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Antiviral Spirooliganones A and B with Unprecedented Skeletons from the Roots of *Illicium oligandrum*

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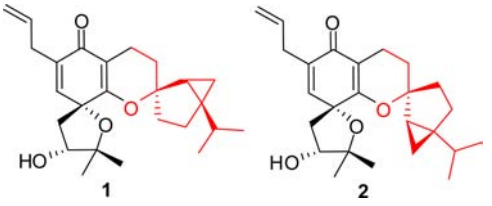
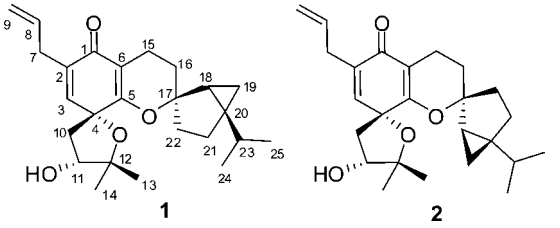
ABSTRACT



Two novel spirooliganones A (1) and (2), a pair of spiro carbon epimers, with a rare dioxaspiro skeleton were isolated from the roots of *Illicium oligandrum*. The structures were fully determined by spectroscopic analysis and chemical methods, especially modified Mosher's method, and X-ray diffraction analysis. Spirooliganone B was found to exhibit more potent activities against coxsackie virus B3 and influenza virus A (H3N2) (IC_{50} 3.70−5.05 μM) than spirooliganone A. The biosynthetic pathway involving a hetero-Diels−Alder reaction of the epimers was proposed.

Plants from the genus *Illicium* have demonstrated a variety of antiviral activities. For example, essential oils from *Illicium verum* show significant activity against the herpes simplex virus type 2 (HSV-2).[1] In view of the potential of this genus to produce new antiviral compounds, we chose to investigate the toxic shrub *I. oligandrum*, which is used in Chinese folk medicine for the treatment of rheumatoid arthritis. Previous investigations of the plant resulted in the isolation of prenylated C_6-C_3 compounds, sesquiterpene lactones, neolignan glycosides, and phenolic diglycosides.[2] However, to this point no antiviral natural products have been discovered from this species.

Our biological and chemical investigations of the lipophilic fraction of the $CHCl_3$ extract from the roots extract have now yielded two new compounds with an unprecedented dispiro skeleton: spirooliganones A (**1**) and B (**2**). They exhibit potent activity against coxsackie virus B3 and influenza virus A/Hanfang/359/95 (H3N2) (IC_{50} 3.70−33.33 μM). Herein, we present the isolation, structure elucidation, and proposed biosynthetic pathways of **1** and **2**, as well as their antiviral activities.



Spirooliganone A (**1**) was obtained as a colorless oil. The molecular formula, $C_{25}H_{34}O_4$, was deduced from HRESIMS and NMR (Table 1) data, indicating a degree

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of unsaturation of nine. Infrared absorption bands at 3394 and 1643 cm^{-1} implied the presence of hydroxyl and carbonyl functionalities, respectively. The ^{13}C NMR and DEPT spectra showed 25 resonances attributable to four methyls, eight methylenes, five methines, and eight quaternary carbons (one α,β-unsaturated carbonyl). Thus, 33 hydrogen atoms could be accounted for; the remaining one was likely from a hydroxyl group.

The ^1H NMR spectrum of **1** displayed two methyl singlets at δ_H 1.25 (H_3-14) and 1.31(H_3-13) and a deshielded singlet at δ_H 6.63 (H-3) suggesting a trisubstituted double bond. $^1H-^1H$ correlation spectroscopy (COSY) and HSQC analysis further revealed six spin systems: **a** (C7−C9), **b** (C10−C11), **c** (C15−C16), **d** (C18−C19), **e** (C21−C22), and **f** (C23−C25) (drawn with thick bonds in Figure 1).

