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Sesquiterpenoids from *Illicium difengpi* and their Chemotaxonomic Significance

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ABSTRACT

Illicium difengpi (family Illiciaceae) is a known medicinal plant used in Ancient Chinese medicines for the treatment of diseases and infections. The purpose of the present study was to investigate the chemical constituents of the stem bark of *Illicium difengpi* and determine their chemotaxonomic significance. The stem barks of *I. difengpi* were purchased from Caitongde Pharmacy and authenticated by Professor Lian-na Sun. The 80 % ethanol extract was fractionated with silica gel column chromatography (C.C.) and compounds were isolated with ODS C.C., silica gel (SiO_2) C.C. and preparative/semipreparative HPLC from the fractions. The chemical structures were elucidated by NMR spectroscopy and mass spectrometry, as well as the comparison of spectra data with that reported in the literature. The present phytochemical investigation on *Illicium difengpi* led to the isolation and characterization of **13** sesquiterpenoids, including five eudesmanes, three cadinanes, two bullatanes and three other types of sesquiterpenoids. All compounds except compound **2** were isolated from this species for the first time. In addition, compounds **1**, **3**, **4**, **5**, **7**, **8**, **11** and **13** have not been reported in any species of the genus Illicium and the family Illiciaceae. Sesquiterpenoid compounds from *I. difengpi* may have the potential to produce new insights into the historical debate concerning the systematic position and phylogenetic relationship of the genus Illicium.

Keywords: *Illicium difengpi, Sesquiterpenoid, Chemotaxomic, Illiciaceae.*

Introduction

The genus Illicium is the only member of the family Illiciaceae and consists of 35 species disjunctively distributed in northeastern America, Mexico, the West Indies, and eastern Asia.[1] The highest concentration of species is in East China (about 30 species). *Illicium difengpi* K.I.B et K.I.M. is a small shrub growing in the mountain areas of southeast China. Its stem bark is listed in Chinese Pharmacopoeia (2015 edition) for its traditional use for rheumatoid arthritis treatment.[2]

The *Illicium* genus has been widely studied and numerous compounds isolated from the various species. Previous studies have revealed the occurrence of monoterpenoids, sesquiterpenoids, diterpenoids and phenylpropanoids from the essential oil,[3-5] sesquiterpenoids, diterpenoids, triterpenoids from fruits and pericarps,[6] and phenylpropanoids, prenylated phenylpropanoids,[7] lignans, neolignans, sesquineolignans, terpene-sesquineolignans[8] and benzoquinones from barks and roots of genus Illicium plants. Among them, prenylated C_6-C_3 compounds, sesquineolignans and secoprezizaane-type sesquiterpenoids are considered to be characteristic chemical markers of *Illicium* species. In previous phytochemical investigation of barks of *I. difengpi*, thirty compounds were isolated including phenylpropanoids and phenylpropanoid glycosides,[9-11] neolignans,[10,12] sesquiterpenoids lactones,[13] triterpene acids[14] and aromatic glycosides.[10] Besides, volatile constituents of different parts of *I. difengpi* including the fruits, pericarps and stem barks have been extensively studied. Over sixty compounds were isolated, including monoterpenoids, sesquiterpenoids and phenylpropanoids.[15-17]

The present study was carried out to investigate the chemical constituents of the stem barks of *Illicium difengpi* and to discuss the chemotaxonomic significance of the isolated compounds.

Materials and Methods

Plant collection and extraction

This study investigated the stem bark of Illicium difengpi, which were purchased from Caitongde Pharmacy, Shanghai, China, in January 2010. Plant material was authenticated by Professor Lian-na Sun (Department of Pharmacognosy, School of Pharmacy, Second Military Medical University) based on morphological characters. Voucher specimen (NO. 20100110) has been deposited in the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, in Shanghai, China.

Air dried stem barks were powdered (40.0 kg) and extracted three times (1:8, 1:8, 1:6) with aqueous ethanol (ethanol/water 8:2) under reflux. The solvent was then evaporated under reduced pressure to obtain a dry residue (1.2 kg). The residue was suspended in water (10 L) and extracted successively with petroleum ether (30-60℃) (3×10 L), EtOAc (3×10 L) and BuOH (3×10 L), affording 40 g, 560 g, and 300 g of each dried fraction, respectively.

