoxy-8-*O*-4′-neolignan-8′-ene (**5**) [13, 14], (+)-*erythro*-(7*S*,8*R*)-Δ$^{8'}$-7-dihydroxy-3,4,5,3′,5′-pentamethoxy-8-*O*-4′-neolignan-8′-ene (**6**) [15], (2*R*)-3-(3,4,5-trimethoxyphenyl)-1,2-propanediol (**7**) [16], (2*R*)-3-(5-methoxy-3,4-methylenedioxyphenyl)-1,2-propanediol (**8**) [17], (2*R*)-3-(3,4-methylenedioxyphenyl)-1,2-propanediol (**9**) [18], (1*R*,2*R*)-1-(4-hydroxy-3-methoxyphenyl)-1,2-propanediol (**10**) [19, 20], 1-(2,6-dihydroxyphenyl)-9-(4-hydroxyphenyl)-1-nonanone (**11**) [21], and 1-(2,6-dihydroxyphenyl)-9-(3,4-dihydroxyphenyl)-1-nonanone (**12**) [22, 23] (**Fig. 1**).

Materials and Methods

Apparatus and chemicals

Optical rotations were measured on a Jasco P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU® 640 spectrophotome-

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Table 1 Cytotoxicity of compounds **2, 4, 5, 11** and **12** against K562 cell lines.

Compound	IC_{50}	
	μg/mL	μM
2	1.2 ± 0.1	3.75 ± 0.31
4	0.7 ± 0.1	2.11 ± 0.30
5	1.0 ± 0.2	2.40 ± 0.48
11	0.8 ± 0.1	2.16 ± 0.27
12	1.7 ± 0.3	4.92 ± 0.87
Cisplatin	0.0076 ± 0.0005	0.0253 ± 0.0017
Crude extracts of *M. fragrans*	9.7 ± 0.5	–

Table 2 Radical scavenging activity of compounds **4, 5** and **10** against DPPH.

Compound	IC_{50}	
	μg/mL	μM
4	13.6 ± 0.5	39.4 ± 1.4
5	71.7 ± 1.8	191.7 ± 4.8
10	24.9 ± 0.5	125.9 ± 2.5
BHT	33.1 ± 0.6	150.6 ± 2.7
Crude extracts of *M. fragrans*	26.4 ± 0.3	–

ter. CD spectra were recorded in MeOH on a Jasco J-180 spectrometer. IR spectra were taken on a Nicolet Nexus 470 spectrophotometer in KBr discs. 1D- and 2D-NMR spectra were recorded on a Jeol JNM-ECP 600 spectrometer using TMS as the internal standard and chemical shifts were recorded as δ values. ESI-MS were measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column (YMC-Pack ODS [A], 20 × 250 mm, 5 μm, 4 mL/min; YMC). TLC and column chromatography (CC) were performed on plates precoated with silica gel GF_{254} (10–40 μm) and over silica gel (200–300 mesh; Qingdao Marine Chemical Factory), and Sephadex LH-20 (Amersham Biosciences), respectively. Vacuum-liquid chromatography (VLC) was carried out over silica gel H (Qingdao Marine Chemical Factory). DPPH (> 99%) and butylated hydroxytoluene (BHT, > 99%) were obtained from J&K Chemical, Ltd. Cisplatin (lyophilized powder) was obtained from Qilu Pharmaceutical Factory. Solvents were distilled prior to use.

Plant material

The fruits of *M. fragrans* Houtt were provided by the Pharmaceutical Factory of Tibetan Medicine of the Tibet Autonomous Region, China. They were identified by Prof. Zhandui from Tibetan Medicine Hospital and further by Dr. Chunxia Zeng from Kunming Institute of Botany. A voucher specimen (KUN-0686056) was deposited in the Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.

