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seco-Sesquiterpenes and acorane-type sesquiterpenes with antiviral activity from the twigs and leaves of *Illicium henryi* Diels

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ABSTRACT

Chemical investigation of an alcohol extract from the twigs and leaves of *Illicium henryi* Diels resulted in the isolation of two new acorane-related *seco*-sesquiterpenes (**1** and **3**), two new acorane-related *seco*-norsesquiterpenes (**2** and **4**), one new 2-*epi*-cedrane sesquiterpene (**5**), eight new acorane-type sesquiterpenes (**6-13**), and a known major constituent of acorenone B (**14**). Their structures were established by interpreting extensive spectroscopic data, including HRESIMS, NMR (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, and HMBC), and NOE difference spectra analysis. The absolute configurations of **1**, **2**, **4**-**7**, **9**, **10**, and **14** were determined by X-ray crystallography, while chemical transformation methods were performed with compound **14** as the starting material to elegantly solve the absolute configuration issue of compounds **8** and **11-13**. Notably, **1** and **2** are *seco*-sesquiterpenes that are related to acorane and possess an unusual ketal-linked hemiacetal in a 6,8-dioxabicyclo[3.2.1]octan-7-ol scaffold ring system. Plausible biosynthetic pathways for compounds **1-14**, which were derived from the acorane skeleton, were proposed. All the isolated compounds (**1-14**) were evaluated for their antiviral and cytotoxic activities.

Keywords : *Illicium henryi* Diels; acorane-type sesquiterpenes; *seco*-sesquiterpenes; chemical transformation; antiviral activity

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1. Introduction

The genus *Illicium* has been regarded as a rich source of sesquiterpenes [1-9], especially *seco*-prezizaane-type sesquiterpenes and prenylated C_6–C_3 compound derivatives [10-16], which have distinct structural features and various carbon skeletons. Some of the sesquiterpenes have exhibited intriguing antiviral, neuroprotective, and neurotrophic activities [7,17-21], while many prenylated C_6–C_3 compounds were found to exhibit increased choline acetyltransferase, cytotoxic and antiviral activities [12-14,22].

Illicium henryi Diels, a toxic shrub that belongs to the genus *Illicium* L., is mainly distributed in southern China. The roots and rhizomes of this species have been used as folk medicine for the treatment of traumatic injuries, arthralgia, and abdominal pain, and its fruits and root bark have been reported to be poisonous [23,24]. To date, there have been a few studies on *I. henryi* [20,21,25-32], and most of these studies have focused on the roots; as a result, some phytochemicals with antiviral activities were discovered [20,28,32]. Herein, we further chemically investigated the twigs and leaves of *I. henryi* and described the isolation, structure elucidation, and antiviral assay of two new *seco*-sesquiterpenes (**1** and **3**), two new *seco*-norsesquiterpenes (**2** and **4**), one new 2-*epi*-cedrane sesquiterpene (**5**), and nine new acorane-type sesquiterpenes (**6-13**), together with one known major constituent of acorenone B (**14**) (Fig. 1). The absolute configurations of **1**, **2**, **4-7**, **9**, **10**, and **14** were determined by X-ray crystallography, while the absolute configurations of **8** and **11-13** were identified using chemical transformation methods. Additionally, plausible biogenetic pathways for these diverse sesquiterpenes were also proposed.

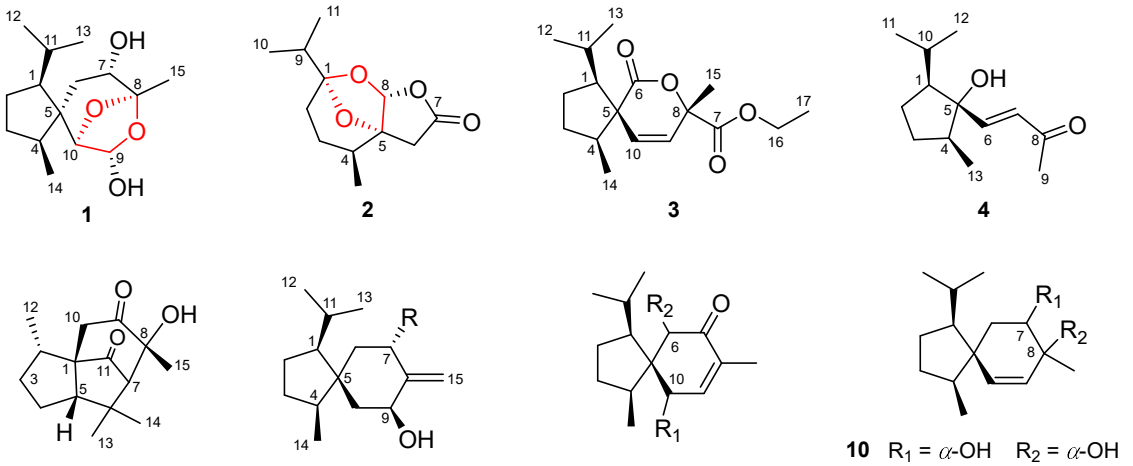


Fig. 1. Chemical structures of compounds **1**–**14**.

2. Results and discussion

Compound **1** was isolated as colorless crystals. Its molecular formula was determined to be $C_{15}H_{26}O_4$ based on the basis of the HRESIMS data $[M + Na]^+$ 293.1720 (calcd for $C_{15}H_{26}O_4Na$: 293.1723),

corresponding to three degrees of unsaturation. The IR spectrum showed absorption bands for hydroxyl groups (3420 cm^{-1}). According to the ^{1}H and ^{13}C NMR along with HSQC data (Tables 1 and 2), 15 carbon resonances attributable to four methyls (δ_C 25.5, 21.4, 19.5, and 17.6), three methylenes (δ_C 42.6, 32.5, and 24.7), six methines (including two mono-oxygenated carbons resonating at δ_C 71.2 and 86.6, respectively, and an acetal carbon resonating at δ_C 97.5), and two quaternary carbons (including a ketal carbon resonating at δ_C 110.0) were revealed. As **1** did not contain any unsaturated bonds, the compound was suggested to be a sesquiterpenoid with a tricyclic ring system. Analyses of the $^{1}H-^{1}H$ COSY spectrum (Fig. 2) revealed the connection of CH_3(12)/CH_3(13)-CH(11)-CH(1)-CH_2(2)-CH_2(3)-CH(4)-CH_3(14) (fragment **a**). In addition, H-1, H_2-3, H-4, H-11, and H_3-14 showed HMBC correlations to a quaternary carbon C-5 (δ_C 47.4), which suggested the presence of a five-membered ring that was substituted with a methyl and an isopropyl group. The COSY spectrum revealed the presence of another two spin systems (fragments **b** and **c**), and the HMBC correlations from H_2-6 and H-10 to C-5 suggested that both were connected to the five-membered ring through C-5. Furthermore, the HMBC correlations from H_3-15 to C-8 and C-7 indicated that CH_3-15 was connected to fragment **b** through C-8. Considering the remaining two degrees of unsaturation, the presence of two oxo bridges lying between C-8 and C-9 and between C-8 and C-10 was deduced from the key HMBC correlations from H-9 and H-10 to the ketal carbon (C-8), leading to the construction of an unusual 6,8-dioxabicyclo[3.2.1]octan-7-ol motif. Herein, the planar structure of compound **1** was assigned as shown in Fig. 2.

Table 1

^{1}H NMR data of compounds **1-5** (500 MHz).

no.	**1**[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]
1	1.50, ddd (8.0, 8.0, 3.5)		2.39, ddd (10.5, 10.5, 9.5)	1.63, ddd (10.5, 10.5, 10.0)	
2	1.62, ddd (8.0, 8.0, 6.0)	Ha 1.68, ddd (13.5, 11.5, 6.0) Hb 1.74, dd (13.5, 6.0)	Ha 2.08, dddd (13.5, 9.5, 9.5, 5.5) Hb 1.44, m	Ha 2.04, m[c] Hb 1.42, ddd (14.0, 11.5, 4.5)	1.50, dd (13.0, 13.0, 7.0)[d]
3	Ha 1.88, dddd (12.5, 9.0, 9.0, 7.5) Hb 1.28, dddd (12.5, 6.0, 6.0, 3.0)	Ha 1.85, ddd (13.5, 5.5, 1.0) Hb 1.38, ddd (13.5, 11.5, 6.0)	Ha 1.93, dddd (13.5, 9.0, 4.5, 4.5) Hb 1.26, m[c]	Ha 1.93, m Hb 1.28, dddd (13.0, 11.5, 11.5, 5.5)	Ha 1.27, ddd (12.0, 12.0, 7.0) Hb 1.72, m[c]
4	2.67, dqd (7.0, 7.0, 2.5)	2.06, septet-like	2.62, ddq (12.0, 8.5, 7.0)	2.06, m[c]	Hα 1.71, m[c] Hβ 1.50, m[c]
5					2.03, dd (10.5, 2.0)
6	Hα 1.73, ddd (15.0, 1.5, 1.5) Hβ 1.68, dd (15.0, 4.5)	Hα 2.67, d (17.5) Hβ 2.75, d (17.5)		6.75, d (15.5)	
7	3.38, dd (4.5, 1.5)			6.46, d (15.5)	2.06, s
8		5.83, s			
9	5.36, d (2.0)	1.97, septet (7.0)	6.07, d (10.5)	2.28, s	
10	4.30, s	0.98, d (7.0)	5.57, d (10.5)	1.36, dsept (10.5, 6.5)	Ha 3.48, d (14.5) Hb 2.26, d (14.5)
11	1.97, septd (7.5, 3.5)	0.98, d (7.0)	1.40, dsept (10.5, 6.5)	0.79, d (6.5)	
12	0.89, d (7.5)	0.99, d (7.0)	0.86, d (6.5)	0.83, d (6.5)	1.37, d (7.0)
13	0.90, d (7.5)		0.82, d (6.5)	0.91, d (6.5)	0.90, s
14	1.02, d (7.5)		0.79, d (6.5)		1.10, s
15	1.45, s		1.72, (s)		1.46, s
16			4.21, qd (7.0, 4.0)		
17			1.27, t (7.0)		

[a] Spectra were recorded in CD_3OD.
[b] Spectra were recorded in $CDCl_3$.
[c] Signals were overlapped.
[d] Signals were observed from 1D NOE spectra.

Table 2

^{13}C NMR data of compounds **1-5** (125 MHz).

no.	1[a]	2[b]	3[b]	4[b]	5[b]
1	57.3, CH	116.9, C	58.1, CH	58.2, CH	58.8, C
2	24.7, CH_2	30.0, CH_2	28.3, CH_2	26.9, CH_2	42.8, CH
3	32.5, CH_2	26.9, CH_2	30.2, CH_2	28.4, CH_2	34.6, CH_2
4	41.2, CH	30.8, CH	47.7, CH	47.7, CH	24.6, CH_2
5	47.4, C	90.4, C	53.6, C	85.2, C	54.2, CH
6	42.6, CH_2	36.2, CH_2	174.1, C	146.4, CH	34.4, C
7	71.2, CH	172.9, CH	170.9, C	129.7, CH	70.6, CH
8	110.0, C	101.5, C	82.7, C	198.2, C	79.8, C
9	97.5, CH	34.1, CH	126.4, CH	28.8, CH_3	207.0, C
10	86.6, CH	17.0, CH_3	124.0, CH	14.6, CH_3	49.6, CH_2
11	28.0, CH	16.9, CH_3	31.3, CH	31.5, CH	214.8, C
12	19.5, CH_3	16.7, CH_3	22.0, CH_3	22.7, CH_3	12.4, CH_3
13	25.5, CH_3		21.3, CH_3	21.4, CH_3	26.4, CH_3
14	17.6, CH_3		15.2, CH_3		27.7, CH_3
15	21.4, CH_3		26.2, CH_3		23.3, CH_3
16			62.3, CH_2		
17			14.2, CH_3		

[a] Spectra were recorded in CD_3OD.
[b] Spectra were recorded in $CDCl_3$.

The relative configuration of **1** was characterized by nuclear Overhauser enhancement (NOE) experiments (Fig. 2). Enhancement of CH_3-12 and CH_3-14 by irradiation of H-10 suggested that the methyl (CH_3-14), isopropyl, and methine (C-10) were on the same side of the five-membered ring, which allowed the configuration of the spiro carbon (C-5) to be identified. The obvious NOEs for H-9 observed after irradiation with H-11 revealed the configuration of C-9, as shown in Fig. 2. However, the configuration of C-7 was still ambiguous based on the NOEs between H-7 and CH_3-15. Fortunately, single crystals of **1** were obtained after a slow crystallization was performed in a mixed methanol and water (10:1) solvent. The absolute configuration of compound **1**, along with the orientation of H-7, was determined by X-ray crystallographic analysis to be 1*S*,4*S*,5*S*,7*S*,8*R*,9*S*,10*R* (Fig. 3), and compound **1** was named illihenin B.

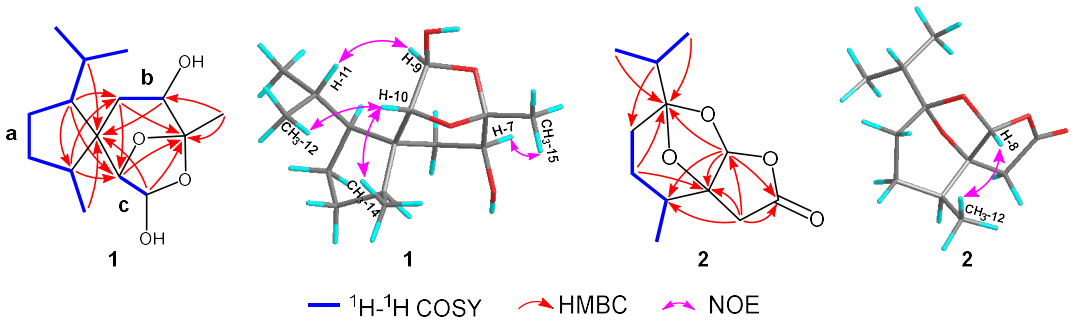


Fig. 2. ^1H-^1H COSY, HMBC, and NOE correlations of **1** and **2**.

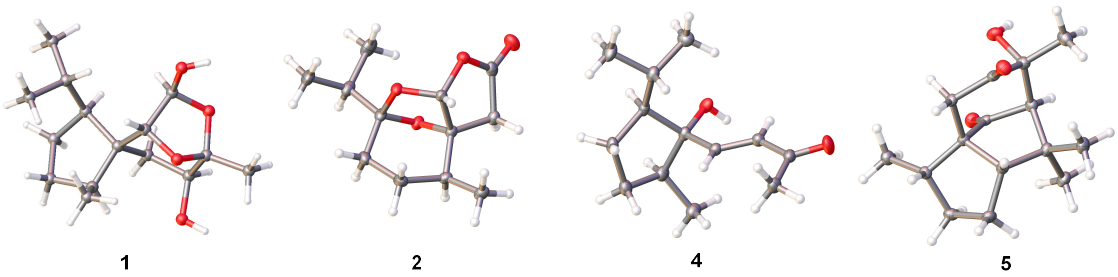


Fig. 3. X-ray crystallographic structures of compounds **1**, **2**, **4** and **5**.