Isolation and structure identification

The dried petroleum ether fraction ($Fr._1$) (40 g) was chromatographed on silica gel column (CC, 80×5 cm; petroleum ether/EtOAc 100:0→0:100) to give four main fractions ($Fr._{1-1}$-$Fr._{1-4}$). $Fr._{1-2}$ was purified by Sephadex LH-20 CC (150×2cm; $CHCl_3$/MeOH 1:1) to yield compound **1** (14.6 mg). The EtOAc fraction ($Fr._2$) (560 g) was chromatographed on silica gel CC (150×10 cm; $CHCl_3$/MeOH 300:1→0:100) to give four main fractions ($Fr._{2-1}$-$Fr._{2-4}$), among which $Fr._{2-2}$ was purified by successive silica gel CC (20×2 cm; petroleum ether/EtOAc 100:1→1:1) to give compound **2** (47 mg). $Fr._{2-3}$ was separated on silica gel CC (35×3 cm; petroleum

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ether/EtOAc 100:1→5:1) to afford compound **5** (65 mg) and a mixture of compound **3** and **4**. The mixture was rechromatographed using semipreparative HPLC system (Agilent 1200 series; YMC HPLC C18 column-5 µm, 250×10 mm, refractive index detector; flow 2 mL/min; mobile phase ACN/H_2O 65:35) to give pure compounds **3** (5 mg) and **4** (8.4 mg). Fr.$_{2\text{-}4}$ was fractionated on silica gel CC (50×4 cm; $CHCl_3$/MeOH 100:0→1:5) to give two main sub fractions (Fr.$_{2\text{-}4\text{-}1}$- Fr.$_{2\text{-}4\text{-}2}$). Fr.$_{2\text{-}4\text{-}1}$ was subjected to silica gel CC (35×3 cm; $CHCl_3$/MeOH 100:0→5:1) to afford compounds **6** (33 mg), **7** (49 mg) and **8** (63 mg). Fr.$_{2\text{-}4\text{-}2}$ was rechromatographed using semipreparative HPLC system (ACN/H_2O 58:42) to obtain pure compounds **9** (2.4 mg), **10** (6.9 mg) and **11** (8.9 mg). The BuOH fraction (Fr.$_3$) (300 g) was chromatographed on silica gel CC (120×8 cm; $CHCl_3$/MeOH 50:1→0:100) to give four main fractions (Fr.$_{3\text{-}1}$-Fr.$_{3\text{-}4}$). Fr.$_{3\text{-}2}$ was subjected to reversed-phased silica gel (C18; MeOH/H_2O 1:4-1:0) and Sephadex LH-20 (150×2 cm; MeOH/H_2O 1:1) CC to finally afford compounds **12** (16.8 mg) and **13** (8 mg).

The identification of isolated compounds was carried out on the basis of spectroscopic experiments. ^{1}HNMR spectra were measured at 600 MHz and ^{13}CNMR spectra at 150 MHz in a Bruker Avance 600 NMR spectrophotometer. HPLC-DAD-MS (Agilent Eclipse Plus C18 column-5 µm, 250×4.6 mm; flow 1 mL/min; mobile phase ACN/0.05% formic acid 15:85→100:0) analysis was performed on an Agilent Technologies 6110 instrument operated in the ±-ESI-MS mode. By analysis of their spectral data and comparison with literature reports, the isolated compounds were identified as dihydropyrocurzerenone (**1**),[18] (-)-α-cadinol (**2**),[19] litseachromolaevanes A (**3**),[20] ent-4(15)-eudesmene-1β,6α-diol (**4**),[21] ent-oplopomone (**5**),[22] (-)-clovane-2,9-diol (**6**),[23] cryptomeridiol (**7**),[24] 1β,4β,7α-trihydroxyeudesmane (**8**),[25] isodunnianin (**9**),[26] oplodiol (**10**),[27] homalamenol A(**11**),[24] bullatantriol(**12**),[28] 1,4,6-trihydroxy-eudesmane (**13**).[29]

Results and Discussion

Thirteen sesquiterpenoids were isolated from the plant and compounds **1** and **3-13** were isolated from the plant for the first time, among them compounds **1**, **3**, **4**, **5**, **7**, **8**, **11** and **13** were isolated from this family for the first time.

