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# Wuweizidilactones A–F: Novel Highly Oxygenated Nortriterpenoids with Unusual Skeletons Isolated from *Schisandra chinensis*

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**Abstract:** A phytochemical study of secondary metabolites produced by *Schisandra chinensis* has led to the isolation of six novel highly oxygenated nortriterpenoids, wuweizidilactones A–F (**1**–**6**). Compounds **3**–**6** possess an unprecedented 3,4-seco-18(13→14)-*abeo*-artane skeleton. Interestingly, structures **3**–**6** have a β-oriented methyl group at the C-14 position. This structural feature corroborates the biogenetic pathway proposed for the formation of 18-norschiartane-type compounds **1** and **2**. The structures of these novel metabolites were established on the basis of their detailed spectroscopic analysis. The structure of **1** was also confirmed by single-crystal X-ray diffraction analysis. For the first time, the absolute configuration of these nortriterpenoids was determined by using a modified Mosher method.

**Keywords:** biogenesis · natural products · structure elucidation · terpenoids · wuweizidilactone

## Introduction

*Schisandra* nortriterpenoids are a structurally intriguing group of highly oxygenated, polycyclic, fused heterocyclic natural products that are produced by plants of the genus *Schisandra*. Our group has isolated almost 40 highly oxygenated nortriterpenoids from a climbing plant of the genus *Schisandra* of the family Schisandraceae.[1] The remarkably varied structures were classified into three main types of nortriterpenoid skeletons, that is, schisanartane, schiartane, and 18-norschiartane.[1e] As a consequence, these structurally complex molecules have attracted great interest from chemists as challenging targets for their total synthesis as well as for biosynthetic studies.[2,3]

*Schisandra chinensis* (Turcz.) Baill., is a climbing plant that is widely found in northeastern China, Korea, and Japan and is known in China by the Chinese name "wu-wei zi". It has been used as sedative and tonic agents in traditional Chinese medicine for a long time. This plant has been reported to contain dibenzocyclooctadiene lignans, which were found to have some important pharmacological effects that include antihepatitis, antitumor, and anti-HIV activities.[4–8] We purified an extract of the aerial part of *S. chinensis* in an attempt to determine if this plant produces nortriterpenoids. This study led to the isolation of six novel, highly oxygenated nortriterpenoids, wuweizidilactones A–F (**1**–**6**) and micrandilactone B (**7**).[1c] Among these nortriterpenoids, compounds **1** and **2** contain the known 18-norschiartane carbon skeleton. We have previously reported three nortriterpenoids that contain 18-norschiartane carbon skeletons.[1b,j] This type of compound was postulated to originate from precursors that contain schiartane carbon skeletons through a sequence of reactions that involve a 1,2-methyl shift followed by oxidation and decarboxylation of the C-14 methyl group.[1e] Interestingly, the β-oriented methyl group at the C-14 position of wuweizidilactones C–F

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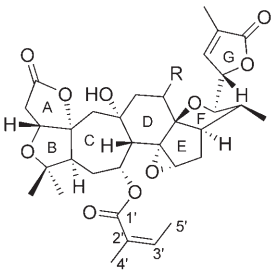
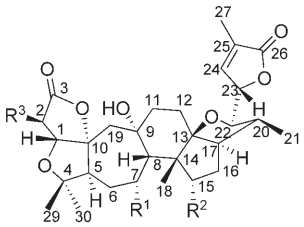
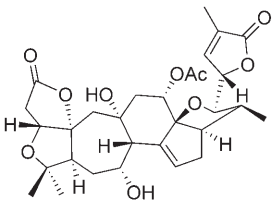


1: R= =O
2: R= α-OAc



| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **3**: | OAc | OH | H |
| **4**: | H | OH | H |
| **5**: | H | OAc | H |
| **6**: | H | OAc | OH |



lancifodilactone A

(**3–6**) suggested that these compounds were key intermediates in the hypothetical biogenetic pathway.[1e] Thus, the unique carbon skeletons of **3–6** not only constitute a new addition to the already impressive architectural diversity of the triterpene class of natural products, but also confirms the biosynthetic pathway we proposed previously. This paper reports the isolation, structure elucidation, and biological properties of these novel nortriterpenoids.

## Results and Discussion

Wuweizidilactone A (**1**) was isolated as colorless crystals. Its molecular formula was determined to be $C_{33}H_{40}O_{11}$ (14 degrees of unsaturation) by means of analyzing $^1H$, $^{13}C$, and distortionless enhancement by polarization transfer (DEPT) NMR spectroscopic data ,and was also verified by high-resolution electrospray mass spectrometry (HR-ESI-MS) data (calcd 651.2208; found: 651.2202 $[M+K]^+$). DEPT results indicated that there were thirty nine protons bound to carbon atoms, and therefore, one hydrogen that can undergo exchange with the solvent was also present. The $^1H$ and $^{13}C$ NMR spectra of **1** showed the coexistence of four singlet and two doublet methyl groups, five methylene groups, four aliphatic $sp^3$ methine carbon atoms, five oxygenated $sp^3$ methine carbons, five $sp^3$ quaternary carbon atoms, one ketone, three ester groups, and two trisubstituted double bonds. This observation suggested that **1** was likely to be an 18-norschiartane-type bisnortriterpenoid[1b] that was substituted by an angeloyl group. This assumption was subsequently confirmed by conducting a set of 2D NMR spectroscopy experiments ($^1H$–$^1H$ COSY, HSQC, HMBC, and ROESY spectra) that provided data for the unequivocal assignment of all $^1H$ and $^{13}C$ NMR signals (Tables 1 and 2).

