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Arisandilactone A, a New Triterpenoid from the Fruits of *Schisandra arisanensis*

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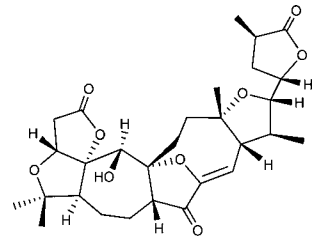
ABSTRACT



Arisandilactone A (**1**)

A phytochemical investigation of the fruits of *Schisandra arisanensis* has yielded a novel triterpenoid, arisandilactone A (1). Compound 1 has an unprecedented skeleton having a 5/5/7/5/8/5-fused hexacyclic ring system. The structure of 1 was elucidated by spectroscopic methods, especially 2D NMR techniques (COSY, HMQC, HMBC, and NOESY) and was confirmed by X-ray crystallographic analysis. A plausible biosynthetic pathway for 1 is also discussed.

The genus *Schisandra* (family, Schisandraceae) has been commonly used in traditional Chinese medicine for a thousand years. This medicinal plant has aroused a lot of interest because of its diverse healing properties and unique chemical structures. Numerous pharmaceutical effects were recently reported including anti-HIV,[1] anti-HBV,[2] antitumor,[3] and antioxidant[4] activities. Several novel skeletons such as C_{18}-dibenzocyclooctadiene lignan, schisanartane-type nortriterpenoid, wuweiziartane-type nortriterpenoid, and kadlongilactone-type triterpenoids and kadsuphilactone A from Schisandraceaous plants[5,6] were also published previously.

S. arisanensis is an endemic plant only distributed in the mountainous area of Taiwan. Previously, 11 new lignans have been isolated and characterized.[7] Herein, we report a novel nortriterpenoid, designated as arisanlactone A (**1**) possessing a 5/5/7/5/8/5-fused hexacyclic ring system, which was isolated from the fruits of *S. arisanensis* after extensive column chromatography.

The freeze-dried fruits of *S. arisanensis* (1.4 kg) were extracted thrice with acetone at room temperature and concentrated under reduced pressure to afford a crude extract (300 g). The crude extract was partitioned between EtOAc and H_2O (1:1) to obtain an EtOAc-soluable layer. After evaporating the organic solvent, this residue was further partitioned between *n*-hexane/MeOH/H_2O (4:3:1) to give a

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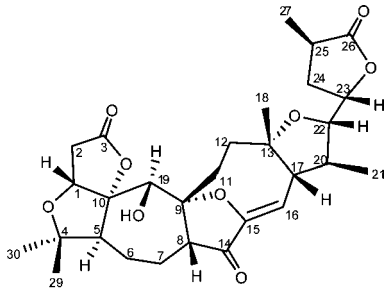
MeOH/H_2O extract. The MeOH/H_2O extract (34.7 g) was subjected to a Si gel flash column (*n*-hexane/EtOAc 40:1 to 0:1) to furnish fractions A−H. Fraction H (2.9 g) was separated on a Sephadex LH-20 and eluted with MeOH to yield a fraction H-1 (1.4 g), which was further chromatographed on a Si gel column (*n*-hexane/CH_2Cl_2/MeOH 100:20:1 to 0:10:1) to give subfraction H-1b. Subfraction H-1b was subjected to a reverse-phase HPLC (MeOH/H_2O 5:3) and further purified by a normal-phase HPLC (*n*-hexane/CH_2Cl_2/MeOH 120:80:2.5) to yield **1** (8.7 mg).



Arisandilactone A (**1**)

Arisandilactone A (**1**) was obtained as a colorless crystal and had the molecular formula $C_{29}H_{38}O_9$ (Δ = 11), as deduced from HRESIMS (*m*/*z* 553.2410 $[M + Na]^+$).[8] The IR spectrum unveiled the presence of hydroxyl (3448 cm^{-1}), ester (1766 cm^{-1}), and olefinic (1653 cm^{-1}) functionalities. The ^1H NMR data of **1** (Table 1) clearly indicated the existence of five methyls, one olefinic methine, and four oxygen-bearing methines. The ^{13}C NMR (Table 1) and DEPT spectra of **1** showed 29 carbon signals, consisting of one conjugated ketone carbonyl, two ester carbonyls, one trisubstituted double bond, four oxymethines, four oxygenated quaternary carbons, five aliphatic methines, six aliphatic methylenes, and five methyls. Due to the fact that **1** showed quite distinctive 1D and 2D NMR data different from those of published nortriterpenoids, the planar structure of **1** was established by assembling of three substructures **X**, **Y**, and **Z** (Figure 1).

