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Chemical Constituents of the Suspension Cell Cultures of *Maytenus hookeri*

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Abstract: Suspension cell cultures of *Maytenus hookeri* Loes. (Celastraceae) in SH media were established from the calli induced from the leaves and young stems of *M. hookeri* on MS media with the supplement of 2 mg/L 2,4_D and 0.1 mg/L KIN (kinetin). Ethyl acetate extract of the cultures showed inhibitory activities against *Penicillium avellaneum* UC_4376 which was sensitive to maytansinoids. Exhaustive isolation of natural products from a large scale of suspension cell cultures did not yield maytansine instead of affording nine compounds including one novel triterpenoid, named 2,3_diacetoxyl maytenusone (**1**), and eight known ones including squalene (**2**), β_sitosterol (**3**), 2′, 3′, 4′_triacetyl_sitoindoside Ⅰ (**4**), salaspermic acid (**5**), maytenonic acid (**6**), 2α_hydroxy_maytenonic acid (**7**), 6,11,12_trihydroxy_8,11,13_abietrien_7_one (**8**) and 11,12_dihydroxy_8,11,13_abietatrien_7_one (**9**) elucidated on the basis of 1D and 2D NMR data. The ^{1}H_NMR and ^{13}C_NMR assignments were made for **1**, **5**, **6** and **7**, while the ^{13}C_NMR assignments for **5** and **6** were revised. The chemical results suggested that the suspension cell cultures of *M. hookeri* did not produce maytansinoids under the reported experiment conditions.

Key words: *Maytenus hookeri*; Celastraceae; suspension cell cultures; maytansine; 2,3_diacetoxyl maytenusone

Maytansinoids are microlides with strong cytotoxic and antineoplastic activities first isolated from *Maytenus serria*[1, 2] and found from various natural sources including a bacterium (*Actinosynnema pretiosum*)[3], mosses[4, 5] and higher plants of three closely related families, Celastraceae, Rhamnaceae[6] and Euphorbiaceae[7]. Based on the broad origins and structure features of maytansinoids, the authors inferred that maytansinoids isolated from higher plants may not be produced by those plants instead by their symbiotic microbes. To verify this speculation and clarify whether plant produces maytansinoids, the tissue cultures of *M. hookeri* were established. Previous studies revealed that this species contains a high concentration of maytansine[8, 9] and has been cultivated in Xishuangbanna Tropical Garden of Botany since the end of 1970's. Meanwhile, phytochemical studies indicated that the wild plant of this species and the cultivations in Xishuangbanna (the original biotope) did not show any difference in maytansine_producing ability. As a likely sustainable alternative supplier of maytansine, the tissue cultures of *M. hookeri* attracted our attention though Kutnuy and coworkers reported that maytansinoids were not detected in the tissue cultures of *M. buchananii*[10].

1 Results and Discussion

The callus of *M. hookeri* was induced on MS media supplemented with 2,4_D and KIN and cultivated under dark at 25 ℃. The nascent calli appeared along with the midrib of the leaves and the cutting_edge of stems were slightly yellow or yellowish to brown. Five_month old calli were hard and non_uniform in color, either brown, yellow, white or green. After eight weeks of cultivation with the addition of active charcoal (1 g/L), the calli became soft and brown. After one year's subculture, the calli were stable not only in color but also in cell growth rate, then a number of calli were chosen for suspension cell cultures in SH liquid media. Both aggregate and single cells were produced four weeks after inoculation. The cultures were subcultivated every four weeks. The average yield of cells was 0.5 g/L DW per day.

In order to determine whether the cultures produce maytansinoids, bioassay with *Penicillium avellaneum* UC_4376 was carried out for the ethyl acetate extract of the suspension cell cultures. After evident inhibitory activity was observed, 7 L of the suspension cell cultures were obtained. The cells were separated from the culture solution through filtration to afford 1 700 g FW of cells that were extracted with 95% ethanol under refluxing. The ethanol extract was partitioned between ethyl acetate and water. The ethyl acetate extract was separated by following the method described by Kupchan[2] to afford CCl_4, $CHCl_3$, base_ and acid_soluble fractions. After the removal of solvents, the cell residue was dried (110 g) and further exhaustively extracted by acetone under refluxing. The cell_free culture solution was concentrated to 1.5 L under vacuum and extracted with ethyl acetate. TLC analysis indicated that the ethyl acetate extracts of fresh cells and culture solution contain similar constituents.

According to the previous researches on plant materials[2], maytensinoids should be in the $CHCl_3$ fraction, but

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Abbreviations: DW, biomass dry weight; FW, biomass fresh weight; KIN, kinetin; MW, molecular weight; SH, Schenk_Hildebrandt.

no inhibition against *P. avellaneum* UC-4376 was observed for this fraction in this work. However, compounds **1**– **4** and **6**, and **7** were isolated from the CCl_4 and base-soluble fractions, respectively. Compounds **3**, **6**, **8** and **9** were isolated from the acetone extract of the dried cells. Compounds **3**, **5** and **6** were obtained from the culture solution. All of their structures were elucidated and **1** was identified to be a new compound.

