New Bis-spirolabdane-Type Diterpenoids from *Leonurus heterophyllus* Sw.

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Twelve natural bis-spirolabdane-type diterpenoids, including eight new, named leoheteronones A—E (3, 6, 8, 9, 11), 15-epileoheteronones B (7), D (10), and E (12), and four known leopersin B (1), 15-epileopersin B (2), leopersin C (4), and 15-epileopersin C (5), together with hispanone (13) and galeopsin (14) were isolated from the aerial parts of the medicinal plant *Leonurus heterophyllus* Sw. (Lamiaceae) grown in Vietnam. Their structures were determined by spectroscopic analyses. The current study emphasized the accumulation of C-15 oxygenated bis-spirolabdane-type diterpenoids of both 13*R* and 13*S* configurations in *L. heterophyllus*.

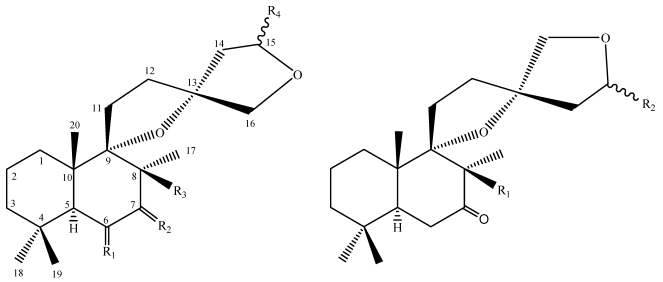
Key words *Leonurus heterophyllus*; Lamiaceae; bis-spirolabdane-type diterpenoid; leoheteronone

Leonurus heterophyllus Sw. (family: Lamiaceae, syn. Labiatae) is used in Vietnamese traditional medicine for the treatment of menstruation and child delivery in gynaecology, high blood pressure, blood stasis, heart disorders, and dysentery.[1)] The rich distribution of bis-spirolabdane-type diterpenoids with variations of functionalities in the plants of the genus *Leonurus* was reported in the previous studies on chemical composition of *L. persicus*,[2—5)] *L. cardiaca*,[6,7)] and *L. sibiricus*.[8,9)] Similarly, two prefuranic labdane-type diterpenoids were isolated from *L. heterophyllus* collected in Guangdong Province, China.[10,11)] However, a systematic chemical investigation on *L. heterophyllus* is necessary for reasons of chemotaxonomic interest. It is noteworthy that bis-spirocyclic labdane-type compounds are not confined to the *Leonurus* species, and so far have been found in the genera of *Leonotis* (Lamiaceae),[12)] *Marrubium* (Lamiaceae),[12)] *Otostegia* (Lamiaceae),[13)] and *Vitex* (Verbenaceae).[14,15)] In our present study, systematic extraction and isolation afforded twelve natural bis-spirolabdane-type diterpenoids (**1**—**12**), eight of which are new (**3**, **6**—**12**), together with hispanone (**13**) and galeopsin (**14**). The known diterpenoids, leopersin B (**1**), 15-epileopersin B (**2**), leopersin C (**4**), 15-epileopersin C (**5**), **13** and **14** are identified on the basis of physical ($[\alpha]_D$), and ^{1}H- and ^{13}C-NMR data.[2,3,16,17)]

Air-dried aerial parts of *L. heterophyllus* were extracted with MeOH by percolation at room temperature. Concentration under reduced pressure yielded an extract, which was divided into *n*-hexane-, ethyl acetate- and 1-BuOH-soluble parts by sequential solvent partitioning with H_2O. Bis-spirocyclic compounds **1**—**12**, and compounds **13** and **14** were obtained by systematic fractionation of *n*-hexane-soluble fraction first over silica gel, then over reversed-phase octadecyl silica (ODS) gel, followed by preparative HPLC. While compounds **3**, **8**, **13** and **14** were obtained in pure form, the other compounds were isolated as epimeric pairs **1**/**2**, **4**/**5**, **6**/**7**, **9**/**10** and **11**/**12** at C-15 position. This phenomenon of the co-occurrence of C-15 oxygenated epimeric pairs has been frequently seen in many examples of bis-spirolabdane-type diterpenoids of the genus *Leonurus*.[2—7,9)]

