Supplementary Materials

Anti-atopic Dermatitis Activity of *Cornus walteri* and Identification of the Bioactive Compounds

Bum Soo Lee [1,†], Ye-Ryeong Cho [2,†], Minju Jeong [3], Yoon Seo Jang [1], Jin-Chul Kim [4], Sanguine Byun [2,*], and Ki Hyun Kim [1,*]

[1] School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

[2] Department of Biotechnology, Yonsei University, Seoul 03722; Republic of Korea

[3] Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Republic of Korea

[4] KIST Gangneung Institute of Natural Products, Natural Product Informatics Research Center, Gangneung 25451, Republic of Korea

***** Correspondence: sanguine@yonsei.ac.kr (S.B.); khkim83@skku.edu (K.H.K.); Tel.: +82-2-2123-5896 (S.B.); +82-31-290-7700 (K.H.K.)

†These authors contributed equally to this study.

General experimental procedure

Optical rotations were measured using a P-2000 polarimeter (Japan Spectroscopic Company, Easton, Maryland, United States of America [USA]). NMR spectra were recorded on a Bruker AVANCE III instrument (Bruker). Preparative and semi-preparative HPLC were performed using a Waters 1525 binary HPLC pump with a Waters 996 photodiode array detector (Waters Corporation, Milford, Connecticut, USA). LC-MS analysis was performed on an Agilent 1200 series HPLC system with a diode array detector and a 6130 Series ESI mass spectrometer using an analytical Kinetex C_{18} 100 Å column (100 mm × 2.1 mm i.d., 5 μm; flow rate:0.3 mL/min) (Phenomenex). Silica gel 60 (230-400 mesh; Merck, Darmstadt, Germany), RP-C_{18} silica gel (Merck, 230-400 mesh), and silica Sep-Pak Vac 6 cc cartridges (Waters) were used for column chromatography. Thin-layer chromatography (TLC) was performed using pre-coated silica gel F_{254} plates and RP-C_{18} F_{254s} plates (Merck), and spots were detected under UV light or by heating after spraying with anisaldehyde-sulfuric acid.

Plant material

CWS were collected from Jeju Island, Korea, in October 2015 and were identified by one of the authors (K. H. Kim). A voucher specimen (SKKU 2015-MC) was deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

NMR and physical data for compounds **1**-**6**.

1. Lupeol (**1**)

Colorless crystals; $[\alpha]_D^{25}$ +23.0 (*c* 0.10, $CHCl_3$); ^1H NMR (700 MHz, $CDCl_3$): δ_H 4.75 and 4.63 (1H each, brs, H-29), 3.22 (1H, dd, *J* = 10.0 Hz; *J* = 4.5 Hz, H-3), 1.66 (3H, brs, H-30), 1.05 (3H, s, H-26), 0.98 (3H, s, H-23), 0.94 (3H, s, H-27), 0.84 (3H, s, H-25), 0.79 (3H, s, H-28) and 0.78 (3H, s, H-24); ^{13}C NMR (175 MHz, $CDCl_3$): δ_C 38.6 (C-1), 27.5 (C-2), 78.8 (C-3), 38.7 (C-4), 55.3 (C-5), 18.3 (C-6), 34.2 (C-7), 40.8 (C-8), 50.4 (C-9), 37.1 (C-10), 20.9 (C-11), 25.2 (C-12), 38.1 (C-13), 42.8 (C-14), 27.3 (C-15), 35.5 (C-16), 92.9 (C-17), 48.2 (C-18), 47.7 (C-19), 150.6 (C-20), 92.8 (C-21), 39.8 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 15.8 (C-26), 14.5 (C-27), 18.1 (C-28), 109.2 (C-29) and 19.2 (C-30); ESIMS (positive-ion mode) *m/z* 427.7 $[M + H]^+$.

2. Betulinic acid (**2**)

White powder; $[\alpha]_D^{25}$ +3.7 (*c* 0.15, MeOH); ^1H NMR (700 MHz, $CDCl_3$): δ_H 4.75 and 4.63 (1H each, brs, H-29), 3.20 (1H, t, *J* = 2.5 Hz, H-3), 3.03 (1H, m, H-19), 1.71 (3H, s, H-30), 0.99 (3H, s, H-26), 0.98 (3H, s, H-25), 0.96 (3H, s, H-24), 0.84 (3H, s, H-22) and 0.77 (3H, s, H-23); ^{13}C NMR (175 MHz, $CDCl_3$): δ_C 38.7 (C-1), 27.4 (C-2), 79.0 (C-3), 38.9 (C-4), 55.3 (C-5), 18.3 (C-6), 34.3 (C-7), 40.7 (C-8), 50.5 (C-9), 37.2 (C-10), 20.9 (C-11), 25.5 (C-12), 38.4 (C-13), 42.4 (C-14), 30.6 (C-15), 32.2 (C-16), 56.3 (C-17), 46.9 (C-18), 49.3 (C-19), 150.4 (C-20), 29.7 (C-21), 37.0 (C-22), 28.0 (C-23), 15.4 (C-24), 16.0 (C-25), 16.1 (C-26), 14.7 (C-27), 180.4 (C-28), 109.7 (C-29) and 19.4 (C-30); ESIMS (positive-ion mode) *m/z* 457.7 $[M + H]^+$.

