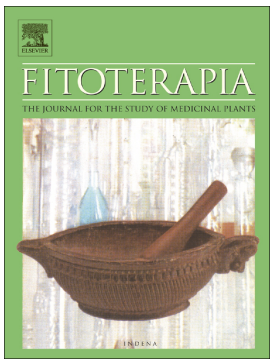

Lophachinins A–E, abietane diterpenes from a Mongolian traditional herbal medicine Lophanthus chinensis

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Lophachinins A–E, abietane diterpenes from a Mongolian traditional herbal medicine *Lophanthus chinensis*

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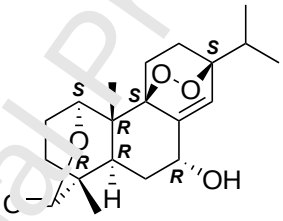
Abstract

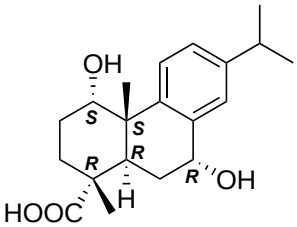
Five new abietane diterpenes, lophachinins A–E (**1**–**5**), and eleven known related diterpenes were isolated from a Mongolian traditional herbal medicine, the aerial parts of *Lophanthus chinensis* (Lamiaceae). The structures of new diterpenes were assigned by spectroscopic analyses. Lophachinins A (**1**) and B (**2**) were abietane diterpene possessing an endoperoxy bridge at C-ring. In contrast, lophachinins C–E (**3**–**5**) had an abietane skeleton with an aromatized C-ring. The absolute configuration of **1** was elucidated by application of the modified Mosher's method, while those of **2**, **3**, and **5** were assigned by chemical conversions. The absolute configuration of lophachinin D (**4**) was deduced by ECD calculation. Anti-inflammatory activity of isolated diterpenes on microglial cells were evaluated.

Graphical abstract:

Lophanthus chinensis



lophachinin A (**1**)



lophachinin C (**3**)

Key words

Mongolian traditional herbal medicine; *Lophanthus chinensis*; Lamiaceae; Abietane diterpene; Anti-inflammatory activity

1. Introduction

Lamiaceae is one of the largest plant families, which includes various medicinally important plants with aromatic and ornamental properties. The plants belonging to the genus *Lophanthus*, being composed of 23 species, are perennial herbs or sub-shrubs distributed over alpine in Turkey, Iran, Afghanistan, central Asia, China, and Mongolia. Although the genus *Lophanthus* is considered to be very closely related to the genus *Nepeta* [1,2], there have been only a few publications of phytochemical studies on *Lophanthus* plants, reporting the presence of essential oils, triterpenes, flavonoids, and polysaccharides [3–6], in spite of the fact that there have been so many phytochemical studies on *Nepeta* plants [7]. Our continuing phytochemical study on Mongolian traditional herbal medicines is aimed at searching specialized metabolites as new natural product leads for therapeutic agents [8–10]. In the course of our ethnobotanical study in Mongolia, we have learned that the aerial parts of *Lophanthus chinensis* Benth. have been used for the treatment of dizziness, fevers, and inflammatory diseases in Mongolia. As part of the research program, we have investigated constituents of the aerial parts of *L. chinensis*, which resulted in the isolation of five new abietane diterpenes, lophachinsins A–E (**1**–**5**), together with eleven known related diterpenes (**6**–**16**). We describe herein the isolation, structure elucidation, and evaluation of anti-inflammatory activity of **1**–**16**.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured by a JASCO P-2200 digital polarimeter. MS were obtained on a Water LCT PREMIER 2695. NMR spectra were measured by Bruker AVANCE 500 and AVANCE 400 spectrometers using the resonances of methanol (δ_H 3.30 and δ_C 49.0), chloroform (δ_H 7.26 and δ_C 77.0), and pyridine (δ_H 8.71 and δ_C 135.5) as internal references. IR spectra were recorded on a

JASCO FT/IR-6200 spectrophotometer, while UV and CD spectra were measured by a JASCO J-1500 spectrophotometer. Column chromatography was performed with silica gel 60N (63-210 μm, Kanto Chemical Co. Inc. Japan), MCI gel CHP-20P (75-150 μm, Mitsubishi Chemical Co., Japan), YMC gel ODS-A (S-50 μm, YMC Co. Ltd., Japan), Sephadex LH-20 (25-100 μm, GE Health Care Bio-Sciences AB, Sweden), and Toyopearl HW-40C (75 μm, Tosoh Co., Japan).

2.2. Plant material

The whole plants of *Lophanthus chinesis* were collected at Ovorhangay province, Mongolia in August 2012. Identification of the plant material was carried out by one of the authors (D. Damdinjav). A voucher specimen (12JM0006) was deposited at the herbarium of Graduate School of Pharmaceutical Sciences, Tokushima University.

2.3. Extraction and isolation

The air-dried aerial parts of *L. chinensis* (1.6 kg) were extracted three times with MeOH (15 L) at room temperature. After removal of the solvent by evaporation, the extract (198.6 g) was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (87.2 g) was further partitioned between *n*-hexane and 90% MeOH aq. The 90% MeOH aq.-soluble materials (60.8 g) were subjected to column chromatography over MCI gel CHP-20P (MeOH/H_2O, 6:4 to 10:0) to give eight fractions (frs. 1–8). Fr. 4 was separated by YMC gel ODS-A (MeOH/H_2O, 6:4 to 10:0) column chromatography to give six fractions (frs. 4.1–4.6). Lophachinin A (**1**, 62 mg) was obtained by crystallization of fr. 4.1 from MeOH. Repeated chromatographic separations of fr. 4.3 by silica gel (*n*-hexane/acetone, 75:25 to 65:35) and Toyopearl HW-40C (MeOH), followed by crystallization from *n*-hexane/$CHCl_3$ (1:1) furnished lophachinin D (**4**, 23 mg). The mother liquor was further subjected to silica gel column chromatography ($CHCl_3$/acetone, 9:1 to 8:2) to give **16** (20 mg). Fr.

