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Bis-sesquiterpenes and diterpenes from *Chloranthus henryi*

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ABSTRACT

Bis-sesquiterpenes, henriols A (**1**), B (**2**), C (**3**), and D (**4**), and three diterpenes, henrilabdanes A (**5**), B (**6**), and C (**7**), together with two known bis-sesquiterpenes and three known labdane diterpenes, were isolated from the ethanol extract of the roots of *Chloranthus henryi*. Their structures and absolute configurations were elucidated by NMR spectroscopic, X-ray crystallographic and CD analyses. Compounds **1**, **5**, **6** and **7** showed moderate hepatoprotective activities with IC_{50} values of 0.19, 0.66, 0.09 and 0.18 μM, respectively. They were not studied further due to the weak effects noted. Compounds **3** and **8** exhibited cytotoxic activities against three types of cancer cell lines including the hepatoma (BEL-7402), human gastric carcinoma (BGC-823), and colon cancer (HCT-8).

1. Introduction

Chloranthus henryi Hemsl. (Chloranthaceae) is a Chinese herb that is widely distributed throughout the south of China, in areas such as Anhui, Zhejiang, Fujian and Jiangxi provinces (Jiangsu New Medical College, 1977). Because of its reputation for facilitating blood circulation and dispersing blood stasis, restoring menstrual flow and subduing swelling, the whole plant has been used in traditional Chinese medicine to treat tumors, rheumatisms, and emmeniopathy (Jiangsu New Medical College, 1977). Previous chemical investigations of *Chloranthus* species resulted in the isolation of several sesquiterpenes (Kawabata et al., 1979, 1981, 1984, 1985; Kawabata and Mizutani, 1988; Uchida et al., 1980; Tahara et al., 1981) and bis-sesquiterpenes (Kawabata et al., 1990, 1993, 1995; Kawabata and Mizutani, 1992; Yang et al., 2007), which are characteristic constituents of the chloranthaceous plants. In previous papers, the isolation and structural elucidation from *C. henryi* of a new sesquiterpene, two new labdane-type diterpenes and a new coumarin were reported (Li et al., 2005; Wu et al., 2006). While searching for hepatoprotective compounds from natural sources using rat hepatic epithelial stem-like cells (WB-F344) with D-galactosamine as a screening system, six hepatoprotective sesquiterpene glycosides were isolated from *Sarcandra glabra* (Chloranthaceae) (Li et al., 2006). The plant *Chloranthus henryi*, from the same family as *Sarcandra glabra*, was also screened for potential hepaprotective effects; its ethanolic extract showed activity, although the IC_{50} value could not be obtained due to the effect of pigments. Further investigation of *C. henryi* by bioactivity-guided fractionation led to isolation of four new lindenane bis-sesquiterpenes, henriols A (**1**), B (**2**), C (**3**), and D (**4**), and three new diterpenes, henrilabdanes A (**5**), B (**6**), and C (**7**), along with two known bis-sesquiterpenes, shizukaol B (**8**), and C (**9**) (Kawabata and Mizutani, 1992) and three known labdane diterpenes 12(*S*),15-dihydroxylabda-8(17),13*E*-dien-19-oic acid (**10**) (Fang et al., 1993), 12(*R*),13(*S*)-dihydroxylabda-8(17),14-dien-19-oic acid (**11**) and 12(*R*),13(*R*)-dihydroxylabda-8(17),14-dien-19-oic acid (**12**) (Inoue et al., 1985). Among them, compounds **1**, **2**, and **510** showed hepatoprotective activities against D-galactosamine-induced toxicity in WB-F344 rat hepatic epithelial stem-like cells. The structural elucidation and the biological activities of compounds **17** are described herein.

2. Results and discussion

Henriol A (**1**) was obtained as colorless square crystals (MeOH). Its molecular formula was $C_{39}H_{42}O_{14}$, as determined by HRFABMS at *m/z* 735.2676 $[M+H]^+$, representing 19° of unsaturation. The IR spectrum of **1** exhibited absorptions of hydroxyl (3435 cm^{-1}) and α,β-unsaturated lactone (1743 cm^{-1}) groups. In the NMR spectroscopic data of **1**, the proton signals at δ 0.55 (1H, *ddd*, *J* = 5.5, 5.0, 4.0 Hz, H-2β), 1.25 (1H, *ddd*, *J* = 8.5, 8.5, 4.0 Hz, H-2α), 1.82 (1H, *m*, H-3), 2.04 (1H, *m*, H-1), 0.76 (1H, *ddd*, *J* = 8.5, 8.5, 4.0 Hz, H-2′α), 1.12 (1H, *ddd*, *J* = 4.5, 4.0, 3.5 Hz, H-2′β), 1.49 (1H, *ddd*, *J* = 8.5, 7.5, 3.5 Hz, H-3′), and 1.68 (1H, *ddd*, *J* = 8.5, 7.5, 4.5 Hz,

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H-1′), together with the corresponding carbon resonances at δ 29.8 (C-1), 10.5 (C-2), 31.2 (C-3), 27.4 (C-1′), 9.4 (C-2′), and 29.2 (C-3′), confirmed the presence of two 1,2-disubstituted cyclopropane rings (Kawabata et al., 1979; Kawabata and Mizutani, 1989; Uchida et al., 1980). The proton signals at δ 0.83 (3H, *s*, H-14) and 0.96 (3H, *s*, H-14′) and the corresponding carbon resonances at δ_C 14.2 and 24.4 indicated the presence of two tertiary methyl groups. The occurrence of these structural fragments implied the presence of two lindenane moieties in the molecule. In the NMR spectra of **1**, except for the signals belonging to the lindenane moieties, the resonances of three methylenes [δ 2.05 and 2.80 (each 1H, *m*, H-h), 2.52 and 2.72 (each 1H, *m*, H-g), and 4.59 and 4.89 (each 1H, *dd*, *J* = 15.0, 6.0 Hz, H-d); δ 29.1(C-h), 29.2 (C-g) and 62.3 (C-d)], three ester carbonyls [δ 167.6 (C-a), 172.0 (C-i) and 173.1(C-f)], a methyl [δ 1.79 (*s*, H-e)], and a trisubstituted double bond [δ 6.62 (*m*, H-c); δ 129.4 (C-b) and 137.1 (C-c)] confirmed that **1** contained a hydroxytiglyl [–OCC(CH_3) = CH–CH_2O–] and a succinyl residue (–$OCCH_2$–CH_2CO–). In addition, these spectroscopic data were similar to those of shizukaol B (**8**) (Kawabata and Mizutani, 1992), indicating that **1** was a lindenane bis-sesquiterpene containing a macrocyclic lactone ring. Comparison of the NMR spectroscopic data of **1** (Tables 1 and 2) with those of **8** highlighted some differences: (i) the upfield chemical shift of C-4 from δ 141.2 to 77.6 and the absence of a methine proton at H-6 (δ 3.95) indicated that C-5 and C-6 were linked by a double bond instead of the 4,5 double bond in **8**; and (ii) the absence from **1** of a carbonyl group (δ 200.7, C-8), replaced by a hemi-ketal carbon (δ 104.2) and a methoxyl group (δ 3.77), implied the existence of another α,β-unsaturated γ-lactone moiety in this compound [δ 123.7 (C-7), 104.2 (C-8), 153.3 (C-11), 10.7 (C-13) and 172.2 (C-12)] (Uchida et al., 1980). This assignment was confirmed by HMBC correlations between H-13 and C-7, C-8, C-11 and C-12 (Fig. 2). The linkage of two units of lindenane was also determined by HMBC correlations between H-15 and C-8′, C-9′, C-10′, C-4, and C-5, and between H-9′ and C-15, and H-9′ and C-6, indicating the same linkage as in compound **8**. Thus, the structure of **1** was elucidated as shown in Fig. 1. To confirm its relative stereostructure, X-ray crystallographic analysis was carried out. The stereochemical image of **1** obtained from the X-ray analysis is shown in Fig. 3. The CD spectrum of **1** was very similar to that of chlorahololide A in the range 200–280 nm (Fig. 4) (Yang et al., 2007), where the first positive Cotton effect ($[\theta]_{270}$ + 16.1) and negative effect ($[\theta]_{209}$ − 26.8) arising from the exciton coupling of two different chromophores of the α,β-unsaturated γ-lactone and the twisted π-electron system indicated a positive chirality of **1**. Thus, the absolute configuration of this compound was established as depicted in Fig. 1.

