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Henrischinins A−C: Three New Triterpenoids from *Schisandra henryi*

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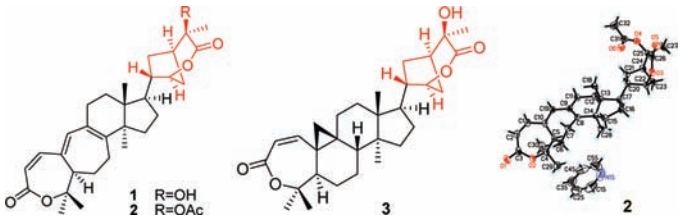
ABSTRACT



Three novel triterpenoids, henrischinins A−C (1−3), featuring the unique motif of a 3-one-2-oxabicyclo[3.2.1]-octane, were isolated from the leaves and stems of *Schisandra henryi*. Their structures were elucidated by spectroscopic methods, and the absolute configuration of 2 was confirmed by a single crystal X-ray diffraction. Compounds 1 and 2 showed weak cytotoxicity against HL-60 cell lines.

The genus of *Schisandra* (family Schisandraceae) is a well-known resource for biosynthesizing structurally diverse *Schisandra* triterpenoids with highly oxygenated, fused heterocyclic skeletons.[1] Over the past decade, considerable effort from our group has been devoted to the discovery of bioactive and novel triterpenoids from the genus *Schisandra*, and this work resulted in the isolation of quite a number of novel *Schisandra* triterpenoids, some of which exhibited significant cytotoxic activity against tumor cell lines[1a,2] and brought a great amount of interest and challenges for biogenetic and synthetic chemistry for years.[3]

In China, there are almost 23 endemic species distributed throughout the country, and many species of this genus have been traditionally used as folk medicine. The fruits of *S. henryi*, which belongs to the *Schisandra* genus, have been used in folk medicine as a surrogate of "Wu-Wei Zi", the fruits of *S. chinensis*, an important traditional Chinese medicine applied as sedative and tonic agents more than 2000 years.[1a,4] Previous studies on the chemical constituents of *S. henryi* conducted by us and other research groups have led to the isolation of a series of triterpenoids and nortriterpenoids,[4] and lignans.[5] In a continuation of our research program to discover structurally unique and bioactive *Schisandra* triterpenoids,[1a] henrischinins A−C

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(1) (a) Xiao, W. L.; Li, R. T.; Huang, S. X.; Pu, J. X.; Sun, H. D. *Nat. Prod. Rep*. **2008**, *25*, 871–891. (b) Alexander, P.; Georg, W. *J. Ethnopharmacol*. **2008**, *118*, 183–187.

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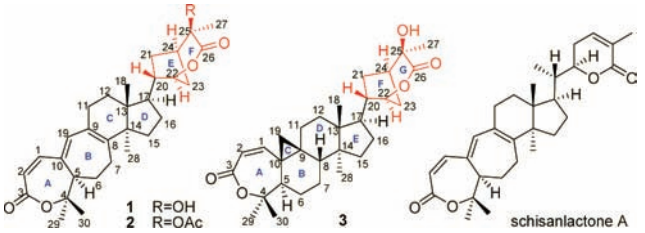
(**1**−**3**), which represented a new class of *Schisandra* triterpenoids featuring an unusual ring system of a five-membered carbon ring, were isolated from the 70% Me_2CO extract of the leaves and stems of *S. henryi*. The structures of these novel metabolites were elucidated by spectroscopic methods, especially 2D NMR techniques. The absolute configuration of **2** was also further confirmed by single-crystal X-ray diffraction. We demonstrate herein the isolation, structure characterization, and cytotoxic activities of these novel triterpenoids.



