Study on Chemical Constituents of *Salacia chinensis* L. Collected in Vietnam

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A new triterpene, 28-hydroxy-3-oxo-30-lupanoic acid (**1**), and a triterpene found for the first time as a natural product, 3-oxo-lupane-30-al (**2**), besides three known triterpenes, 29-nor-21α-H-hopane-3,22-dione (**3**), 21α-H-hop-22(29)-ene-3β, 30-diol (**4**), and betulin (**5**) have been isolated from the *n*-hexane extract of *Salacia chinensis* stems. Their structures were elucidated on the basis of spectral studies.

Key words: Salacia chinensis, Triterpenes, Lupanes, Hopanes, Friedelanes

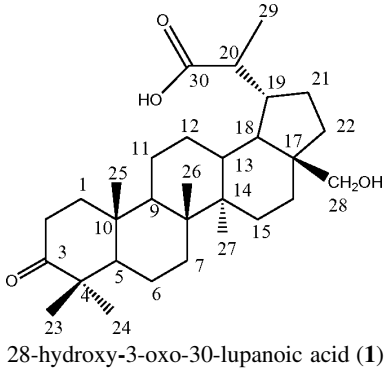
Introduction

Salacia chinensis L. belongs to the family Celastraceae and is widely distributed in Myanmar, Thailand, Malaysia, China, and India. It is a shrub and used, *e. g.*, as an antiinflammatory, antidiabetic, blood tonic, carminative, and emmenagog agent [1]. In Vietnamese traditional medicine, the roots and stems of this species have been used *e. g.* for treatment of rheumatism, backache and depression [2]. A Japanese research group found that the 80 % aqueous methanolic extract of *S. chinensis* collected in Thailand showed hypoglycemic, gastroprotective nitric oxide production inhibitory effects, α-glucosidase and aldose reductase inhibitory and antioxidative activities [3].

This paper deals with the isolation and structure elucidation of a new triterpene, 28-hydroxy-3-oxo-30-lupanoic acid (**1**), and a triterpene found for the first time as a natural product, 3-oxo-lupane-30-al (**2**), besides three known triterpenes, 29-nor-21α-H-hopane-3,22-dione (**3**), 21α-H-hop-22(29)-ene-3β, 30-diol (**4**), and betulin (**5**), from the *n*-hexane extract of *Salacia chinensis* stems.

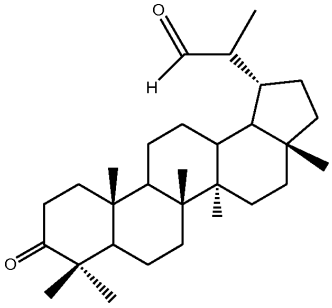
Results and Discussion

Compound **1** showed the molecular ion peak at *m/z* = 471.34761 (calcd. 471.34798 for $C_{30}H_{47}O_4$, $[M–H]^-$) in the negative ESI-HRMS. Its molecular formula has been concluded from the MS, ^{1}H and ^{13}C NMR data as $C_{30}H_{48}O_4$, suggesting that



28-hydroxy-3-oxo-30-lupanoic acid (**1**)

this is a triterpene. The IR spectrum had absorptions at 3490 (OH), 1733 and 1706 cm^{-1} (a ketone in a six-membered ring and a carboxylic group). The ^{1}H NMR spectrum revealed 6 methyl signals as 5 singlets at δ = 0.96, 1.00, 1.05, 1.09, and 1.10 and one doublet at δ = 1.04 with J = 6.7 Hz. The latter together with a double quartet (dq) at δ = 2.72 (J = 3.0, 6.7 Hz), suggested the existence of a >C–CH–CH$_3$ moiety. Furthermore, the typical signals of a -CH$_2$OH group were observed at δ = 3.75 and 3.28 with a geminal coupling constant of 11 Hz. The ^{13}C NMR spectrum indicated 30 carbon atoms, among them 6 methyls, two carbonyls, one of which was a ketone group (δ_C = 220.2) assigned to the 3-oxo group, and the other one was a carboxylic group (δ_C = 179.3), and a hydroxymethyl group at δ_C = 59.8. The analysis of the spectroscopic data and a comparison of the chemical shifts of the tertiary methyl groups

