Electronic Supplementary Material (ESI) for Green Chemistry.

Toward a benign strategy for the manufacturing of betulinic acid

Anna K. Ressmann,[a] Thomas Kremsmayr,[a] Peter Gaertner,[a] Ronald Zirbs,[b] and Katharina Bica[a,*]

[a]Institute of Applied Synthetic Chemistry, Vienna University of Technology, Getreidemarkt 9/163, 1060 Vienna, Austria.

[b]Group for Biologically Inspired Materials, Institute of Nanobiotechnology (DNBT), University of Natural Resources and Life Sciences, Muthgasse 11, 1190 Vienna, Austria.

[*]Corresponding author Katharina Schröder (née Katharina Bica). Email: katharina.schroeder@tuwien.ac.at, Fax: +43 1 58801 16360; Tel: +43 1 58801 163601.

Materials and methods

Commercially available reagents and solvents from Sigma-Aldrich were used, as received without further purification, unless otherwise specified. 1-Ethyl-3-methylimidazolium acetate ([C_2mim][OAc]) was purchased from Iolitec (Heilbronn, Germany). Tetrabutylphosphonium hydroxide solution (40% in H_2O) was purchased from TCI Chemicals (Eschborn, Germany), and the exact concentration was determined *via* titration prior to use. Ionic liquids [C_4mim]Cl, [C_2mim]Me_2PO_4, [C_4mim]$MeSO_3$ and [HN(Et)$_3$]HSO_4 were synthesized according to standard procedures and analytical data was in accordance with literature.[1,2,3,4]

All ionic liquids were dried for at least 24-48 h at room temperature or 50 °C and 0.01 mbar before use and stored under argon.

^{1}H, ^{13}C and ^{30}P NMR spectra were recorded from $CDCl_3$, CD_2Cl_2, d_4-MeOD or d_6-DMSO solutions on a Bruker Advance UltraShield 400 (400 MHz) spectrometer and chemical shifts (δ) are reported in ppm using tetramethylsilane as internal standard coupling constants (*J*) are in Hertz (Hz).

HPLC analysis was performed on a Jasco HPLC unit equipped with a PDA detector.

For the determination of betulin (**1**) a Maisch ReproSil 100 C18 250 x 4.6, 5 µm was used with MeOH:H_2O (0.1% TFA) = 87:13 as solvent and a flow of 1 ml/min; detection was done at 210 nm, at 30 °C column oven temperature, 25 °C tray temperature. Retention times were 7.3 min for the internal standard and 15.7 min for betulin (**1**). For the determination of betulin (**1**), betulinic acid (**2**) and betulinic aldehyde a Maisch ReproSil 100 C18 250 x 4.6, 5 µm was used with MeOH:H_2O (0.1% TFA) = 87:13 as solvent and a flow of 1 ml/min; detection was done at 210 nm, at 30 °C column oven temperature, 25 °C tray temperature. Retention times were 7.3 min for the internal standard, 15.7 min for betulin (**1**), 16.5 min for betulinic acid (**2**) and 28.0 min for betulinic aldehyde.

Microwave reactions were performed on a BIOTAGE Initiator™ sixty microwave unit. The reported times are hold times. For this purpose the reaction mixtures were transferred into a glass pressure microwave tube equipped with a magnetic stirring bar, sealed with a Teflon septum and exposed to microwave irradiation at the required temperature. The absorption mode was set to *„Very high"*.

All reagents were purchased from commercial suppliers and used without further purification unless noted otherwise. Betulin as reference material was isolated from birch bark using extraction with ethanol. It was purified *via* column chromatography.

Chromatography solvents were distilled prior to use. Column chromatography was performed on a Büchi Sepacore Flash System (2 x Büchi Pump Module C-605, Büchi Pump Manager C-615, Büchi UV Photometer C-635, Büchi Fraction Collector C-660) or standard manual glass columns using silica gel from Merck (40-63 µm) with PE/EtOAc or MeOH/CH_2Cl_2 mixtures as eluates.

Figure S1: Biphasic extraction of betulin (2) using hydrophilic ionic liquids

Several other hydrophilic ionic liquids, either in pure form or as aqueous solution, were also tested for the pretreatment of birch bark. Imidazolium derivatives [C_2mim]OAc, [C_2mim]Me_2PO_4, [C_4mim]Cl, and [C_4mim]$MeSO_3$ as well as the low-prize ammonium protic ionic liquid [$HNEt_3$]HSO_4 were evaluated for the biphasic extraction of birch bark using the aqueous solution and *n*-BuOAc.

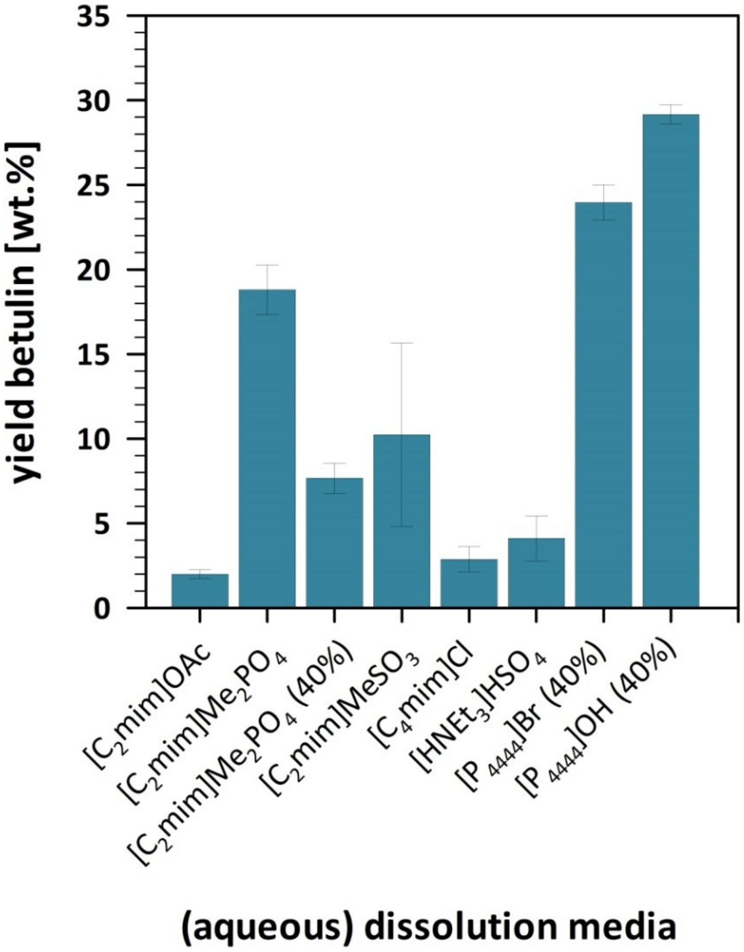


*Figure S1: Biphasic extraction of betulin (**2**) using hydrophilic ionic liquids*

Table S1: Recycling of phosphonium hydroxide solution in the biphasic extraction of betulin (2)

Strategy 1 – Addition of new biomass		**Strategy 2 – Filtration and evaporation**	
Cycle Nr.	Yield betulin [wt.%]	Cycle Nr.	Yield betulin [wt.%]
1	26.8	1	26.5
2	29.5	2	10.0
3	32.5	3	n.d.
4	18.6	4	n.d.

Strategy 1: In a 100 ml flask birch bark (1 g) and 9 g [P_{4444}]OH as 40 wt.% solution in water were stirred for 1 h at room temperature. *n*-BuOAc (30 ml) was added and the mixture was again stirred for 1 h at room temperature. The organic layer was separated and yield was determined via HPLC.Fresh biomass (1 g) was added to the remaining dark slurry. The solution was stirred 1 h at room temperature and *n*-BuOAc (30 ml) was added. This cycle was repeated two more times until the biomass/IL slurry became unable to stir.

