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Limonoids from the Leaves and Twigs of *Walsura yunnanensis*

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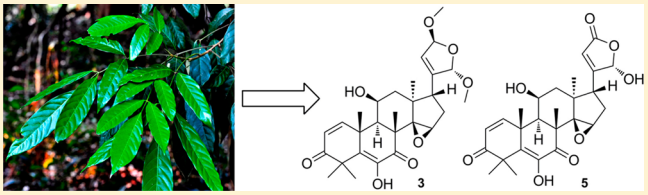
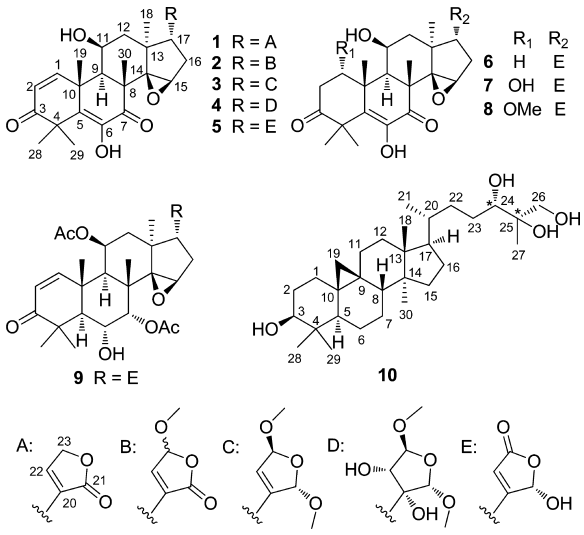
Supporting Information

ABSTRACT: Nine new cedrelone limonoids, namely, walsuranolide B (**1**), 11β-hydroxy-23-*O*-methylwalsuranolide (**2**), yunnanolide A (**3**), yunnanol A (**4**), 11β-hydroxyisowalsuranolide (**5**), 11β-hydroxy-1,2-dihydroisowalsuranolide (**6**), 1α,11β-dihydroxy-1,2-dihydroisowalsuranolide (**7**), 11β-hydroxy-1α-methoxy-1,2-dihydroisowalsuranolide (**8**), and yunnanolide B (**9**), together with a new cycloartane triterpenoid, (24*S**,25*R**)-cycloartane-3β,24,25,26-tetrol (**10**), were isolated from the leaves and twigs of *Walsura yunnanensis*. Their structures were elucidated on the basis of spectroscopic analysis and by comparison with literature data. Compounds **3** and **5** exhibited potent cytotoxicity against five human tumor cell lines with IC_{50} values in the range 2.2−4.2 μM.



Meliaceous limonoids have attracted interest due to their diverse structures and biological activities, such as insect antifeedant, antimalarial, cytotoxic, and 11β-HSD1 inhibitory effects.[1,2] The genus *Walsura* Roxb. (Meliaceae), comprising 30 to 40 species, is distributed primarily in mainland China, India, Malaysia, and Indonesia.[3] Chemical studies conducted in the past decade have shown that this genus is rich in biologically active limonoids. *Walsura yunnanensis* C. Y. Wu is endemic to the Xishuangbanna region of Yunnan Province, People's Republic of China.[3] Earlier investigations have shown the presence of limonoids and phenolic compounds.[2a,4] In a continuing study of the medicinal plants of Xishuangbanna for biologically active secondary metabolites, the leaves and twigs of *W. yunnanensis* were investigated. As a result, 10 new compounds (**1**−**10**) were isolated. This report describes the extraction, isolation, structure elucidation, and cytotoxicity evaluation of these compounds.



RESULTS AND DISCUSSION

The leaves and twigs of *W. yunnanensis* were extracted three times with 95% EtOH. After removing the solvent, the ethanol extracts were suspended in water and then partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc-soluble fraction was purified by column chromatography to produce nine new cedrelone limonoids (**1**−**9**) and one new cycloartane triterpenoid (**10**).

Compound **1** was obtained as colorless needles ($CHCl_3$). Its HREIMS showed a molecular ion peak at *m*/*z* 454.1986 $[M]^+$ (calcd 454.1992), consistent with a molecular formula of $C_{26}H_{30}O_7$, with 16 mass units more than that of the known compound 11β-hydroxycedrelone, isolated from the same plant.[2a] The NMR data of **1** (Tables 1 and 2) were very close to those reported for 11β-hydroxycedrelone, with the only difference being the replacement of signals for the furan ring (δ_H 7.35 (d, J = 1.5 Hz), 7.15 and 6.15 s; δ_C 122.7, 139.5, 110.5, and 143.2) by those for an α,β-unsaturated-γ-lactone ring[5] [δ_H 4.71 (2H, m) and 7.13 (1H, s); δ_C 71.2, 133.3, 149.0, and 174.6] in **1**. This conclusion was confirmed by the HMBC correlations between H-17/C-21 and H-23/C-21 as well as the ROESY correlations between H-11/H-9 and H-9/H_3-18. Thus,