Compound **1** was determined to have the molecular formula $C_{32}H_{44}O_5$ based on the HREIMS data (m/z 508.3177). The ^{13}C-NMR and DEPT spectra (Table 1) showed thirty-two signals for eight methyl, eight methylene, four methine and twelve quaternary carbons including one carbonyl (δ 214.5) and two acetoxyls (δc 168.8, 168.5, δH 20.4, 20.7), indicating a dinor-triterpene. HMBC experiment revealed the ^1H-^{13}C long-range correlations between the protons of six methyl groups (except for the two methyls of acetoxyl groups) and corresponding carbons, suggesting a diacetoxyl dinor-D: A-friedooleana-1, 3, 5(10)-triene[11]. The singlet at δ 6.91 (H-1) showed ^1H-^{13}C long-range correlations with the carbon at δ 138.0s (C-2), 140.0s (C-3), 132.8s (C-5) and 37.1s (C-9), indicating that ring-A was penta-substituted. Therefore, the two acetoxyl groups were located at C-2 and C-3, respectively. This was further supported by the ^1H-^{13}C long-range correlations between the protons at δ 2.00 (H-23) and the carbons at δ 140.0s (C-3), 129.5s (C-4) and 132.8s (C-5). The location of the carbonyl (δ 214.5) was assigned to C-16, C-21 or C-22 through the ^1H-^{13}C long-range correlations between the

Table 1 NMR assignments for compound **1**

Position	^{13}C[a]	^1H[a, c]	^{13}C[b]	^1H[b, c]	HMBC
1	116.1d	6.91 (s)	116.9d	7.27 (s)	C-2, C-3, C-5, C-9
2	138.0s	–	141.2s	–	–
3	140.0s	–	140.0s	–	–
4	129.5s	–	130.0s	–	–
5	132.8s	–	132.6s	–	–
6	28.3t	2.61 (m), 2.82 (dd, 6.4, 17.2)	28.3t	2.57 (m), 2.71 (dd, 6.8, 17.3)	C-5, C-7, C-8, C-10
7	18.0t	1.31 (m), 1.36 (m)	18.3t	1.78 (m, 2H)	C-9
8	42.8d	2.62 (m)	43.2d	1.76 (m)	C-10, C-15, C-25
9	37.1s	–	37.5s	–	–
10	149.7s	–	150.0s	–	–
11	33.6t	0.98 (m), 1.01 (m)	34.1t	1.86 (m, 2H)	C-1, C-10
12	30.0t	1.64 (m), 2.14 (m)	30.1t	1.69 (m, 2H)	d
13	39.6s	–	39.8s	–	–
14	40.0s	–	40.2s	–	–
15	28.1t	1.40 (m), 1.47 (t, 6.0)	28.3t	1.46 (m), 1.40 (m)	C-13, C-14, C-27
16	35.5t	1.35 (m), 1.85 (m)	35.8t	1.19 (m), 1.76 (m)	C-19, C-20, C-29,
17	38.2s	–	38.2s	–	–
18	43.9d	1.66 (m)	44.1d	1.51 (m)	C-13, C-14, C-22
19	31.8t	1.73 (m), 2.23 (m)	32.0t	2.10 (m, 2H)	C-17, C-20, C-21
20	42.3d	2.61 (m)	42.3d	2.53 (m)	C-22
21	214.5s	–	213.9s	–	–
22	53.8t	1.86 (m), 2.98 (br d, 14.4)	54.1t	2.95 (d, 14.0), 1.80 (d, 7.3)	C-16, C-17, C-21, C-28
23	12.5q	2.00 (s, 3H)	12.6q	2.05 (s, 3H)	C-3, C-4, C-5
24	–	–	–	–	–
25	28.1q	1.25 (s, 3H)	28.0q	1.19 (s, 3H)	C-8, C-9, C-10, C-11
26	15.1qe	1.00 (s, 3H)	15.3qe	0.88 (s, 3H)	C-8, C-13, C-14, C-15
27	18.4q	1.26 (s, 3H)	18.3q	1.11 (s, 3H)	C-12, C-13, C-14, C-18
28	32.9q	1.00 (s, 3H)	32.9q	0.94 (s, 3H)	C-16, C-17, C-18, C-22
29	–	–	–	–	–
30	15.2qf	1.00 (d, 7.2, 3H)	15.5qe	1.09 (d, 5.8, 3H)	C-18, C-19, C-20, C-21
AcO-2	168.8f 20.4g	2.32 (s, 3H)	168.6sf 20.2qg	2.36 (s, 3H)	CH_3CO-
AcO-3	168.5f 20.7g	2.29 (s, 3H)	168.9sf 20.6qg	2.31 (s, 3H)	CH_3CO-

a, ^1H-, ^{13}C-NMR and HMBC spectra were obtained at 400 MHz, 100 MHz and 400 MHz, and recorded in $CDCl_3$ at room temperature, respectively. b, ^1H-, ^{13}C-NMR and HMBC spectra were obtained at 500 MHz, 125 MHz and 500 MHz, and recorded in C_5D_5N at 35 ℃, respectively. c, coupling constants are presented in Hz. Unless otherwise indicated, all proton signals integrate to 1H. d, the ^1H-^{13}C long-range correlation were not readily observed because of the overlap of proton signals. e, f, g, the assignments are interchangeable within vertical columns.