4.4 was separated by a Sephadex LH-20 column (MeOH) to give two fractions (frs. 4.4.1 and 4.4.2). Fr 4.4.1 was chromatographed repeatedly over silica gel columns (toluene/*i*-PrOH, 9:1; $CHCl_3$/MeOH, 97:3 to 9:1) to give **7** (27 mg), while crystallization of fr. 4.4.2 from MeOH/H_2O (55:45) yielded lophachinin E (**5**, 305 mg). Repeated chromatographic separations of the mother liquor of fr. 4.4.2 on an ODS column (MeOH/H_2O, 6:4 to 1:0) and on a silica gel column (toluene/*i*-PrOH, 9:1) gave lophachinin C (**3**, 442 mg), **6** (51 mg), and **12** (23 mg). Chromatographies of fr. 4.6 on silica gel columns ($CHCl_3$/MeOH, 98:2 to 97:3; toluene/acetone, 95:5 to 7:3) furnished **8** (3 mg), **9** (10 mg), and **10** (24 mg). Fr. 5 was fractionated by Toyopearl HW-40C column chromatography (MeOH) to give seven fractions (frs. 5.1–5.7). Fr. 5.4 (1.3 g) was applied repeatedly to silica gel columns (toluene/acetone, 95:5 to 6:4; $CHCl_3$/MeOH, 95:5 to 85:15) to give lophachinin B (**2**, 5 mg) and **13** (14 mg). Fr. 6 was separated by Toyopearl HW-40C column chromatography (MeOH) to yield four fractions (frs. 6.1–6.4). Fr. 6.3 was subjected to a silica gel column ($CHCl_3$/EtOAc, 9:5 to 6:4) to give ten fractions (frs. 6.3.1–6.3.10). Crystallization of fr. 6.3.7 from MeOH afforded **11** (842 mg). Silica gel column chromatography (*n*-hexane/acetone, 8:2) of the mother liquor of fr. 6.3.7, followed by purification on an ODS column (MeOH/H_2O, 75:25) gave **14** (27 mg) and **15** (20 mg).

*2.4 Lophachinin A (**1**)*

Colorless amorphous solid; $[\alpha]^{26}_D$ –51.0 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3469, 2971, 1741, and 1634 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and S1); HRESIMS *m/z* 347.1841 ($[M–H]^-$, calcd for $C_{20}H_{27}O_5$, 347.1858).

*2.4.1 Preparation of (S)- and (R)-MTPA esters (**1a** and **1b**) of lophachinin A (**1**)*

A mixture of lophachinin A (**1**, 1 mg), (*R*)- or (*S*)-MTPA chloride (10 μL), and DMAP (0.1 mg)

in anhydrous pyridine (1 mL) was stirred at room temperature for 1h. Reaction mixture was evaporated under reduced pressure to give a residue, which was purified by silica gel column chromatography (*n*-hexane/acetone, 7:3) to afford (*S*)-MTPA ester (**1a**, 0.5 mg) or (*R*)-MTPA ester (**1b**, 1 mg) of **1**.

*2.4.2 (S)-MTPA ester (**1a**) of lophachinin A (**1**)*

Colorless amorphous solid; ^{1}H NMR (C_5D_5N, 500 MHz) δ_H 7.81 (2H, m, Ph), 7.47 (3H, m, Ph), 6.399 (1H, d, *J* = 8.0 Hz, H-7), 6.332 (1H, d, *J* = 1.6 Hz, H-14), 4.767 (1H, brs, H-1), 3.65 (3H, s, OMe), 2.534 (1H, td, *J* = 14.4, 8.0 Hz, H-6a), 2.211 (1H, ddd, *J* = 13.7, 9.6, 4.6 Hz, H-11a), 2.165 (1H, dd, *J* = 14.4, 3.7 Hz, H-5), 1.790 (1H, sept, *J* = 6.8 Hz, H-15), 1.685 (1H, td, *J* = 13.7, 2.4 Hz, H-11b), 1.331 (1H, brt, *J* = 11.3 Hz, H-3a), 1.187 (3H, s, H_3-19), 1.096 (1H, td, *J* = 13.7, 4.6 Hz, H-12b), 1.021 (3H, s, H_3-20), 0.864 (3H, d, *J* = 6.8 Hz, H_3-16), and 0.784 (3H, d, *J* = 6.8 Hz, H_3-17); HRESIMS *m/z* 587.2261 ($[M+Na]^+$, calcd for $C_{30}H_{35}O_7F_3Na$, 587.2233).

*2.4.3 (R)-MTPA ester (**1b**) of lophachinin A (**1**)*

Colorless amorphous solid; ^{1}H NMR (C_5D_5N, 500 MHz) δ_H 7.73 (2H, m, Ph), 7.43 (3H, m, Ph), 6.828 (1H, d, *J* = 1.8 Hz, H-14), 6.423 (1H, d, *J* = 7.6 Hz, H-7), 4.773 (1H, brs, H-1), 3.68 (3H, s, OMe), 2.472 (1H, td, *J* = 14.2, 7.5 Hz, H-6a), 2.241 (1H, ddd, *J* = 13.4, 9.3, 4.2 Hz, H-11a), 1.981 (1H, dd, *J* = 14.2, 3.7 Hz, H-5), 1.932 (1H, ddd, *J* = 12.7, 9.3, 3.0 Hz, H-12a), 1.863 (1H, sept, *J* = 6.8 Hz, H-15), 1.666 (1H, m, H-11b), 1.312 (1H, brt, *J* = 12.0 Hz, H-3a), 1.254 (td, *J* = 12.7, 4.2 Hz, H-12b), 1.066 (3H, s, H_3-19), 1.024 (3H, s, H_3-20), 0.936 (3H, d, *J* = 6.8 Hz, H_3-16), and 0.893 (3H, d, *J* = 6.8 Hz, H_3-17); HRESIMS *m/z* 587.2212 ($[M+Na]^+$, calcd for $C_{30}H_{35}O_7F_3Na$, 587.2233).

*2.5 Lophachinin B (**2**)*

Colorless amorphous solid; $[\alpha]^{19}_{D}$ –48.9 (*c* 0.10, MeOH); IR (KBr) ν_{max} 2966, 1756, 1470, and 1087 cm^{-1};^{1}H and ^{13}C NMR (Table 1); HRESIMS *m/z* 385.1979 ($[M+Na]^+$, calcd for $C_{21}H_{30}O_5Na$, 385.1991).

*2.5.1 Methylation of lophachinin A (**1**)*

A mixture of lophachinin A (**1**, 10 mg), MeI (10 μL), and Ag_2O (0.1 mg) in CH_2Cl_2 (2 mL) was stirred at room temperature for 15 h. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/acetone, 8:2) to give **1c** (3 mg).

*2.5.2 7-O-Methyl lophachinin A (**1c**, lophachinin B)*

Colorless amorphous solid; $[\alpha]^{19}_{D}$ –59.0 (*c* 0.10, MeOH); ^{1}H NMR data was identical with that of lophachinin B (**2**); HRESIMS *m/z* 385.1990 ($[M+Na]^+$, calcd for $C_{21}H_{30}O_5Na$, 385.1991).

*2.6 Lophachinin C (**3**)*

Colorless amorphous solid; $[\alpha]^{23}_{D}$ +31.0 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3457, 2955, 1713, 1390, and 1243 cm^{-1}; ^{1}H and ^{13}C NMR (Table 2); HRESIMS *m/z* 331.1904 ($[M–H]^-$, calcd for $C_{20}H_{27}O_4$, 331.1909).

