Study on Chemical Constituents and Cytotoxic Activities of *Salacia chinensis* Growing in Vietnam

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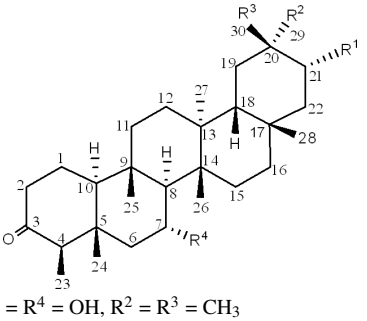
Two new triterpenoids, named 7α,21α-dihydroxyfriedelane-3-one (**1**) and 7α,29-dihydroxyfriedelane-3-one (**2**) have been isolated from the ethyl acetate extract of the stems of *Salacia chinensis* besides the known triterpenoid 21α,30-dihydroxyfriedelane-3-one (**3**). The structures of the isolated compounds were elucidated on the basis of spectral analysis. Eight triterpenoids from this plant have been tested against the four cancer cell lines Hep-G2, LU, KB, and MCF-7. The new compound **1** showed good activity against all four tested cell lines.

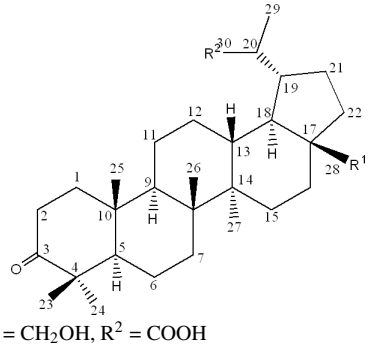
Key words: Salacia chinensis, Triterpenes, Cytotoxic Activities

Introduction

Salacia chinensis L. is growing widely in Myanmar, Thailand, Malaysia, China, India, and Vietnam [1]. This plant is used in the traditional medicine as an anti-inflammatory, antidiabetic, blood tonic, carminative, and emmenagog agent. Previously, we reported the isolation and structure determination of seven triterpenes from the *n*-hexane extract of its stems, namely 28-hydroxy-3-oxo-30-lupanoic acid (**4**), 3-oxo-lupane-30-al (**5**), 29-nor-21α-H-hopane-3,22-dione (**6**), 21α-H-hop-22(29)-ene-3β,30-diol (**7**), betulin (**8**), 29-hydroxyfriedelane-3-one (**9**), and 21α-hydroxyfriedelane-3-one (**10**) [2, 3]. Further investigation led to the isolation of four other triterpenes named friedelane-3-one (**11**), 3β-hydroxyfriedelane (**12**), taraxer-14-ene-3β-ol (**13**), and 3,4-seco-friedelane-3-oic acid (**14**) from the *n*-hexane extract of the leaves of this plant [4]. This paper reports on the structure elucidation of the two new constituents 7α,21α-dihydroxyfriedelane-3-one (**1**) and 7α,29-dihydroxyfriedelane-3-one (**2**) besides the known 21α,30-dihydroxyfriedelane-3-one (**3**) from the ethyl acetate extract of *S. chinensis* stems, as well as on the cytotoxic activities of the isolated triterpenes.



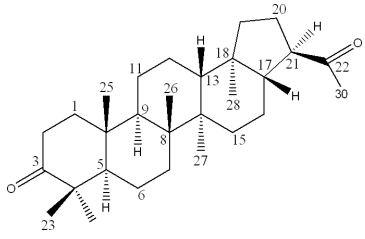
1 $R^1 = R^4 = OH$, $R^2 = R^3 = CH_3$
2 $R^1 = H$, $R^2 = CH_2OH$, $R^3 = CH_3$, $R^4 = OH$
3 $R^1 = OH$, $R^2 = CH_3$ $R^3 = CH_2OH$, $R^4 = H$
3a $R^1 = OAc$, $R^2 = CH_3$, $R^3 = CH_2OAc$, $R^4 = H$



