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Pycnanolide A and B: New Lignan Lactones from the Leaves of *Pycnanthus angolensis* (Myristicaceae)

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ABSTRACT

Pycnanthus angolensis has wide application in ethnomedicine and is thus a rich source of medicinally important natural products. The leaf methanolic extract of *Pycnanthus angolensis* was investigated with the aim of isolating and elucidating the structures of the phytochemical constituents of the plant extract. Isolation and purification of pure compounds was achieved using standard chromatographic techniques. Spectroscopic techniques were used in structural elucidation of new compounds. Two new lignan-lactones, Pycnanolide A and B, were obtained along with four other compounds. The four compounds were identified as β-sitosterol, stigmasterol, phytol and hexadecanoic acid, of which the latter two are encountered for the first time in the plant. Pycnanolide A and B were characterized as (8α, 7'α, 8'β)-3, 4: 3', 4' bis (methylenedioxy) lignan-9, 7'-olide and (8β, 7'β, 8'β)-3, 4: 3',4' bis (methylenedioxy) lignan-9, 7'-olide, respectively. This study helped to characterize the structure of two new lignans-lactones which could form the basis of chemical synthesis and may provide the nucleus for development of new drugs.

Key words: *Pycnanthus angolensis*, Myristicaceae, pycnanolides, lignan-lactones, isolation, structural elucidation

INTRODUCTION

Pycnanthus angolensis (Welw.) Warb., a specie of Myristicaceae plants is commonly known as wild African nutmeg. It is a lowland tree native to West, Central, Southern and East Africa. It is reputed for its analgesic, stomachic, aperative, carminative, anthelmintic, anti-inflammatory, haemostatic and antimicrobial actions in African ethno medicine (Burkill, 2000). Other reported ethno medical indications include its use in the treatment of female sterility, gonorrheal infertility, rheumatism, hemorrhoids, sore throat, rhino pharyngeal and broncho pneumonia. Different parts of the plant have been used to promote healing of wounds, as a poison antidote and for the treatment of leprosy and chronic skin diseases (Burkill, 2000).

Different biological activities of *Pycnanthus angolensis* have been reported in literature. These include: antiplasmodial, anthelmintic, anticancer, antihyperglycemic, phyto-and cytotoxicity and antileishmanial activities (Zirihi *et al*., 2005; Abrantes *et al*., 2008; Gbolade and Adeyemi, 2008; Njoku *et al*., 1997; Fort *et al*., 2000; Onocha *et al*., 2008).

Allantoin (Prista *et al.*, 1960), kombic acid (Lok *et al.*, 1983), flavonoids (Omobuwajo *et al.*, 1992), anticancer and anthemintic dihydroguaiaretic acid (Njoku *et al.*, 1997) were isolated from the plant. Novel anti hyperglycemic and antifungal terpenoid-type quinones (Luo *et al.*, 1999; Fort *et al.*, 2000; Wabo *et al.*, 2007) have also been isolated from this plant. Antiplasmodial lignans (Abrantes *et al.*, 2008) and antimicrobial cyclohignene derivatives (Nono *et al.*, 2010) have also been encountered in the plant.

In continuation of the phytochemical investigation of secondary metabolite constituents of plants (Saleem *et al.*, 2009; Ali *et al.*, 2005, 2011; Onocha *et al.*, 2011a, b), this study described the chemical investigation of the dichloromethane soluble part of the leaf methanolic extract of *Pycnanthus angolensis* and spectroscopic techniques were used in elucidation of the structures of new compounds.