Strategy 2: In a 100 ml flask birch bark (1 g) and 9 g [P_{4444}]OH as 40 wt.% solution in water were stirred for 1 h at room temperature. Water (20 ml) and *n*-BuOAc (30 ml) were added and the mixture was again stirred for 1 h at room temperature. The organic layer was separated and yield was determined via HPLC. The aqueous phase was diluted with 10 ml ethanol and the obtained precipitate was removed via filtration. The solution was concentrated *in vacuo* to a volume of 9 ml and reused with 1 g of biomass.

Figure S2: Evaluation of solvents for the oxidation of betulin

Several solvents, including dimethylcarbonate (DMC), ethyl acetate (EtOAc), diethylcarbonate (DEC), dichloromethane (CH_2Cl_2), *n*-butyl acetate (BuOAc), *tert*-butanol (*t*-BuOH) and acetonitrile (ACN) were evaluated in the oxidation of betulin to betulinic acid. Reactions were performed using 10 mg betulin (**2**) in 1 ml solvent, 1 eq. TEMPO, 3 eq. BAIB for 24 h at room temperature.

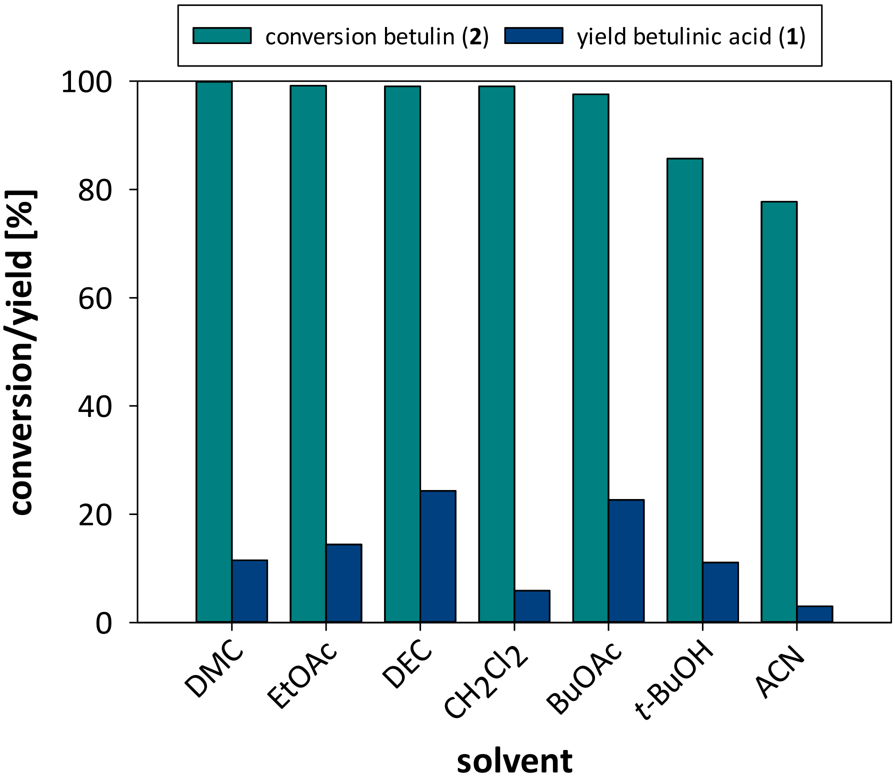


Table S2-S4: Optimization of oxidation of betulin (2) to betulinic acid (1)

Table S2: Variation of oxidation reagents

Entry	TEMPO [eq.]	BAIB [eq.]	Conversion betulin (2) [%]	Yield betulinic acid (1) [%]
1	1	3	99	26
2	0.5	3	99	38
3	0.2	2	73	16
4	0.2	3	100	41
5	0.2	4	100	43
6	0.1	3	98	45
7	0.1	4	95	38
8	0.05	3	95	37

Performed using 10 mg betulin (**2**), 50 eq. water, 2 eq. *t*-BuOH at r.t. for 24 h. Yield was determined *via* RP-HPLC using 1-methyl-1-cyclohexen as internal standard. Performed in triplicates.

Table S3: Concentration dependency of the starting material in BuOAc

Entry	Concentration betulin (2) [mg/ml]	Conversion betulin (2) [%]	Yield betulinic acid (1) [%]
1	15	100	39
2	10	100	43
3	7.5	88	28
4	5	67	13

Performed using 10 mg betulin (**2**), 0.2 eq TEMPO, 3 eq BAIB, 50 eq. water, 2 eq. *t*-BuOH at r.t. for 24 h. Yield was determined *via* RP-HPLC using 1-methyl-1-cyclohexen as internal standard. Performed in triplicates.

Table S4: Influence of the amount of water for the oxidation

Entry	Solvent	Water [eq.]	Conversion betulin (2) [%][b]	Yield betulinic acid (1) [%][b]
1	DMC	0	100	11.5
2	DMC	10	74	0
3	DEC	0	99	24.3
4	DEC	10	100[a]	12.3
5	BuOAc	0	95	22.7
6	BuOAc	10	99[a]	24.2
7	BuOAc	50	99[a]	26.1
8	BuOAc	250	99[a]	30.4[c]
9	BuOAc	2500	10	0

Performed using 10 mg betulin (**2**) in 1 ml solvent, 1 eq. TEMPO, 3 eq. BAIB. [a] Addition of 2 eq. *t*-BuOH. [b] According to HPLC c unidentified byproducts were detected

Figure S3: Impact of acetic acid on the oxidation of betulin (2) to betulinic acid (1)

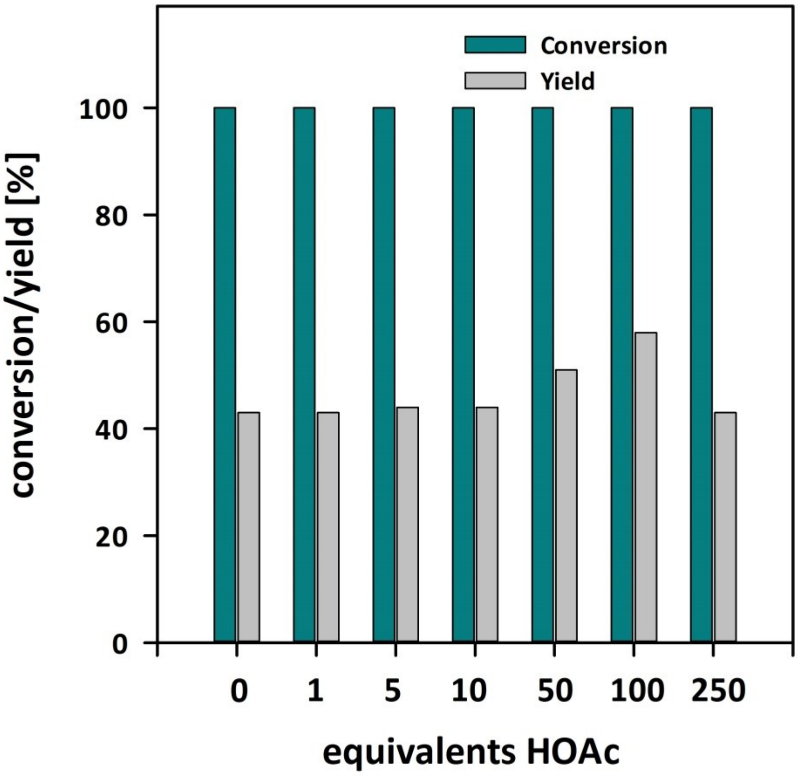


Figure S4-S5: Spectra of isolated betulin (2) (isolated *via* column chromatography)

