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Four new triterpenes from *Ilex cornuta* Lindley

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Abstract: Two new lupane-type triterpenes, 24β-hydroxylupenone (**1**) and 3β-hydroxy-lup-20(29)-en-24-al (**2**), and two new ursane-type triterpenes, 28-formyloxy–3β-hydroxy-urs-12-ene (**3**) and 28-formyloxy-3β-acetoxy-urs-12-ene (**4**), together with 26 known triterpenes (**5–30**) were isolated from the leaves and trunk of *Ilex cornuta* Lindl. The structures of the new compounds were determined by spectroscopic methods, including 1D, 2D NMR (COSY, HMQC, HMBC, and NOESY), and HRMS data.

Key words: *Ilex cornuta*, Aquifoliaceae, triterpene.

Résumé : L'extraction des feuilles et du tronc de *Ilex cornuta* Lindl. a permis d'isoler deux nouveaux triterpènes de la famille du lupane, la 24β-hydroxylupénone (**1**) et le 3β-hydroxylup-20(29)-én-24-al (**2**), deux nouveaux triterpènes de la famille de l'ursane, le 28-formyloxy-3-β-hydroxyurs-12-ène (**3**) et le 28-formyloxy-3-β-acétoxyurs-12-ène (**4**) ainsi que vingt-six triterpèrenes connus (**5–30**). Les structures des nouveaux composés ont été déterminées par des méthodes spectroscopiques, dont des données de RMN 1D et 2D [spectroscopie de corrélation ("COSY"); corrélation de liens multiples hétéronucléaires ("HMBC"); corrélation multi-quantique hétéronucléaire ("HMQC"); spectroscopie de l'effet Overhauser nucléaire ("NOESY")] et des données de spectrométrie de masse à haute résolution (SM-HR). [Traduit par la Rédaction]

Mots-clés : *Ilex cornuta*, Aquifoliacée, triterpène.

Introduction

Ilex cornuta Lindl. (Aquifoliaceae) is an evergreen shrub widely distributed in Korea and China, and is known as Chinese holly.[1] This indigenous plant has been used in Chinese folk medicine for treating dizziness and hypertension.[2] Further, an aqueous extract from its leaves has been used traditionally as a contraceptive, cardiovascular system protection, and antibacterial agent.[3]

Previous phytochemical investigations of *I. cornuta* have shown that its leaves and trunk are a rich source of triterpenoids and flavonoids.[3–7] Some of these isolates have been reported to exhibit various pharmacological effects, such as an increase in coronary blood flow and antihematoblastic coagulation activity.[5,8]

In our continuing search for novel secondary metabolites from Korean medicinal plants, we have investigated the constituents from the leaves and trunk of *I. cornuta*. Column chromatographic separation of the MeOH extract led to isolation of two new lupane-type triterpenes (**1** and **2**) and two new ursane-type triterpenes (**3** and **4**) (Fig. 1), together with 26 known compounds (**5–30**). The structures of these new compounds were elucidated by spectroscopic methods, including 1D and 2D NMR.

Here, we report the isolation of chemical constituents and structural elucidation of compounds **1–30**.

Results and discussion

The 80% MeOH extract of the leaves and trunk of *I. cornuta* was subjected to repeated silica gel column chromatography to afford four new triterpenes (**1–4**), together with 26 known compounds (**5–30**). The structures of the new compounds were established by spectroscopic and chemical means.

