ORGANIC LETTERS

2007
Vol. 9, No. 21
4175−4178

Structural Characterization of Schintrilactone, a New Class of Nortriterpenoids from *Schisandra chinensis*

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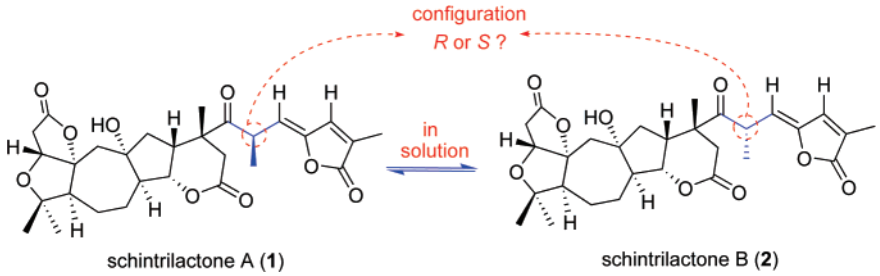
Received July 16, 2007

ABSTRACT



Two new nortriterpenoids, designated as schintrilactones A (1) and B (2), with modified five-membered D ring and δ-lactone E ring, were isolated from *Schisandra chinensis*. Their structures were determined to have a unique carbon skeleton by elucidation of spectroscopic evidence and density functional theory calculations of circular dichroism. Schintrilactones A and B occur as a pair of configurationally unstable and thus slowly interconverting diastereomers.

Plants of the genus *Schisandra* and *Kadsura* (Schisandraceae) have attracted much attention owing to a variety of medicinal properties and biological activities attributed to them. For example, antihepatitis, antitumor, anti-HIV, and inhibiting cholesterol biosynthesis properties have been described for these plants and their constituents.[1−4] One of the most distinguishing features of *Schisandra* and *Kadsura* species studied recently is the discovery of rearranged triterpenoid derivatives endowed with different oxygenated skeletons, most of which can be related biogenetically to cycloartane precursors.[5] Previously reported novel triterpenoids from *Schisandra* and *Kadsura* species including micrandilactones A−C from *S. micrantha*,[6,7] lancifodilactones A, F, and G from *S. lancifolia*,[8−10] rubriflordilactones A and B from *S.*

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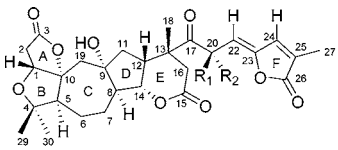
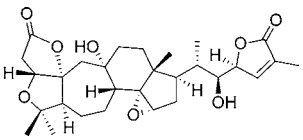
10.1021/ol701679n CCC: $37.00
Published on Web 09/19/2007

rubriflora,[11] kadlongilactones A and B from *K. longipedunculata*,[12] and kadsuphilactone A from *K. philippinensis*[13] exemplify the structural complexity and diversity of compounds produced by plants of the family Schisandraceae. As a result, some of them have been the subject of total synthesis.[14] *Schisandra chinensis* (Turcz.) Baill., widely distributed in the northeastern part of China, Korea, and Japan, has long been used as sedative and tonic agents in traditional Chinese medicine (TCM). Now, the fruits of it have been widely sold as phytomedicinal and dietary supplements in Europe and the United States. In our search for structurally interesting and biologically active natural products from the family Schisandraceae, phytochemical investigation on this plant has resulted in the isolation of micrandilactone B (**3**)[7] and two new nortriterpenoids, schintrilactones A (**1**) and B (**2**), which possess a unique carbon skeleton. Interestingly, compounds **1** and **2** occur as a pair of configurationally unstable and thus slowly interconverting diastereomers.



1 $R_1 = CH_3$, $R_2 = H$
2 $R_1 = H$, $R_2 = CH_3$



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Schintrilactone A (**1**) was obtained as an optically active white solid ([α]19 D +163.4) with a molecular formula of $C_{29}H_{36}O_9$ as established by HR-ESIMS data (found 551.2241 $[M + Na]^+$, calcd for 551.2257), requiring 12 sites of unsaturation. Thirty-five protons were bound to carbons on the basis of DEPT result, thus an exchangeable hydrogen was present. The ^1H and ^{13}C NMR spectra of **1** displayed the existence of four singlets and a secondary methyl, six methylenes, four aliphatic sp^3 methines, two oxygenated sp^3 methines, four sp^3 quaternary carbons, one ketone, three ester groups, and two trisubstituted double bonds. Accordingly, a six-ring structure was required for **1** to fulfill the unsaturation requirement.

