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New Triterpenoid from the Roots of *Calotropis gigantea* (L) Dryand (Asclepiadaceae)

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Abstract Seven triterpenoid derivatives were isolated from the roots of *Calotropis gigantea* (L) *Dryand*, a Chinese traditional medicinal plant. Their structures were established on the basis of their spectroscopic data and comparison with those in the literature. A new triterpenoid acetate derivative, compound **7**, Calotropisis, was isolated and characterized by spectral methods.

Keywords *Calotropis gigantean,* Acelepiadaceae, Triterpenoid derivatives

1. Introduction

Calotropis gigantea (L) *Dryand* (Asclepiadaceae) is mostly distributed in tropical and semitropical areas, mainly in Southwest China and South China in the wild [1-3]. It has been used in traditional medicine for the treatment of various ailments like parasitic diseases, digestive bloating from gas, cough, leprosy, and asthma by the people of the Li nationality, who are indigenous to Hainan island in China. Additionally, the plant is also used as a source of methane through anaerobic fermentation for bio fuel production [4]. This plant has been thoroughly explored for its many medicinal properties [5-7]. The chemistry of *C. gigantea* has been extensively investigated, leading to the isolation, of pregnanes [8, 9], flavonoids [10], a nonprotein amino acid [11], cardenolides [12-14] and terpenes [15-19]. In the present paper, we describe the isolation and structural elucidation of new triterpenoid acetates identified as calotropisis.

2. Experimental

2.1. Instrumentation and Materials

NMR Spectra were recorded on a Bruker-DRX-400-NMR, (^{1}H at 400Hz and ^{13}C at 100Hz) spectrometer (Bruker Biospin Inc., Germany) and chemical shift values are given on a δ (ppm) scale with TMS as internal standard. 2D-NMR experiment was performed using standard Bruker micro-program (XWIN-NMR version 2.6 software. HR-EI-MS experiments were performed using a micro-mass–QTOF micro instrument, with an electro-spray ionization source (eV= 70 V, 80°C) (Waters Ltd., England). Column chromatography was carried out on silica gel (Merck kiesel gel 300-400 mesh, Qingdao Haiyang Chemical Group Company, China), TLCs were carried out on GF_{254} silica gel plates (Merck, Qingdao Haiyang Chemical Group Company, China). All solvents were of commercial grade and used after further purification by simple distillation.

2.2. Plant Material

The Roots of *C. gigantea* were collected in August 2016 from Hunan province, south of China, and the plant was authenticated by prof. Chen Quan Yuan at the college of biology, Northwest Normal University, China, where a voucher specimen (No. 20141016) has been deposited in the herbarium of author's laboratory.

2.3. Extraction and Isolation

The roots of the *C. gigantean* (Linn.) were air-dried for four weeks and ground into a powder. The root (15Kg) was sequentially extracted three times with 17 liters of ethanol at room temperature for 7 days each. Then, the extracts were filtered through cotton and concentrated with a rotary evaporator at 45°C for removal of the organic solvent and dried. A total of 3kg of ethanolic extracts were dissolved in 5L hot distilled water and then prepared by successive partition with petroleum ether (PE) (40-60°C) (fraction **I**), chloroform (fraction **II**), ethyl acetate (fraction **III**) and methanol (fraction **IV**). Each partition step was repeated three times to ensure complete extraction in each case.

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Fraction **I** was evaporated to yield 20g, which was chromatographed on 500g of silica gel (Merck kiesel gel 300-400 mesh) using (PE:C_6H_6) in a gradient elution (30:1-0:1) as the mobile phase. Three sub-fractions were obtained (A_1, A_2 and A_3). Fraction A_1 was again subjected to column chromatography, eluted with PE: EtOAC (gradient 30:1- 20:1) to obtained two further sub-fractions (A_{1-1}–A_{1-2}). Sub-fraction A_{1-1} chromatographed using a PE:EtOAc gradient (30:1-17:1) elution to yield compound **1**. Sub-fraction A_{1-2} was chromatographed using a PE-EtOAc (17:1) elution and then recrystallized with a 1:1 mixture of MeOH:$CHCl_3$ to obtain compound **2**. Fraction A_2 was also subjected to chromatography with a PE: EtOAc gradient (15:1-5-1) elution to yield compounds **3**, **4** and **5** respectively. Fraction A_3 was loaded onto a column and chromatography using a PE:EtOAC gradient (10:1-1:1) elution to provide compounds **6** and **7** respectively.