4 $R^1 = CH_2OH$, $R^2 = COOH$
5 $R^1 = CH_3$, $R^2 = CHO$

Results and Discussion

Compound **1** showed the molecular ion peak at $m/z = 458$ $[M]^+$ in the EI-MS and at $m/z = 481.36494$ $[M+Na]^+$ (calcd. 481.36522 for $C_{30}H_{50}O_3Na$) in the positive ESI-HRMS. Its IR spectrum displayed absorp-



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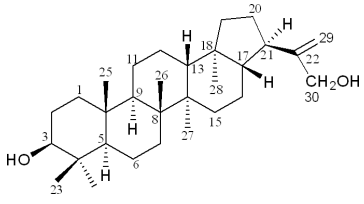

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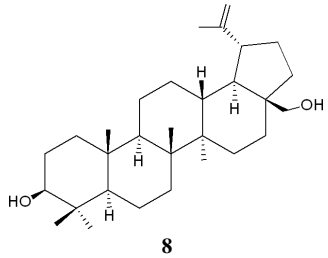

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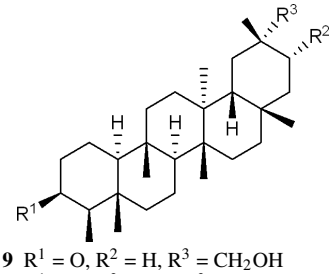
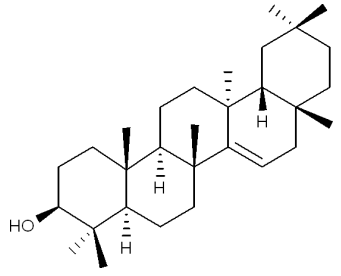


9 R^1 = O, R^2 = H, R^3 = CH_2OH
10 R^1 = O, R^2 = OH, R^3 = CH_3
11 R^1 = O, R^2 = H, R^3 = CH_3
12 R^1 = OH, R^2 = H, R^3 = CH_3

tion bands at 3446 (OH) and 1716 cm^{-1} (>C=O). The ^1H NMR spectrum revealed 8 methyl signals, one of them being a doublet at δ_H = 0.91 (J = 7.0 Hz); the others are singlets at δ_H = 0.80, 0.93, 0.99, 1.08, 1.12, 1.17, and 1.23. Furthermore, the signals of two >CH-OH groups were observed at δ_H = 4.08 (1H, *ddd*, 3.5, 10.5, 10.5 Hz) and 3.70 (1H, *dd*, 4.5, 12.1 Hz). The ^{13}C NMR spectrum showed signals of 30 carbon atoms including one carbonyl (δ_C = 212.3), two hydroxymethines (δ_C = 68.8; 73.8), eight methyls, nine methylenes, four methines, and six quaternary carbon atoms. The analysis of the spectroscopic data and a comparison of the chemical shift of the tertiary methyl



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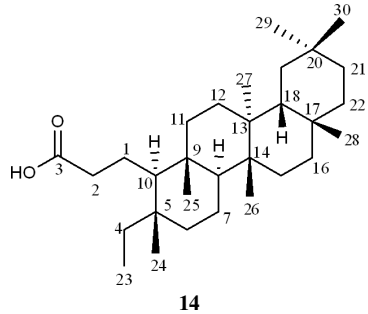