Two proton spin systems were observed from their COSY correlations between H-1 (δ 4.72)/H-2 (δ 2.61, 3.31) and H-5 (δ 2.30)/H-6 (δ 1.56, 1.98)/H-7 (δ 1.70, 2.03)/H-8 (δ 2.35). H-1 and H-2 also showed HMBC correlations to C-3 (δ 175.5) and C-10 (δ 97.1) revealing the presence of a γ-lactone (ring A). Coupled with this, the HMBC correlations of H-19 (δ 3.93) with C-1 (δ 78.8), C-5 (δ 61.8), C-8 (δ 52.5), C-9 (δ 87.3), and C-10 and correlations of H-5 (δ 2.30) with C-10 and C-4 (δ 84.3), along with the proton spin system from H-5 to H-8, could establish rings B and C. Moreover, the HMBC correlations of both H-29 (δ 1.21) and H-30 (δ 1.31) with C-4 and C-5 indicated two geminal methyl groups attached at C-4. Thus, substructure **X** was established accordingly.

(8) $[\alpha]^{25}_D$ +7 (*c* 0.1, MeOH); IR (neat) ν_{max} 3448, 1776, 1653, 1458, 1375, 1068 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; HRESIMS *m*/*z* 553.2410 $[M + Na]^+$ (calcd for $C_{29}H_{38}O_9Na$, 553.2413). Crystal data: orthorhombic system, space group P2(1)2(1)2(1), a = 10.4314(7) Å, b = 15.4544(12) Å, c = 17.8219(16) Å, V = 2873.1(4) Å^3, Z = 4, d = 1.268 Mg/m^3. A crystal of dimensions 0.10 × 0.10 × 0.05 mm was used for measurement on a Siemens SMART CCD XRD. The total number of independent reflections measured was 6513, of which 4745 were observed [R(int) = 0.0556]. Completeness to θ = 67.97°: 99.4%. Absorption correction: semiempirical from equivalents. Max. and min. transmission: 1.00000 and 0.23796. 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 = 0.0754, wR2 = 0.1662. The final X-ray model is shown in Figure 3.

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

no.	δ_H (mult, J in Hz)	δ_C (mult)[b]	HMBC ($^1H-^{13}C$)
1	4.72 (d, 5.7)	78.8 (d)	H-19
2	2.61 (d, 18.3)	34.7 (t)	
	3.31 (dd, 5.7, 18.3)		
3		175.5 (s)	H-1, H-2
4		84.3 (s)	H-5, Me-29, Me-30
5	2.30 (m)	61.8 (d)	H-19, Me-29, Me-30
6	1.56 (m)	22.6 (t)	
	1.98 (m)		
7	1.70 (m)	22.9 (t)	
	2.03 (m)		
8	2.35 (m)	52.5 (d)	H-11, H-19
9		87.3 (s)	H-11, H-19
10		97.1 (s)	H-1, H-2, H-19
11	1.44 (m)	31.3 (t)	H-19
	1.98 (m)		
12	1.20 (m)	33.7 (t)	Me-18
	2.24 (m)		
13		84.9 (s)	Me-18, H-22
14		199.7 (s)	H-7, H-8, H-16
15		153.4 (s)	H-16
16	5.73 (d, 8.6)	111.3 (d)	
17	2.21 (m)	55.6 (d)	Me-18
18	1.17 (s)	27.2 (q)	H-17
19	3.93 (d, 7.9)	68.5 (d)	H-1, H-11
20	3.36 (m)	39.8 (d)	H-17
21	1.08 (d, 6.3)	15.5 (q)	H-17
22	3.52 (d, 9.6)	87.6 (d)	
23	4.52 (d, 8.8)	75.9 (d)	
24	2.03 (m)	33.5 (t)	H-25
	2.45 (m)		
25	2.92 (m)	34.2 (d)	H-24
26		182.8 (s)	H-23, H-25, Me-27
27	1.22 (overlapped)	15.9 (q)	
29	1.21 (s)	21.5 (q)	H-30
30	1.31 (s)	28.4 (q)	H-29
OH	4.03 (d, 7.9)		