Taraxasteryl acetate 1, obtained as white needle crystal, 34 mg, m.p 253-255°C. EI-MS m/z: 468 [M-CH_3], $C_{33}H_{54}O_2$. ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 0.73 (3H, s, H-28), 0.79-083 (9H, s, H-23, 24, 29), 0.85 (3H, t , J= 7.3Hz, H-3'), 0.95 (3H, s, H-26), 0.97 (3H, s, H-25), 1,03 (3H, s, H-27), 2.21 (2H, t, H-2'), 4.05 (1H, dd, J= 8.7, 3.2 Hz, H-3), 4.53 (1H, br s, H-30). ^{13}C-NMR ($CDCl_3$, 100MHz) δ 38.34 (C-1), 23.31 (C-2), 80.61 (C-3), 37.65 (C-4), 55.29 (C-5), 18.18 (C-6), 33.60 (C-7), 40.0 (C-8), 50.03 (C-9), 37.57 (C-10), 21.33 (C-11), 25.8 (C-12), 38.84 (C-13), 41.95 (C-14), 26.58 (C-15), 39.14 (C-16), 34.41 (C-17), 48.59 (C-18), 38.25 (C-19), 154.4 (C-20), 25.65 (C-21), 39.35 (C-22), 27.88 (C-23), 16.60 (C-24), 15.38 (C-25), 16.25 (C-26), 14.06 (C-27), 26.08 (C-28), 19.43 (C-29), 107.23 (C-30), 171.92 (C-1'), 21.16 (C-2').

***α*-amyrin acetate 2**, obtained as white crystal, 154 mg, m.p 259-262°C. EI-MS m/z 468, $C_{32}H_{52}O_2$. ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 0.79 (3H, s, H-28), 0.86 (12H, s, H-23), 0.87(3H, s, H-24), 0.97(3H, s, H-29) ,1.00(3H, s, H-30), 1.06 (3H, s, H-26) 1.24(3H, s, H-25), 0.93 (3H, s, H-26), 0.96 (3H, s, H-25), 1,02 (3H, s, H-27), 2.03(3H, s, O-CH_3), 2.27 (1H, dd, *J* = 7.6, 3.2 Hz, H-18), 4.50 (1H, dd, J= 8.7, 4.3 Hz, H-3), 5.12 (1H, br s, H-12). ^{13}C-NMR ($CDCl_3$, 100MHz) δ 38.45 (C-1), 28.73 (C-2), 80.33 (C-3), 38.45 (C-4), 55.24 (C-5), 18.23 (C-6), 32.85 (C-7), 40.01 (C-8), 47.41 (C-9), 36.80 (C-10), 23.36 (C-11), 124.25 (C-12), 139.49 (C-13), 42.5 (C-14), 26.91 (C-15), 26.85 (C-16), 33.7 (C-17), 59.04 (C-18), 39.59 (C-19), 39.62 (C-20), 31.23 (C-21), 41.5 (C-22), 28.05 (C-23), 15.63 (C-24), 15.47 (C-25), 16.8 (C-26), 23.5 (C-27), 28.38 (C-28), 17.04 (C-29), 21.3 (C-30), 170.94 (C-1'), 21.40 (C-2').