The complete structure of **1** was elucidated by analyzing the 2D NMR data obtained and by comparing these results with the NMR data obtained for lancifodilactone A.[1b] The close similarities between the NMR data for rings A, B, and C with those of a known compound, lancifodilactone A, suggested that **1** has a similar substructure to that of lancifodilactone A. However, different carbon and proton chemical shifts were observed for C-11, C-12, C-14, and C-15 indicating that the structures of **1** and lancifodilactone A differed in rings D and E. The substructures of rings D and E were

Table 1. $^{13}C$ NMR data of wuweizidilactones A–F (**1–6**) in $C_5D_5N$.[a]

| Carbon | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | 81.8 (d) | 82.0 (d) | 82.0 (d) | 82.3 (d) | 82.1 (d) | 87.3 (d) |
| 2 | 35.3 (t) | 35.6 (t) | 36.1 (t) | 37.0 (t) | 37.0 (t) | 73.8 (d) |
| 3 | 174.7 (s) | 174.9 (s) | 175.0 (s) | 175.8 (s) | 175.5 (s) | 177.2 (s) |
| 4 | 84.1 (s) | 84.2 (s) | 84.6 (s) | 85.1 (s) | 85.1 (s) | 85.8 (s) |
| 5 | 54.6 (d) | 53.8 (d) | 52.3 (d) | 58.8 (d) | 58.6 (d) | 58.7 (d) |
| 6 | 30.3 (t) | 30.9 (t) | 31.6 (t) | 29.0 (t) | 29.4 (t) | 29.3 (t) |
| 7 | 69.5 (d) | 70.2 (d) | 70.1 (d) | 25.4 (t) | 25.5 (t) | 25.8 (t) |
| 8 | 46.2 (d) | 46.2 (d) | 58.4 (d) | 57.6 (d) | 57.8 (d) | 57.6 (d) |
| 9 | 79.2 (s) | 75.2 (s) | 72.4 (s) | 70.2 (s) | 70.6 (s) | 70.6 (s) |
| 10 | 97.6 (s) | 98.2 (s) | 99.2 (s) | 99.7 (s) | 100.0 (s) | 100.6 (s) |
| 11 | 54.0 (t) | 41.5 (t) | 39.5 (t) | 38.2 (t) | 38.0 (t) | 38.0 (t) |
| 12 | 202.7 (s) | 70.4 (d) | 35.3 (t) | 35.8 (t) | 34.7 (t) | 34.7 (t) |
| 13 | 94.1 (s) | 90.7 (s) | 94.9 (s) | 93.4 (s) | 93.1 (s) | 93.0 (s) |
| 14 | 72.5 (s) | 69.7 (s) | 55.3 (s) | 55.2 (s) | 53.5 (s) | 53.4 (s) |
| 15 | 56.7 (d) | 55.2 (d) | 79.4 (d) | 78.8 (d) | 81.9 (d) | 81.9 (d) |
| 16 | 26.3 (t) | 26.9 (t) | 33.4 (t) | 33.0 (t) | 30.8 (t) | 30.7 (t) |
| 17 | 41.5 (d) | 43.6 (d) | 52.4 (d) | 53.6 (d) | 53.1 (d) | 53.0 (d) |
| 18 | – | – | 24.6 (q) | 24.0 (q) | 24.3 (q) | 24.1 (q) |
| 19 | 45.2 (t) | 46.1 (t) | 46.9 (t) | 47.7 (t) | 48.6 (t) | 49.8 (t) |
| 20 | 37.8 (d) | 36.6 (d) | 35.9 (d) | 36.0 (d) | 36.0 (d) | 35.9 (d) |
| 21 | 11.9 (q) | 11.7 (q) | 12.8 (q) | 12.5 (q) | 12.1 (q) | 12.0 (q) |
| 22 | 85.6 (d) | 84.9 (d) | 88.9 (d) | 87.9 (d) | 87.2 (d) | 87.0 (d) |
| 23 | 81.5 (d) | 81.4 (d) | 82.4 (d) | 81.8 (d) | 81.4 (d) | 81.2 (d) |
| 24 | 146.3 (d) | 146.7 (d) | 146.8 (d) | 147.2 (d) | 147.0 (d) | 147.2 (d) |
| 25 | 131.2 (s) | 130.9 (s) | 130.7 (s) | 130.5 (s) | 130.5 (s) | 130.4 (s) |
| 26 | 173.9 (s) | 174.1 (s) | 174.4 (s) | 174.5 (s) | 174.4 (s) | 174.5 (s) |
| 27 | 10.7 (q) | 10.7 (q) | 10.8 (q) | 10.9 (q) | 10.8 (q) | 10.9 (q) |
| 29 | 21.7 (q) | 22.1 (q) | 23.0 (q) | 23.6 (q) | 23.7 (q) | 24.1 (q) |
| 30 | 27.9 (q) | 28.4 (q) | 29.1 (q) | 30.0 (q) | 30.0 (q) | 30.1 (q) |

[a] See the Experimental Section for data for the angeloyl or acetyl groups. Chemical shift values $\delta$ are in ppm and the coupling constant $J$ is in Hz (in parentheses).

