EXTRACTION, ISOLATION AND CHARACTERIZATION OF SOME ALKALOIDS FROM THE BARK OF *Pericopsis laxiflora* (FAMILY: FABACEAE)

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Abstract: Extraction and purification of some of the nitrogenous bases present in the bark of *Pericopsislaxiflora*led to the isolation and characterization of three alkaloids. The structures of the isolated compounds on the basis of physical, chemical and spectral techniques, such as IR, UV, ^{1}H-NMR, ^{13}C-NMR, Dept-135 and GC-MS were elucidated as 5,8-Dimethyl-1,2,3,4-tetrahydro-9-acridinamine (1), 4,6-Diaminopyrazolo[3,4-d] pyrimidine (2) and 1,2,3,4,5,6-hexahydro-1,5-methano-8H-pyrido[1,2-a]diazocin-8-one (3).The presence of these nitrogenous bases, with many more yet to be isolated and characterized, is an indication that the plant may provide drugs that will help increase the therapeutic arsenal.

Keywords: Alkaloids, bark, ethanol extract, isolation, *Pericopsislaxiflora*, spectral analysis

Introduction

Alkaloids are nitrogenous bases/amines that usually occur in plants as salts of organic/inorganic acids, sometimes as complexes with tannins but always together with many non-alkaloidal compounds (Golkiewicz and Gadzikowska, 1999). Some of them, especially the alkaline alkaloids mostly exist in organic salts in the form of citrate, oxalate, succinate and tartrate (Yubin *et al*., 2014). The ease with which these compounds are extracted into aqueous acids, combined with their regeneration on treatment with a dilute base helps to separate them from other bioactive compounds in a plant. Usually, medicinal plant-containing alkaloids often contain a variety of alkaloids, so that the need to separate them into individual alkaloids using conventional separation, isolation and purification methods becomes inevitable. This class of phytochemicalsis of special interest because most often they exhibit marked physiological effects in humans and animals (Carey, 2003). *Pericopsis laxiflora* (Benthex Baker) van meeuwen; synonym *Afromosialaxiflora* (Benth. ex Baker) Harms is a savannah, perennial, deciduous shrub or tree belonging to the family Papilionaceae/Fabaceae/Leguminoseae. It is commonly called Satin wood (English), Ayan/Sedun (Yoruba), Makarfo (Hausa) and Abua-ocha (Igbo).

Traditionally, the plant has been reported useful in the treatment of hemorrhoids, headache, rheumatism, arthritis, abdominal pains, sore throat, eye problems, skin diseases, teething pains in childrenand other feverish conditions. It is also used as an antidote against snakebite, intestinal worms, and guinea worms. Parts of the plant are also regarded as a medicine for syphilis, in the treatment of diarrhea and dysentery and as an antibacterial, antimalarial and antiparasitic agent. It is also used as a medication for jaundice and liver diseases (Irvine, 1961; Bouquet and Debray, 1974; Kerharo and Adam, 1974; Ake-Assi, 1988; Neuwinger, 1996; Arbonnier, 2002; Mann *et al*., 2003; Asase *et al*., 2005; Kone *et al.*, 2013; Balde *et al*., 2015; Gera *et al*., 2015; Koffi *et al.*, 2015). Reported biological activity of the various organs of the plant, includes, its usefulness as an antitrypanosomalagent against *Trypanosomabruceibrucei* (Hoet *et al*., 2004) and *T. bruceirhodesiense* (Abiodun *et al*., 2012). The anthelmintic (Kone*et al*., 2005), antibacterial (Quattara*et al*., 2013) and antimicrobial (Okanlawon*et al*., 2015) property of various extracts of the plant has also been reported. The presence of alkaloids,polyphenolics, such as, catechin tannins and flavonoids, cardiac glycosides, sterols and polyterpenes has been detected in the plant (Caimont-Le-Blond, 1957; Oliver, 1960; Quattara *et al*., 2013; Koffi *et al*., 2015; Okanlawon *et al*., 2015).