*2.6.1 Chemical conversion of lophachinin C (**3**) into teideadiol (**3c**)*

A mixture of lophachinin C (**3**, 33 mg), MeI (0.7 mL), and K_2CO_3 (100 mg) in dry acetone (20 mL) was stirred at room temperature for 24 h. After removal of the inorganic salts by filtration, the filtrate was concentrated under reduced pressure to give a residue, which was applied to a silica gel column (*n*-hexane/acetone, 8:2) to give a methyl ester (**3a**, 31 mg) of **3**. A solution of **3a** (20 mg) in

Et_2O (10 mL) was treated with $LiAlH_4$ (30 mg) for 2 h at room temperature with stirring. The reaction mixture was filtered and concentrated to give a residue, which was purified by silica gel column chromatography ($CHCl_3$/acetone, 7:3) to furnish the reduced product (**3b**, 11 mg) of **3a**. A mixture of **3b** (8 mg) and 10% Pd-C (18 mg) in AcOH (10 mL) was stirred under H_2 atmosphere at room temperature for 3 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure to give a residue. Purification of the residue by silica gel column chromatography ($CHCl_3$/acetone, 95:5) gave 7-deoxy derivative (**3c**, 5 mg) of **3b**.

*2.6.2 Methyl ester (**3a**) of **3***

Colorless amorphous solid; $[\alpha]^{25}_D$ +13.5 (*c* 0.10, MeOH); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.26 (1H, brs, H-14),7.22 (1H, d, *J* = 8.0 Hz, H-11), 7.19 (1H, dd, *J* = 8.0, 1.9 Hz, H-12), 4.71 (1H, brs, H-7), 4.37 (1H, brs, H-1), 3.70 (3H, s, 18-OMe), 2.98 (1H, dd, *J* = 13.3, 1.5 Hz, H-5), 2.89 (1H, sept, *J* = 6.9 Hz, H-15), 2.32 (1H, t, *J* = 13.8, 3.7 Hz, H-3a), 2.10 (1H, td, *J* = 13.3, 4.3 Hz, H-6a), 2.07 (1H, m, H-2a), 1.91 (1H, dq, *J* = 14.2, 3.7 Hz, H-2b), 1.61 (1H, brd, *J* = 13.3 Hz, H-6b), 1.47 (1H, ddd, *J* = 13.8, 3.7, 2.8 H, H-3b), 1.30 (3H, s, H_3-19), 1.24 (6H, d, *J* = 6.9 Hz, H_3-16 and H_3-17), and 1.19 (3H, s, H_3-20); HRESIMS *m/z* 369.2043 ([M+Na]$^+$, calcd for $C_{21}H_{30}O_4Na$, 369.2042).

*2.6.3 Reduced product (**3b**) of **3a***

Colorless amorphous solid; $[\alpha]^{26}_D$ +27.1 (*c* 0.10, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ_H 7.28 (1H, d, *J* = 8.4 Hz, H-11), 7.15 (1H, brs, H-14), 7.14 (1H, dd, *J* = 8.4, 2.1 Hz, H-12), 4.66 (1H, brs, H-7), 4.38 (1H, brs, H-1), 3.39 (1H, d, *J* = 11.0 Hz, H-18a), 3.28 (1H, d, *J* = 11.0 Hz, H-18b, overlapped with the solvent signal), 2.85 (1H, sept, *J* = 7.0 Hz, H-15), 2.38 (1H, dd, *J* = 12.3, 3.0 Hz, H-5), 2.14 (tq, *J* = 14.3, 2.2 Hz, H-2a), 1.98–1.88 (4H, m, H_2-3 and H_2-6), 1.76 (1H, dq, *J* = 14.3, 3.4 Hz, H-2b), 1.22 (6H, d, *J* = 7.0 Hz, H_3-16 and H_3-17), 1.16 (3H, s, H_3-20), and 0.92 (3H, s, H_3-19);

HRESIMS *m/z* 341.2098 ([M+Na]$^+$, calcd for $C_{20}H_{30}O_3Na$, 341.2093).

*2.6.4 7-Deoxy derivative (**3c**, teideadiol) of **3b***

Colorless amorphous solid, $[\alpha]^{24}_D$ +83.4 (*c* 0.10, MeOH), ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.20 (1H, d, *J* = 8.2 Hz, H-11), 7.04 (1H, dd, *J* = 8.2, 1.7 Hz, H-12), 6.93 (1H, d, *J* = 1.7 Hz, H-14), 4.34 (1H, brs, H-1), 3.49 and 3.23 (each 1H, d, *J* = 11.0 Hz, H_2-18), 2.86 (2H, m, H_2-7), 2.83 (1H, sept, *J* = 7.0 Hz, H-15), 2.14 (1H, dd, *J* = 12.5, 1.9 Hz, H-5), 2.06 (1H, tdd, *J* = 13.8, 4.2, 2.4 Hz, H-2a), 1.94 (1H, td, *J* = 13.8, 3.7 Hz, H-3a), 1.85 (1H, dq, *J* = 13.8, 3.7 Hz, H-2b), 1.79 and 1.70 (each 1H, m, H_2-6), 1.24 (3H, s, H_3-20), 1.22 (6H, d, *J* = 7.0 Hz, H_3-16 and H_3-17), 1.11 (1H, ddd, *J* = 13.8, 3.7, 2.4 Hz, H-3b), and 0.88 (3H, s, H_3-19); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C 146.2 (C-9), 141.9 (C-13), 137.1 (C-8), 127.9 (C-14), 124.5 (C-12), 123.6 (C-11), 72.0 (C-18), 71.5 (C-1), 43.3 (C-10), 37.4 x 2 (C-4 and C-5), 33.4 (C-15), 30.5 (C-7), 27.9 (C-3), 25.8 (C-20), 23.8 x 2 (C-16 and C-17), 23.6 (C-2), 18.4 (C-6), and 17.3 (C-19); HRESIMS *m/z* 325.2133 ([M+Na]$^+$, calcd for $C_{20}H_{30}O_2Na$, 325.2144).

*2.7 Lophachinin D (**4**)*

Colorless amorphous solid; $[\alpha]^{23}_D$ +19.7 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3402, 2962, 1669, and 1557 cm^{-1}; UV (MeOH) λ_{max} 211 (logε 4.5), 254 (4.1), and 302 (3.4) nm; ECD (MeOH) $\Delta\varepsilon$ (nm) +2.0 (329), –3.0 (300), –1.7 (255), and +9.4 (211); ^{1}H and ^{13}C NMR (Table 2); HRESIMS *m/z* 353.1723 ([M+Na]$^+$, calcd for $C_{20}H_{26}O_4Na$, 353.1729).