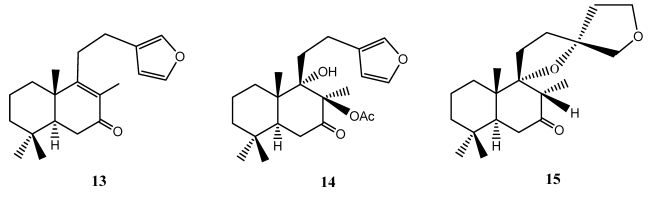
Although the absolute configurations of **1**—**12** were not individually confirmed herein, they are probably of the same normal labdane-type diterpenoids on the basis of the co-occurrence with **13** and **14**, since the formation of bis-spirotetrahydrofuranes involves only C-9 side chain. Additionally, compounds **1**/**2** and **4**/**5** were previously isolated from the same extraction fractions together with 4-β-hydroxymethylpregaleopsin, the absolute assignments of *p*-bromobenzoate derivative of which have been unambiguously determined by single-crystal X-ray crystallographic analysis.[2)] Therefore, the stereochemistry of **1**—**12** is suggested to be presented as in the normal series of labdane-type diterpenoids.

Leoheteronone A (**3**) was isolated as an oil, $[\alpha]_D^{25}$ −42.5°. Its molecular formula was determined to be $C_{23}H_{36}O_6$ by positive-ion high-resolution (HR)-FAB-MS (*m*/*z* 431.2418 $[M+Na]^+$). The IR spectrum indicated the presence of an ester (1746 cm^{-1}) functional group. The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectroscopic data of **3** showed the presence of 23 carbons which were assignable to four tertiary methyl groups [δ_H 1.25 (s), 1.12 (s), 0.82 (s), 0.76 (s); δ_C 15.4, 18.0, 32.7, 21.4, respectively; data were obtained from the observed cross peaks in the heteronuclear single quantum correlation (HSQC) spectrum], a ketone group (δ_C 205.5), an



	R_1	R_2	R_3	R_4
1	H_2	O	OAc	β-OH
2	H_2	O	OAc	α-OH
3	H_2	O	OAc	α-OMe
4	O	β-OH,α-H	H	β-OH
5	O	β-OH,α-H	H	α-OH
6	H_2	O	H	β-OH
7	H_2	O	H	α-OH

	R_1	R_2
8	OAc	α-OMe
9	H	β-OH
10	H	α-OH
11	OAc	β-OH
12	OAc	α-OH



13 **14** **15**

Fig. 1. Chemical Structures of **1**—**15**

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Table 1. ^{1}H-NMR Spectroscopic Data of **3**, **6**—**12** (δ in ppm, J in Hz, 400 MHz, $CDCl_3$)

H	3	6	7	8	9	10	11	12
1a				1.30 m[a]	1.44 m[a]	1.44 m[a]	1.27 m[a]	1.32 m[a]
b	1.42 m[a]	1.40 m[a]	1.40 m[a]	1.24 m[a]	1.30 m[a]	1.30 m[a]	1.20 m[a]	1.26 m[a]
2a				1.62 m[a]				
b	1.57 m[a]	1.55 m[a]	1.55 m[a]	1.57 m[a]	1.56 m[a]	1.56 m[a]	1.57 m[a]	1.57 m[a]
3a		1.40 m[a]	1.40 m[a]	1.40 m[a]	1.41 m[a]	1.41 m[a]	1.40 m[a]	1.35 m[a]
b	1.33 m[a]	1.10 m[a]	1.10 m[a]	1.15 m[a]	1.15 m[a]	1.15 m[a]	1.15 m[a]	1.20 m[a]
5	1.65 dd (14.2, 2.4)	1.90 m[a]	1.90 m[a]	1.69 dd (14.2, 2.4)	1.92 m[a]	1.88 m[a]	1.60 m[a]	1.63 m[a]
6a	2.42 dd (14.2, 11.9)	2.36 dd (14.2, 3.6)	2.34 dd (14.2, 3.6)	2.47 dd (14.2, 11.7)	2.39 m[a]	2.39 m[a]	2.44 m[a]	2.44 m[a]
b	2.26 dd (11.9, 2.4)	2.25 m[a]	2.25 m[a]	2.31 dd (11.7, 2.4)	2.22 m[a]	2.22 m[a]	2.28 m[a]	2.25 m[a]
8		2.61 q (6.8)	2.63 q (6.8)		2.75 q (6.6)	2.68 q (6.6)		
11a	2.16 m[a]	2.20 m[a]	2.07 m[a]	2.25 m[a]	2.15 m[a]	2.15 m[a]	2.25 m[a]	2.25 m[a]
b	1.90 m[a]	1.80 m[a]	1.80 m[a]	2.13 m[a]	1.85 m[a]	1.85 m[a]	2.17 m[a]	2.17 m[a]
12a			2.10 m[a]	2.15 t (5.6)			2.20 m[a]	2.07 m[a]
b	2.11 t (5.1)	2.15 m[a]	1.90 m[a]	1.92 m[a]	2.01 t (7.8)	2.00 t (7.8)	2.15 m[a]	1.95 m[a]
14a	2.16 m[a]	2.27 m[a]		2.26 m[a]			2.37 m[a]	2.24 m[a]
b	2.07 m[a]	2.10 m[a]	2.15 m[a]	2.01 m[a]	2.25 m[a]	1.83 m[a]	1.92 m[a]	2.10 m[a]
15	4.85 t (4.9)	5.48 d (5.1)	5.31 br s	4.90 dd (5.9, 3.8)	5.50 br s	5.36 br s	5.44 d (5.4)	5.34 br s
16a	3.82 d (8.1)	3.87 d (8.7)	4.09 d (9.0)	3.75 d (8.0)	3.88 d (8.8)	4.16 d (8.8)	3.81 d (8.6)	4.04 d (8.5)
b	3.49 d (8.1)	3.83 d (8.7)	3.58 d (9.0)	3.42 d (8.0)	3.81 d (8.8)	3.57 d (8.8)	3.73 d (8.6)	3.45 d (8.5)
17	1.25 s	0.95 d (6.8)	0.90 d (6.8)	1.36 s	1.06 d (6.6)	1.03 d (6.6)	1.29 s	1.34 s
18	0.82 s	0.84 s	0.81 s	0.87 s	0.86 s	0.86 s	0.82 s	0.83 s
19	0.76 s	0.81 s	0.80 s	0.81 s	0.82 s	0.84 s	0.77 s	0.77 s
20	1.12 s	1.06 s	1.08 s	1.17 s	1.13 s	1.06 s	1.11 s	1.11 s
8-OAc	1.99 s			2.04 s			2.00 s	2.01 s
15-OMe	3.29 s			3.33 s				

