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Isolation, characterization and antibacterial activity screening of a new β-carboline alkaloid from *Datura metel* Linn.

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ABSTRACT

Datura metel Linn. (Solanaceae) is a well-known medicinal plant commonly used in phytomedicine to cure diseases and heal injuries. Chemical investigation of the bioactive constituents from the leaves of D. metel afforded a new β-carboline alkaloid (1, 7 dihydroxy-1-methyl 6, 8 dimethoxy β-carboline). The structure was elucidated using NMR spectroscopy in combination with IR and MS spectral data. Antibacterial studies showed that the isolated compound successfully inhibited Pseudomonas aeruginosa, Klebsiella Pneumonia, Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Bacillus subtilis and Salmonella typhi. This result supported the use of this plant in wound treatment.

Keywords: antibacterial, phytomedicine, wound treatment.

INTRODUCTION

Datura metel Linn. (Thorn apple: Devil's trumpet, Solanaceae) has been extensively used for various medicinal purposes throughout the world. The plant is widely used in traditional medicine to cure diseases such as asthma, cough, wound treatment, convulsion, headache and insanity [1,2]. The leaves and seeds are used as anesthetic, antispasmodic, bronchodilator, hallucinogenic and myristic medicines [1,2]. *D. metel* has been extensively used in traditional medicine to cure some ailments such as epilepsy, insanity, asthma, cough, wounds, burns, hemorrhoids, rheumatism and painful menstruation [1,3,4]. These functions are due to many bioactive compounds available in the plant. The solanaceous alkaloids, hyoscyamine and scopolamine [5,6] have been isolated from *D. metel*. Hyoscyamine is the most commonly occurring alkaloid in the solanaceae family and has been associated with varying quantities of hyoscine and in rare cases with traces of atrophine [7]. Scopolamine is found mostly in the leaves while hyoscyamine is produce in the roots but converted to scopolamine in the young leaves [7]. Steroidal constituents, daturasterol and tricyclic diterpene, daturabietarine have been isolated from the bark of *D. metel* along with β-sitosterol [7].. A number of other steroidal compounds have been isolated from regenerated adventitious roots from young leaves of *D.*

metel. The C–28 sterol 3-β-24 dihydro-ergostan 3–25 dienolide, regarded as a precursor of whithanolide and related steroidal lactones, was isolated from *D. metel* [8]. Withanolides are a group of naturally occurring steroids built on the ergostane-type skeleton in which the C–22 and C–26 are oxidized to form a lactone [8].. Withanolide 12-deoxy withan-stramonolide was also isolated [9]. Several studies [10,12] have documented the scientific basis for the efficacy of plants in phytomedicine. The study seeks to ascertain the usefulness of *D. metel* in the treatment of infectious conditions caused by common pathogens. The study involves the isolation, structural elucidation and characterization of the bioactive constituents of some pathogenic bacteria and fungi for possible development of new drugs for the prevention and treatment of infections.

Herein we report for the first time the isolation, characterization and structural elucidation of a β-carboline alkaloid (1, 7 dihydroxy-1-methy 6, 8 dimethoxy β-carboline) from the leaves of *D. metel* and investigate its antibacterial activity.

MATERIALS AND METHODS

General experimental procedure

IR spectra were determined on a Thermo Nicolet Nexus 470 RT–IR spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400FT NMR spectrometer using Tetramethylsilane (TMS) as internal standard. Chemical shifts are expressed in parts per million (ppm). LC–ESIMS spectra were determined in the positive ion mode on a PE Biosystem API 165 single quadruple instrument. HRESIMS (positive ion mode) spectra were recorded on a Thermo Finniga MAT 95XL mass spectrometer. 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 0.25 mm thick silica gel 60 F_{254} aluminum plates (20 × 20 cm Merck, Darmstadt, Germany).

Reagents and solvents like ethanol, chloroform, diethyl ether, hexane were all of analytical grade and were procured from Merck. TLC aluminum sheets silica gel $60F_{254}$ was also purchased from Merck Darmstadt, Germany. The nutrient agar was purchased from Scharian Chemie (APHA), Spain. Ciprofloxacin tablets (Supraflox) 500 mg was purchased from Khandelwal labouratories PVT, India

Plant materials

The fresh leaves of *D. metel* were harvested from the botanical garden of Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria on the 6th February, 2007. Plant samples (fruits, seeds and leaves) were identified by Dr. A. Nmeregini of the Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. A voucher specimen (No: DS/3344) has been deposited at the Forestry Department Herbarium of the University.

