Research Article

Two New Phthalate Derivatives from Bark of *Albizia amara* (Fabaceae)

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ABSTRACT

Two phthalate derivatives with two known compounds were isolated from the chloroform soluble fraction of an acetone extract of *Albizia amara* (Fabaceae). The structures of the isolated compounds were elucidated as,2"-methyl-6-"oxo-6- propoxyhexyl-2'-(methyloctyl) phthalate 1,6"-ethoxy-2"-methyl- 6"oxohexyl)-2'-(methyldocosyl) phthalate **2**, Lupeol **3,** and Octacosyl ferulate **4**by extensive spectroscopic studies, including 1D, 2D-NMR, MS analyses and by comparison with literature review.

Keywords: *Albizia amara* (Fabaceae), Octacosylferulate, phthalate derivatives.

INTRODUCTION

Albizia amara is belonging to the (Fabaceae) family, it is an important medicinal plant found throughout Sudan. The entire plant possesses pharmaceutical constituents of great significance[1], in traditional medicinal the roots are chewed and applied to an eye infection of cattle. It is also used in making soap for washing. Fruits are used as anti-emetic and for treating coughs and malaria[2]. *Albizia amara* has been reported to possess bone fracture healing, Anticancer, antibacterial, antioxidant, and anti-inflammatory activities[3-10]. Many active compounds have been reported from this plant including triterpenes, flavonoids, rare amino acids, lipids, steroids and macrocyclic alkaloids[11], in this study we isolated and structural elucidated of2"-methyl-6-"oxo-6- propoxyhexyl-2'-(methyloctyl) phthalate **1**, 6"-ethoxy-2"-methyl- 6"oxohexyl)-2'-(methyldocosyl) phthalate **2** ,Lupeol**3,** and Octacosylferulate **4. Fig: 1.**

MATERIALS AND METHODS

General experimental procedure

The NMR Spectrum were recorded on a BrukerAvance DRX-400-spectrometer (^{1}H at 400MHz and ^{13}C at 100MHz) and chemical shift values are given on a δ (ppm) scale with TMS as internal standard, 2D-NMR experiment were performed using standard Bruker micro-program (XWIN-NMR version 2.6 software), APCI-MS experiment was performed using micro-mass –OTOF micro instrument, With an atmospheric pressure chemical Ionization source. Column chromatography was carried out on silica gel (Merck kiesel gel 300-400 mech), TLC were carried out on silica gel GF_{254} (Merck), all the chemicals and solvents were commercial grade and used after further purification.

Plant material

The bark of *Albizia amara* were collected in august 2014 from Zalingei area, central Darfur state –Sudan, the plant was authenticated by prof: G, A, Yagoub, department of basic sciences, faculty of agriculture, University of Zalingei. Voucher specimens (No. 20141014), have been deposited in the herbarium of author's laboratory.

Extraction and isolation

The barks of *Albizia amara* were air-dried for four weeks and grinded in to a powder. The bark powder (1Kg) was extracted three times with acetone at room temperature (each 7days × 2L). Then, the filtrates were combined and concentrated in vacuum (Rotary evaporation) for removal of the organic solvent and dried a total of 75g of acetone extracts were subjected to column chromatography (13×100cm column) over silica 1000g, the column eluted with chloroform until no elute come out yield 23g of dried extracts fraction (**I**), and then eluted with ethyl acetate yield 31g, fraction (**II**). Fraction **1** was subjected to CC (50×5cm) on silica gel 200g eluted with petroleum ether -$CHCl_3$ (20:1-1:1) elution to yield thirteen fractions (A to M) , then checked by Thin Layer Chromatography (TLC) used the (PE: $CHCl_3$) 10:1, 5:1, 1:1 as mobile phase, fractions showing similar on TLC were combined together to provided three fractions (**i**, **ii** and **iii**) fraction **i** was loaded on CC eluted again with PE -$CHCl_3$ (5:1) elution and then recrystallized with mixed MeOH- $CHCl_3$ (1:1) to obtained compound **3,** fraction **ii** was further purified on column chromatography used PE: $CHCl_3$(3:1) to afford pure compound **4.** Fraction **iii** was subjected on CC eluted with PE -$CHCl_3$ gradient (3:1, 2:1 and 1:1) elution to yielded compound **1**and **2** respectively.

