

Bis-spirolabdane Diterpenoids from *Leonotis nepetaefolia*

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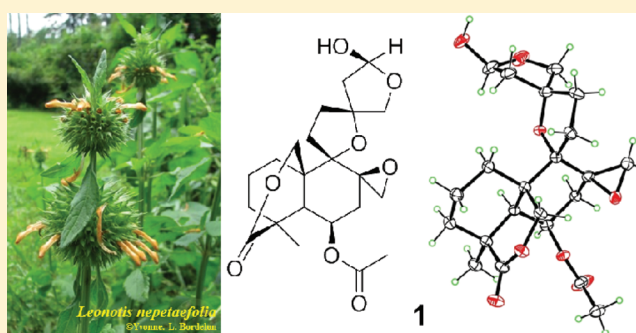
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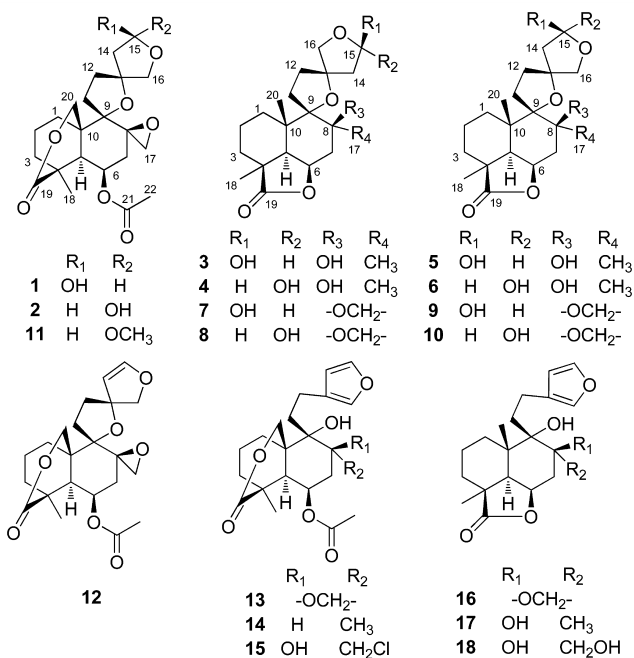
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Supporting Information

ABSTRACT: Ten new bis-spirolabdane diterpenoids, leonepetaefolins A–E (1, 3, 5, 7, 9) and 15-*epi*-leonepetaefolins A–E (2, 4, 6, 8, 10), together with eight known labdane diterpenoids (11–18) as well as two known flavonoids, apigenin and cirsiolol, were isolated from the leaves of *Leonotis nepetaefolia*. The structures of the new compounds were determined on the basis of 1D- and 2D-NMR experiments including ¹H, ¹³C, DEPT, ¹H–¹H COSY, HSQC, HMBC, and NOESY. The absolute configuration of an epimeric mixture of 1 and 2 was determined by X-ray crystallographic analysis. The compounds isolated were evaluated for their binding propensity in several CNS G-protein-coupled receptor assays in vitro.



Leonotis nepetaefolia R. Br. (family: Lamiaceae, syn. Labiatae), also known as Klip Dagga or Lion's Ear, is widely distributed throughout tropical Africa, southern India, and the tropical regions of America. It is traditionally used in Caribbean folk medicine and Ayurvedic herbal medicine to treat a wide array of human diseases such as coughs, fever, stomachache, skin infections, rheumatism, dysmenorrhea, and kidney dysfunction.^{1–4} In Mexico, the name “mota” (marijuana) is given for this plant, which suggests that *L. nepetaefolia* is used as a marijuana substitute.⁵ In addition, a variety of biological activities including antispasmodic,⁵ antibacterial, antifungal,^{6,7} anti-inflammatory,⁸ antioxidative,^{9,10} antiasthmatic, and anti-diarrheal¹¹ activities have been reported for the crude extracts or pure compounds from this plant. Previous phytochemical studies of the plant indicated the presence of labdane diterpenoids,^{2,4,12–17} iridoids,^{9,10} and coumarins.¹⁸ As part of an ongoing effort to search for bioactive constituents from psychoactive plants, CH₂Cl₂–MeOH (1:1) extract of the leaves of *L. nepetaefolia* was investigated. This procedure led to the isolation of 10 new bis-spirolabdane diterpenoids, named leonepetaefolins A–E (1, 3, 5, 7, 9) and 15-*epi*-leonepetaefolins A–E (2, 4, 6, 8, 10), together with eight known labdane diterpenoids, methoxynepetaefolin (11),^{13,16,19} nepetaefolin (12),^{12,15,20} nepetaefuran (13),¹² dubiin (14),²¹ compound 15,¹² leonotinin (16),^{14,20} leonotin (17),²⁰ and LS-1 (18),²² and two known flavonoids, apigenin²³ and cirsiolol.²⁴ Herein, we describe the isolation and structural elucidation of the 10 new bis-spirolabdane diterpenoids 1–10.