From the stem bark and root bark of the plant, Bevan and Ogan (1964) had earlier extractedsome quaternary alkaloids, made up largely of choline and an identical mixture of three non-quaternary alkaloids, in which one of them was identified as N-methylcytisine, which was later confirmed by Adesogan (1976), along with another base, anagyrine. The continuous search for more bioactive compounds, such as alkaloids from plant sources, has therefore, prompted the extraction, isolation and characterization of some nitrogenous bases from the bark of *Pericopsis laxiflora* grown in Nigeria.

Materials and Methods

Collection of plant material

The bark of *P. laxiflora* was collected from a farmland at Gwada village, Shiroro Local Government Area of Niger State, Nigeria in the month of March, 2016. Plant was identified and authenticated by Dr. (Mrs.) Jemilat Ibrahim of the Department of Medicinal Plant Research and Development (MPR&TM) of National Institute for Pharmaceutical Research and Development, Idu (NIPRD).

Extraction of plant material

Air-dried powderedbark of *P. laxiflora* (500 g) wasextracted exhaustively by sonicating with 80% ethanol for 5 days. Extract was concentrated *in-vacuo* to dryness and coded crude ethanol extract of *P. laxiflora* bark (EP, deep brown gummy mass, 27.8% yield).

Test for the presence of alkaloids

A small portion of the extract, EP, was hydrolyzed with 5 cm^3 of 2% aqueous hydrochloricover a steam bath for about 5 minutes and the mixture filtered. 1cm^3eachof the resulting filtrate was treated with 2 drops each of Dragendorff's, Wagner's and Mayer's reagents separately. Precipitation of added reagents in each tube was taken as evidence for the presence of alkaloids (Gonzales and Tolentino, 2014).

Extraction of crude (total) alkaloids from the ethanol extracts (EP)

The crude ethanol extract (EP) was solubilized in water and hydrolyzed with dil. HCl (2N). The mixture was thendefatted with hexane to yield an acidic and a lipophilic portion. The acidic portion was basified with dil. $NH_4OH$and further re-extracted with chloroform to yield an aqueous phase (basic portion) and a chloroform-soluble portion, which was concentrated *in-vacuo*. Traces of water was removed with anhydrous Na_2SO_4 and the portion coded "crude alkaloids of the bark of *P. laxiflora"* (EPa, brownish black powdery mass, 7.8%). The portion was screened for the presence of alkaloids.

Purification of portion EPa

Portion EPa (3 g) was applied to the surface of a prepared glass column packed with 100 g of alumina and eluted sequentially with varying proportions of increasing polarity of

$CHCl_3$: MeOH (100:0 to 0:100). Similarfractions were pooled based on their thin layer chromatographic profile and concentrated *in-vacuo* to yield 6 major fractions, codedEPa_1–EPa_6.All fractions werevisualized under UV light (254 and 366nm) followed by spraying with Dragendorff's reagent for detection of alkaloids.

Isolation of compounds from fraction EPa_3

Purification of fraction EPa_3 (1800mg, 30g of alumina, increasing polarity of $CHCl_3$: MeOH) gave rise to sub-fractions EPa_3A – Epa_3E. Preparative-thin layer chromatographic purification of sub-fraction EPa_3C (silica gel pre coated 50 mm x 100 glass plates, 2 mm thickness, CH_2Cl_2: MeOH: NH_4OH85:15:1) revealed 5 distinct major bands under UV light. Drying, scrapping, triturating and *in vacuo* concentration of each band separately afforded impure compounds that were further purified severally on PTLC using same solvent system. This yielded 3 purecompounds that were coded EPa_3C_1−EPa_3C_3. All compounds were subjected to physical, chemical and spectral characterization.

Physical and spectral characterization of compounds

Melting points were uncorrected and recorded by open capillary method.IR and UV were both recorded in $CHCl_3$ usingFTIR 8400 spectrometerand T60 UV-Visible spectrophotometer, respectively.^{1}H-NMR, ^{13}C-NMR and DEPT-135 spectra were taken in $CDCl_3$ onVarian Gemini spectrometer operating at 400MHz, while, GC-MS was recorded using GCMS-QP 2010 plus Shimadzu.

