Prenylated Benzene Metabolites from *Melicope pteleifolia*

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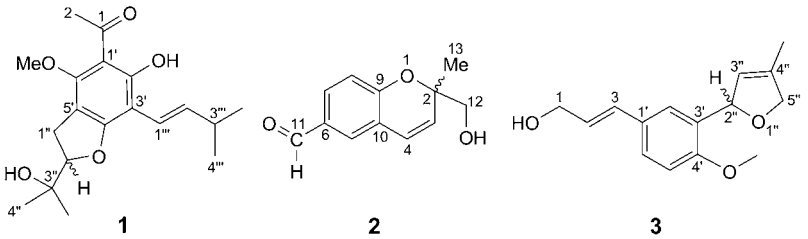
Chemical investigation on the stem and root of *Melicope pteleifolia* afforded three new prenylated benzene metabolites as racemic mixtures, named pteleifolins A–C (**1**–**3**, resp.). Their gross structures were elucidated on the basis of spectroscopic analysis, especially 2D-NMR experiments. An enantiomer resolution of (±)-**1** using chiral HPLC was performed, and the absolute configuration of the enantiomers were determined to be (+)-(*S*)-**1** and (−)-(*R*)-**1** by means of circular-dichroism analysis.

Introduction. – *Melicope pteleifolia* (CHAMP. ex BENTH.) T.HARTLEY (= *Melicope ptelefolia*, classified originally as *Euodia lepta* (SPRENG.) MERR. or *Evodia lepta*, Rutaceae), is a deciduous shrub or arbor distributed in southern China and Southeast Asia [1]. Its whole plant, known as '*San-ya-ku*' in traditional Chinese medicine, has been used as an antipyretic, anti-inflammatory, and analgesic agent to treat trauma, abscess, eczema, dermatitis, and haemorrhoids [2]. The structural richness of the title plant is exemplified by the large variety of secondary metabolites such as chromenes/benzopyrans [3][4], alkaloids [3][5][6], coumarins [7], flavonoids [3][8][9], sesquiterpenoids [3], and acetophenones [10]. Due to our continuing interest in this plant [3], we undertook a phytochemical investigation on the AcOEt fraction of a 95% EtOH extract of the stem and root, resulting in the isolation of three new prenylated benzene metabolites as racemic mixtures, named pteleifolins A–C (**1**–**3**, resp.). We herein describe the isolation and structure elucidation of the three new compounds. An enantiomer resolution of (±)-**1** using chiral HPLC was performed and the absolute configuration of the enantiomers of **1** were determined to be (+)-(*S*)-**1** and (−)-(*R*)-**1** by means of circular-dichroism (CD) analysis.



1 **2** **3**

Results and Discussion. – Compound **1** was obtained as a white powder. The molecular formula $C_{19}H_{26}O_5$ was established from the *quasi*-molecular-ion peak [*M* +

Na]$^+$ at *m/z* 357.1690 in the HR-ESI-MS. The analysis of the 1D- and 2D-NMR spectra allowed us to elucidate the structure of **1** to be 1-{6-hydroxy-2-(2-hydroxypropan-2-yl)-4-methoxy-7-[(1*E*)-3-methylbut-1-en-1-yl]-2,3-dihydro-1-benzofuran-5-yl}ethanone.

The ^{1}H-NMR spectrum in $CDCl_3$ of **1** showed signals for one phenol OH group at δ(H) 14.46 (*s*), a pair of (*E*)-C=C bond H-atoms at δ(H) 6.51 (br. *s*, 2 H) (at δ(H) 6.55 (*dd*, *J* = 16.3, 6.5, 1 H) and 6.48 (*d*, *J* = 16.3, 1 H), when the spectrum was recorded in CD_3OD), one MeO group at δ(H) 3.91 (*s*), one AX_2 spin system at δ(H) 4.70 (*dd*, *J* = 9.3, 8.2, 1 H), 3.27 (*dd*, *J* = 14.8, 8.2, 1 H), and 3.21 (*dd*, *J* = 14.8, 9.3, 1 H), one AcO group at δ(H) 2.62 (*s*), two tertiary Me groups at δ(H) 1.40 and 1.26, and two further Me groups at δ(H) 1.10 (*d*, *J* = 6.8, 6 H). The ^{13}C-NMR spectrum of **1** displayed 18 C-atom signals, which corresponded to one 2′,4′,6′-trioxygenated acetophenone moiety (δ(C) 203.4, 164.4, 164.3, 156.9, 108.2, 108.0, 105.2 (each *s*), and 32.4 (*q*), one MeO group (δ(C) 59.4), and two C_5 units, indicating a diprenylated acetophenone derivative. The IR absorption bands at 2958 and 1612 cm^{-1}, as well as the ^{1}H-NMR signal of the chelated OH group at δ(H) 14.46 (*s*), revealed a 2′-hydroxyacetophenone skeleton for **1**. The MeO group was placed at C(6′), since it exhibited NOE correlation with the AcO Me signal (*Fig.*). An (1-hydroxy-1-methylethyl)dihydrofuran moiety was elucidated based on the similarity of ^{1}H- and ^{13}C-NMR signals to those of acronyculatin B [11] and HMBC experiments (*Fig.*), which is fused along the C(4′)=C(5′) bond as indicated by NOE cross-peak of the MeO with CH_2(1″) (*Fig.*). The remaining ^{1}H- and ^{13}C-signals were ascribed to a (1*E*)-3-methylbut-1-en-1-yl group, attached at C(3′) as indicated by HMBCs (*Fig.*). Accordingly, the gross structure of pteleifolin A was established as **1**.

