

Isolation and Characterization of Estra-17-propoxy, 3', 4' – dimethoxy – 3yl benzoate from the leaves of *Spondias mombin