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Isolation and Characterization of a Phenanthrene Ester from the leaves of *Oxystigma mannii*

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Abstract
This study worked on a lesser known plant Oxystigma mannii and report herein the isolation and characterization of a new Phenanthrene ester, phenyl 8-((3-hydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-17-yl)oxy)octanoate from Oxystigma mannii, a medicinal plant used by the Herbalists of Central Africa for the treatment of different ailments. The structure of the new compound was determined based on the IR, ^{1}HNMR, ^{13}CNMR, DEPT, COSY, HMBC and HSQC spectra. The presence of the isolated compound may be responsible for some of the biological activities exhibited by the plant.

Keywords: *Oxystigma mannii, Phenanthrene ester, **A**ilments*

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I. Introduction

Plants have formed the basis of sophisticated traditional medicinal systems that have been in existence for thousands of years, and they continue to provide humanity with new remedies (Gurib-Fakim, 2006). It has been known that the survival and continued existence of man depends on the efficiency with which man, with all the resources and technology available to him, harnesses, develops and utilizes plants and plant products (Echeme, Ahuchogu and Uchegbu, 2014)

The importance of plants in human and animal health is evident in the increasing presence of natural product drugs in modern medicine. Indeed, natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world today. According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs.

The current advancement in science has made it possible for the isolation of compounds of medical importance from plants that are used in traditional medicinal practices. Different parts of plants such as leaves, roots, stems barks and rhizomes are often extracted using different solvents. In most cases, extracts have been shown to be biologically active both in the *in vitro* and *in vivo* test system (Van-Wyk and Wink, 2004). *Oxystigma mannii* (aka Ntufiak), a forest tree located in fresh water swampy forest of west African growing in Nigeria, Cameroun, Gabon and Equatorial Guinea (Peyeino *et al.*, 2021; Breteler, 1999) is one of the lesser known medicinal plants used in Nigeria to treat different diseases. The stembark of this plant is used locally in treatment of various skin diseases (leprosy, scabies, and fungal skin diseases) and sore throat. This plant is antiseptic, stimulant, expectorant, urogenital, diuretic, for treating diarrhea, and dysentery (Uphof, 1968). The leaves of *Oxystigma mannii* are being used locally to treat malaria, tooth ache etc according to a local source.
In spite of the various uses of this plant as medicine, its constituents have not been fully documented. In this research, a novel compound was isolated and characterized from the leaves of *Oxystigma mannii.*

II. Materials and Methods

2.1 Plant Collection and Identification

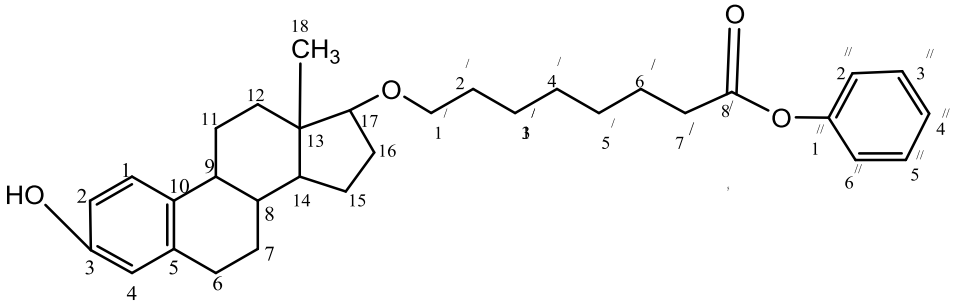
The plant leaves are collected in the month of July, 2019 from a forest edge in Ikono Local Government Area of Akwa Ibom State, Nigeria. It was identified by Mr. Imoh Johnny, a botanist in the Faculty of Pharmacy and Natural medicine, University of Uyo, Nigeria. It was deposited in the University herbarium for referencing with the voucher specimen (**UUPH20i)** for referencing.

2.2 Sample preparation/Extraction

The plant samples (Ntufiak leaves) were washed, air-dried and shredded before being weighed. The leaves extract were prepared by cold maceration. About 1300g of the plant material was poured into a glass container and soaked in methanol (Analytical grade) for 72 hours. The extract was decanted and filtered using

Whatman filter paper No 1. The filtrate were concentrated using Rotary evaporator at a temperature of 25 ^{0}C to yield a greenish-black extract. After total evaporation of the yield at room temperature, a yield of 34.89g was obtained. The crude extract was partitioned between $CHCl_3$ and water and a $CHCl_3$ - soluble fraction (20.5 g) was obtained. 15.0 g of the $CHCl_3$ fraction was then partitioned between petroleum ether (60 – 80 ^{0}C) and aqueous Methanol. 5.0 g of the $CHCl_3$ fraction was then 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 (Uchegbu , Echeme & Iwu (2014)).