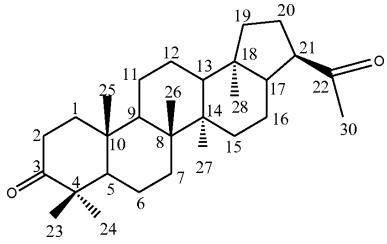


3-oxo-lupane-30-al (**2**)

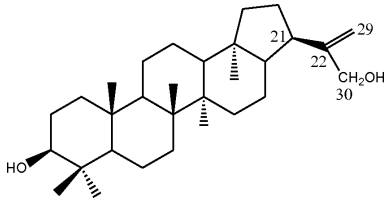
with those of other lupene derivatives [4] suggested the structure depicted for **1**. A study of the CH long-range correlations from the HMBC and the correlation from the ^{1}H-^{1}H COSY spectra confirmed that the carboxylic group was positioned at C-30 [correlation of C-30 (δ_C = 179.3) with H-20 (δ_H = 2.72), H-19 (δ_H = 2.34) and CH_3-29 (δ_H = 1.04)]. The hydroxymethyl group was assigned to C-28. This was confirmed by the $^3J_{CH}$ correlations between 2H-28 (δ_H = 3.75, 3.28) and C-22 (δ_C = 34.4), and C-16 (δ_C = 29.5), and C-17 (δ_C = 47.9). The 3-oxo group was deduced from the $^3J_{CH}$ correlations between C-3 (δ_C = 220.2) and 3H-23 (δ_H = 1.09), 3H-24 (δ_H = 1.05), and 2H-2 (δ_H = 2.52) in the HMBC spectrum. Consequently, the structure of **1** was determined as the new triterpene 28-hydroxy-3-oxo-30-lupanoic acid.

Compound **2** revealed the molecular ion peak at *m/z* = 439 $[M–H]^-$ in the negative ESI-MS. The MS and NMR spectroscopic data suggested the molecular formula $C_{30}H_{48}O_2$. The FT-IR spectrum showed absorptions of ketone and aldehyde groups at 1699 and 2708 cm^{-1}. The ^{1}H and ^{13}C NMR spectral data of **2** were very similar to those of **1** with two exceptions: instead of the signal of the hydroxymethyl group at C-28 in **1**, compound **2** exhibits a methyl group at this position (δ_C = 17.9). Furthermore the carboxylic group at C-30 in compound **1** has been replaced by an aldehyde group [δ_H = 9.63 (s, 1H) and δ_C = 205.0]. Finally, the structure of **2** was determined as 3-oxo-lupane-30-al. Compound **2** was isolated for the first time as a natural product. However, it has previously been synthesized by catalytic hydrogenation (Pd-C, methanol, 1 atm H_2) from 3-oxo-lup-20(29)-ene-30-al, isolated from *Maytenus nemerosa* [5].

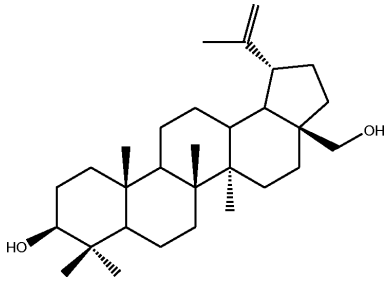
Compounds **3** and **4** have the molecular formula of $C_{29}H_{46}O_2$ and $C_{30}H_{50}O_2$, respectively, according to their MS and NMR spectral data. Their ^{1}H and ^{13}C NMR spectroscopic data are very similar. The



29-nor-21-α-H-hopane-3,22-dione (**3**)



21-α-H-hop-22(29)-ene-3β,30-diol (**4**)



Betulin (**5**)

chemical shifts of the methyl groups in **3** and **4** are typical for a hopane skeleton when compared with other hopane triterpenes [6]. A detailed analysis of the spectroscopic data led to the conclusion that compound **3** was 29-nor-21α-H-hopane-3,22-dione, and **4** was 21-α-H-hop-22(29)-ene-3β,30-diol. Compound **3** has previously been isolated from *Mallotus paniculatus* (Euphorbiaceae) [6] and **4** from *Rhodomyrtus tomentosa* (Myrtaceae) [7]. Their ^{1}H and ^{13}C NMR data are given in the Experimental Section and in Table 1, respectively.

