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Isolation and Characterization of Phenanthrene Methyloctanoate from the Leaves of *Antiaris Africana*

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ABSTRACT: *Plants are sources of bioactive compounds which are used directly as therapeutic agents, as well as starting material for the synthesis of drugs, .Antiaris africana plant is one of such medicinal plants. It is used in the treatment of mental and nervous disorder such as epilepsy. It is also used to treat respiratory infections, syphilis and skin irritants in Nigeria. An Ester, Hexadecahydro – 3, 17 – dihydro – 5, 10, 13, 14 – tetramethyl – 1 H- cyclopenta (α) phenanthren – 17 – yl, 7 – methyloctanoate was isolated and characterized from the leaves of Antiaris africana using IR, ^{1}H NMR, ^{13}C NMR, MS, COSY and DEPT. The presence of the isolated compound in the leaves of A. africana may be responsible for some of the biological activities exhibited by plant.*
KEYWORDS: Natural Product, Ester, *Antiaris Africana*, Diseases

INTRODUCTION

For ages man has used natural products, such as plants, animals, microorganisms, and marine organisms, in medicines to alleviate and treat diseases (Haidan *et al*., 2016). Reports have shown that most countries in Africa and other developing countries rely on plant usage for their primary healthcare. Natural products undergo continual use toward meeting the urgent need to develop effective drugs, and they play a leading role in the discovery of drugs for treating human diseases, especially critical diseases. Nigeria is blessed with many medicinal plants which are used

traditionally to treat different diseases. Recently, Nigeria government is considering integrating herbal medicine as a degree program into the tertiary institution curriculum. The plant *Antiaris africana* is a highly medicinal and a large attractive deciduous timber tree with blotchy grey and white bark. The small, greenish white flowers yield red, velvety fruits. It belongs to the family Moraceae. It has white latex and alternate dissymmetric leaves. The heartwood of the plant is whitish to pale yellow; the texture is moderately coarse and the wood is lustrous. Fresh wood has woolly surfaces. The wood is light in weight and soft. It is used for interior joinery, panelling, moulding, shuttering, furniture, strip flooring, boxes and crates, tool handles, toys and carvings. It is fairly commonly used domestically for light construction and canoes. It is locally popular for drum making. It has a wide usage both in industry (for timber making) and as traditional medicine. Reports showed that the leaves, stems and barks of *Antiaris africana* are used in the treatment of various diseases such as rheumatic and respiratory infections, epilepsy, skin irritant, syphilis etc. Various parts of the plant leaves, stems and barks are used in the treatment of various diseases such as rheumatic and respiratory infection, epilepsy, skin irritant, syphilis etc. The bark extract is used in traditional medicine for the treatment of chest pains. The latex of the plant is used as a purgative. It is also used as a cure for leprosy and sore throat. The leaves and roots are also used to treat mental illnesses. The plant is utilized traditionally in ethnomedicine for epilepsy, lumbago, skin irritant, purgative, nervous disorders (Keay, 1989). An ester named (1E, 3E)-4-(6, 7, 8, 9, 10, 13, 14, 15, 16, 17-decahydro-17-hydroxy-5, 10, 13, 14-tetra methyl-5H-cyclopenta [α] phenanthrene-16-yl) buta-1,3-dienyl-2-phenyl acetate was isolated and characterized from the leaves of *Antiaris Africana* (Onyekwere et al, 2020).

In spite of the various uses of this plant as medicine, its constituents have not been fully documented. In this research, another novel compound was isolated and characterized from the leaves.

MATERIALS AND METHODS

Plant Materials: The leaves of *Antiaris africana* were collected from the Botanical garden of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria and authentication of plant materials was done by Mr.Nduka Ibe of Taxonomy section, Forestry Department.

Sample Preparation: The fresh plant sample was washed and air dried on a laboratory bench on a period of two weeks. The dried leaves were milled into fine powder with Thomas Willey milling machine and then stored in air tight bottles for analysis. 2kg each of the sample were used.

Extraction of the Plant Materials: The leaves of *Antiaris africana* were ground into powder. The powdered samples (2kg) were percolated in ethanol for 48 hours and filtered.