III. Results and Discussions



[1] P**henyl 8-((3-hydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-17-yl)oxy)octanoate**

Compound [1] was eluted with chloroform and methanol at the ratio of 30:70. It was isolated as greenish oil (0.89g). TLC carried on the compound showed one spot (Rf 0.670). The molecular formula of compound [1] was $C_{32}H_{42}O4$ m/z 490 calculated based on the HREIMS m/z 490. The base peak occurred at m/z 219. The structure was proposed based on combined analysis of IR, ^{1}HNMR, ^{13}CNMR, DEPT,COSY,HMBC and HSQC spectra.

The Infrared spectrum (Fig 1) indicated an absorption at 3444.1 cm^{-1} indicating the presence of hydroxyl (OH). The presence of ester carbon-oxygen doubled bond (C = 0) at 1729.5 cm^{-1}. The spectrum depicted carbonyl carbon – oxygen single bond (C – 0) at 1379.1 cm^{-1} , 1282.2 cm^{-1} and 1170.4 cm^{-1}, aromatic ring carbon – carbon double bond was observed at 1461.1 cm $^{-1}$, carbon-hydrogen bond at 2922.2 cm^{-1} and 2855.1 cm^{-1} .

^{1}HNMR spectrum (fig 2) showed the presence of methyl proton labeled 18. The assignment was supported by the high field chemical shift. methylene protons were seen at δ as observed at 0.914 1.121, δ 1.185, δ 1.232, δ 1.315, δ 1.335, δ 1.364, δ 1.389, δ 1.414, δ 1.439, δ 1.479, δ 1.502,1.520 and δ 12.065 were labeled H – 6, H – 7, H – 11, H-12, H-15, H-16, H-1^1, H – 2^1, H – 3^1, H – 4^1, H – 5^1, H – 6^1 and H – 7^1 respectively. The ^{1}HNMR showed a peak at δ 12.065 depicting esther (CH_2 –COOR) which was labeled H – 7^1. Methine protons signals were depicted at δ 1.620 , δ 1.646 and δ 1.669 , labeled H – 8, H – 9 and H – 14 respectively. ^{1}HNMR spectrum indicated the presence of hydroxyl proton, the signal was depicted at δ 4.224 and labeled H-3. Aromatic protons were observed at δ 7.649, δ 7.661, δ 7.667, δ 7.670, δ 7.680, δ 7.692, δ 7.696, δ 7.708, δ 7.718, δ 7.722, δ 7.727 and δ 7.739 indicating the presence of two aromatic rings and labeled H-1, H-2, H-4, H – 2^{11}, H – 3^{11}, H – 4^{11}, H – 5^{11}, and H – 6^{11}. These were confirmed in ^{13}CNMR spectrum (fig 3).

^{13}CNMR spectrum (fig 3) indicated the presence of methyl carbon with the signal observed at δ 13.54 and was labeled C – 18. while methylene carbons signals were depicted at δ 13.92, δ 18.66, δ 22.10, δ 24.42, δ 28.38, δ 28.43, δ 28.55, δ 28.72, δ 28.91, δ 29.04, δ 30.01, δ 31.31 and δ 38.68 and labeled C-6, C-7, C-11, C-12, C-15, C-16, C-1^1, C-2^1, C – 3^1, C – 4^1, C – 5^1, C– 6^1 and C-7^1 respectively. The chemical shifts of methine carbons were seen at δ 38.96, δ 39.24, δ 39.52 and δ 40.07 and are labeled C-8, C-9, C-14 and C-17. The ^{13}CNMR spectrum confirmed the presence of hydroxyl carbon in the compound. The chemical shift was observed at δ 65.01 and it was labeled C-3. ^{13}CNMR spectrum has resonance peaks depicting the presence of aromatic carbons at δ 115.00, δ 116.21 , δ 118.00 , δ 119.10, δ 121.00 , δ 128.10, δ 128.65, δ 128.66, δ 131.50 and δ 131.71. The carbons were labeled C-1, C-2, C-4, C-2^{11}, C – 3^{11}, C – 4^{11}, C – 5^{11}, and C– 6^{11} respectively. The chemical shift at δ 166.96 confirmed the presence of carbonyl carbon and this was labeled C – 8^1. The spectrum shows the chemical shifts of aromatic quaternary carbons at δ 119.10, δ 120.11 and δ 121.00 labeled C-5, C-10 and C- 1^{11}. Another quaternary carbon present was seen at δ 40.07 and labeled C-13.