carbonyl carbon and the protons of two methylenes (δ_C 53.8, δ_H 1.86 and 2.98, and δ_C 31.8, δ_H 1.73 and 2.23), and the methyl at δ 1.00. However, HMBC experiments in $CDCl_3$ could not distinguish C_21 and C_22 though the proton signal of C_30 methyl was a doublet because the signals of three methyl groups at δ 1.00 were heavily overlapped. To resolve this ambiguity, the ^1H_, ^{13}C_NMR and HMBC spectra for **1** were re_obtained in C_5D_5N at 500 MHz, 125 MHz and 500 MHz, respectively, and through changing the recording_temperature the proton signals of all sp^3 carbon_connected methyls were separated at 35 ℃ (Table 1). The HMBC experiments in C_5D_5N at 35 ℃ revealed that the doublet signals of H_30 had ^1H_^{13}C long_range correlation with the carbonyl, unambiguously assigning the carbonyl to C_21. Moreover, the ^1H_ and ^{13}C_NMR data of **1** were similar to those of regeol A[11], indicating that both had the same stereochemistry at C_9, C_13, C_14, C_17, C_18 and C_20. The discrepancy between their ^{13}C-NMR data was attributed to the γ_gauche effects of the C_22 hydroxyl in regeol A onto C_16, C_18, C_20 and C_28, and the inductive effect onto C_17, and the acetylation effects of the hydroxyl groups at C_2 and C_3 in **1** on the ring_A (C_1, C_2, C_3, C_4, C_5 and C_10). Therefore, compound **1** was determined to be 2, 3_diacetoxyl_21_oxo_24, 29_nor_D: A_friedooleana_1, 3, 5(10)_triene (Fig. 1).