Compound **2** (illihenin C) was obtained as crystals with the molecular formula $C_{12}H_{18}O_4$ as determined by HREIMS (*m/z* 249.1095 $[M + Na]^+$, calcd 249.1097), indicating four degrees of unsaturation. The IR spectrum of **2** showed the presence of an ester carbonyl group (1769 cm^{-1}). Based on the HSQC spectra, the 12 carbons displayed in the ^{13}C NMR spectrum (Table 2) could be assigned to three methyl carbons (δ_C 17.0, 16.9, and 16.7), three methylene carbons (δ_C 36.2, 30.0, and 26.9), three methine carbons (including an acetal carbon resonating at δ_C 101.5), and three quaternary carbons (including a ketal carbon resonating at δ_C 116.9 and a mono-oxygenated carbon at δ_C 90.4), which were similar to those of compound **1**. The two units of CH_3(12)/CH_3(13)-CH(11) and CH_2(2)-CH_2(3)-CH-CH_3(14) that were observed in the COSY spectrum were linked via the dioxygenated quaternary carbon C-1, which was supported by the HMBC correlations from H_3-10, H_3-11, and H_2-2 to C-1 and from H-9 to C-1 and C-2. In the HMBC spectrum, a singlet of the acetal proton at δ_H 5.83 (H-8) showed correlations to C-4 and C-5, suggesting a CH(4)–C(5)–CH(8) connection (Fig. 2), which was confirmed by the HMBC correlations from H_2-3 and H-4 to C-5. According to the HMBC correlations from H-8 to the ketal carbon C-1, an ether linkage was positioned between C-1 and C-8, which constructed a seven-membered ring. The residual two carbons C-6 and C-7 were involved in a γ-lactone ring, which was supported by the HMBC correlations from the proton of the acetal group (H-8) to the carbonyl carbon (C-7) and from H_2-6 to C-4, C-5, C-7, and C-8. After analysis of the molecular formula and degree of unsaturation, an ether bridge was deduced to be positioned between the ketal carbon (C-1) and the oxygenated quaternary carbon C-5. Thus, the planar structure of **2** was elucidated to be a novel norsesquiterpenoid that featured the same 6,8-dioxabicyclo[3.2.1]octan-7-ol motif as that of compound **1** (Fig. 2).

The relative configuration of compound **2** was assigned by the decisive enhancement of H_3-12 by the irradiation of H-8 observed in the NOEs experiment, illustrating that CH_3-12 and H-8 were on the same face of the seven-membered ether ring (Fig. 2). To determine the absolute configuration, the CD spectrum was tested (Fig. S28, Supporting Information) and showed a negative Cotton effect at 215 nm for the n→π* transition of the γ-lactone moiety. Based on the extended octant rule [33], the absolute stereochemistry of compound **2** was identified as 1*S*,4*S*,5*R*,8*R*. An X-ray single-crystal structure of **2** (Fig. 3), which was obtained using Cu Kα radiation, permitted the absolute configurations to be unambiguously assigned (CCDC 2161188).

Compound **3** was obtained as a colorless oil with $[\alpha]_D^{20}$ –38.8 (*c* 0.24, MeOH). The HRESIMS and ^{13}C

NMR analysis established the molecular formula as $C_{17}H_{26}O_4$ (*m/z* 295.1901 $[M + H]^+$, calcd 295.1904), requiring five degrees of unsaturation. The IR spectrum revealed the presence of two ester carbonyl groups (1754 and 1735 cm^{-1}). Inspection of the ^1H NMR data (Table 1) indicated the existence of a *cis*-double bond at δ_H 6.07 (d, J = 10.5 Hz, H-9), 5.57 (d, J = 10.5 Hz, H-10) and five methyls at δ_H 1.72 (s), 1.27 (t, J = 7.0 Hz), 0.86 (d, J = 6.5 Hz), 0.82 (d, J = 6.5 Hz), and 0.79 (d, J = 6.5 Hz). The ^1H-^1H COSY cross-peaks allowed the multiplets to be sequenced into the following spin systems: CH_3(12)/CH_3(13)-CH(11)-CH(1)-CH_2(2)-CH_2(3)-CH(4)-CH_3(14), CH(9)-CH(10), and CH_2(16)-CH_3(17) (Fig. 4). Combined with the HMBC correlations from H-11 to C-2 and C-5, from H_3-14 to C-3 and C-5, from H_2-2 to C-3 and C-5, from H-10 to C-1 and C-4, from H-9 to C-5, and from H-1/H-4/H-10 to C-6, a 1-isopropyl-3-methylcyclopentane unit was established, which was linked with the double bond (C-9–C-10) and a carbonyl δ_C 174.1 (C-6) via the common spiro carbon C-5. Furthermore, the HMBC correlations from H_3-15 to C-7, C-8, and C-9 and from H-10 to C-8 indicated that the oxygenated tertiary carbon (C-8) was connected to the methyl group (CH_3-15), the above mentioned double bond, and the remaining carbonyl (C-7), respectively. The HMBC correlations from H_2-16 to C-7 indicated the presence of a chain ester bond between C-7 and the ethoxy group [-O-CH_2(16)-CH_3(17)]. Since only one degree of unsaturation remained, another ester bond between C-6 and C-8 involved in a δ-lactone moiety was reliably deduced. Based on the above results, the planar structure of **3** was established to be a 6,7-*seco*-acorane sesquiterpenoid.

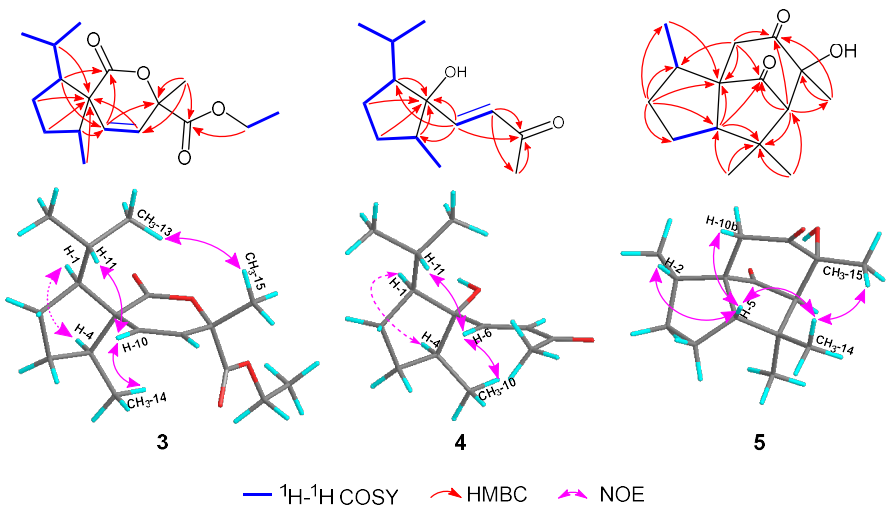


Fig. 4. ^1H-^1H COSY, HMBC, and NOESY correlations of **3-5**.

The configuration of **3** was determined by NOE experiments and CD data. In the NOE experiments, enhancement of H-1 by irradiation with H-4 and enhancement of H-11 and H_3-14 by irradiation with H-10 revealed that CH_3-14 and isopropyl are cofacial with the double bond, while H-1 and H-4 are on the opposite orientation (Fig. 4). The configuration of C-8 was determined based on the enhancement of H_3-13 by irradiation with H_3-15. The CD spectrum of **3** exhibited a negative Cotton effect at 219 nm (Fig. S37, Supporting Information), which indicated that **3** possessed a 1*S*,4*S*,5*S*,8*S* configuration according to the extended octant rule [33]. This result was further supported by the calculated ECD spectra of **3** (Fig. 5).

Therefore, compound **3** was identified as shown in Fig. 1 and named illihenin D.

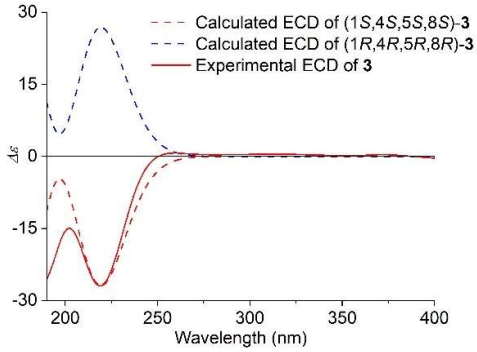


Fig. 5. The experimental ECD spectrum of **3** (the solid line) and the calculated ECD spectrum of **3** (the dashed lines).

With the molecular formula of $C_{13}H_{22}O_2$ based on HRESIMS, illihenin E (**4**) was purified as colorless crystals. The IR spectrum displayed absorptions of α,β-unsaturated ketone (ν_{max} 1685 cm^{-1}) and hydroxy (ν_{max} 3374 cm^{-1}) groups. The signals of three secondary methyls at δ_H 0.91 (d, J = 6.5 Hz), 0.83 (d, J = 6.5 Hz), 0.79 (d, J = 6.5 Hz) and a tertiary methyl group at δ_H 2.28 (s) were observed in the ^1H NMR spectrum (Table 1). Detailed analysis of $^1H-^1H$ COSY and HMBC correlations (Fig. 4) from H-1 and H-4 to C-5 indicated that compound **4** shared a common 1-isopropyl-4-methylcyclopentane unit with **3**. Additionally, the HMBC correlations from H-6/H-7/H_3-9 to C-8 and from H-7 to C-9 suggested the presence of a 3-oxobut-1-en-1-yl, which was attached to C-5 on the basis of the HMBC cross-peaks from H-6/H-7 to C-5 and from H-1/H-4 to C-6. A *trans*-1,2-disubstituted double bond between C-6 and C-7 was revealed by the characteristic coupling constant, $^3J_{H6,\ H7}$ (15.5 Hz). Analysis of the molecular formula and ^{13}C NMR data (Table 2) indicated that the oxygenated quaternary carbon C-5 was substituted with a hydroxyl group. Thus, the planar structure of **4** was identified as a norsesquiterpenoid, as shown in Fig. 4.

The relative configuration of **4** was elucidated by NOE experiments. Irradiation of H-1 enhanced H-4, and irradiation of H-6 enhanced H_3-10 and H-11, which suggested that the isopropyl, CH_3-13, and the 3-oxobut-1-en-1-yl unit were on the same side of the five-membered ring. Single crystals of **4** were obtained in methanol and were subjected to an X-ray diffraction (XRD) experiment with Cu Kα radiation. The absolute configuration was unambiguously determined as (1*S*,4*S*,5*S*) by XRD analysis (Fig. 3) with a Flack parameter of 0.01(15).

Illihenin F (**5**) was obtained as colorless crystals, and its molecular formula was found to be $C_{15}H_{22}O_3$ by HRESIMS data (*m/z* 251.1643 $[M + H]^+$, calculated for $C_{15}H_{23}O_3$ 251.1642). The IR spectrum showed absorption bands at 3430, 1746 and 1713 cm^{-1}, suggesting the presence of hydroxy and carbonyl groups, respectively. The 1D NMR and HSQC data (Tables 1 and 2) of **5** were indicative of two carbonyl groups (δ_C 214.8 and 207.0), four methyls (δ_C 27.7, 26.4, 23.3, and 12.4), three methylenes (δ_C 49.6, 34.6, and 24.6), three methines (δ_C 70.6, 54.2, and 42.8) and three quaternary carbons (δ_C 79.8, 58.8, and 34.4). The above functionalities accounted for two of the five degrees of unsaturation, suggesting that **5** possesses a tricyclic structure. Due to overlapping signals, only two spin systems of CH_3(12)-CH(2) and CH(5)-CH_2(4) were

revealed from the ^{1}H-^{1}H COSY spectrum, which were both linked to C-1 and C-3, and a methyl-substituted cyclopentane unit was constructed based on the HMBC correlations from H_2-3 to C-1/C-2/C-4/C-5, from H_3-12/H_2-4 to C-3 and from H_3-12/H-5 to C-1. On the other hand, the HMBC correlations from H_3-13/H_3-14 to C-5, C-6, and C-7 revealed the presence of a *gem*-dimethyl moiety. In the HMBC spectrum, a singlet for the methyl group at δ_H 1.46 (3H, s, H-15) showed correlations to C-7, C-9 and the sp^3 quaternary carbon C-8, and correlations from H_2-10 to C-1/C-2/C-5/C-8/C-9 were observed. These correlations suggested that C-6, C-7, C-8, C-9, C-10 along with C-1 and C-5 formed a seven-membered ring that was conjugated with cyclopentane. Considering the degrees of unsaturation, the remaining carbonyl group (C-11) was located between C-1 and C-7, which was supported by the key HMBC correlations from H-7/H_2-10 to C-1 and C-11. Therefore, compound **5** was characterized as a cedrane-type sesquiterpenoid, and its planar structure was established, as shown in Fig. 4.

The relative configuration of compound **5** was elucidated by NOE investigations (Fig. 4). In the NOE difference experiments, enhancement of H-2 and H-10b by irradiation with H-5 was observed, and the enhancement of H_3-14 was stronger than that of H_3-13 after irradiation with H-5. Therefore, H-2, H-5, CH_2-10, and CH_3-14 were determined to have *β*-orientations. Additionally, the orientation of CH_3-15 was clearly demonstrated based on the enhancement of H_3-15 by H_3-14 irradiation. The absolute configuration of **5** was finally determined to be 1*S*,2*S*,5*S*,7*R*,8*R* based on the X-ray diffraction crystallographic data [Flack parameter = 0.00(8)] (Fig. 3). Herein, compound **5** was identified as a rare 2-*epi*-cedrane-type sesquiterpenoid that possessed the opposite configuration at C-2 than that of the normal series [34].

Table 3
^{1}H NMR data of compounds **6-9** (500 MHz).

no.	**6**[a]	**7**[b]	**8**[b]	**9**[b]
1	1.24, ddd (11.5, 7.5, 6.0)	1.24, m	1.38, ddd (11.5, 9.0, 6.5)	2.08, m[c]
2	Ha 1.32, m[c]	Ha 1.32, m[c]	Ha 1.62, td (11.5, 4.0)	Ha 1.92, m[c]
	Hb 1.58, ddd (9.5, 7.5, 5.5)	Hb 1.58, m	Hb 1.85, m[c]	Hb 1.48, ddd (11.0, 11.0, 5.5)
3	Ha 1.74, m	Ha 1.71, dd (7.5, 3.0)	Ha 1.85, m[c]	Ha 1.90, m[c]
	Hb 1.29, m[c]	Hb 1.31, m[c]	Hb 1.47, m	Hb 1.24, m
4	2.25, dqd (7.5, 7.0, 5.0)	2.08, dqd (7.5, 7.0, 4.5)	1.85, m[c]	2.04, m[c]
6	1.64, m[c]	Hα 1.78, dd (15.3, 4.5)	Ha 2.80, d (16.5)	4.84, s
		Hβ 1.74, m	Hb 2.12, d (16.5)	
7	4.39, dd (4.5, 4.5)	5.54, dd (4.0, 3.5)		
9	4.51, dddd (12.0, 4.5, 2.5, 2.5)	4.54, dddd (12.0, 5.0, 2.0, 2.0)	6.58, dd (2.5, 1.5)	6.66, d (6.0)
10	Hα 1.87, ddd (12.5, 5.0, 1.0)	Hα 1.99, ddd (12.5, 5.0, 2.0)	4.88, br s	4.43, d (6.0)
	Hβ 1.31, dd (12.5, 12.5)[d]	Hβ 1.33, dd (12.0, 12.0)[d]		
11	1.63, septet (6.5)[d]	1.62, m[c]	1.85, m[c]	2.33, septd (6.5, 6.5)
12	0.84, d (7.0)	0.84, d (6.5)	0.98, d (6.5)	0.95, d (6.5)
13	0.90, d (7.0)	0.91, d (6.5)	1.02, d (6.5)	1.12, d (6.5)
14	1.02, d (7.0)	1.08, d (7.0)	0.92, d (6.5)	0.62, d (6.5)
15	Ha 5.04, br s	Ha 5.20, dd (1.5, 1.5)	1.77, d (2.5)	1.86, s
	Hb 4.97, br s	Hb 4.97, br s		
17		2.03 s		

[a] Spectra were recorded in CD_3OD.
[b] Spectra were recorded in $CDCl_3$.
[c] Signals were overlapped.
[d] Signals were observed from 1D NOE spectra.

