September 2007 Volume 3 Issue 3

Trade Science Inc.

Organic CHEMISTRY

An Indian Journal

Full Paper

OCAIJ, 3(3), 2007 [107-111]

Chemical Investigation Of The Itching Effects Of Hairs Of Mucuna Pruriens (Cow-Hage)

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Received: 18th February, 2007 ; Accepted: 23rd February, 2007

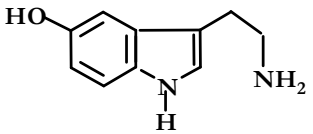
ABSTRACT

The new alkaloid obtained from the hairs of *Mucuna pruriens* has a melting point of 212-214^{0}C with molecular formula $C_{23}H_{24}N_2O_2$. It is readily soluble in cold and hot methanol and Me_2CO but insoluble in water, chloroform, ether, benzene. It yields a crystalline hydrochloride, m.p; 226-227^{0}C00 (dec.) and an acetate, m.p; 244-246^{0}C(dec.). The alkaloid was characterized by IR, UV, ^{1}H-NMR, MS techniques., to deduce the most probable structure for the alkaloid. The name of the most probable structure deduced for the compound is 2-acetyl-3-hydroxy-4-ethenyl-6-(N-cyclopent-2,4-dienyl-piperidine) quinoline(M^+, 360)

INTRODUCTION

The trichomes from the pods of *Mucuna pruriens,* D.C, which constitute cowhage, have long been known to cause intense itching and some pains when applied to human skin. No one has truly characterized the adequate stimulus for the itching effects of the hairs of *Mucuna pruriens.* Broadbent[1] concluded, from pharmacological and histological studies, that itching was not a mechanical effect of the trichomes but was due to the presence of an histamine-liberating substance. In seeking to identify the active component, Armstrong et al [2] have shown 5-hydroxytryptamine to be more effective than histamine as a cutaneous pain-producer in human. It is thus probable that 5-hydroxytryptamine is a factor in the causation of pain following the application of cowhage on to the skin.

Some alkaloids have been isolated from the seed of *Mucuna pruriens,* amongst them are mucunadine, mucunine[3]. Prurienine and prurieninine, with molecular formula, $C_6H_{12}O_2N_2$ and $C_8H_{16}O_2N_2$ respectively, have also been isolated from the seed of *Mucuna pruriens*[3]. Some authors[2,4] have shown 5-hydroxytryptamine (structure below) to be one of the component present in the hairs of *Mucuna pruriens,* and is also responsible for the itching when applied to human skin. Other alkaloids, which have been isolated from the seed of *Mucuna pruriens*, include mucuadine, mucuadinine, mucuadininine, prurienidine and nicotine[5].



5-Hydroxytryptamine

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In the present study, we investigate the active components responsible for the itching effects of the hairs of *Mucuna pruriens,* which belong to the family *Papiilionaceae,* by carrying out extraction and fractionation followed by chemical and spectroscopic characterizations of the extracted compound.

EXPERIMENTAL

Extraction and fractionation

The hairs are scrapped with spatula from the legume of the *Mucuna pruriens*(cowhage) and 100g was shaken sequentially with n-hexane for 2days, followed by ethyl acetate and methylated spirit. The n-hexane extract afforded waxy oil, while ethyl acetate extract gave a solid substance.

The ethyl acetate extract(1.109g) was dissolved in methanol and pre-adsorbed with silica gel, dried over steam bath and submitted to column chromatography. The elutriants being sequence of purified solvents of 100% chloroform with increasing percentages of methanol. The fractions obtained with 5% methanol afforded a solid each and their purities were ascertained with thin layer chromatography (TLC). The solid gave single spot each with identical R_f value of 0.42. They were combined and recrystallized from a mixture of acetone and benzene. After drying, the sample was weighed (0.570g, 60%;). The melting point and the spectroscopic data were determined. M.p : 212- 214^{0}C; IR(KBr), ν(cm^{-1}): $3400_{(m)}$(O-H stret.), $1615_{(s)}$(C=O stret.), $1450_{(m)}$(C=C stret.), $1360_{(m)}$(C-H bend. In CH_3-C), $1260_{(m)}$, $1185_{(w)}$, $1160_{(m)}$ and $1130_{(w)}$(O-H bend. and C- OH stret.); ^{1}H-NMR(CD_3OD) δ : 4.65(O-H, s), 2.69(CH_3CO-, s)5.65(CH_2=C, d), 6.55(H-C=C, t), 6.70(H-Ar, s); MS: m/z(%): 360(M^+, 37); UV: $\lambda^{max.}$(EtOH), nm(ε): 288(1.224×10^5), 214(1.829×10^5).

The pure unknown sample was subjected to various chemical and physical tests. These tests are described below:

Solubility test

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

Color reagent test

The sample was dissolved in methanol and was spotted on a rectangular piece of Whatman No.1 paper alongside with some nitrogenous compounds-pyridine, indole, aniline and non-nitrogenous compounds, such as chlorophenol. This was sprayed with Dragendoff reagent and was left overnight to allow complete dryness. The unknown sample and indole gave deep brown color, aniline gave light brown, pyridine was faint while chlorophenol gave no color.

