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Schincalide A, a Schinortriterpenoid with a Tricyclo[5.2.1.0^{1,6}]decane-Bridged System from the Stems and Leaves of *Schisandra incarnate*

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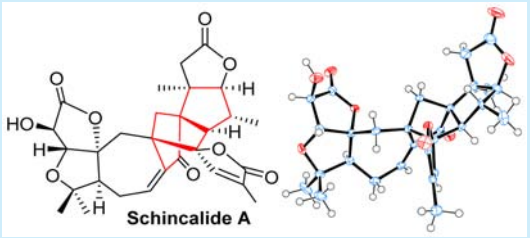
Supporting Information

ABSTRACT: Schincalide A (**1**), an unprecedented schinortriterpenoid possessing a tricyclo[5.2.1.0^{1,6}]decane-bridged system, was isolated from the stems and leaves of *Schisandra incarnate*. The structure with absolute configuration of **1** was determined by extensive spectroscopic analyses and single-crystal X-ray diffraction. A hypothetical biosynthetic pathway of **1** was postulated.



The family Schisandraceae, consisting of two genera, *Schisandra* and *Kadsura*, is mainly distributed in East and Southeast Asia. Only one species of *Schisandra* finds its distribution in North America.[1] Overall, there are about 50 species of this family in the world, 29 of which are distributed in China.[1b] Some species of this family have been used for the treatment of hepatitis, cough, premature ejaculation, chronic dysentery, and insomnia for a long history in China.[1b,2] Since the 1970s, plants of the family Schisandraceae have been a hot topic within the medicinal chemistry and drug discovery communities.[1b] In 1972, nigranoic acid, the first 3,4-seco-cycloartane, was isolated from *S. nigra*, and its NMR spectral assignments were achieved with the aid of computer modeling in 1996.[1b,3] After that, 47 skeletons of triterpenoids were identified from the Schisandraceae plants, with 22 being C_{30} skeletons, 13 being C_{29}, four C_{28}, three C_{27}, one C_{26}, one C_{25}, one C_{24} and two C_{22}. Some of those manifold triterpenoids, especially schinortriterpenoids (SNTs), possess various beneficial bioactivities such as antihepatitis, antitumor, and anti-HIV[1b,4] and have attracted wide attention of phytochemists and pharmacologists. Schinortriterpenoids represent an intriguing class of highly oxygenated and rearranged nortriterpenoids with C_{26} to C_{29} frameworks which were exclusively found in the Schisandraceae plants. The first schinortriterpenoid, micrandilactone A, obtained and identified from *S. miacrantha* in 2003,[5] inspired the discovery of more than 200 structurally interesting schinortriterpenoids afterward. Now, there are up to 16 groups of schinortriterpenoids classified, such as schiartanes, schisanartanes, prschisanartanes, and lancifoartanes.[4] These fascinating molecules have brought great interest and challenges to phytochemists and organic chemists.

Since 2011, our research group has been studying the chemical constituents of the plants of *Schisandra*. Our efforts have led to the isolation of eight new triterpenoids including a unique 6/7/9-fused triterpenoid,[2a,6] eight new lignans including three new lignan glycosides.[7] *Schisandra incarnate* Stapf, a vine plant, is mainly distributed in the west and southwest of Hubei province, China, the stems of which are used for the treatment of traumatic injury, rheumatism, and arthritis in folk medicine. Its chemical constituents have never been reported before. To search for more structurally unique and biogenetically compelling metabolites from the *Schisandra* plants, the stems and leaves of *S. incarnate* were collected from Xingshan County located in the west of Hubei province, China. Our phytochemical investigation on the stems and leaves of *S. incarnate* led to the isolation of schincalide A (**1**), a unique schinortriterpenoid featuring a tricyclo[5.2.1.0^{1,6}]decane-bridged system (Figure 1). This paper reports the isolation, structural elucidation, hypothetical biogenetic pathway, and biological activities of compound **1**.

