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Der Pharmacia Lettre, 2011, 3(1): 221-227
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Anticandidal Activity of Phenanthrenol from *Blighia sapida* Koenig (Sapindaceae) [#]

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ABSTRACT

A new Phenanthrenol; Dodecahydro-1H-cyclopenta[a] phenanthren-10-ol was isolated from the stem bark of Blighia sapida Koenig (Sapindaceae), a medicinal plant used in Nigerian traditional medicine. The structural elucidation was based on analyses of spectroscopic data. The isolate was assessed against clinical strain of Candida albicans. The minimum inhibition concentration (MIC) of this compound (1.6 µg/mL) is the basis of this report.

Keywords: *Blighia sapida*, *Candida albicans*, Phenanthrenol, Minimum inhibitory concentration.

INTRODUCTION

Blighia sapida Koenig is a tropical tree belonging to the Sapindaceae family. It has its origin in West Africa. The fruit aril of *B. sapida* which is edible is reported to reduce the blood sugar content. The plant serves as a component in some local dishes. Previous examinations of the fruit part of this plant revealed the presence of hederagenin, hypoglycin A, hypoglycin B, blighinone [1-4], vomifoliol [5] and non-proteinogenic amino acid: (2S,1'S,2'S)-2-(2'-carboxycyclopropyl) glycine [6]. There is little or no literature on the isolated compound from the stem bark of this plant.

In this paper we report the isolation and elucidation of a new phenanthrenol derivative from the plant and its biological importance as regard its anticandidal properties. This is part of our extensive research aimed at the characterization of the chemical constituents and biological activities of Nigerian medicinal plants and herbs as they are made available [7].

MATERIALS AND METHODS

General

^1H–^1H COSY, HSQC, DEPT, and HMBC were recorded on Bruker Advance 400 MHz NMR spectrometer in $CDCl_3$ with TMS as internal standard. UV spectra were recorded using UV-Visible HP-8453 Spectrophotometer. IR spectra were recorded on a Nicolet FT-IR spectrometer in solution. HR-EIMS spectra were recorded with a JEOL JMS-SX102A (EIMS, 70 eV, gun high 3.0 kV). For chromatography, Silica gel 60 (70-230 mesh) was used and all solvents were distilled prior to use. Whatman precoated silica gel (60A_K6F) plates were used for TLC, with compounds visualized by their UV absorbance at 254 nm and by spraying with 10% (v/v) H_2SO_4 or vanilline-H_2SO_4 followed by heating.

Plant Material

The stem bark of *B. sapida* was collected at a location in Ogbomoso, a town 90km north of Ibadan, Oyo State, Nigeria. It was botanically identified by Mr. T. K. Odewo of the Herbarium, Forestry Research Institute of Nigeria (FRIN), Ibadan. Specimen of the plant was deposited in the herbarium of the Institute. The stem bark was milled using Thomas model 4 Wiley mill and hermetically sealed in plastic bag for use.

Extraction and Isolation

The plant material (2000 g) was exhaustively extracted by percolation with 95% Ethanol, EtOH (5 L) at room temperature for a week. The percolate was evaporated to dryness on a Rotary evaporator at 35 °C to give EtOH residue. The residue EtOH [30 g] was defatted through maceration with n-hexane (3 x 200 mL) to obtained n-hexane extract [9.1 g] after being concentrated in vacuum. The remaining EtOH residue (after being defatted) was extracted with ethyl acetate (3 x 200 mL) by further maceration. The ethyl acetate extract was concentrated to obtained ethyl acetate residue [15.3 g]. The ethyl acetate fraction registered a moderate degree of anticandidal activity at 100 µg/mL concentration tested when compared with ketocanazole. A portion of this EtOAc soluble residue (4.0 g) was fractionated in an open column Silica gel (120 g) eluted with step-wise gradient of increasing percentage of $CHCl_3$ in n-hexane as eluent. Eluants were analyzed on TLC, pooled into 16 fractions and each subjected to anticandidal assay. The anticandidal activity was prominent in a fraction (175 mg) that was eluted with 30% $CHCl_3$ in n-hexane. Further purification of this fraction over silica using pencil column in n-hexane: chloroform (4:1) with two drops of benzene as eluting solvent resulted in the isolation of the active component. The active component afforded a dirty yellow precipitate which was purified by re-crystallizing in 2-propanol. This produced a cream bioactive compound Phenanthrenol [30 mg]. Phenanthrenol, a cream precipitate is a new natural compound. Efforts to isolate a single crystal of the precipitate for X-ray crystallography were unsuccessful.