^{13}C NMR resonances at δ_C 185.4, 133.5, 144.7, 167.4, and 110.0 suggested the presence of a cyclohexadienone group containing an oxygenated olefinic carbon at the β-position; this was confirmed by HMBC correlations from H-3 to C-1, C-2, and C-5. Furthermore, the allyl group (fragment **a**) was determined to be located at C-2 by means of HMBC correlations from H_2-7 to C-1 and C-2. The molecular formula, and the presence of four oxygenated quaternary carbons (δ_C 167.4, 77.7, 85.7, and 89.9), implied the presence of two oxygen bridges in **1**. HMBC correlations from H-11 to C-4 (δ_C 77.7) and C-12 (δ_C 85.7) and from H_2-10 to C-12, as well as the fragment **b**, placed the first oxygen bridge between C-4 and C-12. In addition, a pair of geminal methyl groups (CH_3-13 and CH_3-14) was connected to the quaternary carbon C-12, as evidenced by HMBC correlations from H_3-13 to C-11, C-12, and C-14. Thus, it could be deduced that a C_5 prenyl unit (C10−C14) in a tetrahydrofuran ring system was connected to the allylated cyclohexadienone moiety (a C_6-C_3 unit, C1−C9) through an oxaspiro carbon (C-4). This was supported by HMBC correlations from H_2-10 to C-3 (δ_C 144.7) and C-5 (δ_C 167.4).

Table 1. NMR Data for Spirooliganone A (**1**) in Acetone-d_6

	1	
position	δ_C	δ_H (J in Hz)
1	185.4	
2	133.5	
3	144.7	6.63 (s)
4	77.7	
5	167.4	
6	110.0	
7	33.8	2.96 (2H, d, 7.0)
8	137.0	5.84 (ddt, 17.0, 10.5, 7.0)
9a/9b	116.3	4.98 (d, 10.0)/5.04 (d, 17.0)
10a/10b	44.3	2.60 (dd, 12.5, 7.0)/2.03 (m)[a]
11	78.8	4.37 (m)
12	85.7	
13	28.0	1.31 (s)
14	24.0	1.25 (s)
15a/15b	17.6	2.27 (2H, m)
16a/16b	28.6	1.78 (2H, m)[a]
17	89.9	
18	30.3	1.35 (m)[a]
19a/19b	11.4	0.34 (dd, 5.0, 4.0)/0.49 (dd, 8.0, 5.5)
20	35.4	
21a/21b	27.7	1.73 (m)[a]/2.00 (dd like)
22a/22b	34.1	1.39 (m)[a]/1.75 (m)[a]
23	32.7	1.60 (sept, 6.5)
24	20.6	1.01 (d, 6.5)
25	20.1	0.86 (d, 6.5)

[a] Overlapped.

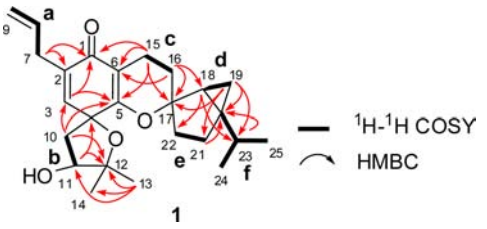

Figure 1. $^1H-^1H$ COSY and key HMBC correlations of **1**.

The remaining 11 carbon resonances, including one oxygenated quaternary carbon (δ_C 89.9), indicated the presence of a monoterpene moiety (C16−C25). Comparison of these ^{13}C NMR data with those of *trans*-sabinene hydrate (previously synthesized from (−)-3-thujol)[3] suggested that **1** possessed a bicyclo[3.1.0]hexane moiety, which was confirmed by the aforementioned two fragments **d** and **e**, as well as HMBC correlations from H-18 to C-22, from H_2-19 to C-17, C-20, C-21, and from H_2-21 to C-20. The isopropyl group (fragment **f**) was assigned to C-20 based upon HMBC cross peaks between H-23 and C-20, as well as between H_2-19/H_2-21 and C-23. Furthermore, HMBC correlations from H_2-15 to C-1, C-5, and C-6, and from H_2-16 to C-17 and C-18, revealed that the bicyclo[3.1.0]hexane moiety was attached to C-6 by the fragment **c**. Analysis of degrees of unsaturation indicated that the bicyclo[3.1.0]hexane moiety was also connected to C-5 through the second oxygen bridge, forming another oxaspiro structure as shown in Figure 1.