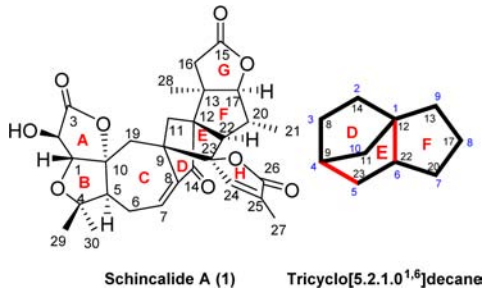


Figure 1. Structure of **1** and its nomenclature of the unique bridged system (number in blue).

The air-dried and powdered stems and leaves of *S. incarnate* (14.5 kg) were extracted seven times with 70% aqueous acetone (7 × 30 L) at room temperature and concentrated under reduced pressure to give a crude extract (1.2 kg), which was then extracted by petroleum ether and EtOAc. The EtOAc part (300 g) was

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chromatographed on a silica gel column with a gradient elution of CH_2Cl_2/Me_2CO (1:0 to 0:1, v/v) to give eight fractions A–H. Fraction E (7.5 g) was subject to further separation on MCI column (MeOH/H_2O, 3:2 to 1:0, v/v), Sephadex LH-20 column (MeOH/CH_2Cl_2, 1:1, v/v), and C18-ODS MPLC (flow rate = 10 mL/min; MeOH/H_2O, 3:2 to 1:0, v/v) to afford subfractions E331–E333. Continuing separation of E332 on reversed-phase semipreparative HPLC (flow rate = 2 mL/min; mobile phase = 70% MeOH/H_2O, v/v; detection wavelength = 210 and 230 nm) yielded **1** (15 mg, t_R 12.1 min).

Schincalide A (**1**), colorless needles, has a molecular formula of $C_{29}H_{32}O_9$ as determined by HRESIMS ($[M + Na]^+$ m/z 547.1923, calcd 547.1944) and ^{13}C NMR data, requiring 14 degrees of unsaturation. The IR spectrum showed absorption bands attributed to hydroxyl groups (3460 cm^{-1}), carbonyl groups (1788, 1765, 1735, and 1720 cm^{-1}), and olefinic groups (1632 cm^{-1}). The UV spectrum showed a λ_{max} at 220 nm in MeOH.

The ^{13}C NMR, DEPT-135, and HSQC spectra of **1** displayed signals for 29 carbons: five methyls, four methylenes, eight methines (including two olefinic and three oxygen-bearing carbons), 12 quaternary carbons (one ketone and three ester carbonyl, two olefinic carbons and three oxygenated carbons) (Table 1), indicating that **1** belonged to the schinortriterpenods.[1b] The ^1H NMR data of **1** clearly demonstrated the existence of one secondary methyl at δ_H 0.99 (d, J = 6.8 Hz) and four tertiary methyls at δ_H 1.06 (s), 1.29 (s), 1.62 (s), and 2.01 (d, J = 1.6 Hz). In the lower field region, three resonances at δ_H 4.30 (s), 4.15 (s), and 4.06 (d, J = 5.8 Hz) were ascribed to three oxygenated methines. Two olefinic proton resonances at δ_H 6.93 (dd, J = 6.2, 8.7 Hz) and 6.77 (d, J = 1.6 Hz), together with the ^{13}C NMR signals (δ_C 148.5, 141.4, 132.2, and 132.2), proposed the existence of two trisubstituted double bonds. Apart from six degrees of unsaturation occupied by four carbonyls and two double bonds, an octacyclic structural unit was required for **1** to fulfill the unsaturation demand. Detailed inspection of 2D NMR of **1** confirmed the presence of eight rings A–H.

