Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Archives of Applied Science Research, 2013, 5 (1):146-150
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

A new alkaloid from the hairs of *Mucuna pruriens*(Cow-Hage)

Olajire Abass. Abiola and Edewor-kuponiyi Theresa Ibibia

Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

---

ABSTRACT

An alkaloid 7-acetyl-5-ethenyl-3-(N-cyclopenta-3", 5"-dienyl-2"-hydroxy piperidine) quinoline responsible for the itching effects of the hairs of Mucuna pruriens was isolated from the ethyl acetate soluble portion of the methanol extract. The structure was determined using spectroscopic techniques.

INTRODUCTION

Mucuna pruriens is a plant of the family of Leguminoseae and is indigenous to tropical countries such as India and Nigeria. It is commonly known as cowhage (Latin) and kiwanch (kapikacchu) (Sanskrit). It is an annual twinning plant with long, thin branches and opposite, lanceolate leaves. The flowers have dark purple colour and occur in drooping racemes. The fruits are curved pods which are thick and leathery. The longitudinally ribbed pod is densely covered with persistent pale-brown trichomes that cause irritating blisters. It has been established that this unique property is as a result of the presence of 5-hydroxytryptamine in the hair (Armstrong *et al*., 1953). It has also been shown that histamine and kinin-like substances may also be responsible for this action (Broadbent, 1953). Some reports have shown that anti-histaminics can afford protection against the itch (Broadbent, 1953). The seeds are black, ovoid and long. Locally *Mucuna pruriens* is used as an anthelmintic. It is used to expel *Ascaris lumbricoides* and *Oxyuris vermicularis*. it is also used externally in the form of ointment as a local stimulant in paralysis. The plant has the following uses in India: thermogenic, anthelmintic, diuretic, emollient, stimulant, aphrodisiac (Kumar *et al*., 1994), purgative, febrifuge and tonic. It is also considered to be useful in the relieve of constipation, nephropathy, strangury, dysmenorrhoea, amenorrhea, elephantiasis, dropsy, neuropathy, consumption, ulcers, helminthiasis and delirium (Warrier, 1995, Dhawan *et al*., 1980, Agharkar, 1991, Sastry *et al*., 1990). *Mucuna pruriens* is also reported to have antineplastic, antioxidant, antidiabetic, antimicrobial, analgesic and anti-inflammatory activities (Sathiyanarayanan *et al*., 2007). The seed powder is used to help in reducing stress, increase secretion of semen, regulate steroidogenesis (Shukla *et al*., 2008, Hernandez-Gonzalez, 2000) and act as a restorative and invigorating tonic (Shukla *et al*., 2007). Chemical constitutents that have been isolated from the plant include L. DOPA (seed), tetrahydroisoquinoline alkaloids, proteins, amino acids, carbohydrates, fats, minerals, lecithins and saponins.

Since the trichomes are the part of the plant that are responsible for the intense itching and pain when it comes in contact with human skin; no one has actually characterized the adequate stimulus for the itching effects of the hairs of *Mucuna pruriens*. In the present study, we investigated the active component responsible for the itching effects of the hairs of *Mucuna pruriens*.

MATERIALS AND METHODS

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. NMR spectra were run on a Bruker AC-300 in CD_3OD.

UV spectra were taken on a Hitachi U-3200 spectrophotometer. MS were measured on a FINNIGAN TSQ-46C MS spectrometer.

Plant material

The plant material used in this study is the leaf of *Mucuna pruriens* (cowhage). This was collected from a traditional health practitioner in Ogbomoso, Nigeria and taken to Department of Pure and Applied Biology of Ladoke Akintola University of Technology, Ogbomoso, Nigeria for identification.

Extraction and isolation

The hairs were scrapped with a spatula from the legumes of *Mucuna pruriens* and 100 g was shaken sequentially with n-hexane for 2 days, followed by ethyl acetate and methanol. The n-hexane extract afforded waxy oil, while the ethyl acetate extract gave a solid substance. The ethyl acetate extract (1.109 g) was dissolved in methanol and pre-absorbed with silica gel, dried over steam bath and subjected to column chromatography. It was eluted with 100% chloroform and increasing percent of methanol. The fraction obtained with 5% methanol afforded a solid which was subjected to thin layer chromatography using the solvent mixture chloroform-methanol (4:1, v/v) giving an R_f value of 0.42. It was recrystalized from a mixture of acetone and benzene, dried in an oven at 110^{o}C, to give a white amorphous powder (0.57 g) with melting point 212-214^{o}C, IR (KBr) v (cm^{-1}): 3400 (m), 1615 (s), 1450 (m), 1360 (m), 1260 (m), 1185 (w), 1160 (m) and 1130 (w); ^{1}HNMR (CD_3OD) δ: 7.92 (s), 2.41(s), 7.12(s), 7.42(t), 5.28(d), 7.56(s), 8.48(s), 4.37 (t), 1.90(t), 1.52 (t), 1.48(t), 2.65(t), 4.28(d), 5.98(d), 5.90(d), 4.8(d), 4.47(s); MS: m/z (%): 360 (M,37); UV: λ_{max} (EtOH), nm (ε): 288 (1.224 X 10^5), 214 (1.829 X 10^5).