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groups with those of other friedelane derivatives [5 – 7] suggested that compound **1** is a friedelanone with two hydroxyl substituents. The locations of the carbonyl as well as of the two hydroxyl groups have been determined by HMBC and NOESY experiments. In the HMBC spectrum the observed correlations between δ_C = 68.8 (C-7) and δ_H = 1.49 (H-8), 1.40 and 2.01 (H_2-6) suggested one hydroxyl group at C-7, which was confirmed by the correlations of δ_H = 4.08 (H-7) and δ_H = 2.01, 1.40 (H_2-6) and 1.49 (H-8) in the ^1H-^1H COSY spectrum. The position of the second hydroxyl group at C-21 was confirmed by correlations between δ_C = 73.8 (C-21) and δ_H = 0.99 (H_3-29), 1.08 (H_3-30), 1.23 (H_3-28), 1.61, and 1.35 (H_2-22) in the HMBC spectrum as well as by the correlations between δ_H = 3.70 (H-21) and δ_H = 1.61 and 1.35 (H_2-22) in the ^1H-^1H COSY spectrum. The 3-oxo group was deduced from the $^3J_{CH}$ correlations between C-3 (δ_C = 212.3) and H_3-23 (δ_H = 0.91), H_2-1 (δ_H = 1.99 and 1.68), and H_2-2 (δ_H = 2.41 and 2.30) in the HMBC spectrum. The configuration of both hydroxyl groups was established as α by the NOESY experiment, which showed NOE correlations between δ_H = 4.08 (H-7) and δ_H = 0.8 (H_3-24), 0.93 (H_3-25) and 1.17 (H_3-26), and between δ_H = 3.70 (H-21) and δ_H = 1.23 (H_3-28) and 1.08 (H_3-30). Consequently, the structure of **1** was determined as the new triterpene 7α,21α-dihydroxyfriedelane-3-one.

Compound **2** was obtained as colorless crystals. Its IR spectrum showed absorptions of hydroxyl and

Table 1. ^{13}C NMR spectral data (125 MHz, CD_3OD) of compounds **1** – **3** and **3a** (δ in ppm).

Position	**1**	**2**	**3**	**3a**
1	21.9	21.9	22.2	22.3
2	41.2	41.2	41.3	41.5
3	212.3	212.3	214.4	212.7
4	58.2	58.3	58.1	58.3
5	42.6	42.6	42.0	42.1
6	52.6	52.3	41.0	41.3
7	68.8	68.8	18.1	18.3
8	57.6	58.7	50.8	52.2
9	39.1	39.1	37.4	37.2
10	58.9	58.9	59.3	59.6
11	35.7	35.9	35.0	35.4
12	30.2	30.4	29.7	30.3
13	40.3	40.1	39.0	39.4
14	39.7	40.4	38.7	38.7
15	33.6	35.4	29.8	31.3
16	36.5	36.1	36.0	35.8
17	31.8	29.7	32.2	32.1
18	43.5	42.4	44.1	43.3
19	36.2	29.5	29.5	31.1
20	34.3	33.5	38.3	37.5
21	73.8	28.0	71.2	71.3
22	47.6	38.3	44.6	42.7
23	6.9	6.9	6.5	6.8
24	15.9	15.9	14.4	14.7
25	19.2	18.9	19.1	18.2
26	19.0	20.8	19.4	18.8
27	19.7	19.0	19.4	18.5
28	32.8	31.9	32.9	32.5
29	25.4	71.4	16.5	21.8
30	31.2	29.1	73.3	71.0
CH_3CO	–	–	–	20.9
CH_3CO	–	–	–	22.1
CH_3CO	–	–	–	170.5
CH_3CO	–	–	–	171.1

ketone groups at 3532 and 1714 cm^{-1}, respectively. The molecular formula of $C_{30}H_{50}O_3$ and the molecular weight of *m/z* = 458 were obtained from the positive high-resolution ESI-MS through the peak at *m/z* = 497.33922 $[M+K]^+$ (calcd. 497.33915). The ^1H and ^{13}C NMR spectra of **2** were very similar to those of **1** with two exceptions. Instead of signals for 8 methyl and two oxygenated methine groups in the spectra of **1**, compound **2** exhibited 7 methyls, one oxygenated methine (δ_H = 4.09, δ_C = 68.8) and one oxygenated methylene (δ_H = 3.37, 3.46; δ_C = 71.4) groups. These spectral data suggested that compound **2** has also a dihydroxyfriedelane-3-one structure like **1** but with a different localization of the hydroxyl groups. The first hydroxyl group was located at C-7 as in **1**, due to the correlations between δ_C = 68.8 (C-7) and δ_H = 1.5 (H-8), 2.02 and 1.39 (H_2-6) in the HMBC spectra as well as the correlations between δ_H = 4.09 (H-7) and 1.5 (H-8), 2.02 and 1.39 (H_2-6) in the COSY spectrum. The second OH group was connected at C-29, due to the cross peaks between: δ_C = 71.4 (C-29) and δ_H = 1.0 (H_3-30), 1.19 (H-19); between δ_H = 3.37 (H-29B) and δ_C = 29.5 (C-19), 33.5 (C-20); between δ_H = 3.46 (H-29A) and δ_C = 29.1 (C-30), 33.5 (C-20) in the HMBC experiments as well as the correlation between H_3-30 (1.0 ppm) and H_3-28 (1.23 ppm) in the NOESY spectrum. The configuration of the 7α-hydroxyl group in **2** was determined by NOE effects of H-7 (4.09 ppm) and H_3-24 (0.8 ppm), H_3-25 (0.92 ppm) and H_3-26 (1.22 ppm). The structure of **2** was thus established as the new triterpene 7α,29-dihydroxyfriedelane-3-one. The NMR spectral data assigned to carbon signals for **1** and **2** are listed in Table 1.