**Abstract in Chinese:**

从五味子（*Schisandra chinensis*）中分离得到了 6 个高度氧化的降三萜，五味子二内酯（wuweizidilactone） A－F （**1－6**）。其中化合物 **3－6** 为四个具有新骨架的降三萜，其 14 位甲基均为 β 构型。这些结构证实了我们以前假设的 18-norschiartane 型骨架三萜的生源合成途径，并通过波谱数据分析以及 X－射线单晶衍射实验确定了所有化合物的化学结构和相对立体构型。另外，通过 Mosher 衍生化法，首次确定了该类降三萜的绝对构型。

Table 2. $^1$H NMR data of wuweizidilactones A–F (**1**–**6**) in $C_5D_5N$.[a]

| Proton | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | 4.29 (d, 5.3) | 4.24 (d, 5.2) | 4.32 (d, 5.2) | 4.24 (d, 5.2) | 4.21 (d, 5.0) | 4.37 (s) |
| 2α | 2.82 (d, 18.1) | 2.78 (overlap) | 2.79 (d, 18.0) | 2.70 (d, 18.0) | 2.69 (d, 18.0) | 4.66 (s) |
| 2β | 3.08 (dd, 18.1, 5.3) | 3.05 (dd, 18.0, 5.2) | 3.03 (dd, 18.0, 5.2) | 2.88 (dd, 18.0, 5.2) | 2.90 (dd, 18.0, 5.0) | |
| 5 | 2.62 (dd, 13.0, 3.3) | 2.62 (dd, 13.1, 3.5) | 2.85 (dd, 13.5, 3.8) | 2.61 (dd, 13.2, 3.3) | 2.56 (dd, 13.2, 3.3) | 2.64 (dd, 13.2, 3.2) |
| 6α | 2.22 (m) | 2.10 (m) | 2.34 (m) | 1.66 (m) | 1.62 (m) | 1.61 (m) |
| 6β | 1.76 (m) | 1.59 (m) | 1.60 (m) | 1.20 (m) | 1.11 (overlap) | 1.20 (overlap) |
| 7α | – | – | – | 2.00 (m) | 1.79 (m) | 1.82 (m) |
| 7β | 5.74 (brd, 7.2) | 5.65 (brd, 8.1) | 5.88 (brd, 8.9) | 2.00 (m) | 1.79 (m) | 1.82 (m) |
| 8 | 3.34 (brs) | 2.90 (brs) | 1.94 (brs) | 1.47 (overlap) | 1.34 (brd, 12.1) | 1.35 (brd, 12.4) |
| 11α | 2.70 (d, 13.2) | 2.21 (overlap) | 1.85 (m) | 1.78 (m) | 1.72 (m) | 1.69 (m) |
| 11β | 3.42 (d, 13.2) | 2.33 (brd, 16.3) | 1.56 (overlap) | 1.49 (overlap) | 1.43 (m) | 1.43 (m) |
| 12α | – | – | 2.63 (m) | 2.50 (overlap) | 2.39 (overlap) | 2.34 (m) |
| 12β | – | 5.44 (brs) | 1.93 (overlap) | 1.93 (m) | 1.86 (m) | 1.80 (m) |
| 15 | 3.83 (brs) | 3.78 (brs) | 3.96 (d, 2.1) | 4.07 (brs) | 5.12 (brs) | 5.12 (brs) |
| 16α | 1.91 (m) | 1.98 (m) | 1.81 (m) | 1.82 (m) | 1.88 (m) | 1.90 (overlap) |
| 16β | 1.62 (m) | 1.57 (m) | 1.81 (m) | 1.74 (m) | 1.55 (m) | 1.51 (m) |
| 17 | 2.84 (m) | 2.54 (m) | 2.87 (m) | 2.86 (m) | 2.50 (overlap) | 2.48 (overlap) |
| 18 | – | – | 1.06 (s) | 1.05 (brs) | 1.04 (brs) | 1.03 (brs) |
| 19α | 2.25 (ABd, 15.5) | 2.22 (overlap) | 2.11 (ABd, 16.3) | 1.95 (ABq, 15.3) | 1.79 (ABd, 15.7) | 2.00 (d, 15.8) |
| 19β | 2.55 (ABd, 15.5) | 2.22 (overlap) | 2.18 (ABd, 16.3) | 1.95 (ABq, 15.3) | 1.88 (ABd, 15.7) | 2.70 (d, 15.8) |
| 20 | 2.67 (m) | 2.78 (overlap) | 2.55 (m) | 2.50 (overlap) | 2.49 (overlap) | 2.49 (overlap) |
| 21 | 0.84 (d, 6.8) | 0.87 (d, 6.8) | 0.92 (d, 6.8) | 0.93 (d, 6.8) | 0.91 (d, 6.5) | 0.90 (d, 6.5) |
| 22 | 3.94 (dd, 9.0, 3.7) | 3.95 (dd, 10.0, 3.2) | 3.81 (dd, 9.0, 3.7) | 3.84 (dd, 9.0, 3.7) | 3.79 (dd, 9.0, 3.7) | 3.78 (brd, 8.8) |
| 23 | 4.91 (brs) | 5.02 (brs) | 5.01 (brs) | 5.03 (brs) | 5.05 (brs) | 5.00 (brs) |
| 24 | 7.14 (brs) | 7.18 (brs) | 7.25 (brs) | 7.28 (brs) | 7.27 (brs) | 7.26 (brs) |
| 27 | 1.74 (brs) | 1.78 (brs) | 1.93 (brs) | 1.93 (brs) | 1.93 (brs) | 1.91 (brs) |
| 29 | 1.06 (s) | 1.00 (s) | 1.17 (s) | 1.14 (s) | 1.11 (s) | 1.15 (s) |
| 30 | 1.21 (s) | 1.20 (s) | 1.30 (s) | 1.28 (s) | 1.24 (s) | 1.25 (s) |

[a] See the Experimental Section for data for the angeloyl and acetyl groups. Chemical shift values $\delta$ are in ppm and the coupling constant $J$ is in Hz (in parentheses).

determined by 2D NMR experiments. The $^1$H–$^1$H COSY correlations (Figure 1) of H-15/H-16/H-17 and HMBC correlations of H-15 ($\delta_H$=3.83 ppm) with C-13, C-14, C-16, and C-17 indicated that there was an epoxy ring between C-14 and C-15. Furthermore, the HMBC cross peaks of $H_2$-11 ($\delta_H$=3.42, 2.70 ppm) with C-9 ($\delta_C$=79.2 ppm), C-12 ($\delta_C$=202.7 ppm), C-13 ($\delta_C$=94.1 ppm), and C-19 ($\delta_C$=45.2 ppm), and of H-17 ($\delta_H$=2.84 ppm) with C-12 allowed us to assign a ketone group at C-12. The presence of an angeloyl group at C-7 was evident from the HMBC correlation of H-7 ($\delta_H$=5.74 ppm) with carbonyl carbon C-1′ ($\delta_C$=166.1 ppm). Thus, the complete structure of **1** was established to be that shown in Figure 1.