Table 4

^{13}C NMR data of compounds **6-13** (125 MHz).

no.	**6**[a]	**7**[b]	**8**[b]	**9**[b]	**10**[a]	**11**[b]	**12**[a]	**13**[b]
1	60.2, CH	58.6, CH	57.1, CH	52.9, CH	60.1, CH	57.6, CH	60.6, CH	59.1, CH
2	26.3, CH_2	25.2, CH_2	27.3, CH_2	25.8, CH_2	23.0, CH_2	24.0, CH_2	22.8, CH_2	21.8, CH_2
3	32.5, CH_2	32.1, CH_2	32.7, CH_2	31.4, CH_2	30.8, CH_2	31.2, CH_2	30.3, CH_2	29.4, CH_2
4	44.1, CH	43.0, CH	45.9, CH	37.0, CH	46.6, CH	47.4, CH	46.7, CH	45.4, CH
5	47.4, C	46.5, C	55.4, C	56.6, C	52.3, C	50.7, C	52.3, C	51.0, C
6	47.1, CH_2	43.6, CH_2	50.5, CH_2	74.2, CH	39.3, CH_2	40.9, CH_2	40.2, CH_2	36.4, CH_2
7	73.9, CH	75.3, CH	199.8, C	201.8, C	72.8, CH	74.2, CH	74.2, CH	77.4, CH_2
8	154.5, C	148.5, C	135.1, C	134.6, C	69.4, C	74.0, C	74.0, C	72.2, C
9	68.2, CH	68.0, CH	148.4, CH	143.2, CH	133.4, CH	133.1, CH	136, CH	133.4, CH
10	38.5, CH_2	37.9, CH_2	70.8, CH	67.4, CH	133.3, CH	129.7, CH	129, CH	128.8, CH
11	29.1, CH	27.8, CH	29.4, CH	29.9, CH	29.8, CH	27.9, CH	29.1, CH	28.1, CH
12	21.6 CH_3	21.3 CH_3	21.8, CH_3	23.2, CH_3	18.3, CH_3	24.3, CH_3	18.8, CH_3	18.2, CH_3
13	25.2, CH_3	24.9, CH_3	24.5, CH_3	24.0, CH_3	25.2, CH_3	20.2, CH_3	25.4, CH_3	25.0, CH_3
14	17.4, CH_3	17.3, CH_3	19.9, CH_3	19.7, CH_3	15.1, CH_3	17.9, CH_3	14.9, CH_3	14.6, CH_3
15	107.4, CH_2	109.9, CH_2	15.1, CH_3	15.5, CH_3	27.0, CH_3	21.5, CH_3	21.8, CH_3	22.9, CH_3
16		170.2, C						172.0, CH_3
17		21.7, CH_3						21.5, CH_3

[a] Spectra were recorded in CD_3OD.
[b] Spectra were recorded in $CDCl_3$.

Compound **6**, colorless crystals, possessed a molecular formula of $C_{15}H_{26}O_2$ as determined by its HRESIMS ion peak at *m/z* 261.1818 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_2Na$, 261.1825). From the ^{13}C NMR and HSQC spectra, a terminal double bond (δ_C 154.5 and 107.4) and two oxygenated carbons (δ_C 73.9 and 68.2) were observed. Combined with the total three degrees of unsaturation, compound **6** was suggested to be a bicyclo sesquiterpenoid. The planar structure of **6** was determined by a combination of ^{1}H–^{1}HCOSY, HSQC, and HMBC spectra (Fig. 6), suggesting an acorane scaffold in **7** that contained two secondary hydroxy groups at C-7 and C-9, which were established by HMBC correlations from the terminal olefinic protons H_2-15 (δ_H 5.04 and 4.97) to C-7 at δ_C 73.9 and C-9 at δ_C 68.2. In the NOE difference experiments, the enhancement of H-1 by H-4 irradiation and the enhancement of CH_3-12 and CH_3-14 by H-10α irradiation indicated that the CH_3-14 and isopropyl groups were *cis*-orientated. In addition, H-9 was assigned to be β-oriented based on the NOEs for H-9 after irradiation with CH_3-14. Furthermore, a small coupling constant of H-7 (dd, J = 4.5, 4.5 Hz) indicated the α-orientation of the 7-hydroxy group. Finally, the X-ray single-crystal structure (Fig. 7) was obtained using Cu Kα radiation [Flack parameter = 0.08(17)]; thus, the absolute configuration of **6** was assigned as 1*S*,4*S*,7*S*,9*S* (CCDC 2161183). Consequently, the structure of compound **6** was defined as (1*S*,4*S*,7*S*,9*S*)-1-isopropyl-4-methyl-8-methylenespiro[4.5]decane-7,9-diol, and the compound was named illihenin G.

When compared to compound **6**, **7** was shown to contain an additional acetyl group, corresponding to the molecular formula $C_{17}H_{28}O_3$, as gleaned from the (+)-HRESIMS ion at *m/z* 303.1926 $[M + Na]^+$ and the NMR data. 2D NMR analysis (Figs. S70-72, Supporting Information), especially the HMBC correlations from H-7 and H_3-17 to C-16, confirmed that the acetoxy group was located at C-7. The absolute

configuration of **7** was verified to be 1*S*,4*S*,5*R*,7*S*,9*S* by X-ray diffraction experiments (Fig. 7). Thus, the structure of compound **7** was established to be 7-*O*-acetylllihenin G.

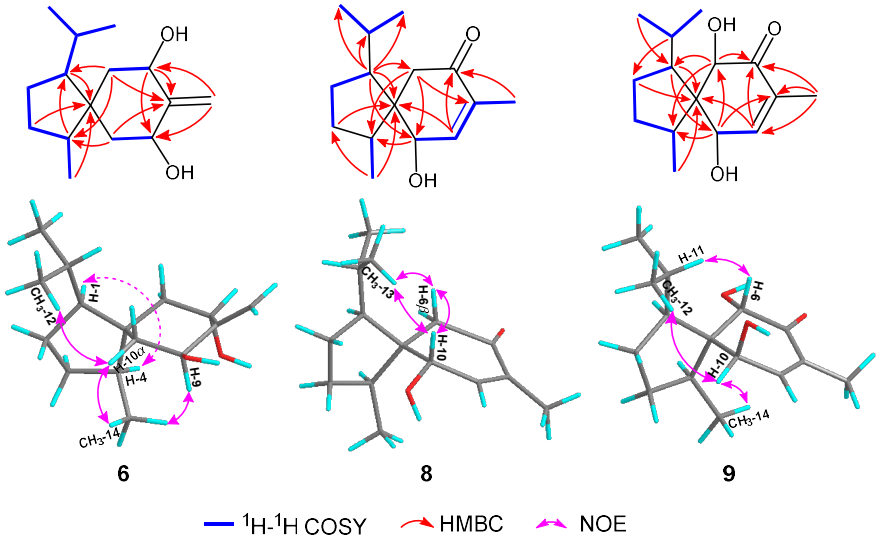


Fig. 6. ^{1}H-^{1}H COSY, HMBC, and NOE correlations of **6**, **8**, and **9**.

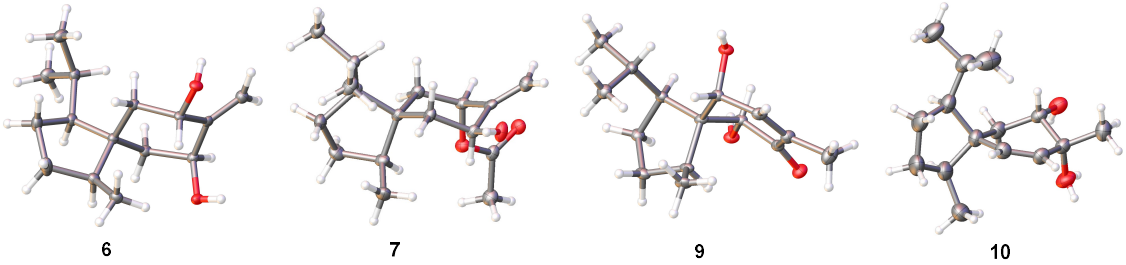


Fig. 7. X-ray crystallographic structures of compounds **6**, **7**, **9**, and **10**.

The molecular formula of compound **8**, $C_{15}H_{24}O_2$, was determined by the (+)-HRESIMS ion at *m/z* 237.1845 $[M+H]^+$ and ^{13}C NMR data. Analysis of the NMR data (Tables 3 and 4) of **8** revealed that its structure is closely related to that of compound **14** except for the presence of an additional hydroxy group. The hydroxy group was placed at C-10 based on the HMBC correlations (Fig. 6) from H-10 (δ_H 4.88, brs) to C-1 (δ_C 57.1), C-4 (δ_C 45.9), and C-6 (δ_C 50.5). Due to the hydrogen signals that highly overlapped in the ^{1}H NMR spectrum (H-2b, H-3b, H-4, and H-11), limited information could be obtained from the NOE difference spectrum (Fig. 6, Fig. S82, Supporting Information) except for the enhancement of H-6*β* and H-10 by CH_3-13 irradiation and the enhancement of H-6*β* by H-10 irradiation, which allowed us to conclude the orientation of the isopropyl and 10-hydroxy groups, as shown in Fig. 6, and the orientation of CH_3-14 was still ambiguous. Considering the similar 1D NMR spectroscopic data due to the 1-isopropyl-4-methylcyclopentane unit (Figs. S129-130, Supporting Information) and biosynthetic pathway, it was deduced that the absolute configurations of C-1 and C-4, which were involved in the five-membered ring in compound **8**, were identical to that of coexisting compound **14**, which was isolated from the twigs and leaves of the plant as a major constituent. To verify this assumption, a simple chemical conversation strategy with

14 as a starting material was proposed, as shown in Scheme 1B.

Previous to those analyses, the absolute configuration of **14** with $[\alpha]_D^{20}$ −13.2 (*c* 0.46, MeOH) needed to be determined. According to the ^{1}H and ^{13}C NMR spectroscopic data (Figs. S129-130, Supporting Information), compound **14** was identified to be a known sesquiterpenoid acorenone B [35], of which the relative configuration was determined by the X-ray crystallographic data of acorenone-B 4-iodo-2-nitrophenyl-hydrazone. Interestingly, a pair of dimeric diastereomers, diacorones A and B (**15** and **16**), were synthesized from **14** under SmI_2-induced reductive conditions [36-39] (Scheme 1A), and both were fortunately isolated as crystals. Hence, the absolute configurations of C-1, C-4, and C-5 in compound **14** were unambiguously assigned as 1*S*,4*S*,5*R* by the X-ray crystallography data of dimers **15** and **16**. (Scheme 1, Figs. S9-10, Supporting Information).

Then, starting from compound **14**, a hydroxy group was introduced straightforwardly by allylic oxidation of the enone using a copper–aluminium mixed oxide catalyst (Scheme 1B) [40]. Finally, 10-hydroxy epimers of acorenone B (**17** and **18**) were obtained, and **8** was assigned to be the 10-hydroxy epimer **17** on the basis of their identical ^{1}H and ^{13}C NMR data (Figs. S146-149, Supporting Information) and CD data (Figs. S150-151, Supporting Information). These results indicated that isopropyl and CH_3-14 in **8** had *cis*-configurations. Furthermore, the absolute configuration of **17** was determined to be 1*S*,4*S*,5*S*,10*S* based on a comparison of its experimental and calculated ECD spectra (Fig. 10). Thus, the absolute configuration of **8** was assigned to be 1*S*,4*S*,5*S*,10*S* by the strategy of chemical transformation from **14**, and the structure of compound **8** was determined to be (1*S*,4*S*,5*S*,10*S*)-10-hydroxyacorenone B.

Compound **9** was assigned a molecular formula of $C_{15}H_{24}O_3$ based on the HRESIMS ion peak at *m/z* 275.1611 $[M + Na]^+$ (calcd for $C_{15}H_{24}O_3Na$, 275.1618). Analysis of the NMR data (Tables 3 and 4) revealed that compound **9** was an acorane sesquiterpenoid that was structurally related to **8**, and the major difference was the presence of an additional hydroxy group at C-6 (δ_C 74.2), which was confirmed by the HMBC correlations (Fig. 6) from oxygenated methine H-6 to C-1, C-4, C-7, and C-10. In the NOE experiments, enhancement of H-2β, H-3β, H_3-12 and H_3-14 by irradiation of H-10 indicated that isopropyl and CH_3-14 had *cis*-configurations and 10-hydroxy was β-oriented. 6-Hydroxy was determined to have an α-configuration, as evidenced by the NOEs for H-11 after irradiation with H-6. The above analysis was verified subsequently by a single-crystal X-ray diffraction experiment [Flack parameter = 0.0(2)], which also assigned the absolute configuration of **9** to be 1*S*,4*S*,5*R*,6*S*,10*R* (Fig. 7). Thus, the structure of **9** was named (1*S*,4*S*,5*R*,6*S*,10*R*)-6,10-dihydroxyacorenone B (**9**).

Compound **10** was isolated as colorless crystals with $[\alpha]^{20}_D$ -24.4 (*c* 0.24, MeOH). The molecular formula was determined by HRESIMS (*m/z* 261.1826 $[M + Na]^+$, calcd for $C_{15}H_{26}O_2Na$, 261.1825) to be $C_{15}H_{26}O_2$. In the ^{1}H NMR spectrum, two olefinic proton signals at δ_H 5.58 (d, J = 10.0 Hz), 5.47 (dd, J = 10.0, 1.2 Hz) indicated the presence of a *cis*-1,2 disubstituted double bond. The same 1-isopropyl-3-methylcyclopentane unit as that of the acorane sesquiterpenoids (**6**-**9**) was present, as confirmed by the ^{1}H–^{1}H COSY and HMBC data (Fig. 8). The COSY correlation of H_2-6/H-7 and the key HMBC correlations from H_3-15 to C-7/C-8/C-9 and from H_2-6/H-10 to the quaternary carbon C-5 at δ_C 52.3 suggested the presence of cyclohexene, which was fused with the five-membered structure via the spiro carbon C-5 (Fig. 8). Additionally, two hydroxy

groups were located at C-7 (δ_C 72.8) and C-8 (δ_C 69.4), which were supported by the HMBC correlations from H_3-15 to C-7 and C-8 and from H-7 to C-8 and C-15. Therefore, the planar structure of **10** was deduced as shown in Fig. 8.

