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Schiglautone A, a New Tricyclic Triterpenoid with a Unique 6/7/9-Fused Skeleton from the Stems of *Schisandra glaucescens*

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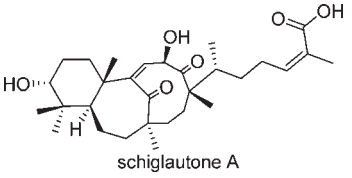
ABSTRACT



schiglautone A

Schiglautone A (1), a unique 6/7/9-fused tricyclic carbon backbone triterpenoid, was isolated from the stems of *Schisandra glaucescens*. Its structure was determined on the basis of spectroscopic analysis and single-crystal X-ray crystallography. A hypothetical biosynthetic pathway of 1 was postulated.

Several plants from the genus *Schisandra* of the family Schisandraceae are widely used in Traditional Chinese Medicine in the treatment of cough, premature ejaculation, chronic dysentery, and insomnia for thousands of years. In addition to the presence of a large number of lignans, *Schisandra* was also found to be rich in triterpenoids with numerous pharmaceutical effects including anti-HIV,[1] anticholesteremics,[2] anti-HBV,[3] and antitumor activities,[4] which has aroused a lot of interest from pharmacologists. In recent years, several novel oxygenated triterpenoid skeletons from *Schisandra*, such as schiartane, 18-norschiartane, 18 (13→14)-abeo-schiartane, schisanartane, preschisanartane, wuweiziartane, and kadlongilactone,[5] also brought great interest and challenges for phytochemists and organic chemists.

Schisandra glaucescens Diels. is a vine plant mainly distributed in the west of the Hubei province and in the southeast of the Sichuan province, China. Its stems have been used for the treatment of various diseases such as

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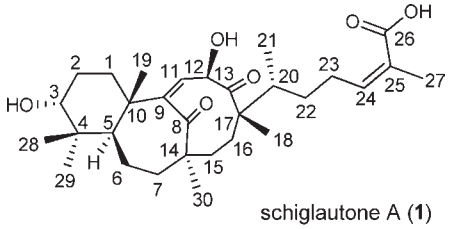
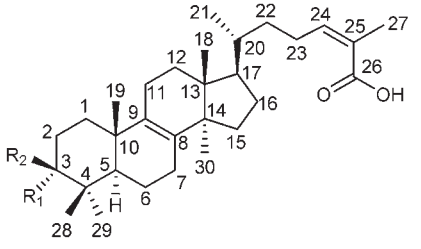
contusion, rheumatism, and arthritis in folk medicine. The chemical constituents and pharmacological potential of *S. glaucescens* have never been reported. In our search for new, potentially biologically active substances from plants, the chemical constituents of the stems of *S. glaucescens* were investigated and a novel triterpenoid possessing an unusual 6/7/9-fused tricyclic ring system was obtained, which was designated as schiglautone A (**1**).

Here, we report the isolation and structure elucidation of (**1**) as well as preliminary results of its biological activity.





The air-dried ground stems of *S. glaucescens* (6.5 kg) were extracted four times with 70% aq. acetone (4 × 25 L) at room temperature and concentrated in vacuo to give a crude extract (750 g), which was partitioned between H_2O and EtOAc. The EtOAc fraction (127 g) was chromatographed on a silica gel column using $CHCl_3$−acetone as elution solvents (from 1:0 to 1:9) to give 14 fractions. Fraction 6 (8.5 g) obtained with $CHCl_3$−acetone of 7:3 was repeatedly chromatographed on a silica gel column (CH_2Cl_2−MeOH, 40:1 to 25:1; Petroleum−EtOAc, 1:1 to 1:2) and Sephadex LH-20 (MeOH; CH_2Cl_2−MeOH, 1:1) columns to yield compound **1** (37.7 mg).

Schiglautone A (**1**) was obtained as a colorless crystal with a molecular formula of $C_{30}H_{46}O_6$ as deduced from HRESIMS data (*m*/*z* 501.3204 $[M + H]^+$, calcd for 501.3216),[6] requiring eight degrees of unsaturation. The ^1H NMR data (Table 1) of **1** clearly indicated the existence of seven methyls, two olefinic methines, and two oxymethines.