Isolation of the Constituents of leaves of *Antiaris africana*

The filtrate from the leaves was concentrated with Rotary evaporator at 40^{o}c to a dark brown crude extract (8.0g). The crude extract was partitioned between $CHCl_3$ and water. 4.0g of the $CHCl_3$ fraction was then partitioned between petroleum ether (60-80^{o}c) and aqueous methanol. 3.0g of the chloroform fraction was subjected to column chromatography over silica gel (200 mesh) and eluted gradually with petroleum ether, petroleum ether : $CHCl_3$ 90:10,80:20,70:30,60:40,50:50,40:60, 30:70,20:80,10:90), $CHCl_3$ then $CHCl_3$–Methanol (90:10,80:20,70:30,60:40,50:50, 40:60,30:70,20:80,10:90) and methanol to yield eight major fractions labeled : F(light yellow oil, 0.5g), G_1 (yellow oil ,0.4g), H (golden yellow oil,0.80g), I (light yellow oil, 0.3g), K (yellow oil, 0.5g), c_2 (yellow oil, 0.40g), g (brown oil 0.1g).

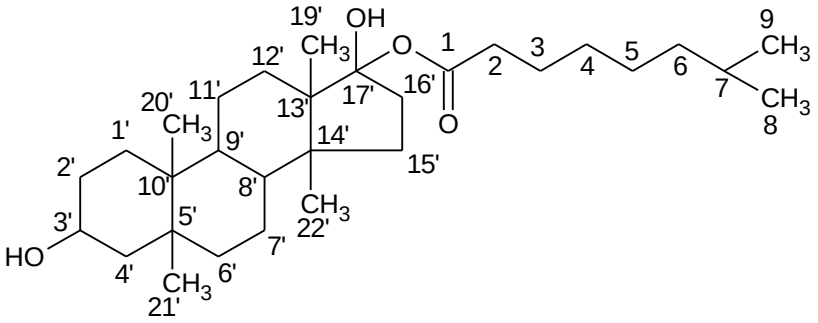
Thin-layer Chromatography

A thin layer of the adsorbent, silica gel (60G) was spread onto the glass plate and allowed to dry. The different sample solutions were spotted each as a small spot near the base of the plate using capillary tube and placed upright in a TLC tank which contains a shallow layer of solvent, the solvent ascends the layer of the adsorbent on the plate. When the thin layer plate has been developed, the plate was removed from the developing tank and allowed to dry until it is free of

solvent. The plate was placed in a chamber containing iodine crystals and left to stand for a short time (Uchegbu *et al*, 2015; Uchegbu *et al*, 2019). Most of the fractions gave a coloured spot each that was clearly visible and their R_f values were calculated.

Chromatographic (partition chromatography, column chromatography, and TLC), spectroscopic (IR, ^{1}HNMR, ^{13}CNMR and MS), 2DNMR techniques were employed to isolate, characterize and identify active constituent from $CHCl_3$ extracts of the leaves.

Results and Discussion



[1] hexadecahydro-3,17-dihydroxy-5,10,13,14-tetramethyl-1*H*-cyclopenta[*a*]phenanthren-17-yl 7-methyloctanoate

Compound [1] was isolated using a mixture chloroform and petroleum ether in the ratio of 70:30. The thin layer chromatography carried out on compound [1] showed one spot. Based on the chromatographic spectra, IR, NMR, MASS, COSY and DEPT, the compound was proposed as hexadecahydro – 3, 17 – dihydro – 5, 10, 13, 14 – tetramethyl – 1 H- cyclopenta (α) phenanthren – 17 – yl, 7 – methyloctanoate with molecular formula C_{30} H_{52} O_4 m/z 476 calculated for m/z 475.3241 and its base peak at m/z 409.3834 calculated for m/z 409 ($C_{25}H_{45}O_4$).

