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Spectroscopic Data of 3-O-Acetyl-betulinic Acid: An Antitumor Reagent

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In this paper, the spectroscopic data of the 3-O-acetyl betulinic acid is reported. This compound was prepared by enzymatic reaction of betulinic acid and acetic anhydride in the presence of lipase from *Candida antarctica* (Novozem® 435) at 54 ºC for 20 h in 79.3 % yield.

Key Words: Betulinic acid, 3-O-Acetyl-betulinic acid, Mass spectrometry, Nuclear magnetic resonance, Antitumor.

INTRODUCTION

Betulinic acid (**1**) is a naturally occurring pentacyclic lupane-type triterpenoid that possess multiple pharmacological activities including inhibition of human immunodeficiency virus (HIV), antibacterial, antimalarial, antiinflammatory, anthelmintic, antioxidant and anticancer properties[1]. Nevertheless, the medicinal uses of betulinic acid in the pharmaceutical industry is strongly limited since it is insoluble in water (0.02 mg/mL), which causes a difficulty in preparation of injectable formulations for biological assays and decreases its bioavailability in the organism. The introduction of polar groups at the C-3 and C-28 positions such as phthalates, amino acids or sugar moieties increases, in certain cases, the hydrosolubility and anticancer activity[2,3].

Previously, 3-O-acetyl betulinic acid (**2**) was synthesized and characterized by conventional methods[4-6]. This compound has been shown better cytotoxicity than betulinic acid against some cancer cell lines (*e.g.* human ovary)[4]. However, analytical data of the compound has not been published in detail yet. We now reported the 1D and 2D NMR and mass spectroscopy data of this compound.

EXPERIMENTAL

Immobilized lipase (triacylglycerol hydrolase, EC 3.1.1.3; Novozym® 435, 10000 PLU/g) from *Candida Antarctica*, supported on a macroporous acrylic resin with a water content of 3 % (w/w) was purchased from Novo Nordisk A/S (Bagsvaerd, Denmark). Chloroform, *n*-hexane was obtained from Fisher chemicals. Betulinic acid was purified from Malaysian *Callistemon speciosus* by previous method[7]. Acetic anhydride was purchased from Acros Organics, Belgium. Ethyl

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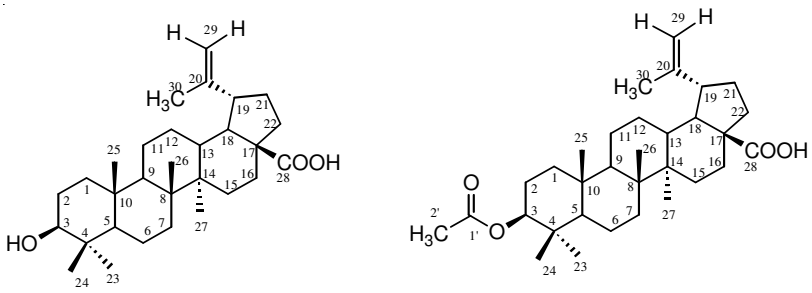
acetate, celite® 545, Na_2SO_4, K_2CO_3 and HCl were purchased from Merck, Germany. All chemicals were of analytical reagent grade.



Betulinic acid (**1**)

3-*O*-acetyl betulinic acid (**2**)

Structure of betulinic acid (**1**) and 3-O-acetyl betulinic acid (**2**)

Preparation of 3-O-acetyl- betulinic acid: 3-O-acetyl-betulinic acid could be prepared by the following procedure. A reaction mixture consisted of betulinic acid (150 mg, 0.328 mmol), acetic anhydride (37.1 mg, 0.364 mmol), Novozym® 435 (0.873 g), celite® 545 (1 g), K_2CO_3 (36 mg) in chloroform (50 mL) and *n*-hexane (50 mL) was magnetically stirred for 20 h at 54 ºC in a thermostated water bath at 150 rpm. After 20 h of reaction, the enzyme was removed by filtration and washed twice with chloroform. The filtrate evaporated to dryness and ethyl acetate was then added and washed separately twice with aqueous solution of HCl and water. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed with gradient on silica gel 60 (*n*-hexane/ethyl acetate, 9:1-5:1, v/v).

NMR spectra were recorded with a Varian Unity Inova 500 NMR spectrometer operating at a resonance frequency of 499.89 MHz for ^{1}H NMR spectra and 125.71 MHz for ^{13}C NMR spectra, respectively.

The electron ionization (EI) mass spectrum of the compound was recorded on Trace GC polaris Q spectrometer (Thermo Finnigan, Japan).