The relative configuration was elucidated from the NOE data and coupling constants (Fig. 8). In the NOE difference spectra of **10**, irradiation with H-10 enhanced the H_3-12 and H_3-14 resonances, indicating that isopropyl, CH_3-14, and CH-10 were cofacial, which was also supported by the observed NOE correlations of H-1 with H-6β and of H-4 with H-6α. In addition, the large coupling constant of $^3J_{H\text{-}6\alpha/H\text{-}7}$ (12.5 Hz) and the NOE correlation of H-7 with H-11 established the β-axial orientation of H-7, and the β-configuration of CH_3-15 was indicated by the strong NOE correlation of H-7 with CH_3-15. Finally, the absolute configuration of compound **10** was determined by a single-crystal X-ray diffraction experiment to be (1*S*,4*S*,5*R*,7*S*,8*R*) (Fig. 7) with a Flack parameter of 0.04(6), which confirmed the above deduction. The structure of **10** was determined to be (1*S*,4*S*,5*R*,7*S*,8*R*)-1-isopropyl-4,8-dimethylspiro[4.5]dec-9-ene-7,8-diol, and the compound was named illihenin H.

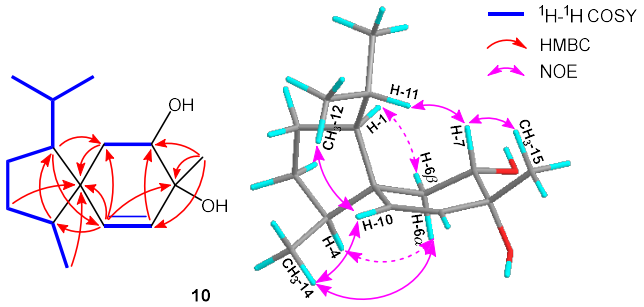


Fig. 8. ^{1}H-^{1}H COSY, HMBC, and NOESY correlations of **10**.

Table 5
^{1}H NMR data of compounds **10**-**13** (500 MHz).

no	**10**[a]	**11**[b]	**12**[a]	**13**[b]
1	1.70, ddd (11.0, 8.5, 3.5)	1.43, ddd (12.0, 7.8, 5.4)	1.66, m[c]	1.70, ddd (11.5, 8.5, 3.5)
2	Hα 1.59, ddd (8.5, 3.0, 3.0)	Hα 1.68, m[c]	1.58, m[c]	Hα 1.62, m[c]
	Hβ 1.55, m	Hβ 1.47, ddd (12.0, 11.5, 6.5)		Hβ 1.54, ddd (16.5, 11.5, 5.5)
3	Hα 1.79, ddt (13.0, 8.5, 5.0)	Hα 1.78, ddd (12.5, 6.5, 3.0)	Ha 1.79, dddd (13.0, 9.0, 9.0, 5.5)	Hα 1.83, ddd (13.0, 9.0, 5.0)
	Hβ 1.27, dtd (13.0, 10.5, 6.0)	Hβ 1.26, m[c]	Hb 1.26, dddd (13.0, 10.5, 10.5, 5.5)	Hβ 1.54, m
4	1.62, m	1.84, m[c]	1.59, m[c]	1.63, m[c]
6	Hα 1.92, dd (12.5, 12.5)	Hα 1.55, ddd (13.2, 4.5, 1.5)	Ha 1.67, m[c]	Ha 1.76, dd (13.0, 13.0)
	Hβ 1.36, ddd (12.5, 4.0, 1.5)	Hβ 1.86, m[c]	Hb 1.49, ddd (13.5, 5.0, 1.5)	Hb 1.64, m[c]
7	3.65, dd (12.5, 4.0)	3.94, dd (12.6, 4.5)	3.91, dd (13.0, 5.0)	5.12, dd (13.0, 5.0)
9	5.58, d (10.0)	5.60, d (10.0)	5.55, d (10.5)	5.62, d (10.5)
10	5.47, dd (10.0, 1.5)	5.36, dd (10.0, 1.5)	5.25, dd (10.5, 1.5)	5.33, dd (10.5, 1.5)
11	1.85, septd (7.0, 3.5)	1.69, septd (6.5, 6.5)	1.98, septd (7.0, 3.5)	2.06, septd (7.0, 3.5)
12	0.77, d (7.0)	0.90, d (6.5)	0.82, d (7.0)	0.84, d (7.0)
13	0.89, d (7.0)	0.83, d (6.5)	0.92, d (7.0)	0.94, d (7.0)
14	0.78, d (7.0)	0.90, d (7.0)	0.75, d (7.0)	0.77, d (7.0)
15	1.25, s	1.26, s	1.14, s	1.25, s
17				2.12, s

[a] Spectra were recorded in CD_3OD.
[b] Spectra were recorded in $CDCl_3$.
[c] Signals were overlapped.

Illihenin I (**11**) was isolated as a white, amorphous powder with $[\alpha]^{20}_{D}$ +21.8 (*c* 0.18, MeOH), while illihenin J (**12**) was a colorless oil with $[\alpha]^{20}_{D}$ -15.6 (*c* 0.43, MeOH). The two compounds shared the same formula ($C_{15}H_{26}O_2$) based on their HRESIMS data. Their ^{1}H and ^{13}C NMR spectroscopic data (Tables 3 and 5) were very close to those of **10**, suggesting that both compounds have the same planar structure as that of **10**. The deduction was corroborated by the HSQC, ^{1}H-^{1}H COSY, and HMBC correlations (Figs. S106-108 and S115-117, Supporting Information). The relative configurations of **11** and **12** were determined by NOE experiments. The same relative configuration of the five-membered rings in compounds **11** and **12** was established from the enhancements of CH_3-13 and CH_3-14 after H-10 irradiation for **11** and the enhancements of CH_3-12 and CH_3-14 after H-10 irradiation for **12** (Fig. 9). Furthermore, the enhancement of CH_3-15 by CH_3-13 irradiation and the enhancement of CH_3-14 by H-7 irradiation in the NOE difference spectra of **11** suggested that OH-7 and OH-8 have *β*- and *α*-orientations, respectively, while the *α*-orientation of OH-7 and *β*-orientation of OH-8 in compound **12** were indicated by the enhancement of CH_3-15 by CH_3-14 irradiation and the enhancement of H-7 by CH_3-13 irradiation in the NOE experiment.

On the basis of biosynthetic considerations, (1*S*,4*S*,5*R*)-**14** could be a reasonable precursor of 1,2-diols **10**-**12** biosynthesis. We thus resorted to chemical transformation from the above mentioned (1*S*,4*S*,5*R*)-**14** to resolve the absolute configurations of compounds **11** and **12**.

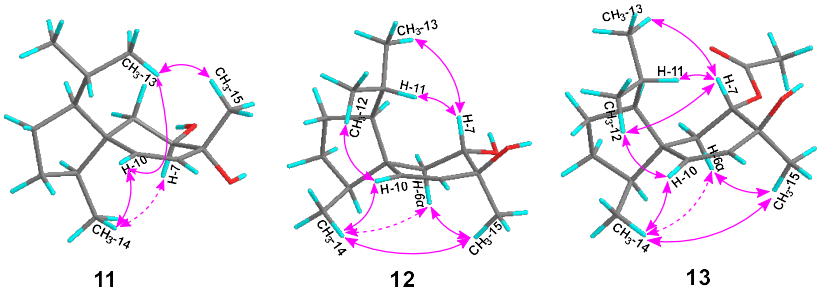
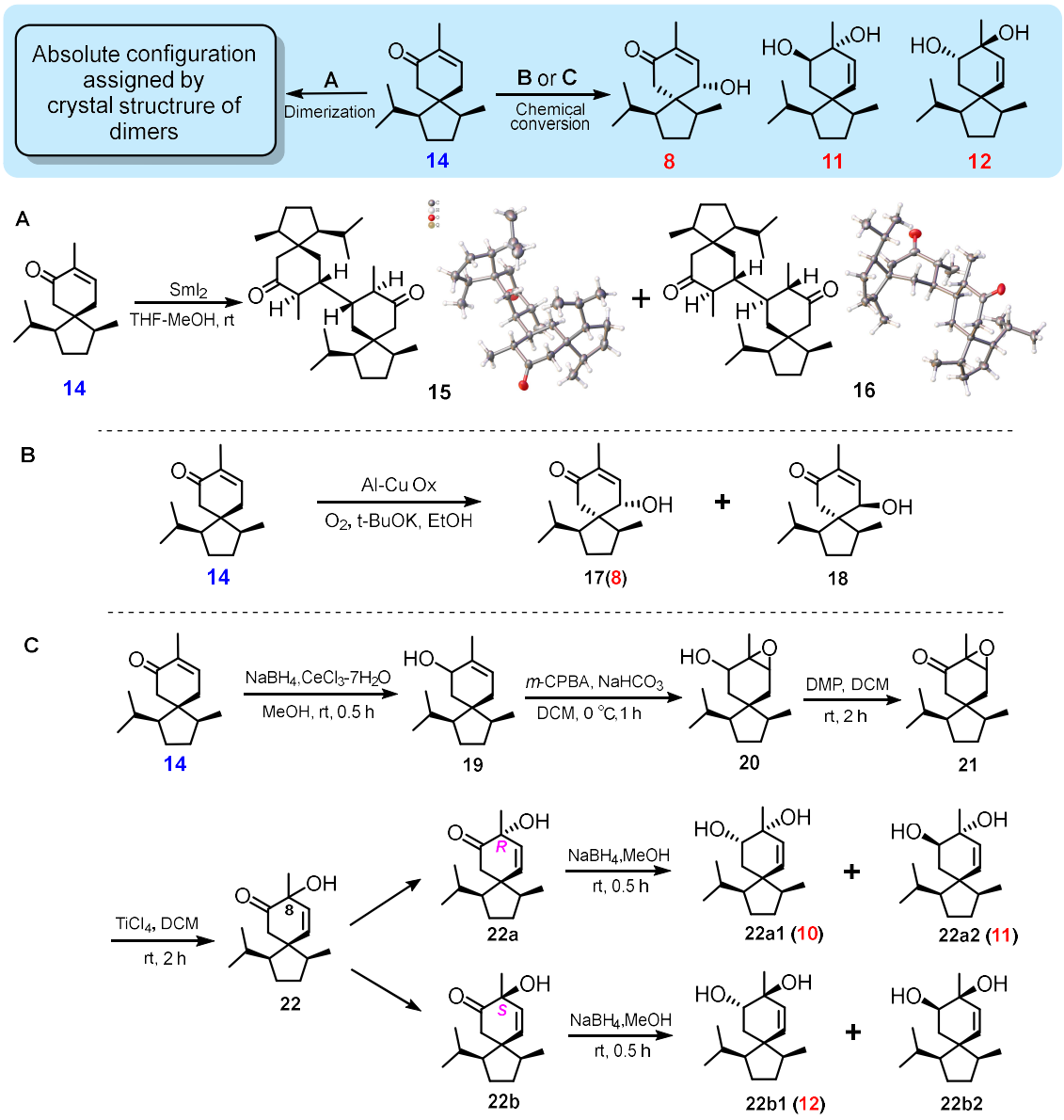


Fig. 9. Key NOE correlations of **11**, **12** and **13**.

We attempted to perform the chemical transformation several times but failed (Fig. S196, Supporting Information) because of the unsatisfactory reaction site or spatial interference of substrates. Finally, a Lewis acid-mediated epoxy-opening/elimination strategy, pivoting *α*,*β*-epoxyketone **20** as a key intermediate, was devised to attain the desired 1,2-diol products (Scheme 1C). To obtain the key *α*,*β*-epoxyketone **20**, the *α*,*β*-unsaturated ketone (1*S*,4*S*,5*R*)-**14** was exposed to Luches reduction conditions to furnish allylic alcohols **18** as a pair of diastereoisomers **18a** and **18b**, which were directly epoxidized by *m*-CPBA to afford epoxy-alcohol isomers **19** (2 steps, 74% overall yield). Due to the introduction of chiral hydroxy and epoxy groups in **14** by reduction and epoxidation, four isomers of **19** could be theoretically produced. However, only a mixture of three isomers was obtained, from which **19a** (Figs. S162-163, Supporting Information) and a mixture of two isomers **19b** (Figs. S164-165, Supporting Information) were isolated by HPLC.

After the mixtures of epoxy-alcohol isomers of **19** were oxidized using Dess-Martin periodinane, an epoxide opening of the corresponding *α*,*β*-epoxyketone **20** using titanium tetrachloride provided a mixture of allyl alcohols (**22a** and **22b**) as well as some chlorohydrin byproducts based on HPLC–MS analysis. The absolute configurations of the chiral alcohols in **22a** and **22b** were assigned to be *8R* and *8S*, respectively,

based on NOE experiments (Figs. S169 and S172, Supporting Information). The absolute configurations of **22a** and **22b** were further confirmed by comparing their calculated ECD spectra and experimental spectra (Fig. 10). The main product, **22a**, was purified by preparative TLC in a 38% yield, while **22b**, which was completely mixed with other coproducts, could only be isolated by preparative HPLC in a 7% yield. Finally, reduction with $NaBH_4$ made it possible to complete the chemical transformation, and four diastereoisomers (**22a1**, **22a2**, **22b1**, and **22b2**) were successfully obtained. According to the ^{1}H and ^{13}C NMR data and ECD data (Figs. S173-193, Supporting Information), three of the four diastereoisomers (**22a1**, **22a2**, and **22b1**) were unambiguously confirmed to be identical to compounds **10-12** (Scheme 1). Thus, the absolute configurations of isolated acorane-type compounds **10-12** and synthetic isomer **22b2** were elegantly determined by chemical transformation, and the structures of **10-12** and **22b2** were assigned as (1*S*,4*S*,5*R*,7*S*,8*R*)-, (1*S*,4*S*,5*R*,7*R*,8*R*)-, (1*S*,4*S*,5*R*,7*S*,8*S*)-, and (1*S*,4*S*,5*R*,7*R*,8*S*)-1-isopropyl-4,8-dimethylspiro[4.5]dec-9-ene-7,8-diol, respectively.



Scheme 1. Chemical transformation strategy to identify the configurations of 1,2-diol epimers **10**, **11** and **12**.

