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ISOLATION AND IDENTIFICATION OF PHYTOCHEMICAL CONSTITUENTS FROM THE ROOTS OF *Calliandra portoricensis*

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ABSTRACT

Calliandra portoricensis is a plant widely used in Nigerian ethnomedicine for the treatment of tuberculosis and other respiratory conditions. In view of its traditional use, the present study is aimed at the isolation and identification of bioactive components or secondary metabolites which may be responsible for its biological activity. The air dried roots was extracted with hexane, ethyl acetate and then methanol via maceration at room temperature. Repeated column chromatography of the ethyl acetate extract over silica gel yielded linalonic acid, spinasterone, lupeol, daucosterol and triacontanyl caffeate. The structures of these compounds were determined by NMR, mass spectrometry and comparison with literature. This is an initial report of these compounds from the plant material.

Key words: Linalonic acid, Triacontanyl caffeate, Daucosterol, Spinasterone, Nigeria

INTRODUCTION

Calliandra portoricensis (Jacq) Benth belongs to the family Mimosaceae. It is a straggling perennial shrub found mostly in West Indies, Panama and Mexico. (Hutchinson, 1937). It is also found in many tropical countries such as Cote de Voire and Nigeria. The plant has a long standing use in Nigerian traditional medicine (Akah and Nwaiwu, 1985, Enwuru *et al*., 2017). The plant is used by traditional herbal medicine practitioners for the treatment of lumbago , constipation, gonorrhea, as a laxative and worm expeller, and convulsion (Adesida, 1976; Akah and Nwaiwu, 1988). Also reported is its anticonvulsant, analgesic, antidiarrheal, antispasmodic and antipyretic activities (Abdulkarim *et al*., 2005) and its extracts have shown anticholigenic, antacid, antiulcer, molluscidal and ovicidal activities in laboratory animals (Aguwal *et al*., 1988). Antimicrobacterial activities of the plants have been reported against *Escherichia coli*., *Staphylococcus aureus*, *Streptococcus faecuim* and *Candida albicans* (Adesina, 1982 and Abdulkarim *et al*., 2005). Phytochemical constituents such as tannins, saponins, flavonoids, cardiac glycosides have been reported to be present in the extracts of the plant (Aguwal *et al*., 1988; Lawal *et al*., 1998.; Orishadipe *et al*., 2004 and Falode *et al*., 2018). However, the phytochemical constituents of these active extracts were not isolated or identified. In this study we report for the first time the isolation of five compounds from the roots of *Calliandra portoricensis* and their spectroscopic identification.

MATERIALS AND METHODS

General

The ^{1}H and ^{13}CNMR (400 MHz) spectra were obtained on a Bruker DPX 400 spectrophotometer using $CDCl_3$ as solvent and TMS as internal standard. Barnstead Electrothermal 9100 melting point apparatus was used to determine melting points, without corrections. TLC was performed on silica gel F_{254} (0.25mm) precoated plates and column chromatographic were carried out in glass columns using silica gel (120-230 mesh, GmbH & Co. KG). Solvents were purchased from commercial sources and redistilled before use. Spots on tlc were visualized with 10% sulfuric acid in ethanol, followed by heating.

Plant Material

The roots of *Calliandra portoricensis* were purchased from a traditional healer in Jos, Plateau State, Nigeria and certified at the School of Forestry Jos. The roots were dried at room temperature for two

weeks and thereafter ground into powder and used for extraction.

Extraction and Isolation

The powdered roots 500 g was extracted with hexane, ethyl acetate and then methanol for 48 hours each. The solvents were recovered using a rotary evaporator at reduced temperature and pressure. 5.0 g of the ethyl acetate extract was dissolved in ethyl acetate and adsorbed on to 15g of silica gel, and allowed to dry at room temperature. A column was packed with 120 g of silica gel in hexane. The adsorbed extract was loaded unto the packed column. The column was eluted with hexane and then with increasing quantities of ethyl acetate in hexane and collecting 100 ml fractions. A total of 180 fractions were collected. All the fractions were examined by tlc and similar fractions were combined to obtain eleven pooled fractions F1 - F11. Fractions F3 and F4 were combined and re-run on a column. The column was similarly eluted and 20 ml fractions were collected and monitored by tlc. On standing fractions F'34, F' 37, F' 57, F'60 and F'67 yielded crystals that were further purified by recrystallization.