a) Average value of unresolved signals (m), assigned on the basis of HSQC experiments.

Table 2. ^{13}C-NMR Spectroscopic Data of **3**, **6**—**12** (δ in ppm, 100 MHz, $CDCl_3$)

C	3	6	7	8	9	10	11	12	15
1	33.2 (−0.6)[a]	32.5 (−6.6)[b]	32.5 (−6.6)[b]	33.3	32.4	32.7	33.5	33.8	39.1
2	17.9 (−0.1)[a]	17.6 (−1.1)[b]	17.7 (−1.0)[b]	17.9	17.8	18.0	18.5	18.6	18.7
3	41.1 (−0.3)[a]	41.5 (−0.2)[b]	41.6 (−0.1)[b]	41.3	41.7	41.5	41.8	41.7	41.7
4	34.2 (−0.2)[a]	33.5 (+0.8)[b]	33.6 (+0.9)[b]	34.5	33.7	33.8	35.0	34.9	32.7
5	49.5 (−0.9)[a]	50.3 (−0.2)[b]	50.3 (−0.2)[b]	50.3	47.5	46.9	50.8	51.0	50.5
6	36.0 (0.0)[a]	35.0 (−5.7)[b]	35.0 (−5.7)[b]	35.9	39.2	39.2	36.5	36.4	40.7
7	205.5 (−0.6)[a]	210.9 (−0.1)[b]	210.1 (−0.9)[b]	205.6	211.1	210.8	206.5	206.6	211.0
8	87.6 (0.0)[a]	46.7 (−0.1)[b]	45.8 (−1.0)[b]	87.5	50.2	50.4	88.2	88.1	46.8
9	96.6 (−1.2)[a]	97.7 (+1.2)[b]	96.2 (−0.3)[b]	96.7	96.4	98.0	98.2	97.4	96.5
10	43.6 (0.0)[a]	42.6 (−0.3)[b]	42.6 (−0.3)[b]	43.5	42.5	42.9	44.1	44.1	42.9
11	28.2 (−0.5)[a]	29.8 (−8.4)[b]	29.5 (−8.7)[b]	28.1	30.0	29.4	29.1	28.7	38.2
12	39.8 (−0.1)[a]	38.9 (+9.2)[b]	38.7 (+9.0)[b]	39.9	34.9	34.9	39.8	37.3	29.7
13	90.8 (−0.6)[a]	90.7 (−0.6)[b]	90.2 (−1.1)[b]	90.7	90.3	90.8	91.1	91.8	91.3
14	46.8 (−0.7)[a]	47.4 (+14.5)[b]	47.5 (+14.6)[b]	46.1	46.2	47.4	47.3	46.9	32.9
15	104.2 (+5.6)[a]	98.8 (+20.7)[b]	99.0 (+20.9)[b]	104.4	98.6	98.8	98.7	98.8	78.1
16	74.4 (−3.8)[a]	78.0 (+10.3)[b]	76.5 (+8.8)[b]	74.4	77.8	76.7	78.5	76.1	67.7
17	15.4 (−0.5)[a]	9.0 (−0.1)[b]	9.3 (+0.2)[b]	15.9	9.3	9.6	16.4	16.7	9.1
18	32.7 (−0.2)[a]	32.9 (+0.2)[b]	32.6 (−0.1)[b]	32.7	32.7	32.8	33.3	33.3	32.7
19	21.4 (−0.1)[a]	21.1 (−0.2)[b]	21.0 (−0.3)[b]	21.6	21.3	21.3	22.0	22.1	21.3
20	18.0 (−0.1)[a]	18.5 (+0.7)[b]	18.5 (+0.7)[b]	18.1	18.6	18.6	18.6	18.6	17.8
8-OAc	168.9 (−0.2)[a]			169.0			171.0	169.6	
	21.3 (−0.1)[a]			21.4			21.9	21.9	
15-OMe	55.0			55.0					