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Table 1. ¹H NMR Data of Compounds 1–10 (δ in ppm, J in Hz, 400 MHz, in CDCl₃)

position	1/2 ^a	3/4 ^a	5/6 ^a	7/8 ^a	9/10 ^a
1a	1.71, m	1.44, m	1.40, m	1.48, m	1.55, m
1b	1.71, m	1.23, m	1.24, m	1.21, m	1.30, m
2a	1.73, m	1.54, m	1.54, m	1.69, m	1.76, m
2b	1.73, m	1.54, m	1.54, m	1.49, m	1.53, m
3a	1.48, m	1.32, m	1.29, m	1.44, m	1.46, m
3b	1.79, m	2.18, m	2.17, m	1.44, m	2.16, m
5	1.96, m	2.08, d (5.6)/2.04, d (5.6)	2.07, d (5.2)/2.01, d (5.2)	2.28, d (4.8)/2.23, d (4.8)	2.28, d (4.4)/2.13, d (4.8)
6	5.12, br s	4.79, dd (13.2, 7.2)	4.77, m	4.79, m	4.82, m
7a	2.60, m/2.52, m	2.31, d (8.4)	2.30, d (8.0)	2.55, m	2.57, dd (17.2, 7.6)/2.61, dd (17.2, 7.6)
7b	1.56, d (16.0)/1.52, d (16.0)	2.31, d (8.4)	2.30, d (8.0)	2.10, m	2.10, m/2.15, m
11a	1.37, m	2.41, m	2.40, m	1.67, m	1.70, m
11b	1.76, m	1.73, m	1.80, m	1.74, m	1.84, m
12a	2.13, m/1.99, m	2.11, m/2.10, m	2.13, m/2.14, m	2.05, m/1.98, m	2.10, m/2.02, m
12b	2.02, m/1.99, m	2.11, m/1.96, m	2.13, m/1.84, m	2.05, m/1.98, m	2.10, m/2.02, m
14a	2.04, m/2.37, dd (13.6, 4.8)	2.48, dd (13.6, 5.6)/2.31, d (9.8)	2.26, m/2.16, m	2.22, m/2.02, m	2.40, m/2.20
14b	2.17, d (13.6)/2.51, m	2.10, m/2.31, d (9.8)	1.79, m/1.91, m	1.86, dd(13.2, 2.0)/2.02, m	1.95, dd (13.2, 2.0)/2.07
15	5.55, br s/5.38, m	5.58, d (5.6)/5.45, d (2.8)	5.57, d (4.4)/5.40, d (3.6)	5.59, d (3.2)/5.41, br s	5.63, d (3.2)/5.43, dd (8.8, 3.2)
16a	3.77, d (8.8)/3.91, d (8.8)	3.80, d (8.8)/4.04, d (8.8)	4.03, d (9.2)/4.23, d (9.2)	3.90, d (9.2)/4.10, d (9.2)	3.89, d (9.2)/4.03, d (9.2)
16b	3.65, d (8.8)/3.55, d (8.8)	3.76, d (8.8)/3.52, d (8.8)	3.97, d (9.2)/3.73, d (9.2)	3.85, d (9.2)/3.65, d (9.2)	3.77, d (9.2)/3.67, d (9.2)
17a	2.28, d (3.2)/2.31, d (2.4)	1.27, s/1.30, s	1.20, s/1.25, s	2.40, d (4.0)/2.45, d (4.0)	2.41, d (4.4)/2.45, d (4.4)
17b	2.53, m/2.53, m			2.74, d (4.0)/2.77, d (4.0)	2.69, d (4.4)/2.72, d (4.4)
18	1.08, s	1.25, s	1.22, s	1.27, s	1.30, s
20a	3.94, d (11.6)	0.98, s/0.99, s	0.96, s/0.98, s	1.03, s/1.05, s	1.08, s/1.10, s
20b	5.01, d (11.6)				
22	1.95, s				

^aAssignments were based on HSQC, ¹H–¹H COSY, and HMBC experiments.

RESULTS AND DISCUSSION

The dried CH₂Cl₂–MeOH (1:1) extract of the leaves of *L. nepetaefolia* was dissolved in 90% aqueous MeOH and partitioned with hexanes and CH₂Cl₂, successively. The CH₂Cl₂-soluble portion was subjected repeatedly to silica gel, Sephadex LH-20, and Lichroprep RP-18 gel column chromatography (CC) and semipreparative HPLC to afford 10 new bis-spirolabdane diterpenoids (**1**–**10**), eight known labdane diterpenoids (**11**–**18**), and two known flavonoids.

Leonepetaefolin A (**1**) and 15-*epi*-leonepetaefolin A (**2**) were obtained as colorless plates, [α]_D²⁵ + 26.2, in a 1:1 epimeric mixture. Their positive-ion HRESIMS gave a pseudomolecular peak at *m/z* 445.1847 [*M* + Na]⁺, consistent with the molecular formula of C₂₂H₃₀O₈, indicating eight degrees of unsaturation. In the IR spectrum, absorption bands of hydroxy (3511 cm⁻¹) and two ester (1735, 1712 cm⁻¹) functional groups were observed. The ¹³C NMR and DEPT spectra of **1/2** showed 22 pairs of carbon signals including two pairs of ester carbonyl carbons (δ_C 170.3/170.4, 176.0/175.8), five pairs of quaternary sp³ carbons (δ_C 40.9/40.8, 41.0 × 2, 55.9/56.0, 86.2/87.2, 90.2/89.7), three pairs of sp³ methines (δ_C 47.0/47.2, 67.7/67.9, 98.5/98.8), 10 pairs of sp³ methylenes (δ_C 20.2/20.3, 23.2/23.5, 32.4 × 2, 33.2/33.5, 36.1/35.1, 39.5/39.6, 45.6/47.0, 47.4/47.3, 73.7/73.9, 77.5/78.1), and two pairs of methyls (δ_C 20.9 × 2, 22.2/22.1). Analysis of the 1D- and 2D-NMR spectra

