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A class of 18(13→14)-*abeo*-schiartane skeleton nortriterpenoids from *Schisandra propinqua* var. *propinqua*

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ABSTRACT

A class of C_{29} triterpene dilactones (**1**–**6**) featuring 18(13→14)-*abeo*-schiartane skeleton have been isolated from the stems of *Schisandra propinqua* var. *propinqua*. The structures of new compounds, propindilactones K–O (**1**–**5**), were determined on the basis of comprehensive spectroscopic means. Biogenetic pathway of **1**–**6** was proposed and then chemically mimicked. The absolute stereochemistries of new compounds were established on biosynthetic consideration coupled with CD experiments. Compound **2** showed promising anti-HBV activity in vitro.

1. Introduction

The fruits of genus *Schisandra* (Schisandraceae) have been widely used in Traditional Chinese Medicine (TCM) as tonic, sedative, and astringent agents for a long time.[1] And recently, they are used commonly in clinical TCM prescription to treat anxious or chronic hepatitis. Phytochemical and pharmacological studies on fruits revealed that it is mainly the dibenzocyclooctadiene lignans that possess various biological activities including antihepatitis, antitumor, and antilipid peroxidation effects.[2] Actually, the stems of *Schisandra* genus are also used for the treatment of rheumatic lumbago, traumatic injury and related diseases in western China. Over 10 years of efforts from our group has been devoted to the secondary metabolite investigation on the stems of about 10 *Schisandra* species, which resulted in a series of structural intriguing nortriterpenoids derived from cycloartane skeleton mainly involving schisanartane, schiartane, and 18-nor-schiartane backbone.[3] These discoveries triggered great interest among phytochemists, synthetic chemists, and pharmacologists.

In order to identify new natural compounds with interesting bioactivities, *Schisandra propinqua* var. *propinqua*, indigenous to Yunnan Province, was phytochemically investigated. Except for dibenzocyclooctadiene lignans,[4] lanostane triterpenoid,[5] schisanartane,[6] and schiartane nortriterpenoids[7] isolated before, a novel group of 18(13→14)-*abeo*-schiartane C_{29} triterpenoids (**1**–**6**) were discovered from this species, which served as important members in *Schisandra* triterpenoid family. Biogenetically, it is the key inter mediate from schiartane to 18-nor-schiartane skeleton along the hypothetic biogenetic pathways,[3d,j] which was chemically mimicked for the first time in this paper. And pharmacologically, it is the first group that was evaluated on anti-HBV activity in vitro and one of them (compound **2**) showed potential anti-HBV activity comparative to that of positive control, which supplied promising nortriterpenoid candidate for further development of this drug plant. Herein we report the isolation, structural elucidation, chemical mimicking, and anti-HBV activity bioassay of five new triterpenoids, propindilactones K–O (**1**–**5**), together with wuweizidilactone D (**6**).[3j]

2. Results and discussion

2.1. Structural elucidation of propindilactones K–O (1–5)

Compounds **1**–**6** were isolated from 70% aqueous acetone extract of the stems of *S. propinqua* var. *propinqua*. Compound **6** was

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identified to be wuweizidilactone D by comparison of its ^{13}C NMR and DEPT spectra with the literature,[3j] which is a 18(13→14)-*abeo*-schiartane C_{29} triterpenoid with 5/5/7/6/5 membered consecutive rings and a β-Me located at C-14. Compounds **1**–**5** are new members of 18(13→14)-*abeo*-schiartane family as determined by comprehensive spectroscopic means including 1D and 2D NMR, together with CD experiments.

Propindilactone K (**1**) was obtained as white crystals from MeOH. The HRESIMS exhibited a pseudo-molecular ion peak $[M-H]^-$ at *m*/*z* 589.2618 corresponding to the molecular formula $C_{31}H_{42}O_{11}$, which was consistent with the NMR data, indicating 11 degrees of unsaturation. The IR spectrum exhibited absorption bands for hydroxyls (3430 cm^{-1}) and carbonyls (1748 and 1638 cm^{-1}). The ^1H, ^{13}C, and DEPT spectra displayed 31 carbon resonances comprising a typical acetyl group (δ_H 1.78, s; δ_C 20.7, 170.3), five methyls (including one secondary methyl and four tertiary ones), six sp^3 methylenes, ten sp^3 methines (six oxygenated at δ_C 87.4, 82.1, 78.6, 76.9, 74.4, and 63.0), two ester carbonyls, and six quaternary sp^3 carbons (five oxygenated at δ_C 99.2, 94.8, 85.1, 72.3, and 57.1). These carbon signal information, coupled with the molecular formula indicated that compound **1** was a highly oxygenated C_{29} nortriterpenoid with eight rings and two hydroxyls. Both characteristic ABX (δ_H 4.27, d, *J*=4.5 Hz, H-1; δ_H 2.74, d, *J*=18.0 Hz, H-2α; δ_H 2.93, dd, *J*=18.0, 4.5 Hz, H-2β) and AB (δ_H 1.90, 2.28, both ABd, *J*=15.5 Hz, H_2-19) spin systems in ^1H NMR spectra (Table 1), in company with ^1H–^1H COSY correlations of H-5/H_2-6/H_2-7/H-8 (Fig. 1) suggested that molecule **1** had the representative 5/5/7 membered A–C rings of *Schisandra* nortriterpenoids.[3d] Two proton spin systems of H_2-11/H-12 and H-15/H_2-16/H-17 in ^1H–^1H COSY (Fig. 1), together with key HMBC correlations from H-12 to C-9, C-13 and ester carbonyl (OAc), and from H_3-18 to C-8, C-13, C-14, and C-15 showed that consecutive 6/5 membered rings (D and E rings) were connected with the seven-membered C ring and Me-18 was located at C-14, while the acetyl group was substituent at C-12 and a hydroxyl at C-15. Taking the above data into account, compound **1** should possess 18(13→14)-*abeo*-schiartane skeleton, comparative to that of wuweizidilactone D (**6**).[3j]