***α*- amyrin caprylate 3**, obtained as white crystal, 43 mg, m.p. 160-163°C. EI-Ms m/z 552, $C_{38}H_{64}O_2$, ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.78 (3H, s, H-28), 0.85 (12H, s, H-23, 24, 29, 30), 0.88 (3H, t, J= 7.3Hz, H-8'), 0.94 (3H, s, H-26), 0.95 (3H, s, H-25), 1,15 (3H, s, H-27), 1.21-1.30 (10H, m, H-3'-7') 2.02 (2H, t, H-2'), 2.76 (1H, m, H-18), 4.49 (1H, dd, J= 8.7, 4.5 Hz, H-3), 5.11(1H, br s, H-12). ^{13}C-NMR ($CDCl_3$, 100MHz) δ 38.76 (C-1), 28.70 (C-2), 80.29 (C-3), 38.14 (C-4), 55.01 (C-5), 18.69 (C-6), 32.56 (C-7), 40.89 (C-8), 47.53 (C-9), 36.80 (C-10), 23.36 (C-11), 124.28 (C-12), 139.58 (C-13), 42.31 (C-14), 27.20 (C-15), 26.38 (C-16), 33.69 (C-17), 59.89 (C-18), 39.24 (C-19), 39.58 (C-20), 31.03 (C-21), 41.50 (C-22), 28.68 (C-23), 16.23 (C-24), 15.71 (C-25), 16.71 (C-26), 23.55 (C-27), 28.07 (C-28), 17.42 (C-29), 21.38 (C-30), 170.94 (C-1'), 34.42 (C-2'), 25.12 (C-3'), 29.24-29.33(C-4', 5', 6'), 23.69 (C-7'), 14.10 (C-8').

***11-oxo-α*-amyrin ethylated 4**, was obtained as white crystal, 31 mg, m.p. 278-281°C. EI-MS m/z 496, $C_{33}H_{52}O_3$. ^{1}H-NMR: (400 MHz, $CDCl_3$): δ 0.79 (3H, s, H-28), 0.86 (12H, s, H-23, 24, 29, 30), 0.88 (3H, t , J= 7.3Hz, H-3') 0.94 (3H, s, H-26), 0.95 (3H, s, H-25), 1,15 (3H, s, H-27), 2.02 (3H, q, H-2'), 2.76 (1H, m, H-18), 4.50 (1H, dd, J= 10.7, 4.5 Hz, H-3), 5.57(1H, s, H-12). ^{13}C-NMR ($CDCl_3$, 100MHz) δ 38.93 (C-1), 28.68 (C-2), 80.89 (C-3), 38.24 (C-4), 55.23 (C-5), 18.92 (C-6), 32.35 (C-7), 40.21 (C-8), 47.20 (C-9), 36.92 (C-10), 199.55 (C-11), 130.36 (C-12), 164.85 (C-13), 42.01 (C-14), 27.11 (C-15), 26.43 (C-16), 33.33 (C-17), 59.03 (C-18), 39.60 (C-19), 39.18 (C-20), 31.22 (C-21), 41.67 (C-22), 28.80 (C-23), 16.25 (C-24), 15.53 (C-25), 16.90 (C-26), 23.67 (C-27), 28.37 (C-28), 17.45 (C-29), 21.27 (C-30), 170.90 (C-1'), 34. 36 (C-2'), 14.24 (C-3').

***11, 12*-Oxidotaraxerol acetate 5**, obtained as colourless needles, 29 mg, m.p. over 300°C. EI-MS, m/z 482, $C_{32}H_{50}O_{23}$. ^{1}H-NMR (400MHz): 0.79, 0.84, 0.88, 0.90, 0 93, 0.98, 1.13, l.29 (each 3H,s, Me x 8), 2.02 (3H, s, OAc) 2.52(1H,d,5=4.4Hz, H-12), 2.98 (d, *J* = 4.7 Hz, 1H), 3.12 (lH, t, J= 44 Hz, H- 11), 4.50 (lH, dd, 5=10.5, 5.8Hz, H-3), 5.56 (lH, dd, 5=8.3, 3 5 Hz., H-15). ^{13}C-NMR ($CDCl_3$, 100MHz) δ 37.99 (C-1), 23.23 (C-2), 80.29 (C-3), 37.62 (C-4), 54.59 (C-5), 18.77 (C-6), 33.12 (C-7), 38.87 (C-8), 51.80 (C-9), 37.45 (C-10), 53.71 (C-11), 58.12 (C-12), 36.46 (C-13), 157.0 (C-14), 118.89 (C-15), 35.20 (C-16), 35.33 (C-17), 48.06 (C-18), 40.21 (C-19), 28.70 (C-20), 36.77 (C-21), 38.20 (C-22), 27.89 (C-23), 17.44 (C-24), 16.51 (C-25), 27.11 (C-26), 30.20 (C-27), 29.90 (C-28), 33.66 (C-29), 19.50 (C-30) 172.13 (C-1'), 20.96 (C-2').