revealed that compounds **1/2** belong to the bis-spirolabdane class of diterpenoids, which possess a spirotetrahydrofuran ring system.^{25–31} An exocyclic epoxide ring [δ_H 2.28 (1H, d, *J* = 3.2 Hz)/2.31 (1H, d, *J* = 2.4 Hz), 2.53 (1H, m)/2.53 (1H, m)]; δ_C 47.4/47.3 (C-17), 55.9/56.0 (C-8)] was indicated by the HSQC and HMBC (H₂-17/C-8) correlations. This epoxide ring was placed at C-8 on the basis of its high-field chemical shift (δ_C 55.9/56.0) and the HMBC correlations between H₂-17 [δ_H 2.28 (1H, d, *J* = 3.2 Hz)/2.31 (1H, d, *J* = 2.4 Hz), 2.53 (1H, m)/2.53 (1H, m)] and C-7 (δ_C 32.4), C-8 (δ_C 55.9/56.0), and C-9 (δ_C 86.2/87.2). In the HMBC spectrum, the presence of cross-peaks between H-6 (δ_H 5.12, br s) and the acetoxy carbonyl carbons (δ_C 170.3/170.4) indicated that an acetoxy group is connected to C-6, which was also supported by the downfield chemical shift of H-6. In view of the eight degrees of unsaturation, there must be an additional ring in the structures of **1/2**. The observation of HMBC correlations between H₂-20 [δ_H 3.94 (2H, d, *J* = 11.6 Hz), 5.01 (2H, d, *J* = 11.6 Hz)] and carbonyl carbons C-19 (δ_C 176.0/175.8) suggested that a δ-lactone ring was present between C-19 and C-20. Comparison of ¹H and ¹³C NMR data (Tables 1 and 2) with the literature values for methoxynepetaefolin (**11**) revealed that the structures of **1/2** are similar to that of **11**,¹⁹ except that the C-15 *O*-methyl group in **11** was replaced by a hydroxy group in **1/2**. This was supported by the significant upfield shift at C-15 (δ_C 98.5/98.8; Δδ_C –6.1/5.8). Furthermore, the HMBC

Table 2. ^{13}C NMR Data of Compounds 1–10 (δ in ppm, 100 MHz, in CDCl_3)

position	1/2 ^a	3/4 ^a	5/6 ^a	7/8 ^a	9/10 ^a
1	33.2/33.5, CH ₂	32.3/32.4, CH ₂	32.8/33.2, CH ₂	28.7/28.9, CH ₂	29.2/29.3, CH ₂
2	20.2/20.3, CH ₂	17.7, CH ₂	17.8, CH ₂	17.9, CH ₂	18.0/17.9, CH ₂
3	39.5/39.6, CH ₂	29.3/29.2, CH ₂	29.4/29.3, CH ₂	28.5/28.4, CH ₂	28.5/28.4, CH ₂
4	40.9/40.8, C	42.0, C	42.1/42.0, C	43.4/43.5, C	43.4/43.5, C
5	47.0/47.2, CH	45.3/45.5, CH	45.3/45.6, CH	45.6/45.8, CH	45.9/46.3, CH
6	67.7/67.9, CH	74.4/74.3, CH	74.6/74.4, CH	74.4/74.0, CH	74.4/74.0, CH
7	32.4, CH ₂	41.0, CH ₂	41.0, CH ₂	32.5/32.4, CH ₂	32.5/32.4, CH ₂
8	55.9/56.0, C	75.4, C	75.4/75.2, C	56.7, C	56.9/56.6, C
9	86.2/87.2, C	92.6/93.7, C	92.8/94.1, C	88.8/90.0, C	88.9/90.3, C
10	41.0, C	39.6/39.7, C	39.6/39.8, C	39.3/39.4, C	39.4/39.5, C
11	23.2/23.5, CH ₂	28.5/28.2, CH ₂	28.2/28.3, CH ₂	24.7, CH ₂	25.0/24.7, CH ₂
12	36.1/35.1, CH ₂	38.0/36.2, CH ₂	37.8/35.9, CH ₂	35.7/34.2, CH ₂	35.9/33.8, CH ₂
13	90.2/89.7, C	88.8/89.2, C	89.5/89.6, C	90.0/89.8, C	90.2/90.1, C
14	45.6/47.0, CH ₂	45.3/44.5, CH ₂	46.8/45.3, CH ₂	48.5/46.7, CH ₂	47.6/45.9, CH ₂
15	98.5/98.8, CH	98.3/98.5, CH	98.8/99.2, CH	99.0/99.2, CH	98.8/99.1, CH
16	77.5/78.1, CH ₂	76.8/75.8, CH ₂	75.3/74.9, CH ₂	76.5/77.1, CH ₂	77.5/78.2, CH ₂
17	47.4/47.3, CH ₂	31.7/31.9, CH ₃	31.6, CH ₃	48.8/49.0, CH ₂	48.9/48.8, CH ₂
18	22.2/22.1, CH ₃	25.9/26.0, CH ₃	25.9/26.0, CH ₃	23.5/23.6, CH ₃	23.6/23.7, CH ₃
19	176.0/175.8, C	182.1/182.2, C	182.4/182.2, C	182.9/182.7, C	182.8/182.5, C
20	73.7/73.9, CH ₂	21.0/21.1, CH ₃	21.0/21.1, CH ₃	21.5/21.7, CH ₃	21.7, CH ₃
21	170.3/170.4, C				
22	20.9, CH ₃				

^aAssignments were based on DEPT, HSQC, and HMBC experiments.

correlations between H₃-18 and C-19, H₂-16 and C-12/C-15, H-15 and C-13/C-16, H₂-14 and C-12/C-16, H₂-11 and C-8/C-10/C-13, H₂-20 and C-1/C-9, and H-6 and C-4/C-5/C-8/C-10 also supported the proposed structures of 1/2 (Figure 1).