Dihydropyrocurzerenone (1): Amorphous. ESI-MS *m/z* 215 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, *δ*, ppm, *J*/Hz): 1.11 (3H, d, *J* = 6.9 Hz, H-14), 2.21 (3H, s, H-15), 2.33 (3H, d, *s*, H-13), 2.34 (1H, m, H-2b), 2.60 (1H, m, H-2a), 2.80 (1H, m, H-5b), 3.28 (1H, dd, *J* = 11.7, 5.3 Hz, H-5a), 7.01 (1H, s, H-9), 7.15 (1H, s, H-12) ; ^{13}C NMR (150 MHz, $CDCl_3$, *δ*, ppm): 133.3 (C-1), 27 (C-2), 31.4 (C-3), 28.6 (C-4), 35.7 (C-5), 130.5 (C-6), 124.4 (C-7), 153.9 (C-8), 109.9 (C-9), 128.9 (C-10), 116.2 (C-11), 140.6 (C-12), 11.2 (C-13), 22.1 (C-14), 20.4 (C-15).

(-)-α-Cadinol (2): Amorphous. ESI-MS *m/z* 223 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.76 (3H, d, J=7.0 Hz, H-13), 0.91 (3H, d, J=7.0 Hz, H-12), 1.05 (2H, m, H-7, 8b), 1.09 (3H, s, H-14), 1.22 (2H, m, H-1, 2b), 1.40 (1H, m, H-9a), 1.58 (1H, m, H-8a), 1.67 (3H, s, H-15), 1.70 (1H, m, H-6), 1.78 (1H, m, H-9b), 1.95 (3H, m, H-2a, 3), 2.14 (1H, m, H-11), 5.50 (1H, brs, H-5); ^{13}C NMR (150 MHz, $CDCl_3$, *δ*, ppm): 49.9 (C-1), 21.9 (C-2), 30.9 (C-3), 134.9 (C-4), 122.3 (C-5), 39.8 (C-6), 46.7 (C-7), 22.6 (C-8), 42.1 (C-9), 72.4 (C-10), 25.9 (C-11), 21.5 (C-12), 15.1 (C-13), 20.7 (C-14), 23.8 (C-15).

Litseachromolaevanes A (3): Colorless gum. ESI-MS *m/z* 257 $[M+Na]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.71 (3H, d, *J* =6.8 Hz, H-13), 0.99 (3H, d, *J* = 6.8 Hz, H-12), 1.73 (1H, m, H-7b), 1.84 (1H, m, H-11), 2.03 (3H, s, H-14), 2.08 (1H, m, H-7a), 2.14 (1H, dd, *J* = 16.8, 8.9 Hz, H-8b), 2.22 (1H, ddd, *J* = 16.8, 8.7, 5.6 Hz, H-8a), 2.23 (3H, s, H-15), 2.57 (1H, ddd, *J* = 11.8, 8.9, 3.6 Hz, H-6), 6.65 (1H, d, *J* = 8.0 Hz, H-1), 6.81 (1H, d, *J* = 1.8 Hz, H-4), 6.84 (1H, dd, *J* = 7.8, 1.9 Hz, H-2); ^{13}C NMR (150 MHz, $CDCl_3$, *δ*, ppm): 115.4 (C-1), 127.3 (C-2), 129.8 (C-3), 128.5 (C-4), 130.0 (C-5), 45.1 (C-6), 26.7 (C-7), 41.8 (C-8), 210.7 (C-9), 151.9 (C-10), 33 (C-11), 20.9 (C-12), 21.3 (C-13), 30.2 (C-14), 20.8 (C-15).