a) Differences of the ^{13}C chemical shifts between **3** and **2** ($\Delta\delta_{C,3-2}$). *b*) Differences of the ^{13}C chemical shifts between **6**/**7** and **15** ($\Delta\delta_{C,6-15}$/$\Delta\delta_{C,7-15}$).

acetal methine group (δ_H 4.85, δ_C 104.2), an isolated oxygenated methylene group [δ_H 3.82 and 3.49 (AB system, J=8.1 Hz); δ_C 74.4], three oxygenated quaternary carbons (δ_C 96.6, 90.8, 87.6), an acetyl group [δ_H 1.99 (s); δ_C 168.9, 21.3], a methoxyl group [δ_H 3.29 (s); δ_C 55.0], seven methylene groups (δ_C 46.8, 41.1, 39.8, 36.0, 33.2, 28.2, 17.9), one methine group (δ_C 49.5), and two quaternary carbons (δ_C 43.6, 34.2). These spectroscopic data, coupled with the six degrees of unsaturation, suggested that compound **3** was a labdane-type diterpenoid possessing two spiro-tetrahydrofuran rings. Close structural features of **3** and 15-epileopersin B (**2**)[2] were exhibited by the similarity of their ^{1}H- and ^{13}C-NMR data, except for the significant downfield shift at C-15 (δ_C 104.2; $\Delta\delta_C$ +5.6) and upfield shift at C-16 (δ_C 74.4; $\Delta\delta_C$ −3.8) (Table 2). This is in agreement with the replacement of the hydroxyl group at C-15 in **2** by a methoxyl group

(δ_H 3.29; δ_C 55.0) in **3**. The relative stereochemistry of **3** was assigned on the basis of nuclear Overhauser enhancement and exchange spectroscopy (NOESY) (Fig. 2). NOEs were detected between Me-18 (δ_H 0.82) and H-5 (δ_H 1.65), Me-18 and H-6α (δ_H 2.26), Me-19 (δ_H 0.76) and H-6β (δ_H 2.42), Me-20 (δ_H 1.12) and H-6β, Me-20 and axial 8-OAc (δ_H 1.99), Me-20 and H_2-11 (δ_H 2.16, 1.90) established the configurations at C-5, C-8, C-9, and C-10 as *S*, *R*, *S*, and *S*, respectively. The configuration at C-13 was determined as *R* from the correlations between H_2-16 (δ_H 3.82, 3.49) and Me-17 (δ_H 1.25), and this also confirmed the α-orientation of Me-17. NOEs between H-15 [δ_H 4.85 (t, J=4.9 Hz)] and H_2-12 [δ_H 2.11 (t, J=5.1 Hz)] and H-15 and the methoxyl group [δ_H 3.29 (s)] facilitated the assignment of the configuration of C-15 as *R*. Thus **3** was determined to be (5*S*,8*R*,9*S*,10*S*,13*R*,15*R*)-8-acetoxy-9,13;15,16-diepoxy-15-methoxylabdan-7-one.