Extraction and isolation of plant materials

Plant materials were treated and analyzed at the Chemistry laboratory, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The leaves (1 kg) were dried on the laboratory bench for 10 days. The dry sample was milled and ground into powder (920 g) using a Thomas Wiley machine (model 5 USA). The powdered plant sample (500 g) was packed into a Soxhlet apparatus (2 L) and extracted exhaustively with 1000 ml ethanol for 24 hrs. The ethanol extract was concentrated using a rotary evaporator at 45°C and left on the laboratory bench for 2 days to obtain a dark-brown oil (30.2 g). The column was packed with silica gel and eluted with

methanol, chloroform and diethyl ether (20: 30: 50) to afford a brown amorphous solid (0.82 mg). The brown amorphous solid was re-crystallized from hexane to afford compound **1**, a brown amorphous solid (0.25 mg). The ^{1}H NMR and ^{13}C NMR of compound 1 were analyzed through the use of a Bruker Avance 400FT NMR Spectrometer and Tetramethyl-Silane (TMS) was used as internal standard determined.

Bioassay

The *in vitro* antibacterial activity of compound **1** was carried out for 24 h culture of seven selected bacteria. The bacteria used were five Gram-negative organisms (*Proteus mirabis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli*) and two Gram-positive strains (*Staphylococcus aureus* and *Bacillus subtilis*). All the test organisms are clinical isolates of human pathogens obtained from the Federal Medical Centre (FMC) Umuahia, Nigeria. Cultures were brought to laboratory conditions by resuscitating the organisms in buffered peptone broth (Scharlan Chemie) and thereafter nutrient agar (peptone 5 g/l and meat extract 3 g/l) and incubated at 30°C for 24 h. The antibacterial activity was performed by a filter paper disc diffusion technique. The medium (7 g nutrient agar in 250 ml distilled water, autoclaved at 115°C for 15 min) was cooled to 50°C. 20 ml of the medium was poured into a sterile Petri dish and allowed to solidify, set for 8 h then observed for contamination. Compound **1** (1 g) was dissolved in 1 ml of absolute ethanol and made up to 10 ml with distilled water to give a concentration of 100 mg/ml (10% dilution). A colony of each test organism was sub-cultured on nutrient broth which contained peptone (5 g/l) and meat extract (3 g/l) and incubated aerobically at 37°C for 8 h. 30 ml of the nutrient broth was used to flood the agar plates. A single sheet of sterilized Whatman No. 1 filter paper disc soaked in Compound **1** (0.02 ml) was used to test for the sensitivity or anti-microbial effect of compound **1**. The plates were incubated at 37°C for 24 h. After incubation, plates were observed for zones of inhibition (in mm diameter). The minimum inhibitory concentration (MIC) was determined [13]. The sensitivity susceptibility of the test bacteria to the standard drug (ciprofloxacin 500 mg) was tested using the incubated agar plate method. The zones of inhibition of ciprofloxacin on the test organisms were measured and compared with those of compound **1** of the same concentration.

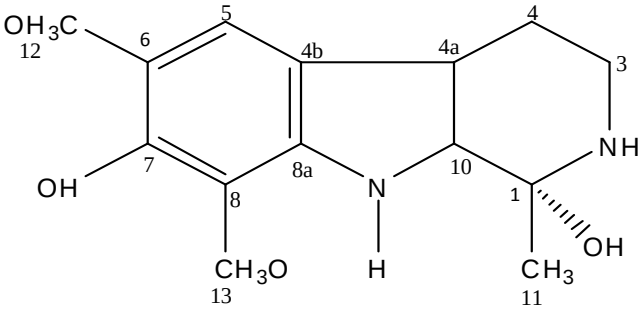
Statistical analysis

All measurements were replicated three times and standard deviations determined. The student's *t*-test at $P< 0.05$ was applied to assess the difference between the means [14].

