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ISOLATION AND STRUCTURAL CHARACTERIZATION OF PHENOLIC GLYCOSIDE AND TRITERPENES IN *CELASTRUS HINDSII* BENTH

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SUMMARY

Chemical investigation of Celastrus hindsii growing in Quang Binh, Vietnam led to the isolation and structural elucidation of glucosyringic acid, lup-20(29)-ene-3β,11β-diol, lup-20(29)-ene-3-one (lupenone) and lup-5,20(29)-diene-3-one. Their structures were determined on the basis of MS, NMR spectra and comparison with reported data.

Keywords: *Celastrus hindsii; Celastraceae; syringic acid, lupane triterpenoids.*

I - INTRODUCTION

Celastrus hindsii Benth is a small trees growing wild or cultivated in Son La, Hoa Binh, Nam Ha, Quang Binh provinces of Vietnam. *Celastrus hindsii* Benth is used as a traditional medicine for the treatment of stomach disease, ulcer, tumors and inflammation in Vietnam [1, 2]. The EtOH extract from the stems of *C. hindsii* shows potent cytotoxicity against *hepatoma*, *colon carcinoma*, as well as against HIV replication in H-9 lymphocytes *in vitro* [2]. Our previous report on phytochemical constitution of the *C. hindsii* leaves dealt with the isolation and structural characterization of three friedelane triterpenes named 3-friedelanol, 3-friedelanone and canophyllol [3]. Further studies on biologically active compounds from this plant, this paper reports the isolation and structural determination of glucosyringic acid (**1**), lup-20(29)-ene-3β,11β-diol (**2**) from stems, lup-20(29)-ene-3-one (lupenone, **3**) and lup-5,20(29)-diene-3-one (**4**) from the leaves. Their structures were elucidated by MS, NMR spectra and comparison with reported data.

II - EXPERIMENT

1. General

FT-IR: IMPACT 410 (Nicolet, Germany). EI-MS: Mass spectrometer 5989B (Hewlett Packard, USA). ESI-MS: LC-MSD-Trap-SL (USA). NMR: BRUKER Avance 500 spectrometer at 499.8 MHz (^{1}H) and 125 MHz (^{13}C, ^{13}C DEPT). Chemical shifts were referenced to internal TMS (δ = 0, ^{1}H), CD_3OD (δ = 49.0, ^{13}C) and $CDCl_3$ (δ = 77.0, ^{13}C). All spectra are recorded in the institute of Chemistry, VAST, Hanoi, Vietnam. CC: Silica gel 60, 0.06 - 0.2 mm (Merck) for the first column, silica gel 60, 40 - 63 μm (Merck) for the following columns. TLC: Silica gel 60 F-254 (Merck).

Table 1: ^{13}C-NMR spectral data of triterpenes **2a**, **2** and **3** [125 MHz, $CDCl_3$, δ (ppm)]

C	**2a** [6]	**2**	**3**
1	39.00	38.07	39.79
2	27.50	25.13	34.53
3	78.60	79.03	216.76
4	39.40	38.89	47.25
5	55.60	53.14	54.31
6	18.10	18.01	18.66
7	35.30	34.14	33.72
8	41.00	41.39	40.86
9	55.70	51.31	48.79
10	37.70	38.89	58.13
11	70.40	75.77	22.54
12	27.70	23.29	24.87
13	37.70	37.61	38.49
14	42.60	42.93	41.98
15	27.50	27.55	27.41
16	55.50	35.60	35.67
17	43.00	42.93	41.98
18	47.70	48.36	41.23
19	47.70	48.00	47.25
20	150.20	150.81	149.81
21	29.80	29.80	30.07
22	39.80	40.02	40.51
23	28.30	27.86	26.43
24	15.50	14.94	14.45
25	16.10	16.25	16.47
26	17.20	14.94	18.52
27	14.50	14.47	14.45
28	18.10	18.05	18.66
29	109.80	109.45	108.40
30	19.40	19.24	18.66

2. Plant material

The leaves of *C. hindsii* were collected in Quang Binh, Vietnam, in October 2005. The species was identified by Mr. Nguyen Quoc Binh, Institute of Ecology and Natural Resources, VAST, Hanoi, Vietnam. A voucher specimen is deposited in the Herbarium of this Institute.