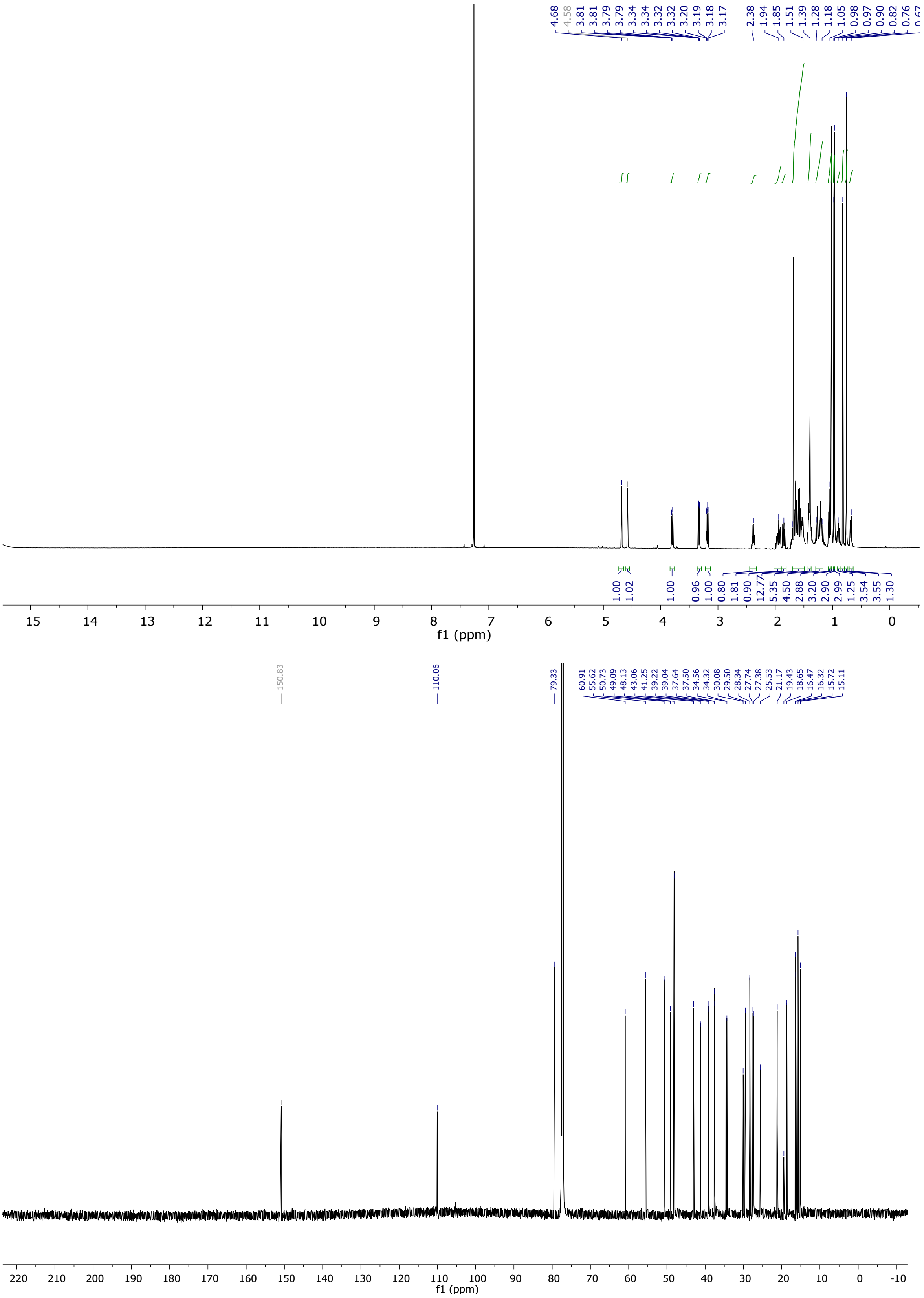


*Figure S4: ^{1}H and ^{13}C NMR spectra of isolated betulin (**2**) of the crude biomass extract (isolated via column chromatography)*

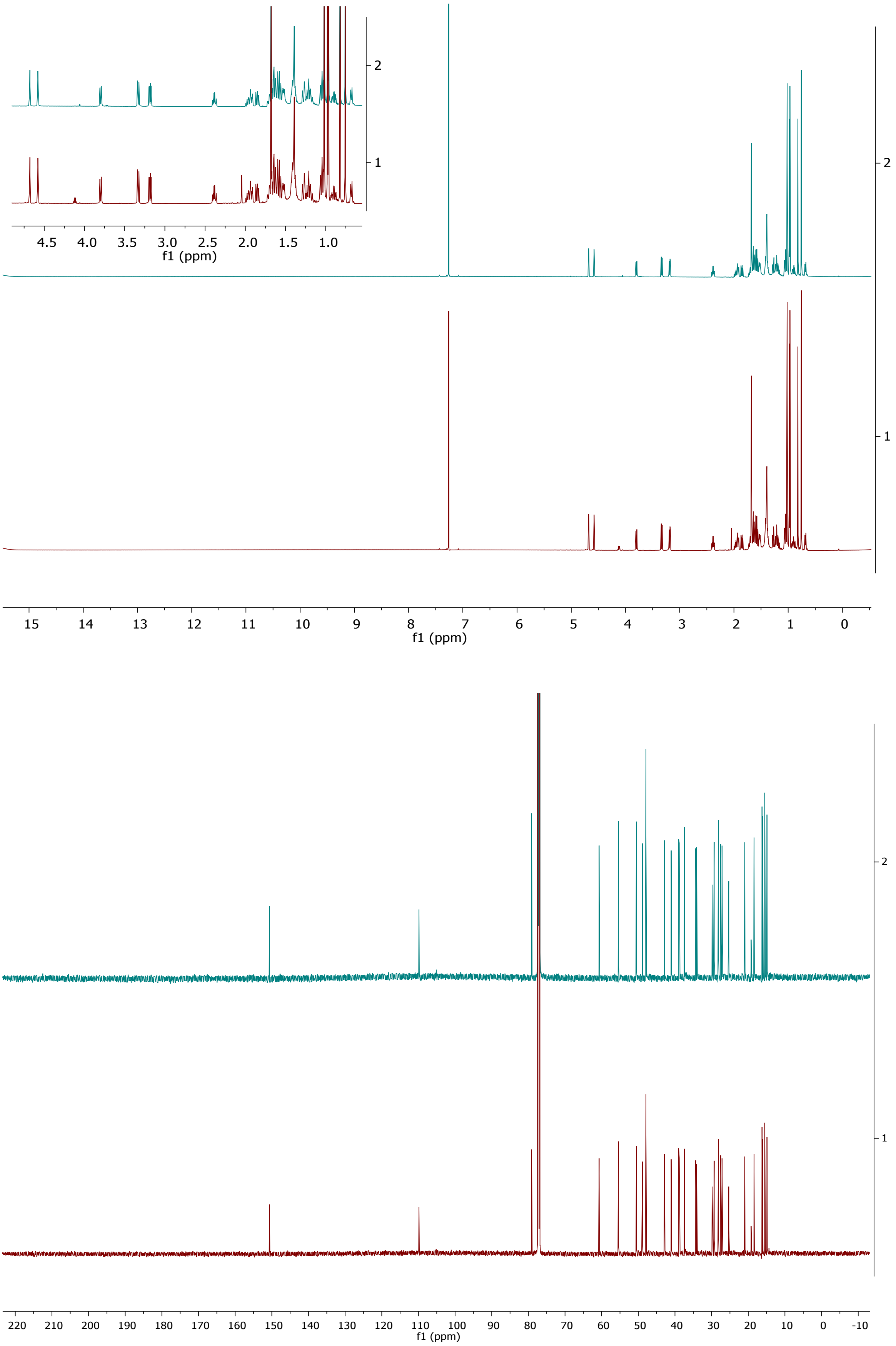


*Figure S5: ^{1}H and ^{13}C NMR spectra of isolated betulin (**2**) of the crude biomass extract (green, isolated via column chromatography) vs. reference betulin (**2**) (brown).*