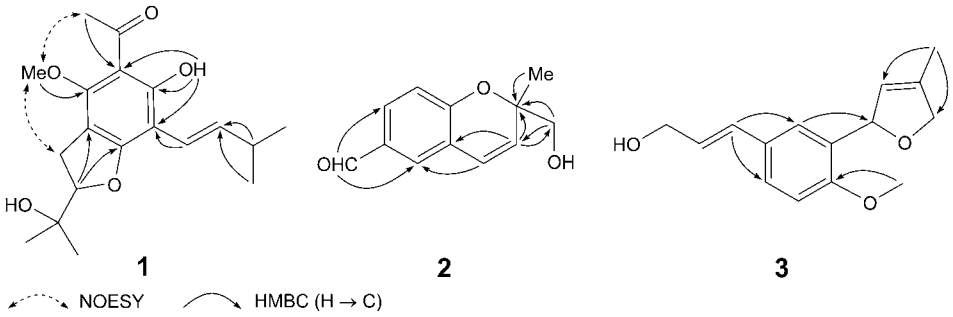


Figure. *Significant 2D-NMR correlations of* **1**–**3**

Compound **1** showed no optical activity, suggesting that **1** might be a racemate. An enantiomer resolution of (±)-**1** by means of chiral HPLC was performed to yield (+)-**1** and (−)-**1** with opposite CD absorptions at 221, 256, and 293 nm. *Nasini* and co-workers reported that acremine N, a similar compound with (+)-(*S*)-configuration, had CD absorptions at 216.4 (− 3.02), 238.0 (+2.82), and 301.4 (− 1.49) nm [12], which were in agreement with those of (+)-**1**, establishing the absolute configurations of the two isomers as (+)-(*S*)-**1** and (−)-(*R*)-**1**.

Compound **2** was obtained as a white amorphous powder, which had the molecular formula of $C_{12}H_{12}O_3$ as deduced from the HR-EI-MS molecular-ion peak at *m/z* 204.0785. The ^{1}H-NMR spectrum of **2** showed signals for an aldehyde H-atom at δ(H)

9.82 (*s*), a 1,2,4-substituted aromatic ring at δ(H) 7.65 (*dd*, J = 8.3, 2.0, 1 H), 7.52 (*d*, J = 2.0, 1 H), and 6.89 (*d*, J = 8.3, 1 H), a pair of coupled olefinic H-atoms at δ(H) 6.53 and 5.67 (2*d*, J = 10.0), one HO–CH_2 at δ(H) 3.69 and 3.67 (2*d*, J = 11.8), one Me group at δ(H) 1.41 (*s*). The above spectral characteristics suggested **2** to be a 2-(hydroxymethyl)-2-methyl-2*H*-chromene with an aldehyde group attached to the aromatic ring [13]. The HMBCs of δ(C) 128.2 (C(5)) with δ(H) 9.82 (H–C(11)) and 6.53 (H–C(4)) disclosed that the aldehyde group was located at C(6) (*Fig.*). The gross structure of **2** was therefore determined to be 2-(hydroxymethyl)-2-methyl-2*H*-chromene-6-carbaldehyde.