The ^{1}H-NMR spectrum showed that leoheteronone B (**6**) and 15-epileoheteronone B (**7**) were isolated as an epimeric mixture in a ratio of 2 : 1. Compounds **6** and **7** were determined to possess the same molecular formula $C_{20}H_{32}O_4$ by negative-ion HR-FAB-MS (*m/z* 335.2231 $[M-H]^-$). The IR spectrum indicated the presence of hydroxyl (3419 cm^{-1}) and ketone (1712 cm^{-1}) functional groups. The ^{1}H- (Table 1) and ^{13}C-NMR (Table 2) spectroscopic data showed two sets of resonances, which were distinguishable by their intensities. From the spectroscopic data, structures closely related to 14,15-dihydroprehispanolone (**15**)[10] were made up for **6** and **7**, except for the presence of additional hydroxyl groups which were deduced to be attached to acetal carbons on the basis of their characteristic chemical shifts [**6**/**7**: δ_C 98.8/99.0; δ_H 5.48 (d, J=5.1 Hz)/5.31 (br s)]. Comparison of the ^{13}C-NMR data for **6**/**7** and **15** (Table 2) showed the major differences in chemical shifts at C-14 (**6**/**7**: δ_C 47.4/47.5; $\Delta\delta_C$ +14.5/+14.6), C-15 (**6**/**7**: δ_C 98.8/99.0; $\Delta\delta_C$ +20.7/+20.9), and C-16 (**6**/**7**: δ_C 78.0/76.5; $\Delta\delta_C$ +10.3/+8.8), which suggested the location of the hydroxyl group at C-15. Furthermore, AB systems for isolated H_2-16 of **6**/**7** were observed in the ^{1}H-NMR spectrum [**6**/**7**: δ_H 3.87, 3.83 (both d, J=8.7 Hz)/4.09, 3.58 (both d, J=9.0 Hz)]. In support of that, similarity of the ^{13}C chemical shifts of the 9,13;15,16-diepoxy moiety in **6**/**7** to those of **1**/**2** has been seen.[2] The configurations of the bis-spirocyclic rings were assigned as 9*R* and 13*R* by observation of the NOESY correlations between H_2-16 protons of **6**/**7** and Me-17 [**6**/**7**: δ_H 0.90/0.95 (both d, J=6.8 Hz)], and between Me-20 [**6**/**7**: δ_H 1.06 (s)/1.08 (s)] and H_2-11 [**6**/**7**: δ_H 2.20 (m), 1.80 (m)/2.07 (m), 1.80 (m)]. By this observation, Me-17 was concluded to occupy α-space, which was supported by the NOEs between axial H-8 [**6**/**7**: δ_H 2.61/2.63 (both ddd, J=6.8, 6.8, 6.8 Hz)] and Me-20. The configuration at C-15 of **7** was determined to be 15*R* by NOESY cross-peak from H-15 [δ_H 5.31 (br s)] to H-12b [δ_H 1.90 (m)]. Accordingly, **6**, which gave no NOE between H-15 and H_2-12, was assigned with 15β-OH (15*S*). Thus the structures of **6**/**7** were determined to be (5*S*,8*S*,9*R*,10*S*,13*R*,15*S*/*R*)-15-hydroxy-9,13;15,16-diepoxy-labdan-7-one, which are new natural compounds. Similar structures were described in a diastereomeric mixture of two semisynthetic C-15 hydroxyepimers.[18] However, they are believed to be of different structures due to a number of significantly different ^{1}H and ^{13}C chemical shifts. In particular, only 20 unassigned carbon signals with up to six oxygenated carbons were reported for the mixture. Furthermore, although the ^{13}C chemical shifts of bis-spirocyclic moiety revealed the possibility of C-15 methoxylated product(s), the expected methoxyl signal(s) did not appear in the publication.[18]