*2.7.1 Calculation for ECD spectrum of lophachinin D (**4**)*

An enantiomer of lophachinin D (**4**) with the 1*S*, 4*R*, 5*R*, and 10*S* configurations was submitted to conformational search with the Molecular Mechanics (MMFF) on Spartan 18 program

(Wavefunction Inc. Irvine, CA.). The initial stable conformers with Boltzmann distributions over 1% were further optimized by DFT calculations at the B3LYP/6-31G(d) level in gas phase on Gaussian 09 program [11]. The optimized stable conformers were subjected to TDDFT calculations at the B3LYP/6-31+G(d,p) level in the presence of MeOH with an IEF-PCM. The resultant rotatory strengths of the lowest 30 excited states for each conformer were converted into Gaussian-type curves with half-bands (0.2 eV) using SpecDis v1.61 [12].

*2.8 Lophachinin E (**5**)*

Colorless amorphous solid; $[\alpha]^{26}_{D}$ +13.4 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3429, 2929, 1725, 1693, 1373, and 1247 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 2); HRESIMS *m/z* 397.2004 ($[M+Na]^{+}$, calcd for $C_{22}H_{30}O_5Na$, 397.1991).

*2.8.1 Chemical conversion of lophachinin E (**5**) into methyl 7α-acetoxydehydroabietate (**5c**)*

A solution of lophachinin E (**5**, 31 mg) in $CHCl_3$/MeOH (1:1, 2 mL) was treated with trimethylsilyldiazomethane (0.6 M solution in *n*-hexane, 1.0 mL) with stirring at room temperature for 1 h. The reaction mixture was concentrated in vacuo to afford a methyl ester (**5a**, 29 mg) of **5**. To a solution of **5a** (25 mg) in pyridine (2 mL), $SOCl_2$ (2 drops) was added, and the mixture was stirred at 0 °C for 1 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc layer was dried over Na_2SO_4 and concentrated to give a residue, which was separated by silica gel column chromatography ($CHCl_3$/acetone, 98:2) to yield the 15,16-dehydro derivative (**5b**, 5 mg) of **5a**. A mixture of **5b** (4 mg) and 10% Pd-C (2 mg) in AcOH (2 mL) was stirred under H_2 atmosphere at room temperature for 5 h. The reaction mixture was filtered and evaporated to give a residue, which was purified by silica gel column chromatography ($CHCl_3$/acetone, 98:2) to give **5c** (4 mg).

*2.8.2 Methyl ester (**5a**) of Iophachinin E (**5**)*

Colorless amorphous solid, $[\alpha]^{21}_{D}$ +4.0 (*c* 0.10, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ_H 7.39 (1H, dd, *J* = 8.4, 2.0 Hz, H-12), 7.29 (1H, d, *J* = 8.4 Hz, H-11), 7.28 (1H, d, *J* = 2.0 Hz, H-14), 5.82 (1H, dd, *J* = 4.0, 1.6 Hz, H-7), 3.64 (3H, s, 18-OMe), 2.51 (1H, dd, *J* = 12.9, 1.3 Hz, H-5), 2.38 (1H, brd, *J* = 13.3 Hz, H-1a), 2.09 (1H, ddd, *J* = 14.8, 12.9, 4.3 Hz, H-6a), 1.86 (1H, m, H-2a), 1.81 (1H, m, H-3a), 1.74 (1H, m, H-2b), 1.67 (1H, m, H-3b), 1.63 (1H, brd, *J* = 12.9 Hz, H-6b), 1.49 (3H, s, H_3-17), 1.48 (3H, s, H_3-16), 1.45 (1H, td, *J* = 13.3, 3.7 Hz, H-1b), 1.27 (3H, s, H_3-19), and 1.18 (3H, s, H_3-20); HRESIMS *m/z* 411.2143 ($[M+Na]^+$, calcd for $C_{23}H_{32}O_5Na$, 411.2147).

*2.8.3 15,16-Dehydroxy derivative (**5b**) of **5a***

Colorless amorphous solid, $[\alpha]^{18}_{D}$ +46.7 (*c* 0.07, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.42 (1H, dd, *J* = 8.4, 2.1 Hz, H-12), 7.27 (2H, m, H-11 and H-14), 5.91 (1H, dd, *J* = 4.3, 1.8 Hz, H-7), 5.33 and 5.06 (each 1H, brs, H_2-16), 3.64 (3H, s, OMe-18), 2.59 (1H, dd, *J* = 13.0, 1.8 Hz, H-5), 2.32 (1H, brd, *J* = 12.5 Hz, H-1a), 2.12 (3H, brs, H_3-17), 2.08 (3H, s, OAc-7), 2.08 (1H, m, H-6a), 1.84–1.64 (5H, m, H_2-2, H_2-3, and H-6b), 1.54 (1H, m, H-1b), and 1.27 (3H, s, H_3-19), 1.18 (3H, s, H_3-20); HRESIMS *m/z* 393.2035 ($[M+Na]^+$, calcd for $C_{23}H_{30}O_4Na$, 393.2042).

*2.8.4 Reduced product (**5c**, methyl 7α-acetoxydehydroabietate) of **5b***

Colorless amorphous solid, $[\alpha]^{18}_{D}$ +24.9 (*c* 0.40, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 7.23 (1H, d, *J* = 8.3 Hz, H-11), 7.18 (1H, dd, *J* = 8.3, 2.0 Hz, H-12), 7.03 (1H, d, *J* = 2.0 Hz), 5.89 (1H, dd, *J* = 4.2, 1.6 Hz, H-7), 3.63 (3H, s, OMe-18), 2.86 (1H, sept, *J* = 6.9 Hz, H-15), 2.59 (1H, dd, *J* = 13.0, 1.8 Hz, H-5), 2.31 (1H, brd, *J* = 12.5 Hz, H-1a), 2.08 (3H, s, OAc-7), 2.07 (1H, ddd, *J* = 14.7, 13.0, 4.2 Hz, H-6a), 1.82 (1H, m, H-3a), 1.81 (1H, m, H-2a), 1.74 (1H, m, H-2b), 1.67 (1H, m, H-3b), 1.65 (1H, m, H-6b), 1.54 (1H, m, H-1b), 1.26 (3H, s, H_3-19), 1.23 (3H, d, *J* = 6.9 Hz, H_3-17),

1.22 (3H, d, J = 6.9 Hz, H_3-16), and 1.18 (3H, s, H_3-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ_H 178.4 (C-18), 170.7 (7-OAc), 147.6 (C-9), 146.6 (C-13), 131.8 (C-8), 128.3 (C-14), 127.2 (C-12), 124.4 (C-11), 70.8 (C-7), 51.8 (18-OMe), 47.3 (C-4), 40.3 (C-5), 37.5 (C-1), 37.3 (C-10), 36.3 (C-3), 33.5 (C-15), 28.2 (C-6), 24.3 (C-20), 24.0 (C-16), 23.8 (C-17), 21.5 (7-OAc) 18.5 (C-2), and 16.4 (C-19); HRESIMS *m/z* 395.2216 ($[M+Na]^+$, calcd for $C_{23}H_{32}O_4Na$, 395.2198);

*2.9. Evaluation for biological activities of **1**–**16***

Lophachinins A–E (**1**–**5**) and known related diterpenes (**6**–**16**) were evaluated for their inhibitory effects on IL-1β production from LPS stimulated microglia cells and antiproliferative activities against human cancer cell lines (MCF-7 and A549) according to the procedures described previously [10].