3. 5α-Stigmast-3,6-dione (**3**)

White crystals; $[\alpha]_D^{25}$ +27.5 (*c* 0.15, MeOH); ^{1}H NMR (700 MHz, $CDCl_3$): δ_H 1.70 (1H, m, H-25), 1.48 (1H, m, H-24), 1.40 (1H, m, H-20), 1.31 (1H, m, H-28), 1.26 (1H, m, H-28), 0.99 (3H, s, H-19), 0.96 (3H, d, *J* = 6.5 Hz, H-21), 0.88 (3H, s, H-29), 0.87 (3H, d, *J* = 6.5 Hz, H-27), 0.85 (3H, d, *J* = 6.5 Hz, H-26), 0.72 (3H, s, H-18); ^{13}C NMR (175 MHz, $CDCl_3$): δ_C 38.0 (C-1), 39.4 (C-2), 209.0 (C-3), 37.0 (C-4), 57.5 (C-5), 211.1 (C-6), 46.6 (C-7), 37.4 (C-8), 53.5 (C-9), 41.2 (C-10), 21.7 (C-11), 38.1 (C-12), 43.0 (C-13), 56.0 (C-14), 24.0 (C-15), 28.0 (C-16), 56.6 (C-17), 12.5 (C-18), 12.0 (C-19), 36.0 (C-20), 18.7 (C-21), 33.8 (C-22), 26.1 (C-23), 45.8 (C-24), 29.1 (C-25), 19.8 (C-26), 19.0 (C-27), 23.1 (C-28) and 12.0 (C-29); ESIMS (positive-ion mode) *m/z* 429.7 $[M + H]^+$

4. 3-*O*-Acetylbetulin (**4**)

White crystals; $[\alpha]_D^{25}$ +25.7 (*c* 0.92, $CHCl_3$); ^{1}H NMR (700 MHz, $CDCl_3$): δ_H 4.70 and 4.65 (1H each, brs, H-29), 4.45 (1H, m, H-3), 3.75 and 3.30 (1H each, d, *J* = 2.0 Hz, H-28), 2.40 (1H, m, H-19), 2.05 (3H, s, OAc), 1.69 (3H, s, H-30), 0.97 (3H, s, H-25), 0.93 (3H, s, H-27), 0.85 (3H, s, H-23), 0.84 (3H, s, H-26) 0.83 (3H, s, H-24); ^{13}C NMR (175 MHz, $CDCl_3$): δ_C 38.4 (C-1), 23.7 (C-2), 80.9 (C-3), 37.8 (C-4), 55.3 (C-5), 37.0 (C-6), 40.9 (C-8), 50.3 (C-9), 18.1 (C-10), 20.8 (C-11), 25.1 (C-12), 42.7 (C-14), 27.0 (C-15), 29.7 (C-16), 46.3 (C-17), 48.7 (C-18), 47.8 (C-19), 150.0 (C-20), 29.7 (C-21), 34.1 (C-22), 16.0 (C-25), 15.9 (C-26), 14.6 (C-27), 60.5 (C-28), 171.9 (C=O), 109.0 (C-29) and 19.0 (C-30); ESIMS (positive-ion mode) *m/z* 485.6 $[M + H]^+$.

5. Betulinic acid methyl ester (**5**)

White crystals; $[\alpha]_D^{25}$ +10.3 (*c* 0.15, CH_2Cl_2); ^1H NMR (700 MHz, $CDCl_3$): δ_H 4.65 and 4.54 (1H each, brs, H-29), 3.65 (3H, s, OCH_3), 3.15 (1H, dd, *J* = 9.5, 5.5 Hz, H-3), 3.03 (1H, m, H-19), 1.70 (3H, s, H-30), 1.10 (3H, s, H-26), 1.01 (3H, s, H-25), 0.92 (3H, s, H-24), 0.83 (3H, s, H-22), 0.79 (3H, s, H-23); ^{13}C NMR (175 MHz, $CDCl_3$): δ_C 38.6 (C-1), 27.4 (C-2), 78.8 (C-3), 38.6 (C-4), 55.1 (C-5), 18.5 (C-6), 34.2 (C-7), 40.7 (C-8), 50.2 (C-9), 37.1 (C-10), 21.1 (C-11), 25.7 (C-12), 38.4 (C-13), 42.4 (C-14), 30.5 (C-15), 32.1 (C-16), 56.3 (C-17), 48.8 (C-18), 39.2 (C-19), 150.1 (C-20), 29.5 (C-21), 37.1 (C-22), 27.8 (C-23), 15.3 (C-24), 16.1 (C-25), 16.5 (C-26), 14.7 (C-27), 176.1 (C-28), 109.2 (C-29) and 19.4 (C-30); ESIMS (positive-ion mode) *m/z* 471.4 $[M + H]^+$.

6. Norphytan (**6**)

White crystals; $[\alpha]_D^{25}$ +1.4 (*c* 0.10, MeOH); ^1H NMR (700 MHz, $CDCl_3$): δ_H 1.45 (4H, m, H-2, H-6, H-10, H-14), 1.30 (2H, m, H-8), 1.25-1.13 (8H, m, H-5, H-7, H-8, H-9, H-11), 1.09-0.97 (8H, m, H-3, H-4, H-12, H-13), 0.80 (12H, d, *J* = 6.5 Hz, H-1, H-15, H-16, H-19), 0.78 (6H, d, *J* = 6.5 Hz, H-17, H-18); ^{13}C NMR (175 MHz, $CDCl_3$): δ_C 19.7, 22.7, 24.5, 24.8, 28.0, 32.8, 37.3, 37.4, 37.5, 39.4; ESIMS (positive-ion mode) *m/z* 269.3 $[M + H]^+$.