Figure S6-S7: Spectra of isolated betulin (2) (isolated *via* crystallization)

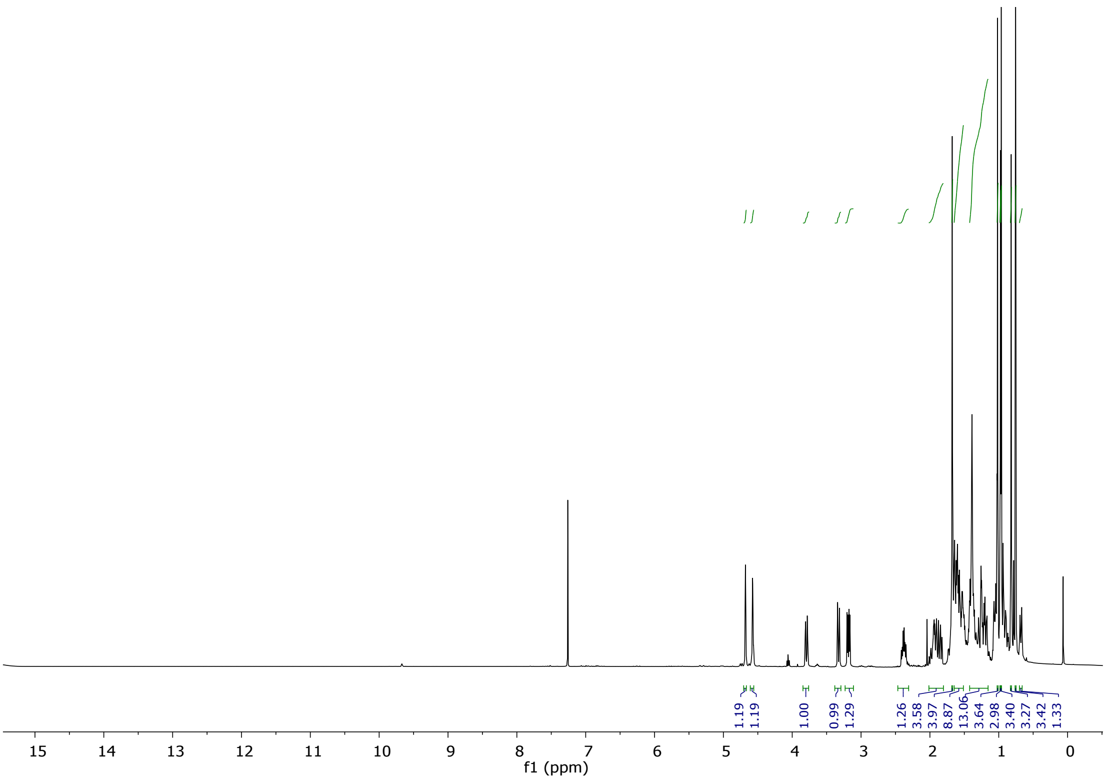


*Figure S6: ^{1}H NMR spectra of isolated betulin (**2**) of the crude biomass extract (isolated via crystallization)*

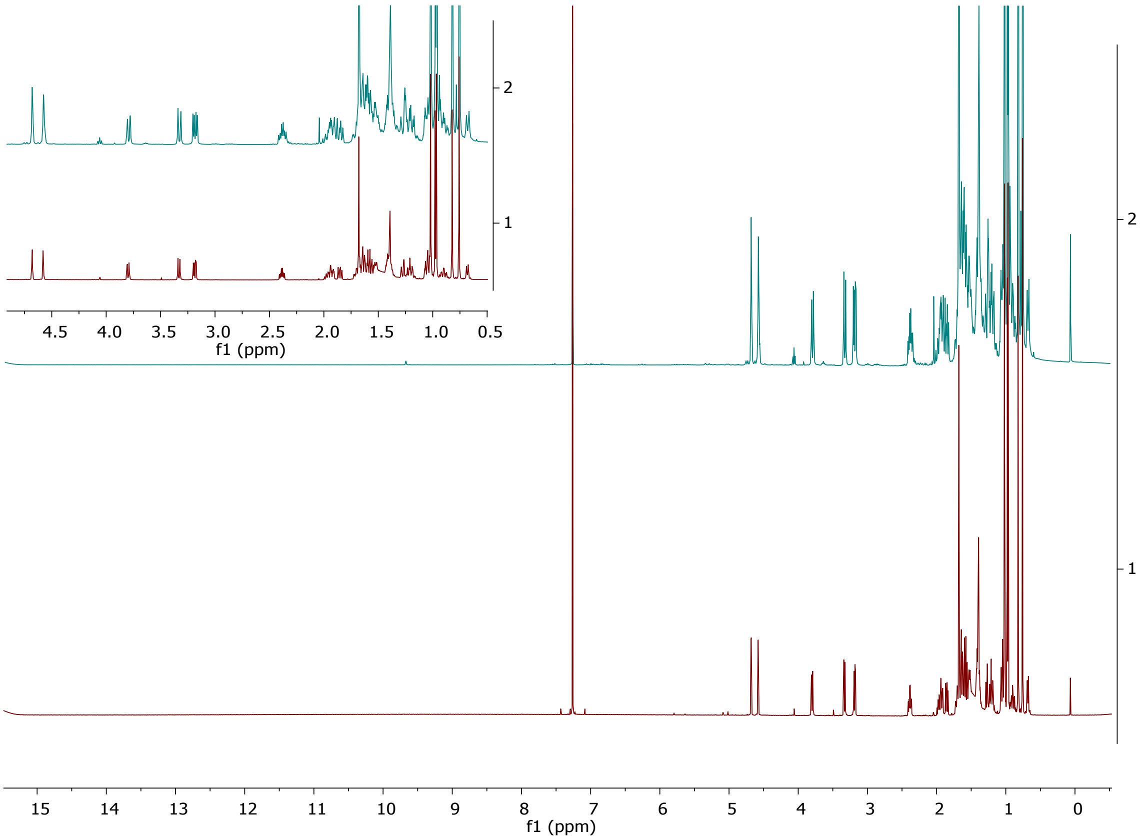


*Figure S7: ^{1}H NMR spectra of isolated betulin (**2**) of the crude biomass extract (green, isolated via crystallization) vs. reference betulin (**2**) (brown)*

Figure S8-S9: Spectra of betulinic acid (1) obtained *via* oxidation of pure betulin (2)