Compound **1** was obtained as a colorless gum whose molecular formula was determined to be $C_{30}H_{48}O_2$ from the $[M + H]^+$ peak at m/z 441.3731 (calculated for $C_{30}H_{49}O_2$: 441.3733) in the high resolution fast atom bombardment HRFAB-MS. The ^1H NMR spectrum of **1** (Table 1) displayed signals for five methyl groups at δ_H 0.79,
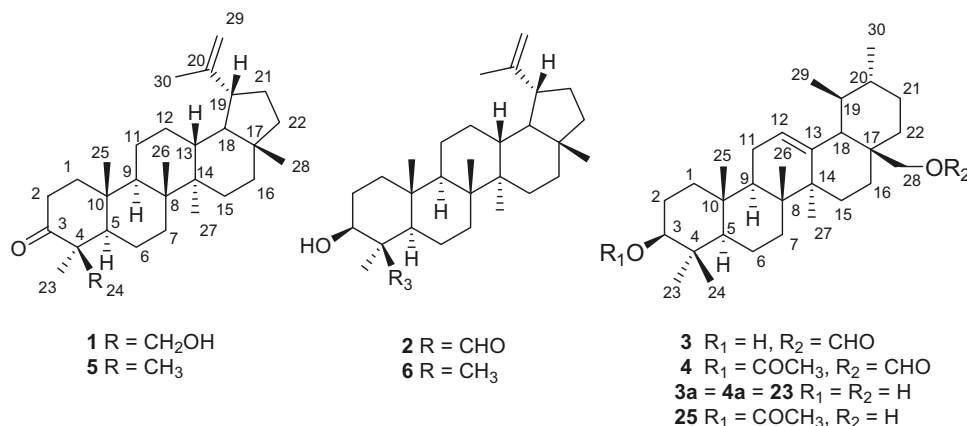
0.87, 0.96, 1.04, and 1.26 (each 3H, s), a isopropenyl group at δ_H 1.68 (3H, s), 4.58 (1H, s), and 4.69 (1H, s), and a hydroxymethyl group at δ_H 3.42 (1H, d, J = 11.0 Hz), and 3.97 (1H, d, J = 11.0 Hz). In the ^{13}C NMR spectrum (Table 2), 30 carbon signals appeared, including six methyl carbons at δ_C 14.7, 15.8, 17.2, 18.2, 19.5, and 22.3, a hydroxymethyl carbon at δ_C 66.0, one ketone carbon at δ_C 221.7, two olefinic carbons at δ_C 109.6, and 151.1, including 10 methylene, five methine, and five quaternary carbons. These data implied that **1** could be a lupane-type triterpene.[9] Comparison of the NMR data for **1** with those of lupenone (**5**),[10] indicated that compound **1** was an analogue of **5**. The main difference was an additional hydroxyl group at C-24, indicating the presence of a hydroxymethyl group in **1** instead of a methyl group in **5**. The HMBC spectrum showed correlations from H-24 to C-3, C-4, and C-23, supporting the presence of the hydroxyl group at C-24 in the structure (Fig. 2). The relative configuration of **1** was supposed to be identical to that of **5** based on the J value and chemical shifts in the ^1H NMR spectrum. The hydroxymethyl group at C-24 was determined to be β-form, as the NOESY experiment showed a correlation between H-24 (δ_H 3.42, and 3.97) and Me-25 (δ_H 0.87) (Fig. 3). On the basis of the NMR data and the spectroscopic data of lupane-type triterprenes isolated from this source,[6] the structure of **1** was determined to be 24β-hydroxylupenone. The oxidation of lup-20(29)-en-3β,24-diol (**11**) by chromium trioxide produced 24β-hydroxylupenone, which was identical to the isolated compound (**1**) based on NMR spectral data.[11,12]

Compound **2** was obtained as a colorless gum whose molecular formula was determined to be $C_{30}H_{48}O_2$ from the $[M + Na]^+$ peak at m/z 463.3553 (calculated for $C_{30}H_{48}NaO_2$: 463.3552) in the HRFAB-MS. The proton and carbon signals in the ^1H and ^{13}C NMR spectra (Table 1 and 2) of **2** were very similar to those of lupeol (**6**).[10] The only difference was the appearance of the formyl group signals (δ_H 9.76; δ_C 206.9), as well as the disappearance of the methyl carbon and proton signals (δ_H 0.94; δ_C 15.4) in **2**.[13] The position of the

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Fig. 1. Chemical structures of compounds **1–6**, **23**, **25**, **3a**, and **4a**.

1 R = CH₂OH
5 R = CH₃

2 R = CHO
6 R = CH₃

3 R₁ = H, R₂ = CHO
4 R₁ = COCH₃, R₂ = CHO
3a = 4a = 23 R₁ = R₂ = H
25 R₁ = COCH₃, R₂ = H

Table 1. ¹H NMR data of compounds 1–4 (CDCl₃, 500 MHz, δ in ppm, *J* in Hz).