Ent-4(15)-eudesmene-1β,6α-diol (4): Amorphous. ESI-MS *m/z* 239 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, *δ*, ppm, *J*/Hz): 0.73 (3H, s, H-14), 0.89 (3H, d, *J* = 6.9 Hz, H-13), 0.98 (3H, d, *J* = 7.0 Hz, H-12), 1.22 (1H, m, H-9b), 1.29 (1H, m, H-8a), 1.30 (1H, m, H-7), 1.53 (1H, m, H-8b), 1.55 (1H, m, H-2a), 1.77 (1H, d, *J* = 9.9 Hz,H-5), 1.88 (1H, m, H-2b), 1.94 (1H, m, H-9a), 2.07 (1H, td, *J* = 13.2, 5.2 Hz, H-3b), 2.27 (1H, m, H-11), 2.36 (1H,ddd, *J* = 12.9, 4.8, 1.7 Hz, H-3a), 3.45 (1H, dd, *J* = 11.4, 4.7 Hz, H-1), 3.72 (1H, t, *J* = 9.7 Hz, H-6), 4.77 (1H, s, H-15), 5.05 (1H, s, H-15); ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm):79 (C-1), 31.8 (C-2), 35 (C-3), 146.2 (C-4), 55.8 (C-5), 67 (C-6), 49.2 (C-7), 18.1 (C-8), 36.2 (C-9), 41.7 (C-10), 25.9 (C-11), 21.1 (C-12), 16.1 (C-13), 11.6 (C-14), 107.8 (C-15).

Ent-oplopomone (5): Colorless needles. ESI-MS *m/z* 239 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.69 (3H, d, *J* = 6.8 Hz, H-12), 0.90 (3H, d, *J* = 6.9 Hz, H-13),1.08 (1H, ddd, *J* = 2.3, 5.0, 14.8 Hz, H-10), 1.14 (1H, m, H-6), 1.20 (3H, s, H-15), 1.37 (1H, m, H-5), 1.40 (1H, m, H-7a), 1.46 (1H, m, H-11), 1.50 (1H, m, H-8a), 1.60 (3H, m, H-1a, 2a, 7b), 1.84 (1H, m, H-8b), 1.81 (1H, ddd, *J* = 2.3, 4.5, 12.0 Hz, H-2b), 1.97 (1H, m, H-1b), 2.20 (3H, s, H-14), 2.66 (1H, ddd, *J*=5.4, 9.4, 11.6 Hz, H-3); ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm):42 (C-1), 28.6 (C-2), 56.9 (C-3), 211.5 (C-4), 55.7 (C-5), 49.4 (C-6), 25.3 (C-7), 22.9 (C-8), 73 (C-9), 46.7 (C-10), 29.5 (C-11), 21.9 (C-12), 15.6 (C-13), 29.5 (C-14), 20.3 (C-15).

(-)-Clovane-2, 9-diol (6): Amorphous. ESI-MS *m/z* 239 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.86 (3H, s, H-13), 0.91 (1H, m, H-12b), 0.96 (3H, s, H-15), 1.03 (3H, s, H-14), 1.08 (1H, m, H-11b), 1.12 (1H, m, H-7b), 1.32 (1H, m, H-6b), 1.39 (1H, m, H-7a), 1.42 (1H, m, H-6a), 1.43 (1H, m, H-5), 1.51 (1H, m, H-3b), 1.56 (1H, m, H-12a), 1.64 (1H, m, H-10b), 1.68 (1H, m, H-11a), 1.72 (1H, m, H-3a), 2.00 (1H, m, H-10a), 3.33 (1H, br s, H-9), 3.79 (1H, dd, *J* = 10.0, 6.0 Hz, H-2); ^{13}C NMR (150 MHz, $CDCl_3$, *δ*, ppm): 43.7 (C-1), 80.4 (C-2), 47.0 (C-3), 36.6 (C-4), 50.1 (C-5), 20.2 (C-6), 32.7 (C-7), 34.2 (C-8), 74.4 (C-9), 25.5 (C-10), 25.9 (C-11), 35.1 (C-12), 24.9 (C-13), 30.9 (C-14), 27.9 (C-15).

