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Schilancitrilactones A–C: Three Unique Nortriterpenoids from *Schisandra lancifolia*

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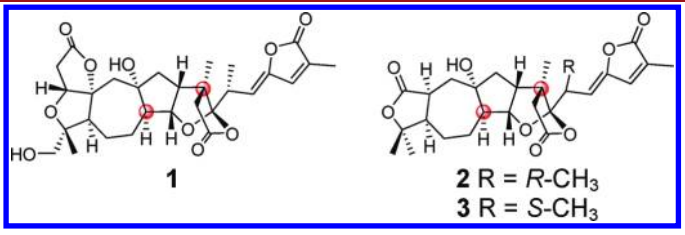
ABSTRACT



Three unique nortriterpenoids, schilancitrilactones A–C (1–3), were isolated from the stems of *Schisandra lancifolia*. Compound 1 possesses a 5/5/7/5/5/5-fused hexacyclic ring system with a C_{29} backbone, while 2 and 3 feature a C_{27} skeleton with a 5/7/5/5/5-fused pentacyclic ring system. Their absolute stereochemistries were established by CD and single-crystal X-ray diffraction experiments. Compound 3 showed anti-HIV-1 activity with an EC_{50} value of 27.54 μg/mL, and 1 exhibited antifeedant activity at 15.73 μg/cm^2.

Schinortriterpenoids (*Schisandra* nortriterpenoids) are a series of naturally occurring polycyclic molecules, which are interesting for study of their structures, bioactivities, and synthesis. From a biogenetic point of view, these compounds are generally supposed to derive from the common cycloartane skeleton by oxidative cleavage, ring rearrangement, loss of carbons, and other reactions, which lead to the formation of 10 distinct skeletons.[1] More interestingly, some of them were found to possess anti-HIV-1 and antitumor bioactivities.[1a,c,2] As a consequence, these exotic schinortriterpenoids have drawn widespread

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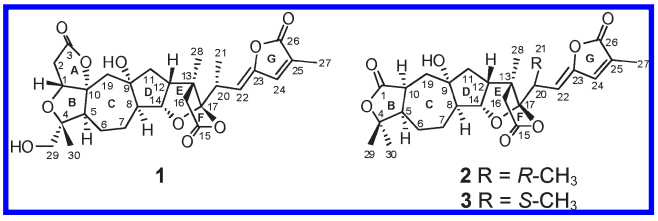
attention and, especially, have brought herculean challenges and ambitious targets for organic synthesis endeavors.[3] Despite the great difficulties in the organic synthesis of these complex molecules for the highly oxygenated and polycyclic scaffolds with many stereogenic centers, the total synthesis of schindilactone A has been accomplished recently.[3a]

Schisandra lancifolia (Rehd. et. Wils.) A. C. Smith, belonging to the genus *Schisandra* of the family Schisandraceae, denotes excellent sources for the discovery of architecturally intriguing nortriterpenoids due to its accumulating high level of this class of secondary metabolites.[1a,e,4] Our further chemical research on the stems of *S. lancifolia* resulted in the isolation of three unique nortriterpenoids, schilancitrilactones A–C (**1**–**3**). Compound **1** possesses a 5/5/7/5/5/5-fused hexacyclic ring system with a C_{29} backbone, while **2** and **3** feature a C_{27} skeleton with a 5/7/5/5/5-fused pentacyclic ring system. It is worthy of note that their structures characterize with an α-orientation hydrogen and an α-orientation methyl at C-8 and C-13 positions, respectively, which are incompatible with those of a common cycloartane triterpenoid.[1a] In addition, the three *cis*-fused five-membered rings (rings D–F), which possess the entire envelope conformations and involve 7 contiguous chiral centers (including 2 quaternary ones), form a structurally rigid tricyclic moiety. Herein, we describe their isolation, structural elucidation including absolute stereochemistries, plausible biogenetic pathway, and biological activities.[5]