Henriol B (**2**) was obtained as colorless needles ($CHCl_3$–MeOH). It was assigned the molecular formula as $C_{35}H_{40}O_{11}$ by HRESIMS. The IR spectrum exhibited absorptions of hydroxyl (3481 cm^{-1}) and α,β-unsaturated lactone (1757 cm^{-1}) groups. The ^1H and ^{13}C NMR spectroscopic data of **2** (Tables 1 and 2) were analogous to those of compound **1**. In the ^1H NMR spectrum of **2**, the absence of two ester carbonyl signals at δ 173.1 and 172.0, and of two methylene resonances at δ 2.5–3.0 indicated that the succinyl part in **1** was not present in **2**. In the HMBC experiment, the methyl group at δ 1.80 (*d*, *J* = 7.0 Hz, H-d) showed a long-range ^1H–^{13}C correlation to the carbon at δ 129.6 (C-b), indicating that the oxygenated methylene (H_2-d) in **1** was replaced by a methyl in **2**. These data indicated that compound **2** lacked a succinyl residue and the γ-hydroxyl of the hydroxytiglyl unit present in compound **1**. Extensive analysis of the 2D NMR spectroscopic data (HSQC and HMBC) supported this deduction. Thus, the structure of **2** was

Table 1
^1H (500 MHz) NMR spectroscopic data for compounds **1**–**4** (**1**, **2** and **4** in CD_3COCD_3 and **3** in $CDCl_3$)

Position	1	2	3	4
1	2.04[a] (1H, *ddd*)	1.85[a] (1H, *ddd*)	2.06[a] (1H, *ddd*)	2.04[a] (1H, *ddd*)
2 α	1.25 (1H, *ddd*, 8.5, 8.5, 4.0)	1.25 (1H, *ddd*, 8.0, 7.5, 4..5)	0.98 (1H, *ddd*, 8.3, 8.0, 4.5)	0.98 (1H, *ddd*, 8.3, 8.0, 4.5)
2 β	0.55 (1H, *ddd*, 5.5, 5.0, 4.0)	0.58 (1H, *ddd*, 5.0, 4.5, 3.5)	0.33[a] (1H, *m*)	0.31 (1H, *ddd*, 4.5, 4.0, 3.0)
3	1.82[a] (1H, *m*)	1.84[a] 1H, (*m*)	1.83[a] (1H, *m*)	1.82[a] (1H, *m*)
6			4.64 (1H, *s*)	3.95 (1H, *d*, 3.0)
9	3.83 (1H, *dd*, 6.7, 1.5)	3.85 (1H, *d*, 7.5)	3.77 (1H, *s*)	3.95 (1H, *s*)
13	1.59 (3H, *s*)	1.55 (3H, *s*)	2.18 (3H, *s*)	1.82 (3H, *s*)
14	0.83 (3H, *s*)	0.86 (2H, *s*)	1.08 (2H, *s*)	1.04 (3H, *s*)
15 α	2.73[a] (1H, *m*)	2.75 (1H, *ddd*, 13.0, 5.5 4.5)	2.78[a] (1H, *m*)	2.86 (1H, *dd*, 16.0, 1.5)
15 β	1.88[a] (1H, *m*)	1.90[a] (1H, *m*)	2.64[a] (1H, *m*)	2.64 (1H, *ddd*, 16.0, 6.0, 4.0)
1′	1.68 (1H, *ddd*, 8.5, 7.5, 4.0)	1.67 (1H, *ddd*, 8.5, 7.5, 4.5)	1.61 (1H, *ddd*, 8.5, 8.0, 4.5)	1.69 (1H, *ddd*, 8.0, 8.0, 4.0)
2′α	1.12 (1H, *ddd*, 4.5, 4.0, 3.5)	1.15 (1H, *m*)	1.32 (1H, *m*)	1.27 (1H, *ddd*, 5.0, 4.0, 4.0)
2′β	0.76 (1H, *ddd*, 8.5, 8.5, 4.0)	0.79 (1H, *ddd*, 8.5, 7.5, 4.0)	0.71 (1H, *ddd*, 9.0, 8.5, 4.0)	0.68 (1H, *ddd*, 8.5, 8.0, 5.0)
3′	1.49 (1H, *ddd*, 8.5, 7.5, 3.5)	1.77 (1H, *ddd*, 9.0, 7.5, 4.5)	1.37 (1H, *ddd*, 8.5, 8.0, 3.5)	1.52 (1H, *ddd*, 9.0, 8.5, 3.5)
5′	2.41 (1H, *dd*, 12.0, 7.0)	2.38 (1H, *dd*, 12.5, 7.0)	1.81 (1H, *dd*, 14.0, 6.5)	1.96 (1H, *dd*, 14.0, 6.5)
6′α	3.02 (1H, *dd*, 17.5, 12.5)	3.00 (1H, *dd*, 17.5, 12.5)	2.84[a] (1H, *m*)	2.84 (1H, *dd*, 18.5, 12.5)
6′β	2.75[a] (1H, *m*)	2.57[a] (1H, *m*)	2.33 (1H, *dd*, 19.0, 12.5)	2.41 (1H, *dd*, 18.5, 6.0)
9′	2.71[a] (1H, *m*)	2.68[a] (1H, *m*)	1.73 (1H, *m*)	1.97[a] (1H, *m*)
13′	4.50 (1H, *d*, 12.0)	4.35 (1H, *m*)	4.48 (1H, *d*, 12.0)	4.70 (1H, *d*, 13.0)
13′	5.13 (1H, *d*, 12.0)	4.35 (1H, *m*)	4.95 (1H, *d*, 12.0)	4.86 (1H, *d*, 13.0)
14′	0.96 (3H, *s*)	1.05 (3H, *s*)	0.77 (3H, *s*)	0.90 (3H, *s*)
15′	3.96 (1H, *d*, 11.5)	4.08 (1H, *s*)	3.71 (1H, *d*, 11.5)	3.78 (1H, *d*, 11.5)
15′	4.50 (1H, *d*, 11.5)	4.08 (1H, *s*)	4.54 (1H, *d*, 11.5)	4.18 (1H, *d*, 11.5)
c	6.62 (1H, *m*)	6.92 (1H, *m*)	6.68 (1H, *m*)	6.87 (1H, *m*)
d	4.59 (1H, *dd*, 15.0, 6.0)	1.80 (3H, *d*, 7.0)	4.77 (1H, *dd*, 14.5, 5.0)	1.84 (3H, *d*, 7.0)
d	4.89 (1H, *dd*, 15.0, 6.0)		5.12 (1H, *dd*, 14.5, 5.0)	
e	1.79 (3H, *s*)	1.84 (3H, *s*)	1.98 (3H, *s*)	1.86 (3H, *s*)
g	2.52[a] (1H, *m*)		2.58[a] (1H, *m*)	
g	2.72[a] (1H, *m*)		2.68[a] (1H, *m*)	
h	2.05[a] (1H, *m*)		2.76[a] (1H, *m*)	
h	2.80[a] (1H, *m*)		2.84[a] (1H, *m*)	
OMe			3.77 (3H, *s*)	3.66 (3H, *s*)
CH_3CO				2.04 (3H, *s*)

