A partial synthesis of 23-hydroxybetulonic acid and 23-hydroxybetulinic acid starting from betulinic acid

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A partial syntheses of the biologically active 23-hydroxybetulonic acid and 23-hydroxybetulinic acid starting from the naturally abundant betulinic acid has been developed in which the key step was the Baldwin's cyclopalladation of the 23-methyl group from a 3-one oxime of betulinic acid, an important drug in Chinese medicine.

Keywords: betulinic acid, 23-hydroxybetulonic acid, 23-hydroxybetulinic acid, cyclopalladation, Baldwin's method

Betulinic acid (**1**), 23-hydroxybetulonic acid (Pulsatillic acid, **2**) and 23-hydroxybetulinic acid (**3**) (Fig. 1) are all naturally occurring pentacyclic triterpenes of the lupane family. Compound **1** is easily obtained from the bark of various trees (up to 2.5% of dry weight),[1] while **2** and **3** were both isolated from the root of *Pulsatilla chinensis* Regel.[2,3] Betulinic acid is one of the most important crude drugs in traditional Chinese medicine and is used for the treatment of amoebic dysentery and malaria.[4] Some evidence shown that **1** possesses a variety of biological activities such as anti-tumour, antibacterial, anti-malarial, anti-inflammatory, anthelmintic antioxidant and anti-HIVactivities,[5–7] whilst **2** and **3** have been proven to have anti-tumour and anti-HIV activities.[8–10] Thus, **2** and **3** showed strong cytotoxic activity on murine melanoma B16 cells with IC_{50} of 22.5 and 32 μg mL^{-1}, respectively.

Compound **3** could also inhibited HMEC proliferation significantly with IC_{50} of 40.44 μg mL^{-1} and arrested growth and induced apoptotic cell death in human leukemia HL-60 cells. This was associated with concurrent down-regulation of Bcl-2 and the telomerase activity.

Recently, we have carried out the structure modification of **3** for new drug screening and excellent results have been obtained.[11–15] However, further pharmacological investigation was hampered by the limited amount of **3** which was available from plant extraction.[16,17] Though **2** and **3** have been identified for many years, no practical semisynthetic approach to these substances has been established to date. We envisioned that **2** and **3** could be achieved through a simple introduction of hydroxyl group on the 23-methyl group of naturally abundant betulinic acid **1**. We noticed a report by Baldwin that an unactivated methyl groups could be functionalised by a cyclopalladation reaction (Baldwin's method).[18,19] This method has been extensively used in the synthesis of natural triterpene compounds.[20,21] These results led us to propose the key step by cyclopalladation of the 23-methyl group from a 3-one oxime functionality of **1** to obtain **2** and **3**. We now report a semisynthetic approach to **2** and **3** starting from **1** by using this method.

Results and discussion

The synthesis of **2** and **3** from betulinic acid **1** is shown in Scheme 1. Treatment of **1** with BnBr in DMF in the presence of K_2CO_3 afforded benzyl betulinate **4**, which was then converted to ketone **5** by oxidation with PCC in CH_2Cl_2. The ketone **5** was converted to the oximated with hydroxylamine hydrochloride in pyridine to give benzyl betulinate 3-oxime **6**. Balwin's method was then applied to introduce a hydroxyl group on the 23-methyl group of **6**. Oxime **6** was first palladated with Na_2PdCl_4 to afford a dimeric organopalladium complex.

Subsequently, acetylation with Ac_2O, oxidation with $Pb(OAc)_4$, and reduction with $NaBH_4$ smoothly gave diacetate **7** in up to 81% yield (for three steps). Finally, hydrolysis of **7** with NaOH in THF and CH_3OH yielded oxime **8**, which was hydrolysed with $TiCl_3$ to give ketone **9**. Selective hydrogenolysis of the benzyl group of **9** with Pd/C in THF produced 23-hydroxybetulonic acid **2** in 75% yield. This step must be carefully monitored by TLC to ensure that the C–C double bond of **9** was not hydrogenated. Reduction of **2** with $NaBH_4$ in CH_3OH and THF smoothly provided 23-hydroxybetulinic acid **3** in good yield. The analytical data of synthetic **2** and **3** are in good agreement with those reported for the natural samples (Tables 1 and 2).