RESULTS AND DISCUSSION

The ethanol extract of dried leaves of *D. metel* was subjected to solvent chromatography. The brown amorphous solid obtained after column chromatography was re-crystallized from hexane to afford compound **1**, a brown amorphous solid (0.25 mg). Thin layer chromatography (chloroform: methanol 7: 3) iodine vapour showed the presence of one spot (R_f 0.65). IR V_{max} 3400 cm^{-1}, (OH) 3368 cm^{-1}, 1683 cm^{-1}, (C=C aromatic) 1382 cm^{-1}, (CN) 1224 cm^{-1} (C-O). HREIMS m/z 280.2356 (M^{+}) calculated for m/z 280 ($C_{14}H_{20}O_4N_2$) and m/z 83.0660 base peak calculated for m/z 83 (C_4H_2ON).

Compound **1** was identified as 1,7-dihydroxy-1-methly 6,8-dimethoxy β-carboline and was assigned the molecular formula m/z 280.2356 calculated for $C_{14}H_{20}O_4N_2$ (m/z 280) with base peak at m/z 83.0660 calculated for C_4H_5ON (m/z 83) on the basis of HREIMS.



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The IR spectrum revealed the presence of hydroxyl, amine, aromatic and ether groups at 3400, 3368, 1683 and 1224 cm^{-1}, respectively. The relative molecular mass of 280.2356 [M+] with base peak at 83.0660 (C_4H_5ON) confirmed compound **1** as 1,7 dihydroxy–4 methyl 6,8 dimethoxy β-carboline. The pattern of fragmentation (**Fig. 1**) showed that compound **1** underwent deprotonation and detachment to afford the base peak of C_4H_5ON (m/z 83.0660). The ^{1}HNMR spectrum (**Table 1**) revealed the presence of a tetrahydro β-carboline system due to signals at δ3.286 (1 Hs), 3.9008 (1 Hs), 6.6775 (1 Hs), 1.30708 (2 Hm), 1.33570 (2 Hm) 4.87716 (1 Hs) and 4.8251 (1 Hs). The spectrum also showed the presence of a methoxy signal at δ3.28619 (3 Hs), 3.30179 (3 Hs); a methyl signal at δ0.88130 (3 Hs) and hydroxyl protons at δ3.52520 (brs) and 3.31814 (brs) attached to the β-carboline system. The aromatic protons were substituted with methoxy and hydroxyl groups except proton H_5, which showed a singlet peak at δ6.6775. The ^{13}CNMR spectrum (**Table 1**) showed the presence of two methene SP^2 carbons at δ49.636 (C_3) and 49.426 (C_4), respectively. Aromatic carbons were observed at δ126.273 (C_{4b}), 143.838 (C_5), 129.029 (C_6), 126.274 (C_7), 129.029 (C_8) and 126.270 (C_{8a}). The methoxy carbons appeared at δ56.417 (C_{12}) and 56.875 (C_{13}). The ^{13}C NMR spectrum also confirmed a tetrahydro β-carboline structure for compound **1**. This analysis confirmed the sample isolated from *D. metel* leaves to be carboline alkaloid (1,7 dihydroxy-1-methyl 6,8 dimethoxy β-carboline) as the measured spectral properties are in accordance with available literature data [15-17]. Bioactive compounds containing β-carboline moiety had earlier been isolated from the sponge genus *Dragmacidon* and were found to exhibit pronounced biological activity such as antimicrobial, anti-tumor, antiviral and insecticidal activity [16]. The isolation of β-carboline alkaloid from *D. metel* leaves therefore shows that β-carboline may be one of its physiological active components.

The β-carboline alkaloid isolated from the leaves of *D. metel* successful inhibited *Proteus mirabis, K. pneumonia, P. aeruginosa, S. aureus, S. typhi, B. subtilis* and *E. coli* (**Table 2**). This result also agreed with the findings of Proksch *et al.* [16], who reported that alkaloids possessing a β-carboline moiety as found in manzamines, didemnolines, eudistomins, fascaplysine, plakortamines and shishijimicins have been found to exhibit pronounced antimicrobial activity. The spectrum of activity of inhibition of compound **1** when compared with standard conventional drug (Ciprofloxacin) is relatively narrow (**Table 2**). However, the level of activity is still good at inhibiting concentration at 100 mg/ml. The range of MIC of compound **1** was 12.5–50 mg/ml (**Table 3**).