HMBC correlations of H-1/C-2, C-3, C-10, C-19; H-2/C-1, C-10; H-19b/C-1, C-10; H-5/C-4, C-10; H-29/C-4, C-5; and H-30/C-4, C-5, comparing to those of the previously reported preschisanartanin N,[8] strongly implicated a similar moiety for two five-membered rings A and B, with a hydroxyl linked to C-2 and two germinal methyls attached to C-4. The HMBC correlations of H-5/C-6, C-10; H-6a/C-5, C-8, C-10; H-6b/C-5, C-7, C-8, C-10; H-19a/C-8, C-9, C-10; and H-19b/C-8, C-9, C-10, coupled with the $^1H-^1H$ COSY correlations of H-6b/H-5 and H-7/H-6a, H-6b, led to the establishment of seven-membered ring C. Subsequently, the HMBC associations of H-11a/C-8, C-9, C-12, C-14; H-7/C-14; and H-19a, H-19b/C-11 exhibited the presence of the third five-membered ring D, which also suggested that ring C fused with ring D through the bridge between C-8 and C-9.

The fourth five-membered ring E, making **1** highly noteworthy, was formed by the unique linkages of C-9–C-23 and C-12–C-22. From the HMBC spectral data, couplings of H-11a/C-9, C-12, C-22, C-23; H-11b/C-22, C-23; H-19a/C-23; and H-22/C-9, C-11, C-14 showed the presence of ring E, which was further proved by single-crystal X-ray diffraction analysis. Then the key $^1H-^1H$ COSY correlations of H-20/H-17, H-21, H-22, along with the HMBC associations of H-17/C-12, C-21, C-22, C-28; H-20, H-21/C-17; H-28/C-12, C-13, C-17; and H-22/C-12, C-20, interpreted the existence of ring F, fused with ring E through the bridge between C-12 and C-22. Consequently, a complex and sterically congested tricyclo[5.2.1.$0^{1,6}$]decane-bridged system constructed by three five-membered rings D–F was established. Furthermore, the HMBC couplings of H-16/C-13, C-15, C-17, C-28; H-17/C-15; and H-28/C-13, C-16, C-17 intimated the moiety of ring G. The eighth ring H, frequently appearing in most SNTs, was implied by the $^1H-^1H$ COSY correlation of H-24/H-27, and the HMBC correlations of H-24/C-23, C-25, C-26, C-27 and H-27/C-24, C-25, C-26. Taking this evidence into account, we assembled the planar substructure of **1** as displayed in Figure 2.

The relative configuration of **1** was partially elucidated on the basis of NOESY spectral data. The key signals of NOESY, H-17/H-21, H-21/H-22, and H-17/H-28 revealed that Me-21, Me-28, H-17, and H-22 shared the same orientation (Figure 3). The

Table 1. NMR Data of Compound 1 ($CDCl_3$)

no.	δ_C, type[a]	δ_H, mult (J in Hz)[b]	COSY[c]	HMBC ($^1H\rightarrow^{13}C$)[c]	NOESY[c]
1	86.0, CH	4.15, s		2, 3, 10, 19	19a, 19b, 29
2	73.0, CH	4.30, s		1, 10	
3	175.4, C				
4	84.1, C				
5	57.2, CH	2.21, dd (3.6, 13.6)	6b	4, 6, 10, 30	30
6a	23.2, CH_2	2.11, ddd (3.6, 8.7, 13.5)	6b, 7	5, 8, 10	7
6b		2.02, ddd (6.2, 13.5, 13.6)	5, 6a, 7	5, 7, 8, 10	7, 29
7	132.2, CH	6.93, dd (6.2, 8.7)	6a, 6b	6, 9, 14	6a, 6b
8	141.4, C				
9	55.3, C				
10	95.3, C				
11a	44.0, CH_2	2.43, d (10.9)	11b	8, 9, 12, 14, 22, 23	
11b		2.33, dd (2.0, 10.9)	11a, 22	22, 23	
12	66.8, C				
13	45.4, C				
14	198.7, C				
15	176.2, C				
16a	40.9, CH_2	2.39, d (18.4)	16b	12, 13, 15, 17, 28	28
16b		2.74, d (18.4)	16a	12, 13, 15, 17, 28	
17	100.0, CH	4.06, d (5.8)	20	12, 15, 21, 22, 28	21, 28
19a	32.3, CH_2	1.99, d (15.7)	19b	5, 8, 9, 10, 11, 23	1
19b		2.30, d (15.7)	19a	1, 5, 8, 9, 10, 11	1
20	39.4, CH	2.56, ddd (5.8, 6.8, 10.1)	17, 21, 22	17, 21, 22, 23	
21	18.6, CH_3	0.99, d (6.8)	20	17, 20, 22	17, 22
22	58.1, CH	2.24, dd (2.0, 10.1)	11b, 20	9, 11, 12, 14, 20, 21, 24	21, 24
23	92.7, C				
24	148.5, CH	6.77, d (1.6)	27	23, 25, 26, 27	22, 27
25	132.2, C				
26	172.5, C				
27	11.0, CH_3	2.01, d (1.6)	24	23, 24, 25, 26	24
28	23.0, CH_3	1.62, s		12, 13, 16, 17	16a, 17
29	20.6, CH_3	1.06, s		4, 5, 30	1, 6b
30	27.6, CH_3	1.29, s		4, 5, 29	5