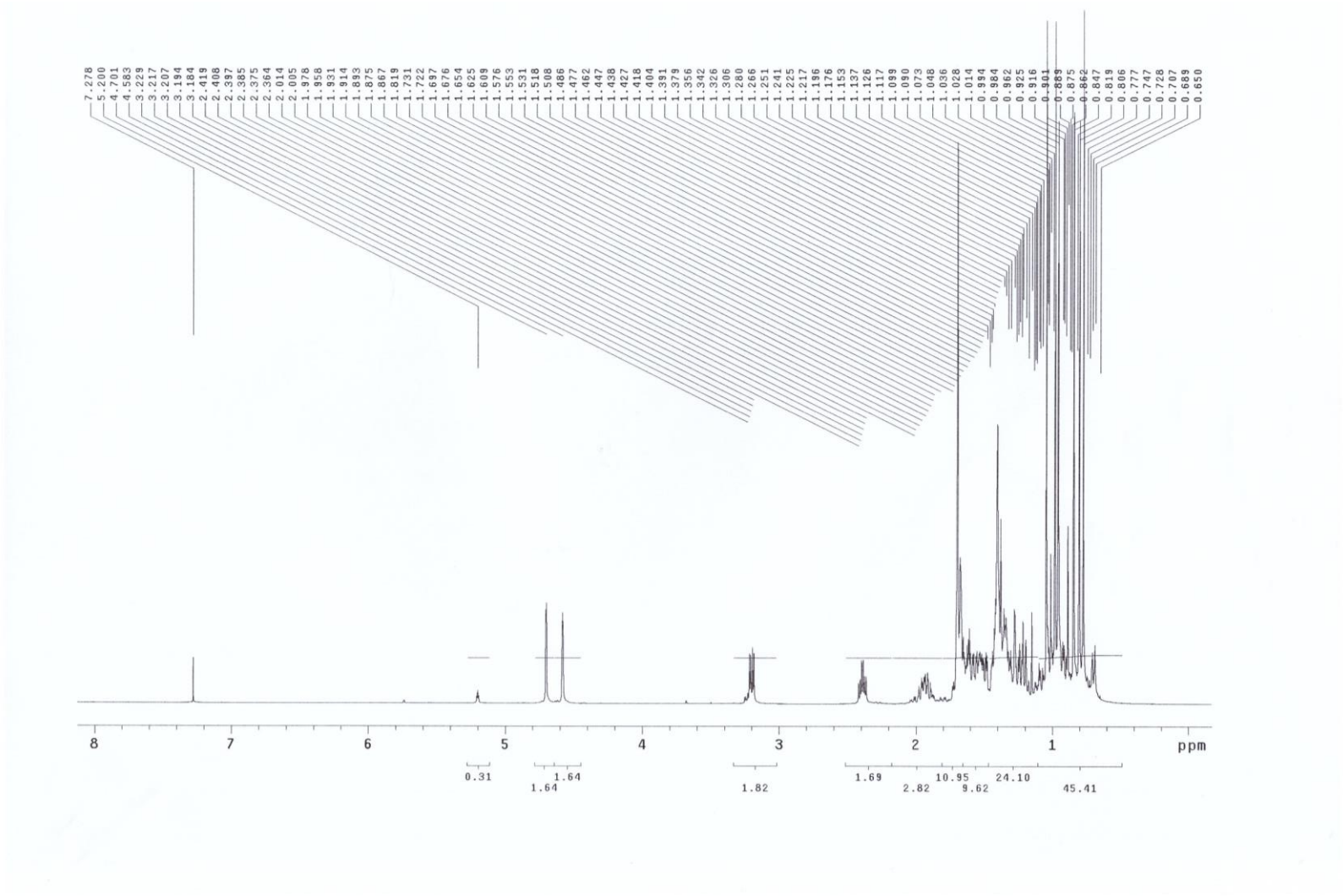

Figure S1. ^{1}H NMR spectrum of lupeol (**1**) ($CDCl_3$, 700 MHz)