The DEPT spectrum (fig 4), confirmed the presence of methylene carbons with their peaks at 18.65 ppm, 22.10 ppm,24.40 ppm, 28.34 ppm, 28.45 ppm, 28.55 ppm, 28.71 ppm, 28.91 ppm, 29.03 ppm, 30.01 ppm, 31.30 ppm, 33.66 ppm. The methylene carbons show negative peaks and are pointing down. Only one methyl

proton was prominent from the spectrum, it's peak points up at 13.92 ppm . Methine peaks were seen and confirmed by the DEPT spectrum. The peaks point down at 39.21 ppm, 39.49 ppm, 39.92 ppm. The spectrum indicated the presence of two aromatic rings in the proposed structure. The presence of carbon bearing hydroxyl group was indicated in the spectrum with its peak facing up at 65.01 ppm.

In the COSY spectrum (fig 5) of compound [1] there are peaks that appear along the diagonal stretching from the upper right to the lower left through the middle of the plot. The interesting peaks are the peaks that do not appear along the diagonal. These peaks indicate which hydrogen is coupled to which other hydrogen. The signal at 0.9 ppm has a cross peak to a quartet with a proton at 1.62 ppm. Signal at 1.5 ppm has a cross peak to 2.3 ppm showing singlet. Proton at 4.22 ppm has a cross peak to a triplet at 1.60 ppm.

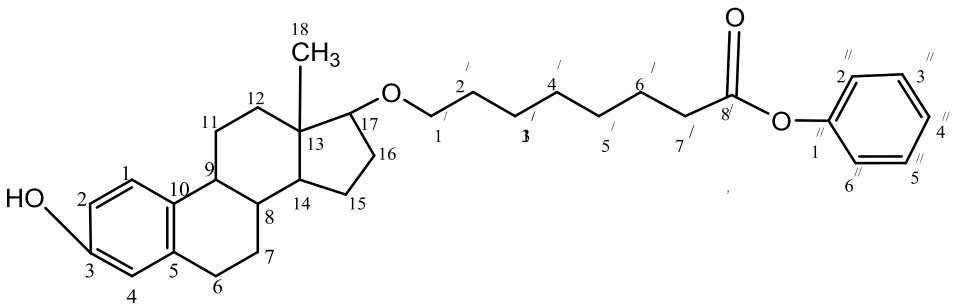
From ^{1}H-^{13}C Heteronuclear Single Quantum Coherence Spectroscopy (HSQC) spectrum (fig 6), the proton signal at 0.9 ppm is correlated to the carbon signal at 14.0 ppm pointing upwards or positively, thus indicating a CH_3 group and splitting triplet. Hence this confirms that there is only one methyl (CH_3) group in the structure. A proton signal seen at 1.22 ppm has a correlation with the carbon at 22.1 ppm facing downwards or negatively, thus indicating a CH_2 group and a singlet. This is confirmed in the ^{1}HNMR and ^{13}CNMR assignments and is labeled C-11. Another correlation occurs between proton at 1.62 ppm and carbon at 31.3 ppm, pointing downwards and depicting a CH_2 group. This confirms the ^{1}HNMR and ^{13}CNMR assignments and is labeled C-$6^{/}$.

The Heteronuclear Multiple Bond Correlation spectrum, HMBC (fig 7) is useful for assigning carbons that have no protons attached to it. From compound [1] above, carbon 5,10,13 and $1^{//}$ have no protons attached to them. C-5 is assigned by HMBC interactions with protons 1,2,4,8,9 and not 3. C-10 is assigned by HMBC interactions with protons1,2,4,8.9 and not 3. C-13 is assigned by HMBC interactions with protons 8,9, 14 and 17. C-$1^{//}$ is assigned by HMBC interactions with protons $2^{//}$ and $6^{//}$, the chemical environment shows it would go downfield than C-5 and C-10 confirming the assignment.

Derivatives of phenanthrene have been reported to possess some biological activities such as anticancer activity, antileukemic activity, antitumor activity. Another research work revealed that phenanthrene derivatives have many distinct therapeutic benefits such as analgestic, antimalarial, antitussive, cytotoxic and anticonstipation (Kwofie and Gupta ,2021). This analysis confirm the use of *Oxystigma mannii* plant by herbalists to treat malaria and other ailments.

The isolated compound is also an ester, esters have many uses in both the living world and industries. Esters are also 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 smell of processed foods. The result of this analysis also indicates that the leaves of *Oxystigma mannii* can be used as a preservative in foods and drugs as well as serve as a natural additive in foods.

The fragmentation pattern of compound [1] is shown in fig [8]. Detachment of the $C_{14}H_{19}O_2$ fragment produced the peak at m/z 219. Another detachment of $C_8H_7O_2$ and $C_{18}H_{24}O_2$ ions from the compound produced the peaks at m/z 135 and m/z 272 respectively.