T

	1		**2**	
Position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	4.77 (brs)	77.0[a]	4.76 (t, 2.5)	77.0[a]
2	1.98, 1.81 (each 1H, m)	25.6	1.97, 1.82 (each 1H, m)	25.6
3	1.93, 1.48 (each 1H, m)	23.4	1.93, 1.47 (each 1H, m)	23.4
4	–	41.7	–	41.7
5	1.80 (dd, 13.9, 4.1)	40.2	1.74 (m)	40.6
6	2.32 (td, 13.9, 8.0)	29.3	2.17 (td, 13.5, 8.2)	24.8
	1.63 (ddd, 13.9, 4.1, 1.5)		1.42 (ddd, 13.5, 4.3, 1.6)	
7	4.89 (m)	66.6	4.46 (brd, 8.2)	75.1
8	–	147.8	–	144.1
9	–	82.1	–	82.1
10	–	41.2	–	41.2
11	2.26 (ddd, 13.4, 9.6, 4.1)	25.0	2.24 (ddd, 13.[illegible], 9.5, 4.2)	24.8
	1.76 (td, 13.4, 2.9)		1.75 (m)	
12	2.10 (ddd, 12.6, 9.6, 2.9)	26.2	2.08 (ddd, 12.6, 9.5, 2.9)	26.0
	1.49 (td, 12.6, 4.1)		1.50 (m)	
13	–	80.5	–	80.6
14	6.71 (d, 2.1)	133.1	6.[illegible] (d, 2.3)	133.8
15	1.95 (m)	32.2	1.92 (sept, 6.9)	32.2
16	0.99 (3H, d, 7.1)	17.1[b]	0.99 (3H, d, 6.9)	17.1[b]
17	0.99 (3H, d, 7.1)	17.3[b]	0.99 (3H, d, 6.9)	17.3[b]
18	–	177.5	–	177.3
19	1.13 (3H, s)	17.8	1.14 (3H, s)	17.8
20	1.01 (3H, s)	18.4	1.01 (3H, s)	18.3
7-OMe			3.39 (3H, s)	56.0

[a] overlapped with the solvent signal, [b] interchangeabl

T

	3		**4**		**5**	
Position	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	4.41 (brs)	71.8	4.42 (brs)	71.4	2.38 (brd, 12.1), 1.46 (m)	39.0
2	2.15 (tdd, 13.9, 3.9, 1.9)	26.1	2.18 (brt, 14.2)	26.1	1.85 (m)	19.7
	1.81 (dq, 13.9, 4.2)		1.82 (dq, 14.2, 3.6)		1.75 (m)	
3	2.48 (td, 13.9, 4.2)	30.8	2.36 (td, 14.2, 3.6)	31.2	1.85 (m)	37.7
	1.41 (ddd, 13.9, 4.2, 3.9)		1.44 (dt, 14.2, 3.6)		1.68 (m)	
4	–	48.1	–	48.3	–	48.2
5	3.99 (dd, 13.3, 1.6)	33.9	3.18 (dd, 14.6, 3.3)	39.5	2.56 (dd, 12.9, 1.7)	41.6
6	2.08 (td, 13.3, 4.0)	31.5	2.76 (dd, 17.4, 14.6)	38.7	2.08 (ddd, 14.6, 12.9, 3.9)	29.1
	1.66 (brd, 13.3)		2.46 (dd, 17.4, 3.3)		1.75 (brd, 14.6)	
7	4.62 (dd, 4.0, 1.6)	69.0	–	201.2	5.82 (dd, 3.9, 1.6)	72.8[a]
8	–	138.2	–	132.8	–	132.9
9	–	144.1	–	152.2	–	149.8
10	–	44.0	–	44.3	–	38.5
11	7.28 (d, 8.8)	125.9	7.47 (1H, m)	126.8	7.30 (d, 8.4)	125.4
12	7.15 (brd, 8.8)	127.6	7.47 (1H, m)	133.1	7.39 (dd, 8.4, 2.0)	126.5
13	–	147.5	–	147.7	–	148.6
14	7.16 (brs)	129.9	7.81 (brs)	125.5	7.27 (d, 2.0)	127.5
15	2.85 (sept, 6.9)	34.9	2.92 (sept, 6.9)	34.9	–	72.7[a]
16	1.22 (3H, d, 6.9)	24.4	1.24 (3H, d, 6.9)	24.2	1.48 (3H, s)	31.7[b]
17	1.22 (3H, d, 6.9)	24.4	1.24 (3H, d, 6.9)	24.2	1.49 (3H, s)	31.9[b]
18	–	182.0	–	183.9	–	181.9
19	1.26 (3H, s)	17.2	1.31 (3H, s)	17.6	1.25 (3H, s)	17.0
20	1.16 (3H, s)	25.6	1.28 (3H, s)	24.3	1.18 (3H, s)	24.7
7-OAc					–	172.6
					2.01 (3H, s)	21.4

[a, b] interchangeable

3. Results and discussion

The MeOH extract prepared from the dried aerial parts of *L. chinensis* collected at Ovorhangay province, Mongolia was partitioned with EtOAc and H_2O. The EtOAc-soluble materials were further partitioned with *n*-hexane and 90% MeOH aq. Repeated chromatographic separations and crystallizations of the 90% MeOH aq.-soluble materials gave lophachinins A (**1**, 62 mg), B (**2**, 5 mg), C (**3**, 442 mg), D (**4**, 23 mg), and E (**5**, 305 mg) (Chart 1), together with eleven known related diterpenes (**6**–**16**) (Chart 2). The structures of the known diterpenes were identified as methyl 15-hydroxydehydroabietate (**6**) [13], 7α-hydroxyteideadiol (**7**) [14], dehydroabietic acid (**8**) [15], 15-methoxydehydroabietic acid (**9**) [16], 7α-hydroxydehydroabietic acid (**10**) [17], 7α-acetoxydehydroabietic acid (**11**) [18], 7α,15-dihydroxydehydroabietic acid (**12**) [19],

12-hydroxydehydroabietic acid (**13**) [20], 7-oxodehydroabietic acid (**14**) [17], karamatsuic acid (**15**) [22], and holophyllin F (**16**) [23] by comparison of their spectroscopic data with those reported in the literature.