Propindilactone K (**1**) had a 13,22-epoxy furan ring (F ring) as that of **6**, since they held similar carbon signals at δ_C 94.8 (s) and 87.4 (d), which were attributed to C-13 and C-22, respectively. An epoxide group was positioned between C-24 and C-25 on the basis of the loss of trisubstituted double bond while the appearance of the greatly upshifted signals at δ_C 63.0 (d) and 57.1 (s) compared with those of **6**, and supported by HMBC correlations from H-22, H-23, and H_3-27 to C-24. Thus, the ^1H and ^{13}C NMR data (Tables 1 and 2), in combination with the HMBC correlation analysis (Fig. 1) furnished the planar structure of **1**.

The relative configuration of **1** was established by ROESY experiment (Fig. 2) together with comparison with data of **6**. Biogenetically, H-8 is tentatively assigned to be β-directed and H-17 to be α-orientation, as *Schisandra* nortriterpenoids are thought to be derived from cycloartane triterpenes.[3d] Accordingly, the ROESY correlations of H-8/H-12 and H-8/H_3-18 indicated that H-12 and H_3-18 were co-facial with H-8 and assigned to be β-orientation, while correlations of H_3-18/H-15, H-15/H_2-16, and H-16α/H-17 suggested that 15-OH was α-orientation. Strong ROESY correlations of H_3-18/H-22 and H-22/H_3-21 indicated that H_3-21 and H-22 were both β-directed. Although the C-22–C-23 bond could rotate to certain extent, the steric bulk of rings F and G made it fairly fixed as judged by the strong ROESY correlations of H-23/H-22 and H-23/H_3-21, which indicated H-23 was β-orientation, the same as that of **6**. Correlations of both H-23 and H_3-27 to H-24 suggested the α-orientation of the epoxide group between C-24 and C-25. The relative configurations of the other chiral centers in compound **1** were found to be the same as those of molecule **6** as depicted.

Propindilactone L (**2**) was a hydroxyl derivative of **1**, since compound **2** showed most similar carbon signals in ^{13}C NMR spectra (Table 2) but 16 mass units higher than that of **1** as deduced

Table 1
^1H NMR spectroscopic data of compounds **1**–**5**[a] in C_5D_5N

Proton	1	2	3	4	5
1β	4.27 (d, 4.5)	4.65 (s)	4.09 (d, 5.6)	4.20 (d, 5.0)	4.16 (d, 5.0)
2α	2.74 (d, 18.0)	4.35 (s)	2.67 (d, 18.0)	2.74 (d, 18.0)	2.68 (d, 18.0)
2β	2.93 (dd, *18.0, 4.5*)		2.89 (dd, *18.0, 5.6*)	2.96 (dd, 18.0, 5.0)	2.91 (dd, 18.0, 5.0)
5α	2.63 (dd, *15.5, 3.5*)	2.66 (dd, *13.5, 3.5*)	2.29–2.33[b]	2.31–2.38[b]	2.35 (dd, 13.5, 4.5)
6α	1.71 (m)	1.70 (m)	1.54 (m)	1.68–1.74[b]	1.66 (m)
6β	1.23–1.31[b]	1.32 (m)	1.18–1.24[b]	1.12 (m)	1.18 (m)
7α	2.05–2.11[b]	2.20 (m)	2.50 (m)	2.19–2.28[b]	1.98–2.06 (m)
7β	2.19 (m)	2.08 (m)	1.81 (m)	2.66 (m)	2.61–2.68[b]
8β	1.78–1.80[b]	1.75–1.80[b]	2.21 (dd, *12.8, 4.8*)	1.70–1.75[b]	1.60 (dd, 11.5, 1.5)
11α	2.71–2.78[b]	2.80 (t like, 13.0)	1.47–1.54[b]	2.18–2.25[b]	1.74 (m)
11β	2.10 (dd, *13.5, 3.5*)	2.12 (dd, *13.5, 3.5*)	1.63 (m)	2.30–2.39[b]	1.86–1.95[b]
12α			1.97 (m)	5.74 (d, 6.0)	2.51 (m)
12β	5.17 (dd, *13.5, 3.5*)	5.27 (dd, *13.5, 3.5*)	1.59 (m)		1.98–2.06[b]
15β	4.08 (d, 3.0)	4.09 (d, 3.0)			4.10 (d, 4.0)
16α	1.74–1.80[b]	1.88 (m)		3.23 (brd, 18.5)	2.67–2.73[b]
16β	1.85–1.92[b]	1.75–1.80[b]		2.88 (m)	3.14 (ddd, 16.5, 4.5, 3.5)
17	2.88 (m)	2.90 (m)	3.10 (d, 11.6)	3.04 (m)	
18	1.10 (s)	1.10 (s)	1.20 (s)	1.29 (s)	1.12 (s)
19α	1.90 (AB d, 15.5)	1.97–2.01[b]	2.03 (AB d, 15.6)	2.01 (AB d, 15.0)	1.86–1.93[b]
19β	2.28 (AB d, 15.5)	3.15 (AB d, 16.0)	2.30 (AB d, 15.6)	2.10 (AB d, 15.0)	2.06 (AB d, 15.5)
20	2.67 (m)	2.67 (m)	2.74 (m)	2.30–2.39[b]	3.09 (m)
21	0.91 (d, 7.0)	0.91 (d, 7.0)	1.32 (d, 7.2)	1.29 (d, 6.5)	1.28 (d, 7.0)
22	3.86 (d, 11.5)	3.86 (d, 10.0)	3.87 (dd, *9.2, 2.0*)	3.87 (dd, 6.5, 2.0)	3.83 (dd, 7.0, 4.5)
23	4.70 (br s)	4.70 (br s)	5.08 (br s)	5.29 (br s)	5.11 (m)
24	4.45 (br s)	4.44 (br s)	7.25 (br s)	7.24 (d, 1.4)	7.22 (br s)
27	2.04 (s)	2.01 (s)	1.89 (s)	1.84 (s)	1.86 (s)
29	1.12 (s)	1.14 (s)	1.00 (s)	1.08 (s)	1.09 (s)
30	1.28 (s)	1.28 (s)	1.21 (s)	1.24 (s)	1.21 (s)