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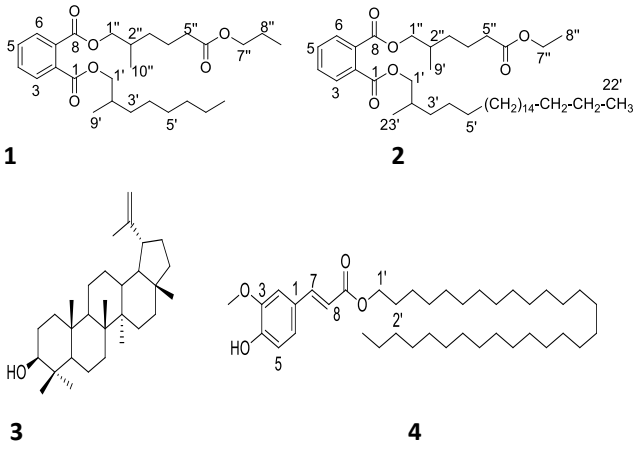

1 **2**

3 **4**

Figure 1: Structures of compounds **1, 2, 3,** and **4**

2''-(methyl pentatonic acid butane ester)-2'-methyloctyl phthalate **1** was obtained as the viscous deep yellowish oil. ^{1}H -NMR (400 MHz, $CDCl_{l3}$) and ^{13}C-NMR (100MHz, $CDCl_3$) **Table: 1,** APCI-MS data showed m/z 463 $[M+H]^+$ suggesting molecular weight 462 corresponding to the molecular formula $C_{27}H_{42}O_6$.

6''-ethoxy-2''-methyl- 6''oxohexyl)-2'-(methyldocosyl) phthalate **2** was obtained as the viscous red yellow oil.^{1}H -NMR (400 MHz, $CDCl_{l3}$) and ^{13}C-NMR (100MHz, $CDCl_3$). **Table: 1**, APCI-MS data showed m/z 663 $[M+H_2O]^+$ suggesting molecular weight 644corresponding to the molecular formula $C_{40}H_{68}O_6$.

Lupeol**3,** was obtained as white crystals; the compound gave purple color with 10% H_2SO_4 in Ethanol test, ^{1}H NMR(400 MHz, $CDCl_{l3}$): δ (ppm) = 0.76 (1H, s, H-24), 0.78 (1H, s, H-28), 0.82 (1H, s, H-25), 0.94 (1H, s, H-27), 0.96 (1H, s, H-23), 1.03 (1H, s, H-26), 1.52 (1H, m, H-2), 1.6 (1H, s, H-30) , 2.37 (1H, m, H-19), 3.18 (1H, dd, J = 11.0; 5.3 Hz, H-3), 4.56 (1H, s, H-29b), 4.68 (1H, s, H-29a) ^{13}C-NMR (100MHz, $CDCl_3$), (C-1) 38.77, (C-2) 27.42, (C-3) 78.97, (C-4) 38.77, (C-5) 55.33, (C-6)18.32, (C-7) 34.28, (C-8) 40.83, (C-9) 50.44, (C-10) 37.22, (C-11) 20.99, (C-12) 25.15, (C-13) 38.06, (C-14) 42.82, (C-15) 27.42, (C-16) 35.58, (C-17) 42.98, (C-18) 48.30, (C-19) 47.96, (C-20) 150.87, (C-21) 29.29, (C-22) 40.02, (C-23) 27.97, (C-24) 15.35, (C-25) 15.99, (C-26) 16.03, (C-27) 14.53, (C-28) 17.98, (C-29) 109.30, (C-29) 19.29. The APCI-MS), m/z 427+H] $^+$ which were correspond to the molecular formula $C_{30}H_{50}O$.

Octacosyl (E)-ferulate **4,** was obtained as white powder, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (1H, d, *J* = 15.9 Hz, H-7), 7.07 (1H, dd, *J* = 8.2, 1.7 Hz, H-6), 7.03 (1H, d, *J* = 1.7 Hz, H-2), 6.91 (1H, d, *J* = 8.2 Hz, H-5), 6.29 (1H, d, *J* = 15.9 Hz, H-8), 5.83 (1H, s, OH), 4.18 (2H, t, *J* = 6.7 Hz, H-1'), 3.93 (3H, s, OCH_3), 1.69 (2H, m, H-2'), 1.39 (2H, m, H-3'), 1.25 (44H, s, H-4' to H-22'), 0.88 (3H, t, *J* = 7.0 Hz, H-28'). The ^{13}C-NMR (100MHz, $CDCl_3$) (C-9) 167.32, (C-4) 147.87, (C-3) 146.73, (C- β7) 144.57, (C-1) 127.05, (C-6) 122.99, (C-α8) 115.69, (C-5) 114.67, (C-2) 109.29, (C-1') 64.58, (C-3-OCH_3) 55.91, (C-26') 31.90, (C-4' to C-25') 29.31- 29.67, (C-2') 28.76, (C-3') 25.98, (C-27') 22.66, (C- 28') 14.08. The APCI-MS data gave m/z 587 which corresponding to $[M+H]^+$ and molecular formula of $C_{38}H_{66}O_4$.

