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ISOLATION AND CHARACTERIZATION OF 4, 15-CHOLESTENE-3-YL BENZOATE FROM THE STEM BARK OF *PENTACLETHRA MACROPHYLLA. (P.Benth)*

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Abstract: Chemical investigation into the bioactive constituents of the stem bark of *Pentaclethra macrophylla P .Bent* (oil bean tree) resulted in the isolation of the steroidal compound 4, 15 cholestene-3-yl benzoate with molecular formulae $C_{30}H_{32}O_2$ and molecular mass 424 . The structure was elucidated using NMR spectroscopy in combination with IR and MS spectral data. The fragmentation pattern of this compound was obtained from the MS data.

Keywords: Pentaclethra *macrophylla*, steroidal compound, spectroscopy. Spectral data, Molecular mass.

INTRODUCTION

Pentaclethra macrophylla is a plant full of phytonutrients, these compounds include alkaloids, saponins, flavonoids and tannins, (Okwu and Aluwo, 2008). The bark of this plant is used to treat leprosy sores while 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 diarrhoea while the pod and leaf extracts are used in the treatment of convulsion. *Pentaclethra macrophylla* is a native of West and Central Africa. It occurs mainly in Nigeria, Cameroon, Cote Divoire, 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. *Pentaclethra macrophylla* tree grows to a height of 21 meters and 60 cm girth. It has a characteristic low branching habit and an open crown which allows substantial light under its canopy. The bole produces a reddish orange colouration after a slash is made. The stem form is crooked and buttressed, some are straight stemmed and less buttressed tree which can pass for timber. The bark is grayish to dark brown, thin and patchy with irregular pieces flaking off (Keay, 1989). The leaves posses a stout angular petiole, the

compound leaves are usually about 20-45 cm long and covered with rusty hairs giving a scurry effect particularly along the upper surface but eventually falls off (Abbiw, 1990).

The plant is a multipurpose tree of West Africa, in Agro forestry and in the tropics. It is recognized by farmers in the south east Nigeria for its soil improvement properties. Its seed is useful as food (Ladipo, 1984). The leaves, bark and root are useful medicines while the trunk is used as timber. 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 (Tico, 2005) .The seed shells are decorative and are often used to craft beads which are worn as necklace, rosaries and sometimes for local dancing apparels (NFT, 1995). Every part of this multipurpose plant has numerous useful applications. The seed of this plant when crushed and eaten with red ants can induce abortion (Abbiw, 1990, Isawumi ,1993, Tico ,2005) .The seed is rich in alkaloids, saponins, flavonoid phenols and tannins (Okwu and Aluwuo, 2008). The pod and leaf are used to treat convulsion. The aqueous leaf and stem bark extract of the plant have antinociceptive, anti inflammatory and cycotoxic activities in mice. The bark, fruits, seed and leaves are used as anthelminitcs, for gonorrhoea treatment and for convulsion as well as analgesics (Bouquet *et al,* 1971; Iwu *et al,* 1999). Works by Okunrubo *et al*, (2009) has demonstrated that the methanolic extract and aqueous fractions of the stem bark of *Pentacletra macrophylla* showed marked antinociceptive activities. Works on aqeous and ethanolic extract of *Pentaclethra macrophylla* have shown that the extracts have antidiarrheal potentials (Akah *et al*,1999). The works of Ugbogu and Akukwe, (2000), on the anti microbial properties of the oil of *Pentaclethra Macrophylla* , *Chrysophyllium albidum and Persea gratissimum seed on E. Coli, P.mirabilis, P. aeruginosa, S.aureus* and *S.epidermidis* have shown that the oils of Pentaclethra.marcphylla have marked anti microbial properties as non of the organisms showed complete resistance to the oil. The leaves of this plant when boiled with bush pepper produce a liquid given for the treatment of fever ,extracts of the leaves seed and bark are used' to treat itching and pains in animals and man and improving the anti inflammatory response (Okorie *et al*, 2006). Oils from the leaves have anti inflammatory qualities and aid in wound management. The seed when ground into paste/lotion renders antimicrobial effects promoting healing while extracts from the bark are applied to leprosy sores .The rich mineral composition of the fermented seed makes it a low cost source of protein. Increase intake of the seed as food increases the hemoglobin value in test 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 in nutritional supplement and pharmaceutical preparation and vitamin supplement in the control of obesity. Though the plant has been used for various ethno-medical applications, its constituents have not been fully documented.