[a] Data of compound **3** were recorded on a Bruker DRX-400 MHz spectrometer, while data of the other four compounds were recorded on a Bruker DRX-500 MHz spectrometer; chemical shift values δ are in parts per million, and the coupling constant *J* is in hertz (in parentheses). Assignments were confirmed by ^1H–^1H COSY, HSQC, and HMBC.

[b] Overlapping signals.

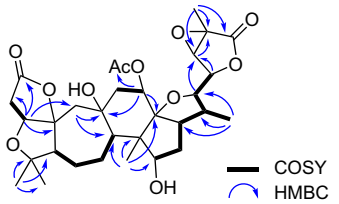


Figure 1. ^{1}H–^{1}H COSY and selected HMBC correlations of **1**.

from the molecular formula $C_{31}H_{42}O_{12}$ of **2**, which was established by the HRFABMS ([M−H]$^-$ at *m*/*z* 605.2618). The main difference was found to be existed in A ring where the methene (δ_C 36.9, t) in **1** was replaced by an oxygenated methine (δ_C 87.0, d) in **2**, which indicated that the new hydroxyl should be located at C-2. Accordingly, the typical ABX spin system in **1** was showed to be replaced by two singlets at δ_H 4.65 (H-1) and 4.35 (H-2) in **2** from the ^{1}H NMR spectra (Table 1). These two proton singlets suggested that the dihedral angel between H-1 and H-2 was near 90°, which further indicated that H-2 should be assigned to be α-orientation, since H-1 was biogenetically assigned as β-directed.[3d] The disappearance of H-1/H-2 cross peak in ROESY spectrum confirmed the deduction. The structure of **2** was thus furnished.

Propindilactone M (**3**) was isolated as an amorphous powder. The HRESIMS showed a pseudo-molecular ion peak at *m*/*z* 527.2261 [M−H]$^-$ corresponding to the molecular formula $C_{29}H_{36}O_9$, requiring 12 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) revealed four tertiary methyls, one secondary methyl, six methenes, seven sp^3 methines (including three oxygenated ones), six quaternary sp^3 carbons (five of them were oxygenated), one carbonyl, two ester carbonyls, and one trisubstituted double bond, which indicated that **3** was a C_{29} nortriterpenoid with eight rings and one hydroxyl. The characteristic ABX and AB spin systems in ^{1}H NMR spectra (Table 1) indicated that molecule **3** had

Table 2
^{13}C NMR spectroscopic data of compounds **1**–**5**[a] in C_5D_5N

Carbon	1	2	3	4	5
1	82.1 (d)	73.5 (d)	81.9 (d)	81.6 (d)	82.0 (d)
2	36.9 (t)	87.0 (d)	35.6 (t)	35.7 (t)	35.8 (t)
3	175.7(s)	177.2 (s)	175.8 (s)	175.0 (s)	175.6 (s)
4	85.1 (s)	85.9 (s)	83.9 (s)	84.4 (s)	84.3 (s)
5	58.7 (d)	59.0 (d)	61.9 (d)	60.9 (d)	61.2 (d)
6	28.7 (t)	28.7 (t)	24.6 (t)	28.3 (t)	28.2 (t)
7	25.5 (t)	25.6 (t)	24.2 (t)	24.2 (t)	24.7 (t)
8	57.5 (d)	57.5 (d)	48.2 (d)	59.0 (d)	57.6 (d)
9	72.3 (s)	72.8 (s)	85.2 (s)	72.0 (s)	73.1 (s)
10	99.2 (s)	99.8 (s)	96.8 (s)	99.3 (s)	99.0 (s)
11	40.5 (t)	40.5 (t)	37.9 (t)	42.3 (t)	40.6 (t)
12	74.4 (d)	74.2 (d)	31.1 (t)	122.0 (d)	18.1 (t)
13	94.8 (s)	95.0 (s)	89.2 (s)	145.6 (s)	138.9 (s)
14	55.3 (s)	55.3 (s)	57.4 (s)	51.0 (d)	55.0 (d)
15	78.6 (d)	78.7 (d)	107.8 (s)	216.5 (s)	77.8 (d)
16	32.7 (t)	32.8 (t)	210.7 (s)	46.0 (t)	42.8 (t)
17	53.3 (d)	53.4 (d)	53.7 (d)	45.5 (d)	131.3 (s)
18	23.6 (q)	23.7 (q)	11.0 (q)	25.1 (q)	23.7 (q)
19	40.4 (t)	40.8 (t)	42.3 (t)	44.8 (t)	46.3 (t)
20	34.7 (d)	34.8 (d)	37.4 (d)	42.0 (d)	35.8 (d)
21	11.4 (q)	11.4 (q)	13.0 (q)	16.9 (q)	16.8 (q)
22	87.4 (d)	87.5 (d)	86.4 (d)	76.1 (d)	75.1 (d)
23	76.9 (d)	76.8 (d)	80.4 (d)	82.9 (d)	83.9 (d)
24	63.0 (d)	63.0 (d)	147.1 (d)	148.5 (d)	148.2 (d)
25	57.1 (s)	57.1 (s)	130.5 (s)	130.6 (s)	130.3 (s)
26	173.0 (s)	173.1 (s)	174.4 (s)	174.9 (s)	174.9 (s)
27	11.3 (q)	11.2 (q)	10.7 (q)	10.8 (q)	10.9 (q)
29	23.6 (q)	23.6 (q)	21.3 (q)	22.6 (q)	22.1 (q)
30	30.0 (q)	30.0 (q)	28.2 (q)	28.7 (q)	28.7 (q)