RESULTS AND DISCUSSION

Compound **1** was obtained as the viscous deep yellowish oil. the APCI-MS data showed proton adduct molecular ion m/z 463.3741 $[M+H]^+$ suggesting molecular ion m/z 462 corresponding to the molecular formula $C_{27}H_{42}O_6$, the ^{1}H NMR spectrum of **1** displayed two signals at δ 7.71 (2H, dd, J = 5.7, 3.3Hz, H-3, H-6) and 7.52 (2H, dd, J = 5.7, 3.3 Hz, H-4, H-5) and two oxygenated methylenes at δ 4.30 (4H, m, H-1', H-1''), giving indication for the phthalate nature of the molecule[12–13], and a signal at *δ* 4.10 ppm (2H, t, *J*=14.7, 6.0 Hz, H-7'') indicated to oxygenated methylene group, along with a methylene triplet at δ 2.30 (2H, t, *J*=7.5 Hz, H-5'') were evident as an indication of a methyl ester group on one side chain[14].The spectrum further showed one methine signal at δ2.03 (2H,m, H-2'') one methylene at δ1.72(2H, m, H-2') and two methylenes at δ 1.44, 1.61(4H, m, H-3', H-3''). A 10 H multiplet at δH 1.27 – 1.30 was assigned to 5 methylenes H-4', H-5', H- 6', H-7' and H-8''. In HMBC spectrum the aromatic protons at δ 7.70 (H-3/H-6) showed cross-peaks to δ 132.29 (C-2, C-7), 130.87 (C-4, C-5), 167.27 (C-1, C-8), indicating the attachment of the phthalate carbonyls (C=O) to C-2 and C-7 at 132.29 ppm. Both oxymethylene protons at δ 4.30 (C1', C-1'') showed cross-peaks to (C-1, C-8) at δ 167.27, which confirmed that both ester chains started with methylene groups, the oxymethylene group at δ 4.10 showed cross peak with (C-6'' and 8'') at δ 173.22 and 19.10 respectively. Whereas, the methyl at δ 0.88 (H-8') showed a cross-peak with δ 22.7 (C-7') and δ 30.65 (C-6') representing a terminal methyl group. **Fig:2.** According to the above evidences and along with comparison to reported literature on similar compounds, the structure of compound **1** elucidated as 2''-(methyl pentatonic acid butane ester)-2'-methyloctyl phthalate. The ^{1}H and ^{13}C NMR data of compounds **2** and **1** are identical. However, the APCI-MS spectra of compound **2** showed a peak at m/z 663 $[M+H_2O]^+$ corresponding to more 13 units (CH_2) than compound **1**. Thus, compound **2**was identified as 6''-ethoxy-2''-methyl- 6''oxohexyl)-2'-(methyldocosyl) phthalate**.** We suggest these two compounds are first time isolated from natural sources. Some previous researches reported that the phthalate derivatives isolated from plant species are antiviral activity against DNA and RNA viruses[18-19]. Thus, the compound **1** and **2** might be antiviral actives. Further studies are needed to determinate the antiviral activities of these compounds. Compound **3**, was obtained as white crystals, the compound gave purple color with 10% H_2SO_4 in Ethanol test, which indicated the presence of triterpene skeleton in this molecule. The atmospheric pressure chemical ionization mass spectrometry (APCI-MS), showed peak at m/z 427[M+H] $^+$ which were correspond to the molecular formula $C_{30}H_{50}O$. The ^{1}H-NMR spectrum of compound **3** showed six tertiary methyl groups δ 0.76 (s, 3H), 0.78 (s, 3H), 0.82 (s, 3H), 0.94 (s, 3H), 0.96 (s, 3H) and 1.03 (s, 3H), represent methyl groups Me-28, 24, 29, 30, 25 and 26

