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ISOLATION AND CHARACTERIZATION OF 13- BENZYL ETHYL METHYL ABIETATE FROM THE LEAF OF *PENTACLETHRA MACROPHYLLA*. (*P.BENTH*)

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Abstract: As part of our study on the bioactive agents from *Pentaclethra macrophylla* tree, P.Benth (oil bean tree), 13- benzyl ethyl methyl abietate was isolated from its leaves. The structure was elucidated using spectra obtained from NMR spectroscopy in combination with IR and MS. its molecular formula is $C_{26}H_{32}O_2$ with molecular weight 376, calculated for M/z 375.29. The IR spectrum showed absorptions of aliphatic carbon, (2924.85, 2855.09cm^{-1}), carbonyl group, (1736. 68 cm^{-1}), aromatic (1457.97 cm^{-1}) and ether (1466.20 cm^{-1}). The fragmentation pattern was also determined

Key words: *pentaclethra macrophylla*, bioactive agents, molecular formula. Fragmentation pattern

INTRODUCTION

Pentaclethra macrophylla contains many phytonutriennts, which include; alkaloids, saponins, flavonoids and tannins, (Okwu and Aluwo, 2008). The extracts from the stem bark of this plant is used to treat leprosy sores. The seed when cooked and fermented is used in preparing delicacies (Asoegwu *et a/*, 2006). The leaf and stem extracts are applied in the treatment of diarrhea while the pod and leaf extracts are used in the treatment of convulsion. *Pentaclethra macrophylla* is a native of both West and Central Africa. It occurs mainly in Nigeria, Cameroon, Cote D ivoire, Togo, Congo, Senegal and Angola. It also occurs in the island of Sao Tome and Principe. It is endemic in the humid and some part of the sub humid zones of West Africa (Keay, 1989).. The pods contain 6-10 flat glossy brown seeds which may vary in size. The seeds are up to 7 cm long (Abbiw, 1990). The seed of this plant when crushed and eaten with red ants can induce abortion, (Abbiw, 1990, Isawumi, 1993, Tico ,2005) .The bark of this tree is used to treat leprosy sores. The seed is rich in alkaloids, saponins, flavonoid phenols and tannins (Okwu and Aluwuo, 2008). . The tree yields forest products for making· household utensils (Okafor, 1987). The mature dispersed seeds are harvested and sold in the market and may serve as a revenue earner. The seed could serve as protein supplement, (Enujiugha and Agbede,2000) .Its richness in vitamins and minerals makes it a highly sought after food supplement for both local consumption and export. The

seed serves as source of oils for candle making, cooking and soap making (Tico, 2005).The fruits, seed and leaves are used as anthelminitcs, for gonorrhea treatment and for convulsion as well as analgesics. (Bouquet *et al*, 1971, Iwu *et al*, 1990). The leaves of this plant when boiled with bush pepper produce a liquid given for the treatment of fever and pains in animals and man and improves the anti inflammatory response. (Okorie, *et al*, 2009). Oil from the leaves has anti inflammatory qualities and aid in wound management. Increased intake of the seed as food increases the hemoglobin value in animals, increased oxygenation of tissue, enhances specific hormone and stimulates the production of red blood cells important in proper cardiac function. The plant is a source of dietary estrogens (phyto estrogens) which can be employed as nutritional supplement as well as pharmaceutical and vitamin supplement in the control of obesity. The seed of *Pentaclethra macrophylla* contains very many organic compounds. Okwu and Aluwo (2008) have identified permacric acid and proline from the seed. The lactans of permaric acid is hydrolyzed under a variety of acidic conditions to afford adipic acid derivative which closes under acidic conditions to give substituted pecolic acid. The root bark is used as a laxative, an enema against dysentery and as a liniment against itching. The seed and leaf extracts have shown both anti-inflammatory and analgesic properties, (Iwu,1993). Agbogidi (2010), has shown that the germination of the seed is highly hindered in soils contaminated with spent lubricating oils but this plant shows soil improvement properties (Akindahunsi, 2004)

AIM OF RESEARCH

The use of the leaf of Pentaclethra macrophylla in the treatment of various diseases by traditional medical practioners in Nigeria is very rampant, their claims of its efficacy has not been properly verified. This work is therefore tailored to identify and structurally elucidate the bioactive components of the leaf of pentaclethra macrophylla.