Solubility test

The sample was placed in the following solvents both at room temperature and at gentle heating: water, methanol, chloroform, diethyl ether, benzene and acetone.

Colour reagent test

The sample was dissolved in methanol and spotted on a rectangular piece of Whatman No. 1 paper alongside with some nitrogenous compounds (pyridine, indole, aniline) and non nitrogenous compound (chlorophenol). This was sprayed with Dragendoff reagent and left overnight to develop.

Sodium fusion test

Sodium fusion solution was prepared using the standard procedure. 0.5 mL of the solution was added to powdered ferrous sulphate (50 mg) in a test tube.

Chemical test

Bicarbonate was added to the sample to determine the presence of carboxylic functional group in the sample. Similarly a few drops of ferric chloride were added to the methanolic solution of the sample to determine the presence of a phenolic group. A Liebman Burchard reaction was also carried out to confirm the presence of triterpene. The chloroform solution of the sample was treated with concentrated sulphuric acid and acetic anhydride to confirm the absence of triterpene.

Derivatization of the sample

Acetylation of the sample

The sample (25 mg) was dissolved in a mixture of pyridine (1 cm) and acetic anhydride and the mixture was allowed to stand at room temperature for 24 hr. The reaction mixture was allowed to stand for 2 hr. The solid that separated out was filtered and dissolved in ether and dried with anhydrous sodium sulphate. It was left to stand overnight to evaporate off to give the acetate derivative (23 mg), m.p: 244-246^{o}C; IR (KBr) appeared bands, (cm^{-1}): 1720 (s),1640 (s), 1640 (s), 1270 (w), 1155 (w), 1110 (w); disappeared bands, v (cm^{-1}): 3400 (m), 1260 (m), 1615 (s); UV (MeOH) λ_{max}, 288 nm, 206 nm.

Methylation of the sample

A suspension of the sample (34 mg) in chloroform (10 mL) was refluxed with silver oxide (75 mg) and methyl iodide (1.5 mL) for 6 hr. The mixture was filtered with a funnel containing beads of silica gel and it was percolated with chloroform. It was evaporated using a rotary evaporator to give an oily material (11 mg), IR (neat), appeared bands, v (cm^{-1}): 2920 (s), 1635 (m), 1725 (s); disappeared bands, (cm^{-1}): 1615 (s); UV (MeOH), λ_{max}, 198 nm;

Amide derivative

The sample was (30 mg) was dissolved in a mixture of acetic acid (1 mL) and acetic anhydride (1 mL) with p-toluene sulphonic acid and left for 24 hrs. Water was added to the reaction mixture and extracted with ether. The ethereal layer was collected and treated with sodium bicarbonate solution to remove any trace of acids. The ethereal

solution was decanted and dried over anhydrous sodium sulphate. This was left overnight for the solvent to evaporate off. The residue was chromatographed on a short column of silica gel to give a gummy material (23 mg), appeared bands, v (cm^{-1}): 1720 (s), 1625 (w), 1280 (w); disappeared bands, v (cm^{-1}): 3400 (m), 1260 (m), 1615 (w).

Hydrochloride of the sample

7.0 mg of the sample was dissolved in methanol and a drop of concentrated hydrochloric acid added. The solution was evaporated over a water bath to yield needle like crystals (5 mg) which was recrystalized from ethanol, mp: 226-227^{o}C.