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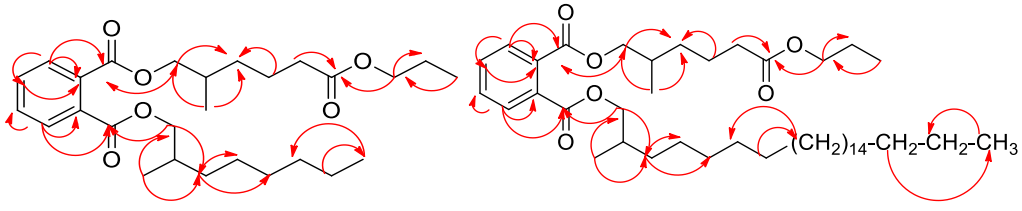
respectively, one vinyl methyl proton (δ 1.67, s, Me-30), one terminal methylene at δ 4.56 and 4.68 (1H, d , 1.9 Hz H_2-29), a CH-α oxymethine proton at δ 3.18 (1H, dd, J = 4.8, 11.4Hz, H-3) and the remaining signals due to methylene and methine protons in high field region (0.68- 2.36) were observed. ^{13}C- NMR chemical shift of compound **3** compared with those reported for Lupeol isolated from *Holarrhena floribunda*[15] were found to be in close agreement.

Compound **4** was obtained as white powder, the APCI-MS data gave m/z 587.5105 which corresponding to $[M+H]^+$ and molecular formula of $C_{38}H_{66}O_4$. The ^{1}H-NMR spectrum of compound **4** showed the signals at δ 6.29 (1H, d, *J*= 15.9 Hz, H-8) and at δ 7.60 ppm (1H, d, J= 15.9 Hz) characteristic of (E)-vinylic system; the signals at δ 6.91 (1H, d, J = 8.2 Hz, H-2), 7.03 (1H, d, J = 1.7 Hz, H-6) and at 7.07 ppm (1H, dd, J= 1.7, 8.2 Hz) typical of a tri-substituted aromatic ring; signal at δ 3.93 ppm (3H, s, H-10), signal at δ H 5.83 ppm attributed to hydroxyl proton. The presence of aliphatic chain esterified can be deduced from δ 4.18 (2H, t, *J* = 6.7 Hz, H-2'), 1.69 (2H, m, H-3'), the intense methylene signals at δ 1.25 (44H, br s), besides the triplet at δ 0.88 (3H, t, *J* = 7.0 Hz H-27'). The ^{13}C NMR spectrum of compound **4** was compared with Octacosyl (E)-ferulate which has been reported [16, 17] , their ^{13}C-NMR data were in good agreement.

Table 1: ^{1}H- and ^{13}C-NMR spectral data of Compound **1**and **2** in $CDCl_3$

Position	δ_C, 1	δ_H, 1	δ_C, 2	δ_H, 2
1/8	167.27	-	167.53	-
2/7	132.29	-	132.28	-
3/6	128.81	7.70 (2H,dd, J = 5.7, 3.3Hz)	128.81	7.71 (2H, dd, *J* = 5.6, 3.2 Hz)
4/5	130.87	7.52 (2H,dd, J = 5.7, 3.3 Hz)	130.87	7.52 (2H, dd, *J* = 5.6, 3.3 Hz)
1', 1"	65.54	4.30(2H, m)	65.54	4.30(2H, m)
2'	39.00	1.72 (H, m)	38.65	1.71 (H, m)
3'	31.90	1.44(2H, m)	30.56	1.43(2H, m)
4'	24.98	1.28 (2H, m)	24.68	1.28 (2H, m)
5'	29.16	1.26(2H, m)	-	-
6'	30.65	1.29(2H, m)	-	-
5'-18'	-	-	29.12-29.67	1.25- 1.30 (2H, m)
19'	-	-	31.88	1.30 (2H, m)
7', 20'	22.55	1.24 (2H, m)	22.67	1.24 (2H, m)
8',21'	13.96	0.88 (3H, t J= 6.9 Hz)	14.09	0.88 (3H, t J= 6.9 Hz)
9', 22'	19.17	0.96(3H, t, J= 7.4 Hz)	19.17	0.96(3H, t, J= 7.4 Hz)
6"	173.22	-	173.54	-
5"	34.04	2.30 (2H t, *J*=7.5 Hz)	34.39	2.30 (2H, m)
4"	24.68	1.61(2H, m)	24.98	1.61(2H, m)
3"	31.90	1.24(2H, m)	30.56	1.24(2H, m)
2"	39.00	2.03(H, m)	38.65	2.03(H, m)
7"	68.80	4.10(2H, t, *J*=14.7, 6.0 Hz)	60.11	4.11 (2H, m
8"	19.10		13.70	0.88(3H, t J= 6.9 Hz)
9"	13.70	0.88(3H, t J= 6.9 Hz)	19.17	0.96(3H,t, J= 7.4Hz)
10"	19.17	0.96(3H,t, J= 7.4Hz)	-	-



1 **2**

Figure 2: The key HMBC correlations observed in compounds **1** and **2**.

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