[a] Data were recorded on a Bruker DRX-500 MHz spectrometer, chemical shift values δ are in parts per million, assignments were confirmed by ^{1}H–^{1}H COSY, HSQC, and HMBC.

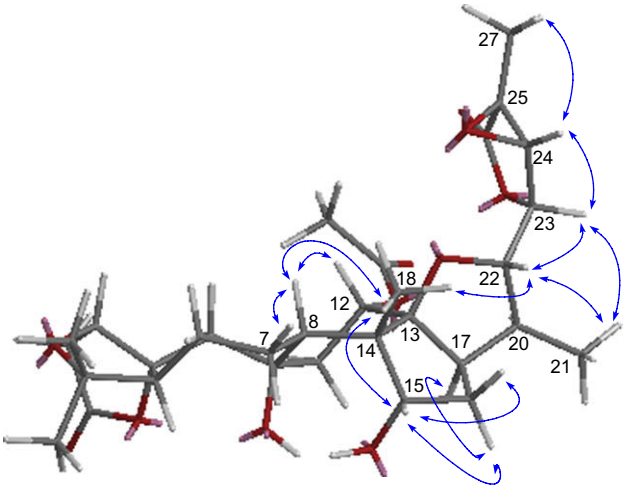


Figure 2. Key ROESY correlations of **1**.

the similar A–C rings as that of wuweizidilactone D (**6**), which was further confirmed by ^{1}H–^{1}H COSY correlations of H-1/H_2-2 and H-5/H_2-6/H_2-7/H-8 (Fig. 3). Signals of the five-membered α-methyl-α,β-unsaturated-γ-lactone ring at δ_C 10.7 (q, Me-27), 80.4 (d, C-23), 147.1 (d, C-24), 130.5 (s, C-25), and 174.4 (s, C-26) were found to be identical to those of **6**. A long proton spin system H-17/H-20/H-22(H_3-21)/H-23/H-24 observed in ^{1}H–^{1}H COSY spectrum (Fig. 3), in combination with two prominent carbon signals at δ_C 89.2 (s, C-13) and 86.4 (d, C-22) suggested that a furan ring maybe also built up between C-13 and C-22 in **3**. Thus, the Me-18 had to migrate to C-14 as in case of **6**. Important HMBC correlations (Fig. 3) from H-8 to C-7, C-9, C-13, C-14, and Me-18, from H_3-18 to C-8, C-13, C-14, and C-15, from H_2-12 and H-17 to C-13, and from H-17 and H-20 to C-16 confirmed that **3** possessed 18(13→14)-*abeo*-schiartane skeleton with 6/5 membered D/E rings and Me-18 at C-14.

Taking 12 degrees of unsaturation and molecular formula into account, there should be one additional epoxide ring built and one hydroxyl group existed among carbons in D and E rings of **3**. Since C-16 was a carbonyl while both C-11 and C-12 existed as two sp^3 methenes without oxidation, the newly built oxo-ring had to cyclize between C-9 and C-15. This deduction was confirmed by the typical hemiketal carbon signal at δ_C 107.8 (s), which was assigned to be C-15. The following pending question was whether this 9,15-oxygen bridge formed above rings D and E or underneath them. To the best of our knowledge, 9-OH in all known nortriterpenoids is α-directed.[3] Then, this 9,15-oxygen bridge was possibly cyclized with α-orientation 9-OH and formed underneath rings D and E, which was absolutely approved by the appearance of the key ROESY cross peaks of H-8 to H-7β, H-12β, and H_3-18 (Fig. 4). Thus, the spatial structure of the newly built 9,15-epoxy furan ring conjunctured with D and E rings was shaped into a cage structure (Fig. 4) with great tension, which resulted in the severely change of related carbon signals among D and E rings, especially of C-8 ($\Delta\delta_C$ −9.4), C-9 ($\Delta\delta_C$ +15.0), and Me-18 ($\Delta\delta_C$ −13.0) on the newly built oxygen ring when compared with that of **6**. At the same time, spatial

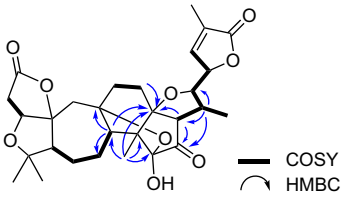


Figure 3. ^{1}H–^{1}H COSY and selected HMBC correlations of **3**.