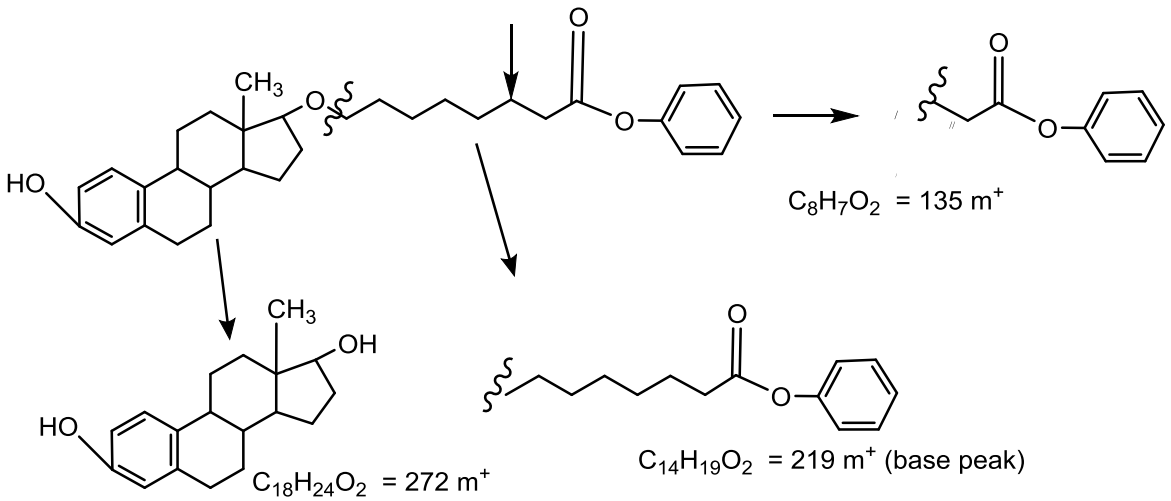


Fig 8 The fragmentation pattern of compound [1]

TABLE 1: INFRARED ANALYSIS OF COMPOUND I

ABSORPTION (Cm-1)	FUNCTIONAL GROUP	TYPE OF COMPOUND
3444.1	-OH	Alcohol
2922.2	-CH	Aliphatic
2855.1	-CH	Aliphatic
1729.5	C=O	Carbohy1 (ester)
1461.1	-C=C	Aromatic
1379.1	C-O	Ether
1282.2	C-O	Ether
1170.4	C-O	Ether

TABLE 2: ^{1}HNMR & ^{13}CNMR CHEMICAL SHIFTS OF COMPOUND (1)

POSITION	CHEMICAL SHIFT(PPM)	TYPE OF CARBON	CHEMICAL SHIFT	MULTIPLICITY	TYPE OF PROTON
1	115.00	Ar-CH	7.649	1Hd	Ar-CH
2	116.21	Ar- CH	7.661	1Hd	Ar – CH
3	65.01	C – OH	4.202	1Ht	Ar – OH
4	118.00	Ar – CH	7.670	1HS	Ar – CH
5	119.10	Ar – C	7.680	-	-
6	13.92	CH_2	1.121	2 Ht	CH_2
7	18.66	CH_2	1.185	2Hq	CH_2
8	38.96	- CH	1.620	1 Hq	CH
9	39.24	- CH	1.646	1 Ht	CH
10	120.11	Ar-C	-	-	-
11	22.10	CH_2	1.232	2Hs	$C_{H2`}$
12	24.42	$-CH_2$	1.315	2Ht	CH^2
13	40.07	C	-	-	-
14	39.52	CH	1.669	1 Hq	CH
15	28.38	CH_2	1.330	2Hq	CH2
16	28.43	CH_2	1.364	2Hq	CH2
17	-	CH-O	4.224	1Ht	OH
18	13.54	CH_3	0.914	1Ht	CH_3
1^1	28.55	CH_2	1.389	2Ht	CH_2
2^1	28.72	CH_2	1.414	2Hq	CH_2
3^1	28.91	CH_2	1.439	2Hq	CH_2
4^1	29.04	CH_2	1.479	2Hs	CH_2
5^1	30.01	CH_2	1.502	2Hs	CH_2
6^1	31.31	CH_2	1.620	2Ht	CH_2
7^1	38.68	CH_2	12.065	2Hs	CH_2
8^1	166.96	-C00R	-	-	-
1^1	121.00	Ar-C	-	-	-
2^1	128.10	Ar – CH	7.698	1H d	Ar-CH
3^1	128.65	Ar – CH	7.708	I H t	Ar - CH
4^1	128.66	Ar – CH	7.718	I H t	Ar-CH
5^1	131.50	Ar- CH	7.722	1 H t	Ar-CH
6^1	131.71	Ar – CH	7.727	I H d	Ar-CH

IV. Conclusion

Oxystigma mannii is a medicinal plant that is not well known in Nigeria. Much work has not been done on it thus it was difficult to get many empirical studies. Hence, the result of this analysis showed that *Oxystigma mannii* leaves can serve as source of quality raw material for pharmaceutical industries for production of useful drugs that can be used for the treatment of cancer, leukemia, tumor, malaria e.t.c..

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