[a] Overlapped with other signals.

Table 2
^{13}C (125 MHz) NMR spectroscopic data for compounds **1**–**4** (**1**, **2** and **4** in CD_3COCD_3 and 3 in $CDCl_3$)

Position	1	2	3	4
1	29.8	29.2	26.1	25.9
2	10.5	10.6	15.8	15.9
3	31.2	31.1	24.7	25.1
4	77.6	77.9	141.2	142.3
5	164.4	164.6	132.5	133.6
6	123.7	124.0	41.5	41.7
7	124.6	124.0	134.6	133.6
8	104.2	104.4	204.6	201.4
9	79.3	78.8	80.6	80.6
10	50.5	50.4	50.2	51.9
11	153.4	154.1	144.3	146.5
12	172.8	172.2	169.1	171.1
13	10.7	10.9	18.9	20.4
14	14.2	14.3	15.2	15.8
15	41.3	41.4	25.5	26.0
1′	27.4	27.8	25.2	25.8
2′	9.4	9.4	11.4	12.3
3′	29.2	29.4	27.9	28.9
4′	77.6	77.9	77.0	77.4
5′	55.8	53.2	61.4	61.6
6′	24.2	21.8	24.0	23.5
7′	175.9	172.8	173.6	172.8
8′	86.3	85.8	93.2	93.6
9′	51.5	51.8	55.6	55.4
10′	46.1	45.2	45.0	45.5
11′	123.4	128.2	123.2	124.3
12″	172.8	169.1	171.8	171.6
13	54.7	54.9	54.8	56.8
14′	24.4	24.5	26.1	26.0
15′	74.4	70.0	72.8	71.7
a	167.6	168.0	167.4	168.1
b	129.4	129.6	129.4	129.2
c	137.1	137.5	136.0	138.1
d	62.3	14.3	61.1	14.4
e	12.8	12.3	13.2	12.3
f	173.1		172.3	
g	29.2		29.5	
h	29.1		29.3	
i	172.0		173.6	173.6
Ome			52.8	52.4
Acetyl				
CH_3				20.1
C=O				170.6

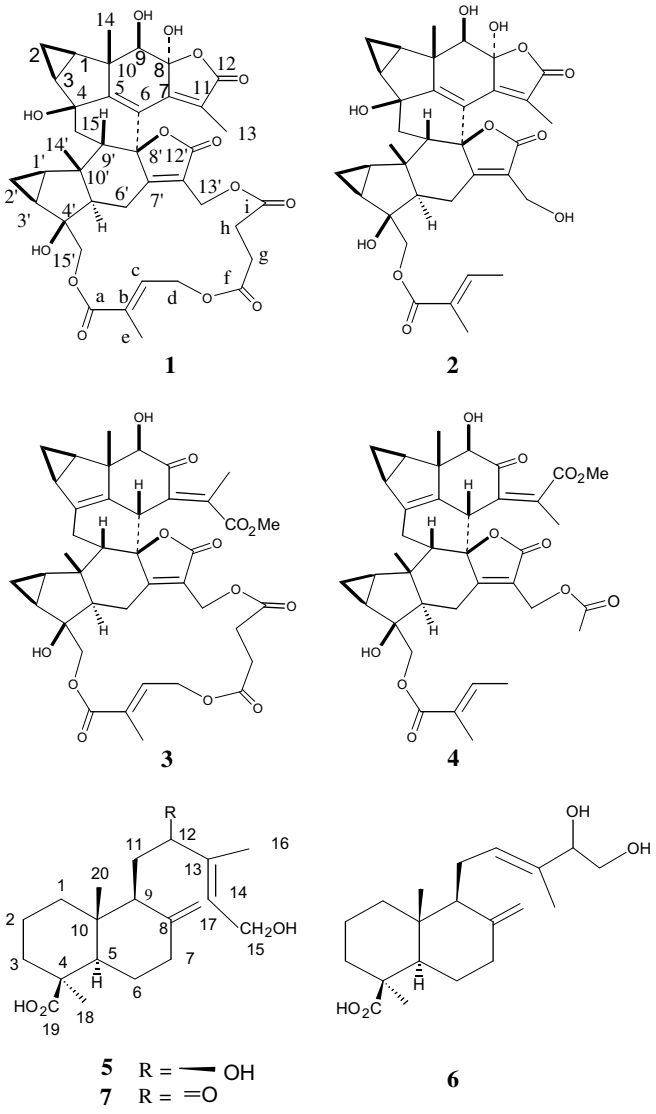

Fig. 1. Structures of compounds **1**–**7**.

elucidated as shown in Fig. 1. The CD spectrum of **2** was very similar to that of **1** in the range 200–280 nm (Fig. 4), indicating the absolute configuration of **2** as depicted in Fig. 1.