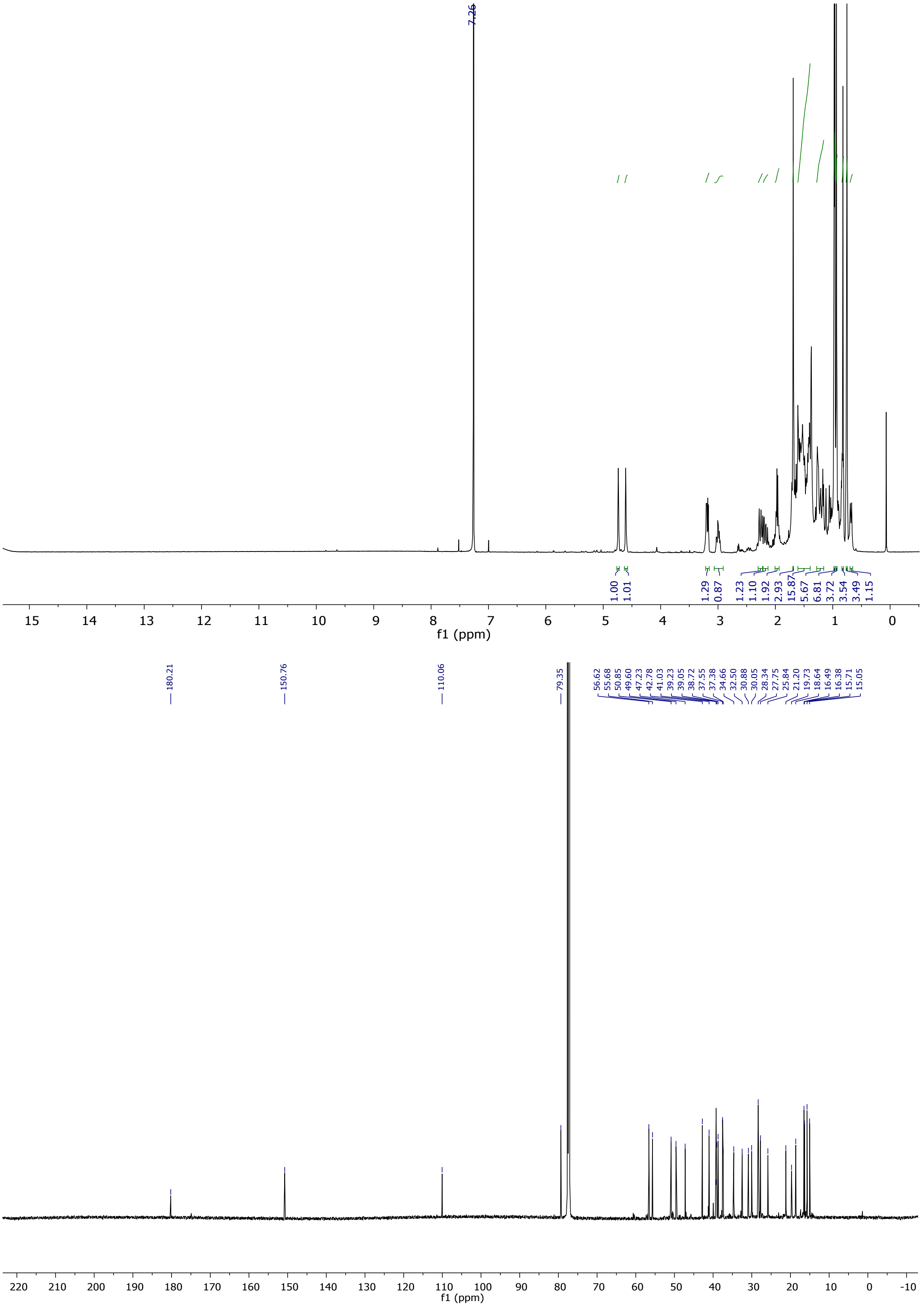


*Figure S8: ^{1}H and ^{13}C NMR spectra of isolated betulinic acid (**1**) obtained via oxidation of pure betulin (**2**) (isolated via crystallization)*

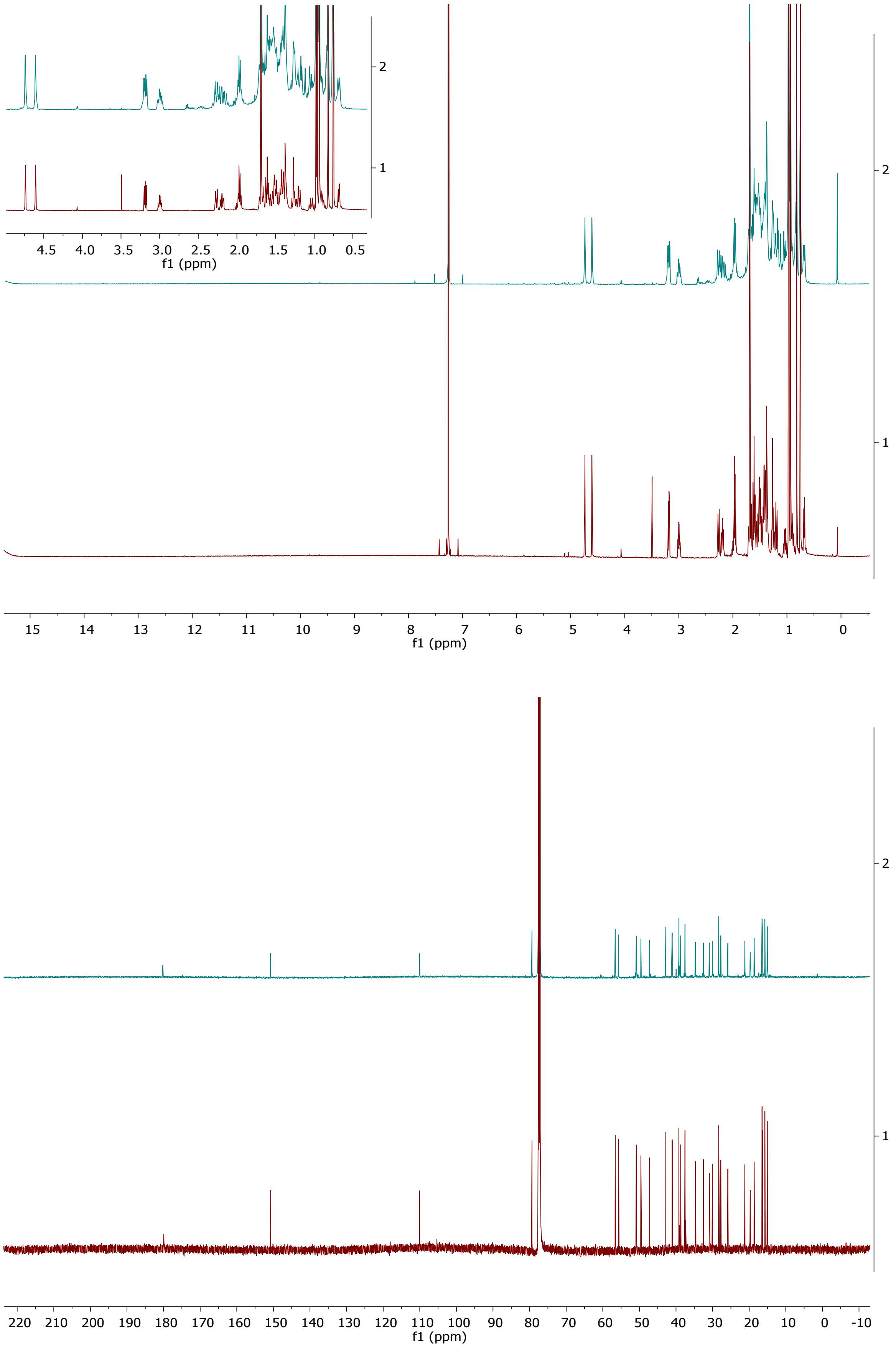


*Figure S9: ^{1}H and ^{13}C NMR spectra of isolated betulinic acid (**1**) obtained via oxidation of pure betulin (**2**) (green, isolated via crystallization) vs. commercially available betulinic acid (**1**) from Sigma-Aldrich (brown)*

Figure S10-11: Spectra of isolated betulinic acid (1) obtained *via* oxidation of crude biomass extract (isolated *via* column chromatography)