The air-dried leaves and stems of *S. henryi* (7.2 kg) were extracted with 70% aqueous acetone at room temperature three times. After removal of the solvent under reduced pressure, the crude extract (450 g) was partitioned between H_2O and EtOAc. The EtOAc part (226 g) was chromatographed over a silica gel column with a gradient elution of $CHCl_3$−acetone (1:0 to 0:1) to give nine fractions, A−I. Fraction E (60 g) was extensively separated over columns of MCI, Sephadex LH-20, and silica gel to afford subfractions E1−E9. Fraction E4 (465 mg) was purified by semipreparative HPLC (Agilent 1100 HPLC system; Zorbax SB-C-18, Agilent, 9.4 mm × 25 cm; MeOH−H_2O 65:35) to yield **1** (4 mg) and **3** (22 mg). Fraction E6 (1.2 g) was purified by semipreparative HPLC (MeCN−H_2O 48:52) to yield **2** (57 mg).

Henrischinin A (**1**) was obtained as an optically active white solid ($[\alpha]_D^{14.2}$ + 92.2). Its molecular formula $C_{30}H_{40}O_5$ assigned through its HRESIMS (*m*/*z* 503.2779, $[M + Na]^+$), requiring 11 degrees of unsaturation. The UV spectrum of **1** showed absorption maxima at 218, 261, and 331 nm, indicating the presence of a big conjugated system. The IR spectrum of **1** showed bands characteristic of a hydroxyl (3439 cm^{-1}) and two carbonyl groups (1736 and 1685 cm^{-1}). The ^{13}C NMR spectrum (Table 1) of **1** displayed the coexistence of five methyl singlets, eight methylenes, one oxygenated and four aliphatic methines, two oxygenated and two aliphatic quaternary carbons, two lactone groups, and three pairs of double bonds (three olefinic methines). Accordingly, a six-ring structure was required for **1** to fulfill the unsaturation requirement. Detailed analysis of the 1D and 2D NMR spectral data for **1** strongly suggested the same substructures for rings A−D with those of schisanlactone A.[6] However, the data for the northern hemisphere of **1** were quite different from those of schisanlactone A.[6] The proton spin systems of H-20/H-22/H_2-23/H-24/H_2-21 deduced from the $^1H-^1H$ COSY spectrum of **1** (Figure 1) indicated the presence

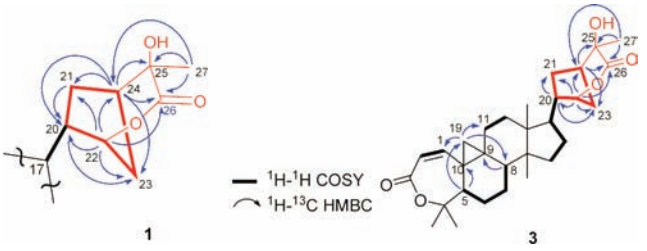


Figure 1. Selected HMBC (H→C) and $^1H-^1H$ COSY (—) correlations of **1** and **3**.

of a five-membered carbon ring E, which was further confirmed by the key HMBC correlations of H-20 with C-21, C-22, and C-23; of H-23α with C-20; and of H-22 and H-24 with C-20, C-21, and C-23. Moreover, a six-membered lactone ring (ring F) was assembled on the basis of the HMBC correlations (Figure 1) of H-22 with C-23 and C-24; of H_3-27 with C-24, C-25, and C-26; and of H-24 with C-22, C-23, C-25, and C-26. The HMBC correlation of the proton at δ_H 4.58 (H-22) to an ester carbonyl group at δ_C 177.1 (C-26) suggested the connection of C-22 (δ_C 85.2) to C-26 through an oxygen bridge. Thus, the motif of the northern hemisphere for **1** was elucidated as a 4-methyl-3-one-2-oxabicyclo[3.2.1]octane moiety. Taking the above data into account, the planar structure of **1** was completely assembled.