Comparison of the ^1H and ^{13}C NMR spectral data for **1** with those of micrandilactone B (**3**) strongly suggested a similar structure for rings A, B, and C of both compounds. This information coupled with the analysis of 2D NMR data confirmed the substructure of rings A, B, and C in **1**. Study of the COSY and TOCSY spectra starting from the proton at δ_H 5.08 (H-14) revealed the presence of a spin-system $-CHCH_2CH_2CHCHCHCH_2-$ (C5/C6/C7/C8/C14/C12/C11). This information coupled with the key HMBC correlations from H_2-11 and H-14 to C-8 and C-9 indicated the presence of a five-membered carbon ring (ring D). Thus, the structure of fragment **I** was constructed (Figure 1). The structure of fragment **II** was assigned as shown in Figure 1 on the basis of the following observations: the long-range couplings observed in the HMBC spectrum (Figure 1); the spin system from H_3-21 to H-22 was readily obtained by COSY data; and the *Z* geometry of the double bond between C-22 and C-23 was deduced from ROESY correlation of H-22 with H-24. Specifically, a base peak at *m*/*z* 137, caused by the fission of the C17−C20 bond, was detected in FABMS. This

Table 1. ^1H and ^{13}C NMR Data of Compounds **1** and **2** (Acetone-d_6, δ in ppm)[a]

	schintrilactone A (**1**)		schintrilactone B (**2**)	
no.	δ_H (*J* in Hz) mult	δ_C	δ_H (*J* in Hz)	δ_C, mult
1	4.23 d (5.3)	81.4	4.24 overlapped	81.3
2α	2.45 d (18.2)	35.5	2.46 d (18.2)	35.6
2β	2.84 overlapped		2.92 dd (18.2, 5.3)	
3		174.6		174.7
4		84.9		85.0
5	2.27 m	61.3	2.32 m	60.0
6α	1.57−1.62 m	21.9	1.55−1.59 m	21.8
6β	1.57−1.62 m		1.55−1.59 m	
7α	1.84 m	24.2	1.85 m	24.1
7β	1.91 m		1.89 m	
8	2.13 m	58.1	2.21 m	56.8
9		79. 4		80.1
10		99.3		99.6
11α	1.52 t like (12.5)	44.5	1.55 overlapped	44.5
11β	1.34 dd (12.5, 7.9)		1.29 overlapped	
12	2.91 m	38.2	3.04 m	38.4
13		50.1		50.5
14	5.08 dd (8.8, 7.6)	83.0	5.01 dd (8.8, 7.2)	83.6
15		169.7		169.9
16α	2.85 overlapped	34.5	2.83 overlapped	34.7
16β	2.32 d (17.4)		2.33 d (17.4)	
17		211.2		211.1
18	1.46 s	22.0	1.26 s	21.9
19α	1.77 ABd (15.5)	40.9	1.85 ABd (15.5)	41.0
19β	2.17 ABd (15.5)		2.10 ABd (15.5)	
20	4.32 m	39.1	4.24 m	39.8
21	1.27 d (6.7)	19.8	1.19 d (6.6)	18.6
22	5.35 d (10.4)	114.1	5.34 d (10.4)	113.0
23		148.5		148.9
24	7.39 d (1.4)	139.2	7.34 d (1.4)	139.0
25		131.1		130.9
26		171.1		170.6
27	1.97 d (1.4)	10.6	1.95 d (1.4)	11.0
29	1.13 s	22.1	1.11 s	22.4
30	1.29 s	28.8	1.29 s	28.9

[a] ^1H NMR at 400 MHz, ^{13}C NMR at 100 MHz, and assignments were based on HSQC, COSY, HMBC, and ROESY experiments.