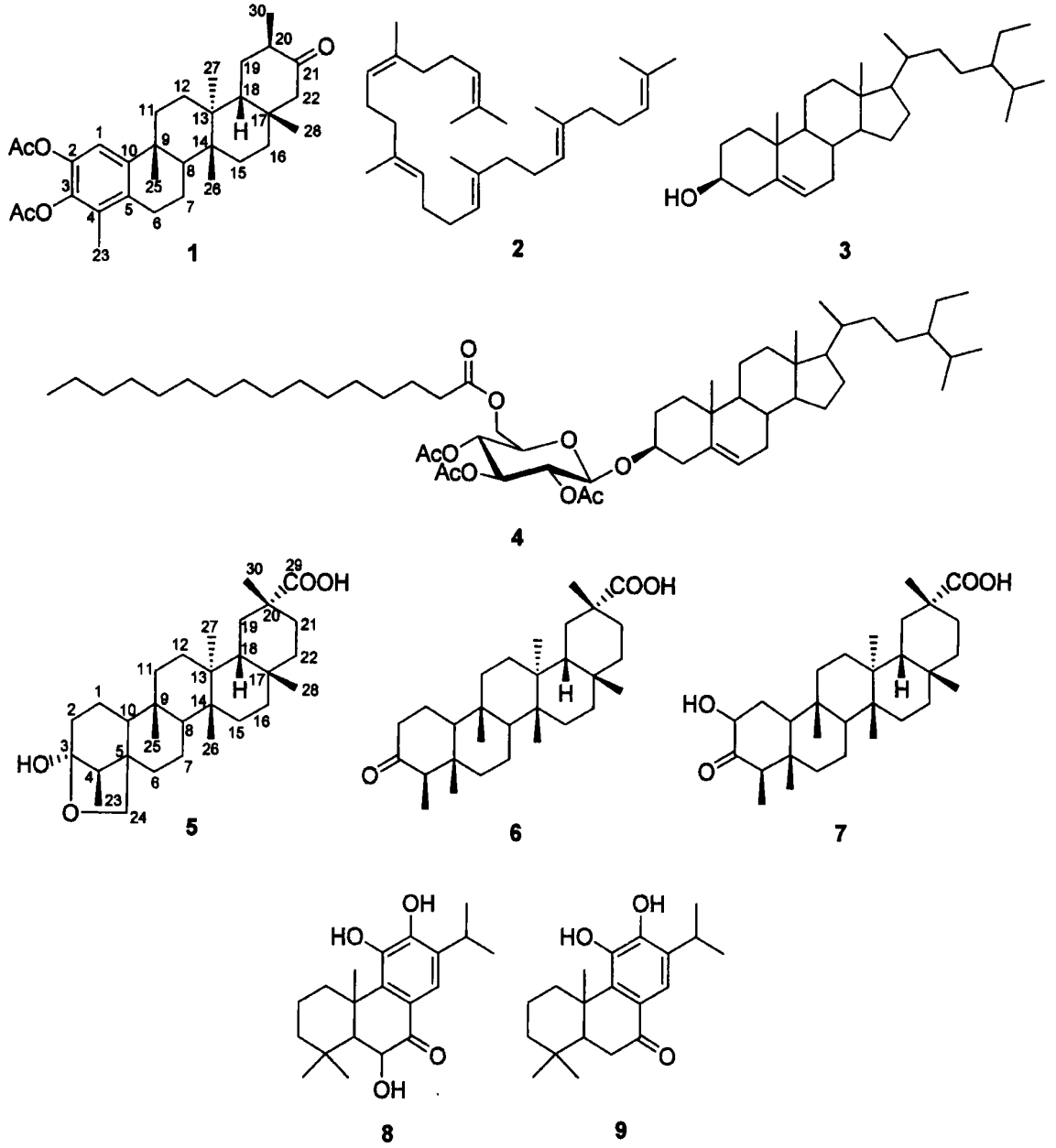

Fig. 1. Structures of compounds **1**– **9**.

Compound **5** was determined to be salaspermic acid[12-14] based on the NMR data (^{1}H_ and ^{13}C_NMR, DEPT, HMQC, and HMBC). Salaspermic is a triterpene lactone which has been isolated from *Salacim acrosperm*[12], *Triptergium wilfordii*[13] and *Kokoona ochracea*[14] of the family Celastraceae since 1979[12], and was active against HIV[13]. The NMR assignments for this compound were not reported until 1994[14]. Ngassapa and coworkers[14] assigned the protonated carbons through the analysis of the ^{1}H_^{13}C HETCOR spectra and the comparison with the ^{13}C_NMR assignments for maytenonic acid[15]. In this study, the ^{1}H_ and ^{13}C_NMR assignments for **5** were carried out on the basis of DEPT, HMQC and HMBC experiments. Our results were consistent with the data reported in reference [14] except for the two methyls at C_26 and C_27 (Table 2).

Compound **6** was determined to have the molecular formula $C_{30}H_{48}O_3$ based on the HREIMS data. The ^{13}C_NMR data and DEPT experiment showed thirty carbon signals for seven methyl, eleven methylene, four methine and eight quaternary carbons including one carboxyl, indicating a triterpene acid. The HMBC experiments revealed the ^{1}H_^{13}C long_range correlations between the protons of seven methyl groups and corresponding carbons, showing that **6** was a friedelane_type triterpene and assigning the resonances for seven methyls at δ 0.71, 0.87 (6H), 0.88, 1.00, 1.10 and 1.26 to H_24, H_23 and H_25, H_26, H_27, H_28 and H_30, respectively. The protons of one methyl (δ_H 0.87, δ_C 6.9), one methyline (δ_H 2.18 and 2.43, δ_C 41.5) and one methine (δ_H 2.23, δ_C 58.3) had ^{1}H_^{13}C long_range correlations with the carbonyl carbon at δ 213.3, establishing the carbonyl to be C_3. The methyl protons at δ 1.27 (δ_C 31.5) showed ^{1}H_^{13}C long_range correlations with the carboxyl carbon at δ 184.9, indicating the carboxyl at C_29 or C_30. The carboxyl group was established to be α_form based on the characteristic shift of the Me_27 at δ 1.00[15]. Therefore, compound **6** was elucidated to be maytenonic acid (3_oxofriedelan_29_oic acid, polpunonic acid) (Fig. 1). The ^{1}H_NMR assignments of seven methyls for **6** were consistent with those in reference [15], but the ^{13}C_NMR assignments were different from the literature data[15-17] (Table 3).

Table 2 NMR assignments for **5**[a]