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Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1−5[a]

	1[b]	2[c]	3[b]	4[c]	5[b]
proton position	(mult., J in Hz)	(mult., J in Hz)	(mult., J in Hz)	(mult., J in Hz)	(mult., J in Hz)
1	7.28, d (10.0)	7.30, d (10.0)	7.32, d (10.0)	7.24[f]	7.32, d (10.0)
2	6.29, d (10.0)	6.30, d (10.0)	6.33, d (10.0)	6.30, d (9.8)	6.31, d (10.0)
9	2.85, s	2.82, s	2.85, s	3.02, s	2.86, s
11	5.04, d (6.3)	5.04, m	5.03, m[f]	5.02, m	5.12, d (6.0)
12α	2.26, dd (15.1, 6.3)	2.20, dd (15.2, 6.2)	2.36, dd (15.0, 5.8)	2.93, dd (15.0, 6.2)	2.34, dd (14.5, 6.0)
12β	2.95, d (15.1)	2.91, d (15.2)	2.30, d (15.0)	2.57, d (15.0)	2.81, d (14.5)
15	4.08, s	4.08, s	4.08, s	4.20, s	4.11, s
16α	2.04, m	2.05, dd (13.3, 11.1)	1.95, dd (13.3, 11.6)	2.56, m	2.06, m
16β	2.14, dd (13.5, 6.5)	2.15, dd (13.3, 6.4)	2.19, dd (13.3, 6.5)	2.25, dd (13.2, 6.2)	2.17, dd (13.4, 6.4)
17	3.02, m	2.98, m	2.64, dd (10.8, 6.5)	2.68, dd (10.7, 6.2)	3.26, dd (10.3, 6.4)
18	0.92, 3	0.90, s	1.05, s	1.70, s	1.01, s
19	1.81, s	1.81, s	1.80, s	1.79, s	1.82, s
21			5.30, s	5.06, s	6.43, s
22	7.13, s	7.01, s	5.73, s	4.65, dd (6.5, 4.4)	6.09, s
23	4.71, m[d]	5.93, s	5.77, s	5.45, d (4.4)	
28	1.88, s[e]	1.87, s	1.892, s	1.89, s	1.878, s
29	1.88, s[e]	1.88, s	1.888, s	1.88, s	1.884, s
30	1.78, s	1.78, s	1.75, s	1.77, s	1.78, s
OMe-21			3.40, s	3.39, s	
OMe-23		3.41, s	3.36, s	3.43, s	

[a]Recorded in C_5D_5N. [b]Recorded at 500 MHz. [c]Recorded at 600 MHz. [d]Double protons. [e]Overlapped by other signal. [f]Overlapped by solvent signal.

the structure of **1** was elucidated as depicted, and this compound has been named walsuranolide B.

Compound **2** was assigned a molecular formula of $C_{27}H_{32}O_8$ on the basis of its HREIMS, or 30 mass units greater than that of **1**. The NMR data of **2** differed from those of **1** (Tables 1 and 2) only in the absence of signals for the oxymethylene (CH_2-23; δ_H 4.71, 2H, m; δ_C 71.2) in the α,β-unsaturated-γ-lactone ring and the presence of signals for dioxymethine [δ_H 5.93 (1H, s); δ_C 103.2] and methoxy (δ_H 3.41, 3H, s; δ_C 56.4) groups. The methoxy group was revealed to be located at C-23 by the HMBC correlation between OCH_3-23 and C-23, although its relative configuration remains undetermined. Further 2D NMR spectroscopic data analysis confirmed this assignment and was used to establish the structure of **2** as the 11β-hydroxy-23-*O*-methyl derivative of walsuranolide, 11β-hydroxy-23-*O*-methyl-walsuranolide.

Compound **3** was obtained as colorless needles ($CHCl_3$). Its molecular formula, $C_{28}H_{36}O_8$, was established by the HREIMS peak at m/z 500.2413 $[M]^+$ (calcd 500.2410), or 16 mass units greater than that of compound **2**. Comparison of its ^{1}H and ^{13}C NMR data (Tables 1 and 2) suggested that **3** differs only in the replacement of the ester carbonyl (δ_C 171.6, C-21) in **2** by a methoxymethine (δ_H 5.30, δ_C 109.7, CH-21; δ_H 3.40, δ_C 55.6, OMe-21). The ROESY correlations between H_3-18/H-23 and H-21/H-17 indicated H-23 and H-21 to be α- and β-oriented, respectively. Therefore, the structure of compound **3** was established as shown, and this compound has been named yunnanolide A.