Germanicol acetate 6, was obtained as white powder, 36 mg, 279-283°C. EI-MS m/z 468, $C_{32}H_{52}O_2$. ^{1}H- NMR (400 MHz, $CDCl_3$): δ 0.77 (3H,s, H-27), 0.84 (3H,s, H-24), 0.85 (3H,s, H-23), 0.92 (3H,s, H-26), 0.93 (3H,s, H-29), 0.97 (3H,s, H-30), 1.05 (3H,s, H-28), 1.09 (3H,s, H-25), 2.01 (3H, s, CH3-CO), 4.48 (1H, dd, J =11.5, 6.2 Hz, H-3), 5.35 (1H, s, H-19) . ^{13}C-NMR ($CDCl_3$, 100MHz) δ 38.29 (C-1), 23.40 (C-2), 80.24 (C-3), 37.52 (C-4), 55.25 (C-5), 18.29 (C-6), 33.60 (C-7), 40.31 (C-8), 50.20 (C-9), 37.04 (C-10), 21.35 (C-11), 26.38 (C-12), 39.60 (C-13), 42.20 (C-14), 26.71 (C-15), 38.16 (C-16), 34.34 (C-17), 48.05 (C-18), 39.25 (C-19), 156.86 (C-20), 25.73 (C-21), 38.84 (C-22), 27.07 (C-23), 16.62 (C-24), 16.32 (C-25), 15.89 (C-26), 14.59 (C-27), 18.90 (C-28), 25.73 (C-29), 109.57 (C-30), 170.73 (C-1'), 21.19 (C-2').

Compound 7, obtained as a colorless powder, 33 mg, m.p. over 300°C, HR-ESI-MS (positive mode) *m/z*= 618.5548[M+NH_4]$^+$, ^{1}H-NMR and ^{13}C-NMR **Table 1**.

3. Results and Discussion

The seven isolated compounds were identified as taraxasteryl acetate (**1**), α-amyrin acetate (**2**), α- amyrin caprylate (**3**), 11-oxo-α-amyrin ethylate (**4**), 1 l, 12-Oxidotaraxerol acetate (**5**), germanicol acetate (**6**), and Calotropisis (**7**). **Figure 1** shows the structures of the triterpenoid isolated.

The six first compounds identified by direct comparison of their ^{1}H- and ^{13}C-NMR spectroscopic data with those reported in the literature [20-26] respectively. Compound **7** was obtained as a colorless powder, (25 mg), molecular formula $C_{37}H_{60}O_{6,}$ HR-ESI-MS (positive mode) showed beak at 618.5548 m/z which correspond to $[M+NH_4]^+$. ^{1}H-NMR spectrum, exhibited six methyl protons δ 0.83-0.87 (12H, s, H-26, 24, 29, 30), 0.96 (3H, s, H-25) and 1,02 (3H, s, H-27), two signal triplet at δ 0.88 (3H, t, J= 7.3Hz, H-5') and 0.93 (3H, t, J= 7.3Hz, H-3") also 1H-NMR spectrum showed extra signals, quartet appeared at δ 4.11 (2H, q, J= 14.1, 7.0, H-2") and triplet at δ 2.27 (2H, t, H-2'), one olefinic proton at δ 5.17 (1H, br s, H-12) and one signals at δ 4.47 (1H, dd, J= 10.7, 4.4 Hz, H-3), were observed in the ^{1}H-NMR Spectrum. The ^{13}C-NMR spectrum also showed six methyl carbons at δ 16.01, 14.66, 16.57, 28.31, 33.46 and 24.32ppm, two olefinic carbons at δ 122.58 and 144.80 ppm, three oxygenated methane carbons at δ 60.14, 65.38 and 73.17 ppm, and two carbonyls at δ 172.08 and 173.07, **Table 1.** The analysis of COSY, and HMBC experiments showed the correlation between methane proton at C-12 (δ_C 122.68) and C-9 (δ_C 47.48), C-14 (δ_C 41.60) and two bonds correlated with C-11(δ_C 23.72) and C-4 (δ_C 38.33). Also the HMBC, **Fig 2 and 3** respectively, showed the connection between methyl proton at δ 0.93 (3H, t, J= 7.3Hz, H-3") and C-2" (δc 60.14) which indicated the presence of ethyl group, the position of it was established from correlation of methylene group at δ_H 4.11(2H, q, J= 7.0, H-2") with C=O -28 at 173.07. Further confirmation was obtained from the upfield shift of carbonyl group at C-28 to 173.07 ppm compared with that of the ursolic acid carbonyl at δ 180.49 [27]. Another correlation between the methylene group at δ 2.27 (2H, t, H-2') with the carbonyl group at δ 172.08 indicated the presence of aliphatic shain on the other side of molecule. According to the above information and by comparison with related reported literatures of related compounds [28], the structure of compound **7** was elucidated as Calotropisis.