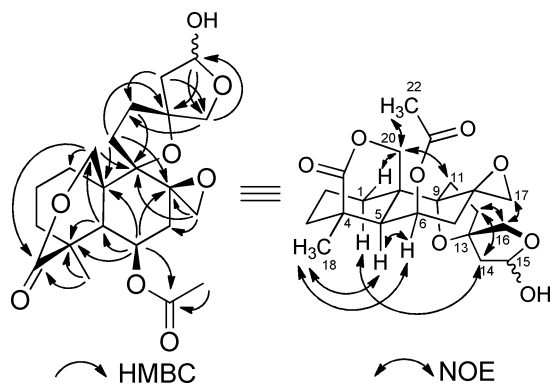


Figure 1. Selected HMBC (arrows point from protons to carbons) and NOE correlations of compounds 1/2.

The relative configurations at C-4, -5, -6, -8, -9, -10, and -13 of 1/2 are the same as those of methoxynepetaefolin (11) on the basis of NOESY data (Figure 1). The orientations of the C-15 hydroxy group were assigned as β in 1 and α in 2 based on the absence/presence of NOE correlation between H-15 and H₂-12. The absolute configurations of 1/2 were defined as 4*S*, 5*R*, 6*R*, 8*R*, 9*S*, 10*S*, 13*R*, and 15*S*/*R* by single-crystal X-ray diffraction analysis (Figure 2). Therefore, the structures of 1/2 were determined to be (4*S*,5*R*,6*R*,8*R*,9*S*,10*S*,13*R*,15*S*/*R*)-6-acetoxy-8,17;9,13;15,16-triepoxy-15-hydroxylabdan-19,20-olide, named leonepetaefolin A (1) and 15-*epi*-leonepetaefolin A (2).

A 2:3 epimeric mixture of leonepetaefolin B (3) and 15-*epi*-leonepetaefolin B (4) was obtained as a colorless gum, $[\alpha]_{\text{D}}^{25} +2.5$. Their positive-ion HRESIMS showed a pseudomolecular ion peak at m/z 389.1934 $[\text{M} + \text{Na}]^+$ and established a

molecular formula of $\text{C}_{20}\text{H}_{30}\text{O}_6$, implying six degrees of unsaturation. The IR spectrum of 3/4 indicated the presence of hydroxy (3400 cm^{-1}) and lactone (1747 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) exhibited duplicate resonances of three tertiary methyl groups (δ_{H} 0.98/0.99, 1.25×2 , $1.27/1.30$; δ_{C} 21.0/21.1, 25.9/26.0, 31.7/31.9, respectively), a hemiacetal methine carbon (δ_{C} 98.3/98.5), three oxygenated quaternary carbons (δ_{C} 92.6/93.7, 88.8/89.2, 75.4×2), an isolated oxygenated methylene (δ_{H} 3.80/4.04, $3.76/3.52$; δ_{C} 76.8/75.8), an oxygenated methine (δ_{H} 4.79×2 ; δ_{C} 74.4/74.3), seven methylenes (δ_{C} 45.3/44.5, 41.0×2 , 38.0/36.2, 32.3/32.4, 29.3/29.2, 28.5/28.2, 17.7×2), one methine (δ_{H} 2.08/2.04; δ_{C} 45.3/45.5), two quaternary carbons (δ_{C} 42.0×2 , 39.6/39.7), and an ester carbonyl carbon (δ_{C} 182.1/182.2). These spectroscopic data suggested that compounds 3/4 are bis-spirolabdan diterpenoids possessing a spirotetrahydrofuran ring system.^{25–31} On the basis of the six degrees of unsaturation, there must be an extra ring in the structures of 3/4. The downfield chemical shift of H-6 (δ_{H} 4.79) suggested that the C-6 hydroxy group was acylated, and therefore a γ -lactone ring between C-6 and C-19 was proposed. The ^1H and ^{13}C NMR data of 3/4 were comparable to those of cyllenin A/15-*epi*-cyllenin A, which were isolated from *Marrubium cylleneum*²⁷ and *M. globosum* ssp. *libanoticum*,²⁹ except for the significant downfield shift at C-7 (δ_{C} 41.0×2 ; $\Delta\delta_{\text{C}}$ +9.3), C-8 (δ_{C} 75.4×2 ; $\Delta\delta_{\text{C}}$ +43.6), and C-17 (δ_{C} 31.7/31.9; $\Delta\delta_{\text{C}}$ +14.5). This is in agreement with the addition of an extra hydroxy group at C-8 in 3/4, which was supported also by the observation of methyl singlets instead of methyl doublets for H₃-17 in the ^1H NMR spectrum. The proposed structures of 3/4 were confirmed by the HMBC correlations between H₃-20 and C-1/C-5/C-9, H₃-18 and C-19, H₃-17 and C-7/C-8/C-9, H₂-16 and C-12/C-15, H-15 and C-13/C-14/C-16, H₂-14 and C-12/C-16, H₂-11 and C-8/C-10/C-13, and H-6 and C-7/C-8/C-10 (Figure 3). The NOESY experiment permitted assignment of the relative configurations of 3/4. In the NOESY spectrum, the absence