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

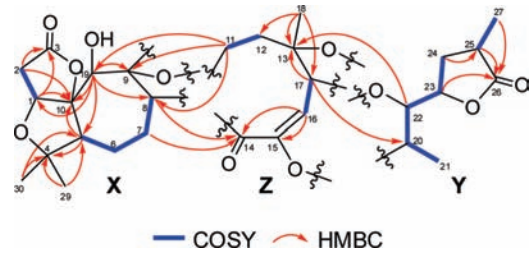


Figure 1. Substructures and selected 2D NMR correlations of **1**.

Through study of the COSY spectrum, two proton sequences between H-21 (δ 1.08)/H-22 (δ 3.52)/H-23 (δ 4.52)/H-24 (δ 2.03, 2.45) and H-25 (δ 2.92)/H-27 (δ 1.22) were obtained, respectively. In the HMBC spectrum, correlations of H-23, H-25, and H-27 with C-26 (δ 182.8) and correlation of H-24 with C-25 (δ 34.2) combined two proton sequences and indicated that there was a γ-lactone ring in the substructure **Y**. In the substructure **Z**, an α,β-unsaturated ketone was elucidated by the upfield chemical shift for C-14 (δ 199.7) and the presence of only one pair of olefinic carbons C-15 (δ 153.4) and C-16 (δ 111.3) in the ^{13}C NMR spectrum. In the COSY spectrum of **1**, the cross peaks of H-11 (δ 1.44, 1.98)/H-12 (δ 1.20, 2.24) and H-16 (δ 5.73)/H-17 (δ 2.21) were also observed. These two proton spin systems were further connected by the HMBC correlations of methyl H-18 (δ 1.17) with C-12 (δ 33.7), C-13 (δ 84.9), and C-17 (δ 55.6) and of H-17 (δ 2.21) with C-13. Consequently, the above 2D NMR spectroscopic analysis established substructure **Z**.

By detailed analysis of the HMBC spectrum, the key correlations of H-17 with C-20 (δ 39.8) and C-21 (δ 15.5) completed the linkage between C-17 and C-20. In addition, the oxymethine H-22 was ^{3}J-correlated with oxygenated quaternary carbon C-13 confirming the assignment of an oxygen bridge between C-13 and C-22. Thus, the substructures **Y** and **Z** were combined unequivocally.

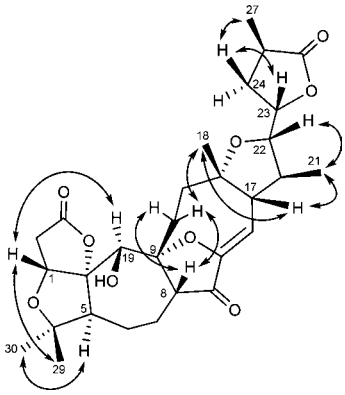


Figure 2. Selected NOESY correlations of **1**.

The HMBC correlation of H-11 with C-8, C-9, and C-19 and correlations of both H-7 and H-8 with C-14 further indicated that substructures **X** and **Z** were connected mutually. On the basis of the above discussion, three substructures were reunited and showed ten degrees of unsaturation. The remaining one degree of unsaturation implied that one ring was required in coincidence with its molecular formula. Finally, the oxygenated quaternary carbons (C-9 and C-15) were connected with only one oxygen between them to agree with its molecular formula.

The relative setereochemistry of **1** was determined on the basis of NOESY correlations (Figure 2) and further confirmed by X-ray crystallographic analysis (Figure 3), assuming one of the geminal methyls (H-29) is located in the