Position	^{13}C	^{1}H[b]	HMBC
1	20.6t	c	–
2	39.2t	2.20 (d, 6.4)	C_1, C_3, C_4, C_10
3	106.1s	–	–
4	54.0d	1.51 (d, 6.4)	C_24
5	47.2s	–	–
6	34.1t	1.70 (br d, 7.2), 1.66 (br d, 7.2)	C_10
7	19.7t	c	–
8	50.3d	1.30 (m)	d
9	37.7s	–	–
10	57.4d	1.14 (m)	C_3, C_6, C_9, C_24
11	34.9t	1.31 (br d, 4.0, 2H)	d
12	29.7t	1.20 (br d, 6.8, 2H)	C_13, C_14, C_27
13	39.6sf	–	–
14	39.4sf	–	–
15	29.6t	1.26 (m, 2H)	C_13, C_14, C_27
16	36.8	1.40 (br d, 7.2)	C_17, C_28,
17	30.5s	–	–
18	44.9d	1.57 (br d, 6.4)	C_13, C_14, C_27, C_28
19	31.0t	2.69 (br d, 11.2, 2H)	C_18, C_20, C_21, C_29
20	40.8s	–	–
21	30.6t	2.54 (br d, 10.4, 2H)	d
22	37.5	1.07 (m), 2.40 (dt, 3.6, 13.6)	C_17, C_18, C_20, C_28
23	8.7q	1.21 (d, 6.8, 3H)	C_3, C_4, C_5,
24	73.1t	3.71 (d, 8.0), 4.25 (d, 8.0)	C_3, C_4, C_5, C_10
25	17.0q	0.90 (s, 3H)	C_8, C_9, C_10, C_11
26	16.7qe	0.83 (s, 3H)	C_8, C_13, C_14, C_15
27	18.2qe	1.23 (s, 3H)	C_12, C_13, C_14, C_18
28	32.2q	1.13 (s, 3H)	C_16, C_17, C_18, C_22
29	181.5s	–	–
30	32.5q	1.42 (s, 3H)	C_19, C_20, C_21, C_29

a, ^{1}H_, ^{13}C_NMR and HMBC spectra were obtained at 400 MHz, 100 MHz and 400 MHz, and recorded in C_5D_5N at room temperature, respectively. b, coupling constants are presented in Hz. Unless otherwise indicated, all proton signals integrate to 1H. c, the ^{1}H_^{13}C correlations were not observed. d, the ^{1}H_^{13}C long_range correlation were not readily observed because of the overlap of proton signals. e, the assignments are different from those in reference [14]. f, the assignments are interchangeable.

Table 3 NMR assignments for **6** and **7**[a]

Position	**6**			**7**	
	^{13}C	^{1}H[b]	HMBC	^{13}C	^{1}H[b]
1	22.3t	1.71 (m), 2.04 (m)	C_10, c	30.9t	2.68 (dt, 3.2, 14.0) 2.13 (dt, 3.8, 14.0)
2	41.5t	2.18 (br d, 14.8), 2.43 (ddd, 2.0, 5.2, 14.8)	C_3, C_4	74.2d	4.40 (t, 3.5)
3	213.3s	–	–	213.6s	–
4	58.3d	2.23 (dd, 6.8)	C_3, C_5, C_23, C_24	52.7d	3.23 (q, 6.8)
5	42.0s	–	–	43.1s	–
6	41.3t	1.26 (m), 1.30 (m)	c	41.2t	1.65 (m), 1.23 (m)
7	18.2t	0.88 (m), 1.48 (br s)	C_14	18.5t	1.40 (m, 2H)
8	50.7d	1.42 (t, 3.6)	c	50.8d	1.46 (br d, 4.0)
9	37.4s	–	–	37.1s	–
10	59.8d	1.52 (dd, 2.8)	C_1, C_25,	52.6d	2.28 (dd, 2.8)
11	36.1t	1.35 (br t, 3.6), 1.42 (br t, 2.8)	C_25	35.4t	1.39 (m, 2H)
12	29.5t	2.20 (br d, 13.6), 2.23 (br d, 13.6)	C_13, C_14, C_27	30.9t	2.13 (m), 1.84 (m)
13	39.2sd	–	–	39.6s	–
14	39.1sd	–	–	39.5s	–
15	29.4t	1.36 (t, 2.8), 1.52 (t, 2.8)	c	29.6t	1.30 (m, 2H)
16	36.6t	1.00 (m), 2.04 (m)	C_17	37.4t	1.05 (m), 2.35 (m)
17	30.1s	–	–	30.5s	–
18	44.2d	1.57 (m)	C_17, C_27	44.8d	1.57 (br d, 6.4)
19	29.3t	1.30 (m), 1.39 (br d, 4.0)	C_13, C_14,	29.8t	1.46 (m), 1.60 (m)
20	40.4s	–	–	40.7s	–
21	30.2t	1.60 (br d, 13.6), 2.36 (br d, 13.6)	C_20, C_30	30.5t	1.40 (m), 2.54 (br d, 14.0)
22	35.3t	1.30 (m), 1.49 (m)	c	36.7t	1.70 (br d, 4.0), 1.39 (br d, 2.8)
23	6.9q	0.87 (d, 6.8, 3H)	C_3, C_4, C_5	6.9q	0.96 (d, 6.8, 3H)
24	14.6q	0.71 (s, 3H)	C_4, C_5, C_6, C_10	14.2q	0.71 (s, 3H)
25	18.4q	0.87 (s, 3H)	C_8, C_9, C_10, C_11	18.6q	0.81 (s, 3H)
26	16.3qe	0.88 (s, 3H)	C_8, C_13, C_14, C_15	16.5q	0.84 (s, 3H)
27	18.0qe	1.00 (s, 3H)	C_12, C_13, C_14, C_18	18.0q	1.21 (s, 3H)
28	31.8q	1.10 (s, 3H)	C_16, C_17, C_18, C_22	32.1q	1.12 (s, 3H)
29	184.8s	–	–	180.5s	–
30	31.5q	1.26 (s, 3H)	C_19, C_20, C_21, C_29	32.4q	1.42 (s, 3H)