Protone	1	2	3	4
1	1.53, m, 1.90, m	1.40, m	1.01, m, 1.65, m	1.02, m, 1.65, m
2	2.40, m, 2.60, m	1.84, m	1.62, m	1.65, m
3α		3.16, dd (11.0, 5.5)	3.21, dd (11.0, 5.5)	4.50, dd (11.0, 5.5)
5	1.58, m	0.99, m	0.72, m	0.85, m
6	1.40, m, 1.50, m	1.46, m	1.42, m, 1.57, m	1.40, m, 1.55, m
7	1.43, m	1.42, m	1.40, m, 1.57, m	1.38, m, 1.57, m
9	1.40, m	1.34, m	1.53, m	1.53, m
11	1.40, m	1.48, m	1.93, m	1.95, m
12	1.69, m	1.70, m	5.16, br s	5.16, br s
13	1.70, m	1.78, m		
15	1.70, m	1.70, m	1.04, m, 1.18, m	1.05, m, 1.17, m
16	1.36, m, 1.53, m	1.38, m, 1.54, m	1.12, m, 1.23, m	1.12, m, 1.23, m
18	1.38, m	1.36, m	1.42, m	1.43, m
19	2.38, m	2.38, m	1.42, m	1.45, m
20			1.00, m	0.95, m
21	1.35, m, 1.90, m	1.30, m, 1.90, m	1.27, m, 1.48, m	1.25, m, 1.48, m
22	1.20, m, 1.40, m	1.20, m, 1.40, m	1.40, m, 1.60, m	1.38, m, 1.60, m
23	1.26, s	1.26, s	1.00, s	0.99, s
24	3.42, d (11.0)	9.76, s	0.79, s	0.88, s
	3.97, d (11.0)			
25	0.87, s	0.77, s	0.95, s	0.95, s
26	1.04, s	1.04, s	1.03, s	1.03, s
27	0.96, s	0.96, s	1.12, s	1.13, s
28	0.79, s	0.79, s	3.74, d (11.0)	3.74, d (11.0)
			4.15, d (11.0)	4.15, d (11.0)
29	4.58, s, 4.69, s	4.57, s, 4.69, s	0.84, d (6.5)	0.81, d (6.5)
30	1.68, s	1.68, s	0.93, d (6.5)	0.94, d (6.5)
CHO			8.08, s	8.08, s
COCH₃				2.04, s

Note: Assignments were based on HMQC, and HMBC experiments.

formyl group in **2** was confirmed by HMBC, which showed long-range correlations between the formyl proton (δ_H 9.76) and the C-3 (δ_C 78.0); the Me-23 (δ_H 1.26) and the C-3 (δ_C 78.0); C-4 (δ_C 52.7) and the formyl carbonyl carbon (δ_C 206.9); as well as the H-3 (δ_H 3.16) and the formyl carbonyl carbon (δ_C 206.9) (Fig. 2). The above data showed that the position of the formyl group was located at C-24. The coupling constants of H-3 (δ_H 3.16, dd, *J* = 11.0, 5.5 Hz) observed in the ¹H NMR spectrum indicated β-orientation of the OH group at C-3 (dd, *J* = 11.0, 5.5 Hz).[10,14] The β-orientation of the formyl group was determined from correlations between the formyl proton (δ_H 9.76)/Me-25 (δ_H 0.77) and H_α-3 (δ_H 3.16)/Me-5 (δ_H 0.99) in the NOESY spectrum (Fig. 3). Thus, the structure of **2** was determined to be 3β-hydroxy-lup-20(29)-en-24-al.

Compound **3** was obtained as a colorless gum whose molecular formula was determined to be C₃₁H₅₀O₃ from the [M]⁺ peak at *m/z* 470.3761 (calculated for C₃₁H₅₀O₃: 470.3760) in the HRFAB-MS. The ¹H NMR spectrum of **3** showed seven methyl signals at δ_H 0.79 (s),