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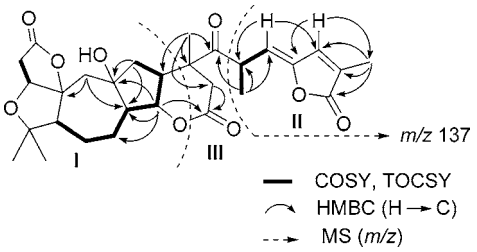

Figure 1. Fragments and key COSY, TOCSY, and HMBC correlations of **1**.

information further corroborated the structure of fragment **II**. Apart from fragments **I** and **II**, there were an isolated methyl (Me-18, δ_H 1.46, s), a methylene (C-16, δ_H 2.85, overlapped and δ_H 2.32, d, $J = 17.4$ Hz), a δ-lactone carbon (C-15, δ_C 169.7), an unoxygenated quaternary carbon (C-13, δ_C 50.1), and a ketone carbon (C-17, δ_C 211.2) to be assigned. Further analysis of the HMBC spectrum showed the following correlations: H_3-21 with C-17; H_3-18 with C-12, C-13, C-16, and C-17; and H-14 and H_2-16 with C-15. These information permitted fragments **I**, **II**, and **III** to be joined to get the gross structure of **1**.

The relative configurations of all of the chiral centers of **1** except for C-20, and the conformation of each ring were elucidated by analysis of the ROESY data, proton coupling constants, and analogy with **3**. The same relative stereochemistry of C-1, C-5, C-9, and C-10, as well as the conformations of rings A−C in **1** as in **3** were deduced from the similar carbon proton chemical shifts, proton coupling constants, and ROESY correlations found in **1**. In particular, ROEs observed from H-14 to H-19β and H_2-6, and from H-8 to H-11α, as well as from H_3-18 to H-12 and H-16β, which were all supported by DFT (density functional theory) calculated interatomic distances of <2.65 Å (Figure 2),

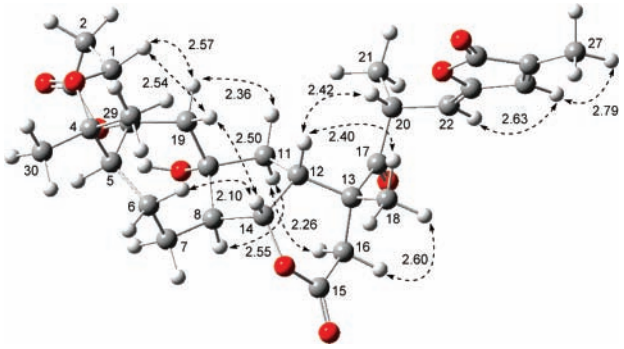

Figure 2. Key ROESY correlations of **1** and corresponding interatomic distance (Å).

established the sequential stereochemistry at C-8, C-14, C-12, and C-13. The DFT calculation and analysis of the MM optimization using the Dreiding force field of **1** showed that there were two possible conformations in the six-membered lactone ring (ring E). In the ROESY spectrum, the H-16α displayed a significant correlation with H-11α, thereby allowing a twist-chair conformation of the six-membered lactone ring (ring E) to be determined as shown in Figure 2.

Schintrilactone B (**2**) had the same molecular formula as that of schintrilactone A (**1**). Side-by-side comparison of their NMR data indicated that **1** and **2** were a pair of C-20 epimers.

Compounds **1** and **2** are the first representatives of this class of metabolites that bear a stereogenic center at C-20. This precluded an assignment of their absolute configuration by an empirical comparison of the circular dichroism (CD) spectrum of **1** or **2** with that of any structurally similar, configurationally known substance. For this reason, quantum chemical CD calculations appeared to be the method of choice.[15] The conformational analysis was performed by means of the semiempirical PM3 method, as implemented in the program package GAUSSIAN 03,[16] starting from preoptimized geometries generated by the MM2 force field in CHEM3D software overlaid with key correlations observed in the ROESY spectrum. The corresponding minimum geometries found were further optimized by DFT calculations at the B3LYP/6-31G(d) level,[17−19] leading to two minimum structures in both cases. For these geometries, CD computations were performed by means of the TDDFT [B3LYP/6-31G(d)] method.[15c,d] The overall spectra of **1** or **2** thus obtained were subsequently compared with the measured experimental CD curve. The results showed that the spectrum calculated for the 20*S*-diastereomer was nearly identical with the experimental one of **2** (Figure 3, right) over the whole range of wavelengths under investigation, whereas the spectrum simulated for the 20*R*-diastereomer exhibited very similar CD behavior compared with the experimental CD curve of **1** (Figure 3, left). The absolute configurations of C-20 in **1** and **2** were thus assigned as *R* and *S*, respectively.