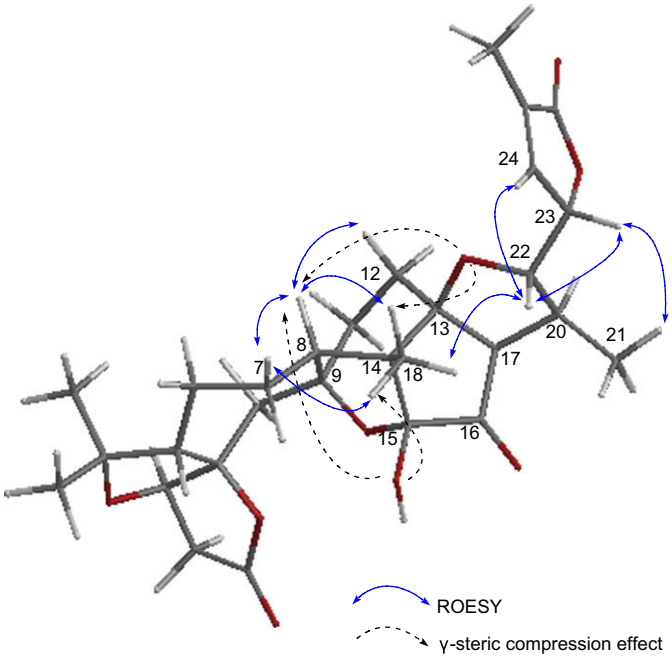


Figure 4. Selected ROESY correlations and γ-steric compression effects of **3**.

readjustment of these atoms led to the γ-steric compression effects from oxygen atoms of 15-OH and 13,22-epoxy ring to both H-8 (δ_H 2.21, $\Delta\delta_H$ +0.87) and H_3-18 (δ_H 1.20, $\Delta\delta_H$ +0.16), which would also resulted in the dramatically upshift of C-8 and Me-18 (Fig. 4). The relative stereochemistry of the other chiral centers was found to be identical to those of **6** by comparison of their ^{1}H NMR coupling constants (Table 1) and ROESY data.

Propindilactone N (**4**) was obtained as colorless crystals from MeOH. Analysis of the ^{1}H, ^{13}C, and DEPT spectra (Tables 1 and 2) indicated that the NMR data of **4** highly resembled those of micrandilactone B (**7**)[3i] in rings A/B/C, C-17 side chain, and α-methyl-α,β-unsaturated-γ-lactone ring F. Considering 11 degrees of unsaturation by HRESIMS ($[M-H]^-$ at *m*/*z* 513.2488, $C_{29}H_{38}O_8$) and a carbonyl signal at δ_C 216.5, as well as the other trisubstituted double bond (δ_C 122.0, d; 145.6, s) displayed in ^{13}C NMR spectrum (Table 2), compound **4** should have another two rings. HMBC correlations from H_3-18 to C-8, C-13, C-14, and C-15 (Fig. 5), in combination with the ROESY cross peaks of H_3-18/H-7β and H_3-18/H-8 indicated that **4** was also a member of 18(13→14)-*abeo*-schiartane family with 6/5 membered D/E rings and the β-Me substituent at C-14. ^{1}H-^{1}H COSY spin systems of H_2-11/H-12 and H_2-16/H-17, together with the HMBC correlations from H-17 to C-12 and C-16 further furnished the whole planar structure of **4** as shown in Figure 5. The relative stereochemistry of **4** was found to be identical to that of **7** by comparison of their ^{1}H NMR coupling constants (Table 1) and ROESY data.