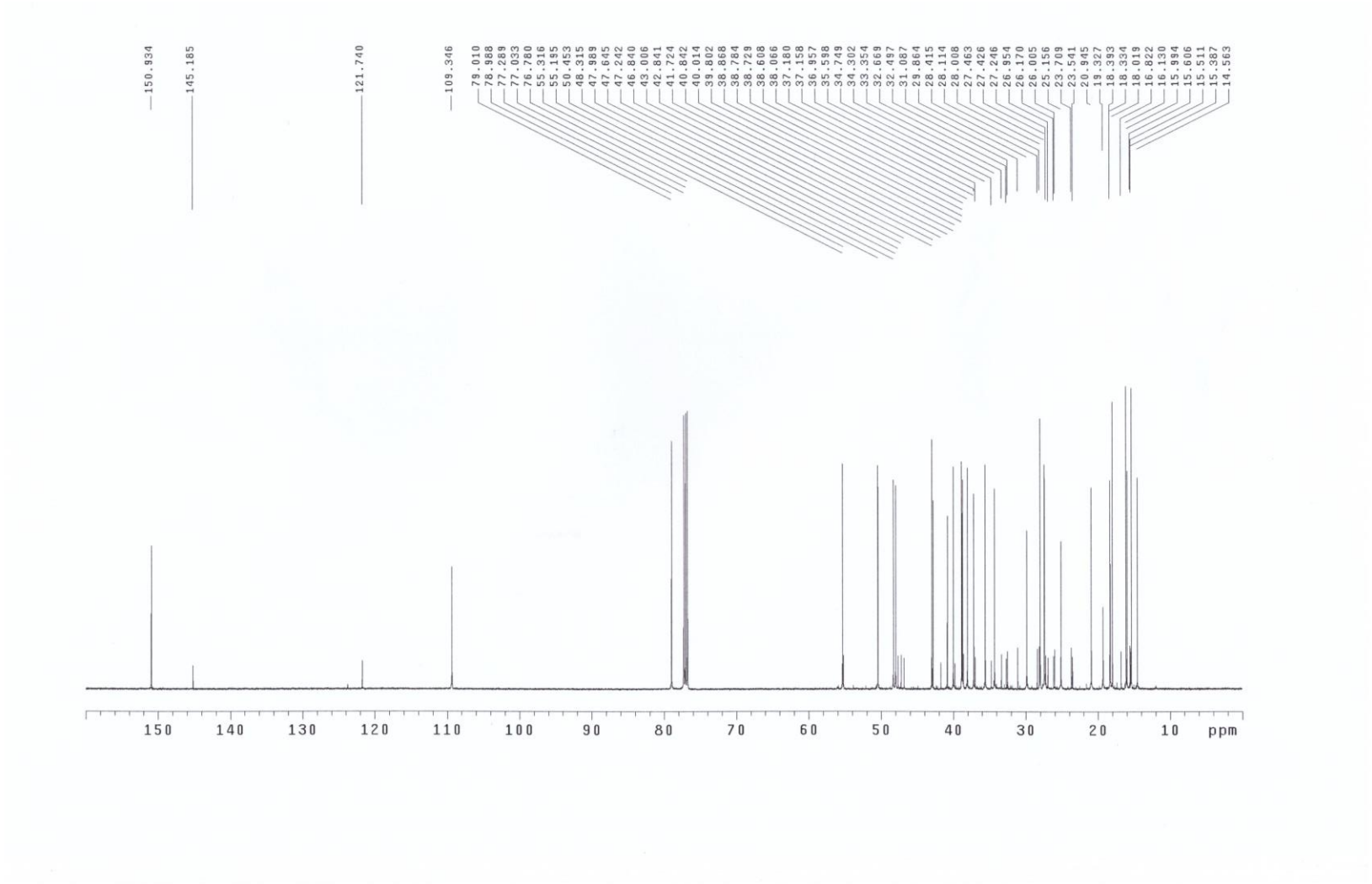

Figure S2. ^{13}C NMR spectrum of lupeol (**1**) ($CDCl_3$, 175 MHz)