0.84 (d, *J* = 6.5 Hz), 0.93 (3H, d, *J* = 6.5 Hz), 0.95 (s), 1.00 (s), 1.03 (s), and 1.12 (s); an oxygenated methine signal at δ_H 3.21 (1H, dd, *J* = 11.0, 5.5 Hz); and a olefinic signal at δ_H 5.16 (1H, br s), as well as a hydroxymethyl group at δ_H 3.74 (1H, d, *J* = 11.0 Hz), and 4.15 (1H, d, *J* = 11.0 Hz) (Table 1). The ¹³C NMR spectrum indicated 30 carbon resonances, which were classified by HMQC experiment as olefinic carbon signals at δ_C 125.8 and 138.0, an oxygenated carbons at δ_C 79.0, a hydroxymethyl carbon at δ_C 70.8, seven methyl, nine methylene, five methine, and five quaternary carbons (Table 2). The ¹H and ¹³C NMR spectra of **3** were very similar to those of 3β,28-dihydroxy-urs-12-ene (**23**),[15] except for the presence of formyl group signals [δ_H 8.08 (1H, s); δ_C 161.3],[16,17] which were located at C-28 based on the HMBC correlation between the H-28 (δ_H 3.74, and 4.15) and the formyl carbonyl carbon (δ_C 161.3) (Fig. 2). The stereochemistry of **3** was established based on the NOESY correlation between H-3 and H-5, and no correlation between H-3 and H-25 indicated that the OH group at C-3 was in β-form (Fig. 3).

Table 2. ^{13}C NMR data of compounds 1–4 (CDCl$_3$, 125 MHz, δ in ppm).

Carbon	1	2	3	4
1	39.5	40.0	39.2	38.6
2	34.5	28.2	28.1	23.8
3	221.7	78.0	79.0	81.1
4	50.9	52.7	38.9	37.9
5	55.5	56.3	55.2	55.5
6	19.4	21.3	18.3	18.4
7	33.8	34.7	32.7	32.9
8	40.9	40.6	40.2	40.2
9	49.8	49.3	47.6	47.8
10	36.8	36.0	36.9	37.0
11	22.1	22.6	23.3	23.6
12	25.4	25.3	125.8	126.0
13	38.4	38.4	138.0	138.3
14	43.1	42.9	42.1	42.2
15	27.6	27.4	25.9	26.2
16	35.7	35.5	23.2	23.5
17	43.2	43.0	38.0	38.2
18	48.5	48.2	54.2	54.4
19	48.1	47.9	39.7	39.5
20	151.1	150.8	39.9	40.2
21	30.1	29.8	30.4	30.6
22	40.2	40.1	35.6	35.8
23	22.3	19.3	28.2	28.2
24	66.0	206.9	15.5	15.9
25	17.2	15.0	15.6	16.2
26	15.8	16.0	17.2	17.5
27	14.7	14.4	23.4	23.3
28	18.2	18.0	70.8	70.1
29	109.6	109.4	17.2	17.5
30	19.5	19.3	21.2	21.4
CHO			161.3	161.5
$\underline{C}OCH_3$				171.2
$CO\underline{C}H_3$				21.4

Alkaline hydrolysis of **3** afforded 3β,28-dihydroxy-urs-12-ene (**3a**), which was identified by comparing its optical rotation value as well as ^1H NMR and MS data.[24] Therefore, the structure of **3** was determined to be 28-formyloxy-3β-hydroxy-urs-12-ene.

Compound **4** was obtained as a colorless gum whose molecular formula was determined to be $C_{33}H_{52}O_4$ from the [M + Na]$^+$ peak at m/z 535.3762 (calculated for $C_{33}H_{52}NaO_4$: 535.3763) in the HRFAB-MS. The ^1H and ^{13}C NMR spectra of **4** were very similar to those of **3**. The only difference was the presence of additional acetyl group signals [δ$_H$ 2.04 (1H, s); δ$_C$ 21.4, and 161.3].[18] The position of the acetyl group was identified by the HMBC spectrum, in which a long-range correlation was observed between the H-3 (δ$_H$ 4.50, dd, J = 10.5, 5.5 Hz) and a carbonyl carbon (δ$_C$ 171.2) (Fig. 2). Alkaline hydrolysis of **4** yielded 3β,28-dihydroxy-urs-12-ene (**4a**), which was identified by comparing its optical rotation value, ^1H NMR and MS data.[15] Hence, the structure of **4** was determined to be 28-formyloxy-3β-acetoxy-urs-12-ene.