Compound **1** was obtained as optically active colorless needle crystals ($[\alpha]_D^{18.1}$ +13.8). The molecular formula $C_{29}H_{36}O_{10}$ was established from the quasimolecular $[M + Na]^+$ ion at *m*/*z* 567.2214 in HRESIMS. The IR spectrum showed absorptions at 3434 and 1769 cm^{-1}, revealing the existence of hydroxyl and carbonyl groups. The ^1H NMR spectrum displayed signals due to one secondary methyl and three tertiary methyls. Two olefinic proton signals at δ_H 4.97 (d, J = 10.5 Hz) and 6.98 (br d, J = 0.8 Hz) suggested the presence of two trisubstituted double bonds (Table S1 in Supporting Information). The ^{13}C NMR and DEPT spectra exhibited signals for 29 carbons, including three carboxylic carbons, four olefinic carbons, five quaternary carbons (four oxygenated ones), six methines (two oxygenated ones), seven methylenes (one oxygenated one), and four methyls (Table 1). By analysis of the HSQC spectrum, all protons were assigned to their respective carbons unambiguously. The above evidence implied that **1** was a highly oxygenated nortriterpenoid.

Table 1. ^{13}C NMR Data for Compounds **1**–**3** ($CDCl_3$, δ in ppm)[a]

position	1	2	3
1	80.8 (d)	179.3 (s)	179.2 (s)
2	34.9 (t)		
3	173.4 (s)		
4	87.1 (s)	85.4 (s)	85.3 (s)
5	52.8 (d)	46.9 (d)	46.8 (d)
6	21.5 (t)	20.4 (t)	20.4 (t)
7	23.7 (t)	23.7 (t)	23.7 (t)
8	53.3 (d)	52.5 (d)	52.3 (d)
9	82.7 (s)	82.1 (s)	82.3 (s)
10	98.4 (s)	39.5 (d)	39.5 (d)
11	41.9 (t)	42.4 (t)	42.4 (t)
12	50.5 (d)	51.7 (d)	52.1 (d)
13	50.4 (s)	50.7 (s)	50.9 (s)
14	84.7 (d)	86.0 (d)	86.5 (d)
15	173.7 (s)	173.7 (s)	173.2 (s)
16	46.7 (t)	46.4 (t)	46.5 (t)
17	121.9 (s)	120.8 (s)	120.8 (s)
19	41.2 (t)	36.0 (t)	36.0 (t)
20	36.1 (d)	36.4 (d)	36.0 (d)
21	16.0 (q)	16.1 (q)	15.7 (q)
22	111.3 (d)	111.3 (d)	113.3 (d)
23	148.4 (s)	148.5 (s)	147.9 (s)
24	137.5 (d)	137.5 (d)	137.8 (d)
25	130.7 (s)	130.8 (s)	130.0 (s)
26	170.3 (s)	170.4 (s)	170.7 (s)
27	10.6 (q)	10.7 (q)	10.6 (q)
28	18.7 (q)	19.2 (q)	19.6 (q)
29	66.7 (t)	24.6 (q)	24.6 (q)
30	17.4 (q)	28.9 (q)	28.9 (q)

[a] Data for compounds **1**–**3** were recorded at 100 MHz, and the assignments were based on DEPT, HSQC, HMBC, COSY, and ROESY experiments.

The gross structure of **1** was initially deduced by comprehensive analysis of its 1D and 2D NMR spectra. The characteristic proton signals appearing as an ABX spin system at δ_H 4.19 (br d, J = 5.0 Hz), 2.69 (d, J = 18.1 Hz), and 2.76 (br dd, J = 18.1, 5.0 Hz) were assigned to H-1, H-2α, and H-2β, respectively.[1b] The existence of a γ-lactone group (ring A) was established by the ^1H–^1H COSY correlation of H-1/H_2-2, together with the HMBC correlations of both H-1 and H_2-2 with C-3 and C-10. The HMBC correlations of H_3-30 (δ_H 1.06) with C-4, C-5, and C-29 and of H-5 (δ_H 2.77) with C-4 and C-10 suggested the presence of ring B. The key HMBC correlations of H_2-19 (δ_H 1.91) with C-5, C-8, C-9, and C-10 and of H-8 (δ_H 2.38) and H_2-11 (δ_H 1.70 and 2.05) with C-9, along with the ^1H–^1H COSY correlations of H-5/H_2-6/H_2-7/H-8/H-14/H-12/H_2-11, indicated the existence of a seven-membered ring (ring C) fused with a five-membered ring (ring D). The above deduction, combined with the HMBC correlations of H_3-28 (δ_H 1.19) with C-12, C-13, C-16, and C-17 and of H_2-16 (δ_H 2.44 and 2.63) with a carbonyl group at δ_C 173.7 (C-15), led to the establishment of the planar substructure **1a** (Figure 1).