The molecular formula of compound **4** was determined to be $C_{28}H_{38}O_{10}$ from its molecular ion peak at m/z 534.2467 $[M]^+$ (calcd 534.2465) in the HREIMS, 34 mass units more than that of **3**. The 1D NMR data of **4** resembled closely those of **3** (Tables 1 and 2), except for the absence of signals for a $\Delta^{20,22}$ double bond and the presence of signals for an oxymethine (δ_H 4.65, dd, J = 6.5, 4.4 Hz; δ_C 79.1; CH-22) and an oxygenated quaternary carbon (δ_C 82.3, C-20). These observations suggested **4** to be the 20,22-dihydroxy-20,22-dihydro derivative of **3**. The ^{1}H−^{1}H COSY correlation between H-22 and H-23 as well as the HMBC correlations between H_2-16/C-20, H-21/C-17, H-21/C-23, OCH_3-21/C-21, and OCH_3-23/C-23 (Figure S25, Supporting Information) confirmed this inference. The ROESY correlations between H-17/H-21 and H-17/H-22 indicated that H-21 and H-22 are β-oriented, while the absence of any ROESY correlation between H-23 and H-21 was consistent with the presence of a H-23α substituent. The structure of **4** (yunnanol A) was thus established as shown.

Compound **5** was obtained as a crystalline solid ($CHCl_3$). Its molecular formula, $C_{26}H_{30}O_8$, was established by the HREIMS peak at m/z 470.1951 $[M]^+$ (calcd 470.1941), showing 16 mass units more than that of isowalsuranolide isolated previously from this plant.[2a] The ^{1}H and ^{13}C NMR data of **5** (Tables 1 and 2) were very similar to those of isowalsuranolide, with a major difference being the replacement of signals for a methylene (δ_C 19.6) in isowalsuranolide by those for an oxymethine (δ_H 5.12, d, J = 6.0 Hz; δc 66.4). These observations suggested that **5** is the 11-hydroxy derivative of isowalsuranolide, and the doublet for H-11 and a singlet for H-9 indicated a cis relationship between H-9 and H-11.[2a] Thus, the structure of **5** was deduced as 11β-hydroxyisowalsuranolide.

Compound **6** was obtained as colorless needles ($CHCl_3$). Its HREIMS revealed a molecular ion peak at m/z 472.2081 $[M]^+$ (calcd 472.2097), consistent with a molecular formula of $C_{26}H_{32}O_8$, having two mass units more than that of compound **5**. The ^{1}H and ^{13}C NMR data of **6** (Tables 2 and 3) were very similar to those of **5**. Differences between the two compounds were the absence of signals for the $\Delta^{1,2}$ double bond and the presence of signals for two additional sp^3 methylenes (δ_C 36.2 and 33.6) in **6**. This compound was therefore assigned as a 1,2-dihydro derivative of **5**. Further 2D NMR spectroscopic data analysis confirmed the structure proposed for **6** as 11β-hydroxy-1,2-dihydroisowalsuranolide.

Compound **7** was obtained as colorless needles ($CHCl_3$), and its molecular formula, $C_{26}H_{32}O_9$, was established from the HREIMS peak at m/z 488.2041 $[M]^+$ (calcd 488.2046),

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Table 2. ^{13}C NMR Spectroscopic Data of Compounds 1−9