The structures of the known compounds (**5–30**) were identified as lupenone (**5**),[10] lupeol (**6**),[10] 3-epi-lupeol (**7**),[14] lupeol acetate (**8**),[19] lupenyl formate (**9**),[20] 3-O-acetylbetulin (**10**),[21] lup-20(29)-en-3β,24-diol (**11**),[22] 3β-hydroxy-lup-20(29)-en-30-al (**12**),[23] 3β-hydroxy-20-oxo-30-norlupane (**13**),[24] betulone (**14**),[25] α-amyrin palmitate (**15**),[26] β-amyrin palmitate (**16**),[27] α-amyrin acetate (**17**),[27] 11-oxo-α-amyrin palmitate (**18**),[28] 11-oxo-β-amyrin palmitate (**19**),[29] 11-oxo-α-amyrin (**20**),[30] 11-oxo-β-amyrin (**21**),[30] 3β,28-dihydroxy-ole-12-ene (**22**),[31] 3β,28-dihydroxy-urs-12-ene (**23**),[15] 3β-acetoxy-28-hydroxy-ole-2-ene (**24**),[32] 3β-acetoxy-28-hydroxy-urs-12-ene (**25**),[18] 3β-acetoxy-13(28)-epoxy-ole-11-ene (**26**),[33] 3β-hydroxy-13(28)-epoxy-urs-11-ene (**27**),[34] 3β-hydroxy-11α-methoxy-urs-12-ene (**28**),[33] 28-nor-urs-12-ene-3β,17β-diol (**29**),[34] and 17β-formyloxy-28-nor-urs-12-en-3β-ol (**30**)[35] by comparison of ^1H and ^{13}C NMR, and MS data with those reported previously.

## Experimental

General experimental procedures

Optical rotations were measured in MeOH using a Jasco P-1020 polarimeter. IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer. HRFAB mass spectra were obtained on a JEOL JMS700 mass spectrometer. NMR spectra, including ^1H-^1H COSY, HSQC, HMBC, and NOESY experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer operating at 500 MHz (^1H) and 125 MHz (^{13}C) with chemical shifts given in ppm (δ). Preparative HPLC was conducted using a Gilson 306 pump with Shodex refractive index detector and Apollo Silica 5 μ column (250 × 10 mm). Silica gel 60 (Merck, 70–230 mesh and 230–400 mesh) and RP-C$_{18}$ silica gel (Merck, 230–400 mesh) were used for column chromatography. Merck precoated Silica gel F$_{254}$ plates and RP–18 F$_{254s}$ plates were used for TLC. Spots were detected on TLC under UV light or by heating after spraying with 10% H$_2$SO$_4$ in C$_2$H$_5$OH (v/v).

Plant material

The leaves and trunk from *I. cornuta* (5.0 kg) were collected on Jeju Island, Korea in March 2011, and the plant was identified by one of the authors (K.R. Lee). A voucher specimen (SKKU-NPL 1108) has been deposited at the herbarium in the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

Extraction and isolation

The leaves from *I. cornuta* (5.0 kg) were extracted with 80% aqueous MeOH under reflux and filtered. The resulting MeOH extracts (550 g) were suspended in distilled water (800 mL × 3) and then successively partitioned with *n*-hexane, CH$_2$Cl$_2$, EtOAc, and *n*-BuOH, yielding 20, 68, 28, and 100 g, respectively. The *n*-hexane soluble fraction (20 g) was chromatographed on a silica gel (230–400 mesh, 500 g) column eluted with *n*-hexane-EtOAc (15:1 – 1:1, gradient system) to yield 10 fractions (H1–H10). Fraction H1 (50 mg) was purified by preparative HPLC (*n*-hexane–EtOAc, 50:1) to give compounds **15** (25 mg) and **16** (6 mg). Fraction H2 (1.5 g) was chromatographed on a silica gel (230–400 mesh, 40 g) column eluted with *n*-hexane–EtOAc (40:1) to give five subfractions (H21–H25). Subfraction H21 (70 mg) was purified by preparative HPLC (*n*-hexane–EtOAc, 30:1) to give **9** (7 mg). Subfraction H22 (200 mg) was separated by preparative HPLC (*n*-hexane–EtOAc, 85:1) to give **8** (7 mg) and **17** (10 mg). Subfraction H23 (40 mg) was separated by preparative HPLC (*n*-hexane–EtOAc, 30:1) to obtain **5** (20 mg). Subfraction H24 (250 mg) was purified by preparative HPLC (*n*-hexane–EtOAc, 50:1) to give **18** (5 mg) and **19** (23 mg). Subfraction H25 (40 mg) was separated by preparative HPLC (*n*-hexane–EtOAc, 30:1) to afford **4** (5 mg). Fraction H4 (1.2 g) was chromatographed on a silica gel (230–400 mesh, 30 g) column eluted with *n*-hexane–EtOAc (40:1) to give three subfractions (H41–H43). Subfraction H42 (50 mg) was purified by preparative HPLC (*n*-hexane–EtOAc, 15:1) to give **7** (12 mg). Fraction H5 (5 g) was chromatographed on a silica gel (230–400 mesh, 100 g) column eluted with *n*-hexane–EtOAc (30:1) and purified further by preparative HPLC (*n*-hexane–EtOAc, 10:1) to give **6** (4 g). Fraction H6 (2 g) was chromatographed on an RP-C$_{18}$ silica gel (230–400 mesh, 60 g) column eluted with 100% MeOH to give three subfractions (H61–H63). Subfraction H61 (200 mg) was purified by preparative HPLC (*n*-hexane–EtOAc, 10:1) to give **2** (4 mg), **11** (20 mg), **24** (27 mg), and **26** (4 mg). Subfraction H62 (100 mg) was purified by preparative HPLC (*n*-hexane–EtOAc, 5:1) to give **3** (7 mg), **10** (4 mg), and **25** (4 mg). Fraction H8 (5 g) was chromatographed on a silica gel (230–400 mesh, 120 g) column eluted with *n*-hexane–EtOAc (40:1) to give six subfractions (H81–H86). Subfraction H81 (150 mg) was purified by preparative HPLC (100% MeOH) to give **1** (4 mg) and **13** (15 mg). Subfraction H83 (120 mg) was separated by preparative HPLC (95% MeOH) to afford **12** (60 mg), **14** (4 mg), and **29** (10 mg). Subfraction H84 (70 mg) was purified by preparative HPLC (*n*-hexane–EtOAc, 4:1) to yield **20**