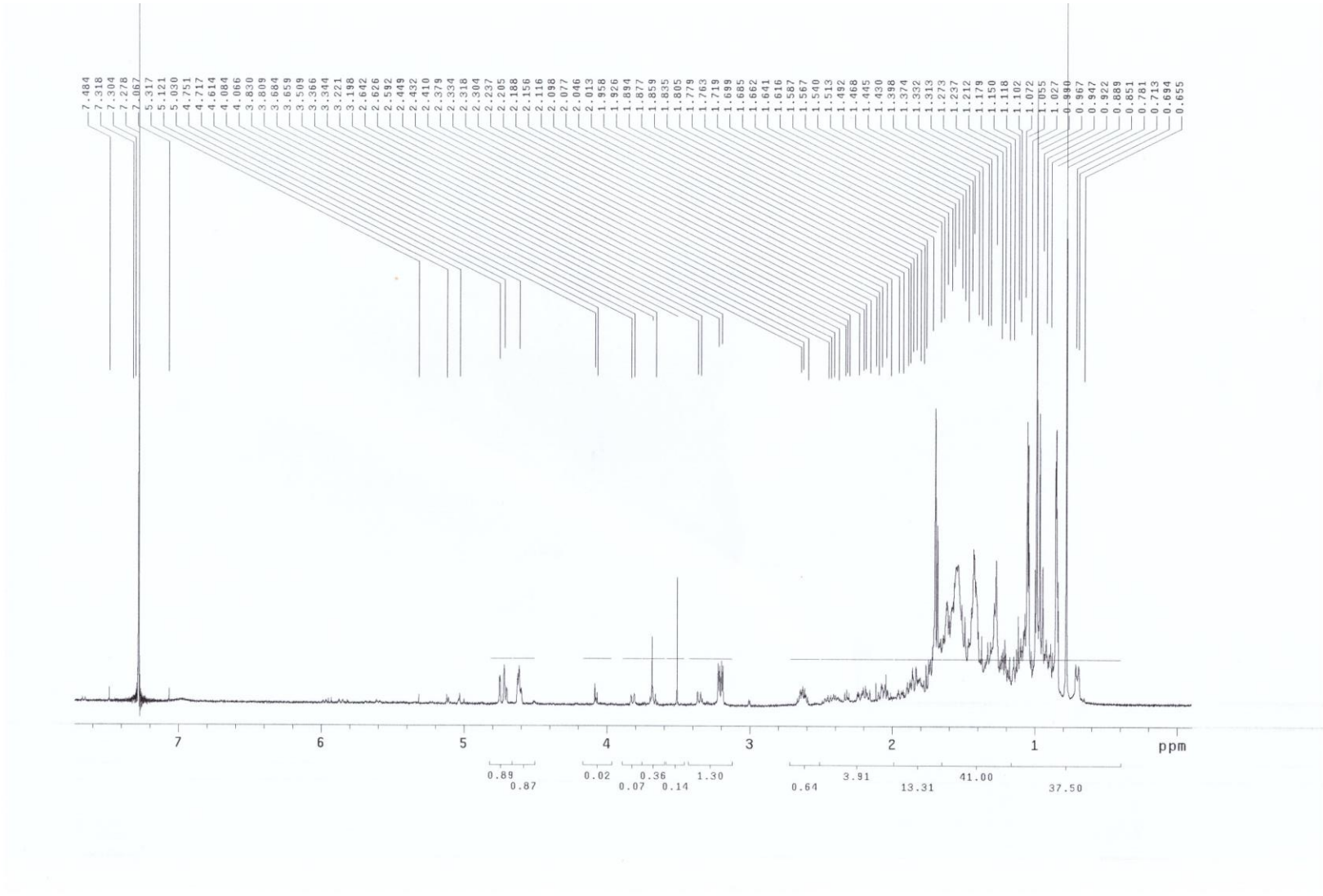

Figure S3. ^{1}H NMR spectrum of betulinic acid (**2**) ($CDCl_3$, 700 MHz)

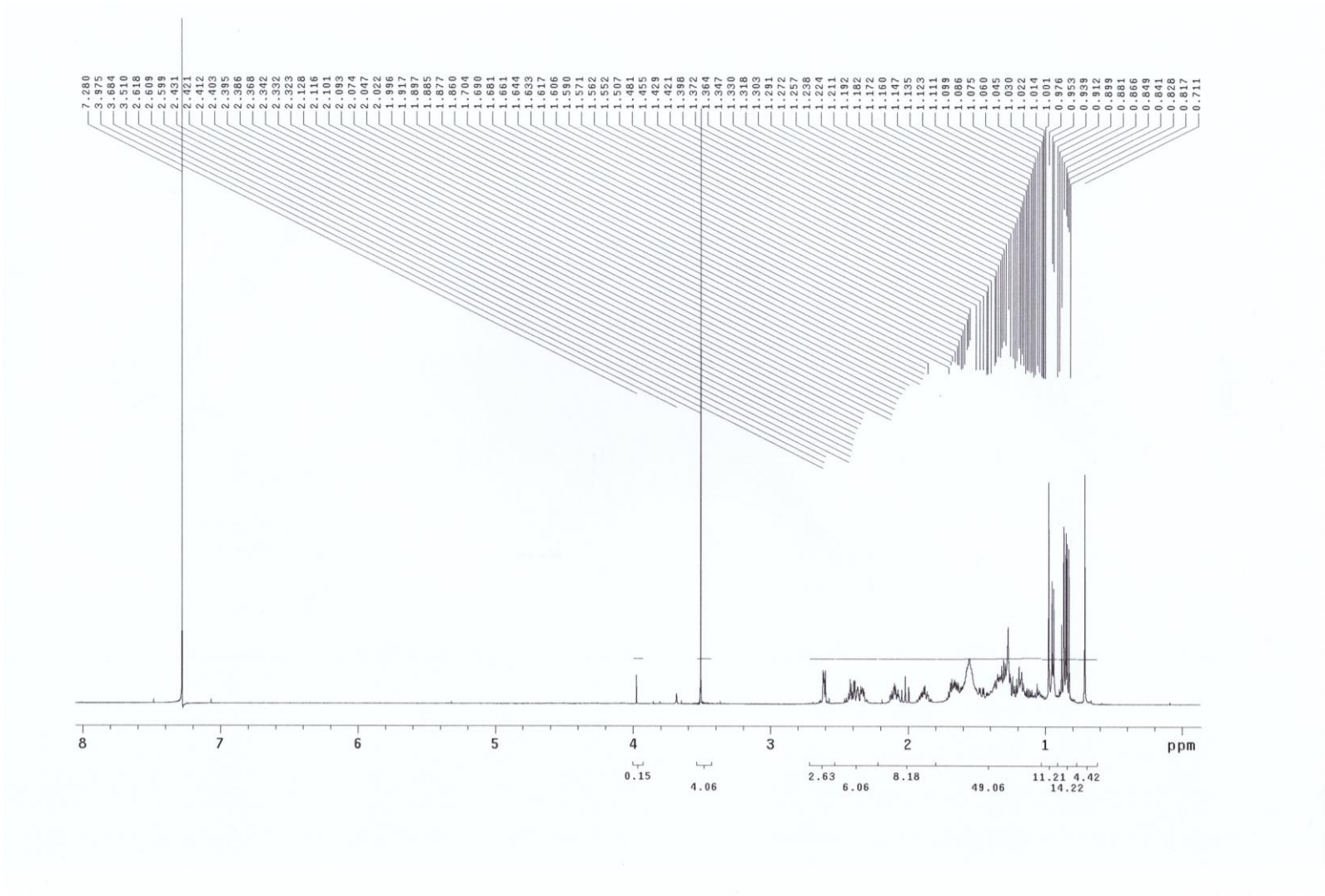

Figure S4. ^{1}H NMR spectrum of 5α-stigmast-3,6-dione (**3**) ($CDCl_3$, 700 MHz)