MATERIALS AND METHOD

Plant materials

The samples were obtained from a farm land in Ezinihitte Mbaise area of Imo state, they were identified by Dr .Nmeregini of forestry department Michael Okpala University of Agriculture Umudike , the voucher specimen were deposited in the Forestry department Herbarium of Michael Okpara University Umudike. The sample were washed with distilled water and room dried .The dried samples were milled with an electric milling machine and stored in air tight plastic bottles and kept for analys**is**

Extraction and Isolation.

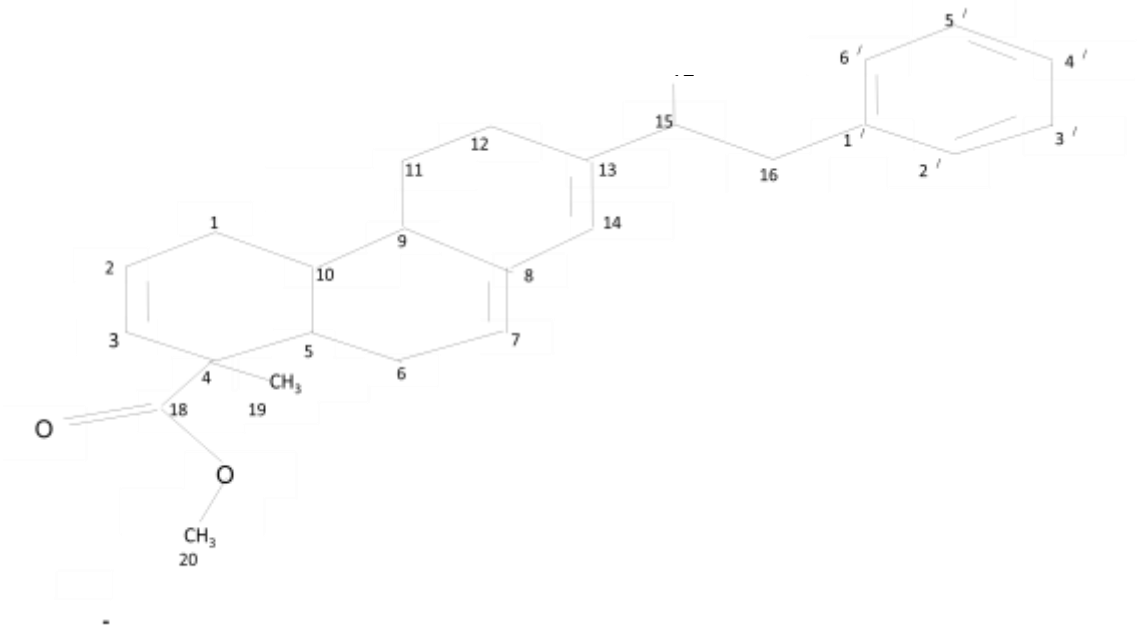
800 g of the ground sample was percolated in 95 % Ethanol for 48 hrs and filtered. The filtrate were concentrated using rotary evaporator regulated at 40 ^{o}C to get a dark extract, (28.6 g). The crude extract was partitioned between chloroform and water to afford chloroform soluble fraction 16.2 g. The Chloroform extract was subjected to column chromatography over silica gel and eluted with Diethyl ether, 100 cm^3, followed by varying volumes of diethyl ether/chloroform mix and later with chloroform /methanol mix and the fractions were labeled. The fractions were subjected to thin layer chromatography using silica gel 60 G and iodine vapour for development. Compound 1 (0.2g) was obtained using diethyl ether /chloroform mix which appeared as only one spot.