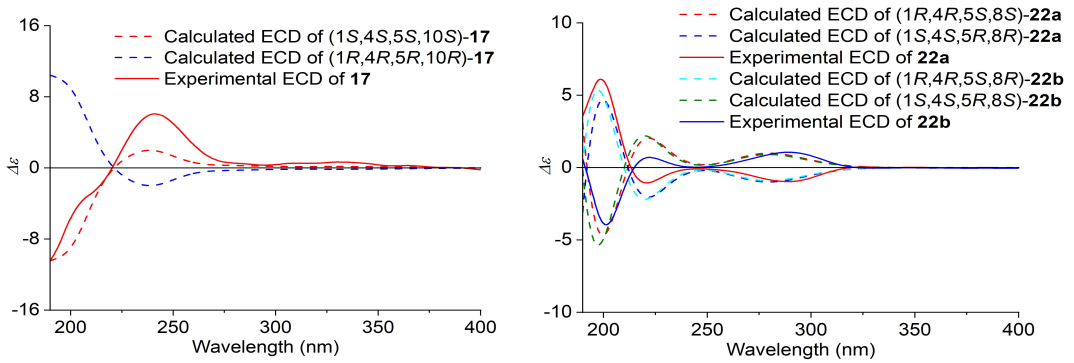
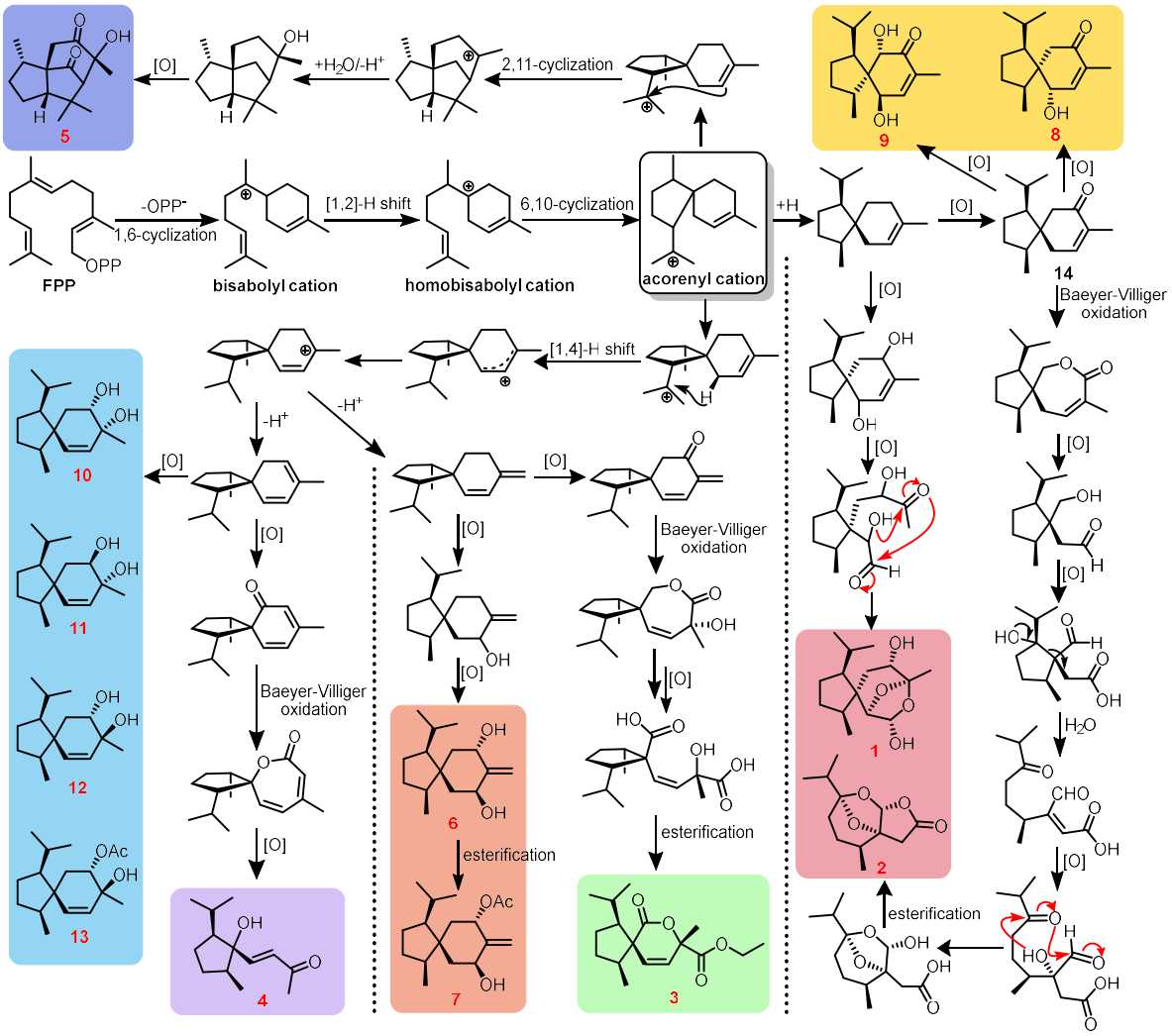


Fig. 10. The experimental ECD spectra of **17**, **22a** and **22b** (the solid lines) and the calculated ECD spectra of **17**, **22a** and **22b** (the dashed lines).

The molecular formula of **13**, $C_{17}H_{28}O_3$, was determined by the ^{13}C NMR and (+)-HRESIMS ion at *m/z* 303.1924 $[M + Na]^+$. Comparing its NMR data (Tables 4 and 5) with those of **12** revealed that **13** was structurally related to **12** but contained an extra acetyl group, which was attached to C-7 by the HMBC correlations (Fig. S126) from H-7 to C-6, C-8, C-15, and C-16 (δ_C 172.0) and from CH_3-17 (δ_H 2.12) to C-16. The NOE experimental data (Fig. 9) were similar to those of **12**, and diagnostic NOEs for H-11, CH_3-13 and CH_3-12 were observed after irradiation with H-7, suggesting that H-7 was *β*-oriented as well. Accordingly, compound **13** was deduced to be (7*S*,8*S*)-7-acetoxy-8-hydroxyillihenin H.

The present investigation on the twigs and leaves of *I. henryi* resulted in the isolation of fourteen sesquiterpenes, including four *seco*-sesquiterpenoids (**1-4**), a 2-*epi*-cedrane sesquiterpenoid (**5**) and nine acorane-type sesquiterpenes (**6-14**). Considering their biosynthesis, these structurally diverse sesquiterpenes were proposed to be derived from a common intermediate, acorenyl cation [41], which could indiscriminately undergo a 2,11-cyclization, a [1,4]-H shift [42], and a reduction by hydride anion transfer; then, the compound could subsequently undergo a series of reactions involving Baeyer-Villiger rearrangement [43], oxidative cleavage [44], esterification, and condensation to yield **1–14**, as shown in Scheme 2. In particular, the intriguing biosynthetic pathway of *seco*-sesquiterpenoids **1** and **2** may involve a more complicated cyclization mechanism, which is speculated to be an unusual hydroxy-triggered cascade reaction that involves an intramolecular aldehyde-ketone cyclization. This plausible biogenetic pathway might be a useful inspiration for the synthesis of these compounds and the structural motif of 6,8-dioxabicyclo[3.2.1]octan-7-ol.



Scheme 2. Proposed biosynthetic pathway of compounds **1-14**.

All the isolated compounds (**1-14**) were evaluated for their antiviral and cytotoxic activities [45]. Compounds **13** and **14** exhibited significant activity against CVB3 virus with IC_{50} values of 33.33 μM and 33.33 μM and selectivity index (SI) values of > 3.0 and > 3.0 (Tables S1, Supporting Information), respectively, compared with that of the positive control ribavirin (IC_{50} = 1688.16 μM and SI = 4.9) but were less effective than those of pleconaril. Interestingly, acetylated derivative **13** showed stronger anti-CVB3 activity than that of its direct precursor, 1,2-diol **12** (IC_{50} > 100 μM), and 1,2-diol isomers **10** and **11**. On the other hand, illihenin C (**2**) and illihenin F (**5**) displayed potential activity against influenza virus A/Hanfang/359/95 (H3N2) with IC_{50} values of 25.87 μM and 33.33 μM and selectivity index (SI) values of 3.9 and 3.0 (Tables S1), respectively, i.e. less promising results than those obtained for the positive controls ribavirin (IC_{50} 5.86 μM and SI > 139.9) and oseltamivir (IC_{50} 4.90 μM and SI > 99.5). In anti-HSV-1 activity, no obvious inhibition was observed for compounds **1-14**. Additionally, the isolated compounds **1-14** at 10.0 μM did not show noticeable cytotoxicity against the human tumor cell lines HCT-116, HepG2, HGC27, MCF7, and U251.

3. Conclusions

In conclusion, thirteen new diverse sesquiterpenes (**1**-**13**), including four *seco*- and/or *nor*-sesquiterpenes (**1**-**4**), a 2-*epi*-cedrane sesquiterpene (**5**), and eight acorane-type sesquiterpenes (**6**-**13**), were isolated and fully characterized from the twigs and leaves of *I. henryi*. The absolute configurations of compounds **1**, **2**, **4**-**7**, **9**, and **10** were determined using X-ray diffraction analysis. The absolute configurations of **8** and **11**-**13** were identified with the help of chemical transformations. Additionally, comparing the calculated and experimental ECD values was conducted to assign the absolute configuration of **3**. Compounds **13** and **14** exhibited significant activity against CVB3 virus, comparing very favourably with ribavirin, a broad-spectrum antiviral used in the clinic. Further, the lack of cytotoxicity against the human tumor cell lines may bode well in terms of tolerance. Analysis of the structures and activities of compounds **10**−**13** indicated that the substituent of the acetyl group may be essential for antiviral activity. It is worth noting that compounds **1** and **2** possess a rare structural motif due to their unique linkage of the ketal to the hemiacetal system. An intriguing biosynthetic route and cyclization mechanism were proposed, which may inspire synthetic and biosynthetic chemists when performing further investigations.

4. Experimental section

4.1. General experimental procedures

Melting points were measured on an XT5B precision microscopic melting point apparatus. Optical rotations were measured on a Rudolph Research Autopol V automatic polarimeter, and UV spectra were obtained on a JASCO V-650 spectrometer. CD spectra were recorded on a JASCO J-815 CD spectrometer. IR spectra were recorded on a Nicolet 5700 FT-IR ATR microscope instrument (FT-IR microscope transmission). 1D and 2D NMR spectra were acquired at 500 or 600 MHz for ^{1}H and at 125 or 150 MHz for ^{13}C using an INOVA 500 MHz or Bruker 600 MHz (Bruker, Germany) spectrometer. Chemical shift values in chloroform-*d* and methanol-d_4 were referenced to the internal signals of residual protons or carbon. HRESIMS data were measured with a Thermo Fisher Scientific Q Exactive Focus LC/MS spectrometer. Column chromatography was performed using silica gel (200-300 mesh and 300-400 mesh, Qingdao Marine Chemical Inc., China), ODS (50 μm, Merck, Germany) and a Sephadex LH-20 column (Amersham Biosciences, Inc., Shanghai, China). Preparative thin-layer chromatography was performed using high-performance silica gel preparative TLC plates ($HSGF_{254}$, glass precoated, Qingdao Marine Chemical Inc. China). Analytical HPLC was performed on a Shimadzu LC-20AT instrument with a Shimadzu SPD-M20A detector, a Shimadzu DGU-20A deaerator, and a Shimadzu SIL-20A sampler using a Diamonsil (250 × 4.6 mm i.d.) C18 column (5 μm). Semipreparative HPLC separations were performed on a Shimadzu LC-6AD instrument with two detectors, an SPD-20A detector and an RID-20A detector, using a YMC (250 × 10 mm i.d.) C18 column (5 μm). TLC separations were carried out on precoated glass silica gel GF_{254} plates. Spots were directly visualized under UV light or by spraying with 5% H_2SO_4 in 95% EtOH, followed by heating. Unless otherwise noted, all chemicals were commercially available and used without further purification.

4.2. Plant material

The twigs and leaves of *Illicium henryi* Diels were collected in Bobai County, Guangxi Province, People′s

Republic of China, in March 2018. The plant was identified by Prof. Song-ji Wei of Guangxi Traditional Medical College. A voucher specimen (S2975) was deposited in the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences.

4.3. Extraction and isolation

The twigs and leaves of *I. henryi* Diels (50 kg) were extracted three times by refluxing with 95% EtOH. The EtOH extract was concentrated under reduced pressure, and the extract (3.4 kg) was mixed with silica gel (100–200 mesh, 7 kg) and then sequentially eluted three times with petroleum ether, CH_2Cl_2, EtOAc and MeOH (each 9 L). The CH_2Cl_2 portion (289 g) was chromatographed on a polyamide column (3 L) with a gradient elution using 40% EtOH, 60% EtOH and 95% EtOH. Then, the 40% EtOH fraction (98.8 g) was fractionated by column chromatography on silica (2 kg) with CH_2Cl_2/EtOAc (100:1 to 1:1) to afford eighteen fractions (DS_1-DS_{18}). Fraction DS_{12} (6.94 g) was divided into four parts ($DS_{12}O_1$–$DS_{12}O_4$) using an ODS column with a gradient elution of MeOH–H_2O (20:80 to 80:20). Fraction $DS_{12}O_3$ (2.18 g) was fractionated by column chromatography on silica (30 g) with PE–EtOAc (5:1 to 1:1) to afford four fractions ($DS_{12}O_3S_1$−$DS_{12}O_3S_4$). Fraction $DS_{12}O_3S_4$ (732 mg) was separated by HPLC with CH_3CN–H_2O-TFA (30:70:0.1) to yield compound **12** (26 mg, t_R = 50 min) and was separated with MeOH–H_2O-TFA (55:45:0.1) to yield compound **1** (5 mg, t_R = 36 min). Fraction DS_{13} (6.0 g) was separated by column chromatography on Sephadex LH-20 with 90% MeOH and provided eight subfractions ($DS_{13}N_1$–$DS_{13}N_8$). $DS_{13}N_2$ (3.14 g) was divided into four parts ($DS_{13}N_2O_1$–$DS_{13}N_2O_4$) using an ODS column with a gradient elution of MeOH–H_2O (50:50 to 70:30). Compound **6** (18 mg, t_R = 33 min) was obtained from fraction $DS_{13}N_2O_4$ (146 mg) and then purified by HPLC with CH_3CN–H_2O (35:65).

The PE portion (228 g) was subjected to a polyamide column (2.2 L) with a gradient elution using 50% EtOH, 70% EtOH and 95% EtOH. Then, the 50% EtOH fraction (130 g) was fractionated by column chromatography on silica (3.5 kg) with PE–EtOAc (500:1 to 1:1) to afford fourteen fractions (PS_1−PS_{14}). Fraction PS_{10} (15.4 g) was chromatographed over an ODS column with a gradient elution of MeOH–H_2O (50:50 to 100:0) to afford six fractions ($PS_{10}O_1$– $PS_{10}O_6$). Fraction $PS_{10}O_3$ (2.2 g) was separated into seven subfractions ($PS_{10}O_3S_1$–$PS_{10}O_3S_7$) after being passed through a silica column (35 g) with the gradient CH_2Cl_2–MeOH as the mobile phase; subfraction 5 (160 mg) was purified by HPLC using 70% MeOH–H_2O to yield **2** (5 mg, t_R = 43 min) and **5** (15 mg, t_R = 32 min). Fraction $PS_{10}O_3S_6$ (600 mg) was separated by column chromatography on Sephadex LH-20 with MeOH and generated four subfractions ($PS_{10}O_3S_6N_1$−$PS_{10}O_3S_6N_4$). Fraction $PS_{10}O_3S_6N_1$ (280 mg) was purified by HPLC to yield **9** (2 mg, t_R = 41 min) with 55% MeOH–H_2O. $PS_{10}O_3S_6N_3$ (160 mg) was purified by HPLC to yield **4** (18 mg, t_R = 27 min) with 55% CH_3CN–H_2O. Fraction $PS_{10}O_3S_7$ (650 mg) was separated by column chromatography on Sephadex LH-20 with MeOH and generated six subfractions ($PS_{10}O_3S_7N_1$− $PS_{10}O_3S_7N_6$). Compounds **10** (18 mg, t_R = 38 min) and **11** (10 mg, t_R = 35.5 min) were obtained from fraction $PS_{10}O_3S_7N_5$ (243 mg) by HPLC with 45% MeOH–H_2O. Fraction $PS_{10}O_6$ (5.3 g) was subjected to a silica gel column (200 ‒ 300 mesh) and eluted with PE–EtOAc (200:1 to 4:1) to produce five fractions ($PS_{10}O_6S_1$–$PS_{10}O_6S_5$). Subfraction 3 (1.5 g) was separated into six subfractions ($PS_{10}O_6S_1D_1$–$PS_{10}O_6S_1D_6$) after being passed through a silica column (45 g) with the gradient CH_2Cl_2–MeOH as the mobile phase. Compounds **7** (8 mg, t_R = 36 min) and **13** (6 mg, t_R = 42 min)

were separated from fraction $PS_{10}O_6S_1D_5$ (409 mg) by HPLC with $CH_3CN–H_2O$ (50:50). Fraction PS_3 (2.3 g) was subjected to an ODS column with MeOH–H_2O (50:50 to 100:0) elution to afford four fractions ($PS_{11}O_1$– $PS_{11}O_4$). Fraction $PS_{11}O_2$ (403 mg) was purified by using thin-layer chromatography and HPLC to yield **3** (5 mg, t_R = 44.3 min) and **8** (5 mg, t_R = 47 min) with 50% $CH_3CN–H_2O$. The main component of fraction PS_1 (50.3 g) was compound **14** (50 g, t_R = 19 min), which was separated partly by HPLC with $CH_3CN–H_2O$ (75:25) to identify its structure.