Conclusions

We have developed a semisynthetic approach to 23-hydroxybetulonic acid (**2**) and 23-hydroxybetulinic acid (**3**) starting from naturally abundant betulinic acid (**1**) in an overall yield of 34% and 29%, respectively. The present contribution should facilitate the structural modification of the natural products **2** and **3** for further pharmacological research, which is proceeding in our laboratory.

Experimental

Melting points were taken on XT-4 micro melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker ACF 300 spectrometer in the indicated solvents (TMS as internal standard). Mass spectra were obtained using Agilent 1100-LC-MSD-Trap/SL and HRMS were obtained using Agilent QTOF 6520. Column chromatography was carried out using silica gel (200–300 mesh).

Benzyl 3β-hydroxy-lup-20(29)-en-28-oate (**4**): BnBr (0.30 mL, 2.5 mmol) was added to a solution of **1** (1.00 g, 2.2 mmol) and K_2CO_3 (0.5 g, 3.6 mmol) in DMF (20 mL). The reaction mixture was stirred for 12 h at room temperature, poured into water (30 mL) and extracted with ethyl acetate (3×20 mL). The combined extract was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 6:1) to give **4** as a white solid (1.10 g, 92%). m.p. 190–193 °C; ESI-MS *m/z*: 547.4 $[M + H]^+$, 564.4 $[M + NH_4]^+$; HR-MS (ESI, M+H) *m/z*: Calcd for $C_{37}H_{55}O_3$: 547.4151, found 547.4154.

Benzyl 3-oxo-lup-20(29)-en-28-oate (**5**): PCC (0.50 g, 2.33 mmol) was added to a solution of **4** (1.05 g, 1.92 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was stirred for 5 h at room temperature. The reaction mixture was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 40:1) to give **5** as a white solid (0.96 g, 92%). m.p. 127–129 °C; ESI-MS *m/z*: 545.4 $[M + H]^+$, 562.5 $[M + NH_4]^+$; HR-MS (ESI, M+H) *m/z*: Calcd for $C_{37}H_{53}O_3$: 545.3995, found 545.3993.

Benzyl 3-hydroxyimino-lup-20(29)-en-28-oate (**6**): Hydroxylamine hydrochloride (0.2 g, 2.9 mmol) was added to a solution of **5** (0.95 g, 1.7 mmol) in pyridine (20 mL. The reaction mixture was stirred at 80 °C for 4 h, cooled to r.t., ethyl acetate (40 mL) was added. The mixture was washed with 10% HCl, water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (petroleum ether/acetone, 16:1) to give **6** as a white solid (0.91 g, 93%). m.p. 232–235 °C; ^{1}H NMR