[a]Recorded at 101 MHz. [b]Recorded at 600 MHz. [c]Recorded at 400 MHz.

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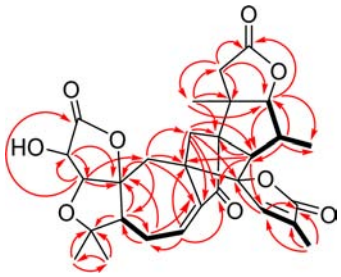


Figure 2. Key $^{1}H-^{1}H$ COSY (bold) and HMBC (red arrows) correlations observed for **1**.

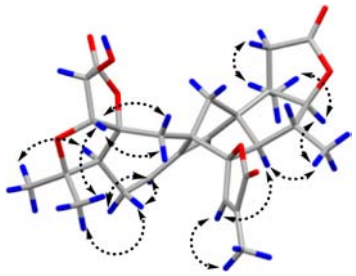


Figure 3. Key NOESY correlations observed for **1**.

NOESY correlations of H-1/H-29 and H-5/H-30 suggested that H-1 and Me-29, and H-5 and Me-30 were positioned on the same face of the molecule. In combination with biogenetic consideration, the relative stereochemistry of six chiral centers (C-1, C-5, C-13, C-17, C-20, and C-22) was established. However, the relative configurations of the other four sp^3 quaternary carbons (C-9, C-10, C-12, and C-23) and one sp^3 methine carbon (C-2) were difficult to determine by NOESY because not enough evidence was observed.

To identify the absolute configuration, **1** was recrystallized in chloroform to afford colorless needle crystals of the orthorhombic space group *P*212121. The X-ray diffraction analysis of **1** with Cu Kα radiation resulted in the Flack parameter of 0.08 (10) and the Hooft parameter of 0.09 (12)[9] for 1784 Bijvoet pairs, allowing an explicit assignment of the absolute configuration of **1** as 1*R*,2*R*,5*S*,9*R*,10*R*,12*S*,13*R*,17*S*,20*R*,22*R*,23*S* (Figure 4). Thus, the structure of **1** was defined and given the name schincalide A.