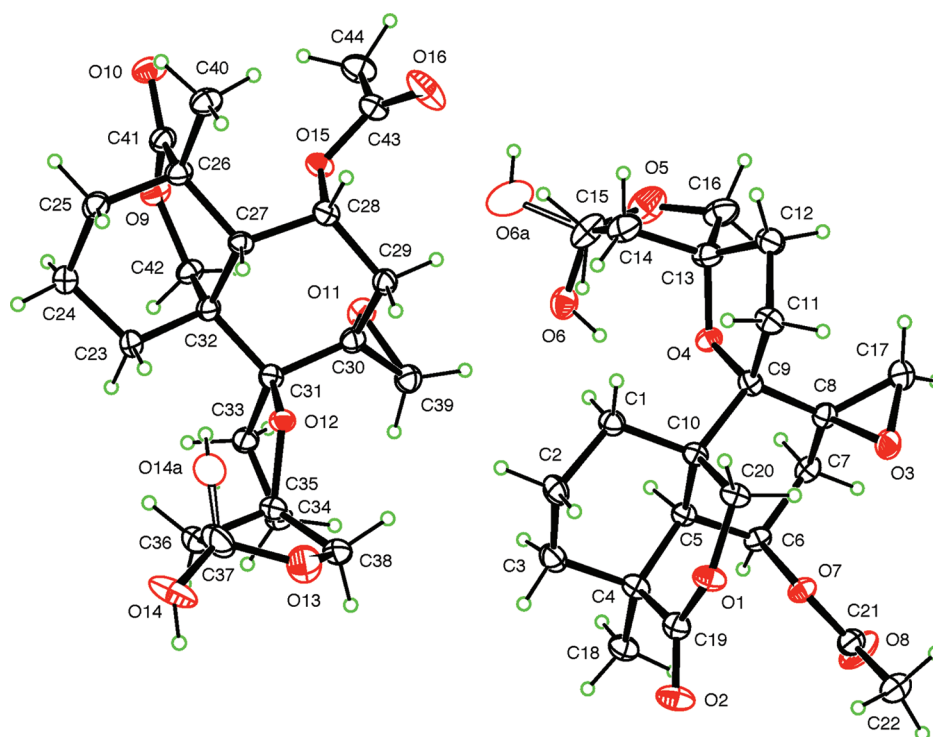


Figure 2. ORTEP drawing of the two independent molecules of compounds 1/2, with 50% ellipsoids. In each case, the O atom represented by the open ellipse is the minor component at the disordered site.

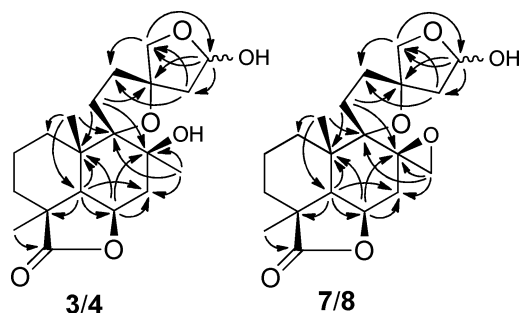


Figure 3. Selected HMBC (arrows point from protons to carbons) correlations of compounds 3/4 and 7/8.

of NOE correlations between H₃-20 and H-5 supported the *trans* fusion of rings A and B in 3/4. NOEs between H-5/H-6, H-5/H-1a, H-5/H₃-18, and H-6/H₃-18 indicated that they are cofacial and α -oriented, while NOE cross-peaks between H₃-20/H-1b and H₃-20/H₂-11 suggested that these protons occupy the opposite side and are thus β -oriented. The NOE correlations between H-1a/H₂-16 as well as H₂-14/H₃-17 suggested the relative configuration at C-13 as shown in 3/4 (Figure 4). This observation also proved the α -orientation of Me-17. Furthermore, an NOE correlation between H-15 and H₂-12 suggested the α -orientation of the C-15 hydroxy group in 4, while the absence of an NOE between H-15 and H₂-12 indicated OH-15 to be β -oriented in 3. Thus, the structures of leonepetaefolin B and 15-*epi*-leonepetaefolin B were proposed as shown in 3/4.

Leonepetaefolin C (5) and 15-*epi*-leonepetaefolin C (6) were obtained as an inseparable epimeric mixture (1:1), $[\alpha]_D^{25} +5.0$. The IR spectrum exhibited absorption bands for hydroxy (3408 cm⁻¹) and lactone (1747 cm⁻¹) groups. The molecular formula, C₂₀H₃₀O₆, of 5/6 was determined to be the same as that of 3/4

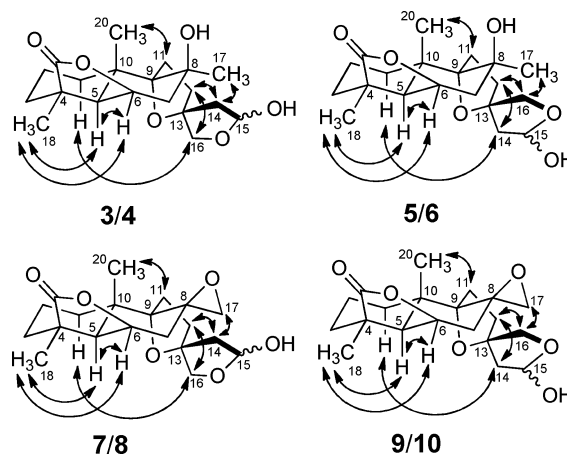


Figure 4. Selected NOE correlations of compounds 3/4, 5/6, 7/8, and 9/10.

by positive-ion HRESIMS (m/z 389.1934 [M + Na]⁺). Slight differences in ¹H and ¹³C NMR spectroscopic data (Tables 1 and 2) of 5/6 in comparison with those of 3/4 suggested that they could be stereoisomeric compounds. 2D NMR experiments including ¹H–¹H COSY, HSQC, and HMBC facilitated the assembly of the structures of 5/6, the same as those of 3/4. The relative configurations at C-4, -5, -6, -8, -9, and -10 were the same as those assigned in 3/4 on the basis of NOESY data (Figure 4). The observation of NOE correlations between H-1a/H₂-14 as well as H₂-16/H₃-17 indicated that compounds 5/6 have the opposite configuration at C-13 compared to that of 3/4. The C-15 hydroxy group was proved to be α -oriented in 6 on the basis of an NOE correlation between H-15 and H₂-12, while no such NOE was observed in 5, suggesting its OH-15 group to be β -oriented. Accordingly, the structures of

leonepetaefolin C and 15-*epi*-leonepetaefolin C were elucidated as shown in 5/6.