RESULTS AND DISCUSSION

Compound 1 was obtained as a white crystalline solid. The ^{1}H NMR spectrum of the compound revealed a multiplet at δ_H 5.18 typical of an olefinic H-5 or H-7 observed for steroids (Thuy *et al.*, 2008). Two other signals for olefinic protons H-22 and H-23 were observed at δ_H 5.35 and 5.04. The proton spectrum also showed two methyl doublets at δ_H 0.82, 0.84 (3H, d J = 6.5 Hz) for an isopropyl unit in the steroid (Keawpradub *et al.*, 2005). Two other methyl groups were observed at δ_H 0.58 and 1.02 and were assigned to the tertiary angular methyl groups at C-18 and C-19, while the two other methyl signals at δ_H 1.03 and 0.81 were for C-21 and C-29. The ^{13}C-NMR spectrum confirmed the presence of a saturated ring ketone at δ_C 212.07 usually at C-3 of a steroid skeleton (Agrawal *et al.*, 1985 and Ragas *et al.*, 2014), three olefinic methine carbons were observed at δ_C 117.7 (C-7), 138.2 (C-22), 129.4 (C-23) and a quaternary olefinic carbon at δ_C 139.5 (C-8). The ^{13}C-NMR spectrum also showed six methyls, nine methylenes, ten methines and four aliphatic quaternary carbon signals. This further confirmed that compound **1** has a steroidal skeleton (Ragasa *et al.*, 2014). Comparison of its chemical shift data (Table 1) with literature reports (Lee *et al.*, 2011 and Ripardo Filho *et al.*, 2012) identified the compound as spinasterone.

Compound 2 was obtained as a white powder. The ^{1}H and ^{13}C-NMR spectra were similar to those of compound **1**. However, the spectra of compound **2** also showed a typical pattern of a glucose moiety with the anomeric proton observed at δ_H 4.41 (Lee *et al.*, 2013 and Benabdelaziz *et al.*, 2014). The sugar was linked to C-3 of the aglycone as indicated by the higher chemical shifts for H-3 and C-3. Comparing the ^{1}H and ^{13}C NMR data for compound **2** (Table 1) and literature data, the compound was identified as daucosterol (Suga *et al.*,1999; Voutquenne *et al.*, 1999 and Lee *et al.*, 2011).

Compound 3 was also obtained as white crystals and had a melting point of 210-212 ^{O}C. The compound gave a purple spot characteristic of a terpenoid on spraying with Vanillin-sulphuric acid reagent and heating. The ^{1}H NMR spectrum (Table 2) showed the presence of two methylene protons at ppm 4.69 (d, J =1.68 Hz) and 4.57 ppm, a methyl attached to a double bond at 1.69 and six other methyl groups between 0.77-1.04 ppm. Proton H-3 and δ H-19 were observed at 3.19 (dd, J = 11.2, 5.0 Hz) and 2.38 (ddd, J = 5.8, 11.0, 11.0 Hz) ppm respectively. The ^{13}C NMR from the Deptq-135 (Table 2) spectrum showed a total of 30 carbon atoms made up of seven methyl carbons, one oxygen bearing carbon, one methylene carbon and one quaternary carbon attached to the methylene group. No carbonyl carbon(s) were observed (confirmed by the absence of a carbonyl carbon in its ^{13}C NMR spectrum). The compound was identified as lupeol based on comparison to literature reports (Igoli and Gray,2008).

Compound 4 was obtained as a pale yellow powder (melting point 188-190 ^{O}C) and Rf = 0.46 on tlc with toluene/ethyl formate/formic acid (5:4:1:). Its UV $_{max\ (CH3CN)}$ (nm) maxima were observed at 205, 240, 292 and 318. The IR