Compound **3** was isolated as colorless needles. It showed the molecular ion peak at *m/z* = 458 $[M]^+$ in the EIMS, the same as that of **1** and **2**. Its IR spectrum indicated the presence of hydroxyl and carbonyl functions at 3421 and 1714 cm^{-1}, respectively. The ^1H and ^{13}C NMR spectra showed similar signals to those of compound **2** with a slight difference in the chemical shifts of the oxygenated methine [δ_H = 3.92 (1H, dd, 4.0, 12.0 Hz), δ_C = 71.2] and the oxygenated methylene group [δ_H = 3.35 (2H, s), δ_C = 73.3]. From a detailed spectral analysis and by a comparison with the data of 21α,30-dihydroxyfriedelane-3-one as well as with those of its diacetate (**3a**), the structure of compound **3** was determined as 21α,30-dihydroxyfriedelane-3-one. This compound has been previously isolated from *Salacia reticulata* (Celastraceae) [8].

Compounds **1**, **2**, **3**, **4**, **6**, **11**, **12**, and **14** were tested for cytotoxicity with four cancer cell lines: liver cancer (Hep-G2), lung cancer (LU), mouth cancer (KB), and breast cancer (MCF-7). Compound **1** was active against all four cancer cell lines tested, with the IC_{50} values 16.22, 19.27, 16.86, and 27.35 $\mu g\ mL^{-1}$, respectively (Table 2). Compounds **4** and **14** were also found to be cytotoxic against all four cell lines with IC_{50} higher than that of **1**. Compound **6** was active against human lung carcinoma cells (LU) (IC_{50} = 117.33 $\mu g\ mL^{-1}$), whereas compounds **3**, **11** and **12** were inactive ($IC_{50} > 128\ \mu g\ mL^{-1}$) (Table 2).

Experimental Section

General

Melting points were determined on a Botius melting point apparatus (Germany). Optical rotation values: Polarimeter

Table 2. Cytotoxic activity of triterpenoids from *Salacia chinensis* L.

Compounds	*In vitro* cytotoxicity IC_{50} (μg mL^{-1})			
	MCF_7[a]	LU[b]	HepG2[c]	KB[d]
7α,21α-dihydroxyfriedelane-3-one (**1**)	27.35	19.27	16.22	16.86
7α,29-dihydroxyfriedelane-3-one (**2**)	> 128	> 128	> 128	> 128
21α,30-dihydroxyfriedelane-3-one (**3**)	> 128	> 128	> 128	> 128
28-hydroxy-3-oxo-30-lupanoic acid (**4**)	69.48	25.77	61.79	62.90
29-nor-21α-H-hopane-3,22-dione (**6**)	> 128	117.33	> 128	> 128
friedelane-3-one (**11**)	> 128	> 128	> 128	> 128
3β-hydroxyfriedelane (**12**)	> 128	> 128	> 128	> 128
3,4-seco-friedelane-3-oic acid (**14**)	77.12	67.07	88.14	83.61
Ellipticine	0.31 – 0.62	0.31 – 0.62	0.31 – 0.62	0.62 – 1.25

[a] Human breast carcinoma; [b] human lung carcinoma; [c] human epatocellular carcinoma; [d] human epidermic carcinoma.