position	1[a]	2[b]	3[a]	4[b]	5[a]	6[a]	7[c]	8[a]	9[d]
1	154.1	154.1	154.1	154.3	154.0	36.2	71.1	81.2	158.2
2	127.5	127.5	127.6	127.4	127.6	33.6	45.0	38.8	125.3
3	204.4	204.4	204.4	204.5	204.4	214.9	214.9	214.1	203.5
4	49.5	49.4	49.5	49.4	49.5	48.8[e]	49.0	48.7	41.0
5	134.1	134.0	134.2	133.8	134.0	140.0	138.4	135.6	48.6
6	144.0	144.0	144.0	144.0	144.1	142.9	145.3	145.1	69.8
7	199.1	199.0	199.0	199.1	198.9	199.5	199.6	199.3	73.7
8	47.5	47.4	47.5	47.5	47.4	47.0	46.8	46.67	44.3
9	46.0	45.8	46.1	45.6	46.1	48.8[e]	40.4	40.2	41.9
10	41.66	41.63	41.7	41.6	41.6	40.1	45.5	44.9	41.7
11	66.5	66.4	66.9	67.0	66.4	66.1	66.3	66.3	63.6
12	47.2	47.0	47.6	47.4	46.5	46.7	46.5	46.72	42.8
13	41.68	41.60	41.8	42.0	42.0	42.1	42.2	42.2	41.1
14	69.6	69.6	69.8	69.5	69.4	69.7	70.0	69.8	71.5
15	56.5	56.3	56.6	56.9	56.2	56.5	56.6	56.4	56.0
16	31.8	31.7	32.5	29.0	31.1	31.2	31.1	31.1	30.7
17	43.3	43.3	45.2	48.9	45.4	45.4	45.4	45.3	42.1
18	22.9	22.9	22.9	23.2	23.0	23.2	22.9	23.0	20.9
19	26.7	26.7	26.7	26.6	26.7	17.9	18.1	17.7	24.0
20	133.3	138.1	144.9	82.3	170.7	170.8	170.8	170.9	169.9
21	174.6	171.6	109.7	111.0	100.3	100.4	100.3	100.4	99.0
22	149.0	146.5	127.7	79.1	120.0	120.0	119.9	119.9	119.4
23	71.2	103.2	107.3	112.6	171.5	171.6	171.6	171.7	170.6
28	27.6	27.6	27.7	27.6	27.6	25.0	25.0	24.6	31.4
29	22.0	22.0	22.0	21.8	22.0	21.5	22.1	21.9	20.1
30	23.6	23.6	23.5	23.8	23.6	24.0	24.1	24.1	21.4
OMe-1								57.2	
OAc-7									170.1
									20.9
OAc-11									169.6
									21.0
OMe-21			55.6	55.2					
OMe-23		56.4	53.2	56.3					

[a]Recorded in C_5D_5N at 125 Hz. [b]Recorded in C_5D_5N at 150 Hz. [c]Recorded in C_5D_5N at 100 Hz. [d]Recorded in DMSO-d_6 at 100 Hz. [e]Overlapped by other signal.

showing 16 mass units more than that of compound **6** (Tables 2 and 3). The ^1H and ^{13}C NMR spectroscopic data observed for **7** were similar to those of compound **6**. However, signals for CH_2-1 (δ_H 2.35 and 1.89; δ_C 36.2) and CH_2-2 (δ_H 2.85 and 2.69; δ_C 33.6) were replaced by proton signals at δ_H 4.68, 3.21, and 3.14 and carbon signals at δ_C 71.1 and 45.0, which suggested that **7** is either a 1- or 2-hydroxy derivative of **6**. The HMBC correlations H-1/C-3, H-9/C-1, and H_3-19/C-1 indicated the hydroxy group is at C-1, and the ROESY correlation H-1/H_3-19 suggested that the hydroxy group is α-oriented. Thus, **7** was determined as 1α,11β-dihydroxy-1,2-dihydroisowalsuranolide.

Compound **8** was obtained as colorless needles ($CHCl_3$), and its HREIMS displayed a molecular ion peak at m/z 502.2205 $[M]^+$ (calcd 502.2203), which corresponded to a molecular formula of $C_{27}H_{34}O_9$, representing 14 mass units more than that of compound **7**. The 1D NMR spectroscopic data of **8** (Tables 2 and 3) were almost identical to those of compound **7**, with the only difference being the presence of an additional methoxy group. The downfield shift of signals for the oxymethine (δ_C C-1, from 71.1 to 81.2) suggested that this methoxy group is located at C-1, which was further supported by the HMBC correlations from OCH_3-1 (δ_H 3.34, s) to C-1 (δ_C 81.2) and H-1 (δ_H 3.83, d, J = 6.1 Hz) to OCH_3-1 (δ_C 57.2). The ROESY correlation H-1/H_3-19 suggested that the methoxy group is α-oriented. Compound **8** was therefore established as 11β-hydroxy-1α-methoxy-1,2-dihydroisowalsuranolide.

Compound **9** was obtained as a white, amorphous powder, and its HREIMS displayed a molecular ion peak at m/z 558.2461 $[M]^+$ (calcd 558.2465), corresponding to the molecular formula $C_{30}H_{38}O_{10}$. Comparison of the NMR spectroscopic data of **9** (Tables 2 and 3) with those of **5** showed that signals for the α-hydroxy-α,β-unsaturated ketone group were absent, whereas those for three methines (δ_H 5.30, dd, J = 12.4, 2.5 Hz; 4.775, m; 4.770, m; δ_C 69.8, 73.7, 63.6) and two acetoxy groups (δ_H 2.10, s; 1.94, s; δ_C 170.1, 20.9; 169.6, 21.0) were observed. These observations suggested **9** to be a 5,6,7-trihydrodiacetate derivative of **5**. Further 2D NMR spectroscopic data analysis (Figures S59−S62, Supporting Information) not only confirmed this assignment but allowed the placement of the two acetoxy groups at C-7 and C-11, respectively, via HMBC correlations of H-7/CH_3CO-7 (δ_C 170.1) and H-11/CH_3CO-11 (δ_C 169.6). The strong ROESY correlation between H-11/H-9 suggested H-11 to be α-oriented, while the ROESY correlations of H-6/H_3-19 and H-7/H_3-30 indicated that H-6 and H-7 are β-oriented. Thus, **9**