(6) $[\alpha]^{25}_D$ −132.5 (*c* 1.89, C_5H_5N); mp 177−179 °C. IR (film/KBr) ν_{max} cm^{-1}: 3454, 1693, 1642, 1457, 1388, 1251; ^1H NMR (C_5D_5N, 400 MHz) and ^{13}C NMR (C_5D_5N, 100 MHz), see Table 1. HRESIMS *m*/*z* 501.3204 $[M + H]^+$ (calcd for 501.3216). Crystal data: monoclinic system, space group *C*2, *a* = 29.890(3) Å, α = 90°, *b* = 7.2742(6) Å, *β* = 112.246(2)°, *c* = 14.7775(13) Å, *γ* = 90°. A crystal of dimensions 0.16 × 0.12 × 0.10 mm^3 was used for measurement on a SMART APEX CCD XRD. Reflections collected: 9658. Independent reflections: 5754 [*R*(int) = 0.0572]. Completeness to *θ* = 25.99°: 99.6%. Absorption correction: None. Max and min transmission: 0.9924 and 0.9879. The structure was solved by direct methods and refined by a full-matrix least-squares on F^2. Final *R* indices [$I > 2\sigma(I)$]: R1 = 0.0612, wR2 = 0.1465. The final X-ray model is shown in Figure 3.

Table 1. ^1H and ^{13}C NMR Data and Key HMBC Correlations of **1**[a]

no.	δ_C (mult)	δ_H (mult, *J* in Hz)	HMBC (from H to C)
1α	29.2 (t)	1.41−1.46 (m)	C-19, C-9, C-3
1*β*		2.36 (td, 13.3, 3.2)	C-19, C-10, C-9, C-3
2α	26.0 (t)	1.80−1.73 (m)	C-3, C-1
2*β*		1.93−1.97 (m)	
3	74.2 (d)	3.57 (brs)	C-5
4	40.1 (s)		
5	41.8 (d)	2.43 (d, 8.9)	C-19, C-9, C-3, C-7
6α	23.1 (t)	1.6 (t, 6.0)	C-14
6*β*		1.36−1.40 (m)	
7α	39.3 (t)	1.48−1.54 (m)	
7*β*		1.19−1.23 (m)	
8	214.7 (s)		
9	152.3 (s)		
10	38.3 (s)		
11	123.1 (d)	5.74 (d, 0.7)	C-12, C-10, C-9, C-8
12	70.5 (d)	5.27 (s)	C-17, C-13, C- C-11, C-9
13	215.9 (s)		
14	50.2 (s)		
15α	34.3 (t)	1.23−1.24 (m)	C-17, C-8
15*β*		2.54 (t, 13.0)	C-30, C-17, C-16, C-8
16α	31.9 (t)	2.21 (t, 14.2)	C-18, C-17, C-15, C-14
16*β*		1.64 (t, 6.0)	C-17, C-13
17	57.2 (s)		
18	16.3 (q)	1.32 (s)	C-22, C-20, C-17, C-13
19	20.7 (q)	1.20 (s)	C-10, C-9, C-5
20	41.6 (d)	1.68−1.72 (m)	C-21, C-17, C-13
21	13.7 (q)	1.02 (d, 6.6)	C-22, C-20, C-17
22a	31.6 (t)	1.26−1.29 (m)	C-24, C-23, C-21
22b		1.86−1.92 (m)	C-24, C-23, C-21, C-17
23a	28.8 (t)	2.75 (td, 15.0, 7.0)	C-25, C-24, C-22, C-20
23b		2.86−2.94 (m)	C-25, C-24, C-22, C-20
24	142.4 (d)	5.98 (tq, 7.2, 0.7)	C-26, C-22, C-27
25	128.4 (s)		
26	170.4 (s)		
27	21.2 (q)	2.05 (d, 0.7)	C-26, C-25, C-24
28	21.8 (q)	0.77 (s)	C-29, C-4, C-3
29	29.2 (q)	1.16 (s)	C-28, C-4, C-3
30	25.8 (q)	1.50 (s)	C-15, C-14, C-8, C-7

[a] Recorded in C_5D_5N, ^1H NMR at 400 MHz and ^{13}C NMR at 100 MHz respectively.

The ^{13}C NMR and DEPT spectrum of **1** showed a total of 30 carbon signals, consisting of two ketone carbonyls, one carboxyl, two trisubstituted double bonds, two oxymethines, four aliphatic quaternary carbons, two aliphatic methines, eight aliphatic methylenes, and seven methyls. Apart from five degrees of unsaturation occupied by two ketone carbonyls, one carboxyl, and two trisubstituted double bonds, a tricyclic structure was required for **1** to fulfill the unsaturation requirement. Comparison of the ^1H and ^{13}C NMR spectrum data of **1** with those of anwuweizic acid (**2**) suggested that they were similar in six-membered ring A and the side chains.[7] The hypothesis of the three carbon rings of **1** mentioned above was confirmed by interpretation of the NMR data of **1**.