MATERIALS AND METHOD

Plant Materials

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

General Procedure for Spectroscopic Determination

IR Spectrum was determined on a thermo Nicolet Nexus 470 FR-IR spectrometer. The ^{I}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 percolated 0.25 mm thick gel 60 F_{254} Aluminum 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.

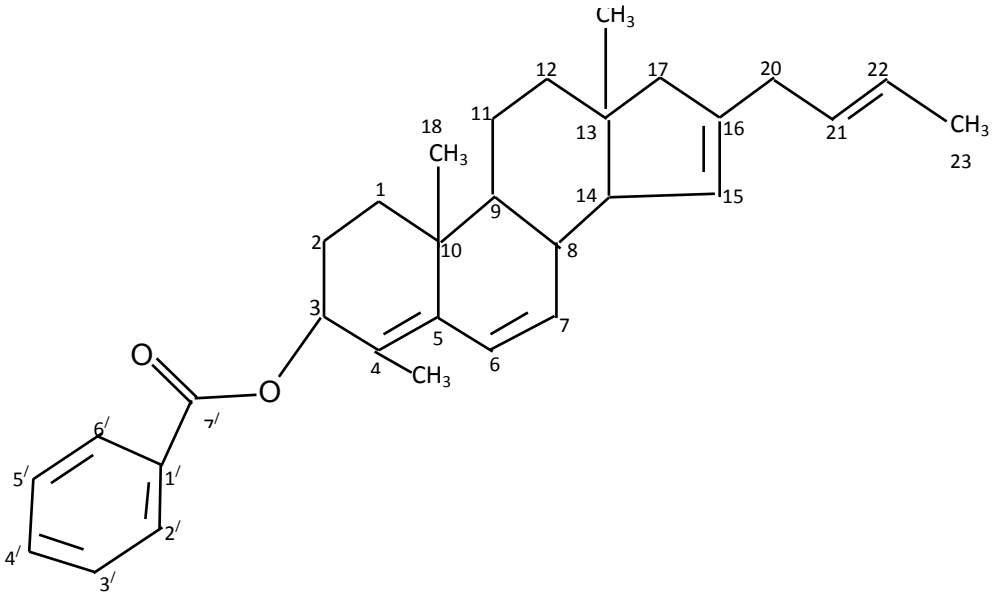
Extraction and Isolation.

800 g of the ground sample was percolated in 95 % Ethanol for 48 hrs and filtered. The filtrate was concentrated using rotary evaporator regulated at 40 ^{o}C to get a dark extract (23.2 g) for *Pentaclethra Macrophylla* bark. The crude extract was partitioned between chloroform and water to afford chloroform soluble fractions 10.4 g for *pentaclethra Macrophylla* bark. The Chloroform extract was subjected to column chromatography over silica gel and eluted with Diethyl ether, 100 cm^3, followed by varying volume mix of diethyl ether/ chloroform as well as chloroform /methanol mix and labeled. The fractions were subjected to thin layer chromatography using silica gel 60 G and iodine vapour for development. Fractions 1 was obtained which weighed (0.53g), a yellow amber oily solution with R_f value (0.623) which appeared as only one spo**t.**

Results and Discussion.