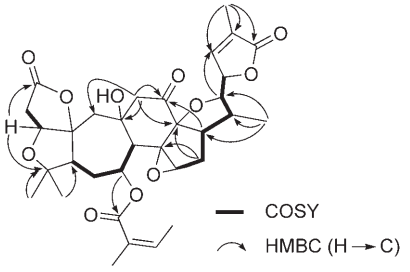



Figure 1. Key correlations of HMBC and $^1$H–$^1$H COSY for **1**.

The relative stereochemistry of **1** was determined by means of ROESY correlations together with X-ray analysis. Biogenetically, these types of triterpenoids are derived from cycloartane triterpenes.[1e] Therefore, H-5 was tentatively assigned to be α-oriented. The cross peaks observed in the ROESY spectrum and X-ray analysis data enabled the relative configuration of all chiral centers to be determined (Figure 2).

Wuweizidilactone B (**2**) was assigned the molecular formula $C_{35}H_{44}O_{12}$, which was deduced by means of HR-ESI-MS analysis (*m*/*z* 679.2720 $[M+Na]^+$). Comparison of the spectroscopic data obtained for **2** with those obtained for **1** revealed that they were quite similar except that the ketone group at C-12 was replaced by an acetoxy group. The presence of the acetyl group was confirmed by the appropriate signals in the $^{13}$C NMR spectrum of **2** ($\delta_C$=170.8, 21.4 ppm). Moreover, the cross peaks in the ROESY spectrum of **2** indicated that the corresponding substituents in **2** had the same orientations as those in **1**. The α-orientation of the acetate group at the C-12 position was confirmed by comparison of the chemical shifts, splitting patterns, and coupling constants of H-12 ($\delta_H$=5.44 ppm) and $H_2$-11 ($\delta_H$=2.33 and 2.21 ppm) of **2** with those of the literature values (H-12: $\delta_H$=5.29 ppm; $H_2$-11: $\delta_H$=2.26, 2.18 ppm) reported for lancifodilactone A, which also has an α-oriented OAc group.[1b]

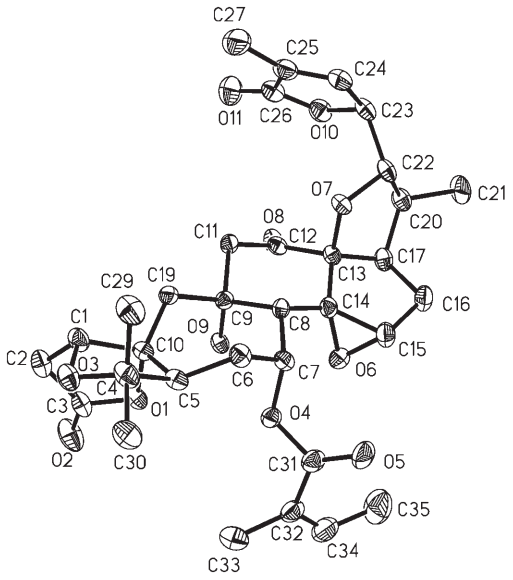



Figure 2. Molecular structure of **1** determined by X-ray analysis.

served for H-5 with H-6 and for H-6 with H-7, as well as the HMBC correlation between H-7 and an ester carbonyl carbon ($\delta_C = 170.7$ ppm), indicated the presence of an acetyl group at C-7. The structure of rings F and G was deduced based on similar carbon and proton chemical shifts to those of **1** (Tables 1 and 2). This deduction was also confirmed by COSY and HMBC correlations (Figure 3) from $H_3$-21 to C-

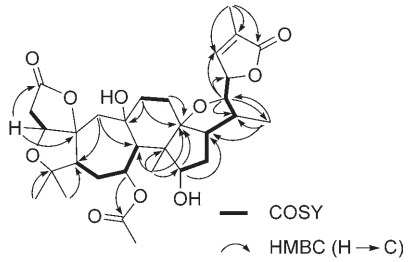



Figure 3. Key HMBC and $^1$H–$^1$H COSY correlations for **3**.

In addition, the strong ROESY correlations of H-12 with H-20 also supported the stereochemistry of C-12.

Wuweizidilactone C (**3**) was obtained as a white solid. A molecular formula of $C_{31}H_{42}O_{10}$ was determined for this compound from the pseudomolecular ion peak at *m/z* 597.2684 $[M+Na]^+$ (calcd for $C_{31}H_{42}O_{10}Na$: 597.2675) obtained by HR-ESI-MS, which is consistent with 11 degrees of unsaturation. The strong absorption bands at $\tilde{\nu} = 3443$, 1781, 1761, 1747, 1657, and 1457 $cm^{-1}$ in the IR spectrum indicated the presence of an OH group, several carbonyl groups, and a double bond. The $^1$H NMR spectrum of compound **3** showed characteristic signals of six methyl groups (five singlets and one doublet), five oxygenated methines ($\delta_H = 4.32$, 5.88, 3.96, 3.81, 5.01 ppm), and an olefinic proton ($\delta_H = 7.25$ ppm) (Table 2). The $^{13}$C and DEPT NMR spectra showed 31 carbon signals, which include the characteristic signals of six methyl groups, five oxygenated methines, four oxygenated quarternary carbons ($\delta_C = 84.6$, 72.4, 99.2, 94.9 ppm), three ester carbonyls ($\delta_C = 175.0$, 174.4, 170.7 ppm), and one trisubstituted double bond ($\delta_C = 146.8$, 130.7 ppm) (Table 1). Therefore, the carbon spectrum is consistent with the molecular formula determined, the IR data, and the $^1$H NMR data analysis mentioned above. Accordingly, a seven-membered ring structure that contained two OH groups was required to correspond with the unsaturation requirement and molecular formula.