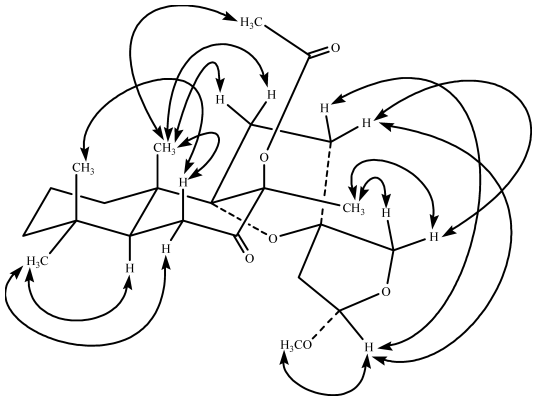

Fig. 2. NOESY Correlations of **3**

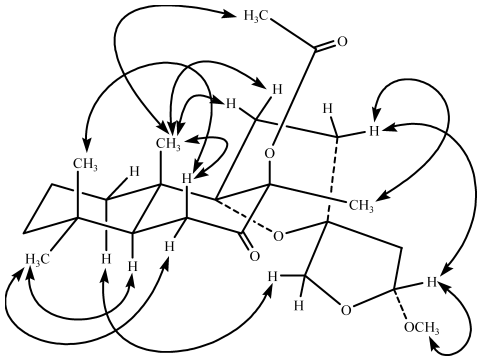

Fig. 3. NOESY Correlations of **8**

Leoheteronone C (**8**) was obtained as an amorphous powder, $[\alpha]_D^{25}$ +24.1°, and has the same molecular formula $C_{23}H_{36}O_6$ as **3** by positive-ion HR-FAB-MS (*m/z* 431.2445 ($[M+Na]^+$). The IR spectrum indicated the presence of an ester (1740 cm^{-1}) functional group. The ^{1}H-NMR data (Table 1) disclosed slight differences at H_2-1 (upfield shifts: δ_H 1.30; $\Delta\delta_H$ −0.12 and δ_H 1.24; $\Delta\delta_H$ −0.18) and Me-17 (downfield shift: δ_H 1.36; $\Delta\delta_H$ +0.11) and close correspondence of the ^{13}C-NMR spectroscopic data (Table 2) with those of **3** was seen, suggesting the isomeric nature of this compound and **3**. In the NOESY spectrum (Fig. 3), correlations were observed in the same manner with **3**, namely, between Me-18 (δ_H 0.87) and H-5 (δ_H 1.69), Me-18 and H-6α (δ_H 2.31), Me-19 (δ_H 0.81) and H-6β (δ_H 2.47), Me-20 (δ_H 1.17) and H-6β, Me-20 and axial 8-OAc (δ_H 2.04), and Me-20 and H_2-11 (δ_H 2.25, 2.13), indicated the stereochemistry of **8** as 5*S*, 8*R*, 9*S*, and 10*S*. However, no NOEs between H_2-16 protons (δ_H 3.75, 3.42) and Me-17 (δ_H 1.36) were detected. Instead, NOESY cross-peak was observed from H-16a (δ_H 3.75) to H-1α (δ_H 1.24), which was consistent with a 13*S* configuration of the uppermost spirocyclic ring. The methoxyl group at C-15 (δ_H 3.33) was assigned as α-positioned (*i.e.*, 15*S*) from the observation of NOESY correlations between H-15 (δ_H 4.90) and H-12b [δ_H 1.92 (m)] and the methoxyl group [δ_H 3.33 (s)]. Thus the structure of **8** was determined to be (5*S*,8*R*,9*S*,10*S*,13*S*,15*S*)-8-acetoxy-9,13;15,16-diepoxy-15-

methoxylabdan-7-one.

Leoheteronone D (**9**) and 15-epileoheteronone D (**10**) were obtained as an epimeric mixture (2 : 5). The IR spectrum indicated the presence of hydroxyl (3419 cm^{-1}) and ketone (1710 cm^{-1}) functional groups. Compounds **9**/**10** showed the same molecular formulae $C_{20}H_{32}O_4$ as those of **6**/**7** by negative-ion HR-FAB-MS (*m*/*z* 335.2193 $[M-H]^-$) and also similar sets of ^{13}C-NMR data (Table 2) to those of **6**/**7**, which indicated the resemblance of stereoisomeric nature of **9**/**10** and **6**/**7**. Slight differences in ^1H and ^{13}C chemical shifts (Tables 1, 2) of **9**/**10** in comparison with **6**/**7** drew our attention to the stereochemistry of spirocyclic rings. The stereochemical assignments of **9**/**10** were provided by NOESY experiment. NOESY correlations were observed between Me-20 [**9**/**10**: δ_H 1.13 (s)/1.06 (s)] and H-8 [**9**/**10**: δ_H 2.75/2.68 (both ddd, J=6.6, 6.6, 6.6 Hz)], Me-20 and H-11a [**9**/**10**: δ_H 2.15 (m)/2.15 (m)], and H-16α [**9**/**10**: δ_H 3.88/4.16 (both d, J=8.8 Hz)] and H-1α [**9**/**10**: δ_H 1.30 (m)/1.30 (m)], instead of the correlation between H-16 and Me-17, observed in 13*R*-spirocyclic structures. The configuration of C-15 was defined as 15*S* for **10** from NOESY cross-peak from H-15 [δ_H 5.36 (br s)] to H_2-12 [δ_H 2.00 (t, J=7.8 Hz)]. Accordingly, **9** was concluded to be 15*R*-epimer of **10**. Therefore, the structures of **9**/**10** were determined to be (5*S*,8*S*,9*R*,10*S*,13*S*,15*R*/*S*)-15-hydroxy-9,13;15,16-diepoxylabdan-7-ones.