MATERIALS AND METHODS

General experimental procedures: Infrared spectra were recorded on JASCO 302-A spectrophotometer in $CHCl_3$ and KBr disk. UV spectra were obtained on a Hitachi UV 3200 spectrophotometer. Electron Impact Mass Spectrometer (EIMS-ionization voltage 70 eV) were measured on a Varian MAT 311 A mass spectrometer and High Resolution EIMS (HREIMS) were measured on a JEOL HX 110 mass spectrometer. 1D and 2D NMR were run on a Bruker AMX 300, 400 and 500 MHZ Nuclear Magnetic Resonance (NMR) spectrometers. The chemical shifts are given in ppm (ð), relative to Tetramethyl Silane (TMS) as an internal standard and coupling constants are in Hz. Column chromatography was carried out on silica gel (70-230 mesh, Merck) and flash silica gel (230-400 mesh, Merck). Thin Layer Chromatography (TLC) was performed on Merck precoated silica gel 60 F_{254} aluminium sheet and spots were detected using cerric sulfate spray reagent and silica gel 60 F_{254} (Merck) for preparative TLC (PTLC).

Plant materials: *P. angolensis* leaves was collected from Barth Road, University of Ibadan, Ibadan and authenticated by Mr. Felix Usang of Federal Research Institute of Nigeria (FRIN) where a voucher specimen (FHI 106464) is deposited.

Extraction, isolation and characterization: Air dried and finely powdered leaves (900 g) were macerated in MeOH for 48 h by filtration and evaporation yielded a crude MeOH extract (60 g) which was re-extracted with hexane followed by dichloromethane. This afforded 10, 15 and 25 g of hexane, dichloromethane and methanol (residue) extracts, respectively.

The dichloromethane extract was subjected to flash chromatography over flash silica gel eluting with hexane and followed by hexane-EtOAc mixture with increasing polarity. A total of 120 fractions of ca, 50 mL each were collected and combined on the basis of TLC analysis giving five mixtures (A-E). Further purification of these mixtures was achieved by repeated column and PTLC. The phytochemical analyses were carried out using standard methods described by Harborne (1993), Egwuche *et al.* (2011), Satnami and Yadava (2011) and Ganesh and Vennila (2011).

RESULTS AND DISCUSSION

The dichloromethane soluble fraction of the methanolic extract of the finely powdered leaves of *P. angolensis* was subjected to flash column chromatography. This afforded five mixtures A-E. The first two mixtures (A and B) contained hydrocarbons; the third mixture (C) contained β-sitosterol and stigmasterol; the fourth mixture (D) contained phytol and hexadecanoic acid while

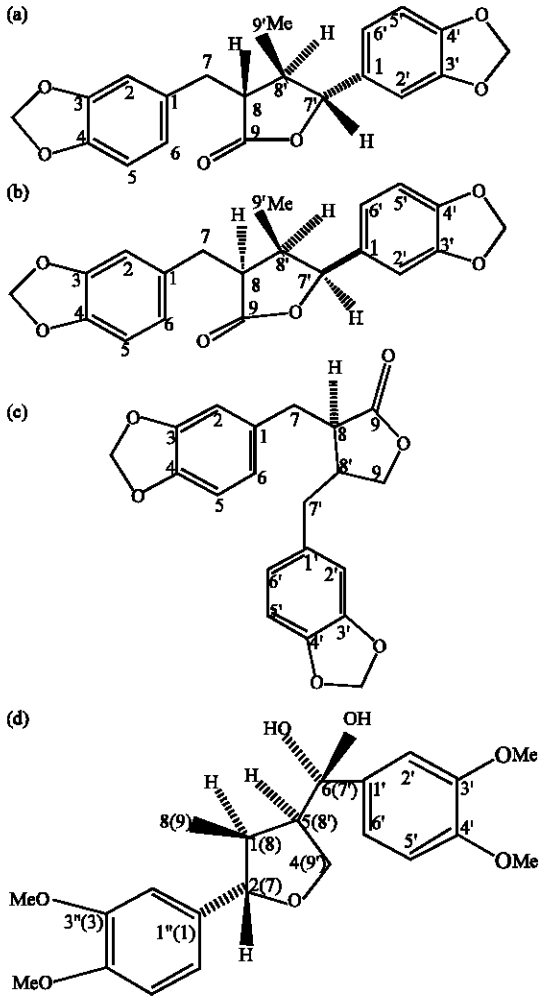

Fig. 1(a-d): (a) Pycnanolide A, (b) Pycnanolide B, (c) Hinokinin and (d) Magnostellin A (Numbering in parenthesis for ease of comparison with 1-3)

