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Isolation of 11β-Hydroxycedrelone from *Walsura yunnanensis* and its Cytotoxic Activity

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11β-Hydroxycedrelone was isolated from the twigs and leaves of *Walsura yunnanensis C. Y. Wu*. Its structure was established on the basis of spectroscopic methods. 11β-Hydroxycedrelone featured a typical rearranged A-ring structure of this compound class. Cytotoxic evaluation showed that its exhibited moderate inhibitory activity against HL-60 cell line with the IC_{50} values of 8.9 μM.

Keywords: 11β-Hydroxycedrelone, *Walsura yunnanensis*, Antitumor activity.

INTRODUCTION

The genus *Walsura* (Meliaceae) comprising about 30-40 species and varieties is naturally distributed in tropical regions such as Southern China, India and Indonesia[1]. Several investigations on this genus have led to the isolation of a number of triterpenoids and tetranortriterpenoids and showed some insect anti-feedants, anti-oxidant, anti-malarial activity and cell protecting activities[2-6]. As a continuous study in our work, searching for the structurally and biologically metabolites from this genus, 11β-hydroxycedre-lone was isolated from the leaves and twigs of *W. yunnanensis*. The structure was established on the basis of spectroscopic methods. It was featured a typical rearranged A-ring structure of this compound class. Cytotoxic evaluation showed that 11β-hydroxycedrelone possessed selective anti-proliferative activity and exhibited moderate inhibitory activity against HL-60 cell line with the IC_{50} values of 8.9 μM. The paper present herein the isolation, structural elucidation and anti-proliferative activity of 11β-hydroxycedrelone.

11β-Hydroxycedrelone, obtained as a white powder, has a molecular formula of $C_{35}H_{46}O_{10}Na$ as determined by the HRESIMS ion at *m/z* 649.2983 $[M + Na]^+$ (calcd for $C_{35}H_{46}O_{10}Na$, 649.2989) with 13° of unsaturation. A detailed account of the structural assignment of 11β-hydroxycedrelone is given as below. The β-furan ring attached to C-17 was observed. The presence of a 14,15-double bond was revealed by the chemical shifts of C-14 at δ_C 158.2 and C-15 at δ_C 120.7 and was confirmed by the mutual HMBC correlations (Fig. 1a) from H-15 to C-13, C-16 and C-17 and from Me-30 and Me-18, to C-14. In the B-ring, a hydroxyl group and a tigloyl moiety (δ_H 6.98, 1H, qd, J = 7.3 Hz, 1.8 Hz, δ_H 1.83, 3H, d, J = 7.3 Hz and δ_H 1.84, 3H, s; δ_C 170.0, 127.5, 140.8, 14.8 and 12.1), were attached to C-7 and C-6, respectively, supported by the HMBC correlations of H-7/C-5, C-6 and C-9 and Me-30/C-7 and C-5/H-6, Me-28, Me-29 and C-1′/H-6, H-3′ and Me-5′. Furthermore, two *O*-acetyl groups were located at C-1 and C-2, which was confirmed by the HMBC correlations from H-1 and H-2 to two ester carbonyl at δ_C 170 and 170.1, respectively. And the HMBC correlations of H-1/C-2 and C-19 further validated the deduction. Then, two remaining oxygenated carbons were tentatively assigned to C-3 and C-11. The chemical shifts of C-3 at δ_C 101.8 and C-11 at δ_C 68.0, indicated that a hemiketal was formed at C-3 *via* an oxygen bridge between C-3 and C-11, which was confirmed by the HMBC correlations from OH-3 (δ_H 6.03, s) to C-2 and C-19 and from H-11 to C-8 and C-12, although on direct HMBC correlations were available. The carbon resonances of the hemiketal moiety in 11β-hydroxycedrelone were consistent with those of Toonaciliatin H[7]. Thus, the planar structure of 11β-hydroxycedrelone was clarified.

EXPERIMENTAL

The leaves and twigs of *W. yunnanensis* were collected from Xishuangbanna of Yunnan Province, People's Republic of China. All solvents used were of the highest commercially available quality.

FT Infrared (IR) spectra were recorded in KBr disks using FD-5DX spectrometer. UV spectra were recorded on a

Shimadzu UV-2550 spectrophotometer. Specific rotations were determined on a Perkin-Elmer 341 polarimeter. ESIMS and HRESIMS were obtained on an Esquire 3000 plus (Bruker Daltonics) and a waters-micromass Q-TOF Ultima Global electrospray mass spectrometer, respectively. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Varian INOVA-400 Spectrometer, using $CDCl_3$ as a solvent; TMS (δ 0.00 ppm) was used as an internal standard. All NMR chemical shifts are reported as δ values in parts per million (ppm) and coupling constants (*J*) are given in hertz (Hz). Semi-preparative HPLC was carried out on a waters 515 pump with a waters 2487 detector (254 nm) and an YMC-Pack ODS-A column (250 × 10 mm, S-5 μm, 12 nm).