Results and Discussion

Extract/portion/fractions/sub-fractions/isolatesallgave brick red-, buff- and yellow- precipitate each with Dragendorff's, Mayer's andWagner's reagents, respectively, indicating thepresence of alkaloids in the plant (Gonzales and Tolentino, 2014). Fractions revealed red, green and blue fluorescence spots under UV light, especially at 366nm, with most spots displaying brown to reddish brown coloration when sprayed with Dragendorff's reagent. The spectral data of the isolated compounds from IR, UV, NMR and GC-MS provedthey were alkaloids/nitrogenous bases of different classes.The UV-visible spectra revealed that their absorptionsoccurred at longer wavelengths. This is not unusual because there is interaction (inductive effect) of the lone pair of electrons on nitrogen with the π-electron system of the ring, causing shiftof the ring's absorption to occur at longer λ (Carey, 2003, Mohan, 2010).

In the ^{1}H- and ^{13}C-NMR spectra,it was observed as usual, that nitrogen, being a strong electronegative specie, shieldedthe neighboring nuclei, so that C-H/C-C bonds are more shielded (higher field/shielded peaks)than N-H protons/bonds (lower field/de-shielded peaks). A common occurrence in the ^{13}C-NMR and DEPT-135^o spectra of all isolated compounds was the presence of more quaternary (disappeared/nulled peaks) than methine/methyl (normal peaks)/methylene (inverted peaks) carbon atoms. GC-MS spectra revealed that the isolated compounds had either an odd- or even- numbered molecular mass. Usually, an odd number of nitrogen atoms correspond to an odd value of the molecular mass ($gmol^{-1}$), while an even number of nitrogen yields an even value of molecular mass (Furniss *et al*., 1989). Their fragmentation patterns showed that cleavages around carbon bonded to nitrogen are a common occurrence. This is because nitrogen, been more electronegative than carbon, is good at stabilizing its adjacent carbocation sites (Carey, 2003).

Elucidation of structures of isolated compounds

Compound 1 (EPa_3C_1)

White flakes, recrystallized from EtOH (10.9mg); melting point 180-182.4^{o}C [lit. 183.5-184^{o}C]; soluble in $CHCl_3$, CH_2Cl_2, EtOAc and Me_2CO, sparingly soluble in MeOH, EtOH andH_2O; TLC(CH_2Cl_2: MeOH4:1), R_f 0.68, UV active; red spot onspraying with Dragendorff's reagent.

IR (cm^{-1}):3460 (N-H asymmetric stretching of amino group), 3310 (N-H symmetric stretching of amino group), 3018 (C-H stretching of benzene), 1588 (benzene ring), 1455, 1350 (methyl groups) and 825 (C-H bending of 1, 4-disubstituted benzene)

$UV_{\lambda max}$ (nm):232 (n-π* transition of substituted quinoline ring), 244 (n-π* transition of hydrogenated acridine ring) and 261 (π-π* transition of substituted benzene).

^{1}H-NMR (ppm):2.67, 2.72 (t, 2H, H-1), 1.56, 1.61 (dtt, 2x2H, H-2 and H-3), 2.323, 2.485 (t, 2H, H-4), 6.98 (d, 1H, H-6), 7.10(d, 1H, H-7), 2.11 (sharp s, 2xCH_3, H-5' and H-8') and 3.91 (d, 2H, H-9)

^{13}C-NMR (ppm): Thirteen strong proton-decoupled peaks, all of the same intensity. 30.7 (C-1), 23.1 (C-2, C-3, C-4), 124.7 (C-5), 124.1 (C-6), 129.2 (C-7), 132.9 (C-8), 140.5 (C-9), 160.8 (C-1'), 109.9 (C-4'), 112.8 (C-9'), 150.1 (C-10'), 20.3 (C-5') and 19.4 (C-8')