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Table 3. ^{1}H NMR Spectroscopic Data of Compounds 6–9

proton position	6[a] (mult., J in Hz)	7[b] (mult., J in Hz)	8[a] (mult., J in Hz)	9[c] (mult., J in Hz)
1α	1.89, m			7.55, d (10.2)
1β	2.35, dd (13.2, 9.6)	4.68, m	3.83, d (6.1)	
2α	2.85, dd (18.8, 9.2)	3.14, m	3.02, d (18.9)	5.93, d (10.2)
2β	2.69, dd (18.8, 9.6)	3.21, m	2.87, dd (18.9, 6.1)	
5				2.43, m
6				5.30, dd (12.4, 2.5)
7				4.775, m
9	2.56, s	3.74, br s	3.45, s	2.29, br s
11	4.92, m	5.05, m[d]	4.92, d (6.0)	4.770, m
12α	2.23, m	2.20, m	2.33, dd (14.5, 6.0)	2.38, m
12β	2.75, m	2.70, m	2.80, d (14.5)	1.68, m
15	4.09, s	4.13, m	4.11, s	3.33, m
16α	2.07, m	2.06, m	2.06, dd (13.3, 10.0)	1.86, m
16β	2.16, m	2.15, m	2.14, dd (13.3, 6.2)	1.91, m
17	3.25, m	3.25, m	3.27, dd (10.0, 6.2)	2.41, m
18	1.02, s	1.07, s	1.07, s	0.92, s
19	1.61, s	1.67, s	1.58, s	1.46, s
21	6.44, s	6.27, s	6.47, s	5.96, s
22	6.10, s	6.03, s	6.08, s	6.01, s
28	1.77, s	2.11, s	1.89, s[e]	1.16, s
29	1.85, s	1.95, s	1.89, s[e]	1.05, s
30	1.74, s	1.82, s	1.77, s	1.34, s
OMe-1			3.34, s	
OAc-7				2.10, s
OAc-11				1.94, s

[a]Recorded in C_5D_5N at 500 Hz. [b]Recorded in C_5D_5N at 400 Hz. [c]Recorded in DMSO-d_6 at 400 Hz. [d]Overlapped by solvent signal. [e]Overlapped by other signal.

was established structurally as depicted and was named yunnanolide B.

Compound **10** was isolated as an amorphous powder, with its molecular formula determined to be $C_{30}H_{52}O_4$ on the basis of the HREIMS data obtained. The ^{1}H and ^{13}C NMR spectra (Table 4) showed this compound to have five tertiary methyl groups (δ_H 0.90, 1.00, 1.66, 1.12, and 1.24), one secondary methyl group (δ_H 1.02), two cyclopropylmethylene protons (δ_H 0.31, d, J = 3.5 Hz; 0.54, d, J = 3.5 Hz), and two typical upfield quaternary carbons (C-9, δ_C 20.3; C-10, δ_C 26.9). These observations indicated that **10** is a cycloartane triterpenoid. Detailed comparison of its NMR data with those of (24*S*)-25-methoxycycloartane-3β,24-diol[6] suggested compound **10** to be its 26-hydroxy analogue. This was supported by the HMBC correlations (Figure 1A) of H_2-26/C-24, H_3-27/C-26, and H-24/C-26. Observation of a strong ROESY correlation between H_3-27 and H-24 and the absence of any ROESY correlation between H_2-23 and H-26 in its preferred configuration suggested the relative stereochemistry as 24*S** and 25*R** (Figure 1B1 and B2). This inference was further supported by comparing its ^{1}H NMR data (in $CDCl_3$; Figure S71, Supporting Information) for H-24 and H_2-26 [ca. δ_H 3.82 (d, J = 11.1 Hz, 1H), 3.46 (m, 2H)] with those of a triterpenoid triol 24*S*,25*R* (or 24*R*,25*S*) isomer [ca. δ_H 3.83 (d, J = 11.2 Hz, 1H); δ_H 3.48 (m, 2H)] that possesses a similar ring D and is a side chain [for the 24*S*,25*S* (or 24*R*,25*R*) isomer]. However, the relevant signals for **10** appeared at ca. δ_H 3.65 (d, J = 11.2 Hz, 1H), 3.49 (d, J = 11.2 Hz, 1H), and δ_H 3.55 (1H).[7] Compound **10** was therefore determined to be (24*S**,25*R**)-cycloartane-3β,24,25,26-tetrol.