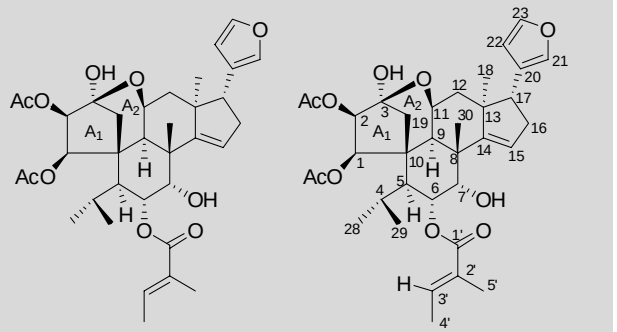
Extraction and isolation: The air-dried powders of trunks of *W. yunnanensis* (4 kg) were extracted three times with 95 % EtOH at room temperature to give an ethanolic extract (127 g), which was partitioned between EtOAc and water to obtain the EtOAc soluble fraction (49 g). The fraction was separated on a column of MCI gel (MeOH:H_2O, 40:60 to 90:10, v/v) to afford eight fractions A-H. Fraction C (8.15 g) was chromatographed on a silica gel column eluted with petroleum ether/ethyl acetate (100:1 to 5:1, v/v) to afford six major fractions. Fraction C6 (285 mg) was purified by a semi-preparative HPLC with 55 % acetonitrile in water to yield compound 11β-hydroxycedrelone (6.5 mg).

11β-Hydroxycedrelone: White powder; $[\alpha]^{24}_D$ + 18 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.49) nm; IR (KBr, ν_{max}, cm^{-1}) 3433, 2926, 1738, 1720, 1651, 1371, 1252, 1030; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table-1; Positive mode ESIMS *m/z* 649 $[M + Na]^+$; HRESIMS *m/z* 649.2983 $[M + Na]^+$ (calcd for $C_{35}H_{46}O_{10}Na$, 649.2989).

Cytotoxicity assay: The cytotoxicity of 11β-hydroxycedrelone was assessed by use of the MTT assay[8-13]. The MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay is a simple non-radioactive colourimetric assay to measure cell cytotoxicity, proliferation or viability. MTT is a yellow, water-soluble, tetrazolium salt. Metabolically active cells are able to convert this dye into a water-insoluble dark blue formazan by reductive cleavage of the tetrazolium ring. Formazan crystals, then, can be dissolved and quantified by measuring the absorbance of the solution at 570 nm and the resultant value is related to the number of living cells.

The effect of 11β-hydroxycedrelone on the cells proliferation efficiency was determined after 24 h incubation with cells. To determine cells proliferation, the A549 cells, HL-60 cells, BEL7404 cell lines and Tca cell lines were individually plated at a density of 1×10^4 cells/well in 96 well plates at 37 °C in 5 % CO_2 atmosphere. After 24 h of culture, the medium in the wells was replaced with the fresh medium containing 11β-hydroxycedrelone of varying concentrations, respectively. The 11β-hydroxycedrelone concentration given upon is the final concentration in the well. Every concentration added five wells as parallel control. After 24 h, 10 μL of MTT dye solution (5 mg/mL in phosphate buffer pH 7.4) was added to each well and incubated for 4 h at 37 °C and 5 % CO_2 for exponentially growing cells and 10 min for steady-state confluent cells. The formazan crystals were solubilized with 100 μL of DMSO and the solution was vigorously mixed to dissolve the reacted dye.