*4.4.1. Illihenin B (**1**)*

Colorless crystals; mp 128-130 ° C; $[\alpha]^{25}_D$ +39.7 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.2) nm; IR ν_{max} 3420, 2956, 2876, 1666, 1473, 1385, 1212, 1160, 1107, 1074, 1049, 1026, 1012, 958, 929, 902, 849 cm^{-1}; The ^1H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* $[M + Na]^+$ 293.1720 (calcd for $C_{15}H_{26}O_4Na$, 293.1723).

*4.4.2. Illihenin C (**2**)*

Colorless crystals; mp 109-110 ° C; $[\alpha]^{25}_D$ –5.4 (*c* 0.38, MeOH); UV (MeOH) λ_{max} (log ε) 204 (1.0) nm; R ν_{max} 3381, 2972, 2961, 2934, 2872, 2853, 2360, 2342, 1769, 1471, 1461, 1340, 1297, 1256, 1245, 1170, 1138, 1119, 1105, 1082, 1076, 1020, 997, 971, 944, 899, 877, 731 cm^{-1}; The ^1H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* $[M + Na]^+$ 249.1095 (calcd for $C_{12}H_{18}O_4Na$, 249.1097).

*4.4.3. Illihenin D (**3**)*

colorless oil; $[\alpha]^{25}_D$ -38.8 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.3), 225 (0.2) nm; IR ν_{max} 2959, 2873, 1754, 1735, 1457, 1382, 1372, 1273, 1254, 1232, 1202, 1153, 1115, 1019 cm^{-1}; The ^1H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* $[M + H]^+$ 295.1901 (calcd for $C_{17}H_{27}O_4$, 295.1904).

*4.4.4. Illihenin E (**4**)*

Colorless crystals; mp 91-93 °C; $[\alpha]^{25}_D$ +63.7 (*c* 0.29, MeOH); UV (MeOH) λ_{max} (log ε) 234 (0.5) nm; IR ν_{max} 3374, 2996, 2957, 2934, 2873, 1685, 1620, 1473, 1446, 1366, 1272, 1255, 1207, 1183, 1114, 993 cm^{-1}; The ^1H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* $[M + H]^+$ 211.1690 (calcd for $C_{13}H_{23}O_2$, 211.1692).

*4.4.5. Illihenin F (**5**)*

Colorless crystals; mp 116-117 °C; $[\alpha]^{25}_D$ -50.7 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 204 (0.35), 300 (0.05) nm; IR ν_{max} 3430, 2977, 2955, 2936, 2873, 1746, 1713, 1475, 1454, 1377, 1278, 1235, 1209, 1188, 1151, 1140, 1100, 1073, 1031, 979, 914 cm^{-1}; The ^1H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* $[M + H]^+$ 251.1644 (calcd for $C_{15}H_{23}O_3$, 251.1642).

*4.4.6. Illihenin G (**6**)*:

Colorless crystals; mp 124-126 °C; $[\alpha]^{25}_D$ +59.7 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.2) nm; IR ν_{max} 3312, 2952, 2871, 1662, 1467, 1422, 1386, 1367, 1324, 1262, 1226, 1171, 1111, 1061, 1040, 994, 912 cm^{-1}; The ^1H and ^{13}C NMR data, see Tables 3 and 4; (+)-HRESIMS *m/z* $[M + Na]^+$ 261.1818 (calcd for $C_{15}H_{26}O_2Na$, 261.1825).

*4.4.7. 7-O-Acetyllillihenin G (**7**)*

Colorless crystals; mp 103-105 °C; $[\alpha]^{25}_D$ +43.7 (*c* 0.48, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.6), 225 (0.15) nm; IR ν_{max} 3458, 2958, 2874, 1740, 1723, 1662, 1471, 1427, 1372, 1240, 1059, 1019, 973, 918

cm^{-1}; The ^{1}H and ^{13}C NMR data, see Tables 3 and 4; (+)-HRESIMS *m/z* $[M + Na]^+$ 303.1926 (calcd for $C_{17}H_{28}O_3Na$, 303.1931).

*4.4.8. (1S,4S,5S,10S)-10-Hydroxyacorenone B (**8**)*

Colorless oil; $[\alpha]^{25}_D$ +59.2 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.5), 238 (0.3) nm; IR ν_{max} 3468, 2954, 2871, 1662, 1461, 1452, 1415, 1378, 1365, 1101, 1058, 1029, 983, 922 cm^{-1}; The ^{1}H and ^{13}C NMR data, see Tables 3 and 4; (+)-HRESIMS *m/z* $[M + H]^+$ 237.1845 (calcd for $C_{15}H_{25}O_2$, 237.1849).

*4.4.9. (1S,4S,5R,6S,10R)-6,10-Dihydroxyacorenone B (**9**)*

Colorless crystals; mp 100-102 °C; $[\alpha]^{25}_D$ -109.7 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 205 (0.9), 243(0.4) nm; IR ν_{max} 3456, 2955, 2926, 2871, 1675, 1463, 1449, 1380, 1362, 1245,1206, 1137, 1108, 1066, 1027, 955, 922 cm^{-1}; The ^{1}H and ^{13}C NMR data, see Tables 3 and 4; (+)-HRESIMS *m/z* $[M + Na]^+$ 275.1611 (calcd for $C_{15}H_{24}O_3Na$, 275.1618).

*4.4.10. Illihenin H (**10**)*

Colorless crystals; mp 95-97 °C; $[\alpha]^{25}_D$ -24.4 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.2) nm; IR ν_{max} 3271, 3015, 2955, 2871, 1457, 1379, 1159, 1141, 1076, 1065, 1040, 979, 970, 923, 761, 734 cm^{-1}; The ^{1}H and ^{13}C NMR data, see Tables 5 and 4; (+)-HRESIMS *m/z* $[M + Na]^+$ 261.1826 (calcd for $C_{15}H_{26}O_2Na$, 261.1825).

*4.4.11. Illihenin I (**11**)*

White amorphous powder; $[\alpha]^{25}_D$ +21.8 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.2) nm; IR ν_{max} 3383, 2952, 2870, 1460, 1375, 1369, 1120, 1083, 1028, 994 cm^{-1}; The ^{1}H and ^{13}C NMR data, see Tables 5 and 4; (+)-HRESIMS *m/z* $[M + Na]^+$ 261.1826 (calcd for $C_{15}H_{26}O_2Na$, 261.1825).

*4.4.12. Illihenin J (**12**)*

Colorless oil; $[\alpha]^{25}_D$ -15.6 (*c* 0.43, MeOH); UV (MeOH) λ_{max} (log ε) 202 (0.3) nm; IR ν_{max} 3391, 3017, 2954, 2931, 2870, 1462, 1386, 1377, 1367, 1126, 1108, 1077, 1059, 1042, 983, 931, 782, 749 cm^{-1}; The ^{1}H and ^{13}C NMR data, see Tables 5 and 4; (+)-HRESIMS *m/z* $[M + Na]^+$ 261.1822 (calcd for $C_{15}H_{26}O_2Na$, 261.1825).

*4.4.13. 7-O-Acetylillihenin J (**13**)*

Colorless oil; $[\alpha]^{25}_D$ +39.8 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.2) nm; IR ν_{max} 3453, 2954, 2871, 1739, 1719, 1463, 1377, 1367, 1243, 1133, 1078, 1031, 982 cm^{-1}; The ^{1}H and ^{13}C NMR data, see Tables 5 and 4; (+)-HRESIMS *m/z* $[M + Na]^+$ 303.1924 (calcd for $C_{17}H_{28}O_3Na$, 303.1931).

*4.5. Synthesis of dimeric compound **14***

A solution of **14** (500 mg, 2.27 mmol) in THF (5 ml) was added to a solution of SmI_2 (4.0 g, 10 mmol) in THF (30 mL) and MeOH (7.5 ml) at room temperature over 2 h. Then brine (3 ml) and citric acid (128 mg, 0.61 mmol) were added. The aqueous layer was extracted with CH_2Cl_2 (5 × 10 ml), and the combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. The crude reaction mixture was purified by column chromatography with PE/EtOAc elution (20:1) to afford diacorone A (**15**, 207 mg, 0.468 mmol, 41%) and diacorone B (**16**, 187 mg, 0.423 mmol, 37%) as colorless crystals. The ^{1}H and ^{13}C NMR data of the products were assigned by ^{1}H-^{1}H COSY, HSQC, and HMBC spectra (Figs. S136-137 and Fig. S139-141, Supporting Information).

*4.5.1. Diacorone A (**15**).* Colorless crystals; mp 95-97°C; IR ν_{max} 3725, 3702, 3624, 3601, 2954, 2871, 1709, 1462, 1379, 1222 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ_H 2.62 (2H, d, *J* = 13.7 Hz, H-6a/6a′), 2.29 (2H, dq, *J* = 12.8, 6.5 Hz, H-8/8′), 2.12 (2H, dd, *J* = 13.7, 1.7 Hz, H-6b/6b′), 1.93 (2H, td, *J* = 11.5, 3.5 Hz, H-9/9′), 1.77 (2H, m, H-3a/3a′), 1.76 (2H, m, H-2a/2a′), 1.71 (2H, m, H-11/11′), 1.70 (2H, m, H-4/4′), 1.65 (2H, dd, *J* = 13.7, 13.7 Hz, H-10a/10a′), 1.45 (2H, ddd, *J* = 13.7, 3.5, 1.5 Hz, H-10b/10b′), 1.36 (2H, ddd, *J* = 16.0, 11.5, 6.0 Hz, H-2b/2b′), 1.28 (2H, m, H-1/1′), 1.25 (2H, m, H-3b/3b′), 1.04 (6H, d, *J* = 6.5 Hz, H_3-15/15′), 1.03 (6H, d, *J* = 6.5 Hz, H_3-12/12′), 0.93 (6H, d, *J* = 6.5 Hz, H_3-13/13′), 0.88 (6H, d, *J* = 7.0 Hz, H_3-14/14′). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 214.4 (C-7/7′), 57.8 (C-1/1′), 52.8 (C-6/6′), 48.8 (C-5/5′), 46.3 (C-8/8′), 46.1 (C-4/4′), 41.7 (C-9/9′), 30.9 (C-3/3′), 29.2 (C-11/11′), 25.5 (C-2/2′), 24.2 (C-12/12′), 22.5 (C-10/10′), 22.1 (C-13/13′), 16.8 (C-14/14′), 12.4 (C-15/15′); (+)-HRESIMS *m/z* $[M + H]^+$ 443.3878 (calcd for $C_{30}H_{51}O_2$, 443.3884).

*4.5.2. Diacorone B (**16**).* Colorless crystals; mp 86-88°C; IR ν_{max} 3725, 3703, 3634, 3604, 2954, 2873, 1709, 1457, 1379, 1224 cm^{-1}; ^1H (500 MHz, $CDCl_3$): δ_H 2.46 (1H, d, *J* = 14.0 Hz, H-6a′), 2.45 (1H, d, *J* = 14.0 Hz, H-6a), 2.39 (1H, dq, *J* = 13.0, 6.5 Hz, H-8), 2.27 (1H, dq, *J* = 13.0, 6.5 Hz, H-8′), 2.20 (1H, d, *J* = 14.0 Hz, H-6b), 2.17 (1H, d, *J* = 14.0 Hz, H-6b′), 1.92 (1H, td, *J* = 12.0, 5.0 Hz, H-9′), 1.83 (1H, ddd, *J* = 13.0, 13.0, 3.0 Hz, H-9), 1.79 (1H, m, H-3a′), 1.76 (1H, m, H-2a′), 1.76 (1H, m, H-3a), 1.74 (1H, m, H-4), 1.74 (1H, m, H-11), 1.73 (1H, m, H-11′), 1.70 (1H, m, H-2a), 1.56 (2H, m, H_2-10), 1.56 (1H, m, H-4′), 1.45 (2H, m, H_2-10′), 1.41 (1H, m, H-2b′), 1.40 (1H, m, H-1), 1.35 (1H, m, H-2b), 1.30 (1H, m, H-1′), 1.28 (1H, m, H-3b), 1.16 (3H, d, *J* = 6.5 Hz, H_3-15′), 1.10 (3H, d, *J* = 6.5 Hz, H_3-15), 1.00 (3H, d, *J* = 6.5 Hz, H_3-12), 0.95 (3H, d, *J* = 6.5 Hz, H_3-12′), 0.90 (3H, d, *J* = 6.5 Hz, H_3-13), 0.84 (3H, d, *J* = 6.5 Hz, H_3-13′), 0.87 (6H, d, *J* = 6.5 Hz, H_3-14/14′).^{13}C NMR (125 MHz, $CDCl_3$): δ_C 215.3 (C-7′), 213.7 (C-7), 57.8 (C-1), 57.2 (C-1′), 53.8 (C-6), 50.5 (C-6′), 48.6 (C-5), 47.8 (C-8),47.6 (C-8′), 47.5 (C-4′), 47.1 (C-5′), 43.8 (C-9), 43.7 (C-4), 42.3 (C-9′), 31.0 (C-3), 30.0 (C-10), 29.5 (C-3′), 28.6 (C-11), 28.3 (C-11′), 26.8 (C-10′), 25.7 (C-2), 24.9 (C-12′), 24.7 (C-12), 24.5 (C-2′), 21.7 (C-13), 21.5 (C-13′), 16.6 (C-14), 14.5 (C-14), 14.3 (C-15′), 13.2 (C-15); (+)-HRESIMS *m/z* $[M + H]^+$ 443.3877 (calcd for $C_{30}H_{51}O_2$, 443.3884).