The relative configuration of **1** was elucidated by ROESY experiment modeling in Chem3D 10.0 (Cambridge Soft, Inc.) as shown in Figure 2. Biogenetically, H-5 and H-17

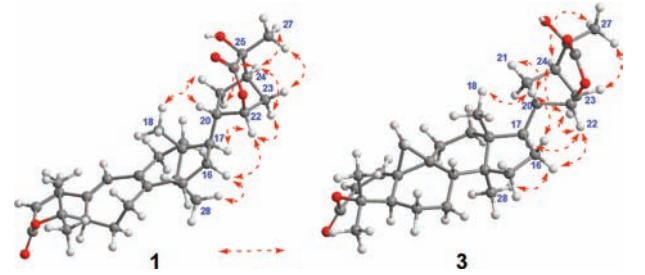


Figure 2. Key ROESY correlations of **1** and **3**.

were tentatively assigned to be α-oriented and Me-18 to be *β*-oriented. The ROESY correlations of H-22 with H-17 and H-23α; of H-16α with H_3-28 and H-22; and of H-24 with

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H-21α, H-23α, and H_3-27 clearly suggested that they were cofacial and arbitrarily assigned as an α-configuration.

Accordingly, the oxygenated substitutes at C-22 and C-25 were assigned to be β-orientated. The correlation of H_3-18 with H-20, of H-20 with H-21β, and of H-23β with H_3-27 implied that H-20 was β-orientated and H_3-27 was assigned to be an α-configuration, respectively. The relative configuration of the remaining stereocenters of **1** was elucidated as the same as that for schisanlatone A[6] by analysis of the ROESY data (Figure 2).

Henrischinin B (**2**) was obtained as colorless and optically active crystals ($[\alpha]_D^{12.5}$ + 321.1). Its molecular formula $C_{32}H_{42}O_6Na$ was deduced by means of HRESIMS analysis (*m*/*z* 545.2873 $[M + Na]^+$). Comparison of the ^1H and ^{13}C NMR data obtained for **2** with those for **1** suggested that **2** was likely to be an acetylation derivative of **1**. The ^{13}C NMR spectrum (Table 1) of **2** showed the signal for C-26 upshifted from δ_C 177.1 in **1** to δ_C 169.9 in **2**, and the signal for C-25 downshifted from δ_C 74.5 in **1** to δ_C 82.5 in **2**, accounting for the shielding and deshielding effects of the acetyl group for C-25 and C-26 in **2**, respectively. In order to confirm the structure of **2** and its absolute configuration, compound **2** was crystallized in pyridine to afford a crystal of the orthorhombic space group $P2_1$, which was analyzed by X-ray crystallography (Figure 3).[8]

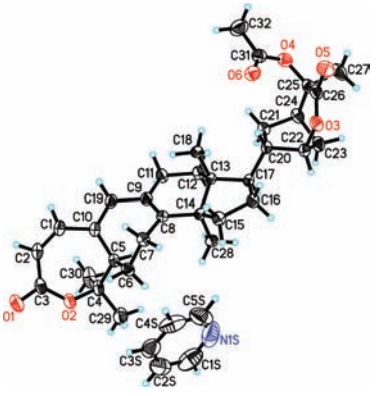


Figure 3. X-ray structure of **2**.

Bearing on six oxygen atoms in the molecule, the final refinement on the Cu Kα data resulted in a Flack parameter of −0.10 (2), allowing an unambiguous assignment of the complete absolute configuration of **2** as shown in its formula.

Henrischinin C (**3**) exhibited an $[M + Na]^+$ ion peak at *m*/*z* 505.2929 in the HRESIMS, corresponding to a molecular formula of $C_{30}H_{42}O_5$. The UV spectrum of **3** showed absorption maxima at 254 nm, indicating the presence of a conjugated system. The IR spectrum of **3** showed bands of a hydroxyl (3347 cm^{-1}), a isolated carbonyl group (1730 cm^{-1}), and an α,β-unsaturated carbonyl group (1657 cm^{-1}). The ^{13}C NMR spectrum (Table 1) with the assistance of DEPT experiments resolved 30 carbon resonances comprising five methyl singlets, nine methylenes, an oxygenated and four aliphatic methines, two oxygenated and four aliphatic quaternary carbons, an ester carbonyl (δ_C 177.3), and an α,β-unsaturated carbonyl group (δ_C 166.9, 120.7, and 150.9). A detailed comparison of the ^1H and ^{13}C NMR data of **3** with those of **1**, together with comprehensive analysis of 2D NMR data, enabled the same moiety of 4-methyl-3-one-2-oxabicyclo[3.2.1]-octane moiety as that of **1**. The main differences between **3** and **1** were in the signals of ring B. The characteristic signals in the ^1H and ^{13}C NMR spectra for **3** (δ_C 28.8, s, C-9; δ_C 33.6, s, C-10; δ_C 31.8, t, C-19, and δ_H 0.82, ABd, *J* = 4.9 Hz, and 1.03, ABd, *J* = 4.9 Hz, H-19) revealed the presence of a three-membered carbon ring,[7] which was further confirmed by