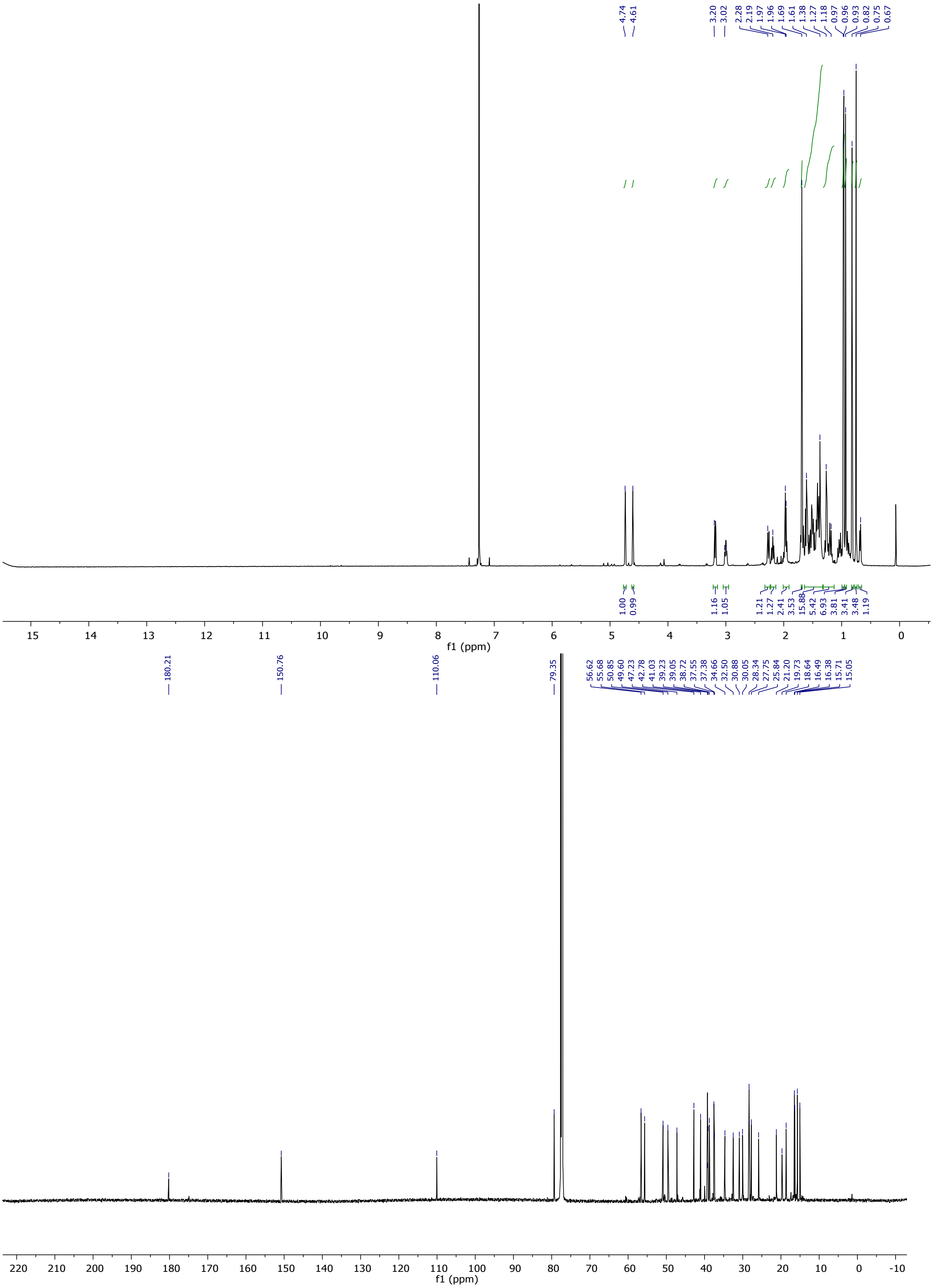
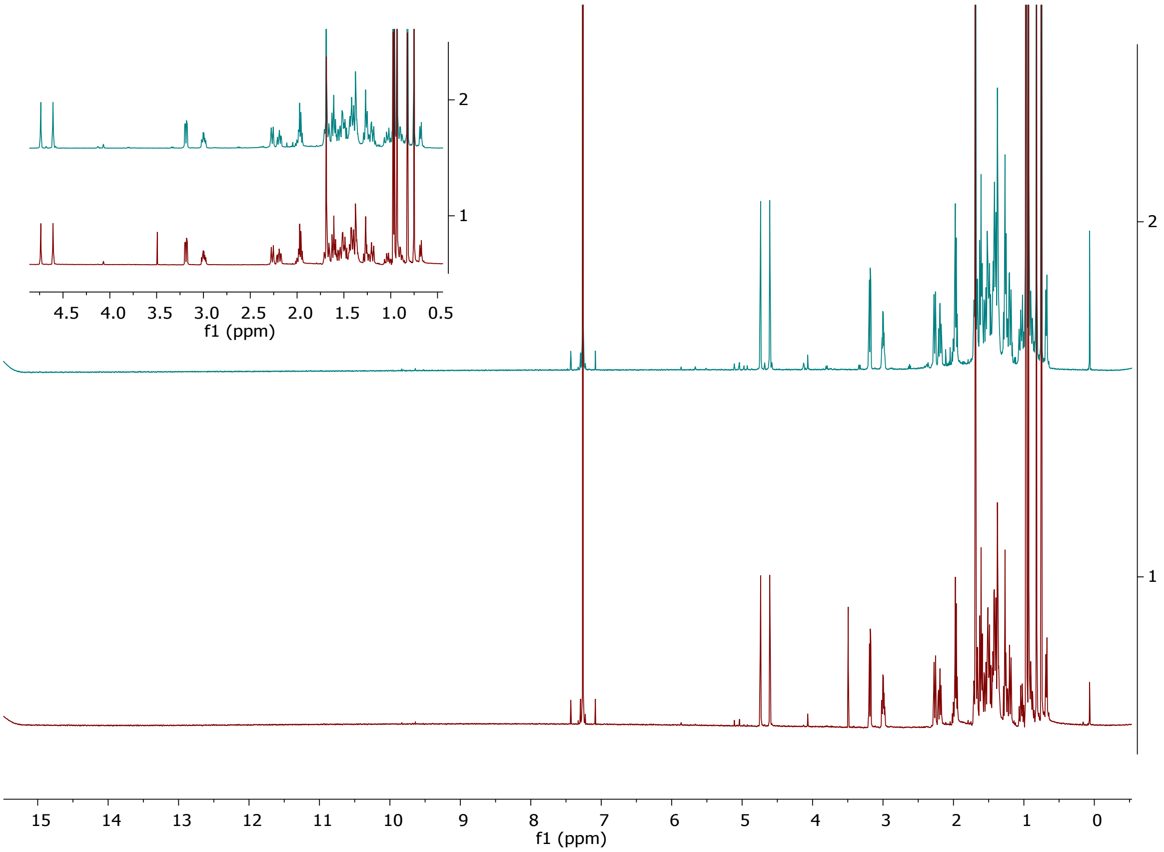


*Figure S10: ^{1}H and ^{13}C NMR spectra of isolated betulinic acid (**1**) obtained after oxidation of crude biomass extract (isolated via column chromatography)*