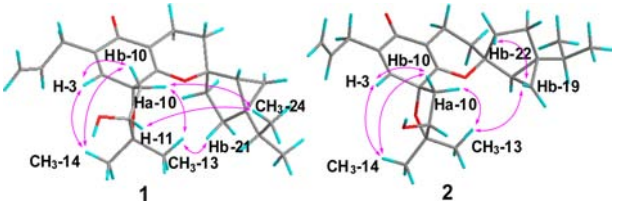

Figure 2. Key NOE correlations of **1** and **2**.

The relative stereochemistry of **1** was deduced from the NOESY spectrum and a series of NOE experiments. The observation of NOESY correlations from H-3 to

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H-10b and H_3-14, and from H-10b to H_3-14, indicated that H-10b and Me-14 were on the same face of the tetrahydrofuran ring. Moreover, a NOESY cross peak was also observed between H-10a and H_3-13, indicating that H-10a and Me-13 were on the same face of the ring, opposite H-10b and Me-14. Strong NOEs were observed for H-10a (δ_H 2.60, dd, J = 12.5, 7.0 Hz) and H_3-13 after irradiation of H-11, but not for H-10b and H_3-14, indicating that 11-OH was on the same face as H-10b and Me-14. Further NOESY correlations from H_3-24 (δ_H 1.01, d) to H-10a and H-11, and from H_3-13 to H-21b (δ_H 2.00), indicated that the tetrahydrofuran and cyclopentane rings were perpendicular to the dihydropyranocyclohexadienone (DHPC) pseudoplane, as depicted in Figure 2. On the other hand, the relative stereochemistry of two spiro skeletons in the molecule was also unambiguously assigned by NOE experiments. Diagnostic NOEs for H_3-24 and H_3-13, but weak ones for H_3-25 and H-23, were observed after irradiation of H-10a (see Figure S11 in SI). This indicated the isopropyl group, rather than the three-membered ring, was close to H-10a. In addition, irradiation of H-11 enhanced the signals for H_3-24 and H-21b (δ_H 2.00), and irradiation of H-23 enhanced H-19b (δ_H 0.49) and H-10a. Collectively, these NOEs suggested that C-10 and C-18 were on the same face of the DHPC pseudoplane.

The absolute configuration at C-11 (*R*) was unambiguously determined by the modified Mosher method (Figure 3).[4] Based upon the assigned relative configuration, the absolute configuration of **1** was established as 4*R*, 11*R*, 17*S*, 18*R*, 20*S*. It is noteworthy that ^{1}H NMR data for MTPA esters of **1** did not provide convincing evidence of the absolute configuration at C-11: both positive and negative $\Delta\delta^{SR}$ values coexisted for the same side of the C-11 stereocenter (see Table S2 in the Supporting Information (SI)). Therefore, the diastereomeric MPA (α-methoxyphenylacetic acid) esters of **1** were prepared, allowing the assignment of the absolute configuration of C-11 as *R* (see Table S3 in SI). This example confirms that MPA esters are more reliable than MTPA esters for assignment of the configuration of chiral carbons in sterically congested spiro-ring environments.[5]

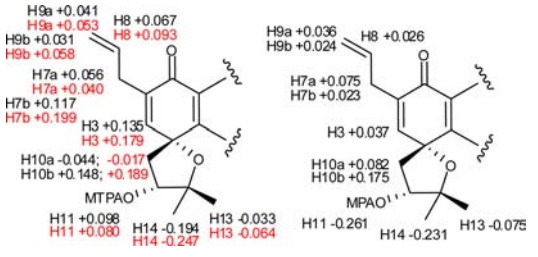

Figure 3. Selected $\Delta\delta^1$H NMR values (normal and red data obtained in pyridine-d_5 and acetone-d_6, respectively) in ppm for MPA (right) and MTPA (left) esters of **1**.