Table 1. ^1H and ^{13}C NMR Data of Compounds **1**−**3** (pyridine-d_5, δ in ppm)[a]

position	1	2	3
1	143.7 (d)	143.7 (d)	150.9 (d)
2	118.4 (d)	118.4 (d)	120.7 (d)
3	166.6 (s)	166.6 (s)	166.9 (s)
4	80.0 (s)	79.8 (s)	84.0 (s)
5	49.3 (d)	49.3 (d)	46.4 (d)
6	39.6 (t)	39.6 (t)	23.7 (t)
7	28.2 (t)	28.1 (t)	24.3 (t)
8	150.7 (s)	150.7 (s)	44.6 (d)
9	129.1 (s)	129.0 (s)	33.6 (s)
10	140.0 (s)	139.8 (s)	28.8 (s)
11	26.8 (t)	26.8 (t)	32.5 (t)
12	30.4 (t)	30.2 (t)	28.3 (t)
13	44.8 (s)	44.9 (s)	45.4 (s)
14	51.7 (s)	51.6 (s)	48.8 (s)
15	31.2 (t)	31.3 (t)	35.1 (t)
16	27.8 (t)	27.8 (t)	28.6 (t)
17	48.8 (d)	48.6 (d)	50.4 (d)
18	17.1 (q)	17.2 (q)	18.5 (q)
19	143.8 (d)	143.8 (d)	31.8 (t)
20	49.1 (d)	48.5 (d)	49.3 (d)
21	31.1 (t)	33.2 (t)	30.2 (t)
22	85.2 (d)	85.2 (d)	85.4 (d)
23	33.6 (t)	34.2 (t)	33.6 (t)
24	46.8 (d)	46.0 (d)	46.7 (d)
25	74.5 (s)	82.5 (s)	74.5 (s)
26	177.1 (s)	169.9 (s)	177.3 (s)
27	28.6 (q)	26.2 (q)	28.6 (q)
28	27.1 (q)	27.1 (q)	18.4 (q)
29	26.2 (q)	26.2 (q)	22.1 (q)
30	29.2 (q)	29.2 (q)	29.3 (q)
31		170.0 (s)	
32		21.2 (q)	

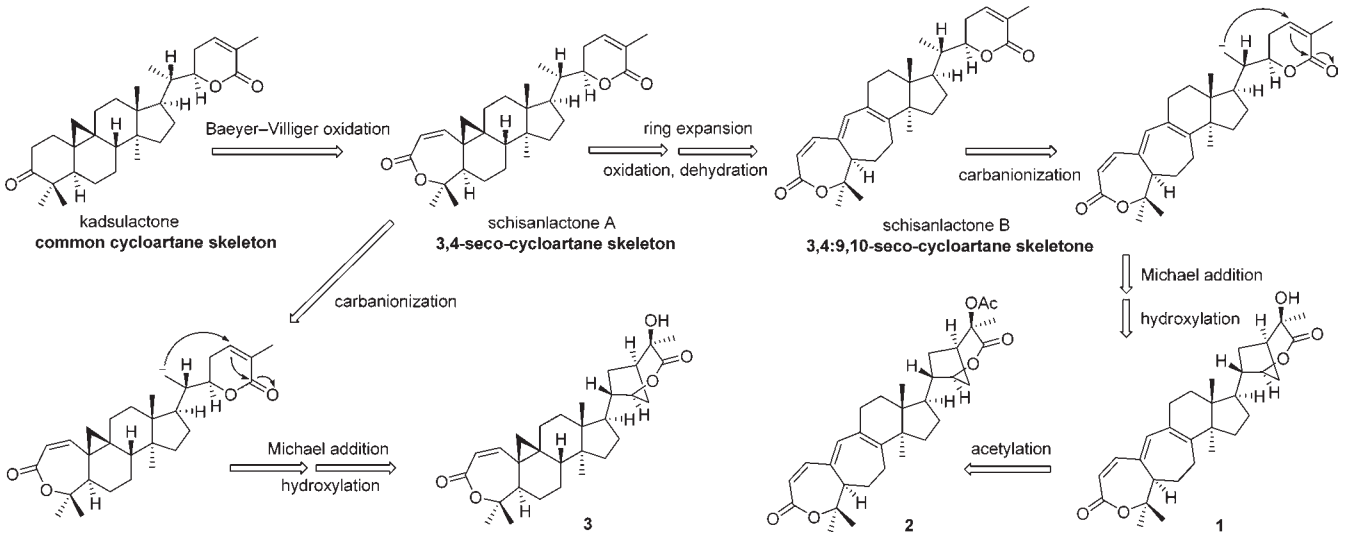
[a] Data of compounds **1**, **2**, and **3** were recorded at 125 MHz, and the assignments were based on DEPT, HSQC, and HMBC experiments.