Fig. 2. Key HMBC (→) of **1–4**.
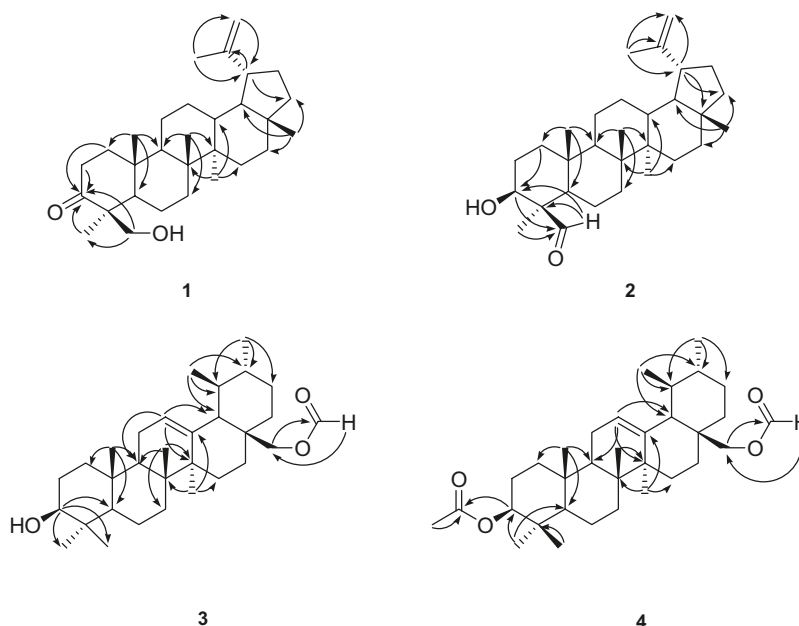
Fig. 3. Key NOESY (⌒) correlations of **1–4**.
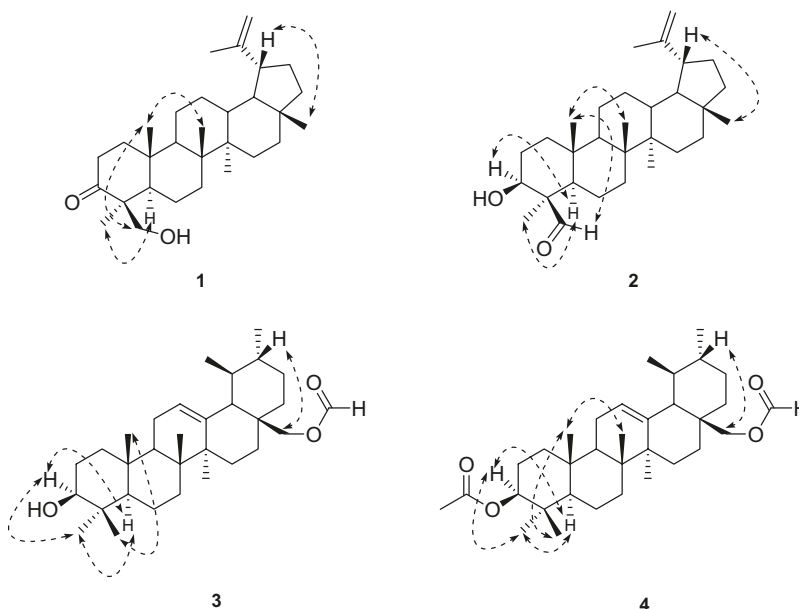
(4 mg), **21** (5 mg), **27** (13 mg), and **30** (13 mg). Fraction H9 (3 g) was chromatographed on an RP-C$_{18}$ silica gel (230–400 mesh, 60 g) column eluted with 100% MeOH and purified by preparative HPLC (*n*-hexane–EtOAc, 4:1) to give **22** (5 mg) and **23** (20 mg). Fraction H10 (1 g) was chromatographed on a silica gel (230–400 mesh, 30 g) column eluted with *n*-hexane–EtOAc (15:1) to give four subfractions (H101–H104). Subfraction H102 (30 mg) was purified by preparative HPLC (*n*-hexane–EtOAc, 4:1) to give **28** (4 mg).