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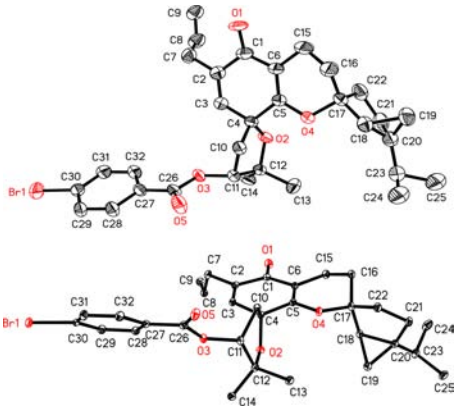
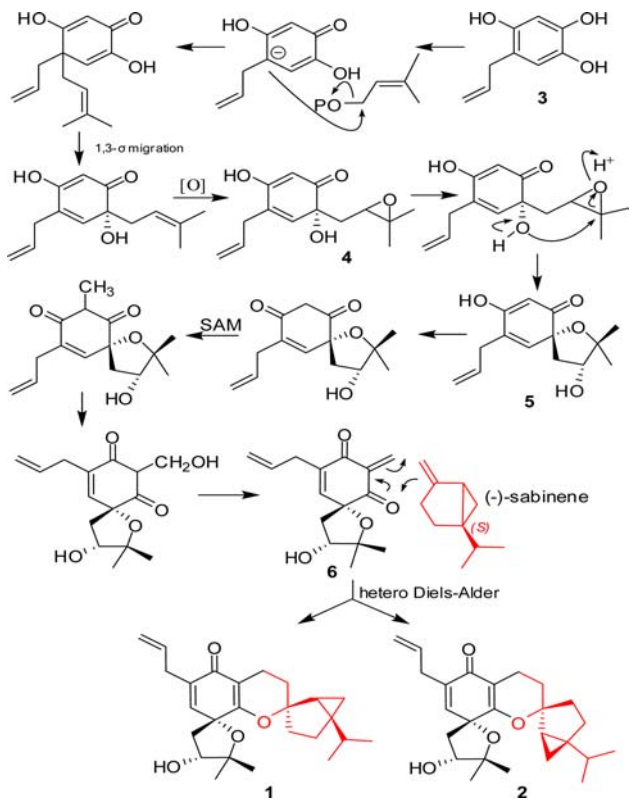


Figure 4. X-ray crystal structures of *p*-bromobenzoate derivatives of **1** (upper) and **2** (below).

Because **1** possessed an unprecedented dispiro skeleton, an X-ray diffraction experiment was necessary to confirm its structure, especially the absolute configuration of C-17, which had been determined by the above indirect methods (Mosher mothod and NOEs). Because a single crystal of **1** could not be obtained, a *p*-bromobenzoate derivative of **1** was prepared by treatment with *p*-bromobenzoyl chloride, a single crystal of which was obtained from acetone−H_2O. Thus, the crystal structure of the derivative (Figure 4) allowed unambiguous assignment of the absolute configuration of **1** (4*R*, 11*R*, 17*S*, 18*R*, 20*S*) based on the Flack parameter (0.00(2)) for anomalous dispersion with Cu Kα radiation. This confirmed our assignment of the C-11 stereochemistry.

Spirooliganone B (**2**) was obtained as a colorless oil. Its molecular formula, $C_{25}H_{34}O_4$, was deduced from HRESIMS and 1D NMR (Table S1 in SI), which was the same as that of **1**. The UV, IR, CD, and NMR spectral data of **2** also resembled those of **1** (see Figures S24, S25, S27, and S28 in SI). The structure was elucidated in the same manner as for **1**. Careful analysis of ^{1}H−^{1}H COSY, HSQC, and HMBC correlations, as well as other NMR data, allowed us to conclude that its structure was similar to **1**. The ^{1}H and ^{13}C NMR chemical shifts from C-1 to C-14 of **2** were nearly identical with those of **1**, suggesting that **2** shared the C-4 and C-11 absolute configurations of **1**. This was supported by the CD and NOE spectra (Figure 2), as well as the Mosher method (see Tables S4 and S5 in SI). The NMR data from C-15 to C-25 of **2**, especially the spiro carbon C-17, were different from those of **1**, suggesting that **2** could be a C-17 diastereomer of **1**, which was supported by obvious NOEs for Hb-19 (δ_H 0.87, dd, 5.5, 3.0 Hz) and H_3-13 (δ_H 1.33, s), instead of H_3-24 and H_3-13, in the NOE spectra of **1**. Finally, the *p*-bromobenzoyl derivative of spirooliganone B (**2**) was again prepared, crystallized, and subjected to X-ray diffraction analysis (Figure 4), enabling unambiguous assignment of the absolute configuration of spirooliganone B (**2**) as shown (4*R*, 11*R*, 17*R*, 18*R*, 20*S*) based on the Flack