Henriol C (**3**) was obtained as colorless oil. Its molecular formula was $C_{40}H_{44}O_{13}$, as determined by positive-mode HRESIMS at *m/z* 755.2668 $[M+Na]^+$, which was identical to that of shizukaol B (**8**) (Kawabata and Mizutani, 1992). The ^1H and ^{13}C NMR spectroscopic data of **3** (Tables 1 and 2) were similar to those of **8** but the proton signals at δ 4.64 (1H, *brs*, H-6) and 2.18 (3H, *s*, H-13) of **3** were shifted downfield compared with the corresponding proton resonances of **8** [δ 3.96 (*d*, J = 3.1 Hz, H-6), 1.96 (3H, *s*, H-13)]. The chemical shifts of C-11 (δ 144.3) and C-8 (δ 204.0) of **3** were also different from those of **8** [δ 147.5 (C-11) and δ 200.7 (C-8)], because of the change of configuration of the C-7/C-11 double bond. When H-6 was irradiated in the NOE difference and NOESY spectra of **3**, enhancements of the methoxyl (δ 3.77) and 14-CH_3 (δ 0.83) groups were observed, respectively. These data indicated that H-6 had a β-orientation and that the configuration of the C-7/C-11 double bond was *E*. Because the configuration of the C-7/C-11 double bond was *E*, the CD spectrum of 3 showed a CD curve with a negative Cotton effect ($[\theta]_{265}$ − 14.6) arising from the exciton coupling of the α,β-unsaturated γ-lactone positive chirality of the compound (Fig. 4). As a result, the structure of **3** was established as represented in Fig. 1.

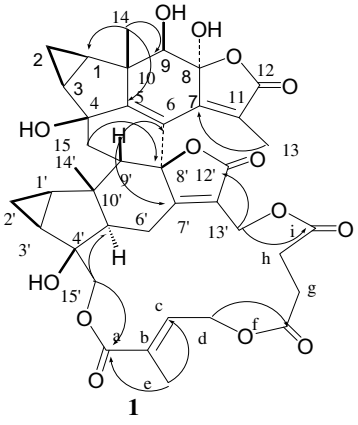

Fig. 2. Key HMBC correlations of Henriol A (**1**).

Henriol D (**4**) was obtained as colorless needles (MeOH), the spectroscopic features of which were similar to those of shizukaol C (**9**) (Kawabata and Mizutani, 1992). The ^1H and ^{13}C NMR spectroscopic data of **4** (Tables 1 and 2) showed an additional acetyl group

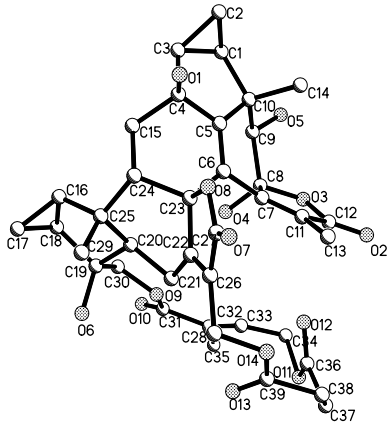

Fig. 3. X-ray structure of henriol A (**1**).

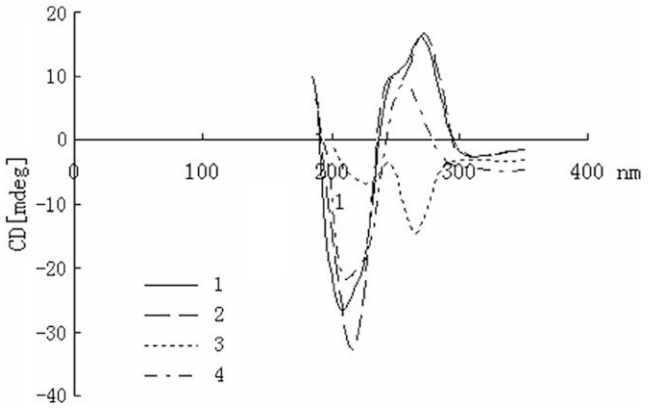

Fig. 4. CD spectra of compounds **1**–**4**.

(δ 2.04, δ 20.4 and 170.6) compared with compound **9**, which was consistent with the molecular formula $C_{38}H_{44}O_{11}$ obtained by HRESIMS. HMBC correlations of the proton signals at δ 4.70 (1H, *d*, *J* = 13.0 Hz, H-13′) and 4.86 (1H, *d*, *J* = 13.0 Hz, H-13′) with the carbonyl carbon (δ 170.6) of the acetyl group indicated that the acetyl group was located at C-13′. The downfield chemical shift of H_2-13′ from δ 4.33 and 4.41 in **9** to δ 4.70 and 4.86 in **4** also supported the above conclusion. The CD spectrum of **4** was very similar to that of **1** in the range 200–280 nm (Fig. 4), indicating the absolute configuration of **4** as depicted in Fig. 1. Thus, the structure of **4** was elucidated as shown in Fig. 1.

Henrilabdane A (**5**) was obtained as colorless needles ($CHCl_3$–MeOH), the molecular formula of which was $C_{20}H_{32}O_4$, as determined by HRFABMS at *m/z* 359.2160 $[M+Na]^+$. The IR spectrum of **5** exhibited hydroxyl (3425 cm^{-1}) and carbonyl (1693 cm^{-1}) absorptions. The ^{13}C NMR spectrum of **5** exhibited 20 carbons, including two olefinic quaternary carbons (δ 141.4 and 150.0), an olefinic methine (δ 129.1) and an olefinic methylene (δ 106.7) attributed to two olefinic bonds (one of which was trisubstituted, the other in an exomethylene group), an oxygenated methylene (δ 58.9), an oxygenated methine (δ 75.0), a carbonyl carbon (δ 178.8) attributed to a carboxyl group, and three methyls (δ 12.1, 13.5, and 29.8). The ^1H NMR spectrum showed three methyl proton signals (at δ 0.64, 1.21 and 1.64) and three olefinic proton resonances [at δ 4.51 (1H, *s*, H-17), 4.86 (1H, *s*, H-17) and 5.53 (1H, *t*, *J* = 6.5 Hz, H-14)]. In the HSQC spectrum, δ 4.51 and 4.86 (H-17) correlated with δ 106.7 (C-17), indicating the presence of an exomethylene group. Analysis of the HMBC spectrum established that the methyl at δ 1.64 (H-16) correlated with δ 141.4 (C-13) and 124.9 (C-14), and that δ 4.09 (1H, *d*, *J* = 6.0 Hz, H-15) correlated with C-13 and C-14, which showed the allylic alcohol moiety ($R_2C=CHCH_2OH$) containing C-13–C-16. This information indicated that **5** could be a labdane diterpene (Fujimoto et al., 1990; Fang et al., 1993). The NMR spectroscopic data of **5** (Tables 3 and 4) were very similar to those of 12,15-dihydroxylabda-8(17),13*E*-dien-19-oic acid (**10**) (Fang et al., 1993) but the carbon signals of C-12 and C-14 of **5** appeared at higher fields (δ 75.0 and 124.9), as did H-17 (δ 4.51). Therefore, **5** was assumed to be an epimer of **10**, differing from **10** only in the configuration of C-12. In the NOE difference spectrum of **5**, H-5 was enhanced by irradiation of H-9, indicating an α-orientation of H-9; in addition, H_2-15 was enhanced by irradiation of H_3-16, indicating an *E*-configuration of the C-13/C-14 double bond (Fig. 5). In the literature, the (12*S*)-