DEPT-135 (ppm): 30.7 (inverted, methylene of a cyclohexane ring), 23.1 (inverted, methylene of a cyclohexane ring), 124.7 (nulled, quaternary of an aromatic ring), 124.1 (normal, methine of an aromatic ring), 129.2 (normal, methine of an aromatic ring), 132.9 (nulled, quaternary of an aromatic ring), 140.5 (nulled, quaternary of a pyridine ring), 160.8 (nulled, quaternary of an aromatic ring), 109.9 (nulled, quaternary of a pyridine ring), 112.8 (nulled, quaternary of a pyridine ring), 150.1 (nulled, quaternary of a pyridine ring), 20.3 (normal, methyl close to an amine group) and 19.4 (normal, methyl substituent).

GC-MS (m/z): 226 (M^+; base peak; $(C_{15}H_{18}N_2)^+$, 211 $(C_{14}H_{15}N_2)^+$, 198 $(C_{13}H_{14}N_2)^+$, 183 $(C_{12}H_{11}N_2)^+$, 168 $(C_{11}H_8N_2)^+$, 154 $(C_{10}H_6N_2)^+$, 128 $(C_9H_6N)^+$, 44 $(C_2H_6N)^+$, 43 $(C_2H_4N)^+$, 42 $(C_2H_3N)^+$and 41 $(C_2H_2N)^+$. It revealed the molecular formula and molecular mass of compound$EPa_3C_1$to be $C_{15}H_{18}N_2$ and 226$gmol^{-1}$, respectively.

Based on the physical, chemical and spectral data obtained for compoundEPa_3C_1in comparison with those reported in literature, the compound was identified as 5,8-Dimethyl-9-amino-1,2,3,4-tetrahydroacridine/Acridin-9-amine, 1,2,3,4-tetrahydro-5,8-dimethyl-/5,8-Dimethyl-1,2,3,4-tetrahydro-9-acridinamine/9-Amino-1,2,3,4-tetrahydro-5,8-dimethylacridine/Tetrahydro-5,8-dimethylaminacrine **(1)**, an aromatic nitrogenous base, which has been detected in the Fabaceae/Leguminoseae family (Rajabudeen *et al*., 2015), while, its derivatives have been reportedand synthesized from several sources (Sondhi *et al*., 2006; Rajabudeen *et al*., 2015).The ease of synthesis, attractive coloration and crystallinity of acridinedrevatives has long attracted the attention of medicinal chemists. This is because of the ability to introduce various substituents unto the basic tricyclic framework, which has given acridines a reputable reputation in the history of chemotheraphy (Stanslas *et al*., 2000) and other biological/pharmacological properties, such as;as antimalarial, antibacterial (Wainwright, 2001) and anti-inflammatory agents (Sondhi *et al*., 2006). The compound has synthetically been reduced to tacrine (1, 2, 3, 4-tetrahydroacridin-9-amine) using a nickel-aluminum alloy catalyst under basic conditions (Kamata *et al*., 2002).

Compound 2 (EPa_3C_2)

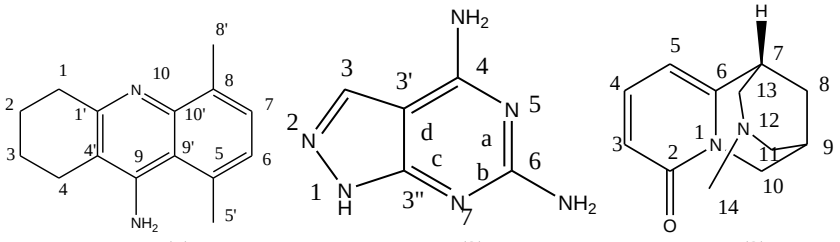
White solid, recrystallized from EtOH (11.4 mg); melting point 128.5-129.3^{o}C; Soluble in $CHCl_3$, CH_2Cl_2, EtOAc and Me_2CO, partially soluble inMeOH, EtOH and H_2O and insoluble in hexane; TLC (CH_2Cl_2: MeOH9:1), R_f (0.62), UV active;deep red onspraying with Dragendorff's reagent.

