Organic LETTERS

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Chiral Resolution and Absolute Configuration of a Pair of Rare Racemic Spirodienone Sesquineolignans from *Xanthium sibiricum*

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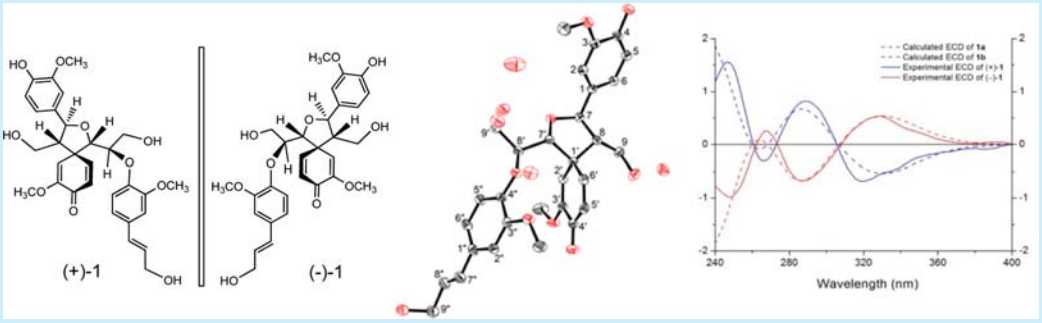
Supporting Information



ABSTRACT: A pair of racemic spirodienone neolignan enantiomers, (±)-sibiricumin A, were isolated from the extract of the fruits of *Xanthium sibiricum*. The resolution of (+)- and (−)-sibiricumin A was achieved by chiral HPLC. The absolute configurations of the racemes were assigned by X-ray and by electronic circular dichroism (ECD). This experiment is the first unambiguous determination of the absolute configuration of spirodienone neolignan.

Xanthium sibiricum Patrin ex Widder (family Asteraceae) is an annual gregarious herb.[1] It is a relatively small genus of plants distributed worldwide, and only four species and two varieties grow in China.[1] Its seeds have been used as a traditional Chinese medicine for the treatment of cancer, fever, headache, nasal sinusitis, and skin pruritus.[2] In particular, the decoction of *X. sibiricum* is highly curative for the treatment of rhinitis. Bioassay experiments have shown that the ethyl acetate extract of *X. sibiricum* exhibits moderate anti-inflammatory activity: that is, it inhibited the ear edema in mice produced by croton oil through subcutaneous injection by 43.5% at a dose of 100 mg/kg. From this active extract, we isolated a pair of racemic tetrahydrofuran spirodienone sesquilignans, named (+)- and (−)-sibiricumin A, (±)-**1** (Figure 1). Tetrahydrofuran type spiro-lignan structures are extremely rare.[3] To the best of our knowledge, only two sesquineolignans (pinobatol[3] and woorenol[4]) with a spiro skeleton have previously been reported. Furthermore, the literature of woorenol and pinobatol only reported the assignment of their planar structures and incomplete determination of their relative configurations.[3,4] Moreover, the absolute configuration of spirodienone lignans has not yet been resolved. In our study, we completed the chiral separation and the assignment of the relative and absolute configuration of racemic **1**, the raceme of (+)-**1** and (−)-**1**, for the first time using X-ray and electronic circular dichroism (ECD). The unambiguous determination of the absolute configuration of the spirodienone sesquineolignan will provide valuable data for future research on this type of rare neolignan.[3,5]

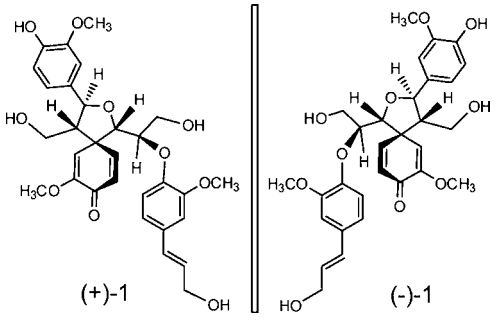


Figure 1. Structures of compound **1**, the raceme (+)- and (−)-**1**.

