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Isolation and Characterization of Estra–2^{II}–en –17–ol, 3yl benzoate from *Mucuna pruriens (Utilis).*

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Abstract
Chemical investigation of the bioactive constituents of the seeds of *Mucuna pruriens (utilis)* resulted in the isolation of a new steroid, Estra–2^{II}–en –17–ol, 3yl benzoate. The structure was elucidated using NMR spectroscopy in combination with IR and MS spectra data.
Keywords: Mucuna, anti-microbial, anti-oxidant, anti-inflammatory, anti-hypertensive.

1. Introduction

Plants have been known to be useful to man; as source of food and drug. They are of paramount importance in the treatment of diseases for man. Nigeria is blessed with many of such plants and they are used as food and in herbal medicine to cure diseases and heal injuries. Some of these plants exhibit anti-microbial, anti-oxidant, anti-inflammatory and anti-hypertensive activities. Such medicinal plants include *Mucuna pruriens* (*utilis*). *M. pruriens* is extensively used in herbal medicine and as food. The seeds have been found to contain high levels of protein and carbohydrate and thus the flour has been recommended to be incorporated into cereals and root/tuber food products to improve their nutritional quality (Udensi and Eke, 2000). The flour is used in soup as soup thickener and to enhance the flavour of the soup. *M. pruriens* is grown widely as a green vegetable, both the green pods and the mature beans are boiled and eaten as food (Burkill, (1995); Duke, (1981)). The seeds are also roasted and ground to make a coffee substitute. Extracts from the seeds have been recommended as one of the best tonics to improve vitality (Manyam et al, 2004).
M. pruriens is highly valued because of its medicinal use. It is used in herbal
medicine as a powerful nervine tonic and an effective aphrodisiac in the treatment of disorders of the male and female reproductive systems (Numida, 2009; Ezueh, 1997; Amin et al, 1996). The seeds are effective in the treatment of parkinson diseases (Manyam et al, 2004; Katzenschlager et al, 2004). Extracts from the seeds have been used to enhance sexual stamina, sensation and libidos in both men and women due to its dopamine inducing properties (Amin et al, 1996; Giuliano and Allard,2001).
Mucuna pruriens seeds have also been found to have antidepressant properties when consumed. It is a very beneficial supplement for body builders as it increases the body's ability to build lean muscle, breakdown fat, and is highly recommended to sportsmen (Ray Sahelian, M.D).
From literature, it is obvious that *Mucuna pruriens* seeds contain some hormones since it is used to build lean muscles and as aphrodisiac by the natives, but isolation has not been made to that effect, thus this study was undertaken to isolate and characterize bioactive constituents of *Mucuna pruriens* seeds.

2. Materials and Method.

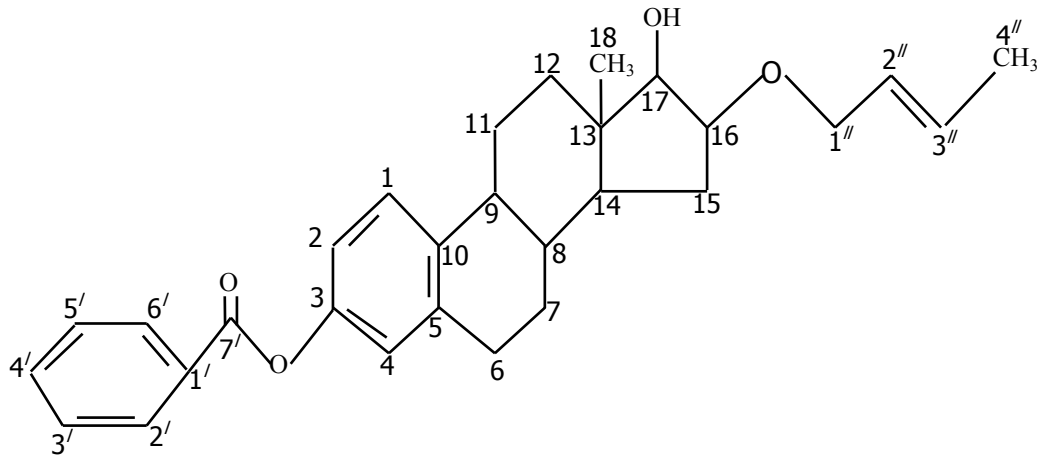
2.1 Plant Materials : The seeds of *M. pruriens* were harvested from the field of National Root Crops Research Institute Umudike ,Abia state, Nigeria. Authentication of plant materials was done by Dr A. Nmeregini of Taxonomy section, Forestry Department, Micheal Okpara University of Agriculture, Umudike, Nigeria.
2.2 Extraction and Isolation of Plant Materials : The seeds were cracked, the testa were removed and the seeds were milled into fine powder with Thomas Willey milling machine and then stored in air tight bottles for analysis. 2kg of the sample was percolated in 98% ethanol for 48hrs, this was then filtered. The filtrate was concentrated with Rotary evaporator at 40^{o}C to a dark brown crude extract (44.3g). The crude extract was partitioned between $CHCl_3$ and water and a $CHCl_3$ - soluble fraction (17.5g) was obtained. 10.0g of the $CHCl_3$ fraction was then partitioned between petroleum ether (60 – 80^{0}C) and aqueous methanol. 5.0g of the $CHCl_3$ fraction was then subjected to column chromatography over silica gel (200 mesh) and eluted gradually with 100ml petroleum ether, then petroleum ether : $CHCl_3$ (90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90;), and 100ml $CHCl_3$; then $CHCl_3$: Methanol (90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90) and 100ml methanol to yield ten major fractions labeled : G (yellow oil, 0.3g), I (yellow oil,0.4g), b (brown oil, 0.3g) c_1 (yellow oil,0.6g), sc_2(yellow oil, 0.60g),d (brown oil 0.32g) f_2 (brown solid, 0.3g), g_2 (brown oil, 0.5g), h (dark brown oil, 0.4g), i (light yellow oil, 0.3g) and i_2 (light yellow oil, 0.4g).

