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Schinarisanlactone A, a New Bisnortriterpenoid from *Schisandra arisanensis*

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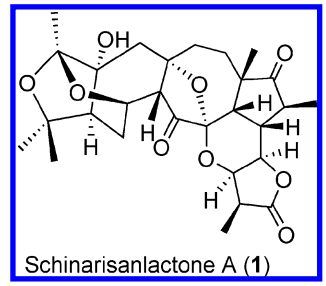
ABSTRACT



A novel triterpenoid, schinarisanlactone A (1), was isolated from the fruits of *Schisandra arisanensis*. Compound 1 possesses an unprecedented skeleton having a 5/7/7/5/7/5/6/5-fused octacyclic ring system. The structure of 1 was determined by 2D NMR techniques (COSY, HMQC, HMBC, and NOESY) and was confirmed by X-ray crystallographic analysis. Schinarisanlactone A (1) exhibited significant anti-HIV activity.

An important review regarding the triterpenoids from the schisandraceae family was reported a couple years ago.[1] Several schisandraceaous triterpenoids of novel skeletons such as kadlongilactone-type triterpenoids,[2] kadsuphilactone A,[3] schisanartane-type nortriterpenoids,[4] wuweiziartane-type nortriterpenoids,[5] and preschisanartanes[6] were published previously. Their biological and pharmaceutical effects including anti-HIV,[7] anti-HBV,[8] antitumor,[9] cytotoxicity,[10] and antiinflammatory[11] activities were also addressed. *S. arisanensis* is an endemic plant only distributed in the mountainous area of Taiwan. Recently a nortriterpenoid, designated as arisandilactone A, possessing

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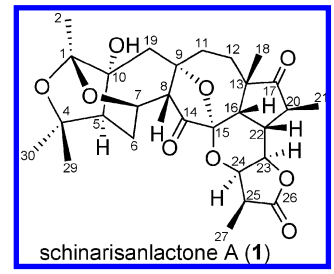
a 5/5/7/5/8/5-fused hexacyclic ring system has been isolated and its structure determined.[12] Herein, we report a novel bisnortriterpenoid, named schinarisanlactone A (**1**), which has an unprecedented 5/7/7/5/7/5/6/5-fused octacyclic ring system with a rare oxygen bridge between C-1 and C-7.

Table 1. ^{1}H and ^{13}C NMR Data for Schinarisanlactone A (**1**)

no.	δ_H(mult, J in Hz)[a]	δc(mult)[b]	HMBC (H→C)
1		108.2 s	Me-2, H-7, H-19
2	1.33 (s)	18.6 q	
4		80.5 s	H-5, Me-29, Me-30
5	2.14 (overlapped)	47.4 d	H-7, H-19, Me-29, Me-30
6α	2.47 (m)	25.0 t	H-5, H-8
6β	2.18 (overlapped)		
7	4.18 (d, 9.2)	67.9 d	H-5, H-8
8	2.68 (overlapped)	59.3 d	H-6, H-11, H-19
9		81.1 s	H-7, H-11, H-19
10		79.3 s	Me-2, H-5, H-6, H-19
11α	2.00 (m)	42.2 t	H-8, H-12, H-19
11β	1.46 (overlapped)		
12α	1.92 (m)	30.4 t	Me-18
12β	1.35 (overlapped)		
13		49.9 s	H-12, Me-18, H-20
14		208.9 s	H-16
15		98.4 s	H-8, H-16, H-22
16	2.48 (d, 6.0)	44.5 d	H-12, Me-18, H-20, H-23
17		219.6 s	H-16, Me-18, H-20, Me-21
18	1.03 (s)	26.3 q	H-12
19α	2.15 (d, 16.4)	44.5 t	H-11
19β	2.58 (d, 16.4)		
20	2.47 (m)	44.6 d	H-16, Me-21, H-22, H-23
21	1.26 (d, 6.4)	14.6 q	H-20, H-22
22	2.68 (m)	40.0 d	H-16, H-20, Me-21
23	4.37 (br s)	74.3 d	H-16, H-20, H-22, H-24
24	4.52 (br s)	68.0 d	H-22, H-23
25	2.82 (m)	41.7 d	
26		176.6 s	H-24, H-25, Me-27
27	1.20(d, 7.2)	7.8 q	H-25
29	1.31 (s)	28.4 q	Me-30
30	1.44 (s)	30.1 q	H-5, Me-29

[a] Measured at 400 MHz (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$. [b] s = C, d = CH, t = CH_2, q = CH_3.

The freeze-dried fruits of *S. arisanensis* (3.1 kg) collected from Nantou county, Taiwan were extracted with ethanol at room temperature, and the crude extract was partitioned between equal volumes of ethyl acetate and water. The EtOAc-soluble portion was further partitioned in *n*-hexane/MeOH/H_2O (4:3:1) to give an aqueous layer. The aqueous residue was chromatographed on a Sephadex LH-20 and eluted with MeOH to yield four fractions (sh-1−4). The second fraction (sh-2, 52 g) was subjected to a Si gel column (*n*-hexane/EtOAc 1:0 to 0:1) to give eight subfractions (sh-2-1−sh-2-8). The fraction sh-2-8 was further purified by a normal-phase HPLC column (*n*-hexane/CH_2Cl_2/MeOH, 30:65:5) and then a reversed-phase HPLC column (MeOH/H_2O, 1:1) to afford **1** (2 mg).