(7) Liu, J. S.; Huang, M. F.; Ayer, W. A.; Bigam, G. *Tetrahedron Lett*. **1983**, *24*, 2355–2358.

(8) Crystal data of henrischinin B (**2**) can be found in the Supporting Information. CCDC-808509 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, U. K.; fax (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

(9) Li, L. N.; Xue, H.; Kangouri, K.; Ikeda, A.; Omura, S. *Planta Med*. **1989**, *55*, 294–296.

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



the HMBC correlations of H-1 and H-5 with C-10 and C-19; of H-8 with C-9 and C-19; and of H-19 with C-1, C-5, C-8, C-9, C-10, and C-11. Portions of the relative configuration of **3** were readily assigned by performance of a ROESY experiment (Figure 2) and comparison with those of **1** and **2**. Likewise, the ROESY correlations of H-22 with H-17/H-23, of H-16α with H_3-28 and H-22, and of H-24 with H-23α and H_3-27 indicated that they were in an α-configuration; correspondingly, the oxygenated substitutes at C-22 and C-25 were assiggned as β-orientated, respectively. H-20 was assigned as a β-configuration, on the basis of a ROESY correlation of H_3-18 with H-20. Furthermore, the ROESY correlations of H-23β with H_3-27 implied that H_3-27 was α-orientated. Thus, the structure of **3** was determined as shown.

The unique biogenetic origin of henrischinins A−C (**1**−**3**) can plausibly be traced back to kadsulactone.[10] Herein we proposed a biogenetic pathway for henrischinins A−C (**1**−**3**) and the relationships between these triterpenoids. This pathway involved a Baeyer−Villiger oxidation, ring expansion, oxidation and dehydrogenation, and Michael addition, followed by hydroxylation and acetylation, starting from kadsulactone or schisanlactones A and B as the precursor (Scheme 1).[1a,7,10]

Compounds **1**−**3** were tested for their cytotoxicity against A-549, HL-60, MCF-7, SMMC-7721, and SW-480 human cancer cell lines. Among them, compounds **1** and **2** showed weak cytotoxicity against HL-60 cell lines with IC_{50} values of 16.5 and 10.5 μM, respectively (Table 2).

Table 2. Cytotoxic Activity of Compounds **1**−**3**[a]

compound	A-549	HL-60	MCF-7	SMMC-7721	SW-480
1	>40	16.5	>40	>40	>40
2	>40	10.5	>40	>40	>40
3	>40	>40	>40	>40	>40
cis-platin	15.6	0.9	14.9	13.8	19.1

[a] Results are expressed as IC_{50} values in μM, data were obtained from triplicate experiments, and *cis*-platin was used as a positive control.

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Supporting Information Available. Detailed method of cytotoxicity test, experimental section, 1D and 2D NMR spectra, UV, IR, optical rotation, and ^{1}H NMR data assignment of compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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