The HRESIMS of **1** ($[M + Na]^+$, m/z 577.2046) combined with the ^{13}C and ^{1}H NMR data indicated a molecular formula of $C_{30}H_{34}O_{10}$ with 14 degrees of unsaturation. The IR spectrum of **1** showed the presence of hydroxyl (3358 cm^{-1}), enone carbonyl (1658 cm^{-1}), and aromatic groups (1633 and 1468 cm^{-1}). The ^{1}H NMR spectrum (Table 1) revealed a number of signals indicating two typical ABX systems and one ABX system different from the typical trisubstituted aromatic ring (δ_H 6.0−7.5 ppm), one trans-substituted olefinic bond at δ_H 6.49 (1H, d, J = 18 Hz) and δ_H 6.23 (1H, dt, J = 18 and 6 Hz), and three methoxyl groups at δ_H 3.93, 3.68, and 3.78 (each 3H, s). The ^{13}C NMR spectrum of **1** revealed 30 carbon signals.

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Table 1. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) Data of 1 in CD_3OD

no.	type	δ_C	δ_H (J in Hz)
1	C	132.7	
2	CH	111.0	7.15 (d, 2.5)
3	C	149.0	
4	C	147.7	
5	CH	116.2	6.82 (d, 8.5)
6	CH	111.0	7.04 (dd, 8.5, 2.5)
7	CH	84.0	5.11 (d, 9.3)
8	CH	62.0	2.85 (dt, 9.3, 5.7)
9	CH_2	60.1	3.45 (d, 5.7)
3-OCH_3	CH_3	56.7	3.93 s
1′	C	56.8	
2′	CH	114.9	6.29 (d, 2.5)
3′	C	153.7	
4′	C	183.9	
5′	CH	129.4	6.17 (d, 8.5)
6′	CH	154.1	7.13 (dd, 8.5, 2.5)
7′	CH	85.5	4.82 (d, 8.4)
8′	CH	80.5	4.42 (dt, 8.4, 3.0)
9′	CH_2	62.6	3.80 overlapped
3′-OCH_3	CH_3	55.5	3.68 s
1″	C	135.0	
2″	CH	116.2	6.82 (d, 2.5)
3″	C	151.0	
4″	C	147.6	
5″	CH	120.5	6.82 (d, 8.5)
6″	CH	120.3	7.04 (dd, 8.5, 2.5)
7″	CH	131.7	6.49 (d, 18.0)
8″	CH	128.4	6.23 (dt, 18.0, 6.0)
9″	CH_2	64.0	4.20 (d, 6.0)
3″-OCH_3	CH_3	56.5	3.78 s

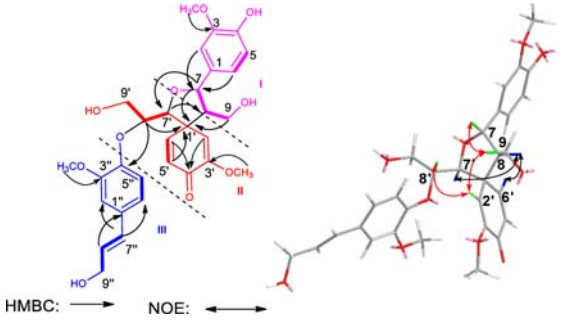

Figure 2. Key HMBC and NOE correlations of **1**.

Interpretation of the ^{1}H, ^{13}C NMR (Table 1), and HSQC spectroscopic data showed the presence of a ketone (δ_C 183.9) carbon, 12 aromatic carbons, six olefinic carbons including two trans-substituted double bond carbons, one sp^3 quaternary carbon (δ_C 56.8), three methoxyls, three oxymethylene carbons, and four methines.