General Procedure for Spectroscopic Determination:

IR Spectrum was determined on a thermo Nicolet Nexus 470 FR-IR spectrometer. The ^{l}H and ^{13}C NMR spectra were obtained on a Bruker Avance 400 FT NMR Spectrometer using tetra methyl silane as internal standard. Chemical shifts are expressed in δ values. LC – ESIMS spectra where determined in the positive ion mode on a PE. Biosystem APl 156 single quadruple instrument. HRESIMS (positive ion mode) spectra were recorded on a thermo Finniga MAT 95 x L Mass spectrometer. Column Chromatography was carried with silica gel (200 – 300 mesh) and the preparation separations was monitored analytically by using thin layer chromatography (TLC) at room temperature on precoated 0.25 mm thick gel 60 F_{254} Aluminium plates 20 x 20 cm Merck, Darmstadt Germany. Reagents and solvents such as Ethanol, chloroform, diethyl ether and Hexane were all of analytical grade and procured from Merck Darmstadt Germany

RESULTS AND DISCUSSION

Compound [1] was isolated as brown oil (0.2) g. From the analysis of its IR, "HNMR, ^{13}CNMR and HREIMS spectra, its molecule formula is $C_{26}H_{32}O_2$ with molecular weight of 376 calculated for M/z 375.29



Compound

1; ***13- benzyl ethyl methyl abietate***

The IR spectrum showed absorptions of aliphatic carbon, (2924.85, 2855.09cm^{-1}), carbonyl group, (1736. 68 cm $^{-1}$) aromatic (1457.97 cm^{-1}) and ether (1466.20 cm^{-1}) .Table (1.0)

Table 1.0 IR absorption analysis of compound [1]

IR absorptions (cm^{-1})	functional group	compound type
2924.85	-CH_2	Aliphatic
2855.09	-CH_2	Aliphatic
1736.68	C=O	Carbonyl
1457.97	C=C	aromatic
1166.20	C=O	ether

Analysis of the HNMR spectrum of compound [1] is shown in table (2.0), the spectrum showed signals of tertiary methyl at δH 0.882 and 0.876. The absorptions of the methylene protons are obtained from the following peaks;

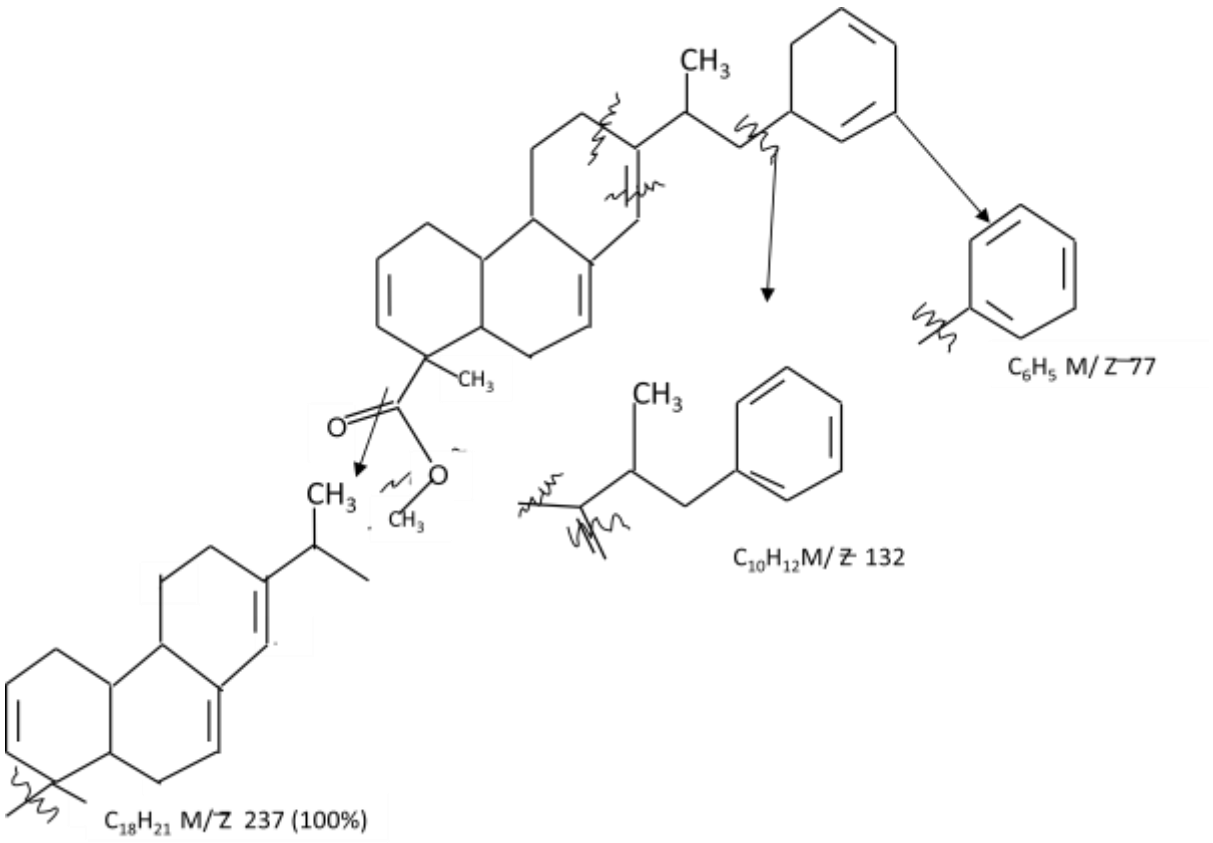
1.266, 1.238, 1.224, 1.213, 1.331 and 1.307. The methine protons showed their absorption peaks at δH 1.603 and 1.523. Analysis of ^{13}CNMR spectrum is shown in table (2.0); the spectrum gave the resonance of the aromatic chemical shifts at δC 125.29 –

