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Monoterpene Indole Alkaloid from *Aframomum Meleguata*

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ABSTRACT: As part of our study on bioactive agents from Nigerian medicinal plants, a new monterpene indole alkaloid (10, 12-dihydroxy-18-ethenyl-4-pyrido-β-carboline **1**) was isolated from the leaves of *Aframomum meleguata*. The structure of **1** was elucidated using NMR spectroscopy in combination with IR and MS spectral data.

Key words: *Aframomum meleguata*, *Monoterpenes,* Indole alkaloid, herbal medicine**.**

INTRODUCTION

Species of the *Zingiberaceae,* (ginger family) have been used for various medicinal purposes in Nigeria (Gill 1992, Iwu 1993), and our systematic studies on Nigerian medicinal plants of this family have revealed marked diversity of the secondary metabolites present and their biological activities (Okwu 2007, Okwu and Njoku 2009). As part of our search for bioactive compounds from endemic species from Nigeria rainforest, *A. meleguata* was studied further after detection of antifungal activity in its aqueous and ethanol leaf extracts (Okwu and Njoku 2009).

A meleguata is commonly known as guinea pepper, meleguata pepper, alligator pepper, guinea grains or grains of paradise. It is a herbaceous perennial plant, native to swampy habitats along the West African Coast (Iwu *et al* 1999). Its trumpet shaped purple flowers develop into 5 to 7 cm long pods containing reddish brown seeds. The seeds have a pungent, peppery taste due to aromatic ketones such as gingerol and paradol (Iwu 1993). *A. meleguata* is used as a fungicide. Antifungal effects of the leaf extract of *A. meleguata* on spore germination and mycelial reduction of the most occurring fungal pathogens causing soft rots of white yam (*Disoscorea rotundata*) tuber have been investigated (Okigbo and Ogbonnaya 2006). Fungi isolated from rotten yams were *Aspergillus flavus, Fusarium oxysporum, Rhizopus stolo-niger, Botryodiplodia theobromae* and *Penicillium chrysogenum*. The ethanolic leaf extract followed by cold water and hot water extracts were most effective on these pathogens (Okigbo and Ogbonnaya 2006). *A meleguata* is very effective against major disease causing micro-organisms such as *Escherichia coli, Candida albicans, Staphylococcus aureus, Pseudomonas aeruginosa, Vibrio cholera, Salmonella spp, Streptococcus spp* and *Neisseria gonorrhea* (Iwu *et al* 1999). The plant has been extensively used in herbal medicine not only for its oxytocic, analgesic, anti-inflammatory and anti-microbial properties but also provides relief in the treatment of human gastro-intestinal, hypermobility and peptic ulceration (Gill 1992). It is generally used as a stimulant and febrifuge. The decoction of the leaves is used for small pox and chicken pox (Gill 1992, Okwu 2005, 2007). The seeds of *A. meleguata* and fruits of *Spondias mombin* are powdered and mixed with cold pap as an anti-fertility agent (Gill 1992, Okwu 2007). It is also used for male sexual impotence (Bitti 2002). Both the leaves and the seeds of *A. meleguata* are used for inflamed condition of throat, fever and exanthemata when crushed and rubbed on the body. The plant is used in phytomedicine to cure rheumatoid arthritis, bronchitis, cough and bone treatment (Okwu 2007). They are also applied for headache as well as made into paste for wounds and sores (Okigbo and Ogbonnaya 2006). The other medicinal uses of *A. meleguata* include aphrodisiae, measles and leprosy (Iwu 1993). It is also taken for excessive lactation and hemorrhage, purgative, galactogogue and as haemostatic agent (Iwu 1993). In addition, the plant possesses anti-diabetic properties (Iwu 1993). *A. meleguata* is used as

carminative, as a cough remedy and lactation aid (Iwu *et al* 1999). Other uses are stomachache bronchitis and dysentery. It is used with lemon grass (*Cymbopogon citrates*) for female hygiene (Iwu *et al* 1999). The seeds, leaves and rhizomes are used in West African herbal medicine as stimulant, carminative and diuretic (Iwu *et al* 1999). It is used to treat snake bites and scorpion stings (Duke 2000). In spite of the various uses of *A. meleguata* in herbal medicine in Nigeria and as spices in food and drug production in Nigeria, the phytoconstituents of this herb have not been fully documented. In this paper, the details of the isolation and structure elucidation of a new monoterpene indole alkaloid (10, 12-dihydroxy-18-ethenyl-4-pyrido-β-carboline) from the leaves of *A. meleguata* was presented.