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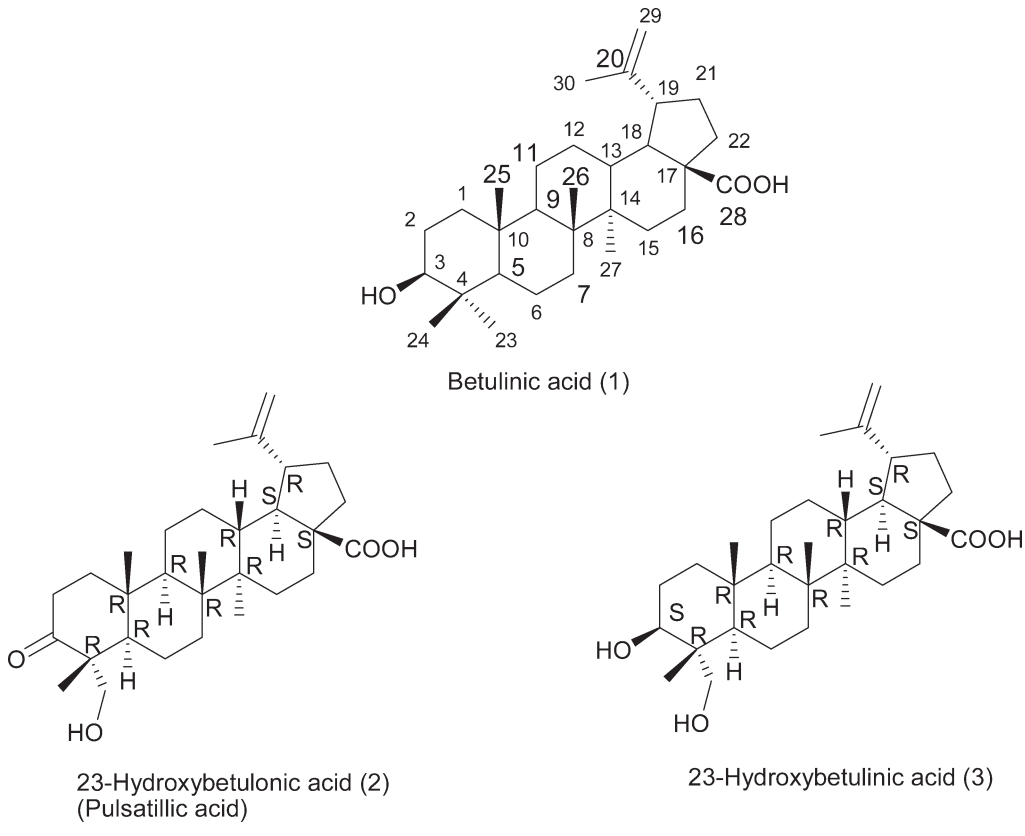


Fig. 1 Structures of pentacyclic triterpenes **1, 2** and **3**.

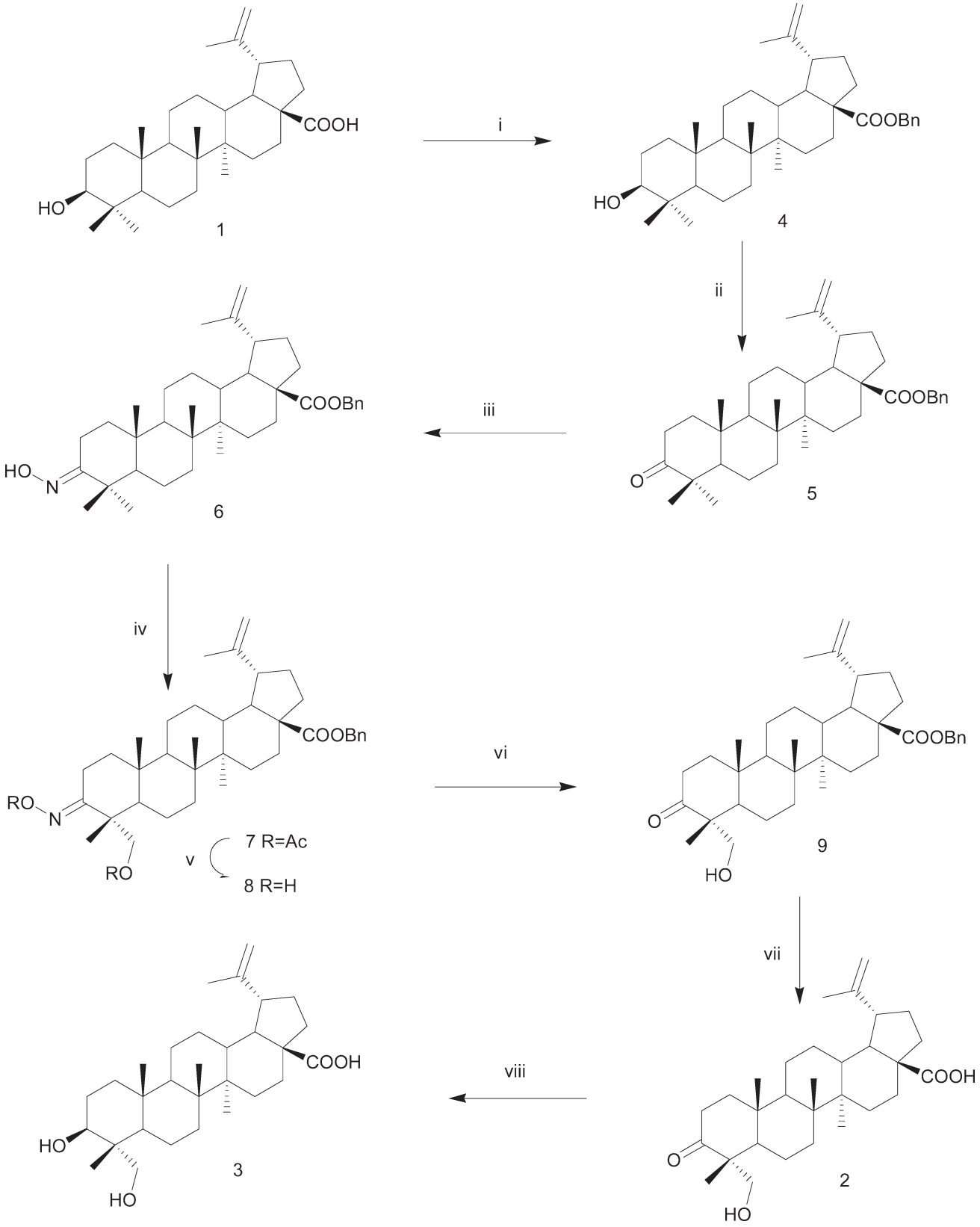
Table 1 ^{13}C NMR spectral data of synthetic **2** and **3** compared with literature (pyridine-d_5, δ)