Table 3
^1H (500 MHz) NMR spectroscopic data for compounds **5**–**7** (5 in CD_3COCD_3, **6** in CD_3OD and **7** in C_5D_5N)

Position	5	6	7
1	1.18[a] (1H, *m*)	1.12[a] (1H, *m*)	1.14 (1H, *ddd*,11.5, 14.0, 4.0)
	1.82[a] (1H, *m*)	1.86[a] (1H, *m*)	1.82 (1H, *m*)
2	1.46 (1H, *m*)	1.47 (1H, *m*)	1.54 (1H, *m*)
	1.86 (1H, *m*)	1.84[a] (1H, *m*)	2.22[a] (1H, *m*)
3	1.08 (1H, *ddd*, 13.0, 11.5, 4.0)	1.09 (1H, *ddd*, 13.5, 13.0, 4.0)	1.08 (1H, *ddd*, 13.5, 13.5, 4.0)
	2.13[a] (1H, *m*)	2.06[a] (1H, *m*)	2.09[a] (1H, *m*)
5	1.41 (1H, *d* 11.5)	1.33 (1H, *dd*, 12.0, 2.5)	1.42 (1H, *d*, 11.0)
6	2.03 (1H, *m*)	1.92[a] (1H, *m*)	2.28[a] (1H, *m*)
	1.93[a] (1H, *m*)	1.92[a] (1H, *m*)	2.14[a] (1H, *m*)
7	1.95[a] 1H, (*m*)	1.83[a] (1H, *m*)	2.44 (1H, *d*, 13.0)
	2.41 (1H, *m*)	2.32 (1H, *m*)	2.41 (1H, *m*)
9	2.13 (1H, *dd*, 11.5, 4.0)	1.69 (1H, *brd*,10.0)	2.85 (1H, *dd*, 10.0)
11	1.50 (1H, *m*)	1.98[a] (1H. *m*)	2.71 (1H, *d*, 15.0)
	1.63 (1H, *m*)	2.25 (1H, *m*)	3.22 (1H, *dd*, 15.0, 10.0)
12	3.95 (1H, *d*, 10.0)	5.33 (1H, *t*, 6.5)	
14	5.53 (1H, *t*, 6.5)	3.90 (1H, *dd*, 10.0, 7.0)	5.53 (1H, *t*, 6.5)
15	4.09 (2H, *d*, 6.0)	3.42 (2H, *m*)	4.68 (2H, *d*, 5.5)
16	1.64 (3H, *s*)	1.56 (3H, *s*)	1.89 (3H, *s*)
17	4.51 (1H, *s*)	4.43 (1H, *s*)	4.50 (1H, *s*)
	4.86 (1H, *s*)	4.77 (1H, *s*)	4.80 (1H, *s*)
18	1.21 (3H, *s*)	1.15 (3H, *s*)	1.34 (3H, *s*)
20	0.64 (3H, *s*)	0.61 (3H, *s*)	0.92 (3H, *s*)

[a] Overlapped with other signals.

Table 4
^{13}C (125 MHz) NMR spectroscopic data for compounds **5**–**7** (**5** in CD_3COCD_3, **6** in CD_3OD and **7** in C_5D_5N)

Position	5	6	7
1	39.8	40.6	39.6
2	20.8	21.2	20.7
3	39.0	39.4	38.6
4	44.6	45.2	44.4
5	56.9	57.5	56.0
6	27.1	27.4	26.7
7	39.5	39.7	38.8
8	150.0	149.7	149.9
9	52.6	57.9	51.5
10	40.7	41.4	40.2
11	31.4	23.7	33.6
12	75.0	129.2	200.6
13	141.5	135.1	142.6
14	124.9	79.1	136.6
15	58.9	66.0	59.7
16	12.1	12.3	11.9
17	106.7	107.9	106.4
18	29.8	29.6	29.6
19	178.8	181.3	179.9
20	13.5	13.4	13.7

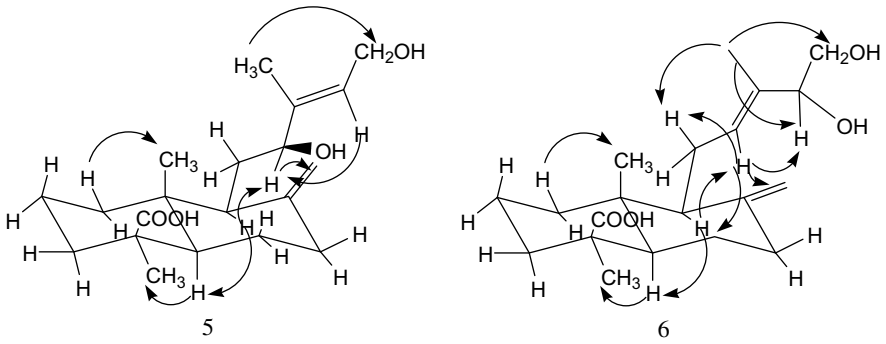

Fig. 5. Key NOE of compounds **5** and **6**.

isomer showed the vinylic proton signal (H-17) at δ 4.72, whereas the (12*R*)-isomer showed the corresponding proton resonance at δ 4.40 (Inoue et al., 1985; Hsieh et al., 1998). The chemical shift of H-17 of **5** was present at δ 4.51 near 4.40. Thus, the C-12 was assigned as 12(*R*); consequently, the structure of **5** was determined as being 12 (*R*),15-dihydroxylabda-8(17),13*E*-dien-19-oic acid.