It is of interest that schintrilactones A (**1**) and B (**2**) are unstable for an extended time period (more than one week) and can interchange slowly in solution. This configurational semistability of C-20 of this pair of new triterpenoids made it rewarding to compare its relative energies with their corresponding enol intermediate. The solvation effect was considered by using methanol, alcohol, acetone, and chloroform in the calculations to resemble the experimental condition using the B3LYP/6-31G(d) method. The polarized continuum model (PCM) was used. Default parameters for the reaction field cavities were used in the PCM model. The relative energies can be seen in Figure 4. The computed energy of the enol intermediate is only 11.7, 11.9, 12.1, and 13.7 kcal/mol higher than that of **1** in methanol, alcohol,

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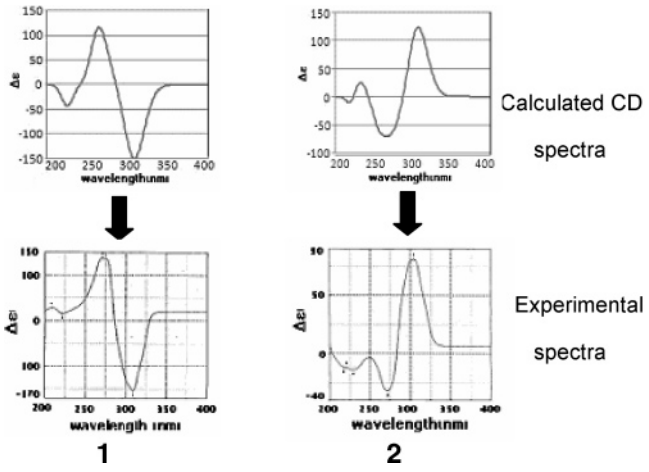
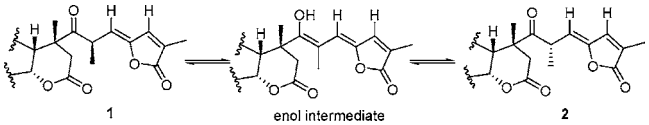


Figure 3. Assignment of the absolute configuration to C-20 in **1** and **2** by comparison of the experimental CD spectra with the spectra calculated for **1** and **2** by using TDDFT methods.

acetone, and chloroform, respectively, which means that it is possible for such an isomerization to occur in solution. The energy difference between **1** and **2** is very small (Figure 4), which is also in agreement with the ratio of 1:1 (**1**/**2**) as found experimentally in the isomeric equilibrium at room temperature.



ΔE (relative to **1**, kcal/mol)

CH_3OH	0.0	11.7	–1.5
C_2H_5OH	0.0	11.9	–1.1
$(CH_3)_2CO$	0.0	12.1	–1.0
$CHCl_3$	0.0	13.7	0.2

Figure 4. Relative energies of **1**, **2**, and enol intermediate at the B3LYP/6-31G(d) level in methanol, alcohol, acetone, and chloroform, using the PCM mode.

We propose a biogenetic pathway (see the Supporting Information) to account for the plausible formation of the modified five-membered carbon ring and six-membered lactone of schintrilactones and whereby is shown the close relationship among these triterpene skeletons of schiartane,[7] pre-schisanartane,[20] and schisanartane,[6] which share a common precursor, namely, artane triterpene. The absolute stereochemistry of micrandilactone B (**3**) was determined by a modified Mosher method.[20] On the biosynthetic consideration, the same absolute configuration is suggested for schintrilactones A (**1**) and B (**2**). As these new, highly oxygenated compounds **1** and **2** with a unique carbon skeleton have not previously been encountered in nature, we proposed the name "wuweiziartane" for this type of skeleton.

The anti-HIV-1 activities of **1**−**3** were tested by a microtiter syncytium formation infectivity assay, using the method previously described, with AZT as a positive control.[21] Compounds **1** and **2** demonstrated anti-HIV-1 activity with EC_{50} values of 17.9 and 36.2 μg mL^{-1}, respectively (AZT: EC_{50} = 2.26 μg mL^{-1}). Compounds **3** showed weak anti-HIV-1 activity with an EC_{50} value of >50 μg mL^{-1}.

Acknowledgment. The authors are grateful to Dr. Hao Xu, the Scripps Research Institute, University of California, for their generous support and helpful discussion on the proposed biogenetic pathway. Financial support of this research was provided by the National Natural Science Foundation of China (No. 20402016) and the Natural Science Foundation of Yunnan Province (No. 2005XY04).

Supporting Information Available: Detailed description of the experimental procedures, copies of the NMR (1D and 2D), and MS spectra of compounds **1** and **2**, and proposed biosynthetic pathways for the formation of wuweiziartane skeleton (**1** and **2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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