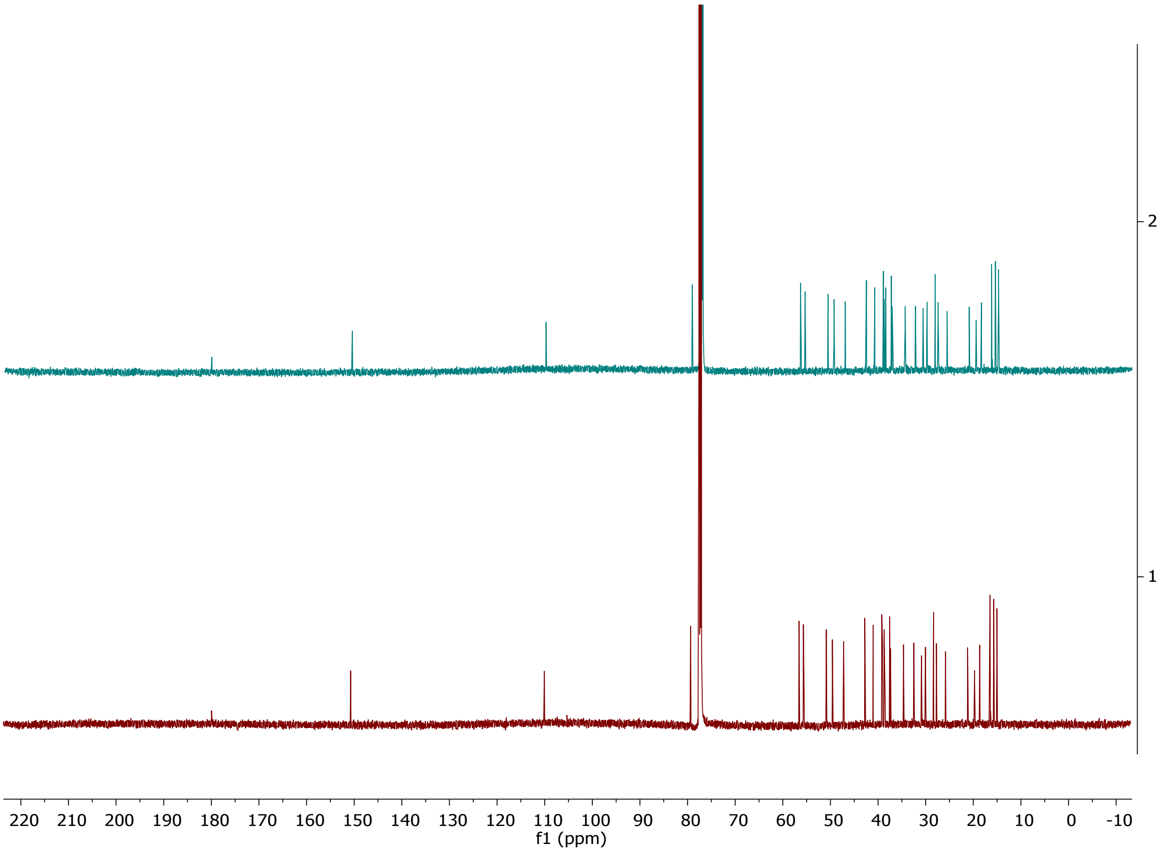
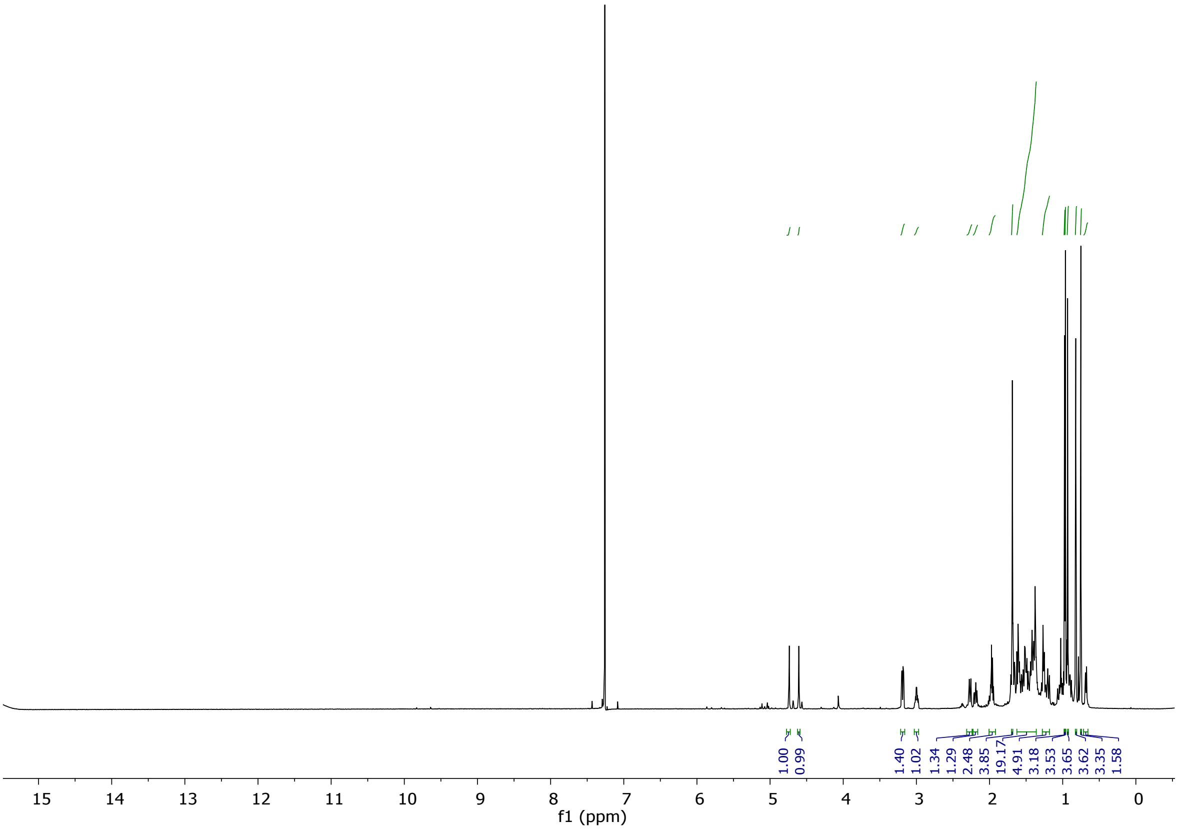


*Figure S11: ^{1}H and ^{13}C NMR spectra of isolated betulinic acid (**1**) obtained via oxidation of crude biomass extract (green, isolated via column chromatography) vs. commercially available betulinic acid (**1**) bought at Sigma-Aldrich (brown)*

Figure S12-S13: Spectra of betulinic acid (1) obtained *via* oxidation of crude biomass extract (isolated *via* crystallization)