Henrilabdane B (**6**) was obtained as colorless needles (MeOH), the molecular formula of which was $C_{20}H_{32}O_4$, as determined by HRFABMS at *m/z* 335.2228 $[M-H]^-$. The IR spectrum exhibited hydroxyl (3336 cm^{-1}) and carbonyl (1687 cm^{-1}) absorptions. The ^1H and ^{13}C NMR spectroscopic data of **6** (Tables 3 and 4) showed an allylic moiety [δ 5.33 (1H, *t*, *J* = 6.5 Hz, H-12); δ135.1 (C-12), 129.2 (C-13)], a carboxy group (δ 181.3) and an exomethylene group [δ 4.48 and 4.82 (each 1H, *s*, H-17); δ 107.9], indicating a labdane diterpene skeleton (Fujimoto et al., 1990; Fang et al., 1993). Compared with the NMR spectroscopic data from **5**, the chemical shift of **6** H-15 was shifted upfield from δ 4.09 to 3.42, the C-9 signal was shifted upfield from δ 57.9 to 52.6 and the C-11 signal was shifted downfield from δ 23.7 to 31.4. The differences in chemical shift between compounds **5** and **6** are ascribable to the location of the double bond. In the HMBC spectrum, the correlations between δ 5.33 (1H, *t*, *J* = 6.5 Hz, H-12) and δ 57.9 (C-9), 23.7 (C-11), between δ 1.69 (1H, *br d*, *J* = 10.0 Hz, H-9) and δ 23.7 (C-11), 135.1 (C-13), 129.2 (C-12), and between δ 1.56 (H-16) and δ 57.9 (C-9), 23.7 (C-11) indicated that the double bond was located at C-12/C-13. The ^1H–^1H COSY correlation of **6** between δ 3.90 (H-14) and δ 3.42 (H-15), and between δ 5.33 (1H, *t*, *J* = 6.5 Hz, H-12) and δ 1.98, 2.25 (each 1H, *m*, H-11) supported this deduction. The relative configuration of **6** was determined by a NOE experiment; when irradiating the proton signal at δ1.69 (1H, *brd*, *J* = 10.0 Hz, H-9), the resonance at δ 1.33 (1H, *dd*, *J* = 12.0, 2.5 Hz, H-5) was enhanced, indicating the α-orientation of H-9 and, thus, the *S*-configuration at C-9; H_2-15 and H-12 were enhanced by irradiation of H-14 (Fig. 5). The chemical shift of C-16 (δ 12.3) indicated that the configuration of the C-12/C-13 double bond is *E* (Fang et al., 1993). Thus, compound **6** was assigned as (9) *S*,14,15-dihydroxylabda-8(17),12*E*-dien-19-oic acid.

Henrilabdane C (**7**) was obtained as colorless crystals ($CHCl_3$–MeOH), and its molecular formula was established as $C_{20}H_{30}O_4$ by HREIMS at *m/z* $[M]^+$ 334.2122. The IR spectrum of this compound exhibited hydroxyl (3425 cm^{-1}) and two carbonyl (1662 and 1714 cm^{-1}) absorptions. The ^1H and ^{13}C NMR spectroscopic data of **7** (Tables 3 and 4) were similar to those of **10** (Fang et al., 1993) but the resonances for the secondary hydroxyl at C-12 (δ 3.90, δ 75.0) were replaced by a keto carbonyl (δ 200.6) in **7**. The HMBC correlations between H-11 (δ 3.22) and C-12 (δ 200.6), H-14 (δ 7.26) and C-12, and H-16 (δ 1.89) and C-12 further supported the above assignments. Hence, the structure of **7** was determined as 12-oxo-15-hydroxylabda-8(17),13*E*-dien-19-oic acid.

Compounds **1**, **2** and **5–10** were tested for hepatoprotective activities against D-galactosamine-induced toxicity in WB-F344 rat hepatic epithelial stem-like cells, using the hepatoprotective activity drug bicyclol as a positive control (Li et al., 2006) (Table 5). Compounds **1**, **5**, **6** and **7** (10 μM) showed moderate hepatoprotective activities with IC_{50} values of 0.19, 0.66, 0.09 and 0.18 μM, respectively and no obvious cytotoxicity was observed under this concentration. They were not studied further due to the weak effects noted. This is, however the first report of any hepatoprotective activity from *Chloranthus* species. Using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method (Carmichael et al., 1987) (Table 6), compounds **1–3**, **5–8** and **10** were tested against several human cancer cell lines including lung adenocarcinoma (A549), stomach cancer (BGC-823), hepatoma (BEL-7402), colon cancer (HCT-8), and hela (A2780) cell lines. Compound **3** showed cytotoxic activities against BEL-7402 and BGC-823 cells, with IC_{50} values of 1.4 and 3.2 μg/mL, respectively. Com-

Table 5
Hepatoprotective effects of compounds **1**, **2** and **5–10** (1×10^{-5} mol/L) against D-galactosamine-induced toxicity in WB-F344 cells[a]

Compound	Cell survival rate (% of normal)	Inhibition (% of control)
Normal	100.0 ± 9.3	
Control	26.4 ± 0.7	
Bicyclol[b]	46.6 ± 8.6*	27.4
1	53.4 ± 5.2**	36.6
2	32.4 ± 0.8**	8.1
5	36.75 ± 0.3**	18.9
6	39.97 ± 1.1*	18.5
7	42.24 ± 1.5*	21.6
8	32.54 ± 0.3**	8.3
9	23.9 ± 1.1	–
10	23.6 ± 8.8	–

[a] Results are expressed as means ± SD (n = 3; for normal and control, n = 6).
[b] Positive control substance.
* $p < 0.05$.
** $p < 0.01$, Significantly different from control after Student's t-test.

Table 6
Cytotoxic activities of compounds **1–3**, **5–8**, and **10**[a]

Compound	IC_{50} (μM)				
	HCT-8	Bel-7402	BGC-823	A 549	A2780
1	>10.00	>10.00	>10.00	>10.00	>10.00
2	>10.00	>10.00	>10.00	>10.00	>10.00
3	>10.00	1.40	3.20	>10.00	>10.00
5	0.54	1.70	5.76	>10.00	>10.00
6	>10.00	>10.00	>10.00	>10.00	>10.00
7	>10.00	>10.00	>10.00	>10.00	>10.00
8	>10.00	>10.00	>10.00	>10.00	>10.00
10	>10.00	>10.00	>10.00	>10.00	>10.00
Adriamycin	0.21	0.48	0.38	0.67	0.42

[a] Values are means of three experiments.

pound **5** showed moderate cytotoxic activities against BEL-7402, HCT-8, and BGC-823 cells, with IC_{50} values of 1.70, 0.54 and 5.76 μM, respectively.

3. Conclusions

Compounds **14** are new lindenane bis-sesquiterpenes that seem to be derived via the same biogenetic route, starting with a Diels–Alder-type reaction between two lindenanes (Kawabata and Mizutani, 1992). Compounds **1** and **2** show two α,β-unsaturated lactone moieties and an unusual double bond at C-5/C-6. These structures differ from those of the previously isolated new lindenane bis-sesquiterpenes from *Chloranthus* species; this might be because of the translocation of the C-4/C-5 double bond and cyclization at C-8/C-12. The X-ray crystallographic analysis of compound **1** was realized, which clarified the stereochemistry of the lindenane bis-sesquiterpene.