Interpretation of the correlations observed in the $^1$H–$^1$H COSY, HSQC, HMBC, and ROESY spectra suggested that compound **3** was a nortriterpenoid substituted by an acetyl group, whose A, B, and C rings were the same as those of compounds **1**, **2**, and lancifodilactone A. In the HMBC spectrum, the proton signals for $H_3$-29 and $H_3$-30 were correlated with one of four oxygenated quarternary carbon (C-4) and methine carbon atoms C-5 ($\delta_C = 52.3$, $\delta_H = 2.85$ ppm). This result, in combination with the $^1$H–$^1$H COSY correlation ob-

17 ($\delta_C = 52.4$ ppm), C-20 ($\delta_C = 35.9$ ppm), and C-22, from $H_3$-27 to C-24, C-25, and C-26, and from H-22 to C-23, C-24, C-20, and C-21. Apart from the $^1$H and $^{13}$C NMR signals in rings A, B, C, F, and G, a tertiary methyl group C-18 ($\delta_H = 1.06$; $\delta_C = 24.6$ ppm), three methylene carbon atoms ($\delta_C = 39.5$, 35.3, 33.4 ppm), one oxygenated methine group C-15 ($\delta_C = 79.4$; $\delta_H = 3.96$ ppm), and a quarternary carbon atom C-14 ($\delta_C = 55.3$ ppm) remained to be assigned. On further analysis of the HMBC spectrum of wuweizidilactone C (**3**), correlations from $H_3$-18 to C-8, C-13, C-14, and C-15, and from H-15 to C-17 and C-13 were observed. These data were used to determine that the methyl group and the oxygenated methine carbon were at C-14 and C-15, respectively. The COSY spectrum showed correlations between the following proton signals: H-15 with H-16, H-11 with H-12. This information, coupled with the HMBC correlations for $H_2$-11 with C-9 and C-13, as well as for $H_2$-12 with C-13, allowed the complete structures of rings D and E to be determined. Therefore, the structure of compound **3** was assigned to be a nortriterpenoid with seven rings that include two γ-lactone rings.

The relative stereochemistry of compound **3** was established by using information obtained from the ROESY spectrum, carbon chemical shifts, and by comparison of its spectroscopic data with those of **1**. The relative stereochemistry of rings A, B, C, F, and G in compound **3** was deduced to be the same as that in **1** from the similar carbon and proton chemical shifts and ROESY correlations found in the spectra of both compounds. In the ROESY spectrum of **3**, correlations from $H_3$-18 to H-7, H-8, and H-22 were unexpectedly observed, as shown in the structure of **3** that was calculated by using DFT (Figure 4).[9,10] These correlations allowed us to place the Me-18, H-7, and H-8 on the same face of the molecule. The H-15 signal in the $^1$H NMR spectrum of **3** was detected as a doublet with a coupling constant of $J = 2.1$ Hz, which suggests an α-orientation of the 15-OH group.

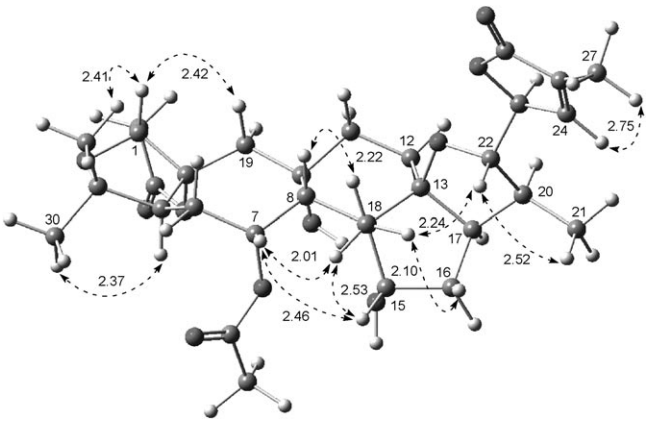



Figure 4. Key ROESY correlations for **3** and their corresponding interatomic distance [Å].

This assignment was confirmed by the ROESY cross peaks of $H_3$-18 with H-15.

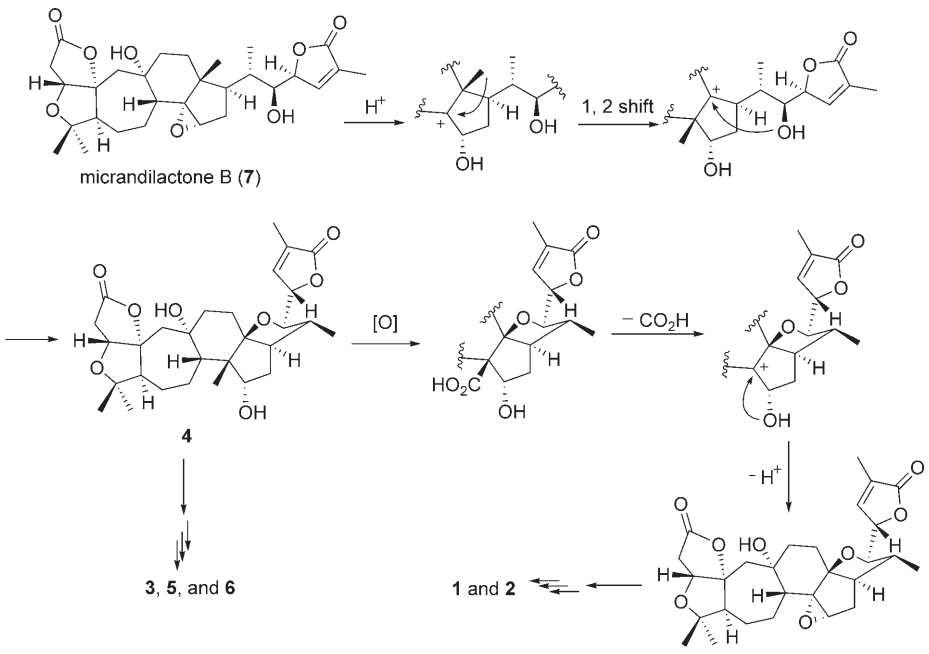
Wuweizidilactones D–F (**4**–**6**) were isolated as amorphous powders. They showed very similar NMR data to those obtained for **3** (Tables 1 and 2), which suggests that they have similar structures. In each of the compounds **4**–**6**, IR bands at $\tilde{\nu}=1759$ to 1781 $cm^{-1}$ indicated the presence of γ-lactone groups.