An inseparable 7:10 epimeric mixture of leonepetaefolin D (7) and 15-*epi*-leonepetaefolin D (8) was obtained as a colorless gum, $[\alpha]_D^{25} +29.9$. The positive-ion HRESIMS of 7/8 exhibited a pseudomolecular ion at m/z 387.1778 $[M + Na]^+$, which is consistent with a molecular formula of $C_{20}H_{28}O_6$, indicating seven degrees of unsaturation. IR absorptions revealed the presence of hydroxy (3424 cm^{-1}) and lactone (1764 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of 7/8 demonstrated two sets of signals, which were distinguishable according to their relative abundance. The close structural similarities between 7/8 and 3/4 were evident from their ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2), except for the significant upfield shifts of C-7 (δ_C 32.5/32.4; $\Delta\delta_C$ -8.5/8.6), C-9 (δ_C 88.8/90.0; $\Delta\delta_C$ -3.8/3.7), and C-8 (δ_C 56.7; $\Delta\delta_C$ -18.7) and significant downfield shift of C-17 (δ_C 48.8/49.0; $\Delta\delta_C$ +17.1). These data, coupled with the seven degrees of unsaturation of 7/8 (one more degree of unsaturation than 3/4), suggested the presence of an exocyclic epoxide ring at C-8 in 7/8, which was supported by the HMBC correlations between H_2 -17 and C-7/C-8/C-9. The HMBC correlations shown in Figure 3 confirmed the planar structures of 7/8. The relative configurations of C-4, -5, -6, -8, -9, -10, and -13 of 7/8 were found to be the same as those of 3/4 based on the NOESY data (Figure 4). In particular, NOE correlations between H_2 -14/ H_2 -17 suggested the relative configurations of C-13 and the epoxide ring as shown in 7/8 (Figure 4). Furthermore, the α -orientation of the C-15 hydroxy group in 8 was deduced from the NOE correlation between H-15 and H_2 -12, while the absence of an NOE correlation between H-15 and H_2 -12 indicated the β -orientation of OH-15 in 7. Thus, the structures of leonepetaefolin D and 15-*epi*-leonepetaefolin D were established as shown in 7/8.

Leonepetaefolin E (9) and 15-*epi*-leonepetaefolin E (10) were obtained as an inseparable epimeric mixture (2:3), $[\alpha]_D^{25} +40.0$, and have the same molecular formula ($C_{20}H_{28}O_6$) as those of 7/8, as indicated by the positive-ion HRESIMS (m/z 387.1788 $[M + Na]^+$). From the IR spectrum, hydroxy (3415 cm^{-1}) and lactone (1765 cm^{-1}) groups were evident. The resemblance of the ^1H and ^{13}C NMR data (Tables 1 and 2) of 9/10 and 7/8 suggested the stereoisomeric nature of these compounds. The molecular structures of 9/10 were similar to those of 7/8 on the basis of 2D-NMR experiments including ^1H - ^1H COSY, HSQC, and HMBC. The relative configurations at C-4, -5, -6, -8, -9, and -10 were assigned as being the same as those of 7/8, which were deduced by a NOESY experiment (Figure 4). Diagnostic NOE correlations between H-1a/ H_2 -14 as well as H_2 -16/ H_2 -17 demonstrated the opposite configurations at C-13 in 9/10 and 7/8. As for 7/8, the absence/presence of an NOE correlation between H-15 and H_2 -12 permitted assignment of the orientation of OH-15 as β in 9 and α in 10. Therefore, the structures of leonepetaefolin E and 15-*epi*-leonepetaefolin E were determined as shown in 9 and 10.

By comparing spectroscopic and specific rotation data with literature values, the 10 known compounds isolated in this investigation were identified as methoxynepetaefolin (11),^{13,16,19} nepetaefolin (12),^{12,15,20} nepetaefuran (13),¹² dubiin (14),²¹ compound 15,¹² leonotinin (16),^{14,20} leonotin (17),²⁰ LS-1 (18),²² apigenin,²³ and cirsiolol.²⁴ It is worth noting that compound 15 is a chlorinated derivative of nepetaefuran (13). This compound was previously obtained by reacting nepetaefolin (12) or nepetaefuran (13) with

phosphorus oxychloride via an $\text{S}_{\text{N}}1$ mechanism.¹² Naturally occurring chlorinated compounds derived from higher plants are rare, consistent with the difficulty of explaining a chlorine source for the biosynthesis of chlorinated compounds in these organisms. In order to test whether compound 15 was obtained as an isolation artifact, a crude methanol extract of *L. nepetaefolia* was analyzed by LC-MS. The result showed that compound 15 could not be detected in this crude extract. Furthermore, nepetaefuran (13) was found to be readily converted into 15 in the presence of HCl. Dichloromethane was used as extraction and partition solvent during the isolation procedure. It is known that dichloromethane contains trace amounts of HCl, especially when stored for a long time and exposed to light. Thus, it is most likely that the chlorinated compound 15 could be an artifact. A similar phenomenon was encountered during the chemical investigation of black cohosh, *Cimicifuga racemosa*.³²