Cryptomeridiol (7): Amorphous. ESI-MS *m/z* 241 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.83 (3H, s, H-14), 1.05 (1H, s, H-8b), 1.13 (1H, s, H-5), 1.18 (1H, s, H-6a), 1.18 (6H, s, H-12,13), 1.19 (1H, m, H-7), 1.56 (1H, s, H-6b), 1.88 (1H, br s, H-8a); ^{13}C NMR (150 MHz, $CDCl_3$, *δ*, ppm): 40.9 (C-1), 20.1 (C-2), 43.3 (C-3), 72.4 (C-4), 54.7 (C-5), 22.6 (C-6), 49.9 (C-7), 21.4 (C-8), 44.5 (C-9), 34.5 (C-10), 73.1 (C-11), 26.7 (C-12), 27.4 (C-13), 18.6 (C-14), 22.5 (C-15).

1β,4β,7α-Trihydroxyeudesmane (8): Amorphous. ESI-MS *m/z* 257 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.94 (3H, d, *J* = 6.9, H-13), 0.95 (3H, d, *J* = 6.9, H-12), 0.98 (3H, s, H-14), 1.14 (3H, s, H-15), 1.40 (1H, m, H-9a), 1.45 (2H, m, H-5, 6a), 1.54 (4H, m, H-3a, 6b, 8), 1.58 (2H, m, H-2a, 11), 1.63 (1H, m, H-9b), 1.69 (1H, m, H-3b), 1.87 (1H, ddd, *J* = 3.9, 12.8 15.4 Hz, H-2b), 3.32 (1H, dd, *J* = 4.0, 11.7 Hz, H-1); ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm):79.3 (C-1), 26.7 (C-2), 39.7 (C-3), 71.5 (C-4), 44.6 (C-5), 29 (C-6), 73.7 (C-7), 29.3 (C-8), 34.6 (C-9), 38.8 (C-10), 39.1 (C-11), 16.8 (C-12), 16.9 (C-13), 11.5 (C-14), 29.8 (C-15).

Isodunnianin (**9**): Colorless prisms. ESI-MS m/z 469 $[M+Na]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.94 (3H, d, *J* = 7.2 Hz, H-15), 1.34 (1H, dd, *J* = 4.0, 6.5 Hz, H-2b), 1.38 (3H, s, H-12), 1.48 (3H, s, H-13), 1.83 (1H, dd, *J* = 2.0, 15.6 Hz, H-8b), 2.22 (1H, dd, *J* = 4.0, 15.6 Hz, H-8a), 2.44 (1H, m, H-1), 2.62 (1H, d, *J* = 20.3, H-10a), 2.63 (1H, m, H-2a), 3.36 (1H, d, *J* = 20.2 Hz, H-10b), 4.51 (1H, d, *J* = 5.3, H-3), 4.59 (1H, d, *J* = 11.9, H-14a), 4.71 (1H, d, *J* = 11.9, H-14b), 5.31 (1H, dd, *J* = 2.4, 4.1 Hz, H-7), 7.51 (2H, t, *J*=7.5, H-3',5'), 7.61 (1H, t, *J*=7.5, H-4'), 8.0 (2H, d, *J*=7.1); ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm):39.6 (C-1), 41 (C-2), 80.7 (C-3), 81.8 (C-4), 47.3 (C-5), 77.6 (C-6), 77.8 (C-7), 29.3 (C-8), 45.1 (C-9), 35.2 (C-10), 170.4 (C-11), 23.4 (C-12), 15.1 (C-13), 65 (C-14), 14.7 (C-15), 170.7 (C-16), 20.9 (C-17), 165.5 (C-18), 128.9 (C-1'), 129.6 (C-2',6'), 129 (C-3',5'), 133.8 (C-4').