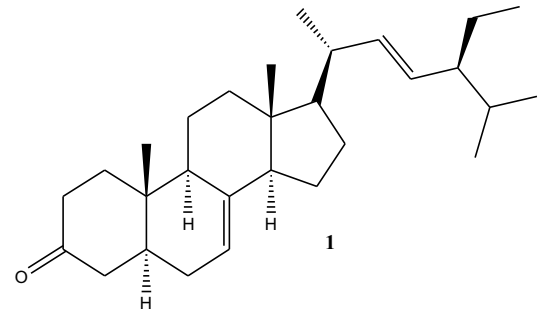
spectrum of the compound gave absorption bands at 3469 (-OH), 2933 (C-H), 1686 (C=O ester), 1642, 1513, 1542 (C=C), 1191 (C-O), 883 (Ar trisub) cm^{-1}. The ^{1}H and ^{13}C-NMR spectra showed aromatic and an alkanyl moiety. In the aromatic region, the proton spectrum showed an ABX spin system with signals at δ_H 6.65 (dd, *J* = 8.2, 2.0 Hz), 6.59 (d, *J* = 2.6 Hz), 6.51(m) respectively and are typical of a 1,3,4 trisubstituted phenyl unit and agrees with the IR spectrum (Fukuoka *et al.*, 1982). Also, two pairs of doublets at δ_H 7.34 (d, *J* = 15.8 Hz) and 6.39 (d, *J* = 15.8 Hz) were observed suggesting a trans propenyl chain. The ^{1}H and ^{13}C NMR indicated an oxygenated methylene group at δ_H 4.16 (δ_C 64.7), a methylene envelope between δ_H 1.25-1.39 (δ_C 23.1-32.4) for overlapping methylene signals (28 x CH_2) and a terminal CH_3 at δ_H 0.90 (δ_C 14.4) for the alkanyl moiety (Keawpradub *et al.*, 2005). In the ^{13}C-NMR spectrum a chemical shift at 167.3 confirmed the presence of an ester carbonyl group. The signals at 144.0 and 115.7 were for two olefinic carbons while the quaternary and phenolic carbons were observed at 146.1, 144.8, 122.7 and aromatic -CH at 116.5,115.7 and 122.0 all from a phenyl unit in the compound. The spectral data in comparison with literature reports confirmed compound **4** to be triacontanyl caffeate.

Analytical Data for Triacontanyl Caffeate (**4**)

White solid mp 188-190°C; TLC: R_f 0.46 (toluene/ethyl formate/formic acid (5:4:1); $UV\lambda_{max\ (CH3CN)}$ (nm) 205, 240, 292 and 318; ESI-MS: 567.5 $[M+Na]^+$ and 543.7 $[M-H]^-$; EI-MS m/z (rel. int.): 544 (3.5) $[M]^+$, 516 (18), 459 (15), 446 (31), 180 (10), 125 (12), 111 (24), 97 (47), 69 (58), 43 (100); Exact Mass (HR EI-MS) m/z 544.4474 $[M]^+$, $C_{35}H_{60}O_4$ requires 544.4492; $IR\nu_{max\ (KBr)}$ (cm^{-1}): 3469 (-OH), 2933 (C-H), 1686 (C=O ester), 1642 (C=C), 1513, 1542 (C=C), 1191 (C-O), 883 (Aromatic tri-subt.); ^{1}H NMR (250 MHz, $CDCl_3$) (ppm): 0.89 (3H, t, J = 7 Hz, $-CH_2$-Me), 1.25 (s, $[CH_2]_{n-2}$]), 1.70 (2H, m, $-OCH_2-CH_2-$), 5.42 (1H, s, -OH), 5.54 (1H, s, -OH), 6.29 (1H, d, J = 15.87 Hz, H-7), 6.88 (1H, d, J = 8.24 Hz, H-6), 7.05 (1H, dd, J = 8.55 and 1.87 Hz, H-5), 7.10 (1H, d, J = 1.87 Hz, H-2) and 7.59 (1H, d, J = 15.87 Hz, H-8); ^{13}C NMR (250 MHz, $CDCl_3$) (ppm): 14.14 (C-35), 22.70 (C-34), 26.01 (C-11), 28.77 (C-12), 29.32-29.72 (C-13…C-32), 31.95 (C-33), 64.78 (C-10), 114.41 (C-2), 115.56 (C-5), 116.08 (C-8) 122.43 (C-6), 127.85 (C-1), 143.76 (C-7), 144.48 (C-3), 146.09 (C-4), 167.62 (C-9).