Compound **5** was obtained as needles. It showed a molecular ion peak at *m/z* = 442 $[M]^+$ in the EI-MS, corresponding to $C_{30}H_{50}O_2$. The ^{1}H and ^{13}C NMR data of **5** are in good agreement with those of 20(29)-lupen-3,28-diol (betulin) [8].

Experimental Section

General

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

Table 1. ^{13}C NMR spectral data of **1** – **4**[a].

Position	**1**	**2**	**3**	**4**
1	39.8	39.6	39.8	39.9
2	34.4	34.1	34.1	27.6
3	220.2	218.0	218.1	79.5
4	47.8	47.1	47.3	39.7
5	55.1	54.9	54.8	56.5
6	19.9	19.7	19.7	19.2
7	33.8	33.7	33.5	35.3
8	41.2	40.8	40.7	41.8
9	49.7	49.7	49.6	49.8
10	37.1	36.9	36.9	38.1
11	21.5	21.3	21.5	22.0
12	27.1	27.3	27.2	27.8
13	37.2	37.5	49.5	44.8
14	43.2	42.9	42.9	43.7
15	26.7[b]	26.5[b]	34.9	28.4
16	29.5[b]	23.7[b]	27.7	36.4
17	47.9	43.1	52.6	51.6
18	48.0	49.4	43.1	43.9
19	40.4	37.9	39.5	40.7
20	41.4	47.4	27.3	32.6
21	23.6[b]	40.5	37.2	39.2
22	34.4	35.3	212.7	155.6
23	26.8	26.7	26.8	28.5
24	21.2	21.1	21.0	15.0
25	16.1	15.9	15.7	16.0
26	15.9	15.8	16.0	16.5
27	14.7	14.3	18.0	16.6
28	59.8	17.9	14.4	18.1
29	9.7	7.4	–	107.0
30	179.3	205.0	29.2	64.9

[a] In CD_3OD, 125 MHz; [b] not exactly assignable to the respective C atoms; values marked with an asterisk may be interchanged within each column.

ter POLAX-2L (Japan). FT-IR: Nicolet IMPACT 410. EI-MS: HP5989B. ESI-MS: AGILENT 1100 LC-MSD trap spectrometer. NMR: Bruker Avance 500 MHz (^{1}H) and 125 MHz (^{13}C, ^{13}C DEPT), TMS (δ = 0.0, ^{1}H) and CD_3OD (δ = 49.0, ^{13}C) as references. Column chromatography (CC): silica gel (70 – 230 and 230 – 400 mesh, Merck). Thin layer chromatography (TLC): DC-Alufolien 60 F_{254} (Merck).

Plant material

Stems of *Salacia chinensis* were collected in Thua Thien – Hue province, Vietnam in March 2006. The species was identified by Dr. Ngo Van Trai, Institute of Materia Medica, Hanoi. A voucher specimen has been deposited in the Herbarium of this Institute (SA 611/04).

The dried and powdered stems of *Salacia chinensis* (2.1 kg) were extracted with 80 % aqueous MeOH at r. t. MeOH was evaporated *in vacuo*, the residue was partitioned with *n*-hexane followed by EtOAc and *n*-BuOH. The *n*-hexane extract (6.81 g) was chromatographed on silica gel with solvents of increasing polarity (0 – 100 % EtOAc in *n*-hexane) to give 16 fractions. The fractions were further purified to afford compounds **1**, **2**, **3**, **4**, and **5**.

Extraction and isolation

*28-Hydroxy-3-oxo-30-lupanoic acid (**1**)*

Fraction 16 (*n*-hexane : EtOAc = 70 : 30) was crystallized from *n*-hexane : $CHCl_3$ = 9 : 1 to give 0.028 g of compound **1** (0.0013 %) as a white powder; R_f = 0.27 (*n*-hexane : EtOAc = 5 : 2). – M. p. 285 – 287 °C. – $[\alpha]_D^{25}$ = +110° (CH_3OH : CH_2Cl_2 = 11 : 1, *c* = 0.1364). – IR (KBr): ν = 3490, 2945, 2866, 1733, 1706, 1455, 1385, 1025 cm^{-1}. – ESI-MS: *m/z* = 471 $[M–H]^-$. – HRMS ((–)-ESI): *m/z* = 471.34761 (calcd. 471.34798 for $C_{30}H_{47}O_4$, $[M–H]^-$). – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.75, 3.28 (each 1H, d, 11.0 Hz, 2H-28), 2.72 (1H, dq, 3.0, 6.7 Hz, H-20), 1.10 (3H, s), 1.09 (3H, s), 1.05 (3H, s), 1.04 (3H, d, 6.7 Hz), 1.00 (3H, s) and 0.96 (3H, s). – ^{13}C NMR: see Table 1.