	2		**3**	
	Ref. 2	Synth.	Ref. 3	Synth.
C1	38.4	38.53	39.2	38.99
C2	36.2	36.60	27.9	27.76
C3	217.2	217.09	73.5	73.31
C4	47.0	46.96	42.9	42.72
C5	49.6	49.76	48.8	48.67
C6	19.9	20.03	18.6	18.44
C7	33.5	33.60	34.5	34.39
C8	40.8	40.94	41.4	40.98
C9	52.4	52.67	51.0	50.86
C10	36.6	36.77	37.4	37.22
C11	21.7	21.82	21.3	21.10
C12	26.0	26.16	26.1	25.98
C13	38.6	38.72	38.6	38.48
C14	42.7	42.88	42.9	42.85
C15	31.1	31.21	31.2	31.06
C16	32.6	32.80	32.9	32.71
C17	56.4	56.62	56.6	56.48
C18	47.5	47.73	47.7	47.63
C19	49.6	49.68	49.7	49.62
C20	151.1	151.28	151.2	151.17
C21	30.0	30.22	30.3	30.14
C22	37.3	37.56	37.6	37.42
C23	68.0	68.2	68.0	67.8
C24	17.2	17.52	12.9	12.77
C25	16.0	16.29	16.8	16.66
C26	15.9	16.08	16.5	16.31
C27	14.6	14.75	14.9	14.75
C28	178.6	178.83	178.9	178.7
C29	109.6	109.97	109.9	109.79
C30	19.3	19.48	19.5	19.31

(CDC_3, 300 MHz): δ 0.79, 0.89, 0.93, 1.03, 1.12, 1.67 (each 3H, s, 23, 24, 25, 26, 27 and 30-CH_3), 2.15–2.29 (3H, m), 2.91–3.02 (2H, m, H-19 and H-2b), 4.60, 4.72 (each 1H, s, H-29), 5.12,5.18 (each 1H, d, J = 12.2 Hz, CH_2-Ar), 7.31–7.52 (5H, m, H-Ar); ESI-MS *m/z*: 560.5 [M + H]$^+$; HR-MS (ESI, M+H) *m/z*: Calcd for $C_{37}H_{54}NO_3$: 560.4104, found 560.4109.

