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Schicagenins A–C: Three Cagelike Nortriterpenoids from Leaves and Stems of *Schisandra chinensis*

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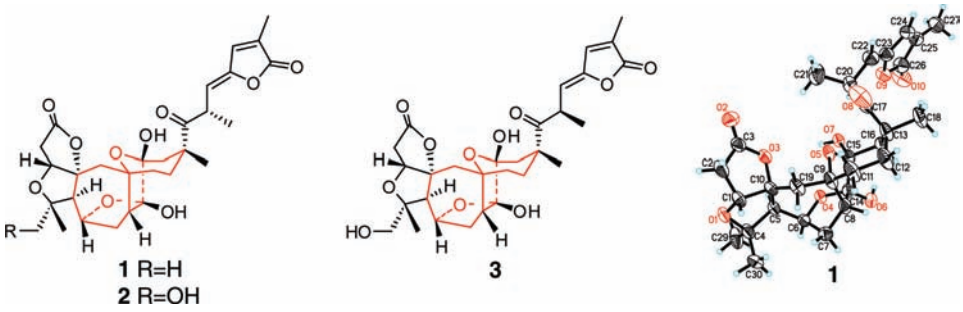
ABSTRACT



Schicagenins A–C (1–3), three unprecedented nortriterpenoids characterized with a tetracyclic oxa-cage motif and C_9 side chain, were discovered from the leaves and stems of *Schisandra chinensis*. Their structures were determined on the basis of extensive spectroscopic analysis, and the absolute stereochemistries were established by single-crystal X-ray diffraction and CD experiments. A plausible biosynthetic pathway of 1–3 was also discussed.

Triterpenoids, biogenetically derived from squalene or related acyclic 30-carbon precursors, are the most ubiquitous, nonsteroidal secondary metabolites in terrestrial and marine flora and fauna.[1] Although more than 20000 members which represent over 100 distinct skeletons of this group have been discovered,[1] new triterpenoid structures with novel skeletons continually emerge through extensive efforts in phytochemistry studies. This large member of natural products came to the foreground of interest of chemical sythesis because of their intriguing skeletons and promising bioactivities.[2]

Schisandra chinensis (Turcz.) Baill., belonging to the genus *Schisandra* of the family Schisandraceae, is an economically and medicinally important species that is endemic to the northeast part of China, Korea, and the far east of Russia. This plant has long been used as a sedative and tonic agent in traditional Chinese medicine, and its fruits have been used for the treatment of hepatitis for over 2000 years in China.[3] Previous investigations on chemical constituents of *S. chinensis* from Tonghua prefecture of the Jilin province in China have led to the isolation of a series of nortriterpenoids.[2c,4] Since the secondary metabolites would plausibly be

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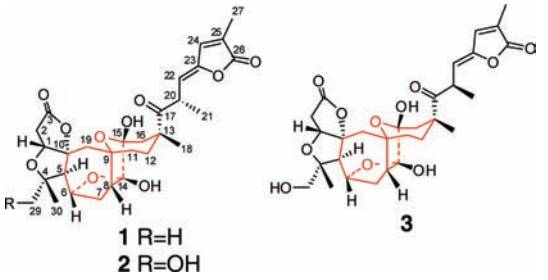
influenced by the ecological environment, we examined the leaves and stems of *S. chinensis* collected in the Yabuli mountain area of Heilongjiang province in China, which is adjacent to the east region of Russia and belongs to the native region of *S. chinensis*. Our current research led to the discovery of three unusual functionalized nortriterpenoids, schicagenins A–C (**1**–**3**),[5] which represented a new class of *Schisandra* triterpenoids characterized with a tetracyclic oxa-cage moiety and a C_9 side chain. In this communication, we report their isolation, structure elucidation including absolute stereochemistries, and cytotoxic activities.



Schicagenin A (**1**) was obtained as an optically active colorless crystal ($[\alpha]_D^{21.7}$ +349.3). Its molecular formula $C_{29}H_{36}O_{10}$ was established from HRESIMS (*m*/*z* 567.220, $[M + Na]^+$), requiring 12 degrees of unsaturation. The IR spectrum indicated the presence of hydroxyl groups (3440 cm^{-1}) and lactone groups (1769 cm^{-1}). The ^{13}C NMR and DEPT spectra of **1** resolved 29 carbon signals comprising two carbonyl groups, one ketone group, two double bonds, six quaternary carbons (five oxygenated ones), five methines (two oxygenated ones), six methylenes, and five methyls (Table 1). These suggested that **1** was a highly oxygenated nortriterpenoid. Considering that the NMR spectrum of **1** was quite different from those of the published nortriterpenoids from the *Schisandra* genus, we first attempted to establish the planar structure of **1** by analyzing its 2D NMR spectroscopic data.