Table 4. ^{1}H and ^{13}C NMR Spectroscopic Data (500 and 125 MHz, C_5D_5N) for Compound 10

position	δ_H (mult., JinHz)	δ_C	position	δ_H (mult., Jin Hz)	δ_C
1α	1.54, m	32.8	16α	1.97, m	28.9
1β	1.25, m		16β	1.68, m	
2α	2.00, m	28.9	17	1.00, s[b]	53.3
2β	1.36, m		18	1.00, s[b]	18.8
3	3.55, dd (4.5, 11.5)	78.3	19a	0.54, d (3.5)	30.4
4		41.5	19b	0.31, d (3.5)	
5	1.32, m	47.8	20	1.57, m	36.8
6α	1.59, m	21.8	21	1.02[c]	19.0
6β	0.79, m		22a	1.82, m	34.5
7α	1.06, m	26.7	22b	1.73, m	
7β	1.30, m		23a	1.99, m	31.6
8	1.49, dd (4.6, 12.3)	48.5	23b	1.89, m	
9		20.3	24	4.19, m	76.6
10		26.9	25		75.1
11α	2.02, m	27.0	26a	4.32, d (10.6)	69.5
11β	1.14, m		26b	4.13, d (10.6)	
12	1.61, m[a]	33.6	27	1.66, s	20.6
13		45.7	28	1.24, s	26.6
14		49.4	29	1.12, s	15.3
15α	1.27, m	36.2	30	0.90, s	19.9
15β	2.15, m				

[a]Double protons. [b]Overlapped by other signal. [c]Signal partially obscured.

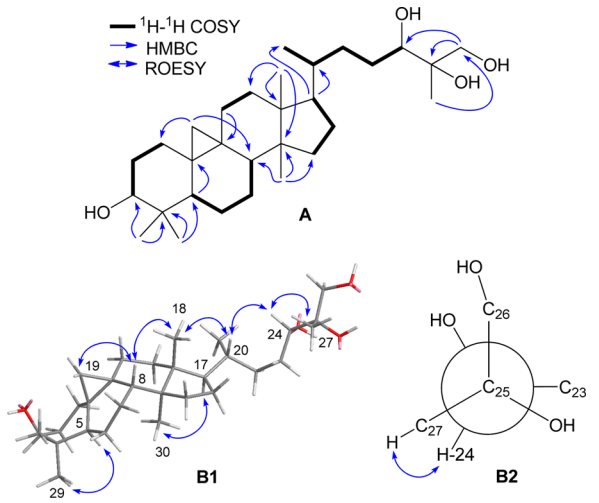

Figure 1. Selected ^{1}H–^{1}H COSY (bold bond), HMBC (A), and ROESY (B1 and B2) correlations of **10**.

Compounds **3**, **5**, **6**, **7**, **9**, and **10** were obtained in sufficient amounts to be evaluated for their cytotoxic activity against human myeloid leukemia (HL-60), hepatocellular carcinoma (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), colon cancer (SW480), and human bronchial epithelial (BEAS-2B) cell lines. Of the substances tested, only compounds **3** and

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5 exhibited potent cytotoxicity against the five human tumor cell lines, with IC_{50} values in the range 2.2−4.2 μM (Table 5).

Table 5. Cytotoxic Activity (IC_{50} μM) of Selected Isolated Compounds for Cancer Cell Lines

compound	HL-60	SMMC-7721	A-549	MCF-7	SW480	BEAS-2B
3	3.6	2.4	3.7	4.2	3.5	5.0
5	3.1	2.2	2.6	3.9	2.4	9.4
cisplatin	1.9	5.3	8.7	>10	>10	>10

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were obtained on an X-4 digital micromelting point apparatus and are uncorrected. Optical rotations were obtained with a JASCO P-1020 polarimeter. UV spectra were measured with a Shimadzu UV-2401A instrument. IR spectra (KBr) were determined on a Bruker Tensor-27 infrared spectrometer. 1D and 2D NMR spectra were recorded on Bruker AM-400, Bruker DRX-500, and Bruker Avance III 600 spectrometers with TMS as an internal standard. EIMS and HREIMS were recorded on an AutoSpec Premier P776 instrument, while ESIMS were measured with a Finnigan MAT 90 mass spectrometer. Semipreparative HPLC was carried out using a Waters system consisting of a 600 pump and a 2996 photodiode array detector. Silica gel (200−300 mesh, Qingdao Marine Chemical Factory, Qingdao, People's Republic of China), Sephadex LH-20 gel (40−70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), and MCI gel (CHP20/P120, 75−150 μm, high-porous polymer, Mitsubishi Chemical Corporation, Tokyo, Japan) were used for column chromatography.