Detailed analysis of the 1D and 2D NMR data allowed the assignment of three C6−C3 substructures, I−III (Figure 2), for **1**. Partial structures I and III were easily established to be two typical C6−C3 units of lignan (I: C1−C9 and III: C1″ -C9″) based on COSY, HSQC and HMBC correlations (Figure 2). However, partial structure II, characteristic of spirodienone lignan, differs from the typical lignan aryl-substitution pattern and will be discussed in greater detail. The cyclohexadienone structure was established based on the three protons at δ_H 6.29 (d, J = 2.5 Hz, H-2′), 6.17 (d, J = 8.5 Hz, H-5′), and 7.13 (d, J = 8.5, 2.5 Hz, H-6′), the six carbon signals at δ_C 56.8 (C-1′), 114.9 (C-2′), 153.7 (C-3′), 183.9 (C-4′), 129.4 (C-5′), and 154.1 (C-6′), and the HMBC correlations from H-2′ and H-6′ to the quaternary carbon at C-4′ (δ_C 183.9). The spin system C7′−C9′ was established by the COSY and HSQC correlations. The C7′-C9′ unit was determined to be attached to C-1′ through C-7′ by the HMBC correlation from H-8′ to

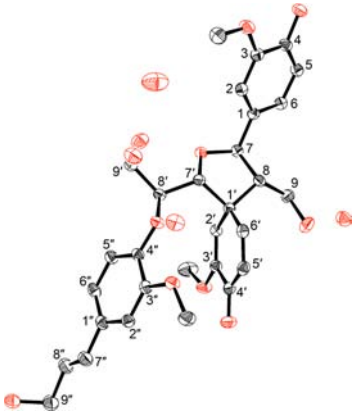

Figure 3. X-ray crystal structure of (±)-sibiricumin A.

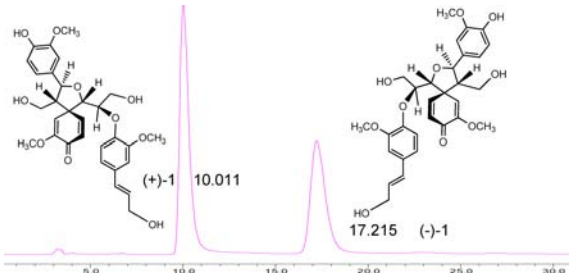

Figure 4. HPLC separation chromatogram of **1** on chiral AD-H column (250 × 10 mm, 5 μm).

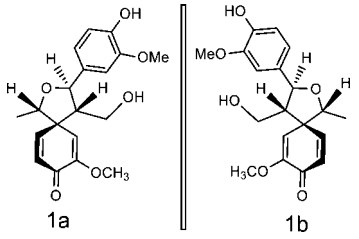

Figure 5. Proposed model compound **1a** and **1b** in the ECD calculation.

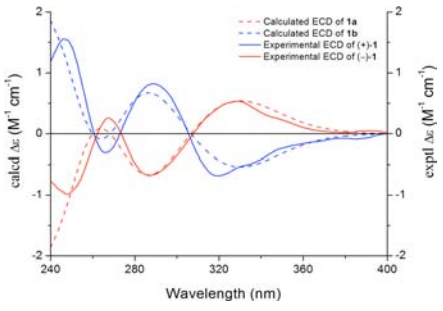

Figure 6. Experimental ECD spectra of (+)-**1** and (−)-**1** and theoretical ECD spectra of **1a** and **1b**.

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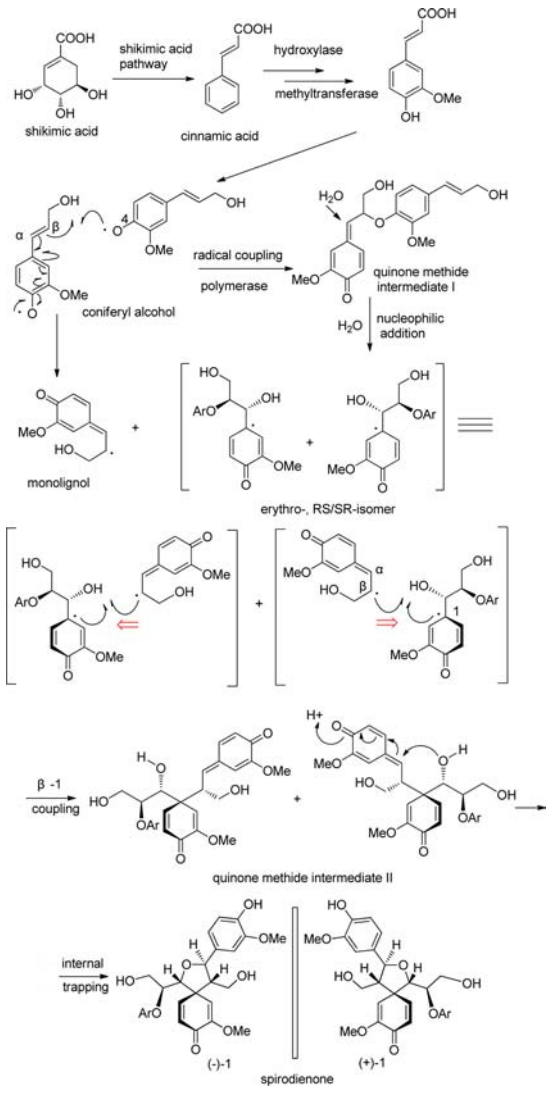
Scheme 1. Plausible Biosynthetic Pathways of (+)-1 and (−)-1