4. Experimental

4.1. General experimental procedures

Optical rotations were determined using a Perkin–Elmer 341 digital polarimeter, whereas UV spectra were taken on a Shimadzu UV-300 spectrophotometer. IR spectra were recorded on a Nicolet-Impact IR 400 spectrometer with KBr pellets. The ^1H and ^{13}C NMR, ^1H–^1HCOSY, HSQC, HMBC, NOE, and NOESY spectra were obtained using an Inova-500 FT spectrometer, at 500 MHz for ^1H and at 125 MHz for ^{13}C, with solvent peaks as references. HRFABMS was performed on an Auto spec Ultima-TOF mass spectrometer, whereas ESIMS were obtained using an Agilent 1100 series LC/MSD Trap SL mass spectrometer. HRESIMS were obtained on an Auto spec Ultima-Tof mass spectrometer. Reversed-phase HPLC was carried out on a Shimadzu LC-6AD instrument using an SPD-10A detector (5 mL/min). A reversed-phase C_{18} column (YMC-Pack ODS-A Φ 20 × 250 mm, 10 μ) was used. Column chromatography (CC) was carried out on silica-gel (100–200, 200–300 mesh, Qingdao Marine Chemistry Company, Qingdao, China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). Kieselgel 60 F_{254} silica-gel plates (Merck, Germany) were used for analytical TLC.

4.2. Plant material

Plant material was collected in the Zhangshu country of Jiangxi province in July 2003 and was identified by Professor Qing-hua Liu of the Faculty of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Nanchang, PR China. A voucher specimen (No. 20030718) is deposited at the herbarium of the Faculty of Pharmacy, Jiangxi University of Traditional Chinese Medicine.

4.3. Extraction and isolation

The dried roots of *C. henryi* (10 kg) were powdered and extracted with EtOH (2 × 100 L) under conditions of reflux for h_1, and after evaporation a crude extract (720 g) was obtained. The latter was further extracted using Et_2O (5 × 2000 mL) to give a Et_2O residue (360 g), which was subjected to silica-gel CC (1.0 kg) eluted with petroleum ether, $CHCl_3$, EtOAc, Me_2CO and MeOH. The EtOAc fraction (128 g) was divided into 12 fractions by silica-gel CC (3.0 kg), using a petroleum ether–acetone gradient (9:1–1:1, v/v) as eluent. Fraction 4 (12.6 g) was separated by silica-gel (200–300 mesh) (500 g), using $CHCl_3$–MeOH (150:1) as the mobile phase to afford henrilabdane C (**7**, 60 mg). Fraction 5 (9.5 g) was separated by silica-gel (500 g) using $CHCl_3$–MeOH (100:1) as the mobile phase to afford henrilabdane B (**6**, 20 mg), and a mixture that was purified by preparative HPLC on ODS [YMC-pack; eluent, MeOH:H_2O (55:45, v/v)] to afford **11** (8 mg) and **12** (6 mg). Repeated chromatography of fraction 6 (9.8 g) using normal-phase silica-gel column (500 g) was carried out, using a $CHCl_3$–MeOH gradient (50:1–25:1 v/v) as eluent to afford henriol D (**4**, 25 mg), shizukaol B (**8**, 800 mg), shizukaol C (**9**, 35 mg) and henrilabdane A (**5**, 130 mg). Fraction 7 (13.2 g) was further purified using a normal-phase silica-gel column (500 g), with $CHCl_3$–MeOH (50:1) as eluent to afford **10** (120 mg), and a partially purified mixture (500 mg) that was further purified by chromatography over a reversed-phase silica-gel column (80 g) [ODS, 65% MeOH:H_2O MeOH:H_2O (55:45, v/v)]] and Sephadex LH-20 (50 g), using $CHCl_3$–MeOH (2:1) as mobile phase to provide henriol A (**1**, 100 mg). Fraction 8 (6.4 g), via normal- and reversed-phase silica-gel column (220 g) chromatography (ODS), afforded a partially purified mixture that was further purified by chromatography over Sephadex LH-20 (50 g), using $CHCl_3$–MeOH (2:1) as the mobile phase to provide henriol B (**2**, 30 mg) and henriol C (**3**, 8 mg).

4.4. Henriol A (*1*)

Colorless square crystals (MeOH), $[\alpha]_D^{15}$ − 29.0 (Me_2CO; *c* 0.10); CD ($c = 1.4 \times 10^{-4}$ mol/L, EtOH): $[\theta]_{270}$ + 16.1 and $[\theta]_{209}$ − 26.8; UV (MeOH) λ_{max} (log ε) 216 (1.82) nm; IR (KBr) ν_{max}: 3435, 2920, 1743, 1695, 1668, 1419, 1356, 1259, 1146, 1092, 947, 924 cm^{-1}; for ^1H NMR (CD_3COCD_3, 500 MHz) and ^{13}C NMR (CD_3COCD_3, 125 MHz) spectroscopic assignments, see Tables 1 and 2; ESIMS *m/z* 757 $[M+Na]^+$; HRFABMS *m/z* 735.2676 $[M+H]^+$ (calc. for $C_{39}H_{43}O_{14}$, 735.2653).

4.5. Crystallography of henriol A (*1*)

$C_{39}H_{42}O_{14}$, $M = 734.77$, monoclinic, space group $P2_1$, $a = 11.858(1)$ Å, $b = 12.812(1)$ Å, $c = 23.092(1)$ Å, $V = 3508.2(4)$ Å^3, $Z = 4$, $d = 1.331$ g/cm^3. A crystal of dimensions 0.10 × 0.20 × 0.30 mm was used for measurements on a MAC DIP-2030 K diffractometer with a graphite monochromator ($w - 2\theta$ scans, $2\theta_{max} = 50.0°$), Mo Kα radiation. The total number of independent reflections measured was 4090, of which 3147 were observed ($|F|^2 \geqslant 8\sigma \geqslant |F|^2$). Final indices: $R_f = 0.061$, $R_w = 0.061$ ($w = 1/\sigma|F|^2$). Hydrogen positions were found from difference Fourier maps and geometric calculations. All calculations were carried out on a PC, using the NOMCSDP program system (Wu and Lu, 1992).

CCDC 679291 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

4.6. Henriol B (*2*)

Colorless needles ($CHCl_3$–MeOH), $[\alpha]_D^{15}$ − 27.0 (Me_2CO; *c* 0.10); CD ($c = 3.9 \times 10^{-4}$ mol/l, EtOH): $[\theta]_{272}$ + 16.7 and $[\theta]_{216}$ 32.8; UV (MeOH) λ_{max} (log ε) 217 (0.58) nm; IR (KBr) ν_{max}: 3481, 2945, 1757, 1714, 1647, 1442, 1383, 1263, 1146, 1082, 968, 904 cm^{-1}; for ^1H NMR (CD_3COCD_3, 500 MHz) and ^{13}C NMR (CD_3COCD_3, 125 MHz) spectroscopic assignments, see Tables 1 and 2; ESIMS *m/z* 659 $[M+Na]^+$; HRESIMS *m/z* 659.2787 $[M+Na]^+$ (calc. for $C_{35}H_{40}O_{11}Na$, 659.2832).