Schinarisanlactone A (**1**),[13] [α] +8 (MeOH), was obtained as colorless needles. The molecular formula $C_{28}H_{36}O_9$ was deduced from ESI-HRMS at *m*/*z* 539.2257 ([M+Na]$^+$), indicating 11 degrees of unsaturation. The IR spectrum showed intense absorption bands at 3457 and 1770 cm^{-1}, suggesting the presence of hydroxyl and ester functionalities. From analysis of DEPT spectra, a total of 28 carbon signals were found and classified into nine quaternary carbons (including two ketone carbonyl, one ester carbonyl, five oxygenated carbons), nine methines (including three oxygenated carbons), four aliphatic methylenes, and six methyl groups. Thus compound **1** contains an octacyclic ring system after deduction of three double bonds. The presence of four tertiary methyl singlets (δ_H 1.03, 1.31, 1.33, 1.44) and two secondary methyl doublets (δ_H 1.20, J = 7.2 Hz; 1.26, J = 6.4 Hz) in the ^{1}H NMR spectrum revealed that compound **1** is a unique bisnortriterpenoid different from nortriterpenoids of the schisanartane type.[14,15]

Analysis of ^{1}H−^{1}H COSY correlations of **1** established three segments of C-5/C-6/C-7/C-8, C-11/C-12, and C-16/C-22/C-23/C-24/C-25/C-27 in addition to C-21/C-20/C-22 (**I**−**III** in Figure 1). In the HMBC spectrum, the correlations of H-16, Me-18, H-20, and Me-21 with C-17 (δ_C 219.6), correlations of H-20 with C-13 (δ_C 49.9) and C-16 (δ_C 44.5), and corrlations of H-24 and Me-27 with C-26 (δ_C 176.6) along with segment **III** revealed the presense of a cyclopentaone (ring F) and a γ-lactone (ring H). Thus, the presence of an octa-ring (C-8 to C-16) and a ketone group located at C-14 was assigned by HMBC correlations of H-11α with C-8 (δ_C 59.3) and C-9 (δ_C 81.1), and of H-12α with C-13 (δ_C 49.9) and C-16 (δ_C 44.5), and correlations of H-8, H-16, and H-22 with C-15 (δ_C 98.4), as well as of H-16 with the C-14 carbonyl carbon (δ_C 208.9) together with segment **II**. At this stage we concluded that **1** possessed the same oxygen bridge between C-9 and C-15 (ring D and E) and between

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(13) $[\alpha]^{25}_D$ +8 (*c* 1.0, MeOH); IR (neat) υ_{max} 3457, 1770, 1455, 1376 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; HRESIMS *m*/*z* 539.2257 [M + Na]$^+$ (calcd for $C_{28}H_{36}O_9Na$, 539.2254). Crystal data: orthorhombic system, Space group $P2(1)2(1)2(1)$, a = 10.33660 (10) Å, b = 10.93000 (10) Å, c = 23.5701 (3) Å, V = 2662.93 (5) Å^3, Z = 4, d = 1.333 Mg/m^3. A crystal of dimensions 0.25 × 0.20 × 0.15 mm^3 was used for measurement on a Siemens SMART CCD XRD. The total number of independent reflections measured was 9595, of which 4848 were observed [R(int) = 0.0248]. Completeness to θ = 68.00°: 100.0%. Absorption correction: Semiempirical from equivalents. Max and min transmission: 1.00000 and 0.85844. The structure was solved by direct methods and refined by a full-matrix least-squares on F^2. Final R indices [$I > 2\sigma(I)$]: $R1$ = 0.0523, $wR2$ = 0.1432. The final X-ray crystallographic model is shown in Figure 4.

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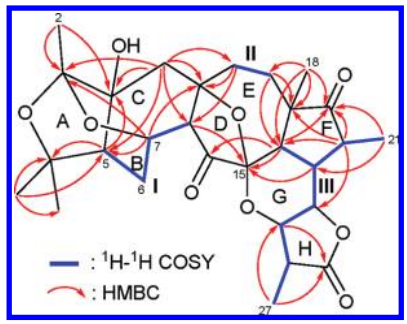


Figure 1. Key ^{1}H−^{1}H COSY and HMBC correlations of **1**.