24-Hydroxylupenone (1)

Colorless gum; $[\alpha]_D^{25}$ + 43.6 (*c* 0.15, CHCl$_3$). IR (KBr) ν_{max}: 3454, 2944, 2867, 1702, 1640, 1457, 1381, 1218, 1041, 883, 758 cm^{-1}. ^{1}H and ^{13}C NMR (see Table 1 and 2). HRFAB-MS *m/z*: 441.3731 [M + H]$^+$ (calculated for C$_{30}$H$_{49}$O$_2$, 441.3733).

3β-Hydroxy-lup-20(29)-en-24-al (2)

Colorless gum; $[\alpha]_D^{25}$ + 9.0 (*c* 0.15, CHCl$_3$). IR (KBr) ν_{max}: 3452, 2944, 1710, 1456, 1380, 1219, 1078, 883, 771, 631 cm^{-1}. ^{1}H and ^{13}C NMR (see Tables 1 and 2). HRFAB-MS *m/z*: 463.3553 [M + Na]$^+$ (calculated for C$_{30}$H$_{48}$NaO$_2$, 463.3552).

28-Formyloxy-3β-hydroxy-urs-12-ene (3)

Colorless gum; $[\alpha]_D^{25}$ + 6.0 (*c* 0.25, CHCl$_3$). IR (KBr) ν_{max}: 3419, 2928, 2869, 1722, 1457, 1379, 1185, 1092, 1043, 997, 769, 663 cm^{-1}. ^{1}H and ^{13}C NMR (see Table 1 and 2). HRFAB-MS *m/z*: 470.3761 [M]$^+$ (calculated for C$_{31}$H$_{50}$O$_3$, 470.3760).

28-Formyloxy-3β-acetoxy-urs-12-ene (4)

Colorless gum; $[\alpha]_D^{25}$ + 13.0 (*c* 0.20, CHCl$_3$). IR (KBr) ν_{max}: 3451, 2929, 2869, 1731, 1459, 1372, 1249, 1028, 771, 610 cm^{-1}. ^{1}H and ^{13}C

NMR (see Table 1 and 2). HRFAB-MS m/z: 535.3762 [M + Na]$^+$ (calculated for $C_{33}H_{52}NaO_4$, 535.3763).

Synthesis of 1 by oxidation of lup-20(29)-en-3β,24-diol (11)[11,12]

Lup-20(29)-en-3β,24-diol (**11**, 10 mg) was dissolved in 10 mL of dichloromethane. After adding 10 mg of pyridinium chlorochromate, the solution was stirred for 2 h at room temperature.[11,12] The reaction mixture was purified through a Silica gel Waters Sep-pak Vac 12cc cartridge (*n*-hexane–EtOAc, 3.5:1) to obtain synthesized **1** (**1a**, 2.0 mg), which was identified by co-TLC, ^{1}H NMR, and HRFAB-MS data.