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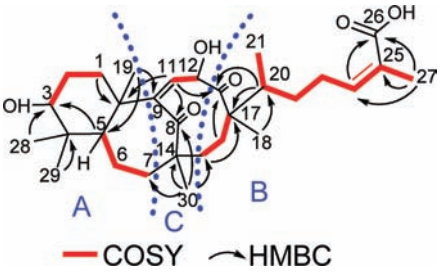


Figure 1. Substructures **A**, **B**, and **C**, COSY and key HMBC correlations of **1**.

Detailed inspection of the 1D and 2D NMR spectra of **1** revealed the presence of signals for three substructures **A**, **B**, and **C** (Figure 1). A six-membered carbon ring (substructure **A** (Figure 1)) was assigned by the HMBC correlations from H-1 to C-19 and C-9, and correlations from H-19 to C-10 and C-5, along with correlations from H-5 to C-19 and C-3, coupled with the COSY correlations between H-1/H-2/H-3 and H-5/H-6/H-7. In addition, the HMBC correlations from both H-28 and H-29 to C-3 and C-4 revealed two geminal methyls attached at C-4.

Substructure **B** is similar to that of the reported anwuweizic acid.[7] Two proton spin systems (H-21/H-20/H-22/H-23/H-24/H-27 and H-15/H-16) were observed from the COSY spectrum. Together with the HMBC correlations of H-21 with C-22, C-20, and C-17; H-20 with C-21, C-17, and C-13; and H-18 with C-22, C-20, C-17, and C-13, the linkage from C-17 to C-13, C-18, and C-20 has been established. The correlations of both H-15 and H-16 with C-17 as well as H-16β with C-13 in the HMBC spectrum proved the connection of the two proton sequences. Meanwhile, the HMBC correlations of both H-27 and H-24 with C-26 and correlations of H-27 with C-26, C-25, and C-24 suggested the location of the carboxyl (C-26) at C-25. The Z geometry of the double bond between C-24 and C-25 was deduced from the NOESY correlation of H-24 with H-27. The above analysis established substructure **B** (Figure 1).

In the substructure **C**, an α,β-unsaturated ketone was elucidated by HMBC correlations from H-11 to C-9 and C-8 and from H-30 to C-14 and C-8. Furthermore, the COSY correlation of H-11/H-12 was also clearly observed.

Finally, the substructures **A**, **B**, and **C** were joined on the basis of the following HMBC correlations: H-19 with C-9; H-11 with C-10; H-30 with C-15 and C-7; and both H-11 and H-12 with C-13.

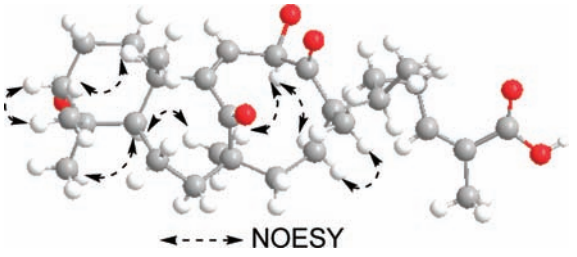


Figure 2. Key NOESY correlations of **1**.

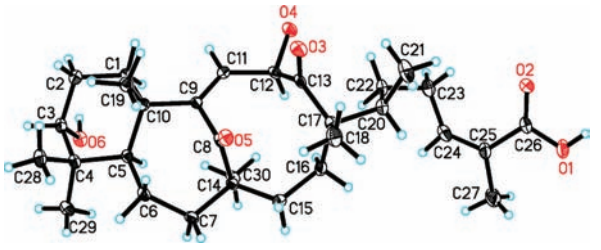
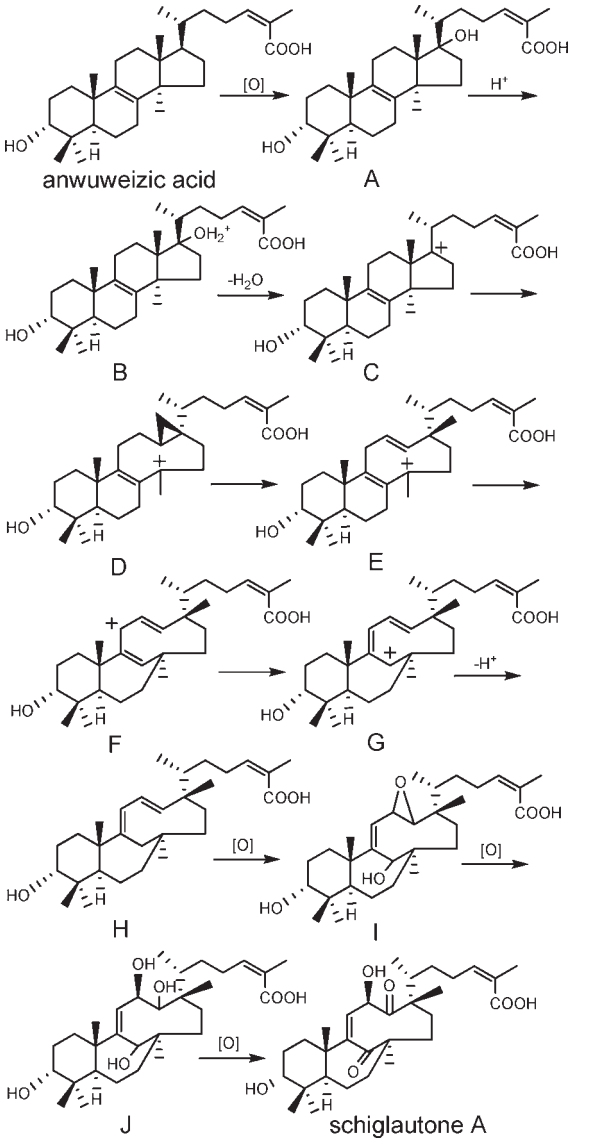