129.89. The methyl carbon chemical shifts are observed at δC 19.15, 19.20 and 77.40. Absorptions of the methine carbons are shown at δC 32.78 and 32.74

Table 2.0 ^{1}HNMR AND ^{13} CNMR Analysis of compound [1]

	δC		δH	
1.	22.90	CH	1.266	2Hd
2.	128.13	=CH	2.300	1 Hm
3.	127.83	=CH	2.257	1Hm
4.	22.55	C	-	-
5.	32.78	CH	1.603	1Hm
6.	22.87	CH_2	1.238	2Hm
7.	126.02	=CH	5.269	1Hm
8.	125.29	=CH	2.347	1Hm
9.	32.74	CH	1.523	1Hm
10.	35.93	C	-	-
11.	22.70	CH_2	1.224	2Hm
12.	22.63	CH_2	1.213	2Hm
13.	128.13	C	-	-
14.	127.83	=CH	2.257	1Hm
15.	19.15	CH_3	0.882	3Hm
16.	22.55	CH_2	1.331	2Hm
17.	21.29	CH_2	1.307	2Hm
18.	137.74	C=O	-	-
19.	19.20	CH_3	0.876	3Hm
20.	77.40	OCH_3	2.347	3Hm
1^1	129.89	C	-	-
2^1	129.89	C	-	-
3^1	129.02	CH	7.052	1Hm
4^1	128.89	CH	5.269	1Hs
5^1	128.28	CH	2.341	1Hm
6^1	128.21	CH	2.308	

The fragmentation pattern of compound [1] is shown below. Detachment of the benzyl fragment from the compound afforded the peak with M/z 77, (C_6H_5). Another detachment of C_9H_9 gave the peak with M/z 117 while the detachment of the fragment $C_{19}H_{24}$ afforded the base peak with M /z 251 calculate for M/z 252. These data suggest that compound [1] is a methyl abietate with benzyl methyl linkage at C_{13}



Fragmentation pattern of compound 1

Benzyl ethyl methyl abietate is an ester derivative of abietic acid. This compound has been found in pine tree, isolatable chromophores, the carboxyl group and the butadiene unit. The compound is chiral with four stereogenic centre and can be precipitated as diamylammonium salt; acidification of the salt liberates abietic acid. The acid is oxidized when stored in an air inclusive container. Berger and Dieter (2009).

Abietic acid has a history of been used in the making of mystic smoke and as part of Greek fire, (liquid fire), a feared incendiary in the Byzantine empire as a weapon of attack and defense. Abietic acid has irritating effect when exposed to the skin. Its usefulness is in soldering where it is added to soldering wire to make flux. Harris and Sanderson (1992)

CONCLUSION

The investigations into the leaf extract of pentaclethra macrophylla has given rise to the isolation of benzyl ethyl methyl abietate. This work has enabled the compound to be fully characterized as its molecular weight and structure has been determined. The

compound so identified is an ester of abietic acid. This compound no doubt plays an important role in the use of the leaf of this plant for treatment of certain disease by traditional medicine practitioners.

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