K. pneumonia, P. mirabilis, P. aeruginosa, S. aureus and E. coli are human commensals and have been incriminated in the infection of wounds [13]Wounds provide environments conducive for the growth of microbial organisms. Evaluation of the effects of compound **1** on clinically isolated microbial contaminants of wounds showed varying levels of inhibitory activity against these microorganisms. Microbial infection of wounds delays healing and causes a more

pronounced acute inflammation reaction which can lead to further tissue injury and damage. However, the antimicrobial activity of compound **1** on these wound isolates may partly contribute to the wound healing effect by eliminating infection and thus permit tissue repair and cell proliferation. *P. mirabis* and *E. coli* are the common cause of urinary track infection and travelers diarrhea [13, 18]. Compound **1** cause varying degrees of inhibition on the growth of these clinical isolates of these organisms. This finding supported the use of the leaves of *D. metel* in the treatment of diarrhea and urogential infections such as gonorrhoea and syphilis in herbal medicine [1,2]. Compound **1** showed inhibition against *K. pneumonia*, *S. aureus* and *P. aergunesa*. This supported the use of *D. metel* leaves for the treatment of wounds for which these pathogens are associated [13]. The leaves of *D. metel* possess phytoconstituents capable of inhibiting the growth of microbial wound contaminants; accelerate wound healing and consequently resulting to cell proliferation. The occurrence of β-carboline in *D. metel* is of significance because this is to the best of our knowledge the first report of its occurrence in any *D. species.*

Table 1: ^{1}H (400MHz) and ^{13}CNMR (75MHz) of Compound 1

	δC		δH		
Position	Chemical Shift	Carbon	Chemical Shift	Multiplicity	Proton
1	79.440	C – OH	3.52520	1 Hbrs	OH
2			4.87716	1 Hs	NH
3	49.636	CH_2	1.30708	2 Hm	CH_2
4	49.426	CH_2	1.33570	2 Hm	CH_2
4a	42.513	C – H	3.28619	1 Hd	
4b	126.273	C			
5	143.838	C	6.6775	1 Hs	C – H
6	129.029	C – O			
7	126.274	C – OH	3.31814	1 Hbs	OH
8	129.029	C – O			
8a	126.270	C			
9			4.8251	1 Hs	NH
10	42.583	C – H	3.9005	1 Hs	C – H
11	24.484	CH_3	0.88150	3 Hs	CH_3
12	56.417	CH_3O	3.28619	3 Hs	OCH_3
13	56.875	CH_3O	3.31179	3 Hs	OCH_3

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

Table 2: Diameter of Zones of Inhibition (mm) of Compound 1 Isolated from *Datura Metel leaves and Ciprofloxacin* (mg/100g)