3. Extraction and Isolation

The dried and powdered leaves of *C. hindsii* (1.7 kg) were extracted with 90% aq. EtOH at room temperature. EtOH was evaporated *in vacuo* at 45□C and the aq. solution was partitioned with *n*-hexane followed by EtOAc and *n*-BuOH. The organic solvents were evaporated *in vacuo* to afford 25.5; 16 and 55g of extracts, respectively.

*a) Glucosyringic acid (**1**)*

The *n*-BuOH extract (55 g) was chromatographed over silica gel with gradient CH_2Cl_2-MeOH (90:10 → 80:20) and then CH_2Cl_2-MeOH-H_2O (80:20:1) to give 5 fractions (F-1 → F-5). Fraction 4 (F-4) was purified by silica gel CC with gradient MeOH-EtOAc-H_2O (5:60:1 → 10:60:2) to give **1** (60 mg, 0.0035%); white powder (MeOH-CH_2Cl_2); ESI-MS: 383 $[M+Na]^+$ (91), 199 $[M+H\text{-glu}]^+$; IR ν_{max}^{KBr} (cm^{-1}): 3524, 3446 (OH), 2927, 2856, 1668, 1598, 1464, 1417, 1385, 1331, 1231, 1131, 1008. ^{1}H- and ^{13}C-NMR, see table 1.

b) Lup-20(29)-ene-3β,11β-diol (2)

The EtOAc extract (16 g) was chromatographed over silica gel with gradient *n*-hexane-EtOAc (20:10) to give 13 fractions (F-1 → F-13). Compound **2** was isolated from fraction 8 (F-8) by crystalization from EtOAc, yield 300 mg (0.017%); EI-MS (*m/z*): 442 $[M]^+$(2), 424 $[M-H_2O]^+$ (9), 257 (14), 232 (9), 219 (13), 203 (17), 189 (25), 121 (57), 107 (68), 81 (88), 69 (76), 55 (100); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.56 (2H, m, H-2), 3.25 (1H, dd, J = 3.4; 12.1 Hz, H-11), 3.42 (1H, dd, J = 4.6; 11.3 Hz, H-3), 1.00 (1H, td, J = 4.8, 7.5 Hz, H-5), 1.33 (1H, m, H-9), 2.37 (1H, dt, J = 11.2, 5.8 Hz), 0.952 (3H, s, Me-23), 0.947 (3H, s, Me-25), 1.04 (3H, s, Me-24), 0.79 (3H, s, Me-26), 0.90 (3H, s, Me-27), 0.75 (3H, s, Me-28), 4,55 (1H, s, H-29A),

4.68 (1H, d, J = 2.1 Hz, H-29B), 1.69 (3H, s, Me-30). ^{13}C-NMR, see table 2.

*c) Lup-20(29)-ene-3-one (3) and lup-5,20(29)-diene-3-one (**4**)*

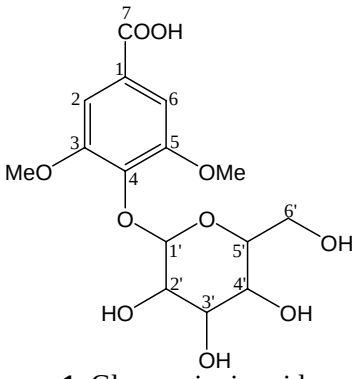
The dried and powdered leaves of *C. hindsii* (600g) were extracted with 90% aq. EtOH at room temperature. EtOH was evaporated *in vacuo* at 45□C and the aq. solution was partitioned with *n*-hexane followed by EtOAc and *n*-BuOH. The *n*-hexane extract (15 g) was chromatographed over silica gel with gradient *n*-hexane-EtOAc (90:10 → 10:90) to give 6 fractions (F-1 → F-6). Compounds **3** + **4** were isolated as white powder from MeOH as a mixture (ratio 1:4) from F-5 (20 mg, 0.0033%).

Lup-20(29)-ene-3-one (**3**): the minor component (*ca* 20 %). ^{13}C-NMR: see table 2.