Scheme 1. Proposed Biogenetic Pathway for **1** and **2**



parameter of −0.023 (11) for anomalous dispersion with Cu Kα radiation.

Spirooliganones A (**1**) and B (**2**), which are epimeric at C-17, feature two oxaspiro structural units which have not previously been reported in a natural product: 7-allyl-2,2-dimethyl-1-oxaspiro[4.5]deca-6,9-dien-8-one and 5-isopropyl-3′,4′-dihydrospiro[bicycle[3.1.0]hexane-2,2′-pyran]. A plausible biogenetic pathway for **1** and **2**, starting from 5-allylbenzene-1,2,4- triol (**3**), a common biosynthetic precursor of prenylated C_6-C_3 compounds,[2e] is proposed in Scheme 1. Compound **3** may be converted to intermediate **4** through prenylation, 1,3-σ migration[6] of prenyl, and oxidation steps, followed by a key epoxyprenyl side-chain cyclization to generate oxaspiro intermediate **5**. Methylation of **5**, followed by oxidation and dehydration, would give **6**. Subsequently, a hetero-Diels–Alder reaction[7] of **6** with monoterpene (−)-sabinene would afford the C-17 epimers **1** and **2**.

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(7) Lawrence, A. L.; Adlington, R. M.; Baldwin, J. E.; Lee, V.; Kershaw, J. A.; Thompson, A. L. *Org. Lett.* **2010**, *12*, 1676–1679.

Compounds **1** and **2** were evaluated *in vitro* for activity against coxsackie virus B3 (CVB3), coxsackie virus A16 (CVA16), influenza virus A/Hanfang/359/95 (H3N2), enterovirus 71 (EV71), and herpes simplex virus 1 (HSV-1).[8] Compounds **1** and **2** showed obvious effect against CVB3 with IC_{50} values of 11.11 and 3.70 μM (see Table S6 in SI), respectively, stronger than that of ribavirin, with their selectivity index (SI) values (3.03 and 4.33, respectively) being comparable to or stronger than that of ribavirin (SI = 3.9). Additionally, compound **2** also showed potential activity against influenza A with an IC_{50} value of 5.05 μM, although its SI value of 4.57 was far less than that of positive control oseltamivir (see Table S9 in SI). However, Compounds **1** and **2** were inactive against EV71 (SHZH98, JS52, H, and BrCr), CVA16, and HSV-1 (see Tables S6, S7, and S8 in SI).

In conclusion, we report two novel antiviral compounds with a rare dioxaspiro skeleton, spirooliganones A and B, from the roots of *I. oligandrum*. Their structures differ only in the absolute configuration of the spiro carbon (C17). This implies that **1** and **2** may be the products of Diels–Alder cycloaddition where the π-face of (−)-sabinene's terminal double bond is attacked from either of two directions. Futhermore, it is worthy of note that **2** shows more potent antiviral activity against coxsackie virus B3 and influenza virus A (H3N2) than **1**, due to their configuration difference. The potent antiviral activity and structurally unique dispirocyclic nature of **2** makes it a promising lead for the development of antiviral agents.

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Note Added after ASAP Publication. The stereochemistry for compounds **1** and **2** contained errors in the version published ASAP August 12, 2013; the correct version reposted September 6, 2013.

Supporting Information Available. Full experimental details, including extraction and isolation, synthesis of MTPA and MPA ester derivatives, X-ray crystal data for *p*-bromobenzoyl derivatives, antiviral assays, MS, IR, UV, CD, and 1D and 2D NMR spectra for **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.