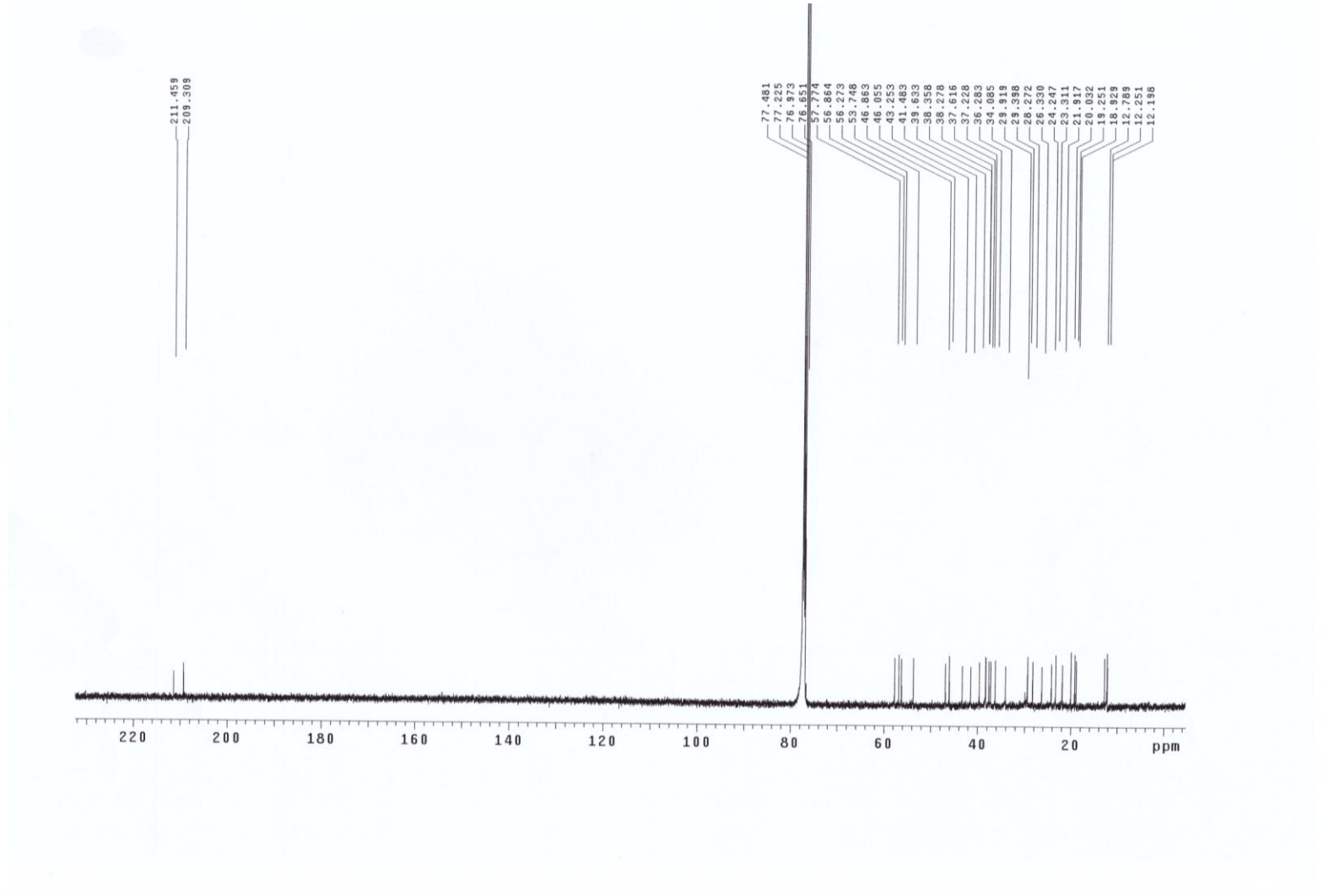

Figure S5. ^{13}C NMR spectrum of 5α-stigmast-3,6-dione (**3**) ($CDCl_3$, 175 MHz)