Chromatographic (partition chromatography, column chromatography, and TLC) and spectroscopic (IR, ^{1}HNMR, ^{13}CNMR and MS) techniques were employed to isolate, characterize and identify active constituents from $CHCl_3$ extracts of the seeds.

3.0 Results and Discussions:

Compound [1] was eluted with methanol and chloroform at the ratio of 70:30. It was isolated as yellow oil (0.6g). Based on combined analysis of HREIMS (m/z 445.41), ^{1}HNMR, ^{13}CNMR and IR spectra, compound [1] was proposed as Estra–2^{II} –en –17–ol, 3yl benzoate with the molecular formula $C_{29}H_{34}O_4$, m/z 446 calculated.

Analysis of the IR is shown in Table 1 and the analysis of ^{1}HNMR and ^{13}CNMR are shown in Table 2. The IR spectrum revealed the presence of hydroxyl, aliphatic, carbonyl, aromatic and ether bands at (3470.52cm^{-1}, 2924.97cm^{-1}, 1741.0cm^{-1}, 3008.59cm^{-1} and 1169.89cm^{-1}) respectively.



[1] Estra-2^{II}-en-17-ol, 3yl benzoate

Table 1: Infra-red Analysis of Estra–2^{II}–en –17–ol, 3yl benzoate [1] from *M. pruriens*

IR Absorption (cm^{-1})	Functional Group	Compound
3470.52	OH	Alcohol
3007.89	C=C	Aromatic
2925.44	– CH_2	Aliphatic
2855.35	– CH_2	Aliphatic
1742.73	C = O	Carbonyl
1167.76	C – O	Ether

Table 2: ^{1}H and ^{13}CNMR Analysis of Estra – 2^{II} – en - 17- ol- 3yl benzoate.

	δC			δH	
1	127.95	CH	5.323	IHd	CH
2	128.12	CH	5.342	IHd	CH
3	130.06	C	_	_	_
4	129.76	CH	5.350	1Hs	CH
5	130.27	C	_	_	_
6	31.59	CH_2	1.231	2Ht	CH_2
7	31.72	CH_2	1.254	2Ht	CH_2
8	33.66	CH	1.607	IHd	CH
9	33.99	CH	1.629	IHd	CH
10	129.76	C	–	–	–
11	31.75	CH_2	1.278	2Hq	CH_2
12	31.84	CH_2	1.285	2Ht	CH_2
13	34.11	C	–	–	–
14	34.14	CH	1.376	IHt	CH
15	31.99	CH_2	1.302	2Ht	CH_2
16	34.75	CH	2.080	1Hd	CH
17	77.11	C–OH	7.265	1Hs	OH
18	14.13	CH_3	0.857	3Hs	CH_3
1^{I}	127.95	C	_	_	_
2^{I}	128.12	CH	5.360	IHd	CH
3^{I}	130.06	CH	5.370	IHt	CH
4^{I}	129.76	CH	5.323	1Ht	CH
5^{I}	130.27	CH	5.342	IHt	CH
6^{I}	129.76	CH	5.350	IHd	CH
7^{I}	173.38	C=O	_	_	_
1^{II}	76.69	CH_2	5.386	2Ht	CH_2
2^{II}	172.92	CH	2.347	IHq	CH
3^{II}	173.34	CH	2.373	IHm	CH
4^{II}	14.18	CH_3	0.857	3Hd	CH_3

S = singlet, d = doublet, t = triplet, q = quartet, m = multiplet

The mass spectrum of compound [1] showed m/z 445.41 which was calculated for $C_{29}H_{34}O_4$ (m/z 446) and base peak m/z 55.18 calculated for C_4H_7 (m/z 55). The ^{1}HNMR spectrum of compound [1] showed presence of methylene protons observed at δH1.231, δH1.254, δH1.278, δH1.285, methine protons at δH 1.607 - δH 2.373 and aromatic protons which showed their signals at δH 5.232 – δH 5.370. The spectrum indicated presence of hydroxyl proton at δH 7.265.