The molecular formula $C_{29}H_{40}O_8$ was assigned to compound **4** based on its HR-ESI-MS and NMR data. It only differed structurally from **3** at C-7. The acetoxy group at C-7 in **3** was replaced by a methylene group ($\delta_C=25.4$ ppm), which resulted in a significant downfield shift of the $^{13}C$ NMR signal of C-5 in **4** compared with that in **3** owing to the absence of a γ-gauche effect between the oxygen atom at C-7 and H-5 in **4**. The molecular formula of wuweizidilactone E (**5**) was determined to be $C_{31}H_{42}O_9$ from HR-ESI-MS analysis. The only differences between the $^1H$ NMR spectra of **4** and **5** were that the spectrum of **4** lacked the methyl signal from an acetyl group and the signal for H-15 was shifted downfield (from $\delta_H=4.07$ in **4** to $\delta_H=5.12$ ppm in **5**). In the $^{13}C$ NMR spectrum of **5**, the acetyl carbon signal ($\delta_C=169.9$, 21.5 ppm) was clearly observed. Accordingly, wuweizidilactone E (**5**) was established to be wuweizidilactone D acetate.

Compound **6** (wuweizidilactone F) was an oxidized derivative of **4**. An additional oxygenated methine carbon was observed at $\delta_C=73.8$ ppm in the $^{13}C$ NMR spectrum of **6** and was assigned to C-2 on the basis of HMBC correlations from H-2 to C-1 and C-10, and from H-1 to C-2. The existence of the hydroxyl group at C-2 led to significant changes in the $^1H$ NMR splitting patterns of H-1 and H-2 and in the $^{13}C$ NMR chemical shifts of C-1, C-2, and C-3 in comparison with those of **4** (Tables 1 and 2). The relative configuration of OH-2 was inferred to be β from the small coupling constant between H-1 ($\delta_H=4.37$ ppm) and H-2 ($\delta_H=4.66$ ppm), which indicates that the dihedral angle between H-1 and H-2 is almost 90°.

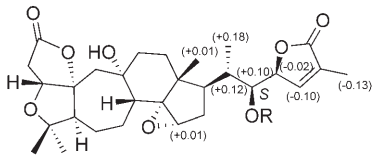
The *Schisandra* nortriterpenoids are derived from cycloartane triterpenoids. Biogenetically the methyl groups at C-13 and C-14 in cycloartane triterpenoids are β- and α-oriented, respectively.[1g,i] Interestingly, the structures of **3**–**6** have a β-oriented methyl group at C-14. This structural feature is incompatible with that of cycloartane triterpenoids. Thus, this methyl group must originate from the methyl group at C-13 by a 1,2-methyl shift. This finding corroborates the biogenetic pathway proposed for the formation of 18-norschiartan-type compounds (Scheme 1).[1e]

Subsequently, we tried to determine the absolute stereochemistry of wuweizidilactones C–F (**3**–**6**) by using the Mosher NMR spectroscopic method.[11] However, approaches to generate α-methoxy-α-trifluoromethylphenylacetate (MTPA) derivatives of **3** were unsuccessful owing to the inherent unreactive character of the sterically hindered alcohol groups. To circumvent this problem, a modified Mosher method was applied to the related known metabolite micrandilactone B (**7**), which contains a reactive secondary alcohol group (Figure 5). Comparison of the $^1H$ NMR




Scheme 1. Hypothetical biogenetic route for the preparation of 18-norschiartane-type compounds.




**7a**: R = (*S*)-MTPA
**7b**: R = (*R*)-MTPA

Figure 5. $\Delta\delta$ ($\delta_S-\delta_R$) values in ppm for MTPA derivatives of **7**.

chemical shifts between the (*R*)- and (*S*)-MTPA esters of **7** led to the assignment of the *S*-configuration at C-22 for micrandilactone B (**7**). As the relative configuration of compound **7** was established by X-ray analysis,[1c] this experiment enabled the absolute configuration of all stereogenic centers of compound **7** to be established, as shown in Figure 5. On the basis of biosynthetic considerations, the same absolute configuration is suggested for compounds **1**–**6**.

The anti-HIV-1 activities of compounds **1**–**7** were tested by means of a microtiter syncytium formation infectivity assay with azidothymidine (AZT) as a positive control by using the method previously reported.[12] Wuweizidilactone A (**1**) and B (**2**) demonstrated anti-HIV-1 activity with $EC_{50}$ values of 26.81 and 28.86 $\mu g\,mL^{-1}$, respectively (positive control: AZT, $EC_{50}=2.26\ \mu g\,mL^{-1}$). Compounds **3**–**7** showed weak anti-HIV-1 activity with $EC_{50}$ values of $>50\ \mu g\,mL^{-1}$. In addition, compounds **1**–**7** were tested for cytotoxicity against K562 and A549 human tumor cells by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method, as previously reported.[13] None of the compounds showed inhibitory activity against the tumor cells and had $IC_{50}$ values of $>100\ \mu g\,mL^{-1}$.