Plant Material. The leaves and twigs of *W. yunnanensis* were collected from Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Science (CAS), Mengla Country, Yunnan Province, People's Republic of China, in October 2012 and were identified by Chun-Fen Xiao (XTBG, CAS). A voucher specimen (no. 2012676) was deposited in the herbarium of XTBG.

Extraction and Isolation. The air-dried, powdered plant material (5.0 kg) was extracted three times (each for 7 days) with EtOH−H_2O (95/5, v/v, 30 L) at room temperature. Removal of solvent of the combined extracts under a vacuum afforded a crude residue (300 g). The residue was then suspended in H_2O (2 L) and partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively. The EtOAc-soluble fraction (100 g) was subjected to silica gel column chromatography (CC) (MeOH−$CHCl_3$ from 0/1 to 1/1) to produce seven fractions (1−7). Fr. 2 (19.8 g) was applied to an MCI gel column (MeOH−H_2O from 3/7 to 4/1) to yield five major fractions (2A−2E). Filtration of the precipitate from Fr. 2A (150 mg) yielded **10** (80 mg). Fr. 2C (500 mg) was chromatographed by Sephadex LH-20 CC, eluted with MeOH−H_2O (from 2/3 to 4/1) to give Fr. 2C1 (15 mg), which was further purified by semipreparative HPLC (MeOH−H_2O from 3/7 to 3/2) to yield **3** (6 mg). Fr. 3 (11.6 g) was separated over an MCI column eluted with MeOH−H_2O (from 2/3 to 4/1) to give six major fractions (3A−3F). The precipitate from Fr. 3A (210 mg) yielded **9** (100 mg). Fr. 3B (1 g) was applied to a silica gel column eluted with petroleum ether−acetone (from 1/0 to 2/1) to give **5** (10 mg) and subfractions 3A1 (50 mg) and 3A2 (15 mg). Subfractions 3A1 and 3A2 were further purified by Sephadex LH-20 CC (MeOH−H_2O from 3/7 to 3/2) and semipreparative HPLC (CH_3CN−H_2O from 2/3 to 7/3) to yield **2** (2 mg), **6** (8 mg), **7** (6 mg), and **8** (2 mg). Fr. 3C (20 mg) and 3D (55 mg) were purified initially by Sephadex LH-20 CC (MeOH−H_2O from 1/4 to 1/1) and then by semipreparative HPLC (CH_3CN−H_2O from 1/4 to 13/7) to yield respectively **1** (6 mg) and **4** (3 mg).

*Walsuranolide B (**1**):* colorless needles ($CHCl_3$); mp 128−130 °C; $[\alpha]^{27}_D$ −3.7 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.20) nm; IR (KBr) ν_{max} 3426, 2935, 1753, 1679, 1628, 1451, 1352, 1251, 1122, 1092, 1054, 1035, 960, 756 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m*/*z* 454.1986 $[M]^+$ (calcd for $C_{26}H_{30}O_7$, 454.1992).

*11β-Hydroxy-23-O-methylwalsuranolide (**2**):* colorless needles ($CHCl_3$); mp 115−117 °C; $[\alpha]^{27}_D$ −2.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.01), 277 (3.84) nm; IR (KBr) ν_{max} 3442, 2924, 1767, 1631, 1456, 1383, 1260, 1095, 1035 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m*/*z* 484.2105 $[M]^+$ (calcd for $C_{27}H_{32}O_8$, 484.2097).

*Yunnanolide A (**3**):* colorless needles ($CHCl_3$); mp 136−138 °C; $[\alpha]^{27}_D$ −32.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.05), 276 (3.13) nm; IR (KBr) ν_{max} 3428, 2925, 1632, 1454, 1379, 1102, 1035 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m*/*z* 500.2413 $[M]^+$ (calcd for $C_{28}H_{36}O_8$, 500.2410).

*Yunnanol A (**4**):* colorless needles ($CHCl_3$); mp 168−170 °C; $[\alpha]^{28}_D$ −24.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 211 (3.89), 278 (3.80) nm; IR (KBr) ν_{max} 3424, 2931, 1679, 1384, 1124, 1034, 992 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m*/*z* 534.2467 $[M]^+$ (calcd for $C_{28}H_{38}O_{10}$, 534.2465).

*11β-Hydroxyisowalsuranolide (**5**):* crystalline solid ($CHCl_3$); mp 126−128 °C; $[\alpha]^{27}_D$ −35.5 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.23), 278 (3.95) nm; IR (KBr) ν_{max} 3426, 2935, 1759, 1678, 1355, 1254, 1122, 1035, 950, 908 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HREIMS *m*/*z* 470.1951 $[M]^+$ (calcd for $C_{26}H_{30}O_8$, 470.1941).