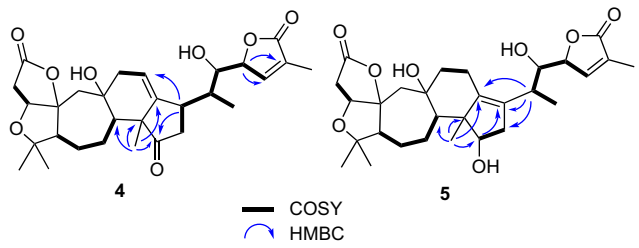
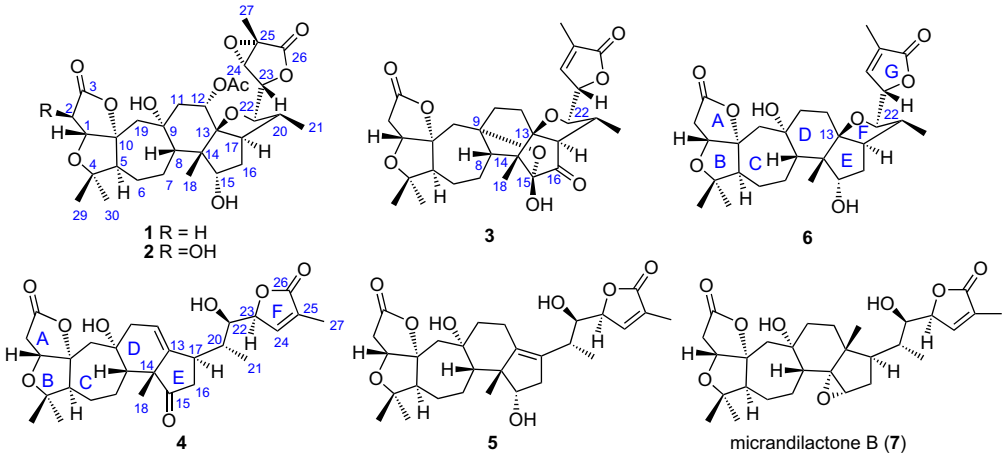
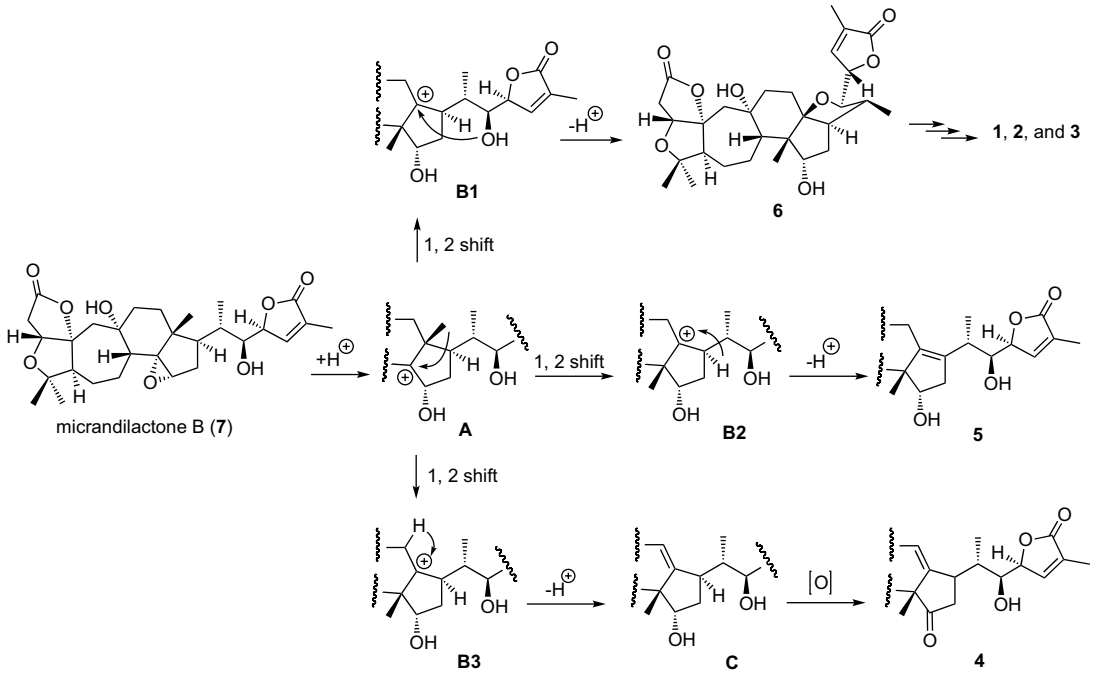


Figure 5. ^{1}H–^{1}H COSY and selected HMBC correlations of **4** and **5**.

Propindilactone O (**5**) was colorless crystals from MeOH. The ^{13}C and DEPT data (Table 2) revealed 29 carbon signals including a quaternary double bond (δ_C 131.3, s; 138.9, s) and an oxygenated methine (δ_C 77.8, d), and the carbon signals among A, B, and F rings were much alike to those of **4**. Three groups of ^{1}H–^{1}H COSY spin systems, H-5/H_2-6/H_2-7/H-8, H_2-11/H_2-12, and H-15/H_2-16, coupled with three groups of key HMBC correlations from H_3-18 to C-8, C-13, C-14, and C-15, from H-15 to C-17, and from H-20 to C-13, C-16, and C-17 accomplished the planar structure of **5**, a 18(13→14)-*abeo*-schiartane nortriterpenoid. The 15-OH was assigned to be α-orientation as established from obvious ROESY correlations of H-15 with H-8 and H_3-18.

The biosynthetic origin of **1**–**6** could be rationalized to micrandilactone B (**7**) (Schemes 1 and 2, discussed below) whose absolute stereochemistry has been established by a modified Mosher method together with X-ray diffraction and then applied to determine the absolute stereochemistry of related nortriterpenoids including **6**.[3j,k] Thus, the absolute configuration of **1**–**3** could be proposed as depicted by correlations with **6** based on the biosynthetic hypothesis (Scheme 1). At the same time, the absolute stereochemistry of **4** and **5** could be determined as shown on the ground of biosynthetic foundation (Scheme 1), which established both *R* configuration of C-20 and C-22. Their CD spectrum similarity with that of **7** further confirmed the same *S* configuration of C-23 whose Cotton effect around 220 nm corresponding to the moiety of α,β-unsaturated-γ-lactone in the side chain (λ_{max} around 213 nm in UV spectra) were $\Delta\varepsilon$ −26.8 at 220 nm for **4**, −59.4 at 215 nm for **5**, and −26.8 at 218 nm for **7**, respectively.





Scheme 1. Hypothetical biosynthetic route of **1**–**6** generated from **7**.