*4.6. Chemical transformation of **14** to **8***

A Cu–Al Ox catalyst (168 mg, 84 mg/mmol substrate) was added to a solution of absolute EtOH (20 mL), and the resultant suspension was stirred at room temperature for 10 min under dry air. To this solution was added **14** (440 mg, 2 mmol) and *t*-BuOK (112 mg, 1 mmol) at room temperature, and the mixture was stirred under dry air for 36 h. A work up was performed by filtering the reaction mixture through a celite pad, and the celite pad was rinsed with MeOH (15 mL). The filtrate was concentrated under a vacuum, and the residue was purified by performing flash column chromatography on silica gel with PE/EtOAc (10:1) to produce alcohol **17** and **18** (172 g, 36% yield) as a yellow oil, in which 80 mg of **14** was recovered.

*4.6.1. (1S,4S,5R,10S)-1-Isopropyl-4,8,10-trimethylspiro[4.5]dec-8-en-7-one (**17**).* The ^1H and ^{13}C NMR data of **17** (Figs S146-147, Supporting Information) were the same as those of compound **8** (Tables 3 and 4); (+)-HRESIMS *m/z* $[M + H]^+$ 237.1845 (calcd for $C_{15}H_{25}O_2$, 237.1849); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 240 (+20.0) nm.

*4.6.2. (1S,4S,5R,10R)-1-Isopropyl-4,8,10-trimethylspiro[4.5]dec-8-en-7-one (**18**).* ^1H NMR (500 MHz,

$CDCl_3$): δ_H 6.68 (1H, brs, H-9), 4.65 (1H, t, *J* = 2.4 Hz, H-10), 2.46 (1H, d, *J* = 16.9 Hz, H-6a), 2.27 (1H, d, *J* = 16.9 Hz, H-6b), 1.90 (1H, m, H-2a), 1.86 (1H, ddd, *J* = 13.0, 6.6, 3.5 Hz, H-3a), 1.77 (3H, brs, H_3-15), 1.69 (1H, t, *J* = 7.8 Hz, H-4), 1.64 (1H, m, H-2b), 1.63 (1H, m, H-11), 1.62 (1H, m, H-3b), 1.42 (1H, m, H-1), 0.99 (3H, d, *J* = 6.9 Hz, H_3-14), 0.89 (3H, d, *J* = 6.9 Hz, H_3-12), 0.88 (3H, d, *J* = 6.9 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 200.1 (C-7), 148.0 (C-9), 134.6 (C-8), 70.0 (C-10), 57.7 (C-1), 54.0 (C-5), 48.9 (C-6), 45.3 (C-4), 31.2 (C-3), 27.4 (C-11), 25.3 (C-3), 24.0 (C-12), 18.3 (C-13), 16.0 (C-14), 15.1 (C-15); (+)-HRESIMS *m/z* $[M + Na]^+$ 237.1845 (calcd for $C_{15}H_{25}O_2$, 237.1849); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 240 (-40.0) nm.

*4.7. Chemical transformation of **14** to 1,2-diols **10-12***

*4.7.1. General procedure for synthesis of (1S,4S,5R)-1-isopropyl-4,8-dimethylspiro[4.5]dec-8-en-7-ol (**19**).*

Sodium borohydride (400 mg, 11.0 mmol) was added to a solution of **14** (2.2 g, 10.0 mmol) and cerium chloride heptahydrate (2.7 g, 11.0 mmol) in MeOH (25 ml) at room temperature. After stirring for 50 min, the reaction was quenched with saturated ammonium chloride. The mixture was extracted with petroleum ether three times, and the organic phase was dried over sodium sulfate, filtered and concentrated. The residue was purified on a silica gel column using PE/EtOAc (15:1) as eluent and furnished 17 (1.92 g, 8.6 mmol, 86%) as a colorless oil, which was separated by HPLC (60%MeCN) to afford a pair of (1*S*,4*S*,5*R*)-1-isopropyl-4,8-dimethylspiro[4.5]dec-8-en-7-ol (**19**) epimers, **19a** (t_R = 25 min) and **19b** (t_R = 27 min).

*4.7.1.1. **19a**.* ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.50 (1H, s, H-9), 4.27 (1H, t, *J* = 6.5 Hz, H-7), 1.85 (1H, br d, *J* = 18.3 Hz, H-10a), 1.83 (1H, dd, *J* = 13.0, 6.0 Hz, H-6a), 1.82 (1H, br d, *J* = 18.3 Hz, H-10b), 1.75 (3H, br s, H_3-15), 1.74 (1H, m, H-3a), 1.65 (1H, p, *J* = 7.0 Hz, H-11), 1.58 (1H, dd, *J* = 13.0, 8.0 Hz, H-6b), 1.57 (1H, m, H-2a), 1.51 (1H, m, H-4), 1.48 (1H, m, H-2b), 1.47 (1H, m, H-1), 1.17 (1H, dtd, *J* = 12.5, 9.0 Hz, H-3b), 0.87 (3H, d, *J* = 7.0 Hz, H_3-12), 0.85 (3H, d, *J* = 7.0 Hz, H_3-13), 0.79 (3H, d, *J* = 6.5 Hz, H_3-14). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 134.7 (C-8), 125.2 (C-9), 69.3 (C-7), 56.2 (C-1), 45.8 (C-5), 45.6 (C-4), 45.2 (C-6), 30.9 (C-3), 28.2 (C-11), 25.7 (C-2), 25.5 (C-10), 22.7 (C-12), 19.4 (C-13), 19.1 (C-15), 15.7 (C-14); (+)-HRESIMS *m/z* $[M + Na]^+$ 245.1878 (calcd for $C_{15}H_{26}ONa$, 245.1876).

*4.7.1.2. **19b**.* ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.46 (1H, br d, H-9), 4.23 (1H, t, *J* = 7.2 Hz, H-7), 1.99 (1H, dq, *J* = 18.0, 3.0 Hz, H-10a), 1.87 (1H, dd, *J* = 12.5, 10.0 Hz, H-6a), 1.73 (3H, brs, H_3-15), 1.72 (1H, m, H-2a), 1.72 (1H, m, H-10b), 1.70 (1H, m, H-3a), 1.65 (1H, m, H-4), 1.65 (1H, m, H-6b), 1.65 (1H, m, H-11), 1.28 (1H, m, H-1), 1.27 (1H, m, H-2b), 1.17 (1H, m, H-3b), 0.96 (3H, d, *J* = 6.7 Hz, H_3-12), 0.85 (3H, d, *J* = 6.7 Hz, H_3-13), 0.80 (3H, d, *J* = 6.2 Hz, H_3-14). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 135.2 (C-8), 125.1 (C-9), 69.6 (C-7), 58.0 (C-1), 46.8 (C-5), 46.0 (C-4), 44.7 (C-6), 31.7 (C-3), 28.4 (C-11), 26.0 (C-2), 25.3 (C-10), 24.7 (C-12), 22.1 (C-13), 19.0 (C-15), 18.6 (C-14); (+)-HRESIMS *m/z* $[M + Na]^+$ 245.1878 (calcd for $C_{15}H_{26}ONa$, 245.1876).

*4.7.2. General procedure for synthesis of (2'S,4S,5'S)-1,2'-dimethyl-5'-isopropylspiro[7-oxabicyclo[4.1.0] heptane-4,1'-cyclopentane]-2-ol (**20**).*

$NaHCO_3$ (1.6 g, 19 mmol) and *m*-CPBA (2.23 g, 13 mmol) were added at 0 °C to a solution of **19** (1.92 g, 8.6 mmol) in CH_2Cl_2 (25 mL). After 1 h at 0 °C, an aqueous solution of sodium thiosulfate (1.0 M, 25 mL)

was added and then extracted with CH_2Cl_2 (20 mL) 3 times. The organic phase was washed with saturated $NaHCO_3$ solution (15 mL) and brine (15 mL), dried over sodium sulfate and evaporated to produce **20** as a white solid (1.62 g, 6.8 mmol, 90%); R_f = 0.4 (PE/EtOAc 5:1). Compound **20** was separated by HPLC to afford **20a** and a mixture of two isomers (**20b**) (Figs. S164-165, Supporting Information).

20a: ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 4.10 (1H, dd, *J* = 5.2, 2.5 Hz, H-7), 3.10 (1H, d, *J* = 5.2 Hz, H-9), 2.18 (1H, dd, *J* = 14.8, 5.2 Hz, H-10a), 1.82 (1H, m, H-2a), 1.82 (1H, m, H-3a), 1.73 (1H, ddd, *J* = 15.6, 5.2, 1.8 Hz, H-6a), 1.66 (1H, d, *J* = 15.6 Hz, H-6b), 1.65 (1H, m, H-11), 1.62 (1H, m, H-4), 1.42 (3H, s, H_3-15), 1.29 (1H, m, H-2b), 1.28 (1H, m, H-1), 1.22 (1H, m, H-3b), 1.16 (1H, dt, *J* = 14.8, 2.3 Hz, H-10b), 1.06 (3H, d, *J* = 6.0 Hz, H_3-14), 0.94 (3H, d, *J* = 6.8 Hz, H_3-12), 0.87 (3H, d, *J* = 6.7 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 69.4 (C-7), 60.8 (C-9), 59.5 (C-8), 58.1 (C-1), 44.0 (C-4), 43.4 (C-5), 40.9 (C-6), 31.9 (C-3), 27.6 (C-11), 24.9 (C-2), 24.7 (C-10), 24.7 (C-12), 21.6 (C-13), 19.1 (C-15), 17.5 (C-14); (+)-HRESIMS *m/z* $[M + Na]^+$ 261.1822 (calcd for $C_{15}H_{26}O_2Na$, 261.1825).

*4.7.3. General procedure for synthesis of (1S,4S,5R)-8-hydroxy-1-isopropyl-4,8-dimethylspiro[4.5]dec-9-en-7-one (**22**).*

A solution of **20** (1.62 g, 6.8 mmol) in dry CH_2Cl_2 (20 mL) was added at room temperature to a solution of Dess-Martin periodinane (3.6 g, 8.5 mmol) in dry CH_2Cl_2 (10 mL), and the mixture was stirred for 2 h. After being concentrated, the residue was purified by chromatography on silica gel (eluent: PE/EtOAc 10:1) to afford **21** as a white solid (1.3 mg, 5.48 mmol, 80.6%); R_f = 0.6 (PE/EtOAc 10:1). A solution of $TiCl_4$ (6.0 mL, 1 M in CH_2Cl_2, 6.0 mmol) was added to a solution of epoxide **21** (1.3 g, 5.48 mmol) in CH_2Cl_2 (30 mL), and the reaction mixture was stirred for 2 h at room temperature. The reaction was quenched with water (25 mL) and extracted with CH_2Cl_2 (25 mL) three times. The combined organic extracts were washed with brine (15 mL), dried over sodium sulfate and evaporated. The residue was purified by chromatography on silica gel eluting with PE/EtOAc (8:1) to produce a pivotal allyl alcohol **22** in a mixture of a pair of epimers, along with some chlorohydrin coproduct. One of the epimers (**22a**) was purified by TLC (eluent: PE/acetone 5:1), while the other one (**22b**) was completely mixed with impurities that could not be separated by silica gel column chromatography. Finally, epimer **22b** was isolated by semipreparative HPLC. The structures of **22a** and **22b** were determined by the ^{1}H, ^{13}C NMR, and 1D NOE spectroscopic data (Figs. S167-174, Supporting Information) together with the calculated and experimental ECD data (Fig. 10).

*4.7.3.1. (1S,4S,5R,8R)-8-hydroxy-1-isopropyl-4,8-dimethylspiro[4.5]dec-9-en-7-one (**22a**).* ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.85 (1H, d, *J* = 10.2 Hz, H-9), 5.51 (1H, dd, *J* = 10.2, 1.7 Hz, H-10), 3.21 (1H, d, *J* = 14.0 Hz, H-6a), 2.18 (1H, dd, *J* = 14.0, 1.8 Hz, H-6b), 1.85 (1H, ddd, *J* = 12.5, 9.0, 8.1 Hz, H-2a), 1.77 (1H, m, H-3a), 1.77 (1H, m, H-4), 1.76 (1H, m, H-4), 1.52 (1H, ddd, *J* = 12.5, 11.0, 3.0 Hz, H-2b), 1.47 (1H, m, H-1), 1.44 (3H, brs, H_3-15), 1.19 (1H, ddd, *J* = 11.2, 6.5, 2.7 Hz, H-3b), 1.01 (3H, d, *J* = 6.7 Hz, H_3-12), 0.93 (3H, d, *J* = 6.7 Hz, H_3-13), 0.69 (3H, dd, *J* = 6.0 Hz, H_3-14) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 213.7 (C-7), 132.7 (C-9), 128.7 (C-10), 74.0 (C-8), 56.4 (C-1), 56.2 (C-5), 47.7 (C-7), 47.4 (C-6), 30.7 (C-3), 29.9 (C-11), 28.9 (C-2), 25.3 (C-12), 23.5 (C-15), 21.4 (C-13), 15.5 (C-14); (+)-HRESIMS *m/z* $[M + Na]^+$ 237.1848 (calcd for $C_{15}H_{25}O_2$, 237.1849); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 201 (-3.94), 222 (+0.70), 289 (+1.06) nm.

*4.7.3.2. (1S,4S,5R,8S)-8-hydroxy-1-isopropyl-4,8-dimethylspiro[4.5]dec-9-en-7-one (**22b**).* ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.83 (1H, d, J = 10.3 Hz, H-9), 5.46 (1H, dd, J = 10.3, 1.5 Hz, H-10), 2.84 (1H, dd, J = 15.1, 2.0 Hz, H-6a), 2.34 (1H, dd, J = 15.1, 1.6 Hz, H-6b), 1.86 (1H, dq, J = 12.5, 11.2, 4.1 Hz, H-3a), 1.80 (1H, ddt, J = 13.0, 4.5, 3.0 Hz, H-2a), 1.72 (1H, dt, J = 10.5, 7.0 Hz, H-4), 1.55 (1H, m, H-11), 1.49 (1H, m, H-1), 1.49 (1H, m, H-2b), 1.43 (3H, s, H_3-15), 1.35 (1H, dtd, J = 12.5, 11.0, 5.1 Hz, H-3b), 0.92 (3H, d, J = 6.9 Hz, H_3-14), 0.79 (3H, d, J = 6.7 Hz, H_3-12), 0.77 (3H, d, J = 6.7 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 213.4 (C-9), 132.2 (C-9), 128.6 (C-10), 73.5 (C-8), 59.1 (C-1), 56.2 (C-5), 46.0 (C-6), 45.5 (C-4), 29.4 (C-3), 29.2 (C-11), 28.9 (C-2), 24.8 (C-12), 23.9 (C-15), 20.4 (C-13), 14.5 (C-14); (+)-HRESIMS *m/z* $[M + Na]^+$ 237.1848 (calcd for $C_{15}H_{25}O_2$, 237.1849); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 198 (+6.11), 221 (-1.05), 289 (-0.97) nm.