The EI-MS spectrum of **3** showed the molecular-ion peaks at *m*/*z* 246, corresponding to the molecular formula $C_{15}H_{18}O_3$, which was agreement with the HR-EI-MS molecular-ion peak at *m*/*z* 246.1259. The ^{1}H- and ^{13}C-NMR spectra of **3** exhibited signals for a 1,2,4-trisubstituted aromatic ring (δ(H) 7.41 (*d*, J = 2.3, 1 H), 7.24 (*dd*, J = 8.4, 2.3, 1 H), and 6.81 (*d*, J = 8.4, 1 H); δ(C) 156.1 (*s*), 131.4 (*s*), 129.5 (*s*), 126.8 (*d*), 124.5 (*d*), 110.5 (*d*)), a prop-2-en-1-ol function (δ(H) 6.55 (*d*, J = 15.8, 1 H), 6.25 (*dt*, J = 15.8, 6.0, 1 H), and 4.28 (br. *s*, 2 H); δ(C): 131.4 (*d*), 126.5 (*d*), and 64.1 (*t*)), one MeO group (δ(H) 3.84 (*s*); δ(C) 55.7), and a C_5 unit with a composition of C_5H_7O (δ(H): 6.07 (*dddq*, J = 5.7, 1.8, 1.8, 1.8, 1 H), 5.58 (*dddq*, J = 1.8, 1.8, 1.8, 1.8, 1 H), 4.71 (*dddq*, J = 12.2, 5.7, 1.1, 1.1, 1 H), 4.63 (*dddq*, J = 12.2, 1.1, 1.1, 1.1, 1 H), and 1.78 (*dddd*, J = 1.6, 1.6, 1.6, 1.6, 3 H); δ(C): 135.9 (*s*), 123.4 (*d*), 83.2 (*d*), 78.4 (*t*), and 12.5 (*q*)), which was attributed to a 4-methyl-2,5-dihydrofuran-2-yl moiety based on a large homoallyl coupling constant (5.7) between H–C(2″) and H–C(5″), the HMBC cross-peaks (*Fig.*), and biogenetic considerations. The HMBCs also established that the prop-2-en-1-ol, MeO, and the C_5 unit were at C(1′), C(4′), and C(3′) of the aromatic ring, respectively, substantiating the planar structure of **3** as (2*E*)-3-[4-methoxy-3-(2,5-dihydro-4-methylfuran-2-yl)phenyl]prop-2-en-1-ol.

Both **2** and **3** were also 1:1 mixture of enantiomers as concluded from their zero optical rotation, and two isolated peaks with equal areas in their chiral HPLC chromatograms.

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh, 400 mesh; *Qingdao Haiyang, Co., Ltd.*, Qingdao, P. R. China), *Sephadex LH-20* (*Pharmacia Biotech AB*, S-Uppsala), and *MCI gel CHP-20P* and *ODS-A gel* (*Mitsubishi Chemical Industries Co., Ltd.*, Japan). TLC: Silica gel *HSGF*$_{254}$ (*Yantai Jiangyou Guijiao Kaifa Co., Ltd.*, Yantai, P. R. China). Semi-prep. HPLC: *Waters* HPLC system, *Waters-2545-HPLC* pump, *Waters-2489* detector, column: *xbridge-C18*, 5 μm, i.d. 10 × 250 mm. Chiral HPLC: *LC-10AT VP PLUS* pump, *SPD-10A VP PLUS* detector, 254 nm, column: *Daicel OD-H*, 5 μm, i.d. 4.6 × 250 mm. Optical rotation: *Perkin-Elmer 341* polarimeter. Circular dichroism (CD): *Jasco J-810* spectropolarimeter. UV Spectra: *Shimadzu UV-2550* spectrophotometer. IR Spectra: *Nicolet-Magna-750-FTIR* spectrometer; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AV-500* instrument at 500 (^{1}H) and 125 MHz (^{13}C); in $CDCl_3$ or CD_3OD soln.; δ in ppm rel. to Me_4Si; J in Hz. ESI-MS and HR-ESI-MS: *Bruker Esquire 3000 plus* and *Finnigan LC QD ECA* mass spectrometers, in *m*/*z* (rel. int.). EI-MS and HR-EI-MS: *Finnigan MAT-95* mass spectrometers; in *m*/*z* (rel. int.).

Plant Material. The chopped stem and root of *M. pteleifolia* was purchased from Bozhou Chinese Materia Medica Market, Bozhou, Anhui Province, P. R. China, in July 2009, and identified by Prof. *Da-Yuan Zhu* at Shanghai Institute of Materia Medica. A voucher specimen (No. 09-1008) was deposited with the Herbarium of Shanghai Institute of Materia Medica.

