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A CONCISE SEMI-SYNTHETIC APPROACH TO BETULINIC ACID FROM BETULIN

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Abstract: Betulin was convert to betulinic acid using two different synthetic routes. The first approach involved an oxidation of betulin using Jones' reagent to betulonic acid and subsequent $NaBH_4$ reduction to betulinic acid. The second approach involved steps utilizing different protecting groups on the alcohol functional groups of betulin and Jones' oxidation to circumvent the isomerization of the secondary alcohol of betulinic acid.

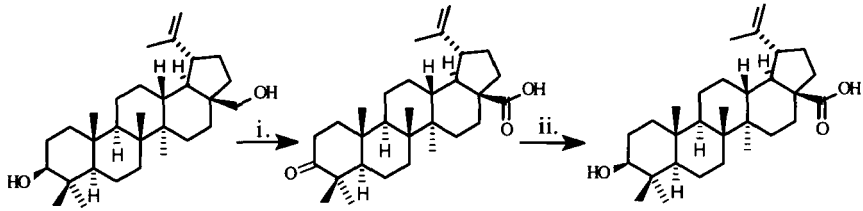
Betulinic acid, 3β-hydroxy-lup-20(29)-ene-28-oic acid (**3**), is a natural product isolated from several genus of higher plants.[1-7] Recently, betulinic acid (**3**) has been demonstrated to possess a remarkably selective antitumor activity against human melanoma (Mel-1, Mel-2 and Mel-4)[1] and an anti-HIV activity in H9 lymphocytic cells.[2] For an extensive biological efficacy study, a large amount of betulinic acid (**3**) was needed.

The bark of white birch, *Betula alba*, contains betulin (25%), lup-20(29)-ene-3β,28-diol (**1**), and betulinic acid (**3**, 0.025%) that is tedious to isolate in quantity to perform an extensive bioassay. It was envisioned that a quantity of betulinic acid (**3**) could be provided from betulin (**1**) through a simple synthetic approach. A number of multi-step synthetic conversion of betulin (**1**) to betulinic acid (**3**), that suffer from a low overall

yield, has been reported.[3,4] Herein we report a concise two step conversion of betulin (**1**) to betulinic acid (**3**), along with a synthetic study.

Crude betulin, $CHCl_3$ extract from the bark of *Betula alba*, was recrystallized thrice from MeOH/$CHCl_3$. Jones' oxidation (CrO_3/H_2SO_4/acetone) of betulin (**1**) gave betulonic acid (**2**)[5-7] in 75% yield after column chromatography over silica gel using pet ether/EtOAc. Betulonic acid (**2**)[5-7] was reduced ($NaBH_4$/THF) to afford α- and β-isomeric mixture of betulinic acid in quantitative yield. The ^{1}HNMR analysis showed that the ratio of the α- and β-isomers was 5:95, respectively, and the β-isomer's ^{1}HNMR was identical with that of the natural betulinic acid (**3**). Recrystallization of α– and β–isomeric mixture of betulinic acid in MeOH afforded the β-isomer betulinic acid (**3**) as colorless needle in 75% yield. The impure betulinic acid (unrecrystallizable α- and β-isomeric mixture of betulinic acid) was reoxidized (Jones' oxidation),[5] reduced ($NaBH_4$), and recrystallized again in MeOH to afford the β-isomer betulinic acid (**3**) in 71% overall yield. The unrecrystallizable mixture of betulinic acid was collected for further process.