Benzyl 3-acetoxyimino-23-acetoxy-lup-20(29)-en-28-oate (**7**): NaOAc (0.17 g, 2.0 mmol) and Na_2PdCl_4 (0.61 g, 2.1 mmol) were added to a solution of **6** (0.9 g, 1.61 mmol) in AcOH (100 mL),. The solution was stirred for 72 h at room temperature, and then ice water (120 mL) was added to give a yellow precipitate. The precipitate was filtered and dried *in vacuo* at 60 °C for 24 h to give a palladium complex. DMAP (10 mg), Et_3N (0.3 mL), and Ac_2O (0.2 mL) were added to a solution of this palladium complex in anhydrous CH_2Cl_2 (30 mL). The reaction mixture was stirred at r.t. for 1 h, washed with water, dried over Na_2SO_4, filtered, and evaporated *in vacuo*. The crude product was dissolved in anhydrous THF (80 mL), and pyridine (0.2 mL) was added. The reaction mixture was stirred for 15 min at room temperature and cooled at –80 °C, and a solution of $Pb(OAc)_4$ (1.2 g, 2.7 mmol) in HOAc (40 mL) was added and again stirred at room temperature for 20 h. To remove the remaining Pd salts, a solution of $NaBH_4$ (0.1 g) in 1 N NaOH solution (10 mL) was added to the reaction mixture. The mixture was stirred for 15 min and filtered. The filtrate was diluted with ethyl acetate (70 mL), and washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, filtered, and evaporated *in vacuo* to give a solid. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 10:1) to give **7** as a white solid (0.86 g, 81%). m.p. 64–67 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.78, 0.86, 0.94, 1.15, 1.68 (each 3H, s, 24, 25, 26, 27 and 30-CH_3), 2.05, 2.16 (each 3H, s, Ac×2), 2.22–2.30 (2H, m), 2.58–2.66 (2H, m), 3.01 (1H, m, H-19), 4.13 (2H, dd, J = 27.5, 11.1 Hz, H-23), 4.60, 4.72 (each 1H, s, H-29), 5.12, 5.17 (each 1H, d, J = 12.3 Hz, CH_2-Ar), 7.31–7.37 (5H, m, H-Ar); ESI-MS *m/z*: 660.4 [M + H]$^+$, 680.5 [M + NH_4]$^+$; HR-MS (ESI, M+H) *m/z*: Calcd for $C_{41}H_{58}NO_6$: 660.4264; found 660.4268.

Benzyl 3-hydroxyimino-23-hydroxy-lup-20(29)-en-28-oate (**8**): 4 N NaOH solution (3 mL) was added to a solution of **7** (0.85 g, 1.3 mmol) in CH_3OH (15 mL) and THF (9 mL). The reaction mixture was stirred at room temperature for 1 h, diluted with ethyl acetate (30 mL), washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography (petroleum ether/ethyl acetate, 16:1) to give **8** as a white solid (0.71 g, 95%). m.p. 224–226 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.79, 0.93, 0.94, 1.01, 1.67 (each 3H, s, 24, 25, 26, 27 and 30-CH_3),



Scheme 1 Reagents and conditions: (i) BnBr, K_2CO_3, DMF, r.t., 12 h (92%); (ii) PCC, CH_2Cl_2, r.t., 2 h, (92%); (iii) $HONH_2.HCl$, pyridine, 80 °C, 4h, (93%); (iv) (a) Na_2PdCl_4, NaOAc, HOAc, r.t., 72 h; (b) Ac_2O, DMAP, Et_3N, CH_2Cl_2, r.t., 1h; (c) $Pb(OAc)_4$, pyridine, HOAc, THF, –78 °C to r.t., 20 h (81% for 3 steps); (v) NaOH, THF, CH_3OH, r.t., 1 h (95%); (vi) $TiCl_3$, HCl, NH_4OAc, H_2O, THF, r.t., 6 h, (75%); (vii) H_2, Pd/C, THF, r.t., (75%); (viii) $NaBH_4$, THF, CH_3OH, r.t., 2 h (87%).