Extraction and Isolation. The stem and root of *M. pteleifolia* (10 kg, dry) was extracted with 95% EtOH at r.t. three times, and the extract was suspended in H_2O and then partitioned successively with petroleum ether (PE), AcOEt, and BuOH. The AcOEt fraction (300 g) was subjected to CC (SiO_2 (2 kg), column i.d. 10 × 80 cm; PE, PE/acetone 100:1–2.5:1 (*v/v*): *Frs. A–M. Fr. K* (18.0 g) was separated by CC (SiO_2 (800 g); PE/acetone 10:1, 5:1, 3:1, 2:1, 0:1 (*v/v*): *Frs. K1–K12. Fr. K5* (91 mg) was purified by CC (*RP-18* (50 g), MeOH/H_2O 4:1 (*v/v*), followed by semi-prep. HPLC (MeCN/H_2O 67:33): **1** (3 mg). *Fr. K6* (439 mg) afforded **2** (2 mg) and **3** (2 mg) after purification by CC (*ODS-A* (100 g); MeOH/H_2O 4:6, 5:5, 6:4, 7:3, 8:2 (*v/v*), followed semi-prep. HPLC (MeCN/H_2O 1:4 for **2** or MeOH/H_2O 1:1 for **3**).

Enantiomer Resolution of (±)-**1**. Racemate (±)-**1** (1.5 mg) was separated by HPLC (pump: *LC-10A VP*, column: *Daicel OD-H*, hexane/^{i}PrOH 94:6): (+)-**1** and (−)-**1** (each 0.5 mg).

Chiral HPLC of **2** *and* **3**. Pump: *LC-10A VP*, column: *Daicel OD-H*, hexane/^{i}PrOH/$NHEt_2$ 90:10:0.1 for (±)-**2**, and hexane/^{i}PrOH 90:10 for (±)-**3**.

*(±)-1-{6-Hydroxy-2-(2-hydroxypropan-2-yl)-4-methoxy-7-[(1*E*)-3-methylbut-1-en-1-yl]-2,3-dihydro-1-benzofuran-5-yl}ethanone* (**1**). Amorphous powder. UV (MeOH): 208 (1670), 258 (4509), 307 (2171). IR: 3430, 2958, 2921, 2850, 1725, 1612, 1434, 1371, 1245. ^{1}H-NMR ($CDCl_3$, 500 MHz): 14.46 (*s*, HO–C(2′)); 6.51 (br. *s*, H–C(1‴), H–C(2‴)); 4.70 (*dd*, *J* = 9.3, 8.2, H–C(2″)); 3.91 (*s*, MeO–C(6′)); 3.27 (*dd*, *J* = 14.8, 8.2, H_a–C(1″)); 3.21 (*dd*, *J* = 14.8, 9.3, H_b–C(1″)); 2.62 (*s*, Me–C(1)); 2.50 – 2.42 (*m*, H–C(3‴)); 1.40 (*s*, Me(5″)); 1.26 (*s*, Me(4″)); 1.10 (*d*, *J* = 6.8, Me(4‴), Me(5‴)). ^{1}H-NMR (CD_3OD, 500 MHz): 6.55 (*dd*, *J* = 16.3, 6.5, H–C(2‴)); 6.48 (*d*, *J* = 16.3, H–C(1‴)); 4.71 (*dd*, *J* = 8.7, 8.4, H–C(2″)); 3.31–3.29 (overlapped by solvent peak, CH_2(1″)); 2.60 (*s*, Me(2)); 2.44–2.33 (*m*, H–C(3‴)); 1.29 (*s*, Me(5″)); 1.27 (*s*, Me(4″)); 1.06 (*d*, *J* = 6.7, Me(4‴), Me(5‴)). ^{13}C-NMR (125 MHz, $CDCl_3$): 203.4 (*s*, C(1)); 164.4 (*s*, C(2′)), 164.3 (*s*, C(4′)), 156.9 (*s*, C(6′)), 141.3 (*d*, C(2‴)), 116.1 (*d*, C(1‴)), 108.2 (*s*, C(1′)), 108.0 (*s*, C(5′)), 105.2 (*s*, C(3′)), 90.7 (*d*, C(2″)), 71.7 (*s*, C(3″)), 59.4 (*q*, MeO–C(6′)); 32.9 (*d*, C(3‴)), 32.4 (*q*, C(2)); 28.4 (*t*, C(1″)), 26.5 (*q*, C(5″)), 24.7 (*q*, C(4″)), 22.9 (*q*, C(4‴), C(5″)). ESI-MS (pos.): 335 ($[M + H]^+$). ESI-MS (neg.): 334 ($[M - H]^-$). HR-ESI-MS: 357.1690 ($[M + Na]^+$, $C_{19}H_{26}NaO_5^+$; calc. 357.1678).