Scheme 1

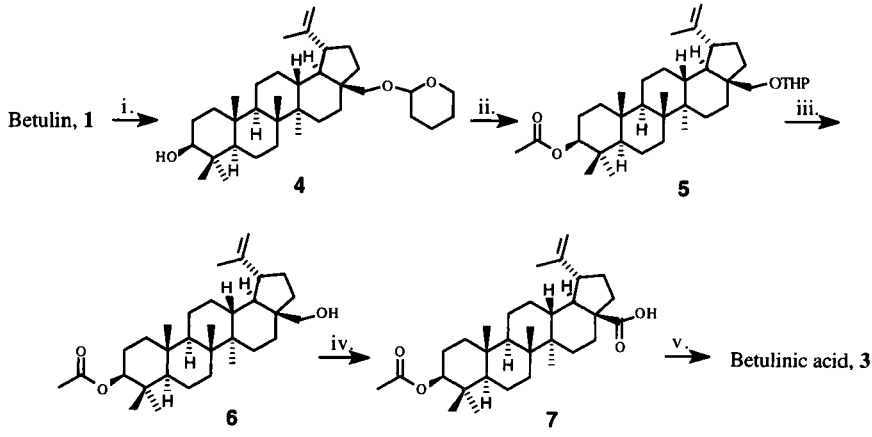


Betulin, **1** Betulonic acid, **2** Betulinic acid, **3**

i. Jones' oxidation (CrO_3/H_2SO_4/acetone/0°C) ii. $NaBH_4$/THF

To circumvent the formation of α-isomer betulinic acid, a synthetic approach which does not involve betulonic acid (**2**) formation, thus, eliminating the reduction step was investigated. The primary alcohol of betulin (**1**) was monoprotected as a THP ether **4** (DHP/CH_2Cl_2/PPTS, 95%) and the secondary alcohol was acetylated (Ac_2O/pyridine, 87%) successively. The THP ether **5** was selectively removed (MeOH/PPTS, 95%) and the primary alcohol acetate **6** was subjected to Jones' oxidation condition (CrO_3/H_2SO_4/acetone) to afford the carboxylic acid acetate **7** (80%). The acetyl group was

Scheme 2



Reagents: i) DHP/CH_2Cl_2/PPTS ii) Ac_2O/pyridine iii) EtOH/PPTS iv) CrO_3/H_2SO_4/acetone v) K_2CO_3/MeOH/H_2O

removed (K_2CO_3/MeOH/H_2O)[8] to give the β-isomer betulinic acid (**3**) in 88% yield. This product was identical to the previously synthesized β-isomer betulinic acid (**3**).

In summary, for a large scale conversion of betulin (**1**) to betulinic acid (**3**), the two step conversion involving Jones' oxidation, $NaBH_4$ reduction, and recrystallization in methanol appears to be the most efficient method. A number of 30 g scale two step conversion has been carried out successfully.

Experimental

General Procedure: Melting points were determined on a Fisher-Johns apparatus and were uncorrected. CIMS was performed on a Finnigan MAT 90 instrument. ^1H NMR and ^{13}C NMR were performed on a Varian XL-300 instrument using standard Varian program.

Preparation of Betulonic acid (2).[5-7] To a solution of betulin (**1**, 1.0 g, 2.26 mmol) in acetone (50 mL) was added freshly prepared Jones' reagent dropwise at 0 °C. The solution was stirred for 1.5 h at 0 °C, quenched with methanol (25 mL), stirred for 5 min, and H_2O (40 mL) was added. The organic solvent was removed under vacuum and the aqueous residue was extracted with EtOAc (2 x 40 mL). The organic layer was separated and washed with water (20 mL), then brine (15 mL). The organic layer was dried ($MgSO_4$), filtered, and solvent was removed under vacuum. The residue was column

chromatographed over silica gel (60 - 200 mesh) using pet ether/EtOAc (4:1) to afford betulonic acid (**3**, 770 mg, 75%). mp 247-249 °C [lit.[5-7] 246-247 °C].

Preparation of Betulinic Acid (3). To a THF (20 mL) containing betulonic acid (**2**, 500 mg, 1.10 mmol) was added $NaBH_4$ (440 mg, 10.0 equiv.) at 0 °C and stirred at rt for 10 h. The reaction was quenched with 2 N HCl (3 mL) solution and THF was removed under vacuum down to 50% volume. The solution was diluted with EtOAc (80 mL) and was washed with H_2O (3 x 5 mL) and brine (5 mL). The organic layer was dried ($MgSO_4$), filtered, and the solvent was removed under vacuum. The white residue (quantitative yield) was dissolved in hot methanol (50 mL) and cooled to rt to induce recrystallization of betulinic acid (**1**, 375 mg, 75%); mp 291-292 °C [lit.[2] 290-293 °C].

Preparation of mono-THP betulin ether 4. To a CH_2Cl_2 solution (15 mL) containing betulin (**2**, 450 mg, 1.016 mmol) was added dihydropyran (DHP, 94 mg, 1.12 mmol) and pyridinium *p*-toluene sulfonic acid salt (PPTS, 30 mg, 0.12 mmol) at rt under N_2 and stirred for three days. After the completion of reaction, 5 mL of sat. $NaHCO_3$ was added. The organic layer was separated and washed with sat NaCl solution (5 mL), dried ($MgSO_4$), filtered, and the solvent was removed under vacuum. The residue was chromatographed over silica gel using pet ether/EtOAc (4:1) to afford mono-THP ether **4** as a diastereomeric mixture (508 mg, 95%). The diastereomeric mixture **4** was submitted to the subsequent reaction without separation. MS (CI) *m/e* (rel intensity) 441 (-THP, 10), 425 (-OTHP, 100), 407 (-OTHP -H_2O, 12).