2.2. Hypothetical biogenetic route for propindilactones K–O (1–6)

Nortriterpenoids with 18(13→14)-*abeo*-schiartane skeleton are thought to be originated from schiartane skeleton via a Wagner–Meerwein rearrangement,[8] in which Me-18 migrated from C-13 to C-14.[2] Firstly, a cyclic carbocation at C-13 was generated, which can rearrange and cyclize further or be neutralized either by deprotonation or water addition without rearrangement. In our study, compounds **1**–**6** and the major component **7**[7] with schiartane skeleton from the same origin strongly suggested a possible biogenetic transformation of **7** into **1**–**6**. When **7** was enzymatically protonated to open the epoxide ring at C-14 and C-15 (**A**), the 1,2 shift of Me-18 would followed to generate a cation at C-13. Then, the OH on the side chain attacked this cation (**B1**) would generate compound **6**, which would further afford **1**, **2**, and **3** by simple post-modifications of oxidation or acetylation. The carbocation intermediate could go through deprotonation directly with proton at C-17 (**B2**) or C-12 (**B3**) to yield compound **5** or intermediate **C**, which would further generate **4** via oxidative process at C-15 (Scheme 1). This proposed biogenetic pathway of schiartane to 18(13→14)-*abeo*-schiartane skeleton was chemical mimicked by a $BF_3 \cdot Et_2O$-catalyzed transformation of **7** to **6** (Scheme 2) for the first time.

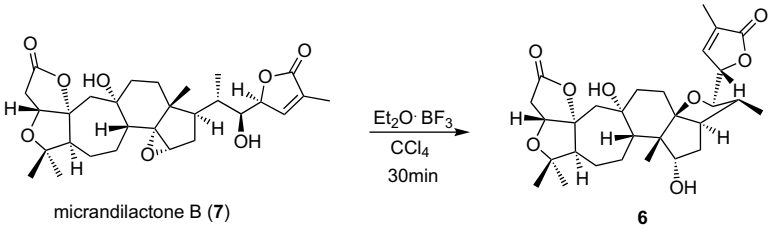
2.3. Bioactive evaluation of compounds 2, 4, and 6

The anti-HBV activities were valuated in vitro among compounds **2**, **4**, and **6** isolated in large amounts (>5 mg) using the Hep G 2.2.15 cell line stably transfected with the HBV genome as reported previously.[9] Anti-HBV activity, cytotoxicity, and selectivity index (SI) are summarized in Table 3. Unfortunately, compound **6** shows neither cytotoxicity nor anti-HBV activity in Hep G 2.2.15 cells. Compound **4** is low cytotoxicity and moderate active to HBsAg with SI value of 1.62, in the range of 1 and 2, while it was inactive to HBeAg. Interestingly, compound **2** exhibit high inhibitory potential against both HBsAg and HBeAg with low cytotoxicity. The SI values of **2** were 2.68 and 1.11, respectively, which were comparative to that of 3TC (2.57 and 1.16, respectively). Anti-HBV evaluation of these compounds suggested that propindilactone L (**2**) was active to inhibit HBsAg and HBeAg in vitro for the first time, which provided candidate for the study of this medicinal plant.

3. Experimental section

3.1. General

Melting points were obtained on an XRC-1 micro melting point apparatus without correction. Optical rotations were carried out on a JASCO DIP-370 digital polarimeter. UV data were obtained on a UV-210A spectrometer and IR spectra were measured on a Bio-Rad FtS-135 spectrophotometer with KBr pellets, whereas CD spectra were recorded on a JASCO J-810 spectropolarimeter. High-resolution electrospray-ionization (HRESIMS) and fast atom bombardment (FABMS) mass spectra were acquired on an API QSTAR time-of-flight mass spectrometer and a VG Autospec-3000 mass spectrometer, respectively. 1D and 2D NMR spectra were taken on



Scheme 2. Chemical correlation of compounds **6** and **7**.

Table 3
Anti-HBV activity, cytotoxicity, and selectivity index of **2**, **4**, and **6**[a]

Compound	CC_{50} (mg/mL)		HBsAg			HBeAg		
			IC_{50} (mg/mL)		SI (average)	IC_{50} (mg/mL)		SI (average)
2	1.173	1.397	0.488	0.471	2.68	1.309	1.061	1.11
4	0.827	0.817	0.553	0.472	1.62	>1.50	>1.50	<0.55
6	>1.900	>1.900	>1.900	>1.900	—	>1.900	>1.900	—
3TC	6.920	6.870	2.670	2.303	2.57	5.94	5.974	1.16

[a] 3TC (lamivudine): positive control; HBsAg: hepatitis B virus surface antigen; HBeAg: hepatitis B virus e antigen; SI (selective index) is the ratio of CC_{50} and IC_{50}. All index were measured on two independent experiments.

a Bruker DRX-400 or DRX-500 NMR spectrometers with TMS as internal standard. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C_{18}, 9.4 mm×25 cm column. Column chromatography (CC) was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), Lichroprep RP-18 gel (40–63 μm, Merck, Darmstadt, Germany), MCI gel (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), and Sephadex LH-20 (Pharmacia). All solvents including petroleum ether (60–90 °C) were distilled prior to use.

3.2. Plant material

The stems of *S. propinqua* var. *propinqua* were herborized in Tengchong County, Yunnan Province, PR China, in July 2006, and identified by Prof. Xi-Wen Li, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 20050823) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, PR China.