the fifth mixture (E) (n-hexane-EtOAc 10:90) yielded semi pure compounds which on further purification by repeated column chromatography and solubility differences afforded Pycnanolide A (1) and B (2) (Fig. 1). The known constituents β-sitosterol, stigmasterol, phytol and hexadecanoic acid-were identified by comparison of their spectral data with published ones as well as with authentic samples (Goodman *et al*., 1973; Bailey *et al*., 1971; Rubinstein *et al*., 1976). Compounds 1 and 2 were characterized as (8α, 7'α, 8'β) -3, 4: 3', 4' bis (methylenedioxy) lignan-9, 7'-olide and (8β, 7'β, 8'β) -3, 4: 3',4' bis (methylenedioxy) lignan-9, 7'- olide, respectively.

Spectral data of compound 1:

- UV λ_{max} nm ($CHCl_3$) (log ε): 191 (6.00), 202 (0.87), 236 (0.15), 286 (0.11), 389 (0.01)
- IR υ_{max} ($CHCl_3$) cm^{-1} : 3745, 2922, 2856, 1832, 1770, 1699, 1649, 1506, 1454, 1245, 1039
- ^{13}C NMR (75.47 MHZ, $CDCl_3$) (Table 1) ^1H NMR (400 MHZ, $CDCl_3$) (Table 2)
- HR EIMS m/z 354.1100 (calcd.for $C_{20}H_{18}O_6$, 354.1103). EIMS m/z (rel. int.) 354 (49), 192 (5), 176 (23), 162 (100), 149 (9), 135 (54), 131 (18), 107 (5), 83 (100), 69 (8), 51 (6)

Table 1: ^{13}C NMR for Pycnanolide A (1) and B (2) (δ (ppm). $CDCl_3$ (75.47 MHZ), CD_3 OD (100.62 MHZ), respectively, Hinokinin (3) (δ (ppm). $CDCl_3$, 25.2 MHZ) (Wenkert *et al*., 1976) and Magnostellin A (4) (δ (ppm). $CDCl_3$, 25.2 MHZ) (Iida *et al*., 1983)

C	1	2	3	4
1	132.8	134.0	131.2	136.4
2	108.4	109.8	108.4	109.6
3	147.9	148.7	147.4	148.6
4	147.2	147.6	145.8	149.1
5	108.7	109.2	107.8	112.2
6	121.3	122.5	121.1	118.4
7	30.7	31.7	34.4	87.8
8	48.4	48.9	46.1	44.0
9	177.1	179.7	177.9	13.0
1'	129.8	131.6	131.0	131.0
2'	105.9	106.9	109.0	109.0
3'	147.9	149.3	147.4	148.3
4'	147.2	149.4	146.0	149.1
5'	108.4	109.2	107.8	111.0
6'	118.6	119.8	121.8	118.0
7'	82.4	83.9	37.9	73.1
8'	38.5	40.1	41.0	48.1
9'	9.3	9.5	70.7	149.0
OCH_2O	100.9	102.2	100.6	-
OCH_2O	101.2	102.5	-	-

Table 2: ^1H NMR data for Pycnanolide A (1) and B (2) (δ (ppm)), $CDCl_3$ (400 MHZ), CD_3 OD (500 MHZ), respectively