Leoheteronone E (**11**) and 15-epileoheteronone E (**12**) were and isolated as an inseparable epimeric mixture (1 : 1). The molecular formulae $C_{22}H_{34}O_6$ of **11**/**12** were determined to be the same as those of **1**/**2** by negative-ion HR-FAB-MS (*m*/*z* 393.2287 $[M-H]^-$). The IR spectrum indicated the presence of hydroxyl (3446 cm^{-1}) and ester (1738 cm^{-1}) functional groups. The close correspondence of ^1H- (Table 1) and ^{13}C-NMR (Table 2) spectroscopic data, which contained duplicate resonances of **11**/**12** and **1**/**2**, suggested that they were stereoisomeric compounds, probably with respect to stereochemistry at C-9 and C-13. In the NOESY spectrum, NOEs were observed between Me-20 [**11**/**12**: δ_H 1.11 (s)/1.11 (s)] and H_2-11 [**11**/**12**: δ_H 2.25 (m), and 2.17 (m)/2.25 (m) and 2.17 (m)] and between H_2-16 [**11**/**12**: δ_H 3.81 and 3.73 (both d, J=8.6 Hz)/4.04 and 3.45 (both d, J=8.5 Hz)] and H-1α [**11**/**12**: δ_H 1.20 (m)/1.26 (m)], thus confirming the assignments of 9*S*,13*S*-configurations of **11**/**12**. Me-19 [**11**/**12**: δ_H 0.77 (s)/0.77 (s)] were placed at the same β-face as Me-20 [**11**/**12**: δ_H 1.11 (s)/1.11 (s)], and Me-18 [**11**/**12**: δ_H 0.82 (s)/0.83 (s)], H-5 [**11**/**12**: δ_H 1.60 (m)/1.63 (m)] and Me-17 [**11**/**12**: δ_H 1.29 (s)/1.34 (s)] at an α-face from the NOESY correlations between Me-18 and H-5, Me-18 and H-6α [**11**/**12**: δ_H 2.28 (m)/2.25 (m)], Me-20 and H-6β [**11**/**12**: δ_H 2.44 (m)/2.44 (m)], Me-20 and axial 8-OAc [**11**/**12**: δ_H 2.00 (s)/2.01 (s)]. The orientation of the hydroxyl group at C-15 was concluded to be 15*S* for **12**, and accordingly 15*R* for **11**, on the basis of the specific NOE between H-15 of **12** [δ_H 5.34 (br s)] and H-12a [δ_H 2.07 (m)]. Therefore, the structures of **11**/**12** were determined to be (5*S*,8*R*,9*S*,10*S*,13*S*,15*R*/*S*)-8-acetoxy-9,13;15,16-diepoxy-labdan-7-ones.

The finding that 13*S* and 13*R* bis-spirolabdane-type diterpenoids co-occurred in the same extract is a rare case in the *Leonurus* genus,[9] and the lack of any 6-oxygenated function in the 13*S* series is unique among *Leonurus* bis-spirocyclic diterpenoids. So far, the *Leonurus* bis-spirocyclic labdanes clearly differ from analogous compounds from *Leonotis* and *Marrubium*[12] by the presence of C-15 oxygenated functions, and from *Otostegia*[13] and *Vitex*[14,15] by the presence of 8-acetoxyl and/or C-7-carbonyl groups. Compounds **3** and **8** may be artifacts formed from **2** and **12**, respectively, since MeOH was used for extraction.

Experimental

General Procedure Optical rotations were measured on a Union Giken PM-101 digital polarimeter. FT-IR spectra were recorded on a Horiba FT-710 spectrophotometer. ^1H- (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded using a JEOL JNM-α 400 NMR spectrometer. Positive-ion and negative-ion HR-FAB-MS were measured on a JEOL SX-102 mass spectrometer with PEG-600 or PEG-400 as a calibration matrix. HPLC was carried out with a JASCO PU-1580 pump and an UV-2075 Plus detector (210 nm) on YMC ODS columns (150×4.6 mm i.d. in analytical and 150×20 mm i.d. in preparative scales) at the corresponding flow rates of 0.5 and 5 ml/min. Silica gel 60 (0.063—0.200 mm, Merck, Germany) and reversed-phase ODS gel (YMC, Japan) were used for open column chromatography. TLC was carried out on Merck precoated TLC plates (silica gel 60 F_{254}), and detected by spraying with 10% H_2SO_4 in 50% EtOH, followed by heating on a hot plate at 200 °C.