The HMBC spectrum showed the following correlations: proton signal at δ_H 1.10 (H-29) with C-4, C-5, and C-30; the methine proton at δ_H 4.19 (H-1) with C-2, C-3, C-10, and C-19; the methine doublet at δ_H 2.46 (H-5) with C-1, C-6, C-7, and C-10; the methine signal at δ_H 4.48 (H-6) with C-8; the proton doublet at δ_H 2.81 (H-8) with C-9 and C-19. This evidence, along with two proton spin systems deduced from $^1H-^1H$ COSY correlations, H-1/H-2 and H-5/H-6/H-7/H-8, led to the establishment of partial structure **1a** (Figure 1).

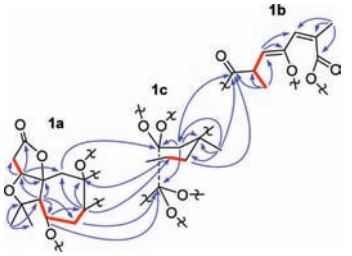


Figure 1. Selected HMBC (H→C in blue) and $^1H-^1H$COSY (— in red) correlations of **1**.

In addition, the HMBC spectrum of **1** also revealed obvious correlations of CH_3-27 (δ_H 1.82) with C-24, C-25, and C-26; of H-22 (δ_H 5.28) with C-23 and C-24; and of CH_3-21 (δ_H 1.81) with C-17, C-20, and C-22. The above evidence, along with a proton spin system deduced from $^1H-^1H$ COSY correlations, H-21/H-20/H-22, led to the establishment of partial structure **1b** (Figure 1). 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 2).

In the ^{13}C NMR spectrum, two hemiketal groups were elucidated by the downfield chemical shift for C-14 and C-15 (δ_C 112.9 and 103.9). The HMBC correlations from CH_3-18 (δ_H 1.08) to C-12, C-13 and C-16, and from CH_2-16 (δ_H 3.36 and 3.12) to C-13, C-14 and C-15, together with the proton spin system H-11/H-12 observed in the $^1H-^1H$ COSY spectrum, suggested the remaining part of the structure may be assigned as structure **1c** (Figure 1).

Table 1. ^{13}C NMR Data of Compound **1**–**3** (Pyridine-d_5, δ in ppm)[a]

position	1	2	3
1	82.4 (d)	82.8 (d)	82.8 (d)
2	35.8 (t)	35.8 (t)	35.9 (t)
3	175.2 (s)	175.1 (s)	175.7 (s)
4	82.7 (s)	86.1 (s)	86.1 (s)
5	63.2 (d)	58.3 (d)	58.0(d)
6	78.1 (d)	78.1 (d)	78.6 (d)
7	31.4 (t)	31.6 (t)	31.6 (t)
8	50.9 (d)	51.0 (d)	50.9 (d)
9	80.2 (s)	80.3 (s)	80.2 (s)
10	96.6 (s)	96.8 (s)	97.1 (s)
11	37.4 (t)	37.5 (t)	37.5 (t)
12	35.4 (t)	35.4 (t)	35.3 (t)
13	50.2 (s)	50.2 (s)	49.8 (s)
14	112.9 (s)	112.8 (s)	112.7 (s)
15	103.9 (s)	103.8 (s)	103.7 (s)
16	47.6 (t)	47.3 (t)	47.7 (t)
17	215.8 (s)	215.7 (s)	216.6 (s)
18	29.8 (q)	29.8 (q)	29.4 (q)
19	45.4 (t)	45.9 (t)	45.8 (t)
20	39.7 (d)	39.8 (d)	40.5 (d)
21	18.9 (q)	18.8 (q)	19.7 (q)
22	116.5 (d)	116.4 (d)	116.4 (d)
23	147.8 (s)	147.9 (s)	149.4 (s)
24	138.6 (d)	138.5 (d)	140.8 (d)
25	129.6 (s)	129.6 (s)	127.3 (s)
26	170.8 (s)	170.8 (s)	171.8 (s)
27	10.4 (q)	10.4 (q)	10.3(q)
29	28.6 (q)	68.3 (t)	68.1 (t)
30	22.1 (q)	18.0 (q)	17.9 (q)

[a] Data for compounds **1**–**3** were recorded at 125 MHz, and the assignments were base on DEPT, HSQC, HMBC, COSY, and ROESY experiments.