The isolated compounds were evaluated for their binding activities to a panel of CNS G-protein-coupled receptors including adrenergic, dopaminergic, histaminic, muscarinic, opioid, and serotonergic receptors and neurotransmitter transporters as previously described.³³ In the primary binding assay, leonepetaefolin D and 15-*epi*-leonepetaefolin D (7/8) at 10 μM inhibited [^3H]SCH23390 binding to the human dopaminergic D_5 receptor (hD_5R) as well as [^3H]QNB binding to the human muscarinic M_3 receptor (hM_3R) by more than 50%. Leonotinin (16) was active on the histaminic H_2 receptor (hH_2R) (>50% inhibition at 10 μM), and methoxynepetaefolin (11) at 10 μM also showed more than 50% inhibition of [^3H]QNB binding to hM_3R (Supporting Information). A secondary binding experiment was performed with several concentrations of these active compounds to determine their K_i values (binding affinities) for hD_5R , hH_2R , and hM_3R . The K_i values of these compounds are more than 10 000 nM for these receptors, which suggested no potential for further evaluation or development.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured on an OptiMelt MPA100 automated melting point system and are uncorrected. Optical rotations were obtained on an AP IV/589-546 digital polarimeter. A PerkinElmer 100 FT-IR spectrometer was used to obtain the IR spectra. The NMR spectra were recorded on an AMX-NMR spectrometer (Bruker) operating at 400 MHz for ^1H and 100 MHz for ^{13}C . The HRESIMS spectra were determined on a Bruker Daltonic micro-TOF instrument fitted with an Agilent 1100 series HPLC and an ESI source. X-ray data were collected using a Bruker Kappa APEX-II diffractometer with $\text{Cu K}\alpha$ radiation. HPLC was performed on a Waters Delta Prep 4000 system (Waters Corporation, Milford, MA, USA), equipped with a 600 controller and a dual-wavelength detector model 2487 monitoring at 210 and 198 nm. Two semipreparative HPLC columns were employed for isolation: column A, Phenomenex Luna 5 μm , C_8 , 100 \AA , 250×10.0 mm; column B, Phenomenex Synergi 4 μm Fusion-RP C_{18} , 80 \AA , 150×10.0 mm. Silica gel 60 (63–200 μm , Sorbent Technologies, Atlanta, GA, USA), LiChroprep RP-18 gel (40–63 μm , Merck, Germany), and Sephadex LH-20 (Pharmacia) were used for open column chromatography. TLC was performed using Merck $\text{Si}_{60}\text{F}_{254}$ plates, sprayed with anisaldehyde reagent solution (prepared by mixing 0.5 mL of anisaldehyde with 10 mL of glacial HOAc, 85 mL of MeOH, and 5 mL of conc. H_2SO_4) and heated with a heat gun.

Plant Material. The ruderal *Leonotis nepetaefolia* is one of only two pantropical and commonly cultivated species of the genus.³⁴ Plant material of *L. nepetaefolia* was purchased from IAmShaman Shop (P.O. Box 12618, Chicago, IL 60612) in April 2010 and authenticated by

one of the coauthors (C.L.B.). The seller provided information that this material was cultivated in Peru. The material was predominantly leaves with a few young stems. A voucher specimen (JLI-LN-201004) is deposited in the Department of Pharmacognosy, School of Pharmacy, University of Mississippi.

Extraction and Isolation. The dried, powdered leaves of *L. nepetaefolia* (400 g) were extracted with CH_2Cl_2 -MeOH (1:1) by percolation at room temperature. After filtration and evaporation, the residue (52 g) was dissolved in 90% aqueous MeOH and partitioned with hexanes and CH_2Cl_2 , successively. The dried CH_2Cl_2 residue (15 g) was subjected to VLC on silica gel eluting with hexanes-EtOAc as solvent system with increasing polarity to yield 11 fractions (A–K). Fractions D and E (2.7 g) were combined and chromatographed over Sephadex LH-20 and silica gel open columns and further purified by repeated semipreparative HPLC [column A, eluting with isocratic 57% MeOH(aq)] to produce **14** (9.2 mg), **15** (21 mg), **17** (55 mg), and **18** (3.0 mg). Fraction F (1.0 g) was subjected to Sephadex LH-20 CC to give seven subfractions, F1–F7. Subfraction F4 (370 mg) was chromatographed over a silica gel open column and further purified by semipreparative HPLC (column A, eluting with isocratic 60% MeOH(aq)) to yield **12** (11 mg), **13** (54 mg), and **16** (35 mg). Subfraction F6 (10 mg) was washed with MeOH to afford pure apigenin (3 mg). Fractions I and J (2.4 g) were combined and precipitated with MeOH to give pure cirsiolol (7.2 mg). The supernatant was subjected to silica gel open column CC to produce an inseparable mixture of **1/2** (42 mg), **11** (7.5 mg), and subfractions I1–I7. Subfraction I3 (420 mg) was subjected to RP-18 gel open column CC [eluting with isocratic 40% MeOH(aq)] and repeated semipreparative HPLC [column B, eluting with 22% MeCN(aq)] to afford inseparable mixtures of **3/4** (10 mg), **5/6** (21 mg), **7/8** (15 mg), and **9/10** (3.0 mg).

Leonpetaeofolin A (1)/15-epi-leonpetaeofolin A (2): colorless plates (MeOH), mp 218–224 °C, $[\alpha]_{\text{D}}^{25} +26.2$ (*c* 0.11, MeOH); IR (film) ν_{max} 3511, 2964, 2935, 1735, 1712, 1242, 1149, 1027, 977, 877 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS *m/z* 445.1847 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{22}\text{H}_{30}\text{O}_8$, 445.1838).