*11β-Hydroxy-1,2-dihydroisowalsuranolide (**6**):* colorless needles ($CHCl_3$); mp 130−132 °C; $[\alpha]^{26}_D$ −55.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.08), 278 (3.86) nm; IR (KBr) ν_{max} 3431, 2925, 1746, 1703, 1679, 1631, 1384, 1253, 1135, 1034 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HREIMS *m*/*z* 472.2081 $[M]^+$ (calcd for $C_{26}H_{32}O_8$, 472.2097).

*1α,11β-Dihydroxy-1,2-dihydroisowalsuranolide (**7**):* colorless needles ($CHCl_3$); mp 120−122 °C; $[\alpha]^{27}_D$ −39.1 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.59), 279 (3.43) nm; IR (KBr) ν_{max} 3441, 2922, 2852, 1632, 1384 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HREIMS *m*/*z* 488.2041 $[M]^+$ (calcd for $C_{26}H_{32}O_9$, 488.2046).

*11β-Hydroxy-1α-methoxy-1,2-dihydroisowalsuranolide (**8**):* colorless needles ($CHCl_3$); mp 135−137 °C; $[\alpha]^{27}_D$ −11.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.11), 279 (3.94) nm; IR (KBr) ν_{max} 3424, 2927, 1761, 1630, 1384, 1092, 1034 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HREIMS *m*/*z* 502.2205 $[M]^+$ (calcd for $C_{27}H_{34}O_9$, 502.2203).

*Yunnanolide B (**9**):* white, amorphous powder; $[\alpha]^{26}_D$ +105.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 221 (4.47) nm; IR (KBr) ν_{max} 3431, 2921, 1767, 1738, 1648, 1379, 1256, 1226, 1120, 1045 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; HREIMS *m*/*z* 558.2461 $[M]^+$ (calcd for $C_{30}H_{38}O_{10}$, 558.2465).

(24S,25R*)-Cycloartane-3β,24,25,26-tetrol (**10**):* white, amorphous powder; $[\alpha]^{26}_D$ +35.3 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3430, 2966, 2936, 2866, 1635, 1449, 1377, 1337, 1287, 1121, 1081, 1043, 1021, 995, 916, 879 cm^{-1}; ^1H and ^{13}C NMR data (C_5D_5N), see Table 4; ^1H NMR ($CDCl_3$, 600 MHz) δ 3.82 (1H, d, *J* = 11.1 Hz, H-26a), 3.47 (1H, m, H-24), 3.46 (1H, d, *J* = 11.1 Hz, H-26b), 3.27 (1H, m, H-3), 1.99 (2H, m, H-11), 1.09 (3H, s, H_3-27), 0.963 (3H, s, H_3-18), 0.96 (3H, s, H_3-28), 0.89 (3H, s, H_3-30), 0.88 (3H, d, *J* = 6.6 Hz, H_3-21), 0.80 (3H, s, H_3-29), 0.55 (1H, d, *J* = 3.9 Hz, H-19a), 0.32 (1H, d, *J* = 4.1 Hz, H-19b); HREIMS *m*/*z* 476.3867 $[M]^+$ (calcd for $C_{30}H_{52}O_4$, 476.3866).

Cytotoxicity Assays. The MTS method was used for assessing the cytotoxicity of the compounds against five tumor cell lines (HL-60 human myeloid leukemia, SMMC-7721 hepatocellular carcinoma, A-549 lung cancer, MCF-7 breast cancer, and SW480 colon cancer). All cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum and 100 U/mL penicillin/streptomycin in a humidified incubator in a 5% CO_2 atmosphere at 37 °C. Then, 100 μL of adherent cells was seeded into each well (1×10^4 cells/well) of 96-well cell culture plates and allowed to adhere for 12 h before test drug addition. Each tumor cell line was exposed to a test compound at concentrations of 0.064, 0.32, 1.6, 8, and 40 μM in DMSO in triplicate for 48 h, with cisplatin as the positive control. After 48 h incubation, 20 μL of MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-

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phenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium] solution was added to each well, which were incubated for another 4 h to give a formazan product. Then 100 μL of 20% SDS was added to each well and incubated 12 h at room temperature for the formazan product to dissolve completely. The OD value of each well was measured at 490 nm using a Biorad 680 instrument. The IC_{50} value of each compound was calculated by the Reed and Muench method.[8]

■ ASSOCIATED CONTENT

Supporting Information

This material (HREIMS, ^{1}H and ^{13}C NMR, and 2D NMR spectra of limonoids (**1**−**9**) and (24*S**,25*R**)-cycloartane-3*β*,24,25,26-tetrol (**10**)) is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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