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of 3-O-acetyl-betulinic acid showed singlets at δ 0.84, 0.85, 0.86, 0.94, 0.98, and 1.70 which indicate the presence of 6 methyl groups of triterpene skeleton. These were attributed to 23-CH_3, 24-CH_3, 25-CH_3, 26-CH_3, 27-CH_3 and 30-CH_3, respectively. The signal at δ 2.05 (3H, s) was due to the protons of acetyl group of the ester carbonyl carbon attached to C-3. The presence of two hydrogens at C-29 position was confirmed by the signals at δ 4.62 (1H, br s) and 4.75 (1H, br s). The double doublet at δ 4.48 (1H, dd, J = 5.5, 10.5 Hz) was assigned as proton attached to the carbon bearing at C-3 position. The signal at δ 3.00 (1H, m)

was due to the hydrogen at C-19 position. The ^{13}C NMR spectrum of 3-O-acetyl-betulinic acid showed the presence of a signal at δ 81.19, which was assigned to C-3 of the desired compound, indicating the presence of a ester group at C-3 position. The ^{13}C NMR spectrum of the compound shows a carboxyl carbon signal at δ 182.13. The ^{13}C spectrum of 3-O-phthalyl- betulinic acid also exhibited the presence of the ester carbonyl carbon which resonated at δ 171.30 ppm. The signals at δ 150.60 ppm and 109.98 ppm due to carbon -carbon double bond between C-20 and C-29, respectively. The signal resonated at δ 21.55 was due to the methyl group of acetate. The ^{13}C NMR chemical shifts of the product are summarized in Table-1. Further confirmation of the compound carried out using the HMBC spectrum (Fig. 1). It was clearly indicated that proton signals for H-30 had correlation with C-19, C-20 and C-29 and H-29 signal was correlated with C-19 and C-30. The correlation of H-23 (methyl) and H-24 (methyl) with C-3 and C-5 was also observed on the spectrum. The HSQC spectrum (Fig. 2) showed that two hydrogen olefinic signals at δ 4.62 and 4.75 are correlated with C-29. The ^{1}H-^{1}H COSY data (Table-1) supported the expected connectivities as shown in Fig. 3. All ^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H COSY and HMBC data of the compound are summarized in Table-1.

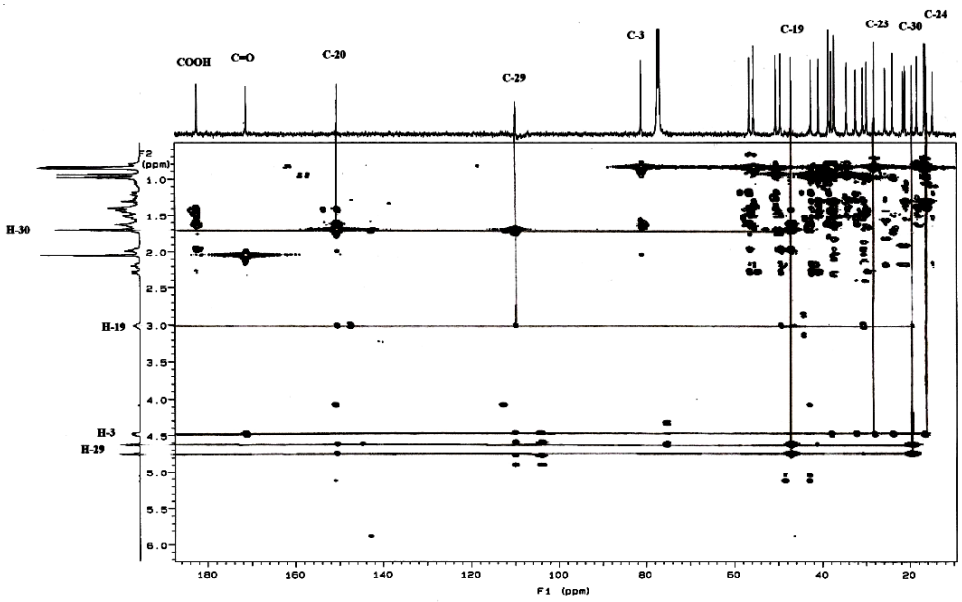


Fig. 1. HMBC spectrum of 3-O-acetyl-betulinic acid

The mass spectral data of 3-O-acetyl-betulinic acid indicated molecular ion at m/z 498 (M^+) and fragments at m/z 483, 456, 438, 423, 395, 248, 220, 203, 189 (Fig. 4). The base peak was observed at m/z 189 which is in agreement with the fragmentation as shown in Fig. 5.