Table 1. ^{1}H-NMR and ^{13}C-NMR data of compound 7 (100 MHz, $CDCl_3$)

Position	δc	δ_H	Position	δc	δ_H
1	38.9	1.60, 1.0(2H, m)	20	31.15	
2	27.46	1.46-138(2H, m)	21	73.17	4.23(1H, dd, J= 10.6,3.0Hz)
3	80.63	4.47(1H, dd, J=8.7Hz)	22	37.28	
4	38.33		23	65.38	4.01(2H, dd, J= 11.5, 5.7Hz)
5	55.20		24	16.01	0.84 (3H, s)
6	18.16		25	14.66	0.96 (3H, s)
7	32.44		26	16.57	0.83 (3H, s)
8	39.59		27	25.76	1,02 (3H, s)
9	47.48		28	28.61	
10	36.99		29	33.46	0.87 (3H, s)
11	23.72	2.04 (2H, d, J=7.0Hz)	30	24.32	0.94 (3H, s)
12	122.58	5.17 (1H, br s)	1’	172.08	
13	144.8		2’	34.28	2.27 (2H, t)
14	41.60		3’	25.23	1.39 (2H, m)
15	26.8		4’	23.33	1.62 (2H, m)
16	26.06		5’	14.23	0.88 (3H, t, J= 7.3Hz)
17	31.92		1"	173.07	
18	47.15	2.17(1H, d, J=7.4Hz)	2"	60.14	4.11(2H, q, J= 7.0Hz)
19	46.41		3"	14.16	0.90 (3H, t, J= 7.3Hz)