IR (cm^{-1}):3523.67 (-NH_2), 3497.45 (N-H), 3033.45 (C-H), 1654.88 (C=N of pyrimidine), 1524.22 (C=C) and 1346.78 (C-N-C of pyrazole)

$UV_{\lambda max}$ (nm):232 (strong, π-π* transition), 295 (Aza-aromatic, n-π* transition, weak)
^{1}H-NMR (ppm):12.9 (s, H-1), 6.99 (s, H-3) and4.00 (d, NH_2 x 2 at C-4 and C-6).
^{13}C-NMR (ppm): 5 strong proton-decoupled peaks, all of the same intensity. 130.7 (C-3), 158.4 (C-4), 160.9 (C-6), 98.6 (C-3') and 151.5 (C-3")
DEPT-135 (ppm):130.7 (normal, methine of a pyrazole ring), 158.4 (nulled, quaternary of a pyrimidine ring), 160.9 (nulled, quaternary of a pyrimidine ring), 98.6 (nulled, quaternary of a pyrazole ring) and 151.5 (nulled, quaternary of a pyrazole ring).
GC-MS (m/z): 150 (M^+; base peak; $(C_5H_6N_6)^+$, 133 $(C_5H_3N_5)^+$, 122 $(C_5H_6N_4)^+$, 108 $(C_5H_6N_3)^+$,107 $(C_5H_5N_3)^+$, 69 $(C_3H_5N_2)^+$, 68 $(C_3H_4N_2)^+$, 55 $(C_2H_3N_2)^+$,43 $(C_2H_4N)^+$ and 41 $(C_2H_3N)^+$.It revealed the molecular formula and molecular mass of compound $EPa_3C_2$to be $C_5H_6N_6$ and 150gmol^{-1}, respectively.
Based on the physical, chemical and spectral data obtained for compoundEPa_3C_2in comparison with those reported in literature, the compound was identified as 4,6-Diaminopyrazolo[3,4-d] pyrimidine/1H-Pyrazolo[3,4-d] pyrimidine-4,6-diamine/Allopurine**(2)**, a fused bicyclic heteroaryl compound containing four nitrogen heteroatoms. It is a pyrimidine derivativein which the pyrimidine ring(a six-membered heterocyclic compound consisting of two nitrogen atoms at positions 1 and 3) has its -d- position fused at positions 3 and 4 of the pyrazole ring. Pyrimidines alongside purines (an isomer of pyrimidine) are weak bases that occur naturally in plants and are the parents of the nucleobases(cytosine, thymine and uracil) that constitute a key structural unit of nucleic acids- DNA and RNA (Seela and Becher, 2001; Carey, 2003). No wonder, such compounds are of interest as a model for biologically active compounds (Chafiq *et al*., 2001; Agrebi *et al*., 2014; Takeara *et al*., 2015). For example, acyclovir, a derivative of pyrazolo[3,4-d]pyrimidine has been reported to be highly active against herpes simplex virus (Cooney *et al*., 1986; Dahlberg *et al*., 1987).

Compound 3 (EPa_3C_3)