Extraction and isolation

The air-dried fruits (4.50 kg) of *M. fragrans* were ground into a powder and extracted with 95% EtOH (3 × 5 L, 24 h each) at room temperature. The extracts were concentrated under reduced pressure. The residue was diluted with water (2 L) and extracted again with cyclohexane (3 × 2 L) and EtOAc (3 × 2 L). The cyclohexane extract (117 g) was subjected to VLC over silica gel H (8 × 12 cm) and eluted with gradient elution of petroleum ether-Me_2CO (v/v 15 : 1, 2000 mL; 8 : 1, 8000 mL; 6 : 1, 6000 mL; 4 : 1, 4000 mL and 2 : 1, 2000 mL). The eluate was collected in portions of 500 mL and eluates containing similar components (TLC) were

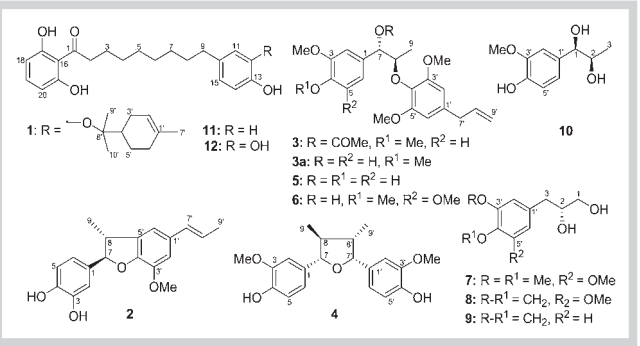

Fig. 1 Chemical structures of compounds **1–12**.

combined to yield 5 fractions (F1–F5). F2 (4 g) was chromatographed over silica gel (3 × 25 cm) and eluted with petroleum ether-$CHCl_3$ (85 : 15, 1500 mL) to afford F2-1 – F2–6, and F2–3 was further separated through semipreparative HPLC (MeOH-H_2O, 65 : 35) to get compound **4** (t_R = 12.0 min, 6.5 mg), **8** (t_R = 6.5 min, 20.3 mg), **9** (t_R = 5.0 min, 14.9 mg). F3 (3 g) was purified by CC over silica gel (2.5 × 25 cm) and eluted with petroleum ether-$CHCl_3$ (4 : 1, 1200 mL), and the final product was compound **5** (12.5 mg). The EtOAc extract (19 g) was subjected to CC over silica gel (5 × 15 cm) and eluted with a step gradient of petroleum ether-Me_2CO (v/v 15 : 1, 300 mL; 10 : 1, 6000 mL; 8 : 1, 600 mL, 6 : 1, 600 mL; 4 : 1, 3000 mL and 2 : 1, 3000 mL). In total, 6 fractions (F1–F6) were obtained. F3 (0.8 g) was chromatographed over Sephadex LH-20 (1.0 × 35 cm) and eluted with $CHCl_3$-MeOH (1 : 1 300 mL) to afford three subfractions, F3-1 – F3-3, and F3-2 was further separated by semipreparative HPLC (MeOH-H_2O, 85 : 15) to give compounds **1** (t_R = 10.5 min, 3.0 mg) and **6** (t_R = 7.3 min, 18.5 mg). F4 (0.6 g) was loaded to the Sephadex LH-20 column (1.5 × 40 cm) and eluted with MeOH (300 mL) to afford compounds **3** (22.0 mg) and **7** (27.0 mg). F5 (0.6 g) was chromatographed over silica gel (1.0 × 20 cm) and eluted with $CHCl_3$-MeOH (19 : 1 400 mL) to afford F5-1 – F5-4, and F5-3 was further separated through semipreparative HPLC (MeOH-H_2O, 75 : 15, 0.1% TFA) to give compounds **2** (t_R = 12.2 min, 7.5 mg), **10** (t_R = 5.5 min, 3.0 mg), **11** (t_R = 16.5 min, 6.7 mg) and **12** (t_R = 14.4 min, 4.2 mg), respectively.