POLAX-2L (Japan). FT-IR: Nicolet IMPACT 410. EI-MS: HP5989B. ESI-MS: AGILENT 1100 LC-MSD Trap spectrometer. HR-ESI-MS: Qstar pulsar (Applied Bioystems). NMR: Bruker Avance 500 MHz. Column chromatography (CC): silica gel (70 – 230 and 230 – 400 mesh, Merck). Thin layer chromatography (TLC): DC-Alufolien 60 F_{254} (Merck).

Plant material

The leaves of *Salacia chinensis* L. were collected in Quang Binh province, Vietnam, in April 2007. The species was identified by Dr. Ngo Van Trai, Institute of Materia Medica, Hanoi. A voucher specimen (No. SC-02) is deposited in the Hanoi University of Technology, Vietnam.

The dried and powdered leaves of *Salacia chinensis* L. (1.8 kg) were extracted with 80 % aqueous MeOH at r. t. After MeOH was evaporated *in vacuo*, the residue was partitioned with *n*-hexane followed by EtOAc and *n*-BuOH. The EtOAc extract (6.5 g) was chromatographed on silica gel with solvents of increasing polarity (0 – 100 % MeOH in dichlomethane) to give 7 fractions. The fractions were further purified to afford compounds **1**, **2** and **3**.

Extraction and isolation

*7α,21α-Dihydroxyfriedelane-3-one (**1**)*

Fraction 3 (0.34 g), eluated with dichloromethane : MeOH = 95 : 5, was rechromatographed over a silica gel column with a mixture of CH_2Cl_2 : MeOH = 95 : 5 followed by crystallization ($CHCl_3$: MeOH = 9 : 1), afforded 80 mg of compound **1** (0.0044 %) as colorless plates. – M. p. 272 – 273 °C. – $[\alpha]_D^{25}$ = +219.8 (c = 0.2, CH_3OH : $CHCl_3$ = 10 : 1). – IR (KBr): ν = 3446, 2928, 1716, 1453, 1386, 1038 cm^{-1}. – EI-MS: *m/z* (%) = 458 (0.7) $[M]^+$, 440 (8) $[M–H_2O]^+$, 422 (5) $[M–2H_2O]^+$, 231 (10), 203 (22), 177 (19), 161 (24), 133 (29), 123 (73), 95 (70), 69 (86), 55 (100). – HRMS ((+)-ESI): *m/z* = 481.36494 (calcd. 481.36522 for $C_{30}H_{50}O_3Na$, $[M+Na]^+$). – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.08 (1H, ddd, J = 3.5, 10.5, 10.5 Hz, H-7), 3.70 (1H, dd, J = 4.5, 12.1 Hz, H-21), 1.23 (s, H_3-28), 1.17 (s, H_3-26), 1.12 (s, H_3-27), 1.08 (s, H_3-30), 0.99 (s, H_3-29), 0.93 (s, H_3-25), 0.91 (d, J = 7.0 Hz, H_3-23), 0.80 (s, H_3-24). – ^{13}C NMR: see Table 1.

*7α,29-Dihydroxyfriedelane-3-one (**2**)*

Fraction 2 (0.18 g) (dichloromethane : MeOH = 98 : 2) was crystallized from a mixture of $CHCl_3$: MeOH (9 : 1) to give 90 mg (0.005 %) of compound **2** as colorless crystals. – M. p. 325 – 327 °C. – $[\alpha]_D^{25}$ = −152.4 (c = 0.2, CH_3OH : $CHCl_3$ = 10 : 1). – IR (KBr): ν = 3532, 2944, 2874, 1714, 1458, 1386, 1197, 1037 cm^{-1}. – HRMS ((+)-ESI): *m/z* = 497.33922 (calcd. 497.33915 for $C_{30}H_{50}O_3K$, $[M+K]^+$). – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.09 (1H, m, H-7), 3.46 (1H, d, J = 10.5 Hz, H-29A), 3.37 (1H, d, J = 10.5 Hz, H-29B), 1.22 (s, H_3-26), 1.18 (s, H_3-28), 1.09 (s, H_3-27), 1.00 (s, H_3-30), 0.92 (s, H_3-25), 0.91 (d, J = 6.5 Hz, H_3-23), 0.80 (s, H_3-24). – ^{13}C NMR: see Table 1.