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(5) For detailed experimental procedures, physical-chemical properties, and ^1H NMR data for compounds **1**–**3**, see the Supporting Information.

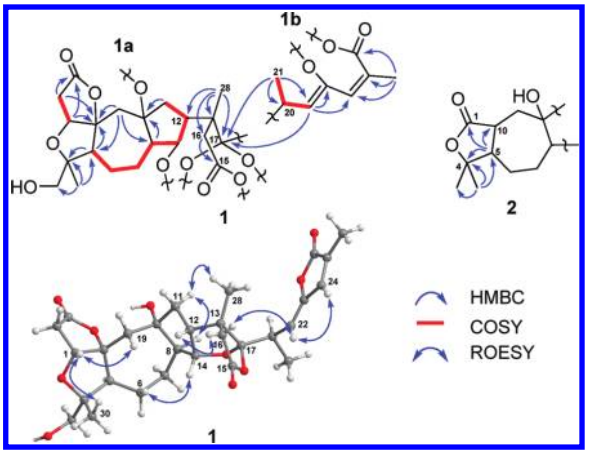

Figure 1. Selected 2D NMR correlations of **1** and **2**.

Furthermore, the secondary methyl (C-21, δ_C 16.0) was found to be located at C-20 by the HMBC correlations from H_3-21 to C-20 and C-22 and the $^1H-^1H$ COSY correlations of H_3-21/H-20/H-22. The tertiary methyl at δ_C 10.6 (C-27) was located at C-25 based on the HMBC cross-peaks of H_3-27 (δ_H 2.01) with C-24, C-25, and C-26. In addition, the presence of conjugated double bonds (C-22/C-23/C-24/C-25) was determined by the HMBC correlations from H-22 (δ_H 4.97) to C-23 and C-24. The *Z* geometry of the double bond between C-22 and C-23 was deduced from the ROESY correlation of H-22 with H-24 (Figure 1). Accordingly, the partial structure **1b** was established as shown (Figure 1).

The linkage of **1a** and **1b** through a carbon−carbon connection of C-17 and C-20 could be deduced from the HMBC correlations from both H_3-21 and H-22 to C-17 (Figure1). In order to establish the absolute stereochemistry of **1**, CD and single-crystal X-ray diffraction experiments were carried out. In the CD spectrum, compound **1** showed a negative Cotton effect at 270 nm ($\Delta\varepsilon = -14.54$), similar to that of arisanlactone A,[6] indicating an *R* configuration of C-20. Combining the X-ray diffraction analysis conducted with Cu Kα radiation, which resulted in a Flack parameter of −0.12(18) (CCDC 861514),[7] we determined the absolute stereochemistry of **1** as 1*R*, 4*R*, 5*S*, 8*R*, 9*S*, 10*R*, 12*R*, 13*R*, 14*S*, 17*R*, and 20*R* (Figure 2).

Compound **2** was isolated as colorless chunk crystals ($[\alpha]_D^{18.1}$ −41.0), and the formula, $C_{27}H_{34}O_8$, was deduced by HRESIMS analysis ($[M + Na]^+$, *m*/*z* 509.2168). Its ^{13}C NMR and DEPT spectra exhibited 27 carbon signals, and most of them were similar to those of **1** (Table 1). The observed differences could be rationalized to the absence of the γ-lactone group (ring A) in the western hemisphere of **2**. These suggested that **2** was a trinortriterpenoid, another new member of the family of schinortriterpenoids.