C-1′. Partial structures I and II were confirmed to be fused through C8−C1′ and C7−O−C7′ to form a tetrahydrofuran type spirodienone structure based on the HMBC correlations from H-9 to C-1′ and from H-7 to C-1′, and from H-7 to C-7′, respectively. The connectivity of partial structures II and III was established to be through C8′−O−C4″ by HMBC correlations from H-8′ to C-4″. Finally, three methoxyl groups were assigned to be located at C-3, C-3′ and C-3″ based on the HMBC correlations. Thus, the planar structure of **1** was established and is shown in Figure 1.

The relative configurations of C-7, C-8, C-1′, and C-7′ were established based on the NOE correlations of H-8/H-7′, H-6′/H-8, H-2′/H-9, H-2′/H-8′, and H-7/H-9 (see Figure 2 and Figure S9 in Supporting Information). The NOE correlation of H-8/H-7′ indicated that H-8 and H-7′ were on the same side of the tetrahydrofuran ring. The NOE correlation of H-6′/H8 determined that the dienone ring was oriented perpendicularly to the tetrahydrofuran ring and that H-6′ was on the same side of H-8. However, H-2′ was determined to be on the opposite side of the tetrahydrofuran ring based on the NOE correlations of H-2′/H-9 and H-2′/H-8′. The NOE correlations of H-7/H-9 and the large coupling constant of $J_{H\text{-}7,H\text{-}8}$ = 9.3 Hz suggested that H-7 is trans to H-8. Thus, the relative configurations of C-7, C-8, C-1′, and C-7′ were established and confirmed to be identical to the configurations of the previously reported woorenol and pinobatol.[3,4] However, the relative configuration of C-8′ of this type of neolignans had never previously been resolved because the single bond between C-7′ and C-8′ inhibited the interpretation of the NOE results. Fortunately, the X-ray experiment allowed us to unambiguously determine the relative configuration of C-8′.

In our study, crystallization of **1** from 10:1 MeOH:H_2O resulted in colorless crystals, which gave an X-ray crystal structure with 1.2 Å resolution (deposition number: CCDC 1017146). X-ray single crystal diffraction of **1** with Mo Kα radiation (Figure 3) confirmed the proposed planar structure and the relative configuration of C-7, C-8, C-1′, and C-7′ deduced by the NOE experiments and also unambiguously determined the relative configuration of C7′ and C8′ to be the *erythro* form. This experiment is the first time that the relative configuration of C-8′ of spirodienone neolignan was fully determined.

It is worth noting that the crystals of **1** have the space group *C*2/*C*; a = 15.4750(4) Å, b = 16.9275(5) Å, c = 23.5749(9) Å, V = 6139.5(3) Å^3), indicating a racemic nature, as also evidenced by the lack of optical activity. Racemate (±)-**1** was successfully separated by HPLC using a CHIRALPAK AD-H column (250 × 10 mm, 5 μm) and a flow rate of 3.0 mL/min to obtain its two optically pure enantiomers, (+)- and (−)-**1** (see Figure 4 and Figure S14 in Supporting Information). Each enantiomer was obtained with enantiomeric excess (ee) ≥ 99%. The first peak eluted from the HPLC was determined as the (+)-enantiomer {$[R]^{25}_D$ = +36° (*c* 0.15, MeOH)} followed by the (−)-enantiomer {$[R]^{25}_D$ = −36° (*c* 0.15, MeOH)}. The components (+)- and (−)-**1** possessed identical MS and NMR spectra, indicating the successful resolution of enantiomers.