*3-Oxo-lupane-30-al (**2**)*

From fraction 4 (*n*-hexane : EtOAc = 95 : 5). Crystallization of this fraction from *n*-hexane gave 0.014 g of compound **2** (0.00067 %), white crystals; R_f = 0.65 (*n*-hexane : EtOAc = 7 : 1). – M. p. 224 – 225 °C. – IR (KBr): ν = 2931, 2866, 2708, 1699, 1462, 1387, 136 cm^{-1}. – ESI-MS: *m/z* = 440 $[M]^+$ ($C_{30}H_{48}O_2$). – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.6 (1H, s, H-30), 2.65 (1H, dq, 3.1, 6.8 Hz, H-20), 2.50 (2H, m), 2.4 (2H, m), 1.10 (3H, s), 1.08 (3H, s), 1.04 (3H, d, 6.8 Hz), 1.03 (3H, s), 0.96 (3H, s), 0.95 (3H, s), 0.81 (3H, s). – ^{13}C NMR: see Table 1.

*29-nor-21α-H-Hopane-3,22-dione (**3**)*

Fraction 7 (*n*-hexane : EtOAc = 90 : 10) was crystallized from *n*-hexane to yield 0.045 g (0.0021 %) of compound **3** as white crystals; R_f = 0.44 (*n*-hexane : EtOAc = 5 : 1). – M. p. 272 – 273 °C. – IR (KBr): ν =2945, 2873, 1701, 1453, 1378, 1164 cm^{-1}. – EI-MS: *m/z* (%) = 426 $[M]^+$ ($C_{29}H_{46}O_2$), 411 (46.8), 340 (15.7), 203 (48.2), 189 (59.3), 163 (63.0), 147 (47.2), 121 (68.9), 107 (73.8), 95 (100.0), 81 (93.5), 67 (71.6), 55 (88.0). – ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.61 (1H, m), 2.44 (2H, m), 2.15 (3H, s), 2.05 (1H, m), 1.87 (2H, m), 0.97 (3H, s). – ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1.

*21α-H-Hop-22(29)-ene-3β,30-diol (**4**)*

Fraction 13 (*n*-hexane : EtOAc = 80 : 20) was crystallized from *n*-hexane : $CHCl_3$ = 15 : 1, giving 0.036 g of **4** (0.0017 %) as white crystals; R_f = 0.34 (*n*-hexane : EtOAc = 5 : 2). – M. p. 246 – 247 °C. – IR (KBr): ν = 3321, 2936, 2865, 1650, 1452, 1040, 915 cm^{-1}. – ESI-MS: *m/z* = 425 $[M + H – H_2O]^+$. – ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD): δ = 4.95, 4.87 (each 1H), 4.05 (2H, s, 2H-30), 1.01, 0.96,

0.93, 0.83, 0.76, 0.74 (each 3H, s). – ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD): see Table 1.

*Betulin (**5**)*

Fraction 10 (*n*-hexane : EtOAc = 80 : 20) was crystallized from *n*-hexane to yield white crystals; R_f = 0.53 (*n*-hexane : ethyl acetate = 5 : 1). – M. p. 217 – 218 °C. – IR (KBr): ν = 3377, 3084, 2934, 2876, 1642, 1459, 1379, 1027 cm^{-1}. – EI-MS: *m/z* (%) = 442 $[M]^+$, 427 (5.2), 411 (24.6), 393 (5.1), 368 (3.9), 234 (17.5), 203 (33.0), 189 (38.2), 175 (18.9), 147 (23.3), 121 (43.2), 107 (51.3), 95 (72.1), 69 (80.5), 55 (100.0). – ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD): δ = 4.68 (1H, s), 4.57 (1H, s), 3.77 (1H, d), 3.38 (1H, m), 3.29 (1H, d), 2.37(1H, m), 1.94 (2H, m).

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