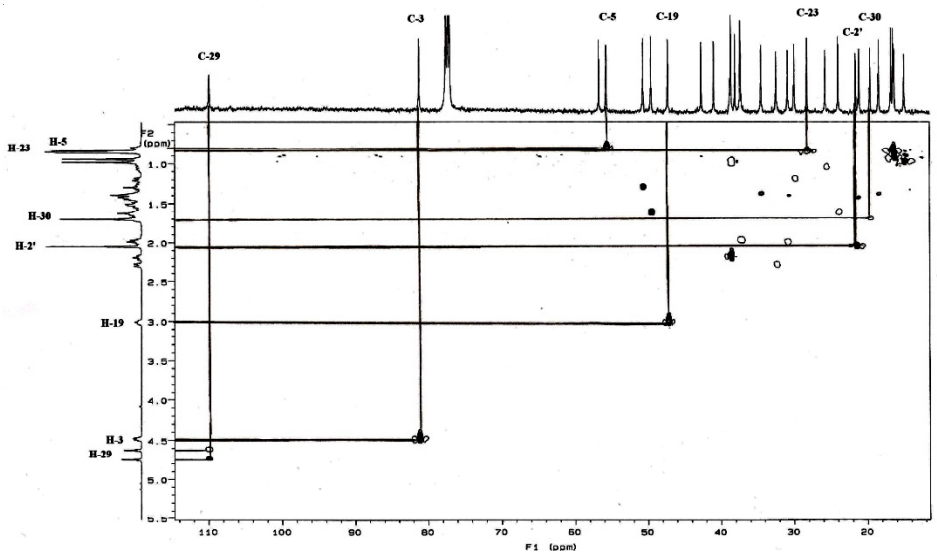


Fig. 2. HSQC spectrum of 3-O-acetylbetulinic acid

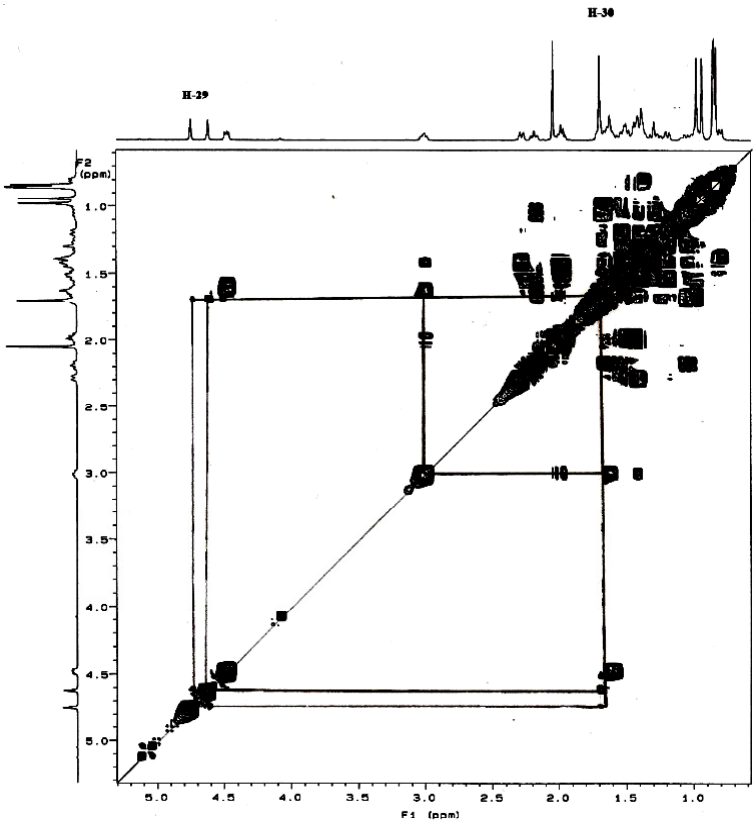


Fig. 3. COSY spectrum of 3-O-acetylbetulinic acid

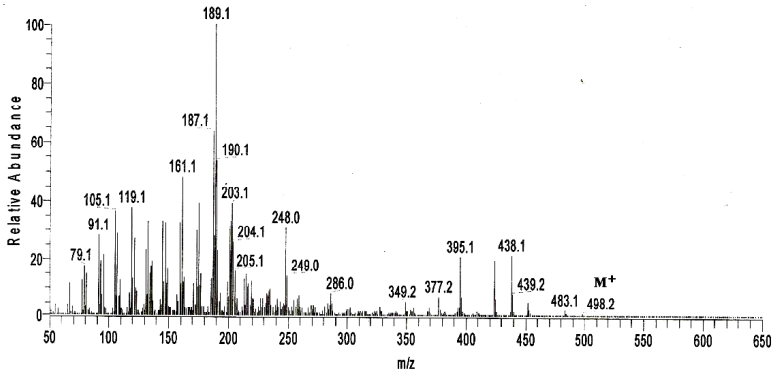


Fig. 4. Mass spectra of 3-O-acetylbetulinic acid

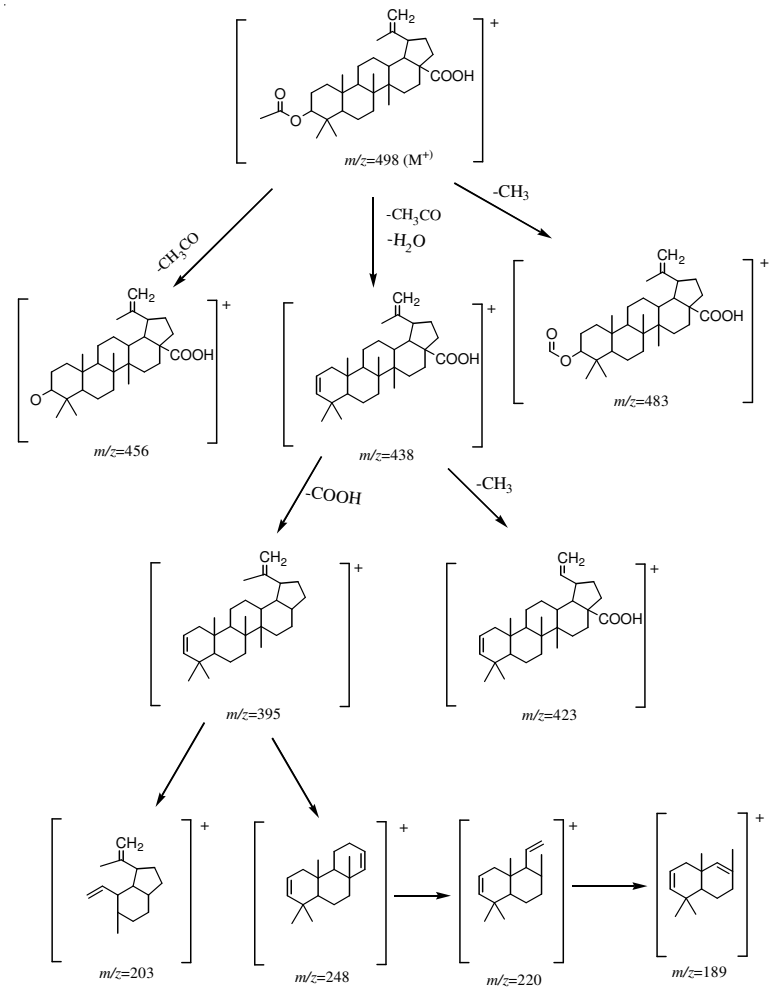


Fig. 5. Main cleavages of 3-O-acetylbetulinic acid obtained under electron ionization conditions

TABLE-1
^{1}H, ^{13}C NMR, ^{1}H-^{1}H COSY AND HMBC DATA OF 3-O-ACETYLBETULINIC ACID

Carbon	δ ^{13}C (ppm)	δ ^{1}H (ppm)	HMBC	COSY
C-1	38.66			
C-2	18.39			
C-3	81.20	4.48 (dd)	H-23, H-24	
C-4	38.03			
C-5	55.65		H-23, H-24	
C-6	21.08			
C-7	34.47			
C-8	40.93			
C-9	50.62			
C-10	37.35			
C-11	23.93			
C-12	25.67			
C-13	38.61			
C-14	42.65			
C-15	30.80			
C-16	32.39			
C-17	56.65			
C-18	49.50			
C-19	47.18	3.00 (m)	H-29, H-30	H-30
C-20	150.60		H-30	
C-21	29.93			
C-22	37.29			
C-23	28.18	0.84 (s)		
C-24	16.28	0.85 (s)		
C-25	16.40	0.86 (s)		
C-26	16.70	0.94 (s)		
C-27	14.89	0.98 (s)		
C-28(COOH)	182.56		H-30	
C-29	109.98	4.62, 4.75 (br s)	H-29	H-30
H-30	19.58	1.70 (s)		
C-30	171.31			
C-1'(C=O of ester) C-2'				
(CH_3 of -OAC)	21.55	2.05 (s)		

ACKNOWLEDGEMENTS

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