Figure 3. Perspective drawing of the X-ray structure of **1**.

Scheme 1. Plausible Biosynthetic Pathway for **1**

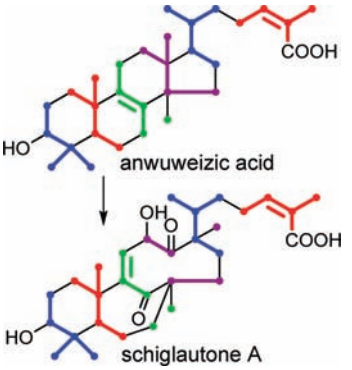


The relative configuration of **1** was mainly deduced by analysis of the NOESY spectrum and analogy with anwuweizic acid (**2**) and epianwuweizic acid (**3**).[8] The fact that H-3 was observed as a broad singlet in the ^{1}H NMR spectrum suggested H-3 was in a β-orientation. Accordingly, key NOESY correlations (Figure 2) of H-3/Me-28/Me-19 suggested that they were on the same face. In contrast, correlations of Me-29/H-5/Me-30/H-12/H-16α indicated that they were on the α-face. In addition, the NOESY cross-peaks of Me-18/H-16β lead to the β-orientation of Me-18. Considering the free rotation of the single bond between C-17 and C-20, the relative stereochemistry of C-21 could not be determined by the NOESY data. Fortunately, a single-crystal X-ray diffraction analysis was performed successfully, which confirmed the planar structure and relative configuration of **1** and allowed assignment of the α-orientation of Me-21 (Figure 3).

Compound **1** has a unique 6/7/9-fused tricyclic triterpenoid skeleton with 30 carbons. It appears to have no precedent among known natural products or synthetic compounds. Triterpenoids from the Schisandraceae family can be classified into three groups on the basis of their different carbon frameworks: lanostane, cycloartane, and *Schisandra* nortriterpenoids.[5] Compound **1** is clearly different from the above three groups in carbon frame. Although a nine-membered-ring enolic cation intermediate was proposed,[1d] such a product has never been verified or isolated from *Schisandra* or other plants. Since the structure of **1** is partially very similar to that of anwuweizic acid, i.e. ring A and the side chain, which was also isolated from the same plant in a large amount, we deduced that **1** was very likely derived from anwuweizic acid (**2**) through the intermediates **A**−**J** (Scheme 1) by a series of biochemical reactions such as acidation, oxidation, hydroxylation, and ring expansion. A hypothetical biosynthetic pathway of **1** was postulated as shown in Scheme 1. Scheme 2 illustrates the possible rearrangement of isoprene units in schiglautone A, which shows the purple isoprene unit in anwuweizic acid has been degraded to two two-carbon units in a nine-membered cycloketone and a methyl group.

(8) Liu, J. S.; Wang, W. G.; Huang, M. F. *Zhongcaoyao* **1990**, *21*, 294.

Scheme 2. Proposed Biogenetic Origin of **1**, Showing Isoprene Units from Anwuweizic Acid



Compound **1** was tested for its inhibitory effects against HeLa, Hep G2, and SGC-7901 cell lines and showed weak cytotoxicities of 25%, 23%, and 13% respectively, with a concentration of 100 μg/mL. 5-Fluorouracil (5-FU) was used as a positive control, which showed 44%, 37%, and 46% inhibition respectively at 100 μg/mL. The cellular proliferation assay was described in the Supporting Information.

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Supporting Information Available. Experimental section, NMR (1D and 2D), MS, IR, CD spectra, X-ray CIF file for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.