3.3. Extraction and isolation

The air-dried stems of *S. propinqua* var. *propinqua* (8 kg) were extracted with 70% aqueous acetone (4×15 L, 3 days each) at room temperature. The solvent was removed in vacuo to afford a crude extract (560 g), which was dissolved in H_2O, and then extracted successively with petroleum ether and EtOAc. The EtOAc-soluble part (250 g) was purified by CC (on SiO_2 with $CHCl_3$/acetone 1→0) to obtain six main fractions (Fr. A–F). Fr. C ($CHCl_3$/acetone 9:1 to 8:2, 29 g) was purified by repeated CC, first on Sephadex LH-20 eluted with MeOH, then on silica gel eluted by PE/i-PrOH in gradient system and followed by recrystallization to afford **2** (6.0 mg) and **6** (20.0 mg) or by semi-prep. HPLC (40–50% aqueous MeOH) to yield **1** (2.4 mg) and **3** (1 mg). Fr. D ($CHCl_3$/acetone 8:2 to 2:1, 45 g) was purified first by CC on silica gel with $CHCl_3$/acetone 4:1 to obtain small fractions of D1, D2, and D3. D2 was then subjected to RP-18 in 30–60% aqueous MeOH gradient system after purified on Sephadex LH-20 eluted with MeOH to afford five mixtures (D2-1 to D2-5). D2-2 (40% aqueous MeOH) was purified on silica gel with PE/i-PrOH 5:1 to give **4** (8.0 mg) and followed by semi-prep. HPLC (40% aqueous MeOH) to yield **5** (4.2 mg).

3.3.1. *Propindilactone K* (***1***)

White crystals, mp 152–153 °C; $[\alpha]_D^{22.8}$ +53.67 (*c* 0.30, MeOH); IR (KBr) ν_{max} 3430, 2972, 2938, 1785, 1748, 1638, 1239, 1028 cm−1; ^1H and ^{13}C NMR data, see Tables 1 and 2, acetyl: δ_H 1.78 (s), δ_C 170.3 (s), 20.7 (q); Negative FABMS: *m*/*z* 589 $[M-H]^-$; Negative HRESIMS: found: 589.2618, calcd 589.2648 for $C_{31}H_{41}O_{11}[M-H]^-$.

3.3.2. *Propindilactone L* (***2***)

White powder, mp 170–171 °C; $[\alpha]_D^{24.9}$ +40.00 (*c* 0.35, MeOH); IR (KBr) ν_{max} 3425, 2971, 2938, 2880, 1786, 1640, 1460, 1381, 1239, 1082, 1026 cm−1; ^1H and ^{13}C NMR data, see Tables 1 and 2, acetyl: δ_H 1.85 (s), δ_C 170.3 (s), 23.6 (q); Negative FABMS: *m*/*z* 605 $[M-H;\ ]^-$; Negative HRFABMS: found: 605.2618, calcd 605.2598 for $C_{31}H_{41}O_{12}$ $[M-H]^-$.

3.3.3. *Propindilactone M* (***3***)

White solid; $[\alpha]_D^{22.1}$ −73.33 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 213.2 (3.70) nm; IR (KBr) ν_{max} 3265, 2934, 1757, 1060 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; Negative FABMS: *m*/*z* 527 $[M-H;\ ]^-$; Negative HRESIMS: found: 527.2261, calcd 527.2281 for $C_{29}H_{35}O_9[M-H]^-$.

3.3.4. *Propindilactone N* (***4***)

Colorless crystals, mp 205–206 °C; $[\alpha]_D^{21.5}$ −36.40 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 260.0 (2.91), 209.4 (4.10) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 301.8 (−12.52), 262.8 (+0.23), 258.4 (−1.34), 220.0 (−26.78), 205.0 (+28.47), 195.2 (+3.94), 193.8 (+4.96) nm; IR (KBr) ν_{max} 3473, 3425, 2974, 2932, 1758, 1086 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; Negative FABMS: *m*/*z* 513 $[M-H]^-$; Negative HRESIMS: found: 513.2486, calcd 513.2488 for $C_{29}H_{37}O_8$ $[M-H]^-$.

3.3.5. *Propindilactone O* (***5***)

White crystals, mp 155–156 °C; $[\alpha]_D^{21.5}$ +36.30 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 279.8 (2.93), 206.0 (4.32) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 214.8 (−59.43), 199.6 (+12.78), 197.0 (+8.99), 194.4 (+14.42), 192.0 (+10.08) nm; IR (KBr) ν_{max} 3420, 2968, 2929, 2874, 1757, 1206, 1185, 1065 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; Negative FABMS: *m*/*z* 515 $[M-H]^-$; Negative HRESIMS: found: 515.2636, calcd 515.2644 for $C_{29}H_{39}O_8$ $[M-H]^-$.

3.4. Transformation of 7 to 6

A solution of $Et_2O{\cdot}BF_3$ (19.6 mg, 0.010 mL) in CCl_4 (1.0 mL) was added dropwise to a solution of compound **7** (14.0 mg, 0.027 mmol) in CCl_4 (5 mL), and the reaction was then stirred at room temperature for 30 min. After quenching the reaction with water, the organic phase was concentrated to give a crude product, which was purified by HPLC semi-preparation (eluted by 45% aqueous MeOH) to afford the desired product **6** (8.1 mg) as identified by ^1H, ^{13}C NMR and TLC check.

3.5. Anti-HBV bioassays

The anti-HBV assays were performed according to the method in the previous report.[9] An antivirus agent, lamivudine (3TC; Glaxosmithkline; Suzhou, China), was used as positive control.

Acknowledgements

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Supplementary data

The HRESIMS, 1D and 2D NMR spectra of **1**–**5**, the UV and CD spectra of **4** and **5**, and the CD spectrum of **7** are available. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2008.10.079.

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