	(δ (mult, J in Hz))	
No.	1	2
2	6.68 (1H, d, J = 1.5)	6.73 (1H, d, J = 1.5)
5	6.75 (1H,d, J = 8)	6.83 (1H, d, J = 7.5)
6	6.71 (1H, dd, J = 8,1.5)	6.76 (1H, d, J = 8,2.5)
7	3.22 (1Hdd, J = 14.7, 4.5, α-H)	3.12 (1H, dd, J = 15.0, 4.6, α-H)
	2.63 (1H, dd, J = 14.7, 10.7, β-H)	2.63 (1H, dd, J = 15.0, 10.8, β-H)
8	3.16 (1H, ddd, J = 10.7,4.5, 4.0)	3.40 (1H, ddd, J = 11.0, 4.8, 4.5)
2'	6.74 (1H, d, J = 2)	6.82 (1H, d, J = 2.5)
5'	6.79 (1H, d, J = 8)	6.81 (1H, d, J = 7.5)
6'	6.70 (1H, dd, J = 8,1.5)	6.75 (1H, dd, J = 7.5, 2.5)
7'	5.39 (1H, d, J = 4.5)	5.50 (1H, d, J = 4.8)
8'	2.66 (1H, m)	2.69 (1H, m)
9'	0.60 (3H, d, J = 7.1)	0.56 (3H, d, J = 7.2)
OCH_2O	5.92 (2H, s)	5.89 (2H, s)
OCH_2O	5.95 (2H, s)	5.93 (2H, s)

Spectral data of compound 2:

- UV λ_{max} nm (MeOH)) (log ε): 196 (6.00), 203 (1.43), 235(0.21), 286 (0.19), 353 (0.02), 390 (0.02)
- IR υ_{max} (KBr) cm^{-1} : 3782, 2923, 1855, 1770, 1727, 1594, 1493, 1443, 1246, 1165, 1039
- ^{13}C NMR (100.62 MHZ, CD_3OD) (Table 1). ^1H NMR (500 MHZ, CD_3OD) (Table 2)
- HR EIMS m/z 354.1100 (calcd. for $C_{20}H_{18}O_6$, 354.1103)

- EIMS m/z (rel. int.) 354 (20), 340 (6.93), 192 (8), 176 (42), 162 (100), 149 (17), 135 (85), 131 (18), 117(12), 82 (100), 69 (17), 56 (7)

Pycnanolide A (1), Cream gum, soluble in chloroform (insoluble in methanol), showed IR absorption bands due to aromatic rings (1649, 1506, 1454 cm^{-1}) and a γ-butyro-lactone (1770 cm^{-1}) (Chen *et al.*, 2007). The UV bands at λ_{max} 202, 236, 286 and 389 nm indicated the presence of a butyrolactone lignan skeleton and substituted benzene chromophores (Lopes *et al.*, 1983; Chen *et al.*, 2007; Ayres and Loike, 2009). The ^{1}H and ^{13}C NMR along with 2D experiments (COSY 45°, HMBC, HMQC) exhibited the characteristic signals of two 3, 4 - methylenedioxy benzyl and butyrolactone lignan moities related to those of lignans isolated from the stem bark of *Pycnanthus angolensis* and earlier encountered in *Chamaecyparis obtuse*-Hinokinin (3) (Abrantes *et al.*, 2008; Wenkert *et al.*, 1976) and Magnostellin A (4) from *Magnolia stellata* (Kato *et al.*, 1986; Iida *et al.*, 1983) (Fig. 1).

The $^{1}H/^{13}C$ NMR spectra revealed the presence of 18 protons and 20 carbon atoms (^{13}C and ^{1}H NMR chemical shifts are given in Table 1 and 2, respectively) and the HREIMS confirmed its molecular formula as C_{20} H_{18} O_6. The existence of a 3, 4-methylenedioxy benzyl and a 3, 4- methylenedioxy phenyl moieties were established by the EI-mass at m/z 135 $[CH_2O_2\text{-Ph- CH}]^+$ and 121 $[CH_2O_2\text{-Ph }]^+$, respectively. This was further supported by the EI-mass characteristic fragment basepeak at m/z 162 attributable to $[CH_2O_2\text{-Ph-CH = CHMe }]^+$, resulting from the cleavage of the 8-8' bond linkage of the two methylene dioxybenzyl units with opening of the lactone ring. This clearly indicates that it contains a secondary methyl at position C-9'. The EI-mass at m/z 192 (m/z 354-162) attributable to the other half of the molecule was also observed.