Oplodiol (10): Amorphous. ESI-MS *m/z* 239 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.99 (3H, s, H-14), 1.06 (6H, d, *J* = 6.9 Hz, H-12,13), 1.21 (3H, s, H-15), 1.33 (1H, dd, *J* = 11.5, 5.9 Hz, H-5), 1.56 (1H, m, H-3b), 1.74 (1H, m, H-3a), 1.80 (1H, m, H-2b), 1.92 (2H, m, H-2a,9b), 2.07 (2H, m, H-6), 2.13 (1H, m, H-9a), 2.33 (1H, d, *J* = 6.8 Hz, H-11), 3.32 (1H, dd, *J* = 11.4, 3.9 Hz, H-1), 5.36 (1H, dd, *J* = 3.4, 2.1 Hz, H-8); ^{13}C NMR (150 MHz, $CDCl_3$, *δ*, ppm): 79.9 (C-1), 26.7 (C-2), 39.4 (C-3), 70.9 (C-4), 46.2 (C-5), 23 (C-6), 141.9 (C-7), 116 (C-8), 40.7 (C-9), 37.6 (C-10), 34.9 (C-11), 21.7 (C-12), 21.1 (C-13), 11.7 (C-14), 29.8 (C-15).

Homalamenol A (11): Amorphous. ESI-MS *m/z* 239 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.99 (1H, m, H-5), 1.06 (3H, s, H-14), 1.13 (3H, s, H-15), 1.27 (1H, m, H-8b), 1.30 (1H, m, H-9b), 1.45 (1H, m, H-3b), 1.59 (1H, m, H-9a), 1.61 (1H, m, H-2b), 1.62 (1H, m, H-3a), 1.63 (6H, s, H-12,13), 1.78 (1H, m, H-2a), 2.06 (1H, m, H-8a), 2.95 (1H, m, H-6), 3.36 (1H, dd, *J* = 11.4, 4.2 Hz, H-1), 5.08 (1H, d, *J* = 9.5 Hz, H-7); ^{13}C NMR (150 MHz, $CDCl_3$, *δ*, ppm): 79.9 (C-1), 27.9 (C-2), 40.6 (C-3), 71.7 (C-4), 59.0 (C-5), 34.9 (C-6), 132.1 (C-7), 29.5 (C-8), 38.6 (C-9), 47.1 (C-10), 128.6 (C-11), 18.1 (C-12), 25.7 (C-13), 14.1 (C-14), 30.6 (C-15).