RESULTS AND DISCUSSION

Table 1: ^{1}HNMR spectral data of isolated alkaloid (33MHz, CD_3OD, δ in ppm)

Position	δ_H
2	8.48(1H, s)
4	7.56 (1H, s)
6	7.12(1H, s)
8	7.92(1H, s)
11	7.42(1H, t)
12	5.28(2H, d)
10	2.41(3H, s)
2'	2.65(2H, t)
3'	1.48(2H, tt)
4'	1.52(2H,tt)
5'	1.98(2H, dt)
6'	4.37(2H, dt)
2"	4.48(1H,d)
3"	5.90(1H, dd)
4"	5.98(1H, d)
5"	4.28(1H, d)
OH	4.47 (1H, s)

Table 2: UV/visible spectral data of isolated alkaloid

Wavelength (λ) (nm)	Molar extinction coefficient (ε) ($dm^3mol^{-1}cm^{-1}$)
214	1.109 X 10^5
288	7.415 X 10^4

Table 3: IR spectral data of isolated alkaloid

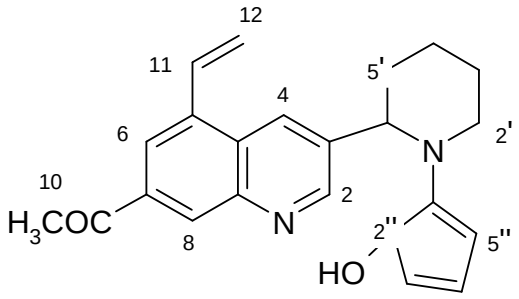
Bands (cm^{-1})	Assignment
3400(m)	OH stretching vibration
1615(s)	C=C, aromatic, C=O stretching vibrations
1450(m)	C=C stretching vibration
1360(m)	C-H bending vibration
1260(m)	OH Bending vibration
1185(w)	C-N stretching vibration
1160(m)	C-C stretching vibration
1130(w)	CH_3 bending vibration

This work was designed to investigate the components responsible for the itching effects of the hairs of *Mucuna pruriens*. The hairs were sequentially extracted with n-hexane, ethyl acetate and methanol. On each extraction, the residues (hairs) were tested on human skin and it was noticed that the itching action reduced immensely after extraction with ethyl acetate. Waxy oil and a white solid were obtained from the n-hexane extract. The ethyl acetate and methanol extracts afforded a solid each. Thin layer chromatography of the ethyl acetate extract with various solvent mixtures resolved well in solvent mixture $CHCl_3$/MeOH (4:1; v/v). Column chromatography was carried out using silica gel as the stationary phase. The column was eluted with chloroform and increasing concentration of methanol. Fractions obtained from 5% methanol gave R_f value of 0.4 and these were combined and recrystalized from a mixture of benzene and acetone. The melting point of the isolated compound was found to be 212-214^{o}C. The compound was subjected to various chemical tests to know the functional groups present. It was found that the compound is phenolic and contains no carboxylic functional group. Other tests carried out include Liebmann Burchard reaction and sodium fusion test. With these tests, it was observed that the isolated compound is not a triterpene and it contains nitrogen. The presence of nitrogen was further confirmed from the colour reaction with Dragendoff reagent which shows that it is an alkaloid. The compound was further characterized using some physical methods that were available. The mass spectrum suggested a molecular mass of 360 for the unknown compound. Also the UV of the compound gave two strong maxima at 214 and 288 nm with molar extinction coefficients of 1.109 X 10^5 and 7.415 X 10^4 dm^3 $mol^{-1}cm^{-1}$ respectively. This indicated that the unknown compound is conjugated.