Compound 5 was obtained from the column chromatography of the ethyl acetate extract on silica gel (eluted with 10% ethyl acetate in hexane) as a pale yellow oil. The ^{1}H NMR showed two methyl singlets at δ_H 2.09 (δ_C 14.2) and 1.30 (δ_C 12.0). The spectral data obtained from its 2D NMR and literature reports identified the compound as linalonic acid (Omori *et al.*, 2011).
Analytical data for Linalonic acid.

^{1}H NMR (500 MHz, $CDCl_3$, 1.39 (3H, s,),1.63 (1H, dddd, *J* = 19.8, 16.5, 8.0, 4.2 Hz), 2.09 (3H, s), 2.28 (1H, m), 2.76 (1H, dd, *J* = 6.7, 4.4 Hz), 3.73 (1H, d, *J* = 4.9 Hz), 5.1 (1H, d, *J* = 10.7), 5.23 (1H, d, *J* = 17.3 Hz), 5.90 (1H, dd, *J* = 17.4, 10.8 Hz), 6.87(1H, t, *J* = 7.5)



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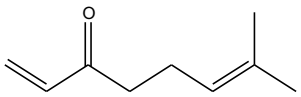

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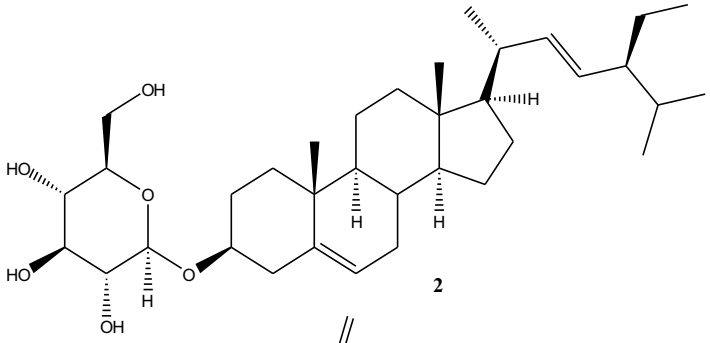

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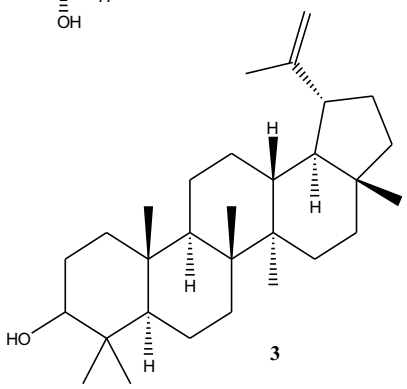

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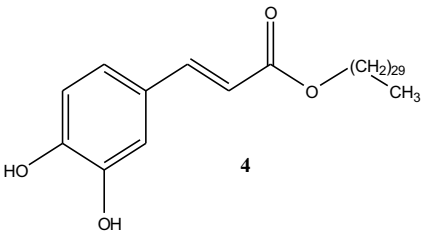

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Table 1: ^{1}H and ^{13}C-NMR data for compound 1 and 2