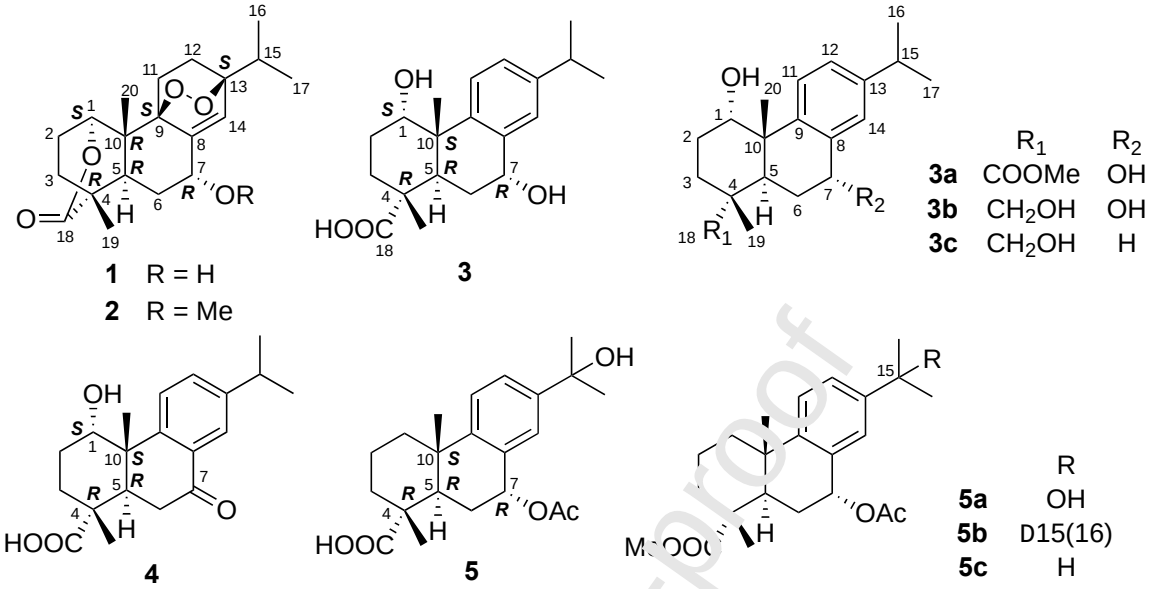


Chart 1. Structures of new diterpenes, lophachinins A–E (**1**–**5**), and their derivatives (**3a**–**3c** and **5a**–**5c**).

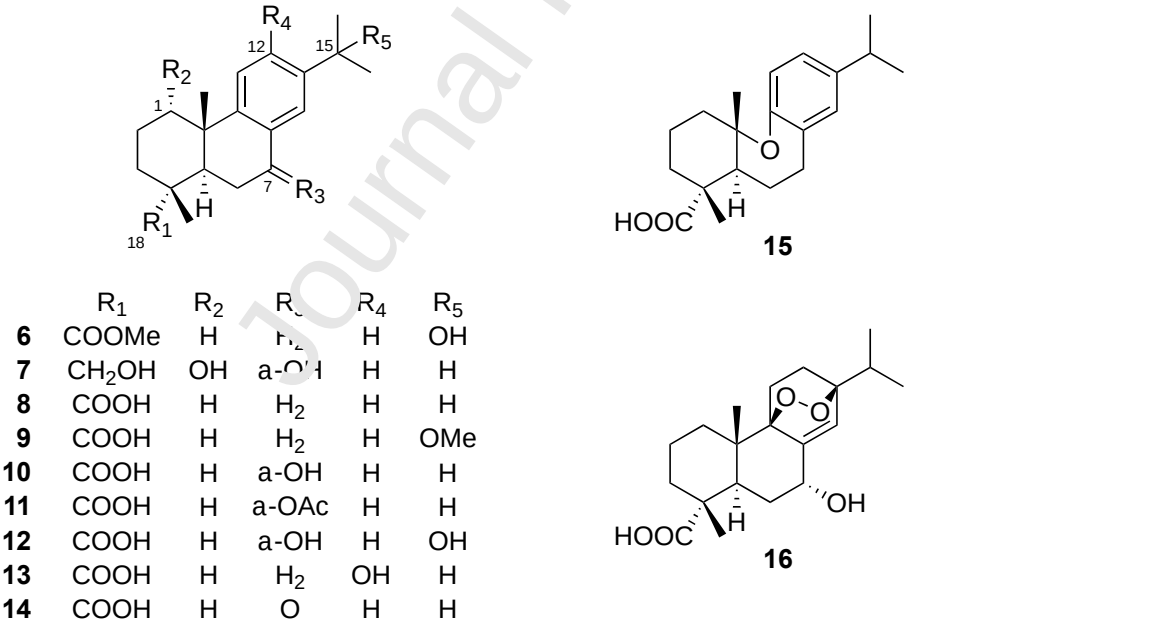


Chart 2. Structures of known diterpenes (**6**–**16**).

Lophachinin A (**1**) was isolated as a colorless amorphous solid. The ^{1}H NMR spectrum showed the resonances due to one trisubstituted olefin, two oxygenated methines, two secondary methyls, and two tertiary methyls (Table 1), while the ^{13}C NMR spectrum indicated the existence of 20

carbons including one carboxyl, two olefinic, and four oxygenated sp^3 carbons. These spectroscopic data and HRESI analysis suggested **1** to be a diterpene with the molecular formula of $C_{20}H_{28}O_5$. The structure of **1** was elucidated by 2D NMR analysis (Fig. 1). ^{1}H-^{1}H COSY cross-peaks of H-1/H_2-2/H_2-3, H-5/H_2-6/H-7, H_2-11/H_2-12, and H_3-16/H-15/H_3-17, along with HMBC correlations of H-14 with C-7, C-8, C-9, C-12, and C-13, H_3-16 with C-13, H_3-19 with C-3, C-4, C-5, and C-18, and of H_3-20 with C-1, C-5, C-9, and C-10, revealed **1** to be an abietane diterpene with oxygen functions at C-1, C-7, C-9, and C-13, a double bond at C-8(14), and a carboxyl group (C-18) at C-4. An HMBC correlation of H-1 with C-18 and an IR absorption at 1741 cm^{-1} revealed the lactone bridge between C-1 and C-18. The ^{13}C NMR chemical shifts of C-9 (δ_C 82.1) and C-13 (δ_C 80.5), together with taking its molecular formula into account, suggested the presence of an endoperoxy bridge between C-9 and C-13 of **1**. This was further supported by resemblance of the chemical shifts for C-9 and C-13 with those of corresponding positions of a structurally related abietane diterpene, holophyllin F (**16**) [23]. Therefore, the planer structure of **1** was assigned as shown in Fig. 1.