The interpretation of the spectral data obtained from the analysis of the sample gave compound 1 which was identified as 4,5- Cholestene -3-yl benzoate which is a steroidal compound and which was obtained as yellow oily

liquid.. The molecular formulae was determined as $C_{30}H_{32}O_2$, this was obtained from the interpretation of its HREIMS (M/z 424.50) calculated for m/z 424, its ^{1}H and ^{13}C NMR spectra



[1] 4,15 Cholestene-3-ylbenzoate

. The IR spectrum of compound [1] showed absorptions at vmax 2925.94cm-^{1} (aliphatic) 2855.88cm^{-1} (aliphatic), 1742.57 (Carbonyl) 1460.15cm^{-1} (aromatic) and 1159.50 (ether). Table 1

Table 1.0 IR absorption of compound [1]

IR absorption	functional group	compound type
2925.94 –	CH_2	Aliphatic
2855.88 -	CH_2	"
1742.57 -	C = O	Carbonyl
1460.15 -	C = C	Aromatic
1159.50 -	C – O	ether

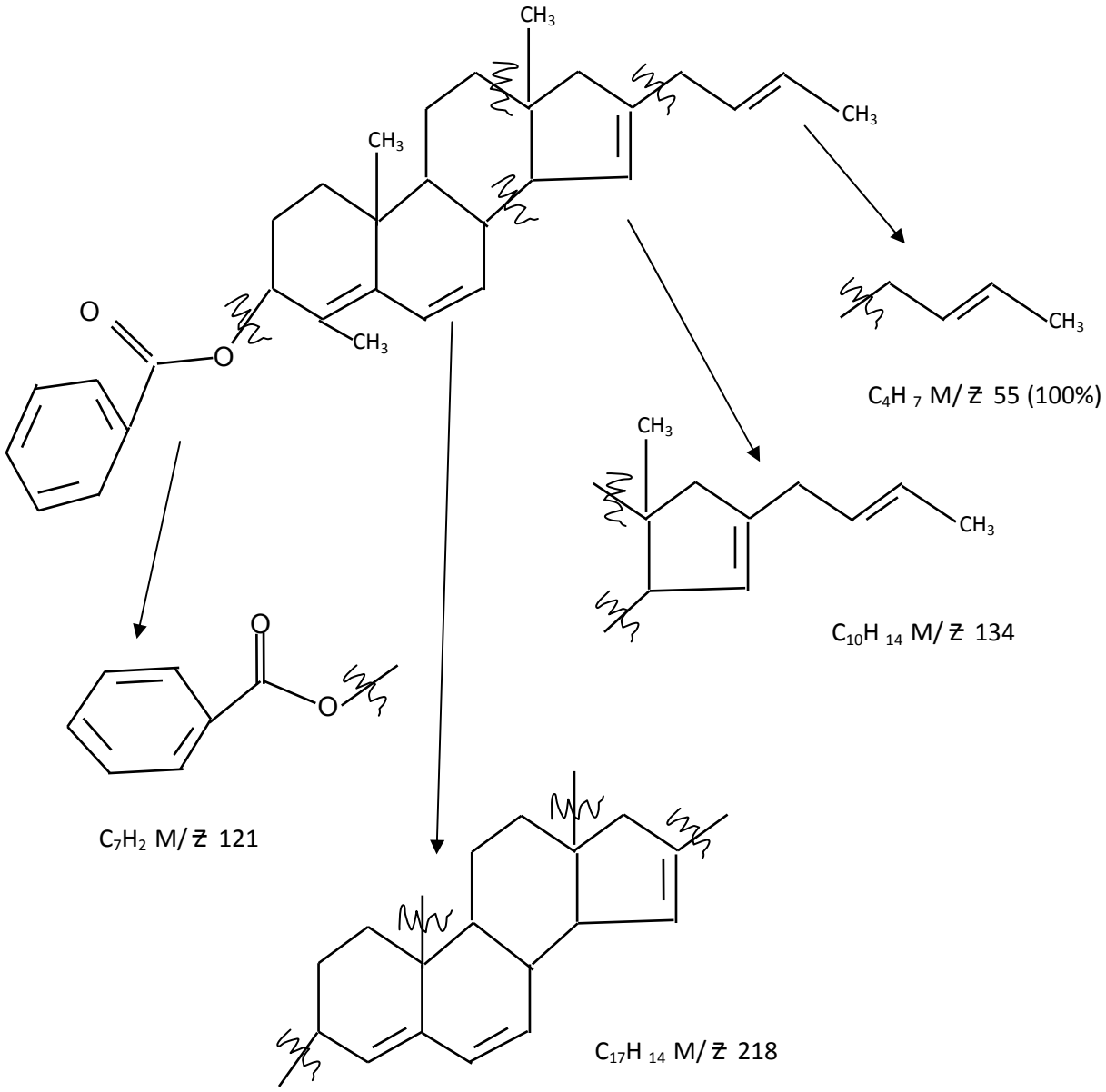
The ^{13}CMR spectrum was interpreted and is shown in table 2.0. The resonance of the aromatic chemical shifts for carbonyl carbon occurred at δc 173.44. The methine shift occur at δc 124.59 and δc 124.55, the methyl shift occur at δc 16.66 and δc 17.83 .The ^{1}HMR spectrum revealed the presence of aromatic protons at δH 7.262. It also showed signals due to tertiary methyl peaks at δH 0.880, 0.877 and 0.854.