Sodium fusion test

Sodium fusion solution of the sample was prepared using the standard procedure, and 0.5mL of the solution was added to powdered ferrous sulphate (50mg) in a test tube. A deep green precipitate was observed, which indicate the presence of nitrogen in the sample.

Chemical test

Bicarbonate was added to the sample, and there was no effervescence, which indicates absence of carboxylic functional group in the sample. Similarly, a few drop of ferric chloride was added to the methanolic solution of the unknown sample, and a blackish color was obtained, which probably indicates presence of 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. It gave brownish oil at the bottom of the test-tube, which showed that triterpene was absent.

Derivatization of the unknown sample

The pure sample was subjected to various chemical reactions to ascertain the presence of some functional groups present in the unknown sample. The following derivatives of the sample were synthesized and characterized by spectroscopic techniques.

Acetylation of the sample

The sample(25mg) was dissolved in a mixture of pyridine(1cm^3) and acetic anhydride(1cm^3), and

the mixture was allowed to stand at room temperature for 24 h. The reaction mixture was added into a crushed ice and then allowed to stand for 2h. The solid that separated out was filtered and dissolved in ether, and was then dried with anhydrous sodium sulphate. This was left overnight to evaporate off, and gave the acetate derivative(23mg, 92%). M.p : 244-246^{0}C(dec.); IR(KBr) appeared bands, ν(cm^{-1}): $1720_{(s)}$(C=O stret. in acetate), $1640_{(s)}$(C=O stret. of the unknown sample), $1270_{(w)}$, $1155_{(w)}$, $1110_{(w)}$ (C-O stret. of acetate) ; disappeared bands, ν(cm^{-1}): $3400_{(m)}$ (O-H stret.), $1260_{(m)}$(O-H bend. /C-OH stret.), $1615_{(s)}$(C=O stret. of the unknown sample); UV(MeOH)$\lambda_{max.}$, 288nm, 206nm.

Methylation of the sample

A suspension of the unknown sample(34mg) in chloroform(10cm^3) was refluxed with silver oxide(75mg) and methyl iodide(1.5cm^3) for 6h. The mixture was filtered with a funnel containing beads of silica gel and it was percolated with chloroform. This was then evaporated under rotary evaporator to 5cm^3, and was left overnight when an oily material separated out(11mg, 32%); IR(neat), appeared bands, ν(cm^{-1}): $2920_{(s)}$(C-H stret.of alkyl), $1635_{(m)}$ (C=C stret.), $1725_{(s)}$(C=O stret. of 6-membered ring ketone); disappeared bands, ν(cm^{-1}): $1615_{(s)}$ (C=Ostret. of the unknown sample); UV(MeOH), $\lambda_{max.}$, 198nm; ^{1}H-NMR(d_5-pyridine), δ : 4.5(O-H, s), 6.4(-C=CH, s), 6.8(-C=CH, d), 7.90(H-Ar, s).

Amide derivative

The unknown sample(30mg) was dissolved in a mixture of acetic acid(1cm^3) and acetic anhydride (1cm^3) with a catalytic amount of p-toluene sulphonic acid(TSA) and left at room temperature for 24 h. Water was then 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(23mg, 77%). IR(neat), appeared bands, (cm^{-1}): $1720_{(s)}$(C=O stret. of ester), $1625_{(w)}$(C=O stret. of the sample), $1280_{(w)}$(C-O stret. in ester); disappeared bands, ν(cm^{-1}): $3400_{(m)}$(O-H stret.), $1260_{(m)}$(O-H bend./C-OH stret. in phenol), $1615_{(w)}$(C=O stret. of the sample).

Hydrochloride of the sample

To an absolute solution of the unknown sample(7mg), a drop of concentrated hydrochloric acid was added. The solution on evaporation over a water bath yielded small needle-like crystals which was recrystallized from absolute alcohol(5mg, 71%), m.p : 226-227^{0}C.

DISCUSSION

This work was designed to investigate the components responsible for the itching action of the hairs of *Mucuna pruriens*. The hairs was first extracted with n-hexane followed by ethyl acetate and lastly with methylated spirit. On each extraction, the residues (hairs) were tested on skin and it was noticed that the itching action reduced immensely after extracting 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 with $CHCl_3$/MeOH mixture. It showed a major spot and a tailing with $CHCl_3$/MeOH(4:1). Thus the column was packed with silica as stationary phase and chloroform as the mobile phase. The column was then eluted with chloroform with increasing concentration of methanol. The solid obtained from ethyl acetate extract was chromatographed using silica as stationary phase and chloroform as mobile phase to separate the components present in the crude extract. Fractions obtained from 5% methanol gave the same R_f value of 0.4 and these were combined together and recrystallized from a mixture of benzene and acetone (unknown sample). The melting point of the unknown sample was found to be 212-214^{0}C. The sample 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(-COOH). Other tests carried out include Liebmann Burchard reaction and sodium fusion test. With these tests, it was found that the compound is

not a triterpene and it contains nitrogen. The presence of nitrogen was further supported from the color reaction with Dragendoff reagent.