IR spectrum revealed V_{max} (3400, 2921.48, 1737.37 and 1244.19 cm^{-1}) for hydroxyl, aliphatic, carbonyl, and ether respectively. IR Spectrum showed the presence of ester and the C = O

absorption due to esters occurred at 1737.37cm^{-1}. In addition strong absorption of C – O stretch occurred at 1244.19 cm^{-1} indicating the presence of ether. The spectrum also depicted the presence of OH at 3400 cm^{-1} and aliphatic functional groups at 2921.48 cm^{-1} and 2851.06 cm^{-1}

The ^{1}HNMR spectrum showed the presence of methyl protons with absorption at δ 0.7906, δ 0.7987, δ 0.8107, δ 0.8300, δ 0.8388 & δ 0.8656 labelled H – 18, H – 19, H – 20, H – 21, H – 8^{1} and H – 9^{1} respectively. The chemical shifts for methylene protons were observed at δ1.2836, δ1.2993, δ–1.3271, δ1.3329, δ1.3393, δ1.3536, δ1. 3582, δ1.3946, δ1.4017, δ1.4225, δ 1.4289, δ1.4928. These were labelled H – 1, H – 2, H – 4, H – 6, H – 7, H – 11, H – 12, H – 15, H – 16, H – 2^{1} and H – 3^{1} H – 4^{1} , H – 5^{1} & H – 6^{1} respectively. Absorptions of the methane protons were seen at δ 37.1507, δ 37.7277, δ 38.2743 & δ 38.2743. These were labelled H – 3a, H – 8, H – 9, and H – 7^{1} . Presence of hydroxyl, OH chemical shifts were observed at δ 4.5023 and δ 5.1811.

The ^{13}CNMR spectrum confirmed the presence of ester. The absorption of ester carbon – oxygen double (C = O) was showed at δ 171.3815. This is labelled C – 1^{1}. The chemical shift for quaternary carbons were also depicted in the spectrum at δ 47.2437, δ 55.2652, δ 121.6507, δ80.9589 & δ145.4859 and were labelled C - 5, C – 10, C – 13, C – 14 and C – 17a respectively. Methyl carbons showed their absorptions at δ 15.5660, δ 16.7032, δ 16.8151, δ 18.2728, δ 21.3246 and δ 23.5424 and labelled C – 18, C – 19, C – 20, C – 21, C – 8^{1} and C – 9^{1} respectively. Absorption of methylene carbons were seen at δ 34.7411, δ 36.8563, δ 23.5827, δ 23.7002, δ 25.9572, δ 26.1463, δ 26.9354, δ 28.0439, δ 28.4027, δ 29.7063, δ 31.0949, δ 32.5025, δ 32.5992 and δ 33.3374. These were labelled C – 1, C - 2, C – 4, C – 6, C - 7, C – 11, C – 12, C -15, C – 16, C – 2^{1} , C – 3^{1}, C – 4^{1} , C – 5^{1} and C – 6^{1} respectively from the spectrum. Methine carbons showed their absorptions at δ 37.7277, δ 38.2743 and δ 38.2743 and were labelled C -3a, C – 8, C – 9, and C – 7^{1} respectively.

COSY (CORRELATION SPECTROSCOPY) analysis is of two types, from ^{1}H-^{13}C COSY spectrum, it showed that proton at δ 1.57 has a cross peak with carbon at δ 38.3 to form CH Proton

at δ 1,55 has a cross peak with carbon at δ 37.7 to form CH. Also proton at δ1.50 has a cross peak with carbon at δ 37.1.

From $^{1}H - ^{1}H$ COSY spectrum, it was observed that the proton at δ 4.50 couples with proton at δ 0.70 and that gives a "cross peak " at (δ 0.70, δ 4.50). Also, proton at δ 2.0 couples with proton at δ 0.8 to give a "cross peak"at (δ 0.8, δ 2.0) . It was observed that proton at δ 5.20 has a " cross peak " with proton at δ 2.0

The DEPT Spectrum depicted carbons attached with odd and even numbers protons. The carbons attached with odd numbers of protons such as CH and CH_3 have a positive phase (Up) and those with even number protons have a negative phase (down). The spectrum confirms the absorbance of methyl gas carbons at δ 15.5621, δ 16.7002, δ 16.8119, δ 18.2699 having positive phases. These were assigned to C – 18, C - 19, C – 20, and C – 21, respectively. Absorbance of methylene carbons were also observed in the spectrum at δ 23.5413, δ 23.5801, δ 25.9542, δ 26.1422, δ 29.7038, δ 32.5953 having negative phases due to even numbers of protons. They were assigned to C - 4, C – 6, C – 7, C – 11, C – 12, C – 15, and C – 16. Methine carbons showed their chemical shift at δ 37.1461 and δ 38.2710 labelled C - 3a and C - 9 respectively.