Table 2 ^{1}H NMR spectral data of synthetic **2** and **3** compared to the literature

	2 Ref. 2 $CDCl_3$	**2** (synth.) $CDCl_3$	**2** (synth.) pyridine-d_5	**3** Ref. 3 $CDCl_3$	**3** (synth.) $CDCl_3$	**3** (synth.) pyridine-d_5
H-24	0.99	0.99	0.81	0.86	0.86	0.88
H-25	0.99	1.00	0.96	0.86	0.86	1.00
H-26	1.01	1.00	1.01	0.94	0.94	1.02
H-27	1.04	1.04	1.04	0.98	0.97	1.06
H-30	1.69	1.70	1.77	1.70	1.69	1.76
H-19	3.04	3.01	3.51	3.02	3.00	3.52
H-23	3.41, 3.64	3.41, 3.64	3.66, 3.95	3.44, 3.74	3.42, 3.72	3.69, 4.16
H-3	—	—	—	3.64	3.62	4.19
H-29	4.62, 4.74	4.62, 4.75	4.75, 4.92	4.63, 4.74	4.61, 4.74	4.74, 4.91

Table 3 Melting point of synthetic **2** and **3** compared with literature

Compound	**2**		**3**	
	Ref. 2	Synth.	Ref. 3	Synth.
M.p.	214–217 °C	219–221 °C	300–302 °C	303–305 °C

2.06–2.30 (3H, m), 3.01–3.10 (2H, m, H-19 and H-2b), 3.48, 3.63 (each 1H, d, J = 11.5 Hz, H-23), 4.59, 4.72 (each 1H, s, H-29), 5.12, 5.17 (each 1H, d, J = 12.3 Hz, CH_2-Ar), 7.31–7.36 (5H, m, H-Ar); ESI-MS m/z: 576.4 $[M + H]^+$; HR-MS (ESI, M+H) m/z: Calcd for $C_{37}H_{54}NO_4$:576.4053, found 576.4049.

Benzyl 3-oxo-23-hydroxy-lup-20(29)-en-28-oate (**9**): A solution of **8** (0.7 g, 1.2 mmol) in THF (25 mL) was added to a buffered solution of $TiCl_3$ (5 mL, 15%-20% $TiCl_3$), concentrated hydrochloric acid (2.7 mL, 37% HCl) and NH_4OAc (2.3 g) in water (9 mL) was added. The mixture was stirred at room temperature for 6 h and extracted with ethyl acetate. The extract was washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The residue was purified by column chromatography (petroleum ether/ethyl acetate, 6:1) to give **9** as a needle (0.51 g, 75%). M.p. 187–189 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.81, 0.95, 0.99, 1.02, 1.67 (each 3H, s, 24, 25, 26, 27 and 30-CH_3), 2.17–2.33 (4H, m), 2.55–2.67 (1H, m), 3.02 (1H, m, H-19), 3.40, 3.63 (each 1H, m, H-23), 4.59, 4.72 (each 1H, s, H-29), 5.12, 5.17 (each 1H, d, J = 12.3 Hz, CH_2-Ar), 7.31–7.37 (5H, m, H-Ar); ESI-MS m/z: 561.4 $[M + H]^+$; HR-MS (ESI, M+H) m/z: calcd for $C_{37}H_{53}O_4$:561.3944, found 561.3949.

3-Oxo-23-hydroxy-lup-20(29)-en-28-oic acid (**2**): A solution of **9** (0.3 g, 0.54mmol), Pd/C (0.04 g, 10% Pd) in THF (10mL) was stirred at room temperature under H_2 at atmospheric pressure. The reaction was monitored by TLC to ensure that **9** mostly reacted but not completely. Then the mixture was filtered and evaporated *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2/CH_3OH, 150:1) to give **2** as a white solid (0.19 g, 75%). m.p. 219–221 °C; $[\alpha]_D^{22}$ +57.7 (c, 0.84, pyridine); ESI-MS: m/z = 469 $[M - H]^-$.

3β,23-Dihydroxy-lup-20(29)-en-28-oic acid (**3**): $NaBH_4$ (0.02 g, 0.55 mmol) was added to a solution of **2** (0.10 g, 0.21 mmol) in CH_3OH (8 mL). The mixture was stirred at room temperature for 2 h and diluted with ethyl acetate (20 mL). The mixture was washed with water, dried over Na_2SO_4, filtered, and evaporated *in vacuo*. The crude product was purified by column chromatography (CH_2Cl_2/CH_3OH, 80:1) to give a white solid (0.087 g, 87%), which was recrystallised (petroleum ether/ethyl acetate/CH_3OH) to give **3** as a white solid (0.043 g). m.p. 303–305 °C; $[\alpha]_D^{22}$ +18.9 (c, 0.27, pyridine); ESI-MS: m/z = 507 $[M + Cl]^-$, 471 $[M - H]^-$.