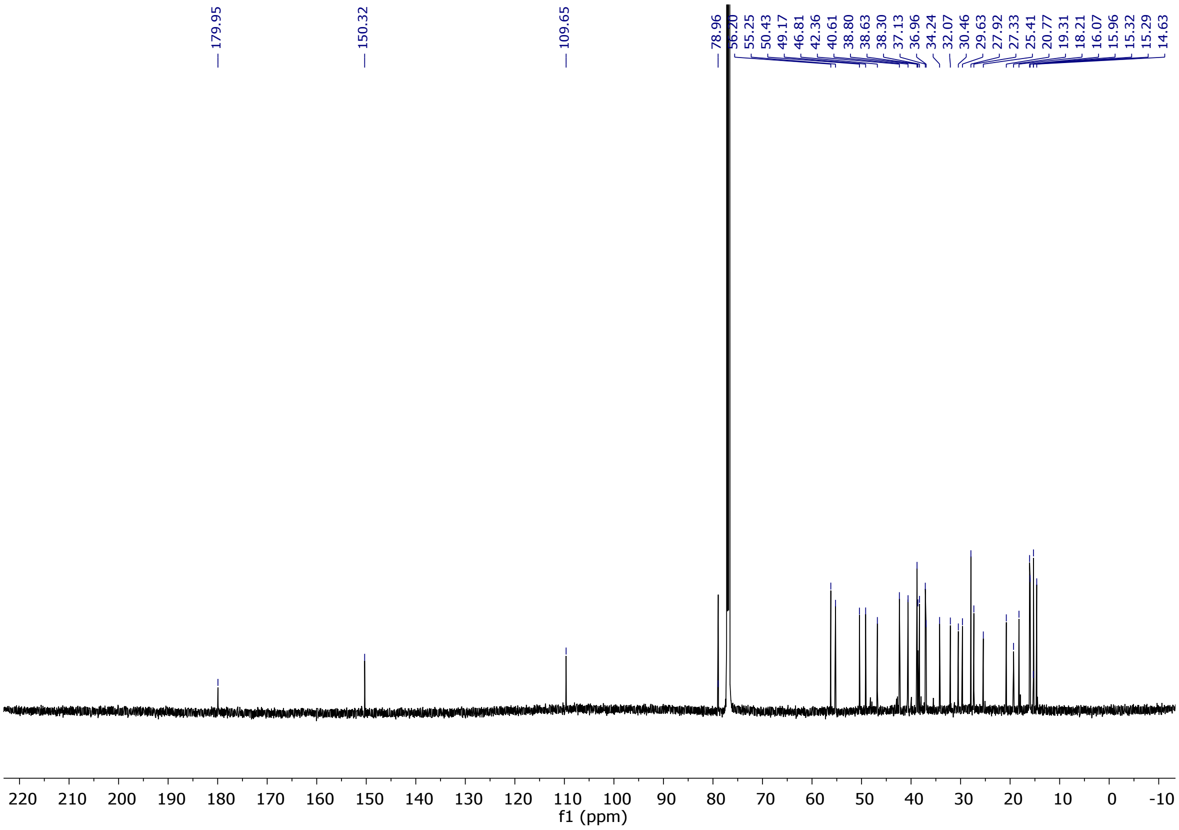
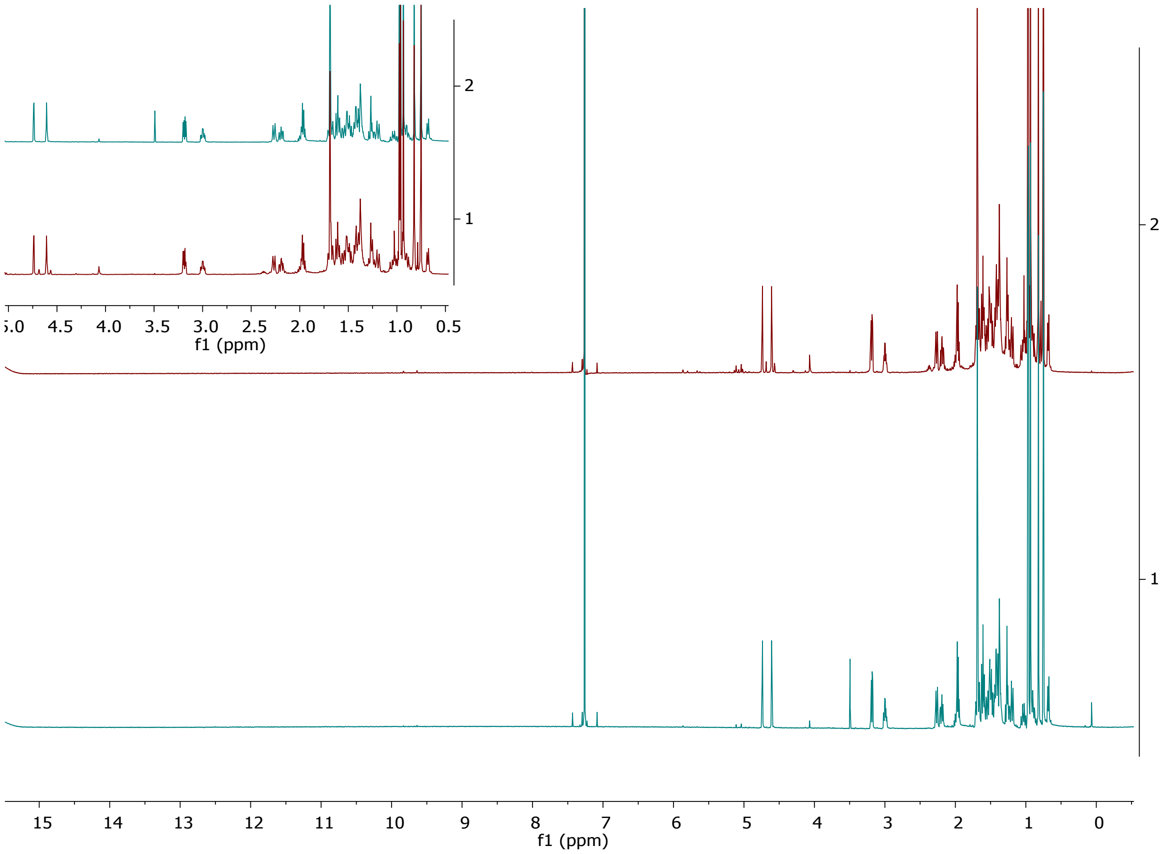


*Figure S12: ^{1}H and ^{13}C NMR spectra of isolated betulinic acid (**1**) obtained after oxidation of crude biomass extract (isolated via crystallization, traces of lupeol could not be separated)*



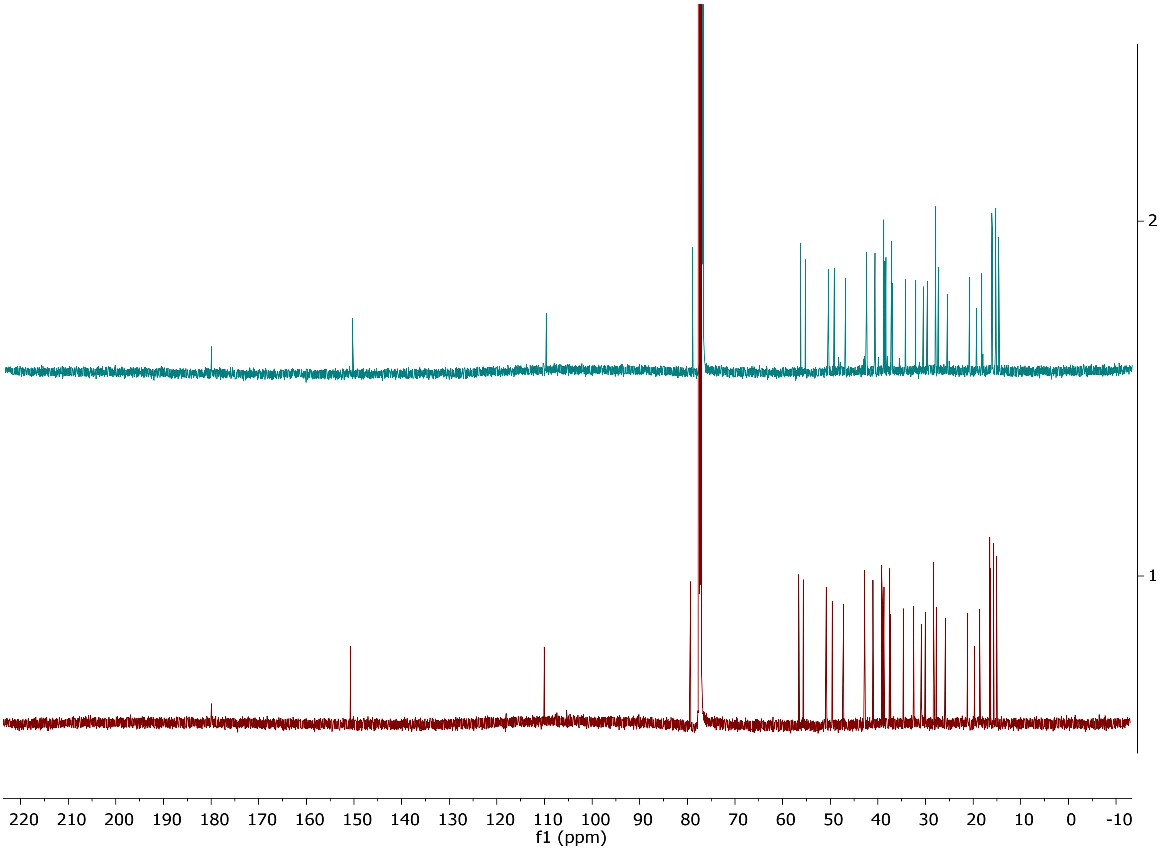


*Figure S13: ^{1}H and ^{13}C NMR spectra of isolated betulinic acid (**1**) obtained via oxidation of crude biomass extract (green, isolated via crystallization) vs. commercially available betulinic acid (**1**) bought at Sigma-Aldrich (brown)*

References

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