C-15 and C-24 (ring G) after detailed analysis of 1D and 2D NMR data and comparison with schindilactone D.[15] HMBC correlations of H-19 with C-1 (δ_C 108.2), C-5 (δ_C 47.4), C-8 (δ_C 59.3), C-9 (δ_C 81.1), and C-10 (δ_C 79.3) and correlations of H-5 with two oxygenated quaternary carbons C-4 (δ_C 80.5) and C-10, and combined with segment **I**, revealed the presence of ring B. Furthermore, the HMBC correlations of both Me-29 and Me-30 with C-4 and C-5 indicated that two geminal methyl groups are attached at C-4. Moreover, the HMBC correlations of H-7/C-1 and Me-2/C-1 and C-10 suggested that an oxepane ring is located between C-1 and C-7 (ring C) and a methyl group (Me-2) is attached at C-1. Therefore, the planar structure of schinarisanlactone A was assigned as **1**, which belongs to an unprecedented 5/7/7/5/7/5/6/5-fused octacyclic ring system with loss of C-3 and has an additional oxygen bridge between C-1 and C-7.

The relative configuration of schinarisanlactone A (**1**) was determined by the analysis of NOESY correlations (Figure 2).

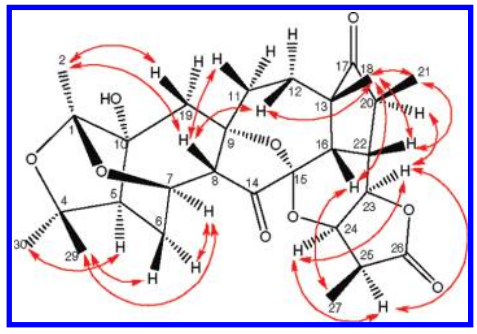


Figure 2. Selected NOESY correlations of **1**.

Assuming that the Me-18 of **1** was in a β-orientation similar to the schisanartane-type nortriterpenoids,[14,15] the NOESY correlations of Me-18/H-12β, H-16, Me-21, H-22, H-16/Me-27, and H-12β/H-8 indicated that they are on the β-face. On the other hand, the NOESY correlations of H-20/H-23/H-24/H-25 suggested that H-20, H-23, H-24, and H-25 are α-oriented.

There are two possible arrangements of 7-oxabicyclo[3.2.2]-nonane (rings B and C) in **1**. One is the β-orientation and the other one is the α-orientation (Figure 3) of the oxgen bridge between C-1 and C-7. However, the latter was eliminated by

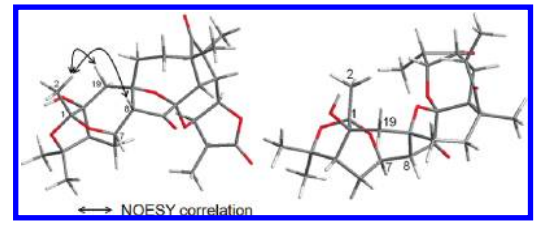


Figure 3. Computer-generated perspective models of **1** with β- (left) and α-oriented (right) oxygen bridge between C-1 and C-7 using MM2 force field calculation.

the key NOESY correlations of H-8/Me-2/H-19. Although no NOESY correlation between Me-2 and H-7 was found, they were both on the same α-face. The geminal methyl groups (Me-29 and Me-30) could be distinguished by the observation of correlation between Me-30 and H-5. The final relative configuration was confirmed by a single-crystal X-ray diffraction analysis, and a perspective drawing of structure **1** is provided in Figure 4. On

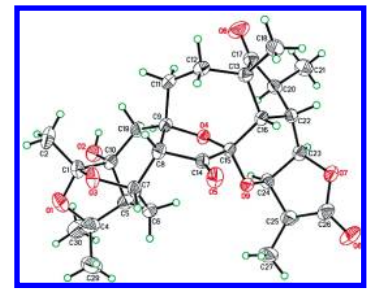
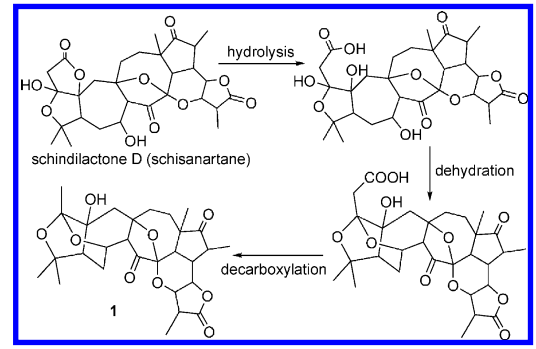


Figure 4. Perspective drawing of the X-ray structure of **1**.

the basis of the above findings, compound **1** was elucidated and a name schinarisanlactone A was given.

A plausible biogenetic pathway for schinarisanlactone A (**1**) is illustrated in Scheme 1. It might be generated

Scheme 1. Plausible Biogenetic Pathway for **1**



from a schisanartane precursor such as schindilactone D.[15] These steps involve hydrolysis of lactone, dehydration with a seven-membered ring formation, and decarboxylation to produce compound **1**.

Schinarisanlactone A (**1**) was tested for in vitro inhibitory activity against the HIV virus. AZT was used as a positive control. It showed significant inhibition (11.8% survival rate) against the HIV virus at 10 μM.

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Supporting Information Available: ^{1}H and ^{13}C NMR, ^{1}H$-^{1}$H COSY, HMQC, HMBC, and NOESY spectra and X-ray crystallographic data (CIF) for **1**, and anti-HIV bioassay method. This material is available free of charge via the Internet at http://pubs.acs.org.

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