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References

1 S.K. Maurya, S. Devi and V.B. Pandey, *Fitothererapy*, 1989, **60**, 468.
2 W.C.Ye, N.N. Ji, S.X. Zhao, J.H. Liu, T.Ye, M.A. Mckervey and E. Stevenson, *Phytochemistrr*, 1996, **42**, 799.
3 W.K. Chen, B.Y. Wang, D.Y. Lu, Q. Lin and L.Y. Lin, *Acta. Chim. Sinica*, 1983, **41**, 739.
4 Jiangsu New Medical College. *The dictionary of traditional Chinese medicine*. Shanghai Scientific & Technical Publishers, 1977. p. 704.
5 E. Pisha, H. Chai, I.S. Lee, T.E. Chagwedera, N.R. Farnsworth, G.A., Cordell, C.W.W. Beecher, H.H.S. Fong, A.D. Kinghorn, D.M. Brown, M.C. Wani, M.E. Wall, T.J. Hieken, T.K. Dasgupta, and J.M. Pezzuto, *Nat. Med*., 1995, **1**, 1046.
6 Y. Perumal and S. Dharmarajan, *Curr. Med. Chem.*, 2005, **12**, 657.
7 M. Rama, K. Vivek, K.S. Sanjay, K.A. Shiv and C.B. An, *Anti-Cancer Agents Med. Chem.*, 2006, **6**, 271.
8 Z.N. Ji, W.C.Ye, G.G. Liu and W.L. Wendy Hsiao, *Life Sci*., 2002, **72**, 1.
9 Wing-Keung, Joyce C.K. Ho, Florence W.K. Cheung, Bonnie P.L. Liu, W.C. Ye and C.T. Che, *Eur. J. Pharmacol.*, 2004, **498**, 71.
10 M.J. Jiang, M. Yang, Y.Y. Zhou, R.J. Zhang, G.X. Cao and G.M. Cai, *Zhongliu Fangzhi Yanjiu*., 2007, **34**, 911.
11 Y. Bi, J.Y. Xu, X.M. Wu, W.C. Ye, S.T. Yuan and L.Y. Zhang, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 1475.
12 Y. Bi, J.Y. Xu, X.M. Wu, W.C. Ye, S.T. Yuan and L.Y. Zhang, *J. Chin. Pharm. Univ.*, 2007, **38**, 108.
13 P. Lan, J. Wang, D.M. Zhang, C. Shu, H.H. Cao, P.H. Sun, X.M. Wu, W.C. Ye and W.M. Chen, *Euro. J. Med. Chem.*, 2011, **46**, 2490
14 J.P. Zhou, D. Li, X.M. Wu, W.C. Ye and L.Y. Zhang, *Chin. Chem. Lett*., 2007, **18**, 1195.
15 P.Q. Zhu, Y. Bi, J.Y. Xu, Z. Li, J. Liu, L.Y. Zhang, W.C. Ye and X.M. Wu, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 6966.
16 K.M. Zhang, J.Q. Jiang and L. Kong, *Chin. J. Nat. Med.*, 2008, **6**, 345.
17 J.F. Hu, Z.L. Ye and F.J. Shen, *Acta Pharmaceut. Sinica*, 1995, **30**, 27.
18 J.E. Baldwin, R.H. Jones, C. Najera and M. Yus, *Tetrahedron.*, 1985, **41**, 699.
19 K. Carr, H.M. Saxton and J.K. Sutherland, *J. Chem. Soc., Perkin Trans. 1*, 1988, 1599.
20 L. Bore, T. Honda and G.W. Gribble, *J. Org. Chem.*, 2000, **65**, 6278.
21 A. Garcia-Granados, P.E. Lopez, E. Melguizo, A. Parra and Y.J. Simeo, *Org. Chem*., 2007, **72**, 3500.