	Spinasterone				Daucosterol			
Position	Experimental		Literature		Experimental		Literature	
	$\delta_{H,}$ (mult.)	δ_C (ppm)	$\delta_{H,}$ (mult.)	δ_C (ppm)	$\delta_{H,}$ (mult.)	δ_C (ppm)	$\delta_{H,}$ (mult.)	δ_C (ppm)
1	1.48 (td), 2.13 (ddd)	38.9	1.44 (m), 2.13 (dddd)	38.8	1.47, 2.12	38.9		37.3
2	2.26 (m)	38. 2	2.28	38.0	2.22	38.2		29.4
3	-	211.7	-	211.7	--	79.2		78.5
4	2.26	44.4	2.24 (m)	44.3	2.23	43.3		38.4
5	1.83	43.0	1.80 (m)	42.3	-	139.5		141.3
6	1.83	30.2	1.83	42.9	5.18	117.7		122.3
7	5.18	117.2	5.18 (m)	116.7	1.83	31.6		31.8
8	-	139.7	-	139.3	1.81	30.4		30.6
9	1.76	49.0	1.77 (m)	48.9	-	49.1		50.7
10	-	34.6	-	34.4	-	37.0		36.8
11	1.55, 1.76	21.9	1.56, 1.75	21.7	1.56, 1.75	21.7		20.4
12	1.27, 2.04	39.5	1.27, 2.04	39.4	1.27, 2.04	40.2		40.3
13	-	43.4	-	43.4	-	41.7		42.7
14	1.76	55.2	1.83 (m)	55.0	1.83	55.9		56.9
15	1.42, 1.54	23.2	1.39,1.50 (m)	23.0	1.56, 1.75	23.9		23.8
16	1.27, 1.68	28.6	1.29, 1.77	28.6	1.29, 1.67	27.6		26.7
17	1.27	56.1	1.30	55.9	1.30	55.6		56.6
18	0.58	12.3	0.58 s	12.0	0.68	12.3	0.67 s	12.4
19	1.02	12.6	1.02 s	12.3	0.97	17.2	0.94 s	19.6
20	2.04	40.9	2.04 (m)	40.9	2.05	34.6		34.5
21	1.03	21.8	1.02	21.8	1.03	20.4	1.00 d	19.4
22	5.35	138.2	5.13 (dd)	137.9	5.16	138.1		32.6
23	5.04	129.7	5.02 (dd)	129.4	5.02	129.3		24.9
24	1.54	51.4	1.55 (m)	51.3	1.56	51.1		46.4
25	1.54 (m)	32.1	1.56 (m)	31.9	1.50	31.9		28.9
26	0.82 (d)	19.1	0.82 (d)	19.1	0.82	19.1	0.94	19.8
27	0.81	20.5	0.84 (d)	21.5	1.18	20.3	0.89	20.1
28	1.18 (m), 1.42 (m)	25.4	1.19 (m), 1.41 (m)	25.5	1.12	21.4	-	21.7
29	0.79	12.4	0.81 (t)	12.3	0.81	12.6	0.87	12.5
1’					4.41 (dd)	101.1		103.0
2’					3.26	73.8		75.8
3’					3.44 (t)	76.7		79.0
4’					3.42	70.5		72.1
5’					3.30	76.1		78.9
6’					4.51 (dd)	62.1		63.2

Table 2: ^{1}H and ^{13}CNMR data for compound **3**

Position	Experimental		Literature	
	δ_H ppm (mult, J)	δ_C (ppm)	δ_H (mult, J)	δC (ppm)
1	0.96, 1.61	38.7	0.95, 1.26	38.7
2	1.23	29.1	1.23	79.0
3	3.12 (dd, *J* = 11.4, 5.03)	79.0	3.16 (dd, *J* = 11.2, 5.2)	79.0
4	-	40.0	-	38.9
5	0.69	55.3	0.65	55.3
6	1.34, 1.49	18.3	1.33, 1.48	18.3
7	1.37	34.3	1.36	34.3
8	-	40.9	-	40.8
9	1.23	50.5	1.23	50.4
10	-	37.2	-	37.2
11	1.34	20.9	1.33	21.0
12	1.53	23.8	1.54	25.2
13	1.59	38.1	1.59	38.1
14	-	43.0	-	42.8
15	1.49	27.4	1.49	27.5
16	1.37,1.44	35.6	1.36, 1.44	35.6
17	-	42.9	-	42.0
18	2.26	48.3	2.25	48.3
19	1.29	48.0	1.28	48.0
20	-	150.9	-	150.8
21	1.23	30.4	1.23	29.9
22	1.18, 1.37	40.9	1.18, 1.36	40.0
23	0.93 (s)	28.0	0.94 (s)	28.0
24	0.72 (s)	14.1	0.73 (s)	14.4
25	0.84 (s)	16.1	0.80 (s)	16.2
26	1.00 (s)	16.3	1.00 (s)	16.0
27	0.92 (s)	15.4	0.92 (s)	14.6
28	0.76 (s)	18.0	0.76 (s)	18.0
29	4.50, 4.62	109.3	4.54, 4.66	109.2
30	1.82 (s)	19.3	1.65 (s)	19.4

CONCLUSION

The results obtained in the present investigation indicates that the roots of *Calliandra portoricesis* is rich in secondary metabolites. This is an initial isolation of linalonic acid, spinasterone, lupeol, daucosterol and triacontanyl caffeate from the plant material.

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