EXPERIMENTAL SECTION

General experimental procedure

The IR spectra were determined on a Thermo Nicolet Nexus 470 FT-IR spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 FT NMR spectrometer using TMS as internal standard. Chemical shifts are expressed in parts per million (ppm). EIMS were performed with a Bruker APEX II mass spectrometer and ESIMS were recorded in the Q-STAR ESI-TOF-MS/MS Spectrometer. HREIMS were obtained on a GCT-MS instrument. Column chromatography was carried out with silica gel (200-300 mesh) and to monitor the preparative separations, analytical thin layer chromatography (TLC) was performed at room temperature on pre-coated 0.25mm thick silica gel 60 F_{254} aluminum plates 20 x 20 cm Merck; Darmstadt, Germany. Reagents and solvents like ethanol, chloroform, diethyl either, hexane were all of analytical grade and were procured from Merck, Darmstadt, Germany.

Plant materials

The fresh leaves of *A. meleguata* were harvested from Ibekuta Ibeku, Okwuato Aboh Mbaise Local Government of Imo State, Nigeria on 15th March 2007. The plant samples (fruits, seeds and leaves) were identified by Dr. A. Nmeregini of Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Nigeria. Voucher specimen No. AF/3355 has been deposited at the Forestry Department Herbarium of the University.

Extraction and isolation plant materials

Plant materials were treated and analyzed at the Chemistry laboratory, Michael Okpara University of Agriculture Umudike, Nigeria. The leaves (1kg) were dried on the laboratory bench for 10 days. The dry sample was milled and ground into powder (950g) using Thomas Wiley machine (model 5 USA). The powdered plant materials were dried and stored in air tight bottles for chemical analysis. The powder plant sample (500g) was packed into a soxhlet apparatus (2 L) and extracted exhaustively with 1000 ml chloroform for 24 h. The chloroform extract was concentrated using a rotary evaporator at 45°C and in a hot air circulating oven to get dark brown oil (10.9g). The column chromatography of the extract was carried out using the chloroform fractions. The column was packed with silica gel and eluted with methanol: chloroform: petroleum ether (20: 30: 50) to get brown oil (0.86mg). The oil gave a single spot on TLC (Rf 0.4667). IR Vmax 3401 cm^{-1} (OH), 3008 cm^{-1} (N-H), 2926 cm^{-1} (-CH_2-), 1244 (-C-N). HEREIMS m/z 281.2439 [M^+]; calculated for m/z 282 C_{17} H_{18} O_2 N_2. ^{1}H NMR and ^{13}C NMR were presented in Table **1**.

RESULTS AND DISCUSSION

Compound **1** was isolated as dark brown oil with strong fluorescence detected at 354 nm which is compatible with an aromatic 5,6-dihydro-β-carboline chromophore, as corroborated by an indole N-H IR band (3008 cm^{-1}). The IR spectrum showed absorptions of Vmax 3401, 3008, 2926 and 1464 cm^{-1}, indicating the presence of –OH, NH, CH_2 and C=C-aromatic functional groups respectively.

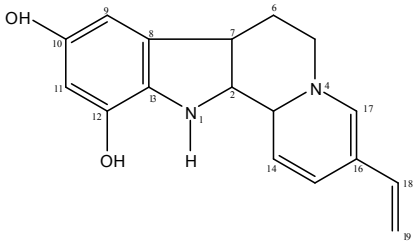

Figure 1: Compound **1** $C_{17}H_{18}O_2N_2$

The HRESIMS of **1** in the positive-ion mode, exhibited a molecular ion peak [M+H] at m/z 281.2439 calculated for $C_{17}H_{18}O_2N_2$ m/z 282. The ^{1}H NMR spectrum (Table 1) revealed the presence of a tetrahydro-β-carboline system due to the signals of δH 4.1675 (1Hs), 4.1397 (1Hs), 2.3275 (2Hs), 2.0204 (2Hd), 1.2775 (1Hs) and 7.4214 (1Hs). This spectrum also shows the presence of the pyridine protons at δH 5.3243 (1Hs) and 5.3281 (1Hs) and 4.1952 respectively.