a, ^{1}H_, ^{13}C_NMR and HMBC spectra were obtained at 400 MHz, 100 MHz and 400 MHz, and recorded in $CDCl_3$ and C_5D_5N for **6** and **7** at room temperature, respectively. b, coupling constants are presented in Hz. Unless otherwise indicated, all proton signals integrate to 1H. c, the ^{1}H_^{13}C long_range correlation were not readily observed because of the overlap of proton signals. d, the assignments are interchangeable. e, the assignments are different from those in reference [15].

Compound **7** was readily determined to be 2α_hydroxyl maytenonic acid (2α_hydroxy_3_oxofriedelan_29_oic acid, 2α_hydroxyl polpunonic acid) based on the inspeetion of NMR data (^{1}H_NMR, ^{13}C_NMR, DEPT, HMQC and HMBC) and the comparison with those of compound **6** (Table 3). Although 2α_hydroxy_3_oxofriedelan_29_oic acid was isolated from *Acanthothamnus aphyllus*[18] in 1994 and from *M. canariensis* in 1995[19], no NMR assignments have been made for this compound. Using DEPT, HMQC and HMBC experiments, the ^{1}H_NMR and ^{13}C_NMR assignments for 2α_hydroxy_3_oxofriedelan_29_oic acid were accomplished (Table 3).

Compounds **2**– **4**, **8** and **9** were determined to be squalene, β_sitosterol[20], 2′, 3′, 4′_triacetyl_sitoindoside Ⅰ, 6, 11, 12_trihydroxy_8, 11, 13_abietrien_7_one[21, 22] and 11, 12_dihydroxy_8, 11, 13_abietatrien_7_one[23], respectively, by comparing with standard samples or literature data.

2 Experimental

2.1 Plant materials

Leaves and stems of *Maytenus hookeri* Loes. were collected from Xishuangbanna, Yunnan Province, China.

2.2 Callus initiation

Fresh leaves and stems were washed with flowing water for 30 min, then sterilized 8 min with 0.1% $HgCl_2$, rinsed three times with sterilized water and cut into small

pieces 5 mm in length. Callus formed and subsequently grew on MS completed media supplemented with 0.9% coconut water, 2 mg/L 2,4-D, 0.1 mg/L KIN. The calli grew under dark at 25 ℃ and transferred to fresh media at 30 days' intervals. One-year old calli were transferred to SH liquid media and cultivated under dark at 25 ℃ and shaken at 100 r/min and subcultivated every four weeks.

2.3 Extraction and isolation

The cells (1 700 g FW) collected fresh from suspension cell cultures were extracted with 95% EtOH under refluxing. The ethanol extract was fractionated according to Kupchan[2] with some modifications. The aqueous layer was first extracted with EtOAc and three parts were obtained: aqueous layer, EtOAc layer and residue. The EtOAc layer was partitioned between EtOAc and 5% aqueous NaOH to give basic and EtOAc layers. The basic layer was neutralized and extracted with EtOAc to afford fraction B (1.0 g). The EtOAc layer was partitioned between EtOAc and 2% aqueous HCl. The EtOAc fraction was washed with water to pH 7.0 and dried over anhydrous Na_2SO_4. After the removal of solvents the EtOAc extract was acetylated with Ac_2O/Py at room temperature overnight. The reaction mixture was evaporated at 40 ℃ under the vacuum and the residue was partitioned with 20% aqueous methanol and carbon tetrachloride to give the CCl_4 portion (fraction A) (3.32 g). The upper layer was diluted to 35% and partitioned by chloroform to give the $CHCl_3$ portion (fraction C) (2.0 g).

Fraction A (3.32 g) was chromatographed on a silica gel (100 g) column eluting with chloroform containing increasing amount of methanol to produce 8 fractions: Aa, Ab, Ac, Ad, Ae, Af, Ag and Ah. Fraction Aa was further subjected to column chromatography eluting with petroleum ether, petroleum ether-acetone (100∶2) to give compound **2** (42 mg). Fraction Af, after repetitive column chromatography eluting with petroleum ether containing increasing amount of acetone (9∶1, 8∶2, 7∶3) to give compounds **1** (14 mg) and **3**. Fraction Ag was purified with column chromatography over Sephadex LH-20 eluted with acetone to give compound **4** (8 mg). In the fraction Ah, compound **6** (49 mg) was recrystallized from acetone.