Electronic circular dichroism (ECD) has proven to be a powerful and reliable method in determining the absolute configuration of natural products.[6] Therefore, the individual absolute configuration of (+)- and (−)-**1** was determined by comparison of the experimental and calculated ECD spectra. In the ECD calculation, the structure was shortened to simplify the computation, as the spirodienone structure is the major chromophore group for the Cotton effect, and the partial structure III and bond C8′−C9′ might generate various conformations but have no significant effect on the CD data, which primarily reflect the spirodienone.[7] A pair of enantiomers (**1a**, **1b**, Figure 5) were proposed to be the model compounds.

Conformational analysis of **1a** and **1b** was performed using the MMFF94 molecular mechanics force field via the MOE software package. The conformers were further optimized using the software package Gaussian 03 at the B3LYP/6-31 g(d) level. The theoretical ECD spectra of **1a** and **1b** were mirror-image and were found to match well with the experimental ECD spectrum of (−)- and (+)-**1**, respectively (Figure 6). Thus, (+)- and (−)-**1** were assigned as (7*S*,8*R*,1′*S*,7′*S*,8′*R*)- and (7*R*,8*S*,1′*R*,7′*R*,8′*S*)-(*E*)-7″-en-4,9,9′,9″-tetrahydroxy-3,3′,3″-trimethoxy-7,7′-epoxy-8′,4″-oxy-8,1′-sesquineolignan-4′-one, respectively.

The biosynthetic precursor of **1** was proposed to be coniferyl alcohol which was produced by shikimic acid pathway (Scheme 1). The β-O-4-coupling reaction of coniferyl alcohol produces a quinone methide intermediate I.[5,8] The quinone methide then adds water to form the β-ether products with two possible isomers in essentially a 50:50 *RS*:*SR* ratio.[1,8] The preformed

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arylglycerol-β-aryl ethers (*RS*/*SR* isomers) can thereafter produce a second quinone methide intermediate II through a β-1-cross-coupling reaction.[5,8] The quinone methide intermediate II may be logically internally trapped by the α-OH to form the spirodienone, (±)-sibiricumin A. It is also possible that the spirodienone neolignan skeleton forms before the third coniferyl alcohol moiety adds to the free hydroxyl group at C-8′ through a β-O-4-coupling reaction to produce (±)-sibiricumin A.

Compounds (±)-**1**, (+)-**1**, and (−)-**1** were examined for their propensity to inhibit NO production in rat polymorphonuclear leukocytes induced by lipopolysaccharides (LPS). Compounds (±)-**1**, (+)-**1**, and (−)-**1** displayed inhibitory effects on NO production of 30, 30, and 33%, respectively, at concentrations of 10 μM. The IC_{50} value of the positive control (dexamethasone) was (2.53×10^{-2} μM) in the same assay.

In conclusion, in this work, we achieved the chiral resolution of a pair of rare racemic spirodienone neolignans, (±)-sibiricumin A, using chiral chromatography, and determined the relative and absolute configuration of the enantiomers by a combination of X-ray and ECD. This is the first time that the relative and absolute configuration of this type of spirodienone neolignan was unambiguously determined. Compounds (+)-**1**, (−)-**1**, and the racemic mixture (±)-**1** showed weak inhibition on NO production in rat polymorphonuclear leukocytes induced by LPS.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, 1D and 2D NMR, MS, IR spectra and X-ray crystal data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

†Y. Shi and Y. Liu contributed equally.

Notes

The authors declare no competing financial interest.

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■ NOTE ADDED AFTER ASAP PUBLICATION

Positive and negative optical rotations were reversed in the version published ASAP October 2, 2014. Therefore, compound (+)-**1** is now labeled (−)-**1** and compound (−)-**1** is now labeled (+)-**1**. All related figures/schemes/graphics in the manuscript and the Supporting Information were replaced and reposted October 17, 2014.