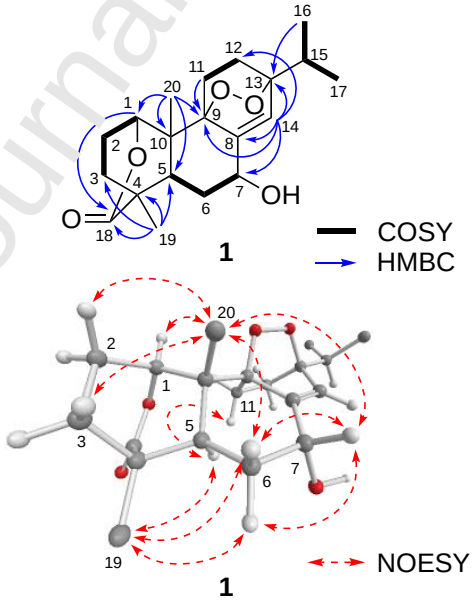


Fig. 1. Selected 2D NMR correlations for lophachinin A (**1**) (protons of methyl groups in 3D model are omitted).

The relative configuration of **1** were elucidated by analysis of the NOESY spectrum measured in C_5D_5N, since there are some overlapped signals in the ^{1}H NMR measured in $CDCl_3$. NOESY

correlations of H_3-19 with H_2-6 and of H_3-20 with H-1, H-2β, H-3β, and H-6β indicated the *trans*-junction of the A/B-rings as well as the β-configurations of H-1, Me-19, and Me-20 (Fig. 1). The β-configuration of the endoperoxy bridge was elucidated by NOESY cross-peaks of H-5/H-11α. In addition, a twisted-boat conformation of the B-ring and the pseudo-equatorial orientation of 7-OH were suggested by a NOESY correlation of H-7/H_3-20. Therefore, application of the modified Mosher's method [24] to the 7-OH was carried out to assign the absolute configuration of C-7. The distribution of Δδ values obtained by ^{1}H NMR analysis for the (*S*)- and (*R*)-MTPA esters (**1a** and **1b**, respectively) of **1** (Fig. 2) indicated the absolute configuration of C-7 to be *R*. Accordingly, the structure of lophachinin A (**1**) was established as shown in Chart 1.

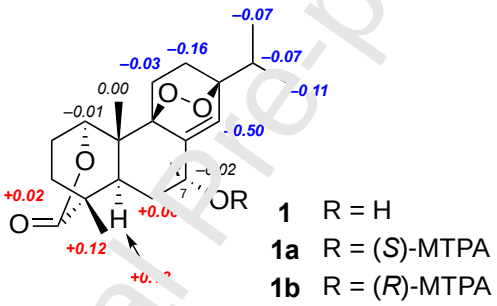
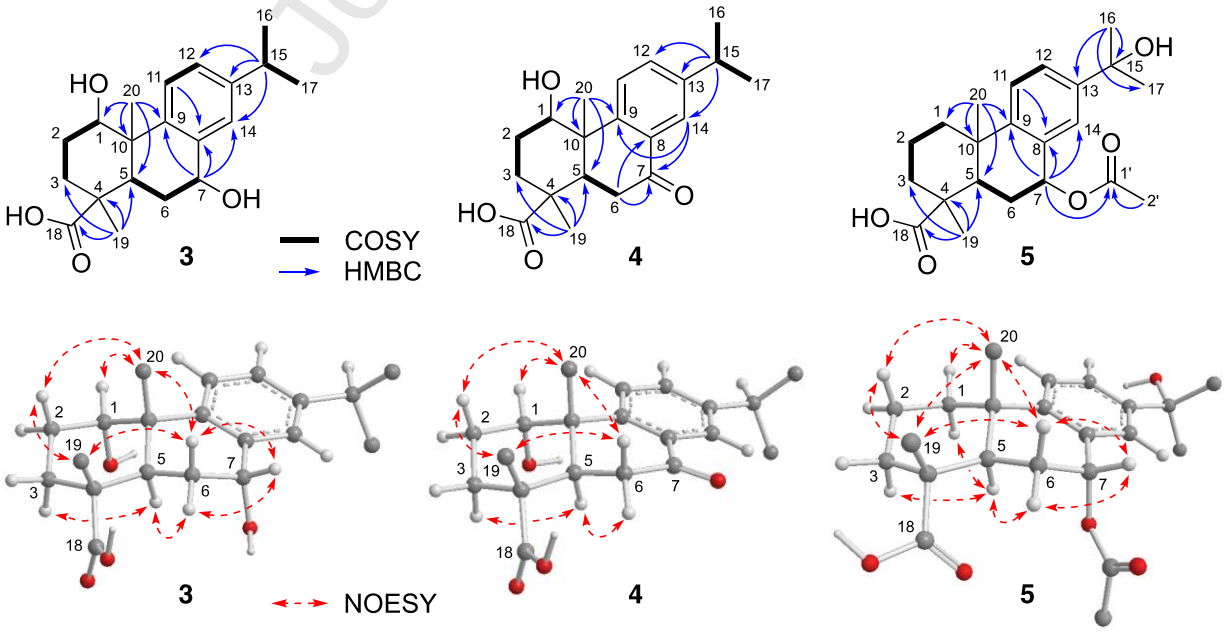


Fig. 2. Distribution of Δδ values [S–R (in ppm)] for the (*S*)- and (*R*)-MTPA esters (**1a** and **1b**, respectively) of lophachinin A (**1**).

Lophachinin B (**2**) gave an $[M+Na]^+$ ion peak at *m/z* 385.1979, which was larger by 14 mass units than that of **1**, indicating the molecular formula of **2** to be $C_{21}H_{30}O_5$. The 1D NMR spectra of **2** resembled with those of **1** (Table 1), except for the observation of one methoxy signal in **2**. The 2D NMR spectra of **2** showed correlations similar to those of **1**, and an HMBC correlation of the methoxy proton signal with C-7 suggested **2** to be the 7-*O*-methyl derivative of **1**. This was confirmed by methylation of **1** with MeI and Ag_2O, giving a product (**1c**) whose ^{1}H NMR spectroscopic data and specific rotation value were identical with those of **2**. Therefore, the structure of lophachinin B (**2**) was characterized as shown in Chart 1.