Identification of isolated compounds

(−)-1-(2,6-Dihydroxyphenyl)-9-[4-hydroxy-3-(p-menth-1-en-8-oxy)-phenyl]-1-nonanone (**1**): white amorphous powder; R_f 0.65, silica gel GF_{254}, $CHCl_3$/MeOH (20 : 1); $[\alpha]_D^{20}$: − 2.7 (*c* 0.16, $CHCl_3$); CD (*c* 0.09, MeOH): $\Delta\varepsilon_{191}$ − 10.1, $\Delta\varepsilon_{193}$ + 14.6, $\Delta\varepsilon_{197}$ − 39.8; IR (KBr): ν_{max} = 3424, 1630, 1597, 1506, 1229 cm^{-1}; HR/ESI-MS: *m/z* = 517.2927 $[M + Na]^+$ (calcd. for $C_{31}H_{42}O_5Na$: 517.2930); ^{1}H-NMR (CD_3OD, 600 MHz): δ = 3.09 (2H, t, *J* = 7.3 Hz, H-2), 1.65 (2H, m, H-3), 1.27–1.38 (8H, m, H-4 – H-7), 1.55 (2H, m, H-8), 2.47 (2H, t, *J* = 7.3 Hz, H-9), 6.73 (1H, d, *J* = 1.4 Hz, H-11), 6.72 (1H, d, *J* = 7.9 Hz, H-14), 6.70 (1H, dd, *J* = 1.4, 7.9 Hz, H-15), 6.32 (2H, d, *J* = 8.0 Hz, H-18 and H-20), 7.18 (1H, t, *J* = 8.0 Hz, H-19), 5.39 (1H, brs, H-2′), 2.11 (1H, m)/1.90 (1H, m) (H-3′), 1.91 (1H, m, H-4′), 2.00 (1H, m)/1.38 (1H, m) (H-5′), 2.09 (1H, m)/1.37 (1H, m) (H-6′), 1.64 (3H, s, H-7′), 1.19 (3H, s, H-9′), 1.22 (3H, s, H-10′); ^{13}C-NMR (CD_3OD, 150 MHz): δ = 209.6 (s, C-1), 45.7 (t, C-2), 25.7 (t, C-3), 30.6 (t, C-4), 30.5 (t, C-5), 30.4 (t, C-6), 30.0 (t, C-7), 32.8 (t, C-8), 36.0 (t, C-9), 134.8 (s, C-10), 125.7 (d, C-11), 150.1 (s, C-12), 143.5 (s, C-13), 116.8 (d, C-14), 124.9 (d, C-15), 111.4 (s, C-

16), 163.4 × 2 (s, C-17 and C-21), 108.3 × 2 (d, C-18 and C-20), 136.8 (d, C-19), 135.0 (s, C-1′), 121.9 (d, C-2′), 32.1 (t, C-3′), 45.3 (d, C-4′), 30.6 (t, C-5′), 30.5 (t, C-6′), 23.6 (q, C-7′), 85.3 (s, C-8′), 24.3 (q, C-9′), 23.4 (q, C-10′).

(7R,8R)-7,8-Dihydro-7-(3,4-dihydroxyphenyl)-3′-methoxy-8-methyl-1′-(E-propenyl)benzofuran (**2**): colorless oil; R_f 0.50, silica gel GF_{254}, $CHCl_3$/MeOH (20 : 1); $[\alpha]_D^{20}$: + 46.3 (*c* 0.04, $CHCl_3$); CD (*c* 0.19, MeOH): $\Delta\varepsilon_{240}$ − 7.85, $\Delta\varepsilon_{256}$ + 8.11, $\Delta\varepsilon_{290}$ + 10.39; HR/ESI-MS: *m/z* = 313.1454 $[M + H]^+$ (calcd. for $C_{19}H_{21}O_4$: 313.1440); ^{1}H-NMR (CD_3OD, 600 MHz): δ = 6.83 (1H, d, *J* = 2.0 Hz, H-2), 6.75 (1H, d, *J* = 8.1 Hz, H-5), 6.73 (1H, dd, *J* = 2.0, 8.1 Hz, H-6), 4.99 (1H, d, *J* = 8.8 Hz, H-7), 3.33 (1H, dq, *J* = 6.6, 8.8 Hz, H-8), 1.33 (3H, d, *J* = 6.6 Hz, H-9), 6.82 (1H, brs, H-2′), 6.77 (1H, brs, H-6′), 6.33 (1H, dd, *J* = 1.8, 15.7 Hz, H-7′), 6.11 (1H, dq, *J* = 6.6, 15.7 Hz, H-8′), 1. 83 (3H, dd, *J* = 1.8, 6.6 Hz, H-9′), 3.83 (3H, s, 3′-OMe); ^{13}C-NMR (CD_3OD, 150 MHz): δ = 133.7 (s, C-1), 114.3 (d, C-2), 146.6 (s, C-3), 146.5 (s, C-4), 116.2 (d, C-5), 119.1 (d, C-6), 94.8 (d, C-7), 46.9 (d, C-8), 18.3 (q, C-9), 133.4 (s, C-1′), 111.1 (d, C-2′), 145.3 (s, C-3′), 147.9 (s, C-4′), 134.7 (s, C-5′), 114.7 (d, 6′), 132.3 (d, C-7′), 123.8 (d, C-8′), 18.5 (q, C-9′), 56.7 (q, 3′-OMe).