## Conclusion

In the present work, we characterized six novel highly oxygenated triterpenoids with unusual nortriterpenoid or bisnortriterpenoid skeletons from aerial parts of *Schisandra chinensis*. From a structural point of view, wuweizidilactones C–F (**3**–**6**) represent a unique class of natural products. Most importantly, compounds **3**–**6** contain a β-oriented methyl group at the C-14 position, which shows that they are key intermediates in the biosynthesis of 18-norschiartane-type triterpenoids. These findings considerably expand the library of isolated compounds for this class of natural products and contribute to our knowledge of triterpenoid chemistry. Additionally, compounds **1**–**7** showed weak anti-HIV-1 activity.

## Experimental Section

**General**: Optical rotations were carried out by using a Perkin–Elmer model 241 polarimeter. IR spectra were measured as KBr pellets by using a Bio-Rad FTS-135 spectrometer. MS data were recorded by using a VG Auto spec-3000 spectrometer or a Finnigan MAT 90 instrument. 1D NMR spectra were recorded by using a Bruker AM-400 and 2D NMR spectra were recorded by using a Bruker DRX-500 instrument in which TMS was used as an internal standard for all measurements. Semipreparative HPLC was performed by using an Agilent 1100 liquid chromatograph equipped with a Zorbax SB-C18, 9.4 mm × 25 cm column. Column chromatography was performed by using silica gel (200–300 mesh; Qingdao Marine Chemical, China), Lichroprep RP-18 gel (40–63 μm; Merck, Germany), or MCI gel CHP 20P (75–150 μm; Mitsubishi Chemical Corporation, Japan).

**Plant material**: The aerial parts of *S. chinensis* were collected in Tonghua prefecture, Jilin Province, P.R. China, in September 2005. The sample was identified by Professor Jun-Lin Yu, and a voucher specimen (KIB 05092106) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

**Extraction and isolation**: Air-dried, powdered aerial parts of *S. chinensis* (4.5 kg) were extracted with acetone at room temperature for two days. After removing the solvents in vacuo at 45 °C, a residue (207 g) was obtained. This residue was dissolved in $H_2O$ (3.0 L) and then extracted successively with petroleum ether (60–90 °C, 2 × 1.5 L) and EtOAc (3 × 2 L). The EtOAc extract (102 g) was purified by column chromatography over MCI gel by using 90% MeOH/$H_2O$ and 100% MeOH as the eluents. The fraction (81 g) that was eluted by using 90% MeOH was further purified by column chromatography over silica gel by using $CHCl_3$/$Me_2CO$ (from 1:0 to 0:1) to give fractions A–E. Fraction C (11.8 g) was purified by column chromatography by using an RP-18 column with MeOH/$H_2O$ (from 30% to 80%) as the eluent to give five main subfractions, C1-C5. Wuweizidilactone A (**1**; 20.5 mg) was obtained from subfraction C2 (1.2 g) after repeated purification steps had been performed that involved column chromatography over silica gel by using $CHCl_3$/$Me_2CO$ (10:1) as the eluent and finally recrystallization from methanol. The remainder of subfraction C2 was separated by semipreparative HPLC by using (55:45) as the mobile phase (3 mL min$^{-1}$) MeOH/$H_2O$ to yield compound **5** (6.0 mg). Separation of subfraction C3 (1.5 g) by silica gel column chromatography by using petroleum ether/isopropyl alcohol (6:1) as the eluent yielded compounds **3** (18.5 mg), **7** (45.0 mg), and a mixture of **2** and **4**. This mixture (48 mg) was finally purified by semipreparative HPLC by using ($CH_3OH$/$CH_3CN$/$H_2O$, 45:10:45) as the eluent to give pure wuweizidilactones B (**2**) (10.0 mg) and D (**4**) (5.5 mg). Compound **6** (6.2 mg) was obtained from subfraction C4 by silica gel column chromatography by using $CHCl_3$/MeOH (20:1) as the eluent.

**Wuweizidilactone A (1)**: Colorless crystals; m.p. 203–204 °C; $[\alpha]_D^{19}=-18.0$ ($c=0.20$ in $CH_3OH$); $^1H$ NMR: see Table 2, angeloyl: $\delta=5.87$ (q, $J=6.4$ Hz), 2.11 (br s), 2.02 ppm (br d, $J=6.4$ Hz); $^{13}C$ NMR: see Table 1, angeloyl: $\delta=166.1$ (s), 127.5 (s), 140.3 (d), 20.9 (q), 15.9 ppm (q); IR (KBr): $\tilde{\nu}=3586$, 2976, 2926, 1781, 1758, 1739, 1710, 1647, 1459, 1386, 1229, 1181, 1149, 1106, 1041 cm$^{-1}$; MS (ESI): $m/z$: 651 $[M+K]^+$; MS (HR-ESI): $m/z$: calcd for $C_{33}H_{40}O_{11}K$: 651.2208; found: 651.2202 $[M+K]$.

**Wuweizidilactone B (2)**: White solid; m.p. 180–181 °C; $[\alpha]_D^{19}=14.7$ ($c=0.12$ in $CH_3OH$); $^1H$ NMR: see Table 2, angeloyl: $\delta=5.80$ (q, $J=6.4$ Hz), 2.07 (br s), 2.05 ppm (br d, $J=6.4$ Hz), acetyl: $\delta=2.03$ ppm (s); $^{13}C$ NMR: see Table 1, angeloyl: $\delta=166.4$ (s), 128.0 (s), 139.4 (d), 21.1 (q), 15.9 ppm (q), acetyl: $\delta_C=170.8$ (s), 21.4 ppm (q); IR (KBr): $\tilde{\nu}=3499$, 2933, 1760, 1730, 1704, 1643, 1457, 1433, 1379, 1233, 1257, 1116, 991 cm$^{-1}$; MS (FAB): $m/z$: 657 $[M+H]^+$; MS (HR-ESI): $m/z$: calcd for $C_{35}H_{44}O_{12}Na$: 679.2730; found: 679.2720 $[M+Na]^+$.