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

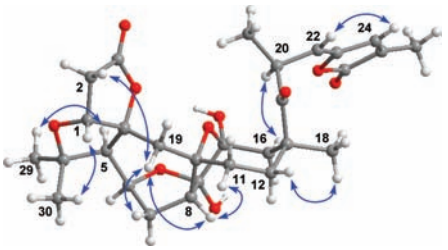


Figure 2. Key ROESY correlations of **1**.

Furthermore, the partial structures **1a** and **1c** were determined to be assembled by carbon−carbon connections of C-8 with C-14 and C-9 with C-11 on the basis of the HMBC correlations H-7 with C-14, H-8 with C-11 and C-14; H-11 with C-9; and H-19 with C-11 (Figure 1).

The HMBC correlation of the proton at δ_H 4.48 (H-6) to a hemiketal group at δ_H 112.9 (C-14) suggested the connection of C-6 (δ_C 78.1) to C-14 through an oxygen bridge. In addition, the key HMBC correlations of H-12, H-16, and H-18 with C-17 permitted partial structures **1c** and **1b** to be connected mutually through a carbon−carbon connection of C-13 and C-17 since C-9, C-14, C-15, C-23, C-24, and C-26 were fully substituted carbons and the 2D NMR spectra did not provide sufficient information to elucidate the connecting patterns of these carbons. Luckily, a single crystal of **1** was obtained from ethanol/H_2O after repeated recrystallization, and an X-ray diffraction analysis conducted using an anomalous dispersion with copper radiation resulted in a Flack parameter of −0.10(2),[6] which not only indicated the connection of C-9 to C-15 and C-23 to C-26 through oxygen bridges but also determined the absolute stereochemistry of **1** to be 1*R*,5*S*,6*S*,8*R*,9*S*,10*R*,-13*S*,14*R*,15*S*,20*S* (Figure 3).

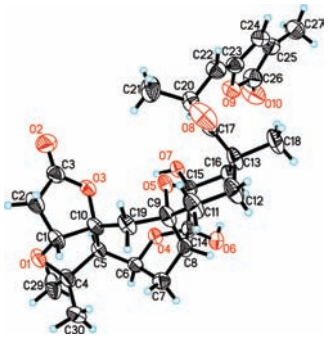


Figure 3. X-ray crystallographic structure of **1**.

Schicagenin B (**2**) was obtained as an optically active white solid ($[\alpha]_D^{21.7}$ +276.0). The HRESIMS (*m*/*z* 583.2151, $[M + Na]^+$) of **2** indicated a molecular formula of $C_{29}H_{36}O_{11}$, which was only one oxygen atom more than that of **1**. The ^{13}C NMR spectrum of **2** was very similar to that of **1** (Table 1). The main difference was the replacement of a methyl (δ_C 28.6) in **1** by an oxygenated methylene (δ_C 68.3) in **2**, which was assigned to be C-29 by the HMBC correlations of H-29 with C-4, C-5, and Me-30. The *Z* geometry of the double bond between C-22 and C-23 was deduced from a ROESY correlation of H-22 with H-24, which was the same as that of **1**. Since compounds **1** and **2** were structurally similar, the absolute configuration of C-20 of **2** could be assigned by an empirical comparison of the circular dichroism (CD) with that of **1**. Thus, the CD spectra of both **1** and **2** were measured, which showed similar Cotton effects in the CD spectra. Compound **1** showed a positive Cotton effect at 307 nm ($\Delta\varepsilon$ = +15.95) and a negative Cotton effect at 270 nm ($\Delta\varepsilon$ = −16.34), and **2** showed a positive Cotton effect at 300 nm ($\Delta\varepsilon$ = +15.95) and a negative Cotton effect at 270 ($\Delta\varepsilon$ = −16.59). Therefore, C-20 of **2** could also be assigned as an *S*-configuration, the same as in **1**. Other chiral centers in **2** could be determined to be identical to those of **1** by comparison of their chemical shifts and analysis of ROESY correlations.

Schicagenin C (**3**) was isolated as a white solid. Its molecular fomula, $C_{29}H_{36}O_{11}$, could be established by the HRESIMS, which showed a $[M + Na]^+$ ion peak at *m*/*z* 583.2150. The close resemblance between the NMR spectra of **2** and those of **3** indicated that **3** was another cagelike nortriterpenoid structurally similar to **2**. The differences of ^1H and ^{13}C NMR (Table S1, Supporting Information) data ($\Delta\delta = \delta$ **3**−**2**) between **2** and **3** of H-20 ($\Delta\delta_H$ −0.42), H-21 ($\Delta\delta_H$ −0.27), H-22 ($\Delta\delta_H$ +0.68), C-17 ($\Delta\delta_C$ +0.9), C-20 ($\Delta\delta_C$ +0.7), C-21 ($\Delta\delta_C$ +0.9), and C-23 ($\Delta\delta_C$ +1.5), along with the coupling constant of H-22 with H-20 reducing from 10.8 Hz in **2** to 6.7 Hz in **3**, and the coupling constant of H-21 with H-20 increasing from 6.3 to 7.0 Hz, suggested that **2** and **3** might be C-20 epimers. This deduction was further comfirmed by comparison of their CD spectra. Compound **3** showed a negative Cotton effect at λ_{max} 300 nm ($\Delta\varepsilon$ = −14.72), while a positive Cotton effect at 270 ($\Delta\varepsilon$ = +4.29). Therefore, C-20 of **3** was assigned as the *R*-configuration.