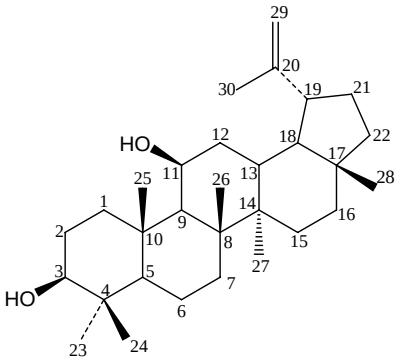
Lup-5,20(29)-diene-3-one (**4**): ^{13}C-NMR (125 MHz, $CDCl_3$, δppm): 34.52 (C-1), 33.05 (C-2), 216.71 (C-3), 46.32 (C-4), 144.25 (C-5), 120.51 (C-6), 46.23 (19), 149.81, 108.40 (C-29).

III - RESULTS AND DISCUSSION

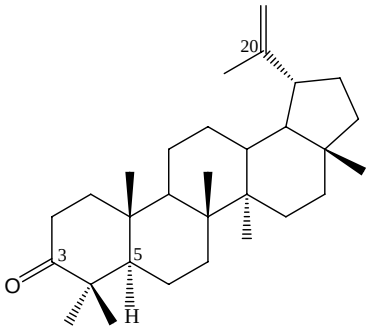
The residue of an ethanol extract of the stems of *C. hindsii* was partitioned with *n*-hexane, ethyl acetate and *n*-butanol, successively. The *n*-BuOH extract, after evaporation of the solvents, was subjected to column chromatography, recrystallization to give **1**.



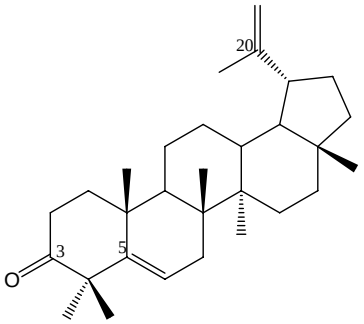
1: Glucosyringic acid



2: Lup-20,29-ene-3β,11β-diol



3: Lup-20(29)-ene-3-one



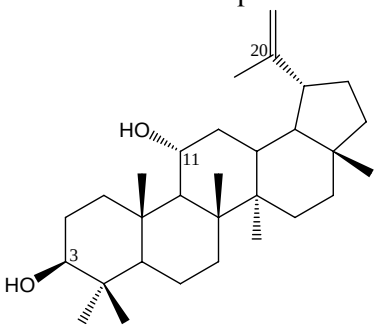
4: Lup-5,20(29)-diene-3-one

The IR spectrum of compound **1** showed the presence of hydroxyl groups (3440 - 3524 cm^{-1}), a carbonyl absorption band (1668 cm^{-1}), aromatic ring (1600) and C-O bond (1008 cm^{-1}). The ESI-MS (positive ions) gave the base peak at *m/z* 383 (100) $[M+Na]^+$, combination with ^{13}C-NMR and APT spectra leading to the molecular formula $C_{15}H_{20}O_{10}$. The ^{13}C-NMR and DEPT spectra showed the presence of a glucose moiety and an aglycone moiety with nine carbon atoms. This was supported by the loss of a 162 mass unit to give a peak at *m/z* 199 $[M+H-162]^+$ in the mass spectrum. The appearance of six signals in the ^{13}C-NMR

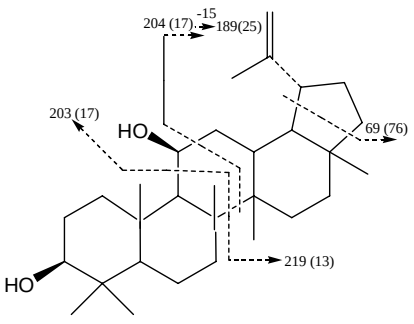
spectrum of the aglycone moiety corresponded to nine carbons, indicating that the molecule must be a degree of symmetry in its structure. This was confirmed by two singlets at δ 7.39 (H-2 and H-6) and 3.92 (6H, 2xOCH_3), therefore two methoxyl groups were attached at C-3&C-5. The ^{1}H-NMR spectrum displayed one doublet at δ5.28 (d, J = 8.0 Hz, H-1') and the ^{13}C-NMR signal at δ_C104.29 (C-1'), suggesting that β-D-glucose moiety was attached to C-4. This was confirmed by the CH long-range correlation of C-4 and anomeric proton H-1' (δ_C169.66)/δ_H5.09), whereas C-7 shows correlations to both H-2, H-6 (δ_C169.66/δ_H7.39) in the HMBC spectrum. Therefore, the structure of **1** was elucidated as glucosyringic acid. This compound was isolated from the roots of *Rododendron molle*. In a preliminary in *vitro* bioassay glucosyringic acid inhibited significantly the proliferation of murine B lymphocytes at a concentration of 1x 10^{-6} M [4].