The compound was characterized further by use of some physical methods available. The mass spectrum suggested the molecular mass of 360 for the unknown compound. Also, the UV of the sample gave two strong maxima at 214 nm and 288nm with molar extinction coefficients of 1.109×10^5 and $7.415\times10^4 dm^3\ mol^{-1}\ cm^{-1}$ respectively. This really indicated that the unknown compound is strongly conjugated. The shift in the second maximum from 288 nm to 328 nm, which occurred on raising the pH to 8 really negated the possibility of having an indole alkaloid such as tryptophan, tryptamine and indican[6]. The IR spectrum of the sample shows a band at $3400cm^{-1}$, which indicates the presence of O-H as already confirmed from the chemical test; also the signal at $1615cm^{-1}$ shows the presence of carbonyl group(>C=O) in the unknown compound., largely reduced as a result of being in conjugation with the rings and/or due to hydrogen bonding between the O-H and >C=O of the unknown compound. The proton NMR(CD_3OD) gave singlet at δ values of 6.70, 6.55 and a broad singlet at δ value of 4.65. It also gave a doublet at δ 5.63 (J=5Hz). The signal at δ4.65(s), which was observed for the compound disappeared on addition of D_2O, suggesting it to be proton of O-H. The proton NMR did not give any signal at δ 2.1-3.0 region for the proton in $N\text{-}CH_3$ environment, which suggested the absence of alkylated tryptamine in the unknown sample.

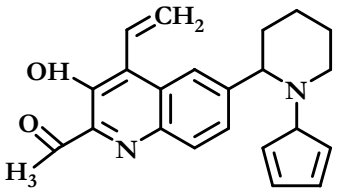
Carrying out some reactions on the unknown sample did further confirmation of all these functional groups. The sample was acetylated with acetic anhydride in the presence of pyridine. The product isolated was treated with ferric chloride and it was found that phenol is absent, indicating that the hydrogen of the O-H of phenol had been successfully replaced with $-COCH_3$. The product melted at 244-246^{0}C(dec.). When the IR of the acetylated product was taken, some new bands appeared at $1720cm^{-1}$ and $1640cm^{-1}$ while a band at $3400cm^{-1}$ due to O-H stretching vibration disappeared as well. What could be observed here was that >C=O absorption at $1615cm^{-1}$ in the unknown compound in the sample shifted to $1640cm^{-1}$ on acetylating, 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 288nm and 205nm.

The IR spectrum of the unknown compound in the sample did not show the presence of -NH or $-NH_2$, to confirm this further, the sample was treated with acetic anhydride in the presence of acetic acid with a catalytic amount of p-toluene sulphonic acid. A gummy compound isolated was treated with ferric chloride and it was found that phenol was absent. The IR spectrum of the gummy material was similar to that of the acetylated product. Hydrochloride of the sample was also prepared and this melted at 226-227^{0}C. This melting point however, did not agree with any of the hydrochloride derivatives of all the alkaloids so far obtained from hairs and seeds of *Mucuna pruriens*.

The unknown sample was also methylated with methyl iodide in the presence of silver oxide. The product isolated was treated with ferric chloride and the product was found to be phenolic. The product was also dissolved in methanol and was spotted on a Whatman No.1 filter paper and sprayed with Dragendoff reagent. It was found that no color 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 $2920cm^{-1}$, due to C-H stretching vibration of alkyl and at $1725cm^{-1}$ due to >C=O stretching vibration of the unknown compound, now free of H-bonding. The absorption at $1615cm^{-1}$ for the >C=O stretching vibration of the unknown compound in the sample was no longer observed. The UV of the methylated product was taken and this gave a maximum absorption at 198nm. This indicated that a new product was formed. The proton NMR(Py-d_5) gave singlet at δ values, 7.90, 6.40 and a broad singlet at δ 4.5. It also gave a signal at δ 6.80 (d). The signal at δ 4.5(s), which was observed for the methylated product, disappeared on addition of D_2O, also suggesting it to be the proton of O-H. The proton NMR did not give any signal at δ 2.1-3.0 region for the proton in $N\text{-}CH_3$ environment, which further suggested the absence of nitrogen in the methylated product, thus indicating that a degradation had likely occurred.

CONCLUSION

The extractive obtained from the hairs of *Mucuna pruriens* shows itching activity. The alkaloid responsible for the itching has been shown to have molecular formula $C_{23}H_{24}N_2O_2$ from the spectroscopic and chemical methods. The probable structure that is consistent with the molecular formula is:



2-Acetyl-3-hydroxy-4-ethenyl-6-(N-cyclopent-2, 4-dienyl-piperidine) quinoline (M^+, 360)

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