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(7) Flack, H. D. *Acta Crystallogr., Sect. A* **1983**, *39*, 876–881.

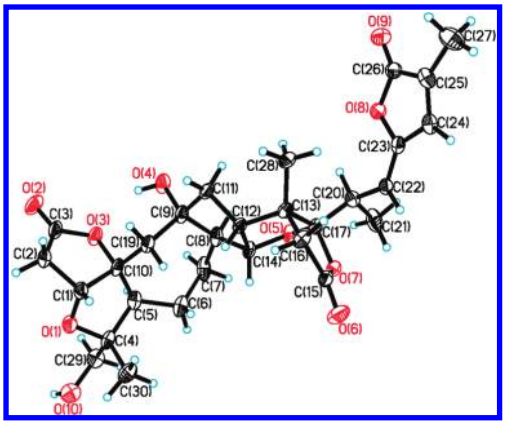


Figure 2. X-ray crystallographic structure of **1**.

Comprehensive assignments of carbon signals of **2** through extensive analysis of NMR spectra indicated that the signals at δ_C 179.3 and 39.5 were ascribed to C-1 and C-10, respectively. Furthermore, the presence of a γ-lactone ring (ring B) in **2** might be deduced by the HMBC correlations of H_3-30 (δ_H 1.44) with C-4, C-5, and C-29 and of H-5 (δ_H 2.47) with C-1, C-4, and C-10 (Figure 1). The CD spectrum of **2** showed a negative Cotton effect at 268 nm ($\Delta\varepsilon = -9.64$), similar to that of **1**, indicating an *R* configuration of C-20 in **2**, as well. Moreover, a single-crystal X-ray diffraction analysis was conducted with Cu Kα, which resulted in a Flack parameter of 0.00(19) (CCDC 861515). Thus, the absolute stereochemistry of **2** was established to be 5*R*, 8*R*, 9*S*, 10*S* 12*R*, 13*R*, 14*S*, 17*R*, and 20*R* (Figure 3).

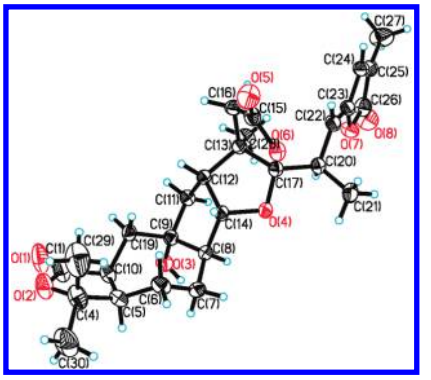
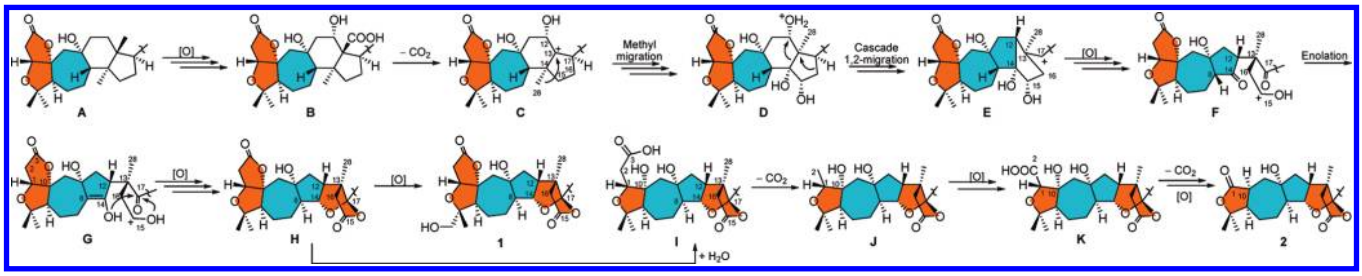


Figure 3. X-ray crystallographic structure of **2**.