4.7. Henriol C (*3*)

Colorless oil, $[\alpha]_D^{15}$ − 107.0 ($CHCl_3$; *c* 0.10); CD ($c = 1.4 \times 10^{-4}$ mol/l, EtOH): $[\theta]_{265}$ 14.6, $[\theta]_{226}$ 6.9 and $[\theta]_{244}$ 3.5; UV (MeOH)

λ_{max} (log ε) 220 (2.22) nm; IR (KBr) ν_{max}: 3498, 2925, 1753, 1712, 1691, 1437, 1377, 1275, 1174, 1076, 995 cm^{-1}; for ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectroscopic assignments, see Tables 1 and 2; ESIMS *m/z* 755 $[M+Na]^+$; HRESIMS *m/z* 755.2668 $[M+Na]^+$ (calc. for $C_{40}H_{44}O_{13}Na$, 755.2680).

4.8. Henriol D (**4**)

Colorless needles (MeOH), $[\alpha]_D^{15}$ − 179.0 (*c* 0.10, Me_2CO); CD ($c = 1.6 \times 10^{-4}$ mol/l, EtOH): $[\theta]_{257}$ + 8.9 and $[\theta]_{210}$ 21.9; UV (MeOH) λ_{max} (log ε) 218 (0.530) nm; IR (KBr) ν_{max}: 3467, 2947, 1763,1736, 1691, 1645,1439, 1363, 1257, 1155, 1084, 987, 918 cm^{-1}; for ^1H NMR (CD_3COCD_3, 500 MHz) and ^{13}C NMR (CD_3COCD_3, 125 MHz) spectroscopic assignments, see Tables 1 and 2; ESIMS *m/z* 699 $[M+Na]^+$; HRESIMS *m/z* 699.2806 $[M+Na]^+$ (calc. for $C_{38}H_{44}O_{11}Na$, 699.2781).

4.9. Henrilabdane A (**5**)

Colorless needles from $CHCl_3$–MeOH, m.p. 185–186 °C; $[\alpha]_D^{15}$ − 61.2 (MeOH; *c* 0.40); IR (KBr) ν_{max}: 3425 (OH), 2943, 1693 (C=O), 887 cm^{-1}; for ^1H and ^{13}C NMR (CD_3COCD_3, 500 MHz and 125 MHz) spectroscopic assignments, see Tables 3 and 4; HRFABMS at *m/z*: 359.2160 $[M+Na]^+$, calc. for $C_{20}H_{32}O_4Na$, 359.2198; ESIMS *m/z*: 359 $[M+Na]^+$.

4.10. Henrilabdane B (**6**)

Colorless needles from MeOH, m.p. 185–186 °C; $[\alpha]_D^{15}$ − 42.6 (MeOH; *c* 0.20); IR (KBr) ν_{max}: 3336 (OH), 2943, 1687 (C=O), 887 cm^{-1}; for ^1H and ^{13}C NMR (CD_3OD, 500 MHz and 125 MHz) spectroscopic assignments, see Tables 3 and 4; ESIMS *m/z*: 359 $[M+Na]^+$; HRFABMS at *m/z*: 335.2228 $[M-H]^-$, calc. for $C_{20}H_{31}O_4$, 335.2228.

4.11. Henrilabdane C (**7**)

Colorless needles from $CHCl_3$–MeOH, m.p. 226–227 °C; IR (KBr) ν_{max}: 3400 (OH), 2943, 1714 (C=O), 1662 (C=O), 891 cm^{-1}; for ^1H and ^{13}C NMR (C_5D_5N, 500 MHz and 125 MHz) spectroscopic assignments, see Tables 3 and 4; HREIMS *m/z* 334.2122 $[M]^+$, calc. for $C_{20}H_{30}O_4$, 334.2144; EIMS *m/z*: 334 $[M]^+$.

4.12. Protective effect against cytotoxicity induced by D-galactosamine in WB-F344 cells

Hepatoprotective activity was assayed as described by Li et al., 2006, using bicyclol as a positive control. The hepatoprotective effects of compounds **1**, **2** and **5**–**10** were determined using an MTT colorimetric assay in WB-F344 cells, with some modification. Each cell suspension of 1×10^4 cells in 200 μL of Dulbecco's Modified Eagles Medium containing fetal calf serum (3%), penicillin (100 units/mL) and streptomycin (100 μg/mL) was planted in a 96-well microplate and precultured for 24 h at 37 °C under a 5% CO_2 atmosphere. Fresh medium (200 μL) containing bicyclol and test samples was added and the cells were cultured for 1 h, then exposed to 40-mM D-galactosamine for 24 h. The cytotoxic effects of test samples were measured simultaneously in the absence of D-galactosamine. The medium was changed to a fresh one containing 0.5 mg/mL of MTT. After 3.5 h of incubation, the medium was removed and DMSO (150 μL) was added to dissolve formazan crystals. The optical density (OD) of the formazan solution was measured on a microplate reader at 492 nm. Inhibition (%) was obtained by the following formula:

$$\text{Inhibition } (\%) = [(OD_{(sample)} - OD_{(control)})/(OD_{(normal)} - OD_{(control)})] \times 100$$

All values were expressed as ±SD. The Student's *t*-test for unpaired observations between normal or control and tested samples was carried out to identify statistical differences; p values <0.05 were considered significantly different. The survival rate of compound up the control indicated that this compound showed the hepatoprotective activity against D-galactosamine-induced toxicity in WB-F344 rat hepatic epithelial stem-like cells. The IC_{50} value was calculated from a dose-dependent curve.

4.13. Cytotoxicity assay

The cytotoxic activity of compounds **1**–**3**, **5**–**8** and **10** were evaluated using lung adenocarcinoma (A549), stomach cancer (BGC-823), hepatoma (BEL-7402), colon cancer (HCT-8) and hela (A2780) cell lines. Adriamycin was used as a positive control. Following 72 h of continuous treatment of the cells with the samples, the supernatant was doffed off and 0.1 mL of MTT (0.5 mg/mL in RPM1640) was added after each well had been carefully washed with RPM1640. The cell growth was measured with an MTT assay procedure (Carmichael et al., 1987) and the IC_{50} values were calculated from a dose-dependent curve from A549, BGC-823, BEL-7402, HCT-8 and A2780 cell lines.

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