The shift in the second maxima from 288 nm to 328 nm, which occurred on raising the pH to 8 negated the possibility of having an indole alkaloid such as tryptophan, tryptamine and indican (Rapport *et al*., 1948). The IR spectrum of the compound showed bands at 3400 cm^{-1}, which indicates the presence of OH as already confirmed from the chemical test; also the signal at 1615 cm^{-1} showed presence of carbonyl group in the unknown compound. It is largely reduced as a result of being in conjugation with the rings and/or due to hydrogen bonding between the C=O and OH. The proton NMR (CD_3OD) gave singlet each for the aromatic protons at δ value of 7.12, 7.92, 7.56 and 8.48 at positions 6, 8, 4 and 2 respectively; triplets for methylene protons at δ 2.65, 1.48, 1.52 and 1.90 at positions 2', 3', 4' and 5' respectively; a singlet at δ 4.80 and 4.37 for methine protons at positions 6' and 2''. The ethylenic protons gave doublet at δ 5.28 (J = 5 Hz), 5.90, 5.88 and 4.28 at positions 3'', 4'', 5'' and 12 respectively, and a triplet at δ 7.42 at position 11. The signal at δ 4.47 (s) disappeared on addition of D_2O suggesting it to be proton from OH. The methyl proton occurred at δ 2.41 at position 7''. There was no signal at 2.1 – 2.5 indicating absence of an alkylated tryptamine in the unknown compound. The unknown compound was acetylated with acetic anhydride in the presence of pyridine. The isolated compound when treated with ferric chloride showed absence of phenol indicating that the hydrogen of the OH has been successfully replaced with an acetyl group The product gave a melting point of 244-246^oC. The IR of the acetylated product showed some new bands at 1720 cm^{-1} and 1640 cm^{-1} while the band at 3400 cm^{-1} due OH stretching vibration disappeared. This shows that the stretching vibration due to C=O shifted from1615 cm^{-1} to 1640 cm^{-1}on acetylation indicating that the C=O of the acetylated product was probably free of hydrogen bonding. The UV of the acetylated product gave two maxima at 288 and 205 nm. The IR spectrum of the unknown compound did not show presence of –NH or $–NH_2$. This was confirmed when the compound was treated with acetic anhydride in the presence of acetic acid and p-toluene sulphonic acid. The product isolated gave a negative test for phenol when treated with ferric chloride. The IR of the product was similar to that of the acetylated compound. The hydrochloride of the isolated compound was prepared and has a melting point of 226-227^oC. This melting point however did not agree with any of the hydrochloride derivatives of all the alkaloids so far obtained from hairs and seed of *Mucuna pruriens*. The isolated compound was also methylated with methyl iodide in the presence of silver oxide. The product isolated was treated with ferric chloride and gave a positive test for phenolic. The product was also dissolved in methanol and was spotted on a Whatman No. 1 filter paper and sprayed Dragendoff reagent. It was observed that no colour developed on the paper after drying, indicating the absence of nitrogen in the methylated derivative. In the IR of the methylated product, some new bands appeared at 2920 cm^{-1}, due C-H stretching vibration of alkyl and 1725 cm^{-1} due to C=O stretching vibration of the isolated compound, now free of H-bonding. The absorption at 1615 cm^{-1} for the C=O stretching vibration of the isolated compound was no longer observed. The UV of the methylated product gave a maximum absorption at 198 nm. This showed that a new product was formed. The proton NMR (Py-ds) gave singlet at δ values, 7.90, 6.40 and a broad singlet at 4.5. It also gave a signal at δ 6.80 (d). The signal at δ at 4.5 (s), which was observed for the methylated product disappeared on addition of D_2O, also suggesting it to be the proton of OH. The proton NMR did not give any signal at δ 2.1-2.5 region for the proton in $N-CH_3$ environment, which further suggested the absence of nitrogen in the methylated product, thus indicating that degradation had likely occurred.

CONCLUSION

The compound isolated from the hairs of *Mucuna pruriens* showed itching property and was identified as an alkaloid with molecular formula $C_{23}H_{24}N_2O_2$ using both spectroscopic and chemical methods. The proposed structure that is consistent with the molecular formula is given in scheme 1.



7-acetyl-5-ethenyl-3-(N-cyclopenta-3'',5''-dienyl-2''-hydroxypiperidine)quinoline

Scheme 1: Proposed structure of isolated compound with itching property

REFERENCES

[1] D Armstrong; RML Dry; CA Keele; JW Markham, *J Physiol*. **1953**; 120 (3):326–351.

[2] JL Broadbent. *Bristish Journal Pharmacol., chemother*., **1953**;8, 263.
[3] MM Rapport; AA Green; IA Page, *J. Biol. Chem*., **1948**;176, 1243.
[4] M Hernandez-Gonzalez, *Physiol. Behaviour*, **2000**; 71: 51-56.
[5] AKV Kumar; KK Srinivasan; T Shanbhag; SG Rao,. *Indian Drugs* **1994**; 31, 321-327.
[6] L Sathiyanarayanan; A Arulmozhi, *Pharmcog. Rev*. **2007**; 1, 157-162.
[7] KK Shukla; AA Mahdi; MK Ahmad; SN Shankhwar; S Rajender; SP Jaiswar, *Fertil. Steril.* **2008**; 6, 1934-1940.
[8] PK Warrier; VPK Nambiar; C Ramankutty, Indian medicinal plants **1996**; Vol. 4. p. 68–72. Orient Longman, Chennai, India.
[9] CST Sastry; YY Kavathekar. Plants for reclamation of wastelands. Publications and Information Directorate, New Delhi. **1990**; p. 317–318.
[10] SP Agharkar, Medicinal plants of Bombay presidency. **1991**; p. 1–2. Scientific Publ. Jodhpur. India.
[11] BV Dhawan; MP Dubey; BN Mehrotra, RP Rastogi; JS Tondon, *Ind. J. Expt. Biol.* **1980**; 18, 594-606.