Anticandidal assay

In-vitro anticandidal activity was performed using the agar-well diffusion assay as describe by Hufford et al., [8] with some modifications. Clinical isolate of *C. albicans* designated as UCH was used for the evaluation of anticandidal activity. The organism was grown in Sabouraud-dextrose broth (SDB) for 24 h at 37^0C. The cells were harvested by centrifugation (2000 rpm, 3 min). The cells were then washed and suspended in sterile 0.9% saline to give a final

concentration of 10^6 cfu/ mL. Disposable petri-dishes containing Sabouraud-dextrose agar were streaked with the suspension (10^6 cfu/mL) of *C. albians* UCH using sterile cotton swabs. Cylindrical plugs were removed from agar plates by means of a sterile cork borer to produce wells with a diameter of 10 mm. To the well was added 100 µL of test solution. The activity was recorded as the width in mm of the inhibition following incubation of the plates at 37^0C for 24 h. The antifungal agent used as positive control was ketocanazole (at 0.5 µg/mL). The positive control solution was prepared as describe for the test sample.

Minimum Inhibitory Concentration (MIC) determination

The method used to determine the mic was the twofold serial broth dilution assay in yeast nitrogen broth (Difco). The ethyl acetate fraction that paved way for the phenanthrenol was initially tested using a concentration of 100 µg/mL in the first tube. The test compound was added to sterile Sabouraud-Dextrose broth as a solution in DMSO. The inoculum for the mic determination was prepared as describe by Clark et al., [9]. With the help of calibrated sterile wire loop, 10 µL of the 10^6 cfu/mL suspension of clinical isolate of *C. albicans* from the University College Hospital (UCH, Ibadan) was used as inoculums for each tube. The mic value was taken as the lowest concentration of compound that inhibited the growth of the test organisms after 24 and 48 h of inoculation at 37^0C. The antifungal agent used as positive control was ketocanazole. The positive control solution and test was as described for the test sample.

RESULTS AND DISCUSSION

The isolation of Compound 1 (Phenanthrenol) was guided by *in vitro* anticandidal assays using repetitive open column chromatography to separate bioactive ethyl acetate extract [4.0 g]. The purified Compound 1 was obtained as a cream precipitate (Mpt. 196-198 °C). The infrared and ultraviolet spectra indicated the presence of conjugated system. The IR of Compound 1 also exhibited bands characteristic of alcohol (3538 cm^{-1}) and double bonds (1600 cm^{-1}). The structure and NMR data of compound 1 were first deduced by comparing with the corresponding data of cholesterol and further confirmed by the HSQC and HMBC spectra. The standard ^{13}C NMR spectrum as well as polarization transfer (DEPT) experiment of Compound 1 depicted twenty-seven carbon atoms. The ^{13}C NMR (DEPT) showed resonance for three quaternary carbons, ten methines, ten methylenes and four methyl groups giving an attached proton formula of $C_{27}H_{41}$ (Table 1). One important piece of evidence for the structural elucidation came from the high resolution exact mass measurement of Compound 1, providing a dehydrated molecule of m/z 416, indicating a molecular formula of $C_{27}H_{44}O_3$ (Cal. 416.637, found 416.1). This molecular formula possesses a double bond equivalent of six, which was consistent with the exact mass measurements. The clustering of signals between 0.85 and 1.8 is a characteristic of triterpenes moiety.

The ^{1}H-^{13}C 2D NMR shift correlated measurement (HSQC and HMBC) of Compound 1 showed the presence of three protons (δ 5.00, H-18; δ 5.01, H-3; and δ 5.34, H-11) which are not directly attached to any carbon. This fact suggested the presence and attachment of heteroatoms like oxygen. The downfield shifted resonance at δ5.34 indicated that this hydroxyl group was attached to double-bond. Two oxy-methine groups were also recognized at δc 72.2 and δc 57.1 suggesting cyclic and aliphatic oxy-methine groups respectively. The ^{1}H NMR spectrum

contained a proton signal at δ 6.53 (1H, d, H-12) that was directly coupled to ^{13}C signals at δ122.1. A ^{1}H-^{13}C 2D NMR shift correlated measurement (HMBC) showed long range coupling between H-12 and C-13/ C-14; H-17 and C-20/ C-21; H-6 and C-19/ C-5; H-14 and C-7/ C-15 which is consistent with and independently confirming the structure of Compound 1 (Figure 1). The NMR data of rings A, D, and the side chain were comparable with those of cholesterol. However, the resonances of C5, C6, C7, C8, C9, C11, C12 and C13 differed. There was no evidence of correlation or connectivity between methyl protons or carbon (C19) with C10/H10 or C5/H5. This implied that the methyl group (C19) is not likely to attach to C10 or C5 as in the case of cholesterol. The detailed HMBC correlations are shown in Table 1. The quaternary centre assignments (8, 9, and 11) were confirmed by utilizing selective low power decoupling techniques (irradiation at H for H-10, H-12, H-7, and H-14). The unsaturations of rings A and B are common in steroids such as estrange, ergosterol, cholesterol and their derivatives. However, unsaturation in ring C is unusual.