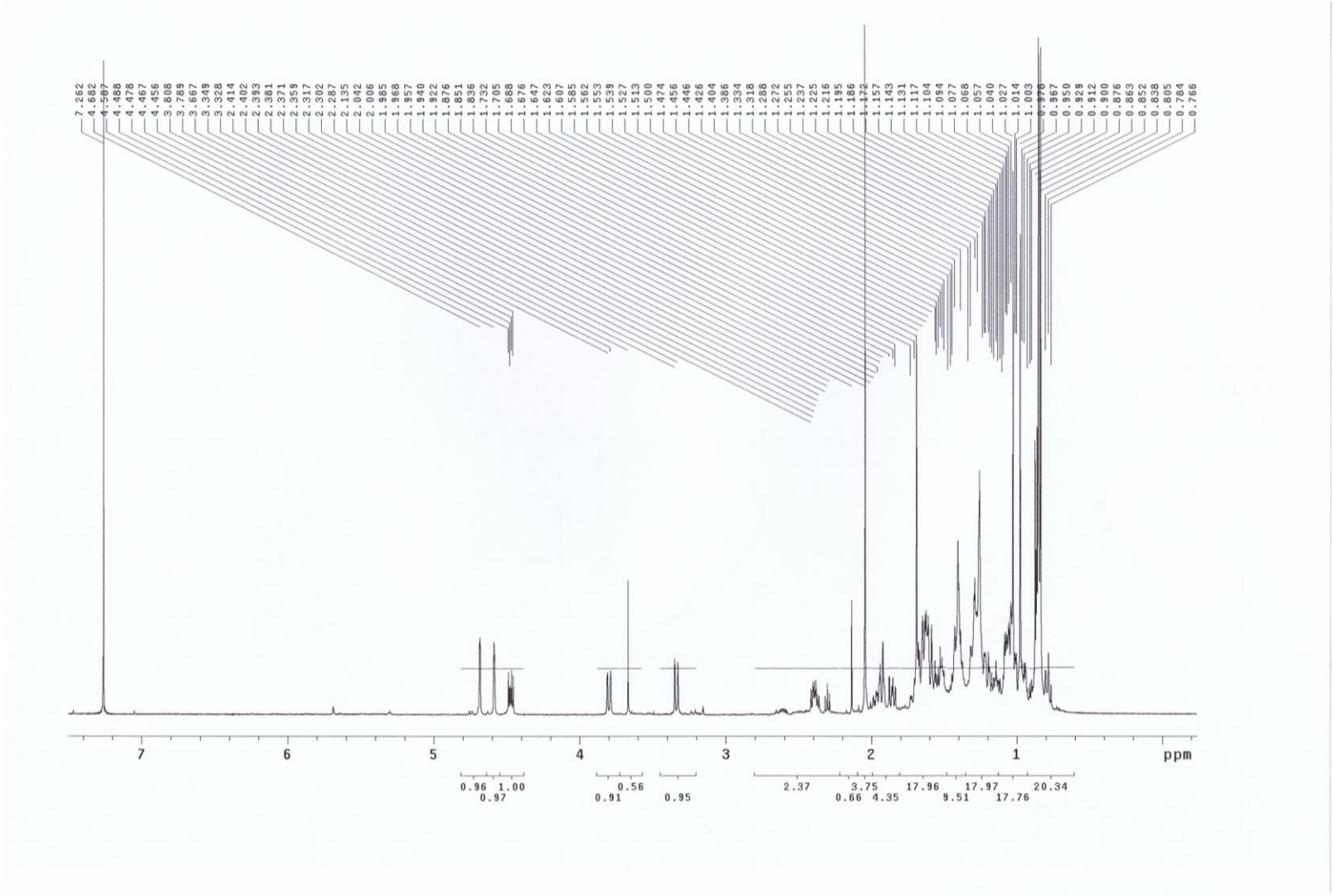

Figure S6. ^{1}H NMR spectrum of 3-*O*-acetylbetulin (**4**) ($CDCl_3$, 700 MHz)