**Wuweizidilactone C (3)**: White solid; m.p. 210–211 °C; $[\alpha]_D^{19}=-3.1$ ($c$ 0.15 in $CH_3OH$); $^1H$ NMR: see Table 2, acetyl: $\delta=2.17$ ppm (s); $^{13}C$ NMR: see Table 1, acetyl: $\delta=170.7$ (s), 20.9 ppm (q), detailed COSY, HMBC, and ROESY data are given in Table S1 in the Supporting Information; IR (KBr) $\tilde{\nu}=3520$, 2976, 2934, 1781, 1761, 1747, 1657, 1457, 1383, 1245, 1231, 1069, 1022, 934 cm$^{-1}$; FABMS: $m/z$: 575 $[M+H]^+$; MS (HR-ESI): calcd for $C_{31}H_{42}O_{10}Na$: $m/z$: 597.2675; found: 597.2684 $[M+Na]^+$.

**Wuweizidilactone D (4)**: Amorphous powder; $[\alpha]_D^{19}=-9.8$ ($c$ 0.11 in $CH_3OH$); $^1H$ and $^{13}C$ NMR: see Tables 1 and 2; IR (KBr) $\nu_{max}=3424$, 2968, 2931, 1759, 1629, 1459, 1383, 1253, 1242, 1189, 1065, 1022 cm$^{-1}$;

FABMS: $m/z$: 513 $[M+H]^+$; MS (HR-ESI): calcd for $C_{29}H_{40}O_8Na$: $m/z$: 539.2620; found: 539.2603 $[M+Na]^+$.

**Wuweizidilactone E (5)**: Amorphous powder; $[\alpha]_D^{19}=1.5$ ($c$ 0.12 in $CH_3OH$); $^1H$ NMR: see Table 2, acetyl: $\delta=2.09$ ppm (s); $^{13}C$ NMR: see Table 1, acetyl: $\delta=169.9$ (s), 21.5 ppm (q); IR (KBr) $\bar{\nu}=3440$, 2972, 2932, 1760, 1728, 1453, 1373, 1245, 1189, 1069, 1025, 1001 $cm^{-1}$; FABMS: $m/z$: 559 $[M+H]^+$; MS (HR-ESI): calcd for $C_{31}H_{42}O_9Na$: $m/z$: 581.2726; found: 581.2719 $[M+Na]^+$.

**Wuweizidilactone F (6)**: Amorphous powder; $[\alpha]_D^{19}=8.5$ ($c$ 0.16 in $CH_3OH$); $^1H$ NMR: see Table 2, acetyl: $\delta=2.11$ ppm (s); $^{13}C$ NMR: see Table 1, acetyl: $\delta=170.0$ (s), 21.6 ppm (q); IR (KBr) $\bar{\nu}=3441$, 2971, 2933, 1759, 1454, 1374, 1246, 1218, 1708, 1000, 929 $cm^{-1}$; FABMS: $m/z$: 575 $[M+H]^+$; MS (HR-ESI): calcd for $C_{31}H_{42}O_{10}Na$: $m/z$: 597.2675; found: 597.2670 $[M+Na]^+$.

**X-ray crystallographic analysis of 1**: Formula: $C_{33}H_{40}O_{11}$; $M_r=588.63$; monoclinic; space group: $P2_12_12_1$; $a=13.076(1)$, $b=23.303(1)$, $c=23.609(1)$ Å; $V=7193.9(7)$ Å$^3$; $Z=4$; $\beta=107.67(1)°$; $\rho_{calcd}=1.199$ g cm$^{-3}$; crystal dimensions: $0.20\times0.40\times0.60$ mm. The total number of independent reflections measured was 8637, of which 4228 were observed ($|F|2=8\sigma|F|2$). The final indices were $R_f=0.0736$, $Rw=0.2125$ ($w=1/\sigma|F|2$), $S=0.988$. Crystal structure measurements were made by using a MAC DIP-2030 K diffractometer with graphite-monochromated $Mo_{K\alpha}$ radiation. The data were collected by using the $\omega-2\theta$ scan technique to a maximum $2\theta$ value of 50.0°. The crystal structures were solved by direct methods by using SHELX-86,[14] expanded by using difference Fourier techniques, and refined by the program and method NOMCSDP[15] and full-matrix least-squares calculations. The non-hydrogen atoms were refined anisotropically and hydrogen atoms were included at their calculated positions. CCDC-638440 contains crystallographic data for **1**. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

**Preparation of (*R*)- and (*S*)- MTPA esters of 7**: Micrandilactone B (**7**) was derivatized with (*R*)- and (*S*)-MTPACl. Anhydrous $CH_2Cl_2$ (0.5 mL) was added under a nitrogen atmosphere at room temperature to micrandilactone B (3.0 mg). Following an incubation period (5 min) that involved vigorous stirring, one crystal of dimethylaminopyridine and $C_5H_5N$ (10 μL) were added. The reaction components were stirred for 20 min before (*R*)- or (*S*)-MTPACl (2 μL) was added. The reaction was incubated (30 min) before $C_5H_5N$ (300 μL) was added. The reaction was dried in vacuo and subsequently purified by column chromatography over an RP-18 column by using MeOH/$H_2O$ (45%) as the eluent. The purified derivatives were dried and analyzed by means of $^1H$ NMR and $^1H$–$^1H$ COSY spectroscopies.

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