Apart from the spectroscopic data in support of the elucidation of Phenanthrenol, we attempted to propose the biosynthetic pathway for the compound as illustrated in Fig. 2 to evaluate its plausible biogenesis. Hydroxylation of the ring B and C of the hypothetical precursor was presumed to have occurred enzymatically. The induction of acetyl unit might have occurred in ring B through CoA ester with acetyl CoA or acetic acid followed by enzymatic hydroxylation. We therefore think that Fig. 2 shows the most plausible pathway to Phenanthrenol in *B. sapida*. While this pathway satisfied the results of our Phenanthrenol, the exact sequence of steps remains unknown, as do the specific intermediate structures. This can be examined by isotopic labeling and enzymatic studies. Phenanthrenol exhibited a significant zone of inhibition against the tested strain of *C. albicans*. The minimum inhibition concentration (mic) of phenanthrenol was found to be 1.6 µg/mL of the strain in yeast nitrogen broth. The current drug of choice for disseminated *Candidiasis* is amphotericin B with MIC value of 0.39 µg/ml. Phenanthrenol could be a promising potential new antifungal drug especially if subjected to structural adjustment reactivity (SAR). Studies are currently in progress to evaluate the *in vivo* efficacy and toxicity of phenanthrenol and the results will be reported elsewhere.

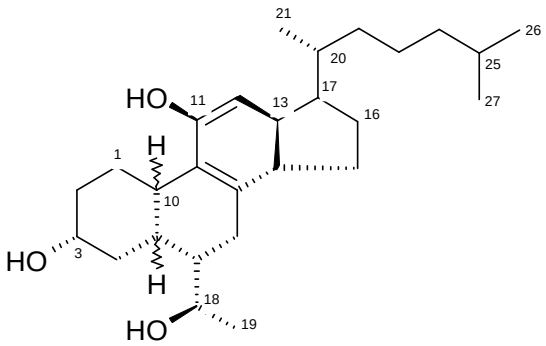

Fig 1: Structure of Phenanthrenol

Dodecahydro-1H-cyclopenta[a] phenanthren-10-ol [Phenanthrenol]
Mp: 196-198ºC; R_f: 0.58; 30 mg (1.5 x 10^{-5}% of dried weight)
^{13}C NMR (100 MHz, $CHCl_3$): see Table 1.; **^{1}H NMR** (400 MHz, $CHCl_3$): Table 1

UV λmax ($CHCl_3$) 251 nm;
IR (Nujor): 3538 (OH); 1051 (C-O); 1600 (C=C) and 2958 (CH str)
EIMS (low and high resolution): m/z 398 (80%)-H_2O, 317; 300; 224
CIMS m/e 190.2, 189.2, 188.1, 121.3, 69, 42.9

Table 1: ^{1}H NMR and ^{13}C NMR spectral data for Phenanthrenol (400 MHz, and 100 MHz, $CDCl_3$)

S/N	**δC[a]**	**δC[b]**	**M**	**HMBC (H to C)**	**δH**
1	30.1	t	37.5	2,3,5,9,10	1.52, 2H, m
2	32.0	t	31.6		1.93, 2H, m
3	72.2	d	71.3	2,4,5	3.81, 1H, m
3-OH	-	-	-		5.01, 1H, m
4	34.3	t	42.4		
5	46.6	d	141.2		2.0, 2H, m
6	37.6	d	121.3	4,5,7,8,10,19	1.90, 1H, m
7	24.4	t	32.0	5,9,18	1.89, 2H, bd
8	138.7	s	160.5		
9	129.6	s	50.5		
10	56.4	d	36.5	8,9	2.66, 1H, m
11	141.1	s	21.2		
11-OH	-	-	-		5.34, 1H, s
12	122.1	d	28.3	9,11,13,14,17	6.53, 1H, d
13	50.5	d	42.4		2.65, 1H, bm
14	42.6	d	56.9	7,8,9,12,13,15	2.73, 1H, bm
15	28.6	t	24.3		1.77, 2H, m
16	29.5	t	40.0		1.33, 2H, m
17	36.9	d	56.5	12,13,14,15,16,20,21	1.79, 1H, m
18	57.1	t	12.0	5,6,7	4.10 1H, m
18-OH	-	-	-		5.00, 1H, m
19	12.5	q	19.4	6,18	1.10, 3H, bd
20	44.2	d	35.8		
21	12.6	q	18.8	17,20,22	1.12, 3H, bd
22	32.3	t	36.4		
23	26.4	t	24.1	20,25	1.59-1-10
24	36.5	t	39.6		6H, m
25	24.7	d	28.0		
26	20.1	q	22.5		
27	20.1	q	22.6	25	0.85, 6H, bd