Cream colored crystals, recrystallized from Et_2O (14mg); melting point 134-136^{o}C [lit. 135-137^{o}C]; soluble in $CHCl_3$, CH_2Cl_2, EtOAc, Me_2CO, MeOH, EtOH andH_2O and sparingly soluble in Et_2O; TLC(CH_2Cl_2: MeOH 9:1), Rf 0.55, UV active;deep red on spraying with Dragendorff's reagent.
IR (cm^{-1}):3285 (NH-), 3002 (aromatic C-H stretching), 2775 (quinolizidine alkaloid), 1658 (3^o amide/aromatic C=O/α-pyridone ring), 1475 (CH_2 scissoring/CH_3 bending) and 662 (O-C-N bending)
$UV_{\lambda max}$ (nm):228 (π-π* transition), 309 (presence of amide)
^{1}H-NMR (ppm): 6.14 (d, 1H, H-3), 6.33 (t, 1H, H-4), 5.12 (d, 1H, H-5), 1.98 (m, 1H, H-7), 1.46 (m, 2H, H-8), 2.01 (m, 1H, H-9), 2.25 (d, 2H, H-10), 2.98 (d, 2H, H-11), 2.21 (d, 2H, H-13) and 2.69 (s, 3H, H-14).
^{13}C-NMR (ppm): 12 strong proton-decoupled peaks, all of the same intensity. 158.7 (C-2), 115.4 (C-3), 134.9 (C-4), 102.6 (C-5), 149.9 (C-6), 32.6 (C-7), 33.8 (C-8), 24.7 (C-9), 48.8 (C-10), 59.1 (C-11), 55.3 (C-13) and 43.6 (C-14)
DEPT-135 (ppm):158.7 (nulled, quaternary, amide-like), 115.4 (normal, methine of an aromatic ring), 134.9(normal, methine of an aromatic ring), 102.6 (normal, methine of an aromatic ring), 149.9 (nulled, quaternary of an aromatic ring), 32.6 (normal, methine of a piperidine ring), 33.8 (inverted, methylene of a piperidine ring), 24.7 (normal, methine of a piperidine ring), 48.8 (inverted, methylene of a piperidine ring), 59.1 (inverted, methylene of a piperidine ring), 55.3 (inverted, methylene of a piperidine ring) and 43.6 (normal, methyl, aliphatic-N)
GC-MS (m/z): 240 (M^+; $C_{12}H_{16}N_2O)^+$, 98 (base peak; $C_5H_8NO)^+$, 84 $(C_4H_6NO)^+$, 70 $(C_3H_4NO)^+$,69 $(C_3H_3NO)^+$, 68 $(C_3H_2NO)^+$, 57 $(C_2H_2NO)^+$, 44 $(C_2H_6N)^+$, 43 $(C_2H_4N)^+$, 42 $(C_2H_3N)^+$, 41 $(C_2H_2N)^+$. It revealed the molecular formula and molecular mass of compound EPa_3C_3 to be $C_{12}H_{16}N_2O$ and 240gmol^{-1}, respectively.
Based on the physical, chemical and spectral data obtained for compoundEPa_3C_3in comparison with those reported in literature, the compound was identified as 1,5-Methano-8H-pyrido[1,2-a][1,5]diazocin-8-one,1,2,3,4,5,6-hexahydro-3-methyl(1R)/N-Methylcytisine/12-Methylcytisine/Cytisine, 12-methyl/(1R)-1,2,3,4,5,6-Hexahydro-1, 5-methano-8H-pyrido [1, 2-a]diazocin-8-one/Caulophylline**(III)**, an alkaloid that has been reported, isolated and characterized from several sources (Bevan and Ogan, 1964; Adesogan, 1976; Keller and Hatfield, 1979; Barlow and Johnson, 1989; Woldemicheal and Wink, 2002; Wang *et al*., 2011; Perez *et al*., 2012; Mathi *et al*., 2015). This nitrogenous base of the lupinane group, in association with other alkaloids is a common occurrence in the Papilionaceae/Fabaceae family (Cromwell, 2013).



(1) **(2)** **(3)**

Conclusion

Extraction, fractionation and preparative thin layer chromatographic separation and purification of the alkaloids present in the bark of *Pericopsis laxiflora*, a plant belonging to the Papilionaceae/Fabaceae family; a family rich in alkaloids, afforded three nitrogenous bases that were characterized and structurally elucidated as 5,8-Dimethyl-1,2,3,4-tetrahydro-9-aminoacridine, 4,6-Diaminopyrazolo [3,4-d] pyrimidineand 1,2,3,4,5,6-hexahydro-1,5-methano-8H-pyrido [1,2-a] diazocin-8-one. Further work will focus on the isolation and characterization of more alkaloidal bases from the plant and the biological/pharmacological efficacyof such isolates will also be determined.

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