(+)-Erythro-(7S,8R)-Δ$^{8'}$-7-acetoxy-3,4,3′,5′-tetramethoxy-8-O-4′-neolignan (**3**): colorless oil; R_f 0.60, silica gel GF_{254}, $CHCl_3$/MeOH (15 : 1); $[\alpha]_D^{20}$: + 15.4 (*c* 0.30, $CHCl_3$); CD (*c* 0.13, MeOH): $\Delta\varepsilon_{202}$ + 8.48, $\Delta\varepsilon_{244}$ − 2.56, $\Delta\varepsilon_{280}$ − 1.19. IR (KBr): ν_{max} = 3430, 1625, 1510, 1178, 1223, 1023, 935 cm^{-1}; HR/ESI-MS: *m/z* = 429.1923 $[M - H]^-$ (calcd. for $C_{24}H_{29}O_7$: 429.1913); ^{1}H-NMR ($CDCl_3$, 600 MHz): δ = 6.88 (1H, d, *J* = 1.4 Hz, H-2), 6.80 (1H, d, *J* = 8.3 Hz, H-5), 6.84 (1H, dd, *J* = 1.4, 8.3 Hz, H-6), 5.86 (1H, d, *J* = 3.2 Hz, H-7), 4.43 (1H, dq, *J* = 3.2, 6.4 Hz, H-8), 1.32 (3H, d, *J* = 6.4 Hz, H-9), 6.39 (2H, brs, H-2′ and H-6′), 3.33 (2H, d, *J* = 6.4 Hz, H-7′), 5.96 (1H, m, H-8′), 5.08 (1H, dd, *J* = 1.8, 8.7 Hz, H-9′*Z*), 5.11 (1H, dd, *J* = 1.8, 15.2 Hz, H-9′*E*), 3.86 (3H, s, 3-OMe), 3.84 (3H, s, 4-OMe), 3.78 (6H, s, 3′,5′-diOMe), 2.18 (3H, s, 7-OCOMe); ^{13}C-NMR($CDCl_3$, 150 MHz): δ = 130.4 (s, C-1), 109.9 (d, C-2), 148.5 (s, C-3), 148.3 (s, C-4), 110.6 (d, C-5), 119.0 (d, C-6), 76.4 (d, C-7), 79.9 (d, C-8), 14.3 (q, C-9), 135.6 (s, C-1′), 105.2 × 2 (d, C-2′ and C-6′), 153.2 × 2 (s, C-3′ and C-5′), 133.5 (s, C-4′), 40.4 (t, C-7′), 137.1 (d, C-8′), 115.8 (t, C-9′), 55.8 × 2 (q, 3,4-diOMe), 55.7 × 2 (q, 3′,5′-diOMe), 22.1 (q, 7-OCOMe), 170.1 (s, 7-OCOMe).

(−)-1-(2,6-Dihydroxyphenyl)-9-[3,4-dihydroxyphenyl]-1-nonanone (**12**): colorless crystals (MeOH), mp 128–129 °C; R_f 0.50, silica gel GF_{254}, $CHCl_3$/MeOH (20 : 1); IR (KBr): ν_{max} = 3419, 2932, 2851, 1627, 1520, 1228 cm^{-1}; HR/ESI-MS: *m/z* = 381.1692 $[M + Na]^+$ (calcd. for $C_{21}H_{26}O_5Na$: 381.1678); ^{1}H-NMR (CD_3OD, 600 MHz): δ = 3.08 (2H, t, *J* = 7.3 Hz, H-2), 1.65 (2H, m, H-3), 1.25–1.36 (8H, m H-4 – H-7), 1.52 (2H, m, H-8), 2.41 (2H, t, *J* = 7.8 Hz, H-9), 6.59 (1H, d, *J* = 2.4 Hz, H-11), 6.64 (1H, d, *J* = 7.8 Hz, H-14), 6.45 (1H, dd, *J* = 2.4, 7.8 Hz, H-15), 6.33 (2H, d, *J* = 8.3 Hz, H-18 and H-20), 7.16 (1H, t, *J* = 8.3 Hz, H-19); ^{13}C-NMR (CD_3OD, 150 MHz): δ = 209.7 (s, C-1), 45.7 (t, C-2), 25.7 (t, C-3), 30.6 (t, C-4), 30.6 (t, C-5), 30.5 (t, C-6), 30.3 (t, C-7), 32.9 (t, C-8), 36.3 (t, C-9), 135.8 (s, C-10), 116.5 (d, C-11), 145.9 (s, C-12), 144.0 (s, C-13), 116.2 (d, C-14), 120.6 (d, C-15), 111.4 (s, C-16), 163.4 × 2 (s, C-17 and C-21), 108.3 × 2 (d, C-18 and C-20), 136.8 (d, C-19).