[a]*Phenanthrenol measured in $CDCl_3$,*
[b]*Cholesterol measured in Dioxane/$CDCl_3$, -not determined, M-Multiplicity.*

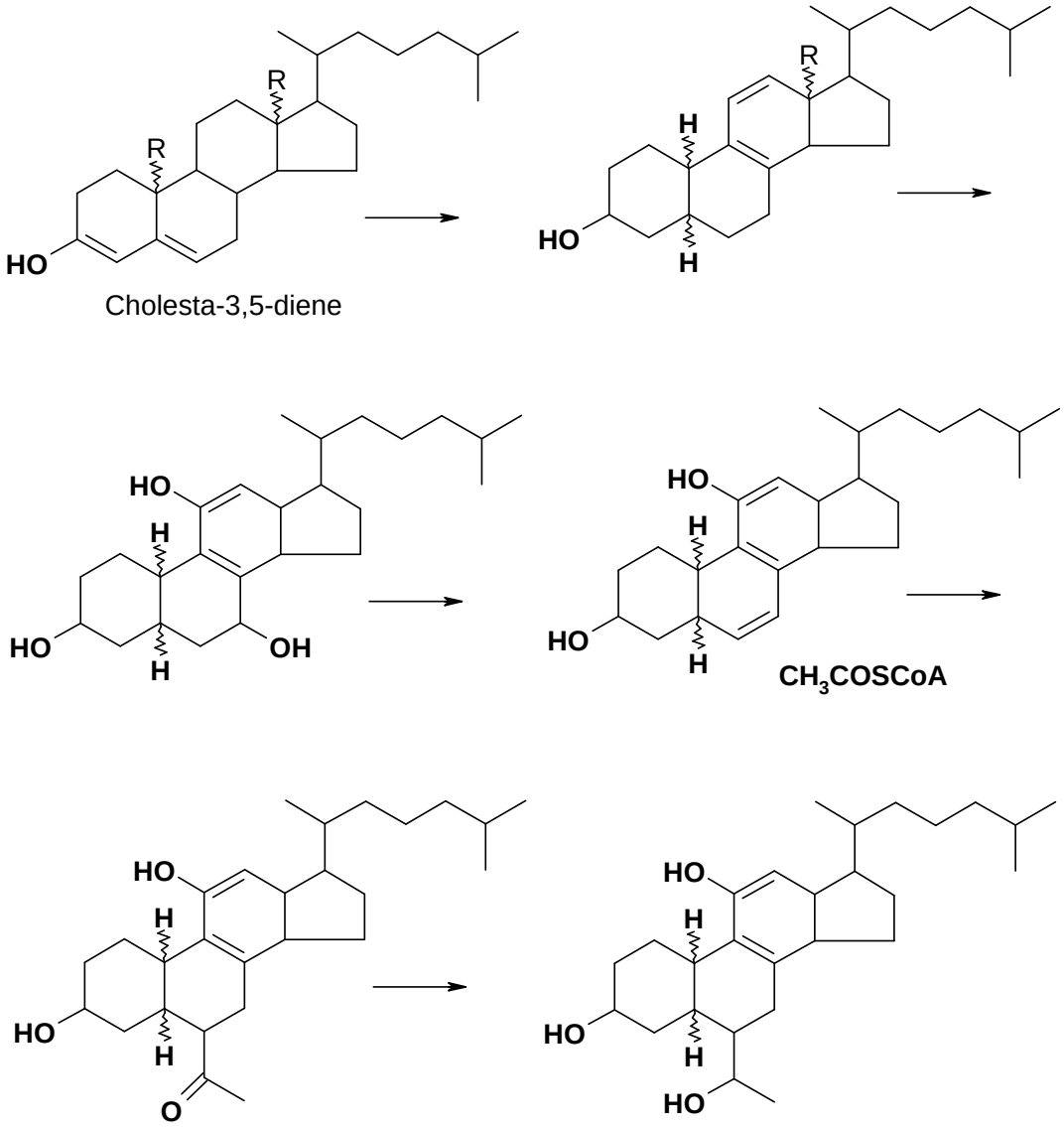

Fig 2: Proposed Biosynthesis of Phenanthrenol

Acknowledgements
IAO is grateful to Prof. Njar formerly of the University of Ibadan, Nigeria for the spectra analysis.

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