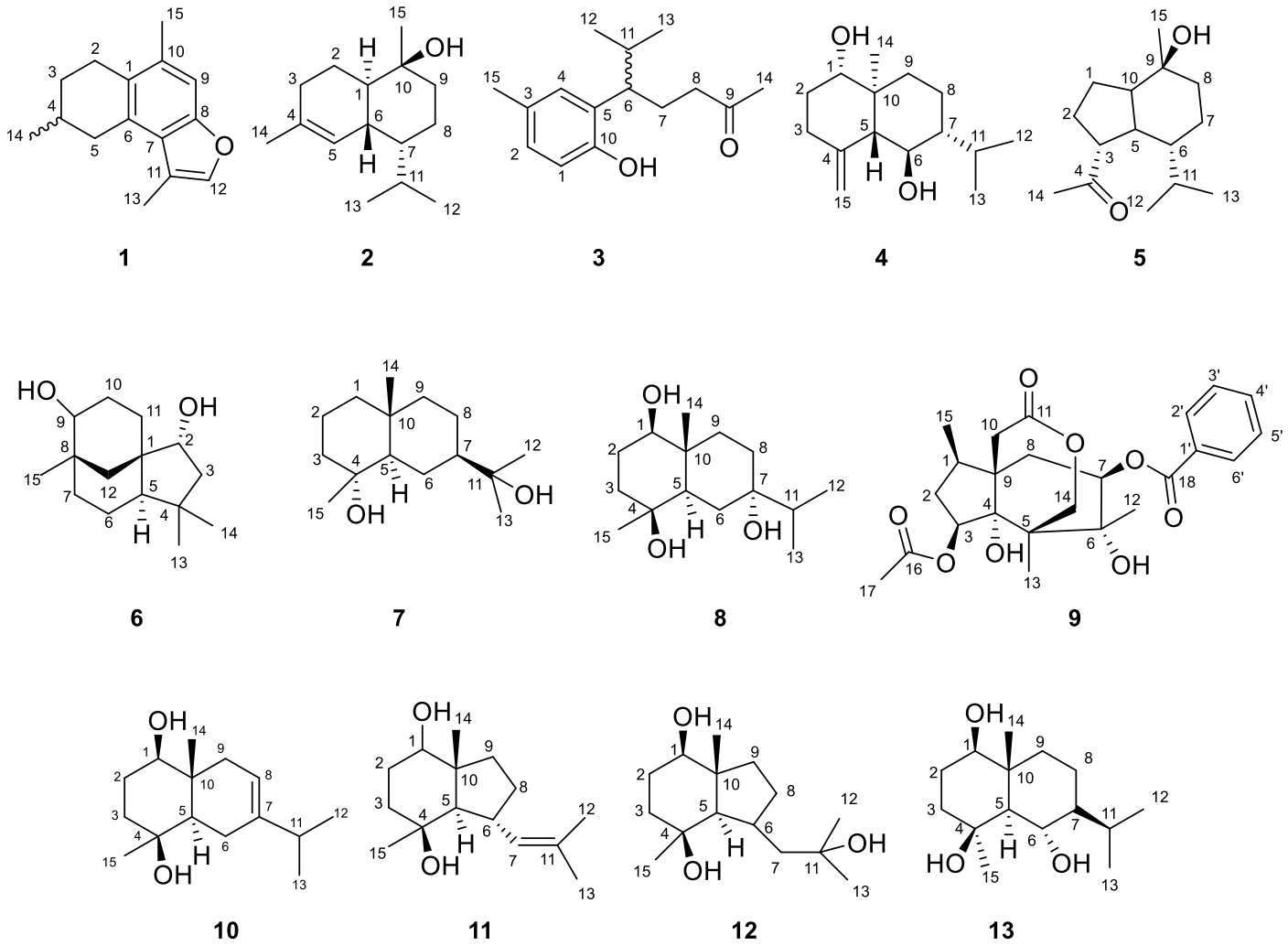

Figure 1: Chemical structures of compounds **1-13**

Bullatantriol (12): Amorphous. ESI-MS *m/z* 257 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 1.11 (1H, d, J = 10.6 Hz; H-5), 1.43 (6H, s, H-12, 13), 1.52 (1H, m, H-9a), 1.56 (1H, m, H-3a), 1.56 (1H, overlap, H-7b), 1.56 (3H, s, H-14), 1.60 (3H, s, H-15) 1.87 (1H, m, H-3b), 1.94 (1H, m, H-2a), 1.98 (1H, m, H-9b), 2.32 (2H, m, H-8), 2.38 (1H, br d, *J* = 13.8 Hz; H-7a), 2.45 (1H, m, H-2b), 2.80 (1H, m, H-6), 3.73 (1H, dd, *J* = 4.0, 11.3 Hz, H-1); ^{13}C NMR (150 MHz, $CDCl_3$, *δ*, ppm): 80.7 (C-1), 27.9 (C-2), 42.0 (C-3), 72.5 (C-4), 60.3 (C-5), 33 (C-6), 52.2 (C-7), 33.5 (C-8), 28.7 (C-9), 48.2 (C-10), 72.6 (C-11), 32 (C-12), 30.3 (C-13), 15.1 (C-14), 30.0 (C-15).

1,4,6-Trihydroxy-eudesmane (13): Amorphous. ESI-MS *m/z* 257 $[M+H]^+$. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 0.95 (6H, d, *J* = 6.9 Hz, H-12, 13), 0.99 (3H, s, H-14), 1.14 (3H, s, H-15), 1.38 (1H, m, H-9b), 1.46 (2H, m, H-5, 6b), 1.56 (1H, m, H-3b), 1.58 (3H, m, H-6a, 8), 1.62 (2H, m, H-2b,11), 1.67 (1H, m, H-9a), 1.71 (1H, m, H-3a), 1.89 (1H, m, H-2a), 3.31 (1H, dd, *J* = 11.4, 3.9 Hz, H-1); ^{13}C NMR (150 MHz, $CDCl_3$, *δ*, ppm): 80.1 (C-1), 27.7 (C-2), 42.4 (C-3), 72.8 (C-4), 57.8 (C-5), 70.0 (C-6), 53.1 (C-7), 19.4 (C-8), 39.2 (C-9), 42.1 (C-10), 27.0 (C-11), 21.7 (C-12), 16.1 (C-13), 14.0 (C-14), 34.5 (C-15).