Chemical transformation

9 mg (0.02 mmol) of **3** was stirred with 6 mg of KOH in 6 mL of MeOH and refluxed for 2 h until **3** was consumed. The reaction mixture was cooled to room temperature, 10 mL of H_2O were added and the mixture was extracted with 30 mL of CH_2Cl_2. After drying over anhydrous Na_2SO_4 and evaporation under reduced pressure, the residue from the CH_2Cl_2 layer was purified by semi-preparative HPLC to give compound **3a** (7 mg, 90% yield).

(+)-Erythro-(7S,8R)-Δ$^{8'}$-7-hydroxy-3,4,3′,5′-tetramethoxy-8-O-4′-neolignan (**3a**): colorless oil, $[\alpha]_D^{20}$: + 14.2 (*c* 0.25, $CHCl_3$). CD (*c* 0.12, MeOH): $\Delta\varepsilon_{208}$ + 5.58, $\Delta\varepsilon_{244}$ − 6.04, $\Delta\varepsilon_{273}$ − 1.54; ^{1}H-NMR ($CDCl_3$, 600 MHz): δ = 6.95 (1H, d, *J* = 1.2 Hz, H-2), 6.80 (1H, d, *J* = 8.3 Hz, H-5), 6.76 (1H, dd, *J* = 1.2, 8.3 Hz, H-6), 4.81 (1H, br.s, H-7), 4.34 (1H, dq, *J* = 2.6, 6.6 Hz, H-8), 1.12 (3H, d, *J* = 6.6 Hz, H-9), 6.46 (2H, s, H-2′ and H-6′), 3.37 (2H, d, *J* = 6.6 Hz, H-7′), 5.98 (1H, m, H-8′), 5.11 (1H, br.d, *J* = 8.8 Hz, H-9′*Z*), 5.14 (1H, dd, *J* = 1.5, 15.2 Hz, H-9′*E*), 3.89 (3H, s, 3-OMe), 3.86 (3H, s, 4-OMe), 3.87 (6H, s, 3′,5′-diOMe), 4.12 (1H, d, *J* = 1.8 Hz, 7-OH); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ = 132.6 (s, C-1), 109.2 (d, C-2), 147.9 (s, C-3), 148.8 (s, C-4), 110.7 (d, C-5), 118.1 (d, C-6), 72.8 (d, C-7), 82.3 (d, C-8), 12.8 (q, C-9), 136.1 (s, C-1′), 105.4 × 2 (d, C-2′ and C-6′), 153.5 × 2 (s, C-3′ and C-5′), 132.9 (s, C-4′), 40.6 (t, C-7′), 137.1 (d, C-8′), 116.2 (t, C-9′), 55.9 (q, 3-OMe), 55.8 (q, 4-OMe), 56.1 × 2 (q, 3′,5′-diOMe).

Biological assays

Cytotoxic activities against K562 cell lines and antioxidative activities were evaluated by the MTT method [24] and by the DPPH scavenging assay [25], respectively.

In the MTT assay, the cell lines were grown in RPMI-1640 supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. These cell suspensions (200 µL) at a density of 5×10^4 cell mL^{-1} were plated in 96-well microtiter plates and incubated for 24 h under the above conditions. Next, 2 µL of the test compounds in MeOH at different concentrations were added into each well and further incubated for 72 h under the same conditions. MTT solution (20 µL, 5 mg/mL in RPMI-1640 medium) was added into each well and incubated for 4 h. Old medium (150 µL) containing MTT was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a SpectraMax Plus plate reader at 540 nm.