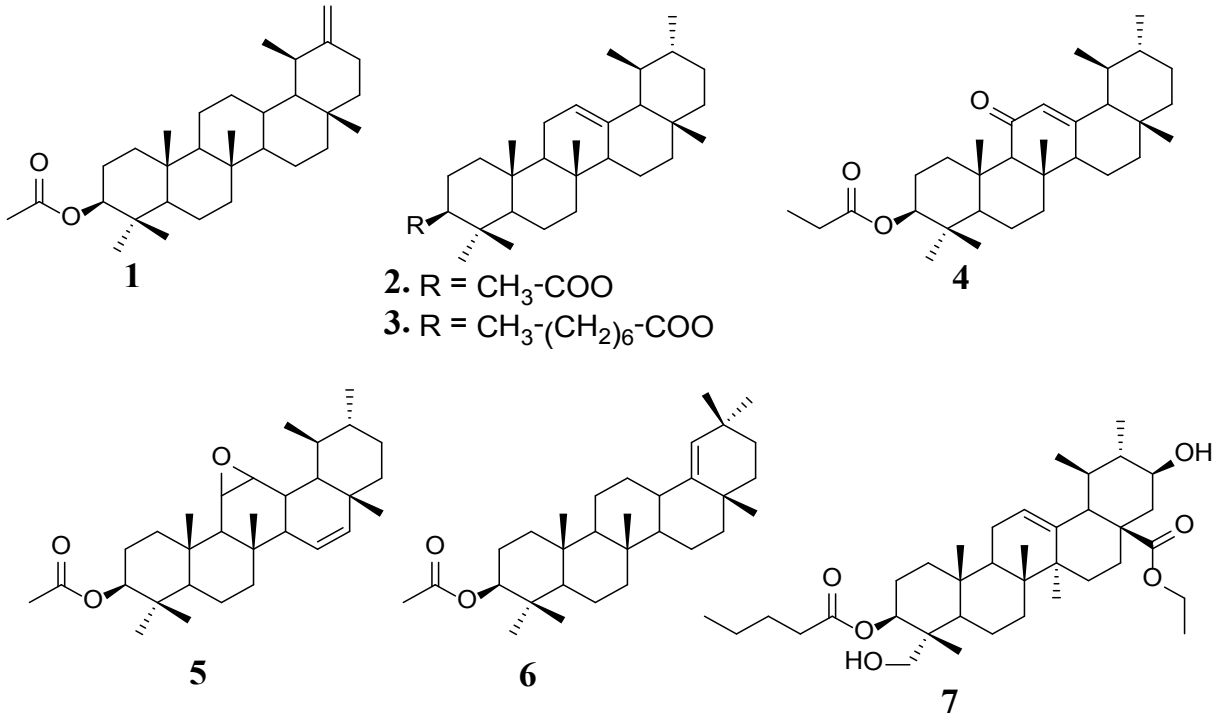

Figure 1. Structures of compounds **1–7**

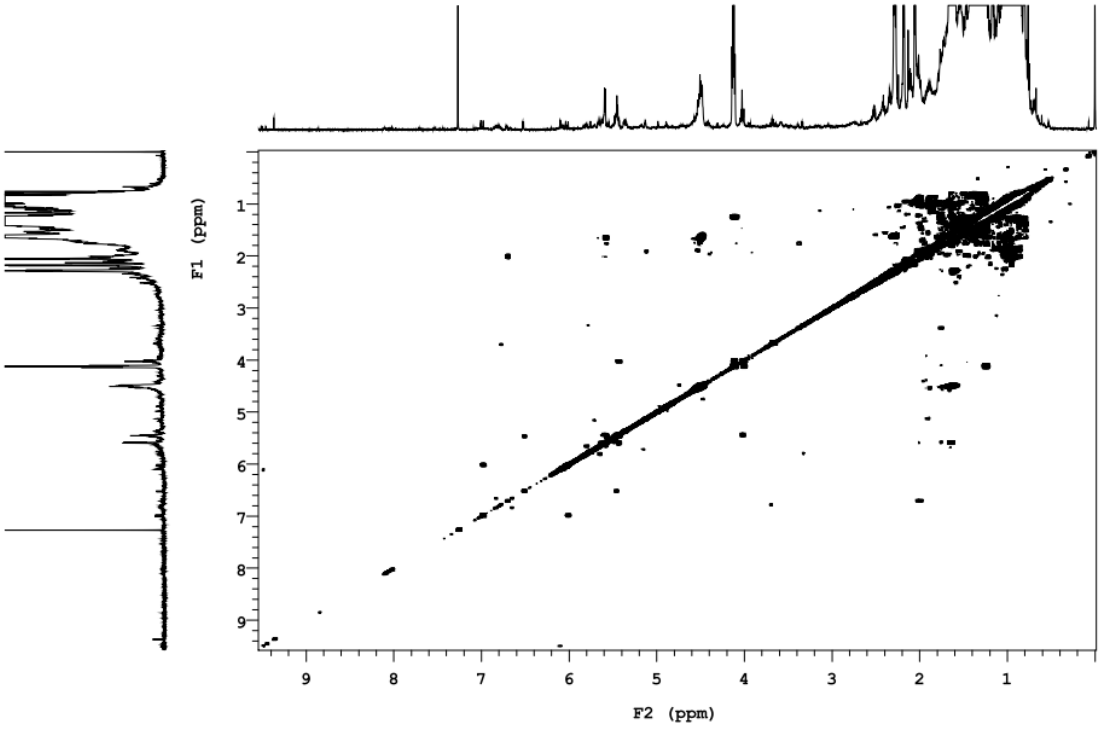

Figure 2. ^{1}H-^{1}H COSY spectra of compound 7

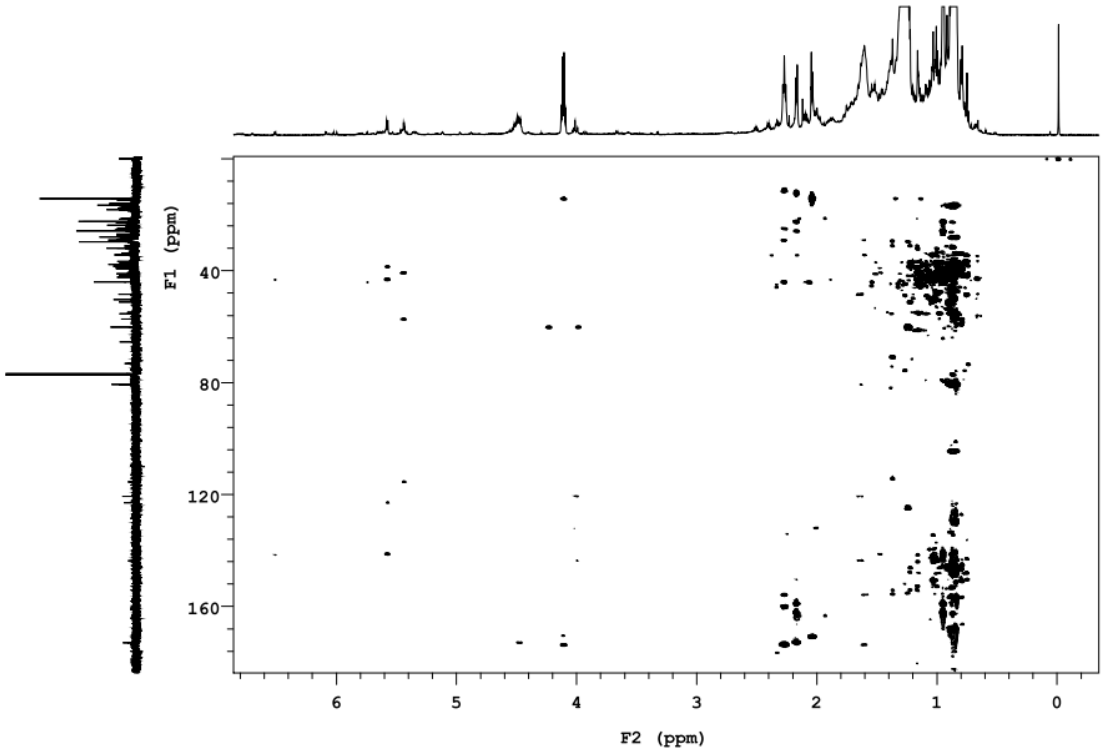

Figure 3. HMBC spectra of compound 7

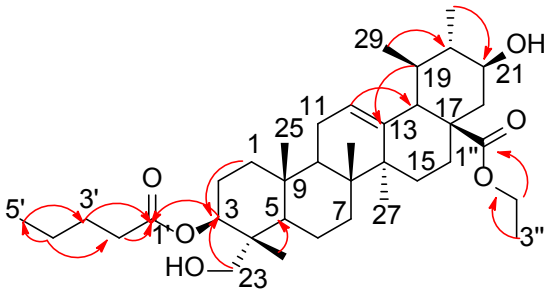

Figure 4. The key HMBC correlations observed in compound **7**

4. Conclusions

Based on the results of our present study, roots of *C. gigantean* contain triterpenoid derivatives, as the principal secondary metabolites. A new triterpenoid acetate derivative, calotropisis with six known compounds were separated and their structures identified by spectral analysis.

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