The isolated compound is an ester. Esters have many uses in both the living world and industries. Esters are used as food and drug preservatives because they can prevent the growth of microorganisms such as molds and yeast. They have sweet fruit smells. Their properties make them suitable for the preparation of cosmetics and perfumes. These esters are used as food additives to improve the flavor and aroma of processed foods.

CONCLUSION

The novel compound isolated from the leaves of this plant being an Ester and a phenanthrene derivative possesses biological activities. Reports showed that derivatives of phenanthrene possess some biological activities such as anticancer activity, anti-leukemic activity, antitumor activity etc. Esters have many uses in both the living world and industries (Onyekwere *et al*, 2020), thus the

isolated compound may be the reason why the leaves of *Antiaris africana* is used in treatment of many diseases traditionally.

Table 1: Infrared Analysis (cm^{-1}) of Compound [1]

IR ABSORPTION (CM^{-1})	FUNCTIONAL GROUP	BOND TYPE
3400	OH	ALCOHOL
2921.48	CH	ALIPHATIC
2851.06	CH	ALIPHATIC
1737.37	C = 0	CARBOXYL (ESTER)
1244.19	C - O	CARBONYL

Table 2: ^{1}H and ^{13}CNMR Analysis of Compound [1]

	^{13}C CHEMICAL SHIFT	TYPES OF CARBON	^{1}H CHEMICAL SHIFT	TYPE OF CARBON	MULTIPLICITY
1^{I}	34.7411	CH_2	1.2836	CH_2	2Ht
2^{I}	36.8563	CH_2	1.2993	CH_2	2Ht
$3a^{I}$	37.1506	CH	1.5006	CH	1Hm
$3b^{I}$	-	-	4.5023	OH	1Hs
4^{I}	23.5829	CH_2	1.3160	CH_2	2Hd
5^{I}	47.2437	Cq	-	-	-

6 [I]	23.7002	CH_2	1.3271	CH_2	2Ht
7 [I]	25.9572	CH_2	1.3329	CH_2	2Hq
8 [I]	37.7277	CH	1.5455	CH	1Ht
9 [I]	38.2743	CH	1.5718	CH	1Hs
10 [I]	55.2652	Cq	-	-	-
11 [I]	26.1463	CH_2	1.3393	CH_2	2Hq
12 [I]	26.9354	CH_2	1.3456	CH_2	2Ht
13 [I]	121.6507	Cq	-	-	-
14 [I]	80.9589	Cq	-	-	-
15 [I]	28.0439	CH_2	1.3536	CH_2	2Ht
16 [I]	28.4029	CH_2	1.3582	CH_2	2Ht
17 [I]	145.4859	Cq	-	-	-
17b	-	-	5.1811	OH	1Hs
18 [I]	15.5660	CH_3	0.7906	CH_3	3Hs
19	16.7032	CH_3	0.7987	CH_3	3Hs
20	16.8151	CH_3	0.8107	CH_3	3Hs
21	18.2728	CH_3	0.8300	CH_3	3Hs
1	171.3815	C = O(ESTER)	-	-	-
2	29.7063	CH_2	1.3946	CH_2	2Hs
3	31.0949	CH_2	1.4017	CH_2	2Hs
4	32.5025	CH_2	1.4225	CH_2	2Hs
5	32.5992	CH_2	1.4289	CH_2	2Hs
6	33.3574	CH_2	1.4928	CH_2	2Hs
7	38.2743	CH	1.5817	CH	1Hs

8	21.3246	CH_3	0.8388	CH_3	3Hs
9	23.5424	CH_3	0.8656	CH_3	3Hs

s- singlet, d- doublet, t- triplet, q- quartet

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