In the DPPH assay, samples to be tested were dissolved in MeOH and the solution (160 µL) was dispensed into wells of a 96-well microtiter tray. 40 µL of the DPPH solution in MeOH (1.5×10^{-4}) were added into each well. The mixture was shaken and left to stand for 30 min. After the reaction, the absorbance was determined at 520 nm, and the percent inhibition was calculated. IC_{50} values denote the concentration of sample required to scavenge 50% of the DPPH free radicals.

Supporting information

^{1}H- and ^{13}C-NMR spectra of compounds **1** and **2** are available as Supporting Information.

Results and Discussion

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{31}H_{42}O_5$ by positive HR/ESI-MS at *m/z* = 517.2927 $[M + Na]^+$ (calcd.: 517.2930). The IR spectrum showed hydroxy, carbonyl, C=C and aromatic ring absorption bands at 3424, 1716, 1630 and 1506 cm^{-1}, respectively. Its 1D-NMR spectra were similar to those of diphenylnonanonoid compound **12** except for additional 11 protons consisting of one vinyl proton, three methyl protons, and seven upfield protons (**Fig. 1S**, Supporting Information) and 10 carbons consisting of two vinyl carbons, one oxygenated quaternary carbon, one meth-

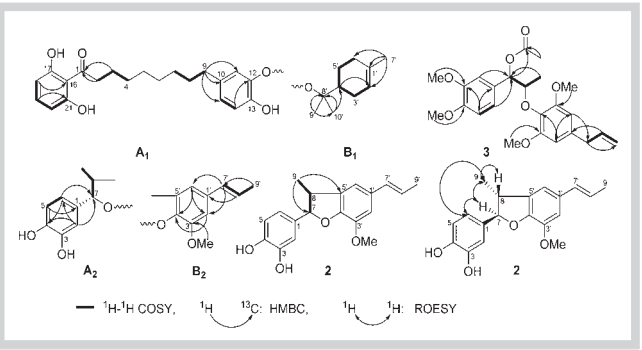

Fig. 2 The key HMBC, ^{1}H-^{1}H COSY, and ROESY correlations of **1**–**3**.

ine carbon, three methylene carbons, and three methyl carbons (**Fig. 2S**, Supporting Information). These data revealed that **1** was composed of a diphenylnonanonoid moiety $\mathbf{A_1}$ and a $C_{10}H_{17}O$ moiety $\mathbf{B_1}$ that was further deduced as *p*-menthenol by 2D-NMR spectra. ^{1}H-^{1}H COSY allowed the construction of the proton spin system: H-2′/H-3′/H-4′/H-5′/H-6′. The HMBC correlations from H-7′ (δ = 1.64, s) to C-1′ (δ = 135.0, s), C-2′ (δ = 121.9, d) and C-6′ (δ = 30.5, t) suggested the connectivity of C-1′ and C-6′, and fixed the 7′-CH_3 to C-1′. HMBC correlations between H-9′ (δ = 1.19, s) with C-8′ (δ = 85.3, s), C-10′ (δ = 23.4, q) and C-4′ (δ = 45.3, d), and between H-10′ (δ = 1.22, s) with C-8′, C-9′ (δ = 24.3, q) and C-4′ confirmed that 9′-CH_3, 10′-CH_3 and C-4′ were attached to the oxygenated quaternary carbon C-8′. Thus, the *p*-menthenol moiety B_1 was assembled. On comparing with those of **12**, the H-11, H-15, C-11 and C-15 were shifted downfield by + 0.14, + 0.25, + 9.2 and + 4.3 ppm, respectively, due to the substitution effects on the *o*- and *p*-positions. These observations showed that moieties A_1 and B_1 was linked together *via* C_{12}-O-$C_{8'}$ that was further supported by the key HMBC correlations between H-14 (δ = 6.72, d, J = 6.0 Hz) with C-12 (δ = 150.1, s), and between H-9 (δ = 2.47, t, J = 7.3 Hz) with C-10 (δ = 134.8, s), C-11 (δ = 125.7, d), and C-15 (δ = 116.8, d) (**Fig. 2**). Therefore, the structure of compound **1** was established as (−)-1-(2,6-dihydroxyphenyl)-9-[4-hydroxy-3-(*p*-menth-1-en-8-oxy)-phenyl]-1-nonanone.