Cagelike natural products are a special class of complex molecules and are mainly attributed to iridoids,[7] sesquiterpenoids,[8] diterpenoids,[9] alkaloids,[10] xanthones,[11]

(6) The crystal data of schicagenin A (**1**) can be found in the Supporting Information. Deposition no. CCDC 825583.

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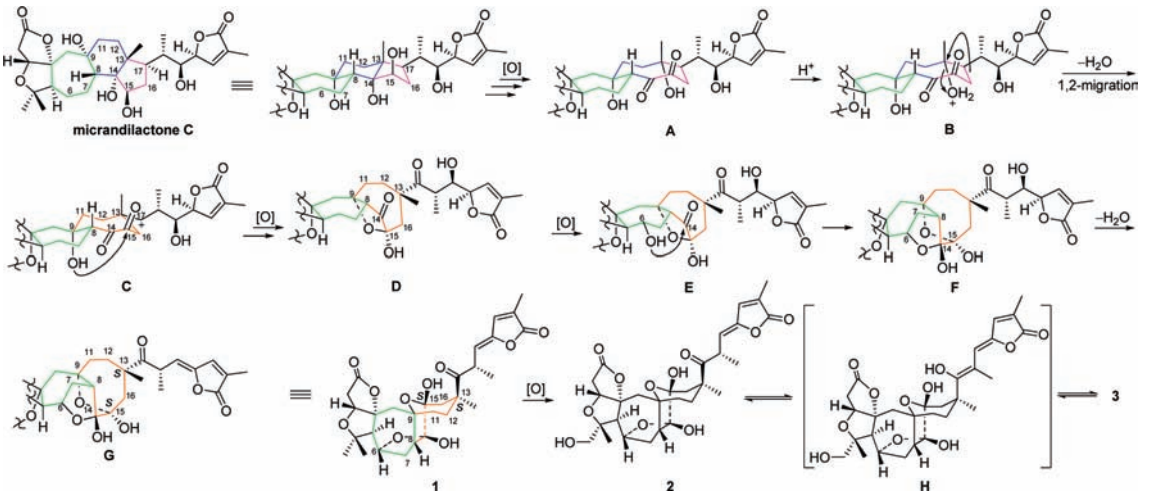
Scheme 1. Hypothetical Biogenetic Pathway of **1**−**3**



and phlorglucinod derivatives.[12] Some of them exhibit significant biological activities, including antitumor,[7a] antibiotic,[14] and neurite outgrowth-promoting[8] activities.

Therefore, they have brought great interest and challenges for synthetic chemists.[15] From the literature research, there are extremely rare reports on natural cagelike triterpenoids. Even a series of triterpenoids grouped as *Schisandra* nortriterpenoids have been isolated and elucidated from plants of genus the *Schisandra* thus far;[2c,16] schicagenins A−C (**1**−**3**) still represent a new class of *Schisandra* nortriterpenoids for their novel carbon skeleton, featuring an tetracyclic oxacage structure core and the C_9 side chain formed by highly oxygenation and rearrangement. As we reported, *Schisandra* nortriterpenoids are derived biogenetically from the cycloartane triterpenoids, and schiartane skeleton nortriterpenoids are considered to be the first class on the biosynthetic pathway because they still maintained the core cycloartane skeleton in their structues.[2c] Herein, we proposed a plausible biogenetic pathway of schicagenins A−C (**1**−**3**) from the schiartane-type nortriterpenoid micrandilactone C through intermediates A−H (Scheme 1). This pathway involves oxidation, dehydroxylation, 1,2-migration, and ring-closure, followed by dehydroxylation to form their structures. The formation of intermediate C from intermediate B by rearrangement involving the 1,2-migration is the key step to form the C_9 side chain and the unique carbon skeleton.

Compounds **1**−**3** were tested for the in vitro cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 human cancer cell lines using the MTT method.[17] However, none of them showed obvious inhibitory activity against those cells with $IC_{50} > 40\ \mu M$.

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

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