4.7.4. General procedure for synthesis of 1,2-diol epimers **22a1**, **22a2**, **22b1**, *and* **22b2**.

Sodium borohydride (15 mg, 0.4 mmol) was added to a solution of **22a** (66 mg, 0.3 mmol) in MeOH (2 ml) at room temperature. After stirring for 30 min, the reaction was quenched with water (1 mL). The mixture was concentrated and purified with TLC using PE/EtOAc (4:1) as the solvent to furnish **22a1** (32 mg, 0.134 mmol, 44.8%) and **22a2** (21 mg, 0.088 mmol, 30.1%) as colorless oils; R_f =0.35 (**22a1**) and 0.25 (**22a2**) (PE/EtOAc 5:1). **22b1** and **22b2** were obtained using **22b** (66 mg, 0.3 mmol) with the same procedure. Purification by TLC using PE/EtOAc (4:1) as the solvent produced **22b1** (39 mg, 0.164 mmol, 54.6%) and **22b2** (16 mg, 0.067 mmol, 22.4%) as colorless oils; R_f =0.25 (**22b1**) and 0.35 (**22b2**) (PE: EA 5:1).

*4.7.4.1. (1S,4S,5R,7S,8R)-1-Isopropyl-4,8-dimethylspiro[4.5]dec-9-ene-7,8-diol (**22a1**).* The ^{1}H and ^{13}C NMR data of **22a1** were the same as those of compound **10** (Tables 5 and 4, Figs. S175-178, Supporting Information); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 190 (-8.5) nm.

*4.7.4.2. (1S,4S,5R,7R,8R)-1-Isopropyl-4,8-dimethylspiro[4.5]dec-9-ene-7,8-diol (**22a2**).* The ^{1}H and ^{13}C NMR data of **22a2** were the same as those of compound **11** (Tables 5 and 4, Figs. S181-184, Supporting Information); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 190 (-8.5) nm.

*4.7.4.3. (1S,4S,5R,7S,8S)-1-Isopropyl-4,8-dimethylspiro[4.5]dec-9-ene-7,8-diol (**22b1**).* The ^{1}H and ^{13}C NMR data of **22b1** were the same as those of compound **12** (Tables 5 and 4, Figs. S187-190, Supporting Information); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 198 (+28.5) nm.

*4.7.4.4. (1S,4S,5R,7R,8S)-1-Isopropyl-4,8-dimethylspiro[4.5]dec-9-ene-7,8-diol (**22b2**).* ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.71 (1H, d, J = 10.1 Hz, H-9), 5.56 (1H, dd, J = 10.2, 1.4 Hz, H-10), 3.71 (1H, dd, J = 12.5, 4.5 Hz, H-7), 1.95 (1H, dd, J = 12.8, 12.8 Hz, H-6a), 1.88 (m, 1H, H-4), 1.77 (1H, dd, J = 11.5, 8.8 Hz, H-3a), 1.76 (1H, m, H-6b), 1.64 (1H, dt, J = 13.2, 6.6 Hz, H-11), 1.52 (1H, ddd, J = 13.0, 4.5, 1.5 Hz, H-2a), 1.47 (1H, m, H-1), 1.43 (1H, m, H-2b), 1.34 (3H, s, H_3-15), 1.25 (1H, m, H-3b), 0.93 (3H, d, J = 6.8 Hz, H_3-12), 0.84 (3H, d, J = 6.8 Hz, H_3-14), 0.83 (3H, d, J = 6.8 Hz, H_3-13); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 133.3 (C-9), 131.6 (C-10), 71.6 (C-7), 68.9 (C-8), 57.2 (C-1), 50.8 (C-5), 47.0 (C-4), 40.7 (C-6), 31.6 (C-3), 28.9 (C-11), 26.5 (C-12), 25.4 (C-2), 23.9 (C-15), 21.3 (C-13), 18.0 (C-14) (Figs. S193-194, Supporting Information); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 190 (+33.5) nm.

4.8. X-ray crystallographic data

Crystallographic data for the structures of compounds **1**, **2**, **4**−**7**, **9**, **10**, **15** and **16** were deposited in the Cambridge Crystallographic Data Centre Database (deposition numbers CCDC 2161192, 2161188, 2161184, 2161193, 2161183, 2161189, 2161187, 2161190, 2161196, and 2161194). Copies of the data can be obtained free of charge from CCDC, 12 Union Road, Cambridge, CB2 1EZ, U.K. (fax: +44 1223 336033; e-mail: deposit@cced.cam.ac.uk)

*4.8.1. Crystal data for illihenin B (**1**).* $C_{15}H_{26}O_4$ (M =270.36 g/mol): orthorhombic, space group $P2_12_12_1$ (no. 19), a = 9.89990(10) Å, b = 10.16240(10) Å, c = 29.6426(3) Å, V = 2982.25(5) Å^3, Z = 8, T = 100.00(10) K, μ (Cu Kα) = 0.693 mm^{-1}, *Dcalc* = 1.204 g/cm^3, 19075 reflections measured (9.2 ° ≤ 2Θ ≤ 150.408 °), and 5993 unique reflections (R_{int} = 0.0264, R_{sigma} = 0.0237), which were used in all calculations. The final R_1 was 0.0313 (I > 2σ(I)), and wR_2 was 0.0798 (all data). Flack parameter: -0.11(5), Hooft parameter: -0.07(14).

*4.8.2. Crystal data for illihenin C (**2**).* $C_{12}H_{18}O_4$ (M =226.26 g/mol): monoclinic, space group $P2_1$ (no. 4), a = 6.28860(10) Å, b = 6.31210(10) Å, c = 14.5166(3) Å, β = 92.988(2) °, V = 575.442(18) Å^3, Z = 2, T = 100.00(10) K, μ (CuKα) = 0.802 mm^{-1}, *Dcalc* = 1.306 g/cm^3, 5854 reflections measured (12.21 ° ≤ 2Θ ≤ 146.944 °), and 2235 unique reflections (R_{int} = 0.0203, R_{sigma} = 0.0213), which were used in all calculations. The final R_1 was 0.0252 (I > 2σ(I)), and wR_2 was 0.0656 (all data). Flack parameter: 0.01(7).

*4.8.3. Crystal data for illihenin E (**4**).* $C_{13}H_{22}O_2$ (M =210.31 g/mol): monoclinic, space group $P2_1$ (no. 4), a = 5.90980(10) Å, b = 7.19200(10) Å, c = 14.6509(2) Å, β = 95.794(2) °, V = 619.530(16) Å^3, Z = 2, T = 100.00(10) K, μ(CuKα) = 0.578 mm^{-1}, *Dcalc* = 1.127 g/cm^3, 8224 reflections measured (12.14° ≤ 2Θ ≤ 147.98°), and 2444 unique reflections (R_{int} = 0.0158, R_{sigma} = 0.0134), which were used in all calculations. The final R_1 was 0.0250 (>2sigma(I)) and wR_2 was 0.0664 (all data). Flack parameter: 0.01(15).

*4.8.4. Crystal data for illihenin F (**5**).* $C_{15}H_{22}O_3$ (M =250.32 g/mol): orthorhombic, space group $P2_12_12_1$ (no. 19), a = 5.92475(7) Å, b = 14.6207(2) Å, c = 15.3503(2) Å, V = 1329.71(3) Å^3, Z = 4, T = 100.01(10) K, μ (Cu Kα) = 0.684 mm^{-1}, *Dcalc* = 1.250 g/cm^3, 9203 reflections measured (8.352° ≤ 2Θ ≤ 148.846°), and 2686 unique reflections (R_{int} = 0.0308, R_{sigma} = 0.0280), which were used in all calculations. The final R_1 was 0.0288 (I > 2σ(I)), and wR_2 was 0.0753 (all data). Flack parameter: 0.00(8).

*4.8.5. Crystal data for (7S,9S)-dihydroxyillihenin H (**6**).* $C_{15}H_{26}O_2$ (M =238.36 g/mol): orthorhombic, space group $P2_12_12_1$ (no. 19), a = 6.42920(10) Å, b = 12.6136(2) Å, c = 16.7555(2) Å, V = 1358.79(3) Å^3, Z = 4, T = 100.00(10) K, μ(CuKα) = 0.581 mm^{-1}, *Dcalc* = 1.165 g/cm^3, 10145 reflections measured (8.78 ° ≤ 2Θ ≤ 147.44 °), and 2674 unique reflections (R_{int} = 0.0286, R_{sigma} = 0.0241) which were used in all calculations. The final R_1 was 0.0301 (>2sigma(I)) and wR_2 was 0.0729 (all data). Flack parameter: 0.08(17).

*4.8.6. Crystal data for (7S,9S)-7-acetoxy-9-hydroxyillihenin H (**7**).* $C_{17}H_{28}O_3$ (M =280.39 g/mol): triclinic, space group P1 (no. 1), a = 8.0977(2) Å, b = 9.3044(2) Å, c = 10.3067(3) Å, α = 89.315(2) °, β = 89.207(2) °, γ = 89.802(2) °, V = 776.42(3) Å^3, Z = 2, T = 100.00(10) K, μ(CuKα) = 0.633 mm^{-1}, *Dcalc* = 1.199 g/cm^3, and 23218 reflections measured (8.58° ≤ 2Θ ≤ 146.88°), 5872 unique (R_{int} = 0.0296, R_{sigma} = 0.0219), which were used in all calculations. The final R_1 was 0.0425 (>2sigma(I)) and wR_2 was 0.1107 (all data). Flack

parameter: 0.00(18).

*4.8.7. Crystal data for (6S,10R)-6,10-dihydroxyacorenone B (**9**).* $C_{15}H_{24}O_3$ (M =252.34 g/mol): monoclinic, space group $P2_1$ (no. 4), a = 9.7313(3) Å, b = 6.9436(2) Å, c = 11.0613(3) Å, β = 115.321(4) °, V = 675.61(3) Å^3, Z = 2, T = 100.00(13) K, μ(CuKα) = 0.674 mm^{-1}, *Dcalc* = 1.240 g/cm^3, and 10874 reflections measured (8.84 ° ≤ 2Θ ≤ 144.78 °), 2503 unique (R_{int} = 0.0484, R_{sigma} = 0.0278), which were used in all calculations. The final R_1 was 0.0402 (>2sigma(I)) and wR_2 was 0.1059 (all data). Flack parameter: 0.0(2)

*4.8.8. Crystal data for (7S,8R)-dihydroxyillihenin G (**10**).* $C_{15}H_{26}O_2$ (M =238.36 g/mol): trigonal, space group $P3_212$ (no. 153), a = 22.5496(3) Å, c = 15.35043(18) Å, V = 6759.71(19) Å^3, Z = 18, T = 100.01(10) K, μ(Cu Kα) = 0.526 mm^{-1}, *Dcalc* = 1.054 g/cm^3, 59359 reflections measured (4.524° ≤ 2Θ ≤ 154.564°), and 9372 unique reflections (R_{int} = 0.0576, R_{sigma} = 0.0323), which were used in all calculations. The final R_1 was 0.0411 (I > 2σ(I)), and wR_2 was 0.1112 (all data). Flack parameter: 0.04(6).

*4.8.9. Crystal data for diacorone A (**15**).* $C_{90}H_{150}O_6$ (M =1328.10 g/mol): monoclinic, space group $P2_1$ (no. 4), a = 11.65590(10) Å, b = 24.2847(3) Å, c = 14.9959(2) Å, β = 102.4380(10) °, V = 4145.11(8) Å^3, Z = 2, T = 100.00(10) K, μ (CuKα) = 0.483 mm^{-1}, *Dcalc* = 1.064 g/cm^3, 58352 reflections measured (7.04 ° ≤ 2Θ ≤ 150.4 °), and 16508 unique reflections (R_{int} = 0.0459, R_{sigma} = 0.0461), which were used in all calculations. The final R_1 was 0.0510 (>2sigma(I)) and wR_2 was 0.1436 (all data). Flack parameter: -0.08(18).

*4.8.10. Crystal data for diacorone B (**16**).* $C_{30}H_{50}O_2$ (M =442.70 g/mol): tetragonal, space group $P4_3$ (no. 78), a = 10.14050(10) Å, c = 26.2081(4) Å, V = 2694.97(6) Å^3, Z = 4, T = 100.00(10) K, μ (CuKα) = 0.495 mm^{-1}, *Dcalc* = 1.091 g/cm^3, 19678 reflections measured (8.72 ° ≤ 2Θ ≤ 147.4 °), and 5316 unique reflections (R_{int} = 0.0274, R_{sigma} = 0.0222), which were used in all calculations. The final R_1 was 0.0409 (>2sigma(I)) and wR_2 was 0.1058 (all data). Flack parameter: 0.0(3).

4.9. Quantum Chemical ECD Calculations

Conformational searches for compounds **3**, **8**, **22a** and **22b** were performed with Spartan 14 software using the molecular mechanics force field (MMFF94). Conformations with Boltzmann distributions over 1.5% were further used for ECD calculations. The dominant conformations were optimized at the ωB97XD/TZVP level in acetonitrile. The energy, oscillator strengths, and rotational strengths of the excitations were calculated using the TDDFT methodology at the Cam-B3LYP/TZVP level in MeOH, and ECD spectra were then simulated by the Gaussian function with a half-band width of 0.45 eV. The final ECD spectra for **3**, **8**, **22a** and **22b** were obtained by averaging the calculated data of the lowest energy conformers for each structure according to their Boltzmann distribution.

4.10. Antiviral Assay

African green monkey kidney (Vero) cells, Madin-Darby canine kidney (MDCK) cells, Coxsackie viruses (Cox B3, Nancy strain), influenza A (A/Hanfang/359/95, H3N2) and HSV-1 were all from the Institute of Virology, Chinese Academy of Preventive Medicine.

4.10.1 Cytotoxicity assay

The cytotoxicity of all tested compounds in the presence of Vero and MDCK cells was monitored by

cytopathic effect (CPE). The Vero and MDCK cells (2.5×10^4/well) were plated into a 96-well plate. After 24 h, the monolayer cells were incubated in the presence of various concentrations of test compounds. After 48 h of culture at 37 °C and 5% CO_2 in a carbon dioxide incubator, the cells were monitored by CPE. The median toxic concentration (TC_{50}) was calculated by Reed and Muench analyses.

4.10.2. Anti coxsackie and HSV-1 activity assays

Confluent Vero cells grown in 96-well microplates were infected with 100 median tissue culture infective doses (100 $TCID_{50}$) of Cox B3 and HSV-1. After 2 h of adsorption at 37 °C, the monolayers were washed with phosphate buffered saline (PBS) and incubated at 37 °C in maintenance medium (MEM plus 2% fetal bovine serum (FBS)) with or without different concentrations of the tested compounds. A viral CPE was observed when the viral control group reached 4+ (4+, about 75-100% cells exhibited CPE; 3+, about 50-75% cells exhibited CPE; 2+, about 25-50% cells exhibited CPE;1+, about 1-25% cells exhibited CPE), and the IC_{50} (the compound concentration required to inhibit 50% of virus-induced CPE) was determined based on the CPE scores by Reed and Muench method [46].

4.10.3. Anti-influenza A activity assay

Confluent MDCK cells grown in 96-well microplates were infected at 100 $TCID_{50}$ with influenza A (A/Hanfang/359/95, H3N2). After 2 h of adsorption at 37 °C, the monolayers were washed with PBS and incubated at 37 °C in maintenance medium with or without different concentrations of test compounds. A viral CPE was observed when the viral control group reached 4+, and the antiviral activity of the tested compounds was determined by Reed and Muench analyses.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi. org/10.1016/j.bioorg.2022.xxxxxx.

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