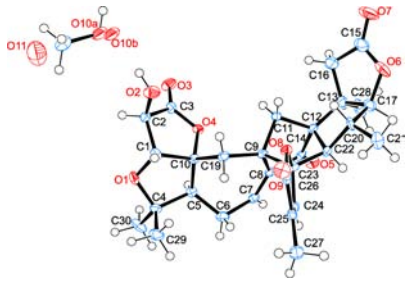
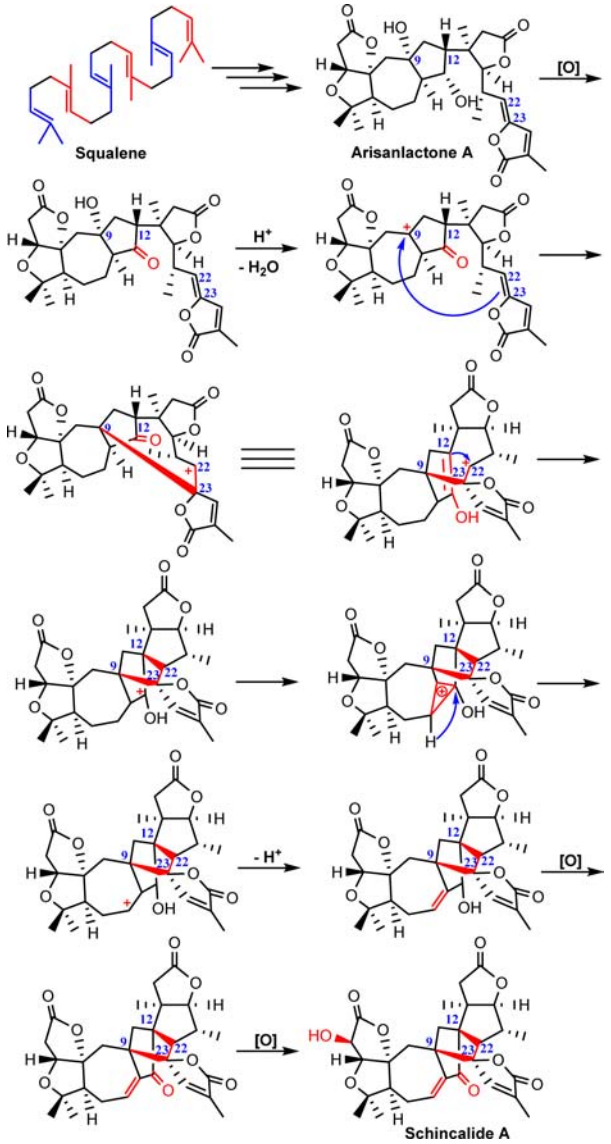


Figure 4. Perspective drawing of the X-ray structure of **1**.

Schincalide A was recognized as the first example of rearranged nortriterpenoid with a tricyclo[5.2.1.$0^{1,6}$]decane-bridged system. Two new bridges between C-9 and C-23 and between C-12 and C-22 made compound **1** noteworthy. The methyl group at C-13 of compound **1** was α-axial. There were only six SNTs possessing an α-methyl at C-13 having been reported before,[4,10] the skeletons of which were supposed to be derived from 3,4:9,10-disecocycloartane by decarboxylation at C-18 and the 1,2-methyl shift of Me-28 at C-14.[4] Since the structure of **1** was found to share similar structural units of rings A–C, G, and H, and the same stereochemistry at C-13 with arisanlactone A[4,10b] obtained from the fruits of *S. arisanesis*, we proposed that **1** was very likely to derive from arisanlactone A by a series of biochemical reactions such as oxidation, acidition, and nucleophilic addition. A hypothetical biogenetic pathway of **1** was postulated as shown in Scheme 1.

Scheme 1. Hypothetical Biogenetic Pathway to 1



Cytotoxicity of **1** was tested using the MTT method against the HepG2 human hepatocellular carcinoma cell line, A2780 human ovarian cancer cell line, and Panc02 murine pancreatic cell line, but no activity was detected with IC_{50} > 40 μM. Additionally, **1** was tested for in vitro immunosuppressive activity and exhibited weak inhibition with 36.76% against ConA-induced T-cell proliferation and 11.89% against LPS-induced B-cell proliferation at 50 μg/mL concentration. Cyclosporin A and mycophenolate mofetil were used as positive controls. The cellular proliferation assay is described in the Supporting Information.

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■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02197.

Experimental details and full NMR, MS, IR, CD spectra of schincalide A (PDF)
X-ray data of schincalide A (CIF)

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Notes

The authors declare no competing financial interest.

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