Leonpetaeofolin B (3)/15-epi-leonpetaeofolin B (4): colorless gum, $[\alpha]_{\text{D}}^{25} +2.5$ (*c* 0.08, MeOH); IR (film) ν_{max} 3400, 2933, 1747, 1204, 1142, 989, 923 cm^{-1} ; ^1H and ^{13}C NMR data see Tables 1 and 2; positive-ion HRESIMS *m/z* 389.1934 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{30}\text{O}_6\text{Na}$, 389.1940).

Leonpetaeofolin C (5)/15-epi-leonpetaeofolin C (6): colorless gum, $[\alpha]_{\text{D}}^{25} +5.0$ (*c* 0.08, MeOH); IR (film) ν_{max} 3408, 2935, 1747, 1205, 1142, 988, 923 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS *m/z* 389.1934 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{30}\text{O}_6\text{Na}$, 389.1940).

Leonpetaeofolin D (7)/15-epi-leonpetaeofolin D (8): colorless gum, $[\alpha]_{\text{D}}^{25} +29.9$ (*c* 0.11, MeOH); IR (film) ν_{max} 3424, 2946, 1764, 1197, 1115, 1003, 924 cm^{-1} ; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS *m/z* 387.1778 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{28}\text{O}_6\text{Na}$, 387.1784).

Leonpetaeofolin E (9)/15-epi-leonpetaeofolin E (10): colorless gum, $[\alpha]_{\text{D}}^{25} +40.0$ (*c* 0.18, MeOH); IR (film) ν_{max} 3415, 2946, 1765, 1197, 1115, 1003, 925 cm^{-1} ; ^1H NMR and ^{13}C NMR data, see Tables 1 and 2; positive-ion HRESIMS *m/z* 387.1788 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{20}\text{H}_{28}\text{O}_6\text{Na}$, 387.1784).

X-ray Crystallography of 1 and 2. The crystal structure and absolute configuration of a disordered cocrystal of **1** and **2** were determined using data collected at *T* = 90 K with $\text{Cu K}\alpha$ radiation (λ = 1.54178 Å) on a Bruker APEX-II CCD diffractometer, equipped with an Oxford Cryostream cooler. Structures were solved using the program SHELXS-97³⁵ and refined anisotropically by full-matrix least-squares on F^2 using SHELXL-97.³⁵ All H atoms were visible in difference maps, but were placed in idealized positions for the refinements, with torsional parameters refined for all OH and methyl groups. There are two independent molecules in the asymmetric unit, and both have substitutional disorder at C-15, with both α - and β -OH groups present. For each independent molecule, a population parameter was refined, constraining the populations of the two epimers to sum to unity. One of the independent molecules is 72.6%

R, while the other is 41.3% *R* at C-15; overall, the crystal studied was 57.0% *R*. This may differ from the composition of the bulk sample. The absolute configuration was determined by refinement of the Flack parameter³⁶ based on resonant scattering of the light atoms and computation of the Hooft parameter,³⁷ yielding a probability of 1.000 that the reported configuration is correct. Crystal data: **1/2**, leonpetaeofolin A/15-epi-leonpetaeofolin A, $\text{C}_{22}\text{H}_{30}\text{O}_8$, M_r = 422.46, monoclinic space group $P2_1$, a = 10.8606(5) Å, b = 11.4963(5) Å, c = 15.4999(7) Å, β = 90.154(2)°, V = 1935.26(15) Å³, Z = 4, D_x = 1.450 Mg m^{-3} , θ_{max} = 69.6°, R = 0.029 for 6869 data having $I > 2\sigma(I)$, $wR2$ = 0.077, and GOF = 1.041 for all 6922 data and 571 refined parameters. The Flack parameter is 0.07(9) and the Hooft parameter is 0.08(4) for 3180 Bijvoet pairs.

It should be noted that the two independent molecules are very nearly related by a 2_1 screw axis orthogonal to the monoclinic symmetry direction. Thus, there is a strong pseudosymmetry, closely resembling orthorhombic $P2_12_12_1$. The structure can be refined in $P2_12_12_1$ to nearly the same *R* value, 0.031. However, this model requires only one independent molecule with a single *R/S* ratio and a β -angle symmetry-constrained to exactly 90°. However, averaging the intensity data under orthorhombic symmetry yields R_{int} = 0.059 vs 0.028 for monoclinic, and the β -angle clearly differs from 90° by 77 standard uncertainties. This has been confirmed using several different crystals, on two diffractometers with different radiations, and persists over a range of temperatures from 90 K to at least 220 K. Thus, we believe that the lower symmetry is correct, although both $P2_1$ and $P2_12_12_1$ models lead to the same conclusions about the identity of the compounds, the mix of epimers, and the absolute configuration.

Radioligand-Binding Assays. Radioligand-binding assays at human cloned GPCRs and transporters were performed by using the resources of the NIMH-PDSP at the University of North Carolina at Chapel Hill. Detailed online protocols are available for all assays at the NIMH-PDSP Web site (<http://pdsp.med.unc.edu/pdspw/binding.php>). Initial screening assays were performed by using 10 μM of isolated compounds for quadruplicate determinations, and the percent inhibition of specific bindings was determined. Where 10 μM test compounds inhibited more than 50% of specific binding, their affinities (K_i) were further performed by using six concentrations of unlabeled ligand spanning a 10 000-fold dose range. K_i values were calculated by using GRAPHPAD PRIZM.

■ ASSOCIATED CONTENT

● Supporting Information

1D- and 2D-NMR spectra of compounds **1–10** and primary CNS receptor binding studies data for isolated compounds are available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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