Compound **2** was obtained as a colorless oil. Its molecular formula was determined as $C_{19}H_{20}O_4$ from its positive HR/ESI-MS at *m/z* = 313.1454 $[M + H]^+$ (calcd.: 313.1440). Two phenylpropyl moieties A_2 and B_2 in **2** were elucidated by its 1D- and 2D-NMR spectra (**Fig. 2**), and the *E*-configuration of $\Delta^{7''}$ (**Fig. 1**) was deduced from the large coupling constant of $J_{7'',8''}$ (15.7 Hz). The connection between C-8 and C-5′ was deduced from the HMBC correlation between H-9 (δ = 1.33, d, J = 6.6 Hz) and C-5′ (δ = 134.7, s). C-7 and C-4′ were bridged through an oxygen to form the dihydrobenzofuran ring. ROESY experiments showed correlations between H-7 and H-9, and between H-6 and H-9, while no correlation between H-7 and H-8 was detected (**Fig. 2**). These data, together with the large coupling constant of $J_{7,8}$ (8.8 Hz) in dihydrofuran ring, allow us to establish the *trans*- configuration that was the same as (7*S*,8*S*)-kachirachirol B [26,27]. The opposite specific rotation ($[\alpha]_D$: + 46.3 in $CHCl_3$) of compound **2** to (7*S*,8*S*)-kachirachirol B ($[\alpha]_D$: − 60.0 in $CHCl_3$) suggested that they are the enantiotopic isomers. The CD Cotton effects showed the negative absorption ($\Delta\varepsilon$ − 7.85) at the short wavelength (240 nm) and then positive absorptions ($\Delta\varepsilon$ + 8.11, + 10.39) at the long wavelength (256, 290 nm) similar to (+)-licarin A but opposite to (−)-licarin A [28]. Thus, the structure of **2** was elucidated as (7*R*,8*R*)-7,8-dihydro-7-(3,4-dihydroxyphenyl)-3′-methoxy-8-methyl-1′-(*E*-propenyl)benzofuran.

The constitution of compound **3** was elucidated as 7-acetoxy-3,4,3′,5′-tetramethoxy-8-*O*-4′-neolignan-8′-ene by analysis of its HR/ESI-MS, IR, 1D-NMR and 2D-NMR spectra (**Fig. 2**) [10]. The relative configuration of **3** was elucidated as *erythro* according to the small coupling constant of $J_{7,8}$ (3.2 Hz) and the upfield shift of $\delta_{C\text{-}9}$ (δ = 14.3, CH_3) [29]. The absolute configuration of **3** was deduced as (7*S*,8*R*) from its specific rotation ($[\alpha]_D$: + 15.4) [14] and the negative Cotton effects in the CD spectrum at 243 ($\Delta\varepsilon$ − 22.4) and 280 ($\Delta\varepsilon$ − 4.4) nm [30], which was further supported by the chemical transformation. After being subjected to basic hydrolysis in KOH-MeOH, compound **3** gave the neolignan (**3a**), the structure of which was identified by its ^{1}H- and ^{13}C-NMR spectra and specific rotation ($[\alpha]_D$) [14]. This is the first time that the absolute configuration of **3** has been determined.

The cytotoxicities of the compounds **2**–**6** and **11, 12** were evaluated with K562 cell lines using the MTT method [24]. Compounds **2, 4, 5, 11** and **12** displayed moderate cytotoxicity with IC_{50} values of 3.75, 2.11, 2.40, 2.16 and 4.92 μM (**Table 1**), respectively (cisplatin, positive control, IC_{50} = 25.3 *n*M).

The antioxidative activities of the compounds **3**–**6** and **9**–**11** were examined with the DPPH assay [25]. All the compounds showed radical scavenging activity in a concentration-dependent manner, among which compounds **4, 5** and **10** were more active than or close to the positive control, BHT (IC_{50} = 150.6 μM), with IC_{50} values of 39.4, 191.7 and 125.9 μM, respectively (**Table 2**).

Compounds **1**–**12** comprise three structural types, i.e., diphenylnonanonoids, phenylpropanoids and bisphenylpropanoids (lignans and neolignans) which were also the main categories of compounds from *M. fragrans* in 60 literature references. The antioxidative activity of compounds **4** and **10** (IC_{50} = 13.6 and 24.9 μg/mL, respectively) corresponds to that of the crude extracts (IC_{50} = 26.4 μg/mL) (**Table 2**), showing that **4** and **10** are probably the main active components.

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