Plant Material The aerial parts of *L. heterophyllus* were collected from Dai Yen Village, Hanoi, Vietnam, in May 2004 and identified by Professor Vu Van Chuyen at Hanoi College of Pharmacy (Hanoi, Vietnam). A voucher specimen (No. HCTN 2004-5) is deposited in the Herbarium of the Hanoi College of Pharmacy.

Extraction and Isolation of Compounds 1—14 The powdered air-dried aerial parts of *L. heterophyllus* (2.0 kg) were extracted with MeOH by percolation at room temperature. After filtration and evaporation, the MeOH extract was suspended in H_2O and extracted with *n*-hexane, ethyl acetate, and 1-BuOH. The *n*-hexane-soluble part (64.9 g) was subjected to a silica gel column using *n*-hexane with increasing amounts of EtOAc to afford four pooled fractions: fraction 1 (18.1 g, *n*-hexane–EtOAc, 10 : 1), fraction 2 (37.5 g, *n*-hexane–EtOAc, 4 : 1), fraction 3 (4.1 g, *n*-hexane–EtOAc, 2 : 1), and fraction 4 (0.5 g, *n*-hexane–EtOAc, 1 : 1). Open column chromatography of fraction 1 on silica gel (*n*-hexane–EtOAc, 10 : 1) gave **13** (10 mg). Silica gel open column chromatography (*n*-hexane–EtOAc, 4 : 1) of fraction 2 gave **14** (408.4 mg). Fraction 3 was subjected to ODS gel open column chromatography (MeOH–H_2O, 4 : 1) and repeated ODS gel preparative HPLC (MeOH–H_2O, 4 : 1) to afford **3** (35.1 mg), and **8** (8.3 mg), and inseparable mixtures of **1**/**2** (67.4 mg), **4**/**5** (33.2 mg), **6**/**7** (17.7 mg), **9**/**10** (8.6 mg), and **11**/**12** (7.6 mg).

Leoheteronone A (**3**): Yellowish oil, $[\alpha]_D^{25}$ −42.5° (c=3.51, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 2954, 2873, 1746, 1470, 1369, 1245. ^1H- and ^{13}C-NMR: see Tables 1 and 2. Positive-ion HR-FAB-MS: *m*/*z* 431.2418 $[M+Na]^+$ (Calcd for $C_{23}H_{36}O_6Na$: 431.2410).

Leoheteronone B and 15-epileoheteronone B (**6**/**7**): White amorphous powder. IR ν_{max} (film) cm^{-1}: 3419, 2950, 2872, 1712, 1468, 1366, 1253. ^1H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: *m*/*z* 335.2231 $[M-H]^-$ (Calcd for $C_{20}H_{31}O_4$: 335.2222).

Leoheteronone C (**8**): White amorphous powder, $[\alpha]_D^{25}$ +24.1° (c=0.83, $CHCl_3$). IR ν_{max} (film) cm^{-1}: 2954, 2873, 1740, 1463, 1369, 1215. ^1H- and ^{13}C-NMR: see Tables 1 and 2. Positive-ion HR-FAB-MS: *m*/*z* 431.2445 $[M+Na]^+$ (Calcd for $C_{23}H_{36}O_6Na$: 431.2410).

Leoheteronone D and 15-epileoheteronone D (**9**/**10**): White amorphous powder. IR ν_{max} (film) cm^{-1}: 3419, 2948, 2872, 1710, 1465, 1365, 1253. ^1H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: *m*/*z* 335.2193 $[M-H]^-$ (Calcd for $C_{20}H_{31}O_4$: 335.2222).

Leoheteronone E and 15-epileoheteronone E (**11**/**12**): White amorphous powder. IR ν_{max} (film) cm^{-1}: 3446, 2956, 2873, 1738, 1470, 1369, 1246. ^1H- and ^{13}C-NMR: see Tables 1 and 2. Negative-ion HR-FAB-MS: *m*/*z* 393.2287 $[M-H]^-$ (Calcd for $C_{22}H_{33}O_6$: 393.2277).

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