The molecular formula of **3** was assigned to be $C_{27}H_{34}O_8$ by HRESIMS, which was the same as that of **2**. It was found that the NMR data of **2** and **3** were very similar (Table 1 and Table S1). Side-by-side comparison of their NMR data suggested that the minor differences may result from the distinctness of the side chains in the eastern hemisphere of **2** and **3**. Likewise, the double bond between C-22 and C-23 of **3** was *Z* geometry, judging from the ROESY correlation of H-22 with H-24.

Scheme 1. Hypothetical Biogenetic Pathway of **1** and **2**



Therefore, the reason for the differences of the side chain could be rationalized to the dissimilar configuration of C-20. The absolute configuration of C-20 of **3** could be disclosed by an empirical comparison of the CD with that of **2**. The positive Cotton effect at 271 nm ($\Delta\varepsilon = +24.29$) of **3** was reverse to the negative Cotton effect of **2** [268 nm ($\Delta\varepsilon = -9.64$)]. Therefore, C-20 of **3** could be assigned as *S* configuration, and the absolute configuration of **3** was further confirmed by single-crystal X-ray diffraction analysis using Cu Kα radiation (CCDC 861516) (Figure 4).

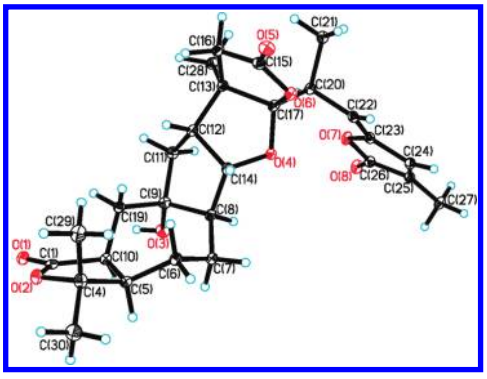


Figure 4. X-ray crystallographic structure of **3**.

From a structural point of view, the hydrogen at C-8 and the methyl group at C-13 and C-14 in the common cycloartane skeleton are *β*-, *β*-, and α-orientation, respectively.[1a] However, the structural characteristics of compounds **1**–**3** are inconsistent with those of cycloartane-type triterpenoids. It is reasonable to presume that the α-orientation hydrogen at C-8 might be formed through the enolation of C-8/C-14, and the α-orientation methyl at C-13 might be derived from a 1,2-methyl migration of the methyl group at C-14. A plausible biogenetic pathway of **1** and **2**, starting from a 3,4-*seco*-cycloartane triterpenoid (A), is proposed (Scheme 1). A cascade 1,2-migration is thought to be responsible for constructing the 7/5 ring system backbone from intermediate D to intermediate E.

The anti-HIV-1 activity of **1**–**3** was evaluated by the inhibition assay for the cytopathic effects of HIV-1 (EC_{50}) with AZT as a positive control ($EC_{50} = 0.005$ μg/mL and $CC_{50} > 200$ μg/mL) by using the method previously reported.[8] Compound **3** showed anti-HIV-1 activity with EC_{50} value of 27.54 μg/mL and a therapeutic index (TI) more than 7.26, while **1** and **2** were not bioactive with EC_{50} value > 100 μg/mL.

Among terpenoids functioning as protective substances for insects in the natural world,[9] meliaceous limonoids, which are known as tetranortriterpenoids, exhibit remarkable activity against a broad range of insect species.[10] Such biological knowledge is particularly lacking for schinortriterpenoids, and this special class of metabolites may play an important role as protective substances. Therefore, the antifeedant effect of **1**–**3** against a generalist plant-feeding insect cotton bollworm (*Helicoverpa armigera*) was assayed as described previously.[11] Compound **1** showed weak antifeedant activity, with an antifeedant index (AI%) of 14.1% at 15.73 μg/cm^2. Given that it is interesting to explore the biogical functions of schinortriterpenoids in chemical defense, we will continually make great effort to discover the effects of this class of compounds in the natural world.

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Supporting Information Available. Detailed experimental procedures, physical-chemical properties, 1D and 2D NMR, MS, IR, UV, CD, and ORD spectra, and X-ray crystal structures (CIF) of compounds **1**–**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.