Fraction B (1.0 g) was column chromatographed with silica gel (30 g) and eluted with the mixtures of petroleum ether and acetate ethyl (9∶1, 8∶2, 7∶3), and further purified with column chromatography over Sephadex LH-20 eluted with acetone to give compound **7** (5 mg).

After being extracted with 95% EtOH the fresh cells were air-dried (110 g) and extracted three times (6 h each time) with acetone under refluxing to yield 1.0 g of extract (yellow gum, fraction E). Fraction E was chromatographed on a silica gel column (26 g) eluted with petroleum ether-acetone (9∶1, 8∶2, 7∶3) to afford eight fractions (E_1, E_2, E_3, E_4, E_5, E_6, E_7, E_8). Fraction E_3 mainly contained β-sitosterol by comparing with the standard sample on TLC and was subjected to column chromatography over Sephadex LH-20 eluted with acetone to produce **3**. Fraction E_5 was subjected to column chromatography over silica gel eluted with the gradient mixtures of chloroform and acetone (100∶1, 100∶2).

The fractions combined on the basis of TLC results were subjected to column chromatography over Sephadex LH-20 eluted with acetone to give the mixture of compounds **8** and **9** (18 mg). Compound **6** (176 mg) was recrystallized from E_6 in acetone. The supernatant of E_6 was isolated by column chromatography over Sephadex LH-20 eluted with acetone to yield **7** (10 mg).

The culture solution (7 L) was concentrated to 1.5 L under the vacuum and extracted three times with ethyl acetate to produce 1.5 g of black gum. The black gum was mixed with 2.0 g of silica gel (Walk gel) and chromatographed over silica gel (50 g) eluted with chloroform, chloroform-methanol (100∶2, 100∶5) and methanol to give five fractions (e_1, e_2, e_3, e_4, e_5). The fraction e_2 was further separated into fractions e_2a, e_2b, e_2c and e_2d with column chromatography over silica gel (10 g) eluted with chloroform-methanol (100∶3). TLC results showed that fraction e_2a was the same as **3**. Compound **6** (98 mg) was recrystallized from fraction e_2b in chloroform. Compound **5** (10 mg) was precipitated from fraction e_3 in methanol.

The whole amount of **3** isolated in this study was 70 mg.

2.4 Microbiological assay

Hanka's method was applied[24] in the activity detection for the EtOAC extract.

The inhibitory activities against *P. avellaneum* UC-4376 were observed by analyzing the inhibitory zone.

2.5 Identification

Compound 1 Amorphous powder. $[\alpha]_D^{28} = -41.8°$ (*c* 1.3, $CHCl_3$). EIMS (70 eV) m/z (%): $[M^+]$ 508 (50), 466 (75), 424 (100), 409 (58), 388 (90), 36.0 (20). HREIMS (70 eV) m/z: 508.317 7 (Calcd for $C_{32}H_{44}O_5$, 508.318 8). See Table 1 for ^{1}H-NMR and ^{13}C-NMR data.

Compound 2 Colorless oil. EIMS (70 eV) m/z (%): $[M^+]$ 410 (43), 367 (12), 341 (35), 328 (13), 299 (11), 286 (5), 273 (15), 260 (6), 231 (18), 218 (16), 203 (27), 191 (40), 177 (25), 163 (25), 149 (57), 137 (78), 121 (65), 109 (58), 95 (75), 81 (100), 69 (98), 55 (39). The molecular formula $C_{30}H_{50}$ was determined from the ^{1}H-NMR and ^{13}C-NMR spectra together with the EIMS data. The ^{13}C-NMR and DEPT showed fifteen carbon signals including four methyls (δ_H 1.68, s, 3H, δ_H 1.60, s, 9H, δ_C 25.7, 17.7, 16.01 and 15.98), five methylenes (δ_H 2.01, m, 10H, δ_C 39.73, 39.71, 28.2, 26.7 and 26.6), three methines (δ_H 5.15, δ_C 124.4, 124.3 and 124.2, sp^2) and three quaternary (δ 135.1, 134.9 and 131.2, sp^2) carbons, indicating three double bonds and molecular constitution $C_{15}H_{25}$ (MW 205). According to its molecular weight (410), a symmetrical unsaturated hy-

drocarbon structure with eight methyls was obvious, so compound **2** was determined to be squalene.

Compound 3 EIMS (70 eV) m/z (%): $[M]^+$ 414 (100), 396(27), 381 (18), 367(3), 354 (5), 329 (20), 303 (34), 289 (5), 273 (15), 255 (15), 231 (12), 213 (16), 145(17), 109 (14), 69 (25). Comparing with standard sample, **3** was determined to be β_sitosterol[20].