Data of (−)-(*R*)-**1**. $[\alpha]_D^{22} = -33$ (*c* = 0.012, MeOH). CD (*c* = 0.012, MeOH): 221 (+4.1), 256 (−6.0), 293 (+4.8).

Data of (+)-(*S*)-**1**. $[\alpha]_D^{22} = +33$ (*c* = 0.009, MeOH). CD (*c* = 0.009, MeOH): 221 (−3.1), 256 (+4.6), 293 (−3.5).

*(±)-2-(Hydroxymethyl)-2-methyl-2*H*-chromene-6-carbaldehyde* (**2**). Amorphous powder. UV (MeOH): 257 (1224), 302 (204). IR: 3415, 2971, 2925, 2850, 2740, 1685, 1641, 1598, 1571, 1486, 1440, 1367, 1261. ^{1}H-NMR (500 MHz, $CDCl_3$): 9.82 (*s*, H–C(11)); 7.65 (*dd*, *J* = 8.3, 2.0, H–C(7)); 7.52 (*d*, *J* = 2.0, H–C(5)); 6.89 (*d*, *J* = 8.3, H–C(8)); 6.53 (*d*, *J* = 10.0, H–C(4)); 5.67 (*d*, *J* = 10.0, H–C(3)); 3.69 (*d*, *J* = 11.8, H_a–C(12)); 3.67 (*d*, *J* = 11.8, H_b–C(12)); 1.41 (*s*, Me–C(2)). ^{13}C-NMR (125 MHz, $CDCl_3$): 190.9 (*d*, C(11)); 158.5 (*s*, C(9)); 132.4 (*d*, C(7)); 130.4 (*s*, C(6)); 128.2 (*d*, C(5)); 127.8 (*d*, C(3)); 124.2 (*d*, C(4)); 121.1 (*s*, C(10)); 116.8 (*d*, C(8)); 81.2 (*s*, C(2)); 69.2 (*t*, C(12)); 23.5 (*q*, C(13)). EI-MS: 204 (1, M^+), 173 (100), 144 (6), 115 (6), 91 (2), 77 (1). HR-EI-MS: 204.0785 (M^+, $C_{12}H_{12}O_3^+$; calc. 204.0786).

*(±)-(2*E*)-3-[4-Methoxy-3-(2,5-dihydro-4-methylfuran-2-yl)phenyl]prop-2-en-1-ol* (**3**). Amorphous powder. UV (MeOH): 212 (6396), 262 (5166). IR: 3411, 2940, 2838, 1718, 1606, 1496, 1249. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.41 (*d*, *J* = 2.3, H–C(2′)); 7.24 (*dd*, *J* = 8.4, 2.3, H–C(6′)); 6.81 (*d*, *J* = 8.4, H–C(5′)); 6.55 (*d*, *J* = 15.8, H–C(3)); 6.25 (*dt*, *J* = 15.8, 6.0, H–C(2)); 6.07 (*dddq*, *J* = 5.7, 1.8, 1.8, 1.8, H–C(2″)); 5.58 (*dddq*, *J* = 1.8, 1.8, 1.8, 1.8, H–C(3″)); 4.71 (*dddq*, *J* = 12.2, 5.7, 1.1, 1.1, H_a–C(5″)); 4.63 (*dddq*, *J* = 12.2, 1.1, 1.1, 1.1, H_b–C(5″)); 4.28 (*s*, CH_2(1)); 3.84 (*s*, MeO–C(4′)); 1.78 (*dddd*, *J* = 1.6, 1.6, 1.6, 1.6, Me–C(4″)). ^{13}C-NMR (125 MHz, $CDCl_3$): 156.1 (*s*, C(4′)); 135.9 (*s*, C(4″)); 131.4 (*d*, C(3)); 131.4 (*s*,C(3′)); 129.5 (*s*, C(1′)); 126.8 (*d*, C(6′)); 126.5 (*d*, C(2)); 124.5 (*d*, C(2′)); 123.4 (*d*, C(3″)); 110.5 (*d*, C(5′)); 83.2 (*d*, C(2″)); 78.4 (*t*, C(5″)); 64.1 (*t*, C(1)); 55.7 (*q*, MeO–C(4′)); 12.5 (*q*, Me–C(4″)). EI-MS: 246 (M^+, 43), 215 (24), 191 (100), 115 (13), 91 (8), 83 (16), 77 (6). HR-EI-MS: 246.1259 (M^+, $C_{15}H_{18}O_3^+$; calc. 246.1256).

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