These structural features was collaborated by the ^{1}H NMR/^{13}C NMR that showed the presence of two methylenedioxy moieties whose protons appeared as singlets at δ 5.92 (2H, s) and 5.95 (2H, s), respectively, while the carbons resonated at δ 100.9, 101.2 ($2OCH_2O$) and 147.9, 147.2, 147.9, 147.2 (C-3, 4 and C-3', 4'), respectively. Furthermore, six aromatic proton signals observed at δ 6.68 (1H, d, J = 1.5 Hz, H-2), 6.70 (1H, dd, J = 8,1.5 Hz, H-6'), 6.71 (1H, dd, J = 8,1.5 Hz, H-6), 6.74 (1H, d, J = 2 Hz, H-2'), 6.75 (1H,d, J = 8 Hz, H-5), 6.79 (1H, d, J = 8 Hz, H-5'), respectively established the presence of two tri-substituted aromatic rings. The oxymethine at δ 5.39 (1H, d, J = 4.5 Hz, H-7'), two methines at δ 3.16 (1H,ddd, J = 10.7, 4.5, 4.0 Hz, H-8) and 2.66 (1 H, m, H-8'), respectively and the secondary methyl at δ 0.60 (3H, d, J = 7.1 Hz, H-9') further establishes the butyrolactone moiety.

In addition, the typical AX spin system at δ 2.63 (1H, dd, J = 14.7, 10.7, βH-7) and 3.22 (1H, dd, J = 14.7, 4.5 Hz, αH-7) established the presence of two chemically non equivalent geminal protons whose couplings to each other and to the adjacent proton at δ 3.16 (1H, ddd, J = 10.7, 4.5, 4.0 Hz, H-8) was also evident in the COSY 45° spectrum. A proton spin system $-CH_2$-CH-CH (Me) -CH- which located the saturated carbons at positions 7, 8, 8' (9'), 7', respectively and further established the location of the secondary methyl at position 9' was clearly observed in the COSY 45° spectrum of 1. The spectrum exhibited COSY cross peaks which represents couplings between H-7 [δ 3.22(1H, dd), 2.63 (1H, dd)] and H-8 [δ 3.16 (1H, ddd)]; between H-8 and H-8' [δ 2.66 (1H, m)]; between H-8' and H-9' [δ 0.60 (3H, d)] and between H-8' and H-7' [δ 5.39 (1H, d)] (Hu *et al.*, 2005). One bond proton-carbon connectivities were confirmed by the HMQC NMR experiments and ^{2}J and ^{3}J long range proton-carbon couplings were indicated through the Heteronuclear Multiple-bond Connectivity (HMBC) NMR experiments.

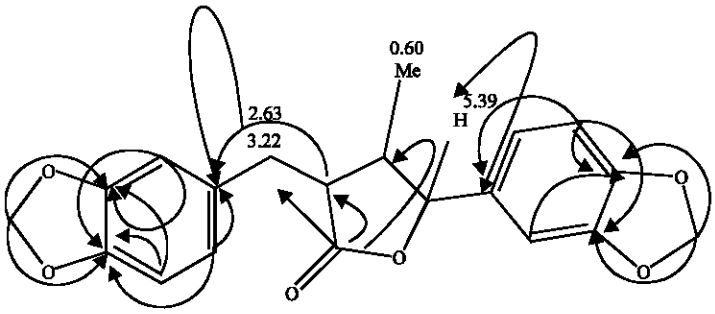

Fig. 2: HMBC correlations for Pycnanolide A (1) and B (2)