*21α,30-Dihydroxyfriedelane-3-one (**3**)*

Fraction 3 (0.34 g) was rechromatographed over a silica gel column with a mixture of CH_2Cl_2 : MeOH = 95 : 5. Crystallization ($CHCl_3$: MeOH = 9 : 1) afforded 180 mg (0.01 %) of compound **3** as colorless crystals. – M. p. 285 – 286 °C. – IR (KBr): ν = 3421 – 3300, 2929, 2879, 1714, 1460, 1383, 1039 cm^{-1}. – EI-MS: *m/z* (%) = 458 (1) $[M]^+$, 440 (10), 410 (2), 302 (4), 273 (12), 231 (10), 175 (12), 161 (15), 121 (41), 95 (51), 81 (61), 55 (100). – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.92 (1H, dd, J = 4.0, 12.0 Hz, H-21), 3.35 (2H, s, H_2-30), 1.16 (6H, s), 1.04 (3H, s), 0.88 (6H, s), 0.87 (3H, d, J = 7.0 Hz), 0.72 (3H, s). – ^{13}C NMR: see Table 1.

Acetylation of diol **3** with Ac_2O-pyridine (1 : 1) at r. t. for 24 h gave 21α,30-dihydroxy-3-friedelanone diacetate (**3a**).

*21α,30-Dihydroxyfriedelane-3-one diacetate (**3a**)*

Colorless crystals (*n*-hexane : $CHCl_3$ = 9 : 1). – M. p. 175 – 178 °C. – IR (KBr): ν = 2936, 1733, 1465, 1385, 1239, 1022, 759 cm^{-1}. – EI-MS: *m/z* = 542 $[M]^+$ ($C_{34}H_{54}O_5$). – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.09 (1H, dd, J = 4.5, 12.5 Hz, H-21), 3.95 (1H, d, J = 10.9 Hz, H-30A), 3.86 (1H, d, J = 10.9 Hz, H-30B), 2.07 and 2.01 (each 3H, s, OAc),

1.28, 1.12, 1.02, 0.94, 0.87, 0.73 (each 3H, s, Me) and 0.88 (3H, d, J = 6.5 Hz, H-23). – ^{13}C NMR: see Table 1.

Assay of cytotoxic activities using Hep-G2, LU, KB and MCF-7 cell lines

The human cancer cell lines supplied by the American Type Culture Collection (ATCC) were maintained in a suitable medium adding FBS and were incubated at 37 °C in a humidified atmosphere of 5 % CO_2.

The Hep-G2 (the human epatocellular carcinoma), KB (the human mouth epidermal carcinoma) and MCF_7 (the human breast carcinoma) cell lines were maintained in RPMI-1640 culture medium with 10 % fetal bovine serum (FBS).

The LU (the human lung carcinoma) cell line was maintained in DMEM culture medium with 10 % fetal bovine serum (FBS).

The cell line was cultured at 37 °C in an atmosphere of 5 % CO_2 in air (100 % humidity). The cells were treated in triplicate at various concentration of the natural compounds (1 and 10 μg mL^{-1}) and incubated for 72 h at 37 °C in an atmosphere of 5 % CO_2. The cell growth inhibition was determined by the MTT assay. After incubation for 72 h, the media was removed, and the cells were incubated with 10 μL of media containing 5 mg/mL stock solution of MTT in 40 μL RPMI-1640 (for Hep-G2, KB, MCF_7) or in 40 μL DMEM (for LU). After incubation for 4 h at 37 °C in an atmosphere of 5 % CO_2, the formazan crystals formed were dissolved by adding 150 μL of DMSO per well. The optical density was measured at 570 nm. The number of viable cells was proportional to the extent of formazan production. % CI = [1–(OD_{570} treated/OD_{570} control)] × 100[18].

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