The ^{13}C NMR spectrum (Table 1) confirmed a tetrahydro-β-carboline monoterpenoid structure for **1**. The ^{13}C NMR showed the presence of a terminal vinyl group for **1** at δC 128.134 and the presence of the signals for the aromatic carbon at δC 130.312, 129.798, and 129.798. Apart from the molecular ion peak at m/z 281.2439, the high resolution mass spectrum gave fragment peaks at m/z 280 and 279 respectively due to proton migration and

rearrangement. Detachment of the hydroxyl group produces the peak at m/z 264.2449 calculated for $C_{17}H_{15}ON_2$ (m/z 263). Further fragmentation afforded the base peak m/z 83.9525 and the peak m/z 81.0706 calculated for $C_5H_7N_2$ (m/z 81). The pattern of fragmentation of compound **1** is shown in figure **2**. This analysis confirmed compound **1** isolated from the leaves of *A. meleguata* to be a monoterpenoid indole alkaloid 10,12-dihydroxy-18-ethenyl-4-pyrido-β-carboline. Indole alkaloids containing a monoterpenoid moiety as in **1** is unusual. The isolation of monoterpenoid indole alkaloid from *A. meleguata* supported the use of this herb in phytomedicine for the treatment of rheumatoid arthritis. This work therefore shows that monoterpenoid indole alkaloid is one of the physiologically bioactive constituents of *A. meleguata*. The occurrence of monoterpenoid indole alkaloid in *A. meleguata* is of significance because this is to the best of our knowledge the first report of its occurrence in any *Aframomum* species. These findings supported the use of *A. meleguata* as raw materials for food and pharmaceutical industries.

Table 1: ^{1}H (400 MHz and ^{13}C NMR (400 MHz) Chemical shifts of Compound 1

Position	δ H		δ C	
1	4.1675	1Hs		
2	1.2553	1Hs	14.166	CH
3	4.1397	1Hs	22.761	CH
4				
5	2.3275 – 2.7705	2Hd	24.816	CH_2
6	2.0204 – 2.0573	2Hd	24.975	CH_2
7	1.2775	1Hs	14.144	CH
8			127.986	C
9	7.4214	1Hs	130.312	CH
10	4.1397	1Hbs	129.798	C
11	7.4214	1Hs	130.312	CH
12	4.1531	1Hbs	129.798	C
13				
14	5.3243	1Hs	127.986	CH
15	5.3281	1Hs	128.154	CH
16			130.101	C
17	5.3360	1Hs	130.312	CH
18	5.3434	1Hs	129.788	CH
19	4.1952	2Hs	128.134	$=CH_2$

s = singlet, d = doublet, m = multiplet, t = triplet, bs = broad singlet

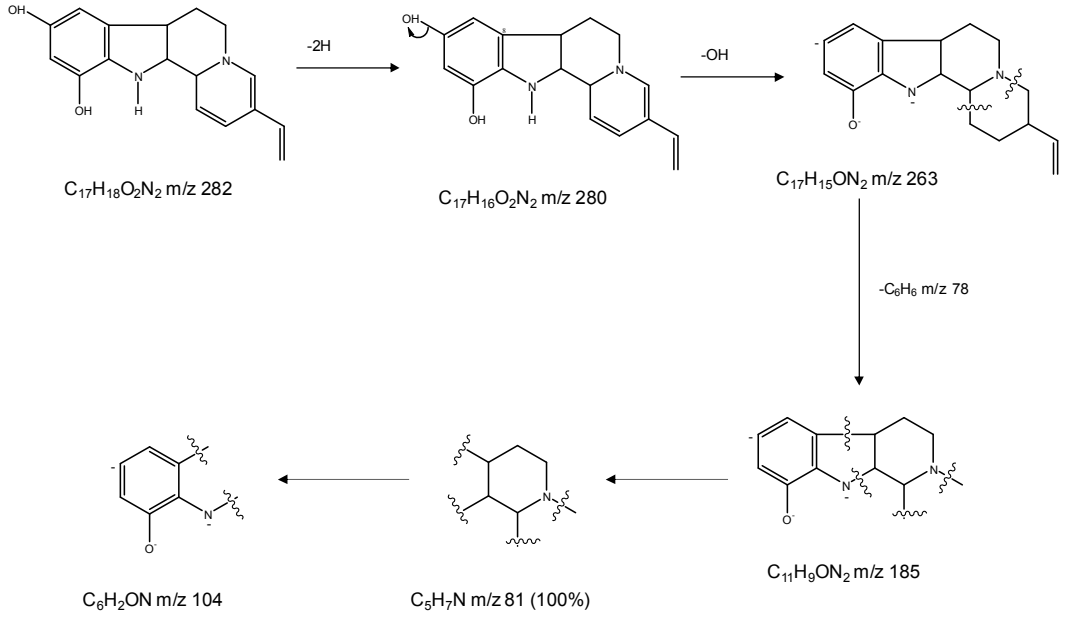

Figure 2: Fragmentation pattern of compound 1

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