TABLE-1
^{1}H NMR AND ^{13}C NMR SPECTROSCOPIC DATA OF 11β-HYDROXYCEDRELONE



Protons	11β-Hydroxy-cedrelone[a]	Carbons	11β-Hydroxy-cedrelone[b]
1	5.24 (d, 3.6)	1	85.7
2	4.80 (d, 3.6)	2	86.6
	-	3	101.8
4	2.39 (m)	4	27.6
5	2.12 (m)	5	40.8
6	5.31 (dd, 12.0, 2.4)	6	72.4
7	4.08 (s)	7	72.1
	-	8	43.3
9	2.24 (d, 6.4)	9	37.2
	-	10	48.8
11	4.47 (dd, 15.3, 8.7)	11	68.0
12	2.30 (dd, 12.8, 8.7); 1.66 (dd, 12.8, 8.7)	12	39.6
	-	13	46.3
	-	14	158.2
15	5.60 (d, 2.6)	15	120.7
16	2.60 (dd, 15.7, 12.1); 2.43 (m)	16	34.1
17	2.92 (dd, 10.6, 7.1)	17	51.0
18	0.77 (s)	18	19.3
19	2.14 (m)	19	36.8
	-	20	123.7
21	7.26 (s)	21	139.9
22	6.29 (s)	22	111.1
23	7.39 (s)	23	142.7
28	0.83 (d, 7.3)	28	18.2
29	1.09 (d, 7.3)	29	25.0
30	1.44 (s)	30	28.0
3-OH	6.03 (s)		
1-OAc	2.14 (s)	1-OAc	170.0, 21.3
2-OAc	2.14 (s)	2-OAc	170.1, 21.7
	-	1′	170.0
	-	2′	127.5
3′	6.98 (qd, 7.3, 1.8)	3′	140.8
4′	1.83 (d, 7.3)	4′	12.1
5′	1.84 (s)	5′	14.8

[a]Data were measured in $CDCl_3$ at 400 MHz
[b]Data were measured in $CDCl_3$ at 100 MHz

The absorbance of each well was read on a micro-plate reader (DYNATECH MR7000 instruments) at 570 nm. The spectrophotometer was calibrated to zero using culture medium without cells. We selected inhibitory effect to evaluate side effects of the silica coated fluorescent 11β-hydroxyce-drelone to cells proliferation. The inhibitory effect of 11β-hydroxycedrelone was calculated as percentage inhibition in comparison to the value obtained in untreated well to which no 11β-hydroxycedrelone was added.

RESULTS AND DISCUSSION

The relative stereochemistry of 11β-hydroxycedrelone was established on the ROESY experiment (Fig. 1b). The ROESY correlations of Me-18/H-9, Me-18/H-11, H-9/H-1, H-9/H-11, H-1/H-2 and H-2/OH-3 indicated that H-1, H-2, OH-3, H-11, Me-18 and H-9, were cofacial and were randomly assigned to be α-oriented and the C-3/C-10 hemiketal group was in β-orientation. Subsequently, the ROESY correlations of H-17/H-12b, H-19/Me-30, Me-30/H-7, H-7/H-6 and H-6/Me-30 indicated that they were cofacial and β-oriented. Therefore, the structure of 11β-hydroxycedrelone was thus fully assigned as depicted.

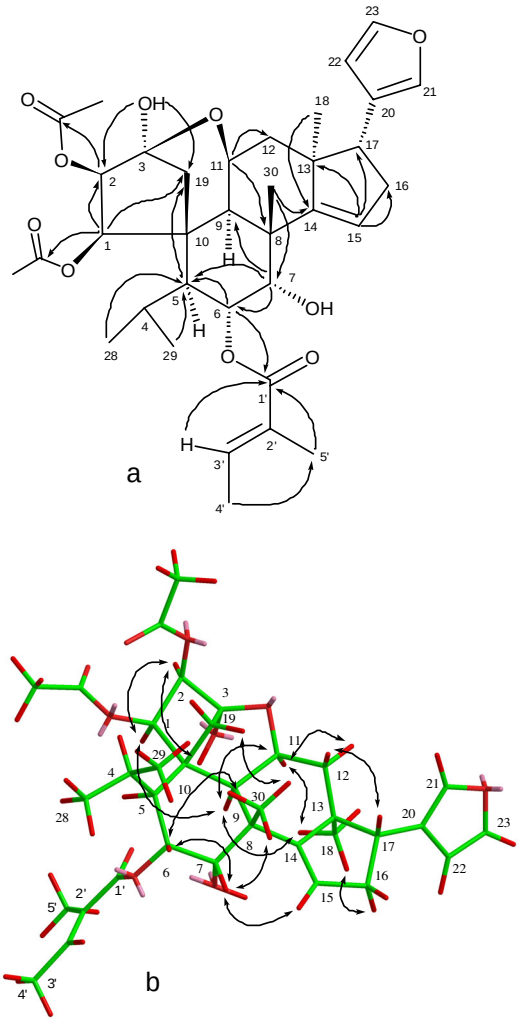


Fig. 1. (a) Selected HMBC (H → C), and (b) ROESY (H ↔ H) correlations of 11β-hydroxycedrelone

11β-Hydroxycedrelone was tested for the antitumor activitiy against four different cancer cell lines: human lung cancer cell line (A549), human premyelocytic leukemia (HL-60), human liver cancer cell line (BEL7404) and human tongue cancer cell line (Tca) by using the MTT. It was found that 11β-hydroxycedrelone possessed selective anti-proliferative activity and exhibited moderate inhibitory activity against HL-60 cell line with the IC_{50} values of 8.9 μM.

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