1a: Colorless gum; $[\alpha]_D^{25}$ + 15.0 (*c* 0.07, CHCl$_3$). IR (KBr) ν_{max}: 3425 (OH), 1705 (C = O), 1650 (C = C) cm^{-1}. ^{1}H NMR (CDCl$_3$, 500 MHz) δ: 0.78 (3H, s, H-24), 0.86 (3H, s, H-25), 0.95 (3H, s, H-27), 1.03 (3H, s, H-26), 1.25 (3H, s, H-23), 1.67 (3H, s, H-30), 2.36 (1H, m, H-19), 3.42 (1H, d, *J* = 11.0 Hz, H-24a), 3.96 (1H, d, *J* = 11.0 Hz, H-24b), 4.58 (1H, s, H-29a), 4.68 (1H, s, H-29b). ^{13}C NMR (CDCl$_3$, 125 MHz) δ: 14.6 (C-27), 15.8 (C-26), 17.2 (C-25), 18.2 (C-28), 19.5 (C-30), 19.6 (C-6), 22.0 (C-11), 22.3 (C-23), 25.4 (C-12), 27.6 (C-15), 30.1 (C-21), 33.8 (C-7), 34.6 (C-2), 35.7 (C-16), 36.9 (C-10), 38.4 (C-13), 39.5 (C-1), 40.2 (C-22), 40.9 (C-8), 43.1 (C-14), 43.2 (C-17), 48.2 (C-19), 48.5 (C-18), 49.8 (C-9), 51.0 (C-4), 55.5 (C-5), 66.0 (C-24), 109.6 (C-29), 151.1 (C-20), 221.7 (C-3). FAB-MS m/z: 441 [M + H]$^+$.

Alkaline methanolysis of compounds 3 and 4[15]

Compounds **3** and **4** (each 1.5 mg) were hydrolyzed with 0.5 mol/L KOH in MeOH (1 mL) at room temperature for 1 h. And then, H$_2$O (3 mL) was added and the mixture was extracted three times with CHCl$_3$, and the CHCl$_3$ extract was evaporated in vacuo. The CHCl$_3$ extract was purified through a Silica gel Waters Sep-pak Vac 12cc cartridge (*n*-hexane–EtOAc, 5:1) to give **3a** (0.8 mg), and **4a** (0.7 mg) (same as **23**), which were identified by ^{1}H NMR, MS, and optical rotation value.

3a (= **4a**): Colorless gum; $[\alpha]_D^{25}$ + 18.0 (*c* 0.04, CHCl$_3$). IR (KBr) ν_{max}: 3350 (OH), 2910 (C-H) cm^{-1}. ^{1}H NMR (CDCl$_3$, 500 MHz) δ: 0.79 (3H, s, H-24), 0.81 (3H, d, *J* = 6.5 Hz, H-29), 0.92 (3H, d, *J* = 6.5 Hz, H-30), 0.95 (3H, s, H-25), 0.99 (3H, s, H-23), 1.00 (3H, s, H-26), 1.10 (3H, s, H-27), 3.20 (1H, d, *J* = 10.5 Hz, H-28a), 3.23 (1H, dd, *J* = 11.5, 5.0 Hz, H-3), 3.55 (1H, d, *J* = 10.5 Hz, H-28b), 5.14 (1H, br s, H-12). ^{13}C NMR (CDCl$_3$, 125 MHz) δ: 15.6 (C-25), 15.7 (C-24), 16.7 (C-26), 17.3 (C-29), 18.3 (C-6), 21.3 (C-30), 23.2 (C-27), 23.3 (C-11), 23.4 (C-16), 26.0 (C-2), 27.2 (C-15), 28.1 (C-23), 30.6 (C-21), 32.8 (C-7), 35.2 (C-22), 36.9 (C-10), 38.0 (C-1), 38.7 (C-4), 39.3 (C-19), 39.4 (C-20), 40.0 (C-8), 42.0 (C-14), 47.6 (C-9), 47.7 (C-17), 54.0 (C-18), 55.2 (C-5), 69.9 (C-28), 79.0 (C-3), 125.0 (C-12), 138.7 (C-13). HRFAB-MS m/z: 443 [M + H]$^+$.

Supplementary data

Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjc-2012-0411.

Acknowledgements

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