Lophachinin C (**3**) was obtained as an optically active colorless amorphous solid {$[\alpha]_D$ +31.0 (*c*

0.10, MeOH)}, of which molecular formula was assigned as $C_{20}H_{28}O_4$ by the HRESIMS. The ^{1}H NMR spectrum showed the signals due to three aromatic protons, two oxygenated methines, two secondary methyls, and two tertiary methyls (Table 2), while the ^{13}C NMR spectrum displayed 20 carbon resonances including those arising from one carboxyl group, one trisubstituted benzene ring, and two oxygenated methine carbons. Analysis of the 2D NMR spectra (Fig. 3) implied **3** to be a typical abietane diterpene with an aromatized C-ring, hydroxy groups at C-1 and C-7, and carboxyl group (C-18) at C-4. NOESY correlations of H_3-20/H-1, H_3-20/H-2β, H-2β/H_3-19, H_2-6/H-7, H-6β/H_3-19, and H-6β/H_3-20 indicated the β-orientations of H-1, H-7, Me-19, and Me-20 (Fig. 3), while the α-orientation of H-5 was disclosed by NOESY correlations of H-5/H-6α and H-3α/H-6α. In addition, the absolute configuration of **3** was confirmed by chemical conversion of **3** into teideadiol, whose absolute configuration was previously determined as 1*S*, 4*R*, 5*R*, and 10*S* [24]. Thus, reduction of a methyl ester (**3a**) of **3** by $LiAlH_4$ gave an alcohol (**3b**), whose 7-hydroxy group was further removed by catalytic reduction with H_2/Pd-C to yield the 7-deoxy derivative (**3c**) of **3b**. The derivative (**3c**) was identified as teideadiol by comparisons of its spectroscopic data as well as specific rotation value with those reported in the literature [14,25,26]. Consequently, the structure of **3** including its absolute configuration was confirmed as shown in Chart 1.



models are omitted).

Lophachinin D (**4**) gave a sodiated molecular ion at *m/z* 353.1723 ($[M+Na]^+$, Δ –0.6 mmu) in the HRESIMS, suggesting the molecular formula of $C_{20}H_{26}O_4$. The ^{1}H NMR spectrum of **4** resembled with that of **3** (Table 2), except for the absence of the oxymethine proton signal assignable to H-7, but instead the ^{13}C NMR spectrum disclosed a resonance due to a ketone carbonyl group. In addition, the presence of an abietane diterpene structure similar to **3** was provided by 2D NMR analyses (Fig. 3), where the existence of the ketone carbonyl group at C-7 was confirmed by HMBC correlations of H-14 and H_2-6 with C-7. The relative configuration of **4** was concluded to be the same as that of **3** by NOESY analysis (Fig. 3). The absolute configuration of **4** was deduced by comparison of the ECD spectrum with TDDFT calculated spectrum. Thus, the experimental spectrum of **4** was well correlated with the calculated spectrum of an enantiomer with the 1*S*, 4*R*, 5*R*, and 10*S* configurations (Fig. 4). Accordingly, the structure of **4** was assigned as shown in Chart 1.

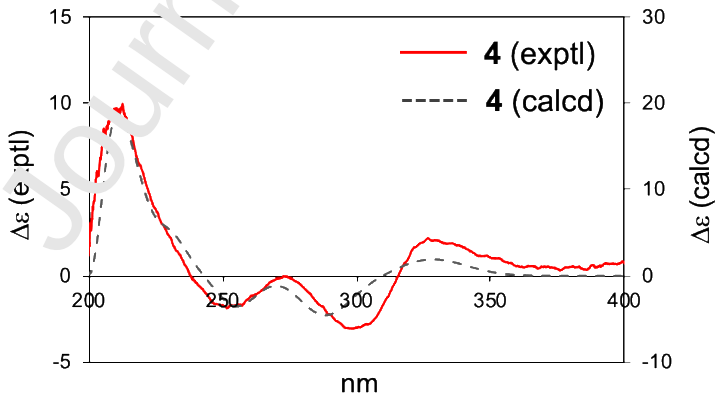


Fig. 4. Experimental and calculated ECD spectra of lophachinin D (**4**).

Lophachinin E (**5**) was isolated as an optically active colorless amorphous solid {$[\alpha]_D$ +13.4 (*c* 0.10, MeOH)}. The ^{1}H and ^{13}C NMR spectra suggested **5** to be an abietane diterpene structurally related to **3** and **4** with an acetoxy group (Table 2), while the ^{1}H-^{1}H COSY and HMBC correlations were suggestive of **5** to be 7-acetoxy-15-hydroxydehydroabietic acid (Fig. 3). The NOESY analysis

(Fig. 3) indicated the α-configuration of the acetoxyl group at C-7. The absolute configuration of **5** was confirmed by chemical conversion of **5** into methyl 7α-acetoxydehydroabietate, whose absolute stereochemistry was previously determined [27]. Thus, treatment of methyl ester (**5a**) of **5** with $SOCl_2$ in pyridine gave a 15-dehydro derivative (**5b**), which was further treated with H_2/Pd-C to yield a reduced product (**5c**). The ^{1}H and ^{13}C NMR data and specific rotation value of **5c** were in good agreement with those reported for methyl 7α-acetoxydehydroabietate [27]. Accordingly, the structure of lophachinin E (**5**) was characterized as shown in Chart 1.

Based on a traditional usage of *Lophanthus chinensis* in Mongolia, the isolated diterpenes (**1**–**16**) were evaluated for their anti-inflammatory activity on microglial cells. Thus, lophachinins A (**1**) and B (**2**) and some known diterpenes (**7**, **8**, **9**, **10**, **12**, and **13**) demonstrated moderate inhibitory effects on IL-1β production from LPS-treated microglial cells at 100 μM (% of IL-1β production: 81.6, 48.2, 89.8, 48.7, 69.4, 76.0, 89.2, and 72.4, respectively) without cytotoxicity against microglial cells. The diterpenes **1**–**16** were also evaluated for their antiproliferative activity against human cancer cell lines (MCF7 and A549), showing no cytotoxicity against both cell lines (IC_{50} >100 μM).

4. Conclusion

Phytochemical study on the MeOH extract from the aerial parts of a Mongolian traditional herbal medicine, *Lophanthus chinensis*, resulted in the isolation of five new abietane diterpenes, lophachinins A–E (**1**–**5**), and eleven known abietane diterpenes (**6**–**16**). The structures of **1**–**5** were elucidate by extensive spectroscopic analysis and chemical conversions. Lophachinins A (**1**) and B (**2**), and **16** possessed an endoperoxy bridge at C-ring, whereas the others had an aromatized C-ring. Highly oxygenated abietane diterpenes were shown to present in this plant. Lophachinins A (**1**) and B (**2**) and some diterpenes exhibited moderate anti-inflammatory activity.

Acknowledgment

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Conflict of Interest

[FITOTE-D-20-00948]

The authors declare no conflict of interest.

Supplementary Data

Supplementary Material

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