Chemotaxonomic significance

In the present study, thirteen sesquiterpenoids isolated from *I. difengpi* can be divided into six types: eudesmane (**4, 7, 8, 10, 13**), muurolane (**1, 2, 3**), bullatane (**11, 12**), secoprezizaane (**9**), oplopanone (**5**), clovane (**6**). All compounds except compound 2 were isolated from this species for the first time. In addition, compounds 1, 3, 4, 5, 7, 8, 11 and 13 have not been reported in any species of the genus *Illicium* and the family *Illiciaceae*.

Illicium (*Illiciaceae*) is one of the basal lineages of angiosperm. It was classified in the family *Magnoliaceae* in the early taxonomic literature[30] mainly based on morphological characters. However, rapidly accumulating genetic data and improved analysis of these data provided new knowledge of phylogeny. The angiosperm phylogeny group suggested that Illiciaceae could be optionally included in Schisandraceae which is comprised in Austrobaileyales.

Sesquiterpenoid compounds from *I. difengpi* may have the potential of providing new insights into the historical debate concerning the systematic position and phylogenetic relationship of the genus *Illicium*. Compounds **5**,[31] **8**,[25] **10** [32] and **11** [25] have been previously obtained from several species of genus *Magnolia*, which may indicate a genetic relationship between genera *Illicium* and *Magnolia*. However, *Illicium* was subsequently excluded from the *Magnoliaceae* by Smith[33] and Bailey and Nast,[34] and assigned familial rank as the *Illiciaceae*; this has received wide support. Sy *et al.*[35] had reported phytochemical evidence, analyzing the distribution of phenylpropanoids and neolignans as well as cycloartane triterpenoids in selected plant genera, to corroborate this classification.

Besides, the presence of secoprezizaane-type sesquiterpenoids (compound **9**, which was previously obtained in *Illicium simonsii*) is almost entirely restricted to the family *Illiciaceae*; this unique presence strengthen the taxonomic position as independent family and confirms the suggestion that these compounds could be considered as important chemotaxomic markers of this family.[30]

The presence of sesquiterpenoids muurolane (compounds **1, 2, 3**) and oplopanone (compound **5**) and absence of these compounds in other species allow them to be considered as distinct chemical markers and may be used to differentiate *I. difengpi* from other members of the genus. The common presences in plants and skeleton variations of sesquiterpenoids contribute a lot to the taxonomy of the genus *Illiciaceae*. The occurrence of sesquiterpenoids in family *Illiciaceae* has been analyzed by Zhao and Luo.[24, 36] This paper is illustrative about trends concerning the distribution

of sesquiterpenoids in *Illicium* species and provides dendrogram of the genus *Illicium*. This result is in line with Lin's classification. Lin had divided this genus into two sections, viz.: *Illicium sect. Illicium* (*I. difengpi* belongs to this section) and *Illicium sect. Cymbostemon* (*I. tashiroi* belongs to this section) based on morphological character of flower buds and tepals.[37, 38] Our study reports unequivocally the presence of six more types of sesquiterpenoids in *I. difengpi* and yield information of systematic importance. As far as compound **9** and compounds **6**, **10** and **12** are concerned they have been isolated in species of *I. simonsii* and *I. tashiroi*, respectively.[26, 39] The cooccurrence of compound **6**, **10** and **12** may suggest a close relationship between *I. difengpi* and *I. tashiroi*.

Conclusion

From the chemical constituents obtained from *I. difengpi* especially the sesquiterpenoids, we could get a new clue to strengthen the taxonomic position of *Illiciaceae* as an independent family and yield information of systematic importance within this family. To fully elucidate the systematic correlation of genus *Illicium* plants, further phytochemical investigations are necessary.

Conflict of Interest

No conflict of interest associated with this work.

Authors' declaration

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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