Preparation of mono-THP ether betulin acetate 5. To a 8 mL pyridine solution containing the diastereomeric mixture of THP ether **4** (280 mg, 0.53 mmol) was added acetic anhydride (110 mg, excess) and stirred for 36 h at rt. The pyridine was removed under vacuum and the residue was diluted with EtOAc (40 mL) and washed with H_2O (2 x 5 mL) and sat. NaCl (5 mL). The organic layer was dried ($MgSO_4$), filtered, and the solvent was removed under vacuum. The residue was chromatographed over silica gel using pet ether/EtOAc (4:1) to give a diasteromeric mixture of mono-THP ether betulin acetate **5** in 87% yield (263 mg). The diastereomeric mixture **5** was submitted to the subsequent reaction without separation. MS (CI) *m/e* (rel intensity) 569 ($M + H^+$, 8), 509 (-CH_3COOH, 26), 485 (-THP, 12), 468 (-OTHP, 10), 425 (-CH_3COOH -THP, 100) 409 (-CH_3COOH -OTHP, 8), 407 (17).

Preparation of betulin acetate alcohol 6. To a 5 mL methanol solution containing the diastereomeric mixture of mono-THP ether betulin acetate **5** (260 mg, 0.457 mmol) was added PPTS (10 mg, 0.04 mmol) and was stirred for 36 h at rt. The reaction solution was quenched with sat. $NaHCO_3$ (5 mL), and extracted with EtOAc (50 mL). The organic layer was separated and washed with H_2O (2 x 100 mL), dried ($MgSO_4$), filtered, and the solvent was removed under vacuum. The residue was chromatographed over silica gel

using pet ether/EtOAc (4:1) to afford the betulin acetate alcohol **6** in 95% yield (210 mg). mp 258-259 °C [lit.[3,4] 259-260]; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 171.04, 150.45, 109.72, 80.91, 60.52, 55.34, 50.27, 48.770, 47.79, 47.76, 42.68, 40.90, 38.35, 37.77, 37.26, 37.05, 34.13, 33.95, 29.70, 29.12, 27.92, 27.00, 25.13, 23.67, 21.34, 20.81, 19.04, 18.16, 16.48, 16.16, 15.95, 14.70; MS (CI) *m/e* (rel intensity) 485 ($M + H^+$, 4), 467 (-H_2O, 27), 425 (-CH_3COOH, 100), 407 (22).

Preparation of betulinic acid acetate 7. To an acetone (10 mL) solution containing betulin acetate alcohol **6** (170 mg, 0.35 mmol) was added freshly prepared Jones' reagent (1.0 mL) dropwise at 0 °C while stirring. The solution was stirred at 0 °C for 1.5 h, and was quenched with methanol (5 mL). After stirring for 5 min, 7 mL H_2O was added. The organic solvent was removed under vacuum and the aqueous residue was extracted with EtOAc (2 x 10 mL). The organic layer was separated and washed with H_2O (2 X 5 mL), and sat NaCl (5 mL). The organic layer was dried ($MgSO_4$), filtered, and the solvent was removed under vacuum. The residue was chromatographed over silica gel using pet ether/EtOAc (4:1) to afford the betulinic acid acetate **7** in 80% yield (140 mg). mp 288-290 °C [lit.[4] 289-291 °C]; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 182.12, 171.08, 150.36, 109.74, 80.93, 56.38, 55.38, 50.36, 49.22, 46.93, 42.39, 40.66, 38.39, 38.36, 37.78, 37.09, 37.04, 34.20, 32.13, 30.54, 29.67, 27.93, 25.41, 23.68, 21.33, 20.82, 19.33, 18.14, 16.46, 16.17, 16.02, 14.64; MS (CI) *m/e* (rel intensity) 499 ($M + H^+$, 43), 453 (-CO_2H_2, 21), 439 (-CH_3COOH, 100).

Removal of the acetyl group.[8] Betulinic acid acetate **7** (80 mg, 0.16 mmol) was stirred in aqueous methanol solution containing K_2CO_3 (excess) for 24 h to give the betulinic acid (**3**) in 88% yield after column chromatography (pet ether/EtOAc, 4:1). mp 291-292 °C [lit.[2] 290-293 °C].

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