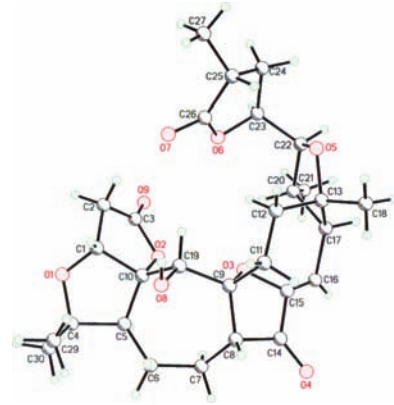
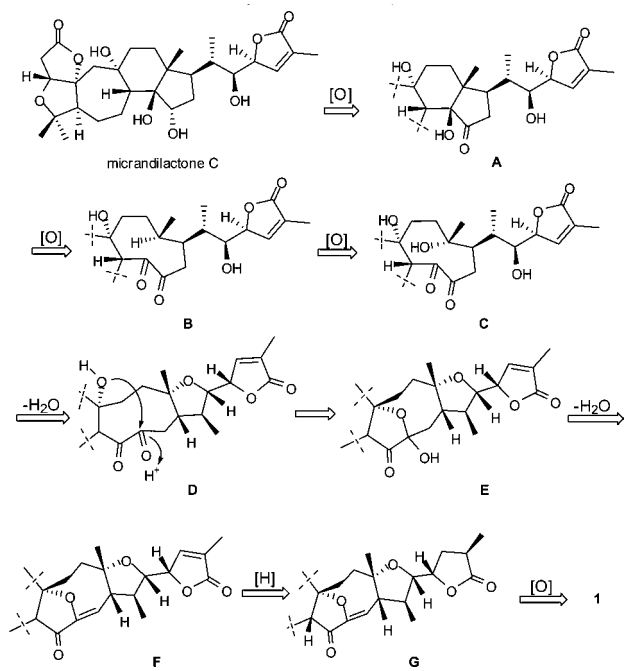


Figure 3. Perspective drawing of the X-ray structure of **1**.

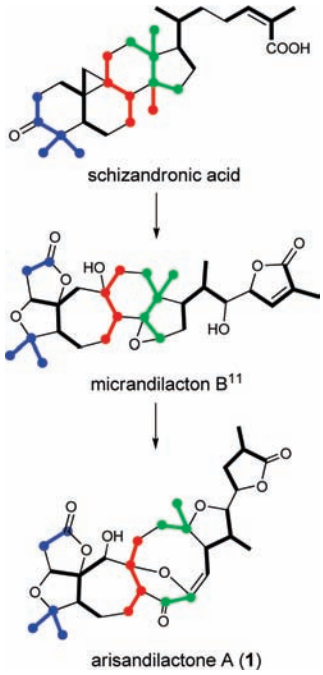
β-orientation. The NOESY spectrum of **1** showed correlations of H-29/H-1/H-19 indicating the proximity of correlated proton pairs in space. Conversely, NOE cross-peaks were found between H-30 and H-5, suggesting that they were on the α-face. Considering the biogenetic pathway, the oxygenated substituent at C-9 was frequently on the α-face leading to the β-orientation of the C-9/C-11 bond. Accordingly, the presence of NOESY correlations between H-8/H-11/Me-18 displayed that H-8 and Me-18 were in β-orientation. In turn, the NOESY cross-peaks of Me-18/H-17/Me-21/H-22 and H-23/H-24β/Me-27 suggested β-orientation of these protons. On the basis of the above findings, structure **1** was identified as arisandilactone A.

A plausible biogenetic pathway of **1** was postulated as shown in Scheme 1 based on the biosynthetic pathway from schiartane-type nortriterpenoid to preschisanartane-type nortri-

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



Scheme 2. Biogenetic Pathway of **1**, Showing Isoprene Units from Schizandronic Acid[10]



terpenoid.[5,9] Arisanlactone A (**1**) might be derived from micrandilactone C through the intermediates **A**−**G**. This pathway involves oxidation, oxidation with ring expansion, hydroxylation, dehydration with five-membered ring formation, dehydration to hemiketal, and dehydration to produce **1**. Scheme 2 illustrates the final backbone of isoprene units in **1**, which shows the green isoprene has been degraded to produce two and three carbon units in a nine-membered cycloketone from schizandronic acid through the intermediate micradilacton B.

Compound **1** exhibited weak inhibitory activity (14.0 ± 3.30%) in vitro against HSV-1 virus replication at 100 μM. Acyclovia was used as a standard compound, which showed 97.0 ± 1.0% inhibition at 2.5 μM. The plaque reduction assay is described in the Supporting Information.[12]

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Supporting Information Available: 1D and 2D NMR spectra and X-ray CIF file for compound **1** and plaque reduction assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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