The HMBC spectrum (Fig. 2) exhibited a number of cross peaks representing long range heteronuclear interactions between the quaternary carbons and the protons. The downfield oxygen-bearing carbons at δ 147.9 and 147.2 were identified as the oxygen-bearing aromatic carbons, i.e., C-3/3' and C-4/4', respectively and they exhibited HMBC interactions with the protons resonating at δ 6.68, 6.74 (H- 2, 2') and 6.75, 6.79 (H-5, 5'). This established that the aromatic methine carbons C-2/C-5 and C-2'/C-5' were bound to the oxygen-bearing aromatic quaternary carbons C-3/4 and C-3'/4', respectively. This was further supported by the cross peak the oxygen-bearing aromatic carbons at δ 147.9 and 147.2 caused with the dioxymethylene protons at δ 5.92 (2H, s) and 5.95 (2H, s). Similarly, cross peaks between aromatic C-1 (δ 132.8) and protons resonating at δ 6.71 (H-6), 3.22 (H-7 α), 2.63(H-7 β), 3.16 (H-8) and between aromatic C-1' (δ 129.8) and protons resonating at δ 6.79 (H-5'), 5.39 (H-7') were also observed in the HMBC spectrum. In addition, the carbonyl carbon at δ 177.1 which exhibited identical long range couplings with the non equivalent geminal protons at δ 2.63 (1H, dd, J = 14.7,10.7, βH-7) and 3.22 (1H, dd, J = 14.7, 4.5 Hz, αH-7) also caused cross peaks with the methine protons at δ 3.16 (1H, ddd, J = 10.7, 4.5, 4.0 Hz,H-8) and 2.66 (1 H, m, H-8'), respectively. Thus with the HMBC and HMQC connectivities together with the HREIMS and COSY 45°, the chemical shifts of all the protons and carbons could be assigned unambiguously (Table 1).

The relative stereochemistry at the C -8, C-7' and C-8' stereo centres was established as (8α, 7'α, 8'β) by comparison of chemical shifts and coupling constants of 1 with those of 3, 4 and known related 3, 4-methylenedioxy benzyl butyrolactone lignans, (Chen *et al*., 2007; Moss, 2000; Hu *et al*., 2005; Ayres and Loike, 2009; Iida *et al*., 1983; Wenkert *et al*., 1976). This was further supported on the basis of the analysis of the NOESY data. In this spectrum, H-8 at δ 3.16 and H-9' at δ 0.60 showed NOE correlations with the geminal proton H-7 β at δ 2.63. This establishes that C-8 proton and C-8' methyl are β oriented. The protons of H-7' and H-8 at (δ 5.39 and 3.16, respectively) showed correlation with H-9' δ 0.60 while clear effects was also observed between protons of H-7' and H-9' further establishing the β orientation of C-8,7' protons and C-8' methyl. There was also a clear NOE correlation between H-8' at δ 2.66 and the geminal proton H-7α at δ 3.22. This interaction is possible only when the C- 8' proton is α oriented. No NOEs were found between H-8 and 8'; H-8' and 9' or H-7' and 8'. Important NOESY interactions are presented around the structure (Fig. 3a). Thus the structure of 1 was deduced as (8α, 7'α, 8' β) -3, 4: 3', 4'-bis (methylenedioxy) lignan-9,7'-olide. To the best of our knowledge, this is the first time this lignan-lactone is being reported.

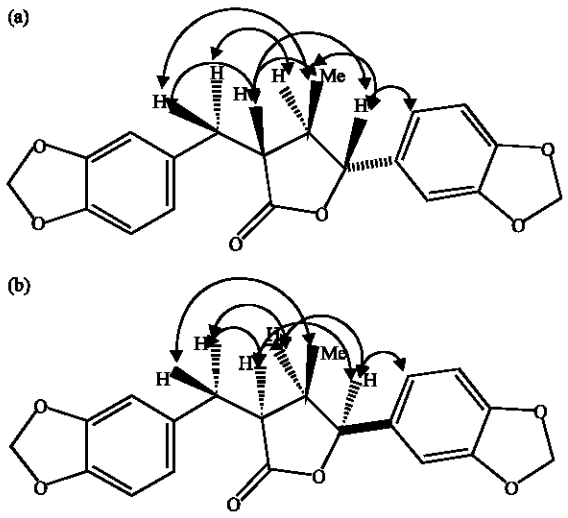

Fig. 3(a-b): NOESY correlations for Pycnanolide A (1) and (b) NOESY correlations for Pycnanolide B (2)