The ^{13}CNMR spectrum depicted the presence of aromatic carbon atoms, their chemical shifts were observed at δ127.95 - δ130.27. The carbonyl chemical shifts appeared at δ173.38. The methyl carbons showed their signals at δ14.13 and δ14.18. The signals of the methylene carbons were observed at δ31.59 – δ31.99 while the chemical shifts of the methine carbons appeared at δ 33.66 – δ 34.25.

Fragmentation pattern of compound [1] is shown in fig (1). Detachment of C_4H_7 ion from the compound afforded the base peak m/z 55.18 calculated for m/z 55. The cleavage between the two oxygen atoms in the benzoate gave the peak at m/z 105. Also detachment of C_5H_5O ion from the compound afforded another prominent peak m/z 81.20 calculated for m/z 81. The isolated compound has the skeleton of Estradiol, a sex hormone. The proton in the second hydroxyl is replaced by phenyl oxide. Estradiol protects against brain injury, neurodegeneration and cognitive decline. It protects against stroke injury (Dubal et al, 2000). Thus, this compound isolated from *M. pruriens* has biological and physiological activities. The presence of this compound in *Mucuna pruriens* seed may be the reason why the natives use this plant in the treatment of disorders of the male and female reproductive systems, parkinson disease and increase libido in both men and women (Numida, 2009; Ezueh, 1997; Amin et al, 1996). Estradiol has been found to reduce anxiety and depression – like behavior of aged female mice (Walf and Frye, 2005). The isolated compound may also be the reason *mucuna pruriens* seeds are used by the herbalists as anti- depressant.

Conclusion

The results presented here showed that *M. pruriens* plant can serve as a pharmacodynamic agent. The plant can be seen as a potential source of useful drugs and can serve as source of quality raw material for pharmaceutical

industries. Thus this contributes to the scientific evidence for the use of this medicinal plant in traditional medicine for the treatment of diseases like pains, depressions, erectile dysfunction, parkinson disease, e.t.c.

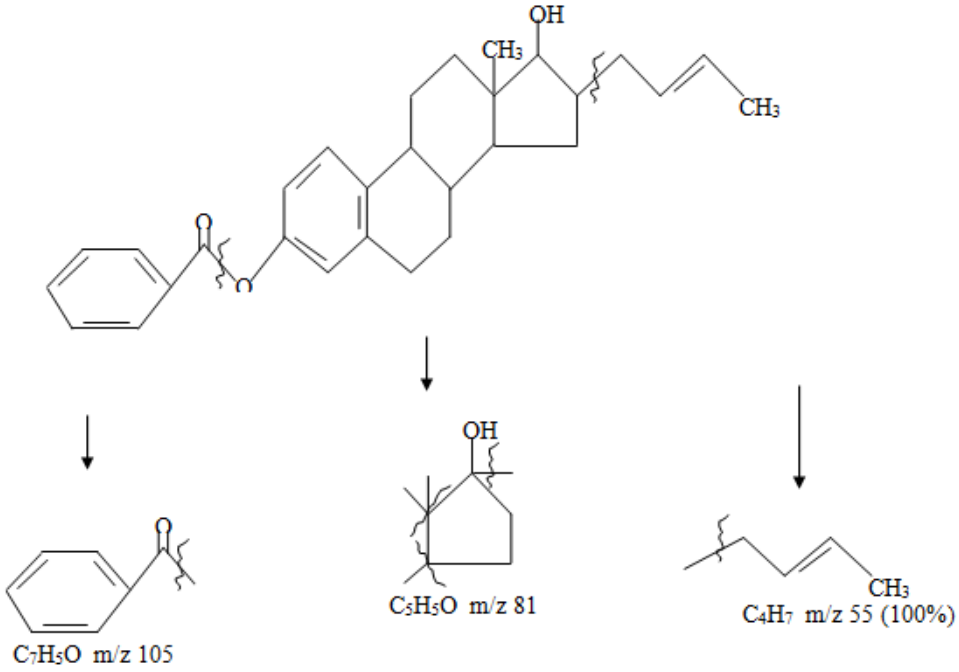


Fig 1: fragmentation Pattern of Compound [1].

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