Compound **2** was isolated from the EtOAc extract of the stems. The IR spectrum indicated a hydroxy (3363 cm^{-1}) and olefinic methylene (>C=CH_2) group (1641, 3070 cm^{-1}). The EI-MS showed a molecular ion peak at *m/z* 442 (2) $[M]^+$, combination with ^{13}C-NMR and APT spectra leading to the molecular formula $C_{30}H_{50}O_2$. The EI-MS displayed three important fragment ions at *m/z* 219 (13), 203 (17), 189 (25), suggested that **2** is a lupan-triterpene skeleton [5]. The peak at *m/z* 203 indicated fragment ion of ring A-B with C-3 hydroxy group; ion at *m/z* 219 was formed by the C-ring cleavage indicated that the second hydroxy was in the fragment of ring D-E as shown in *Scheme 1*. The ^{13}C-NMR and DEPT spectra showed the presence of signals for 30 carbons including 7xCH_3, 10xCH_2, 7xCH, 6xCq. The ^{1}H- and ^{13}C-NMR spectral data for **2** was similar to those of **2a** except the significant changes in the chemical shifts at C-11 and its neighbour: signal of C-11 shifted downfield (Δδ 5.4 ppm), C-9 and C-12 were shifted upfield (Δδ4.4 ppm). Significantly changes in the chemical shifts of C-11, C-9 and C-12 can only be explained when the configuration of C-11 is different to those of **2a**. The orientation of the hydroxyl group was clarified by NOESY spectrum. The correlations between H-11*α*/H-3*α*, Me-27, Me-30 indicated that the relative configuration of OH-11 is *β*. Therefore, the structure of **2** was determined as lup-20,29-ene-3*β*,11*β*-diol. This compound was isolated for the first time from *Dodonaceae attennuata* [6]. Its epimer, lup-20,29-ene-3*β*,11*a*-diol (nepeticin) shows antibiotic and blood cholesterol reducing activity [6].

The NMR spectra indicated that compounds **3+4** were isolated as a mixture with a 1:4 ratio from the *n*-hexane extract of the leaves of *C. hindsii*, determined by the integrals in the ^{1}H-NMR. The ^{13}C-NMR spectrum of **3** and **4** is similar to those of **2**, except the appearance of a ketone group (δ216.76/216.71). In the ^{13}C-NMR spectrum, only the major component **4** showed two double bonds at δ144.25, 120.51, 149.81, 108.4 of C_5=C_6 and C_{20}=C_{29}. The structure of **3** was identified as lup-20(29)-ene-3-one (lupenone) by comparison of its ^{13}C-NMR spectral data with reported data [5], whereas the structure **4** was suggested as lup-5,20(29)-dien-3-one with the help of ACD/^{13}C-NMR software [6, 7].



2a: Lup-20(29)-ene-3β,11α-diol



Scheme 1: EI-MS spectral fragmentation of **2**

Table 2: ^{13}C- and ^{1}H-NMR spectral data of **1** [125 MHz, CD_3OD, δ (ppm)]

C	δC	δH (J in Hz)	HMBC correlation
1	127.61		H-2, H-6
2,6	108.81	7.39, 2H	5-OMe
3,5	153.76		H-2, H-6, OMe C-5/H-6, C-3/H-2
4	139.82		H-1', H-2, H-6
7	169.66		H-2, H-6
1'	104.29	5.09 d (7.5)	H-2'
2'	75.28	3.56 m	H-1', H-3'
3'	77.34	3.52 m	H-2', H-4', H-5'
4'	70.86	5.52 m	H-3', H-5'
5'	77.91	3.33 m	H-4', H-6'
6'	62.05	3.72 dd (4.9; 11.5) 3.72 dd (4.9; 11.5)	H-5'
OMe	57.33	3.92 s	H-6

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