Compound 4 Colorless gum. Three main fragments were observed at m/z 239, 397 and 527 in the FABMS. The ^{1}H_NMR and ^{13}C_NMR spectra indicated the presence of three acetoxyls and one carbon_carbon double bond. It was the same on TLC as the acetylated product of sitoindoside Ⅰ which was isolated from the calli of *M. hookeri* (unpublished data), therefore, **4** was determined to be 2′, 3′, 4′_triacetyl_sitoindoside Ⅰ.

Compound 5 Colorless needles. EIMS (70 eV) m/z (%): $[M^+]$ 472 (50), 454 (3), 426(10), 358 (2), 318 (3), 289 (8), 235 (8), 189 (7), 155 (13), 135 (13), 125 (100). HREIMS m/z: 472.354 6 (Calcd for $C_{30}H_{48}O_4$, 472.355 3). See Table 2 for ^{1}H_ and ^{13}C_NMR data.

Compound 6 Colorless needles. $[\alpha]_D^{28}$ − 61.2° (*c* 1.45, $CHCl_3$). EIMS (70 eV) m/z (%): $[M^+]$ 456 (60), 441 (15), 410 (24), 342 (19), 273 (74), 250 (35), 231 (33), 155 (69), 109 (100). HREIMS m/z: 456.362 2 (Calcd for $C_{30}H_{48}O_3$, 456.360 3). See Table 3 for ^{1}H_ and ^{13}C_NMR data.

Compound 7 Colorless needles. EIMS (70 eV) m/z (%): $[M^+]$ 472 (15), 454 (8), 426 (15), 368 (6), 289 (30), 250 (13), 235 (16), 189 (26), 155 (45), 135 (38), 121 (60), 109 (100), 81 (69), 69 (64). See Table 3 for ^{1}H_ and ^{13}C_NMR data.

Compound 8 Amorphous powder. ^{1}H_NMR ($CDCl_3$, 400 MHz) δ 0.92, 0.95 and 1.38 (s, 3H each), 1.27 and 1.25 (d, J = 5.0 Hz, 3H each), 1.53 (s, 3H). ^{13}C_NMR ($CDCl_3$, 100 MHz) δ 19.0 (C_2), 21.9 (C_19), 22.5 and 22.7 (C_16 and C_17), 27.3 (C_15), 33.4 (C_4), 18.6 (C_18), 55.0 (C_5), 36.4 (C_1), 41.0 (C_3), 40.0 (C_10), 73.0 (C_6), 125.3 (C_8), 138.7 (C_9), 143.0 (C_11), 146.5 (C_12), 131.8 (C_13), 118.0 (C_14), 200.0 (C_7). The HMBC experiments showed that δ 7.16 (H_14) had ^{1}H_^{13}C long_range correlations with C_12, C_13 and C_7, and the proton at δ 3.05 (15_H) was related with C_12, C_11, C_14, C_16, C_17, indicating that isopropyl is connected with C_13. EIMS (70 eV) m/z: $[M^+]$ 332, $C_{20}H_{28}O_4$. Therefore, compound **8** was determined to be 6α_hydroxydemethylcryptojaponol[21, 22], all the data were consistent with those in literature.

Compound 9 As it was mixed with **8**, the ^{1}H_ and ^{13}C_NMR spectra showed that the ratio of the two compounds was about 7∶3 (**8**/**9**). The EIMS at m/z 316 indicated that **9** had one less hydroxyl than **8** did. The ^{13}C_NMR signals at δ 35.5, 50.2 and 19.8 were assigned to C_6, C_5 and C_18, respectively. Therefore, compound **9** was determined to be demethylcryptojaponol[23], all the data were consistent with those reported previously.

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云南美登木悬浮培养细胞的化学成分

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摘要: 利用组织培养方法以云南美登木(*Maytenus hookeri* Lose.)未木质化的茎和嫩叶为材料诱导出愈伤组织, 经过继代培养建立了悬浮细胞培养系。培养物的乙酸乙酯提取物显示抗橙色青霉(*Penicillium avellaneum* UC_4376)生长的生物活性。对培养物进行化学成分研究, 分离鉴定了 9 个化合物: 2, 3_diacetoxyl maytenusone (**1**)、角鲨烯 (squalene, **2**)、β_谷甾醇 (β_sitosterol, **3**)、2′, 3′, 4′_triacetylsitoindoside Ⅰ (**4**)、salaspermic acid (**5**)、美登酮酸 (maytenonic acid, **6**)、2α_羟基美登酮酸(2α_hydroxy_maytenonic acid, **7**)、6, 11, 12_trihydroxy_8, 11, 13_abietrien_7_one (**8**) 和 11, 12_dihydroxy_8, 11, 13_abietrien_7_one (**9**), 其中化合物 **1** 为新化合物。通过 2D NMR 对化合物 **5**– **7** 的 NMR 数据进行了全指定, 并修正了化合物 **5** 和 **6** 的部分碳谱数据指定。

关键词: 云南美登木; 卫矛科; 悬浮细胞培养; 美登木素; 2, 3_diacetoxyl maytenusone

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