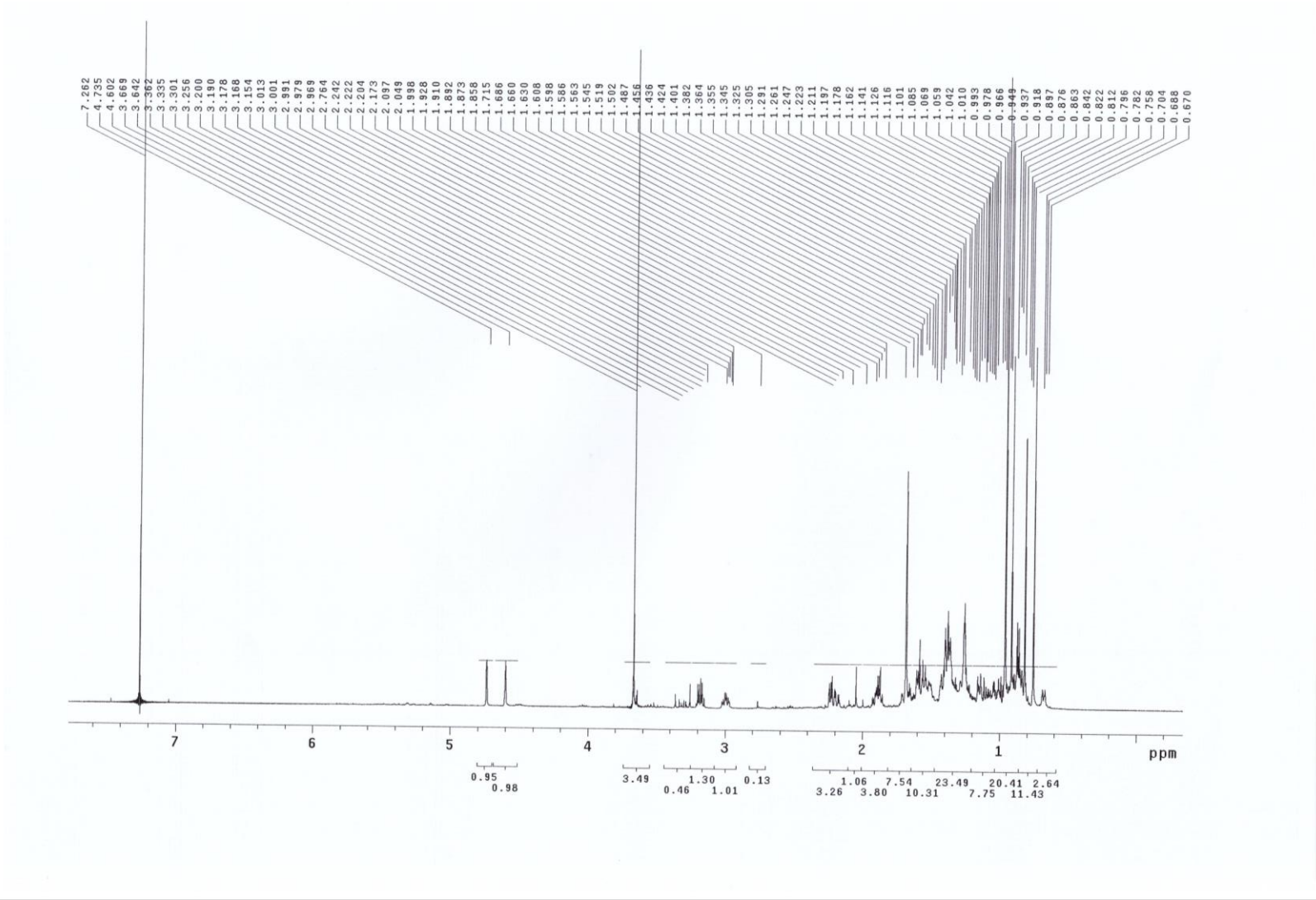

Figure S7. ^{1}H NMR spectrum of betulinic acid methyl ester (**5**) ($CDCl_3$, 700 MHz)

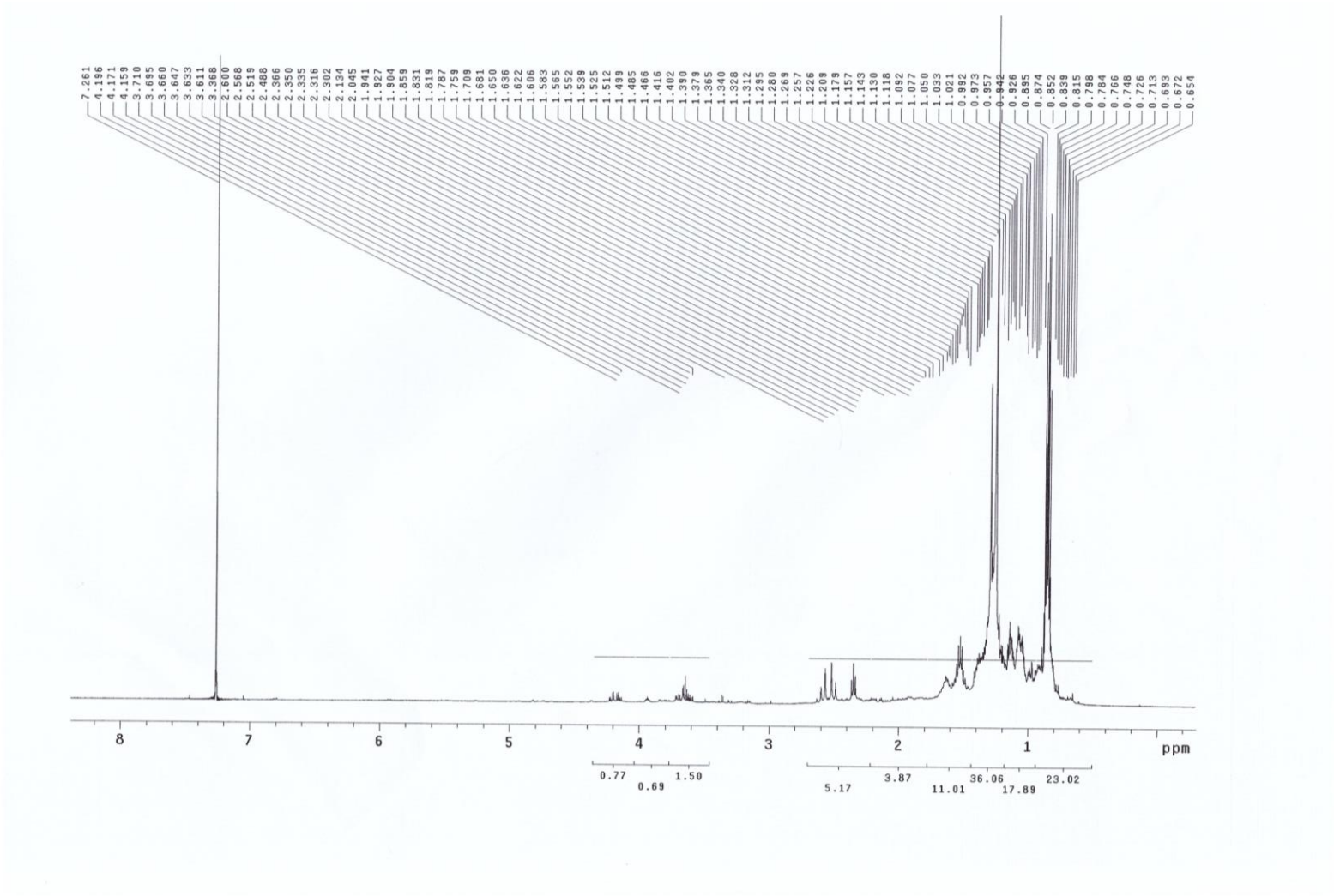

Figure S8. ^{1}H NMR spectrum of norphytan (**6**) ($CDCl_3$, 700 MHz)