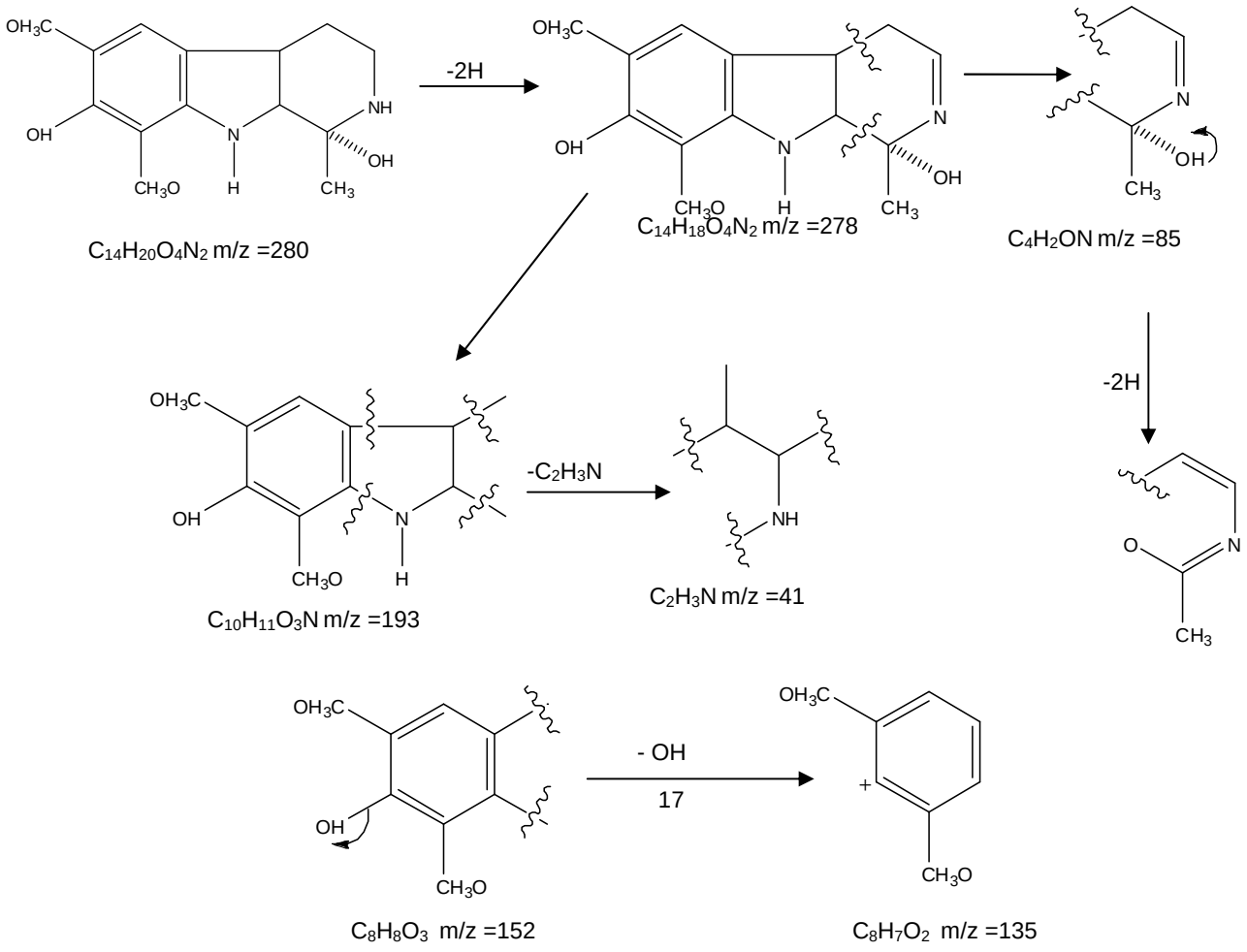
Test Organisms	Compound 1 from *Datura Metel* leaves	Ciprofloxacin
Proteus mirabis	5.0 ± 0.10	35.0 ± 0.01
Klebsiella pneumonia	7.0 ± 0.20	12.0 ± 0.10
Pseudomonas aeruginosa	11.0 ± 0.20	14.0 ± 0.01
Staphylococcus aureus	6.0 ± 0.02	25.0 ± 0.11
Solmonella typhi	7.0 ± 0.11	23.0 ± 0.10
Bacillus subtilis	10.0 ± 0.02	30.0 ± 0.10
Esherichia coli	5.0 ± 0.10	11.0 ± 0.20

Data are means ± Standard deviation of triplicate determination

Table 3: Minimum inhibitory concentration of Compound 1 Isolated from the leaves of *Datura Metel* on the Pathogens mg/ml

	Concentration of Compound 1 mg/ml Zone of inhibition (mm)					
Pathogens	100	50	25	12.5	6.25	**Mic mg/ml**
Proteus mirabis	5.0	4.0	3.0	1.0	-	12.5
Klebsiella pneumonia	7.0	6.0	4.0	1.0	-	12.5
Pseudomonas aeruginosa	11.0	8.0	4.0	1.0	-	12.5
Staphylococcus aureus	6.0	4.0	2.0	-	-	25
Solmonella typhi	7.0	5.0	2.0	-	-	25
Bacillus subtilis	10.0	7.0	4.0	2.0	-	12.5
Escherichia coli	5.0	3.0	-	-	-	50.0

Data are means of triplicate determinations
- No Zone of Inhibition.



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