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

	δC		δH	
1.	29.83	CH_2	1.293	2HM
2.	29.62	CH_2	1.285	2HM
3.	32.93	CH	1.598	1HM
4.	135.05	=CH	2.312	1HM
5.	130.15	C	-	-
6.	124.59	=CH	2.286	1Ht
7.	124.55	=CH	2.109	1HM
8.	39.51	CH	1.440	1HM
9.	39.51	CH	1.435	1HM
10.	39.59	C	-	-
11.	29.41	CH_2	1.255	2HM
12.	29. 41	CH_2	1.233	2HM
13.	37.42	C	-	-
14.	37.24	CH	1.435	1HM
15.	124.40	CH	2.038	1HM
16.	124.10	C	-	-
17.	29.25	CH_2	1.380	2HS
18.	19.88	CH_3	0.880	3HS
19.	17. 81	CH_3	0.877	3HS
20.	28.11	CH_2	1.334	2HM
21.	124.59	=CH	2.022	1HM
22.	124.55	=CH	2.009	1HM
23.	16.16	CH_3	0.854	3HM
1^{1}	130.15	C	-	-
2^{1}	124.59	CH	2.262	1HS
3^{1}	124.55	CH	2.076	1Hd
4^{1}	124.40	CH	2.055	1Hs
5^{1}	124.10	CH	2.038	1Hd
6^{1}	119.22	CH	2.022	1Hm
7^{1}	173.44	C=O	-	-

The fragmentation pattern of compound [1] showed that it undergoes cleavage at C_{16} to produce the base peak C_4H_7 (M/≠ 55). The detachment of the phenyl acetate gives the peak at M≠ 121 and the peak at M/≠ 218 was as a result of

the tetracyclic skeleton..These values wee obtained directly from HREIMS data indicates the detachment of these fragments



Fragmentation Pattern o f compound [1].

Generally steroids and steroidal compounds are known for the roles in the treatment of cancer ,arthritis , allergies and in birth control. The bark of this plant is used to treat itching and pains in animals and man and improving the anti inflammatory response. Okorie *et al* ,(2009).We are optimistic that this compound is involved in alleviating cell inflammation which may also be responsible for the ethno medical use of the bark of *pentaclethra macrophylla* for the treatment of diseases associated with inflammations of cells.

CONCLUSION

The compound, 4, 15- Cholestene 3- yl benzoate isolated from the stem bark of pentaclethra macrophylla is a novel compound with many promising potentials as this compound with its derivatives and modifications will play an important role in the management of cancer, allergies and inflammations. We are continuing the work on this compound and its modifications

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