The HREIMS confirmed that compound 2 (Pycnanolide B) had the same molecular formula with compound 1. It was obtained as a yellow gum, soluble in methanol (insoluble in chloroform) and showed IR absorption bands due to aromatic ring (1594, 1493, 1453 cm^{-1}) and a γ-butyrolactone (1770 cm^{-1}) (Chen *et al.*, 2007). The UV bands at λ_{max} 203, 235, 286 and 390 nm also indicated the presence of substituted benzene chromophore. The $^{1}H/^{13}C$ NMR confirmed the presence of 20 carbons and 18 hydrogen atoms with features similar to compound 1 except that the 3, 4 and 3', 4' signals appeared at δ 149.4, 149.3, 148.7, 147.6, respectively. Full assignments of ^{1}H and ^{13}C NMR signals (Table 1) were accomplished using HMBC (Fig. 2), HMQC, HREIMS, COSY 45° and comparison with compound 1.

The chemical shift δ 3.40 (1H, ddd) and coupling constants (J = 11.0, 4.8, 4.5) of H-8 in 2 is different from that of H-8 (δ 3.16, 1H, ddd, J = 10.7,4.5, 4.0; β-oriented) in 1 suggesting very strongly that 2 maintains a different stereochemistry at the C-8 position. The proposed stereochemistry of 2 was supported by its NOESY data (Fig. 3b). In the NOESY spectrum, H-8 at δ 3.40 exhibited NOE correlations with the geminal proton H-7α (δ 3.12) and H-8'(δ 2.69) thereby establishing the α-orientation of C-8 and 8' protons. Similarly, the chemical shift (δ 5.50 (1H, d) and coupling constant (J = 4.8) of H-7' in 2 is different from that of H-7' (δ 5.39,1H, d, J = 4.5; β-oriented) in 1 indicating a different stereochemistry at the C-7' position. This is supported by clear effects observed between H-7' (δ 5.50) and protons at δ 2.69 and 3.40 (H-8' and 8, respectively). Further supporting the α-orientation of the C-8, 7' and 8' protons are the NOE crosspeaks observed between H-8' (δ 2.69) and the geminal proton H-7α (δ3.12). NOE interaction was also observed between H-9' at δ 0.56 and geminal proton H-7 β at δ 2.63. This interaction is possible only when the C-8' methyl is β oriented. No NOEs were observed between H-8 and H-9'; H-8' and 9' or H-7' and 9'. From the above evidence compound 2 which is also being reported for the first time, was characterized as (8β, 7'β, 8'β) - 3, 4: 3', 4'-bis (methylenedioxy) lignan-9,7'-olide.

CONCLUSION

Lignans are a group of important plant metabolite that has received much attention as a result of their pharmacological activities since the discovery of podophyllotoxin. A number of them have been shown to have a preventive role in breast cancer due to their phytoestrogenic activity. A number of them also exhibit antineoplastic properties and pronounced cytotoxic activity. Considerable synthetic efforts have been made notably in the area of podophyllotoxin and its relatives which includes anticancer drugs in clinical use. Some of these lignans have been used as starting compounds for the production of semi synthetic anticancer drugs. Several anticancer agents have been developed as a result of this type of research and many new exciting molecules capable of arresting cancerous cell growth have given insight into structural feature and types of molecules worth modifying synthetically. This study has led to the isolation and elucidation of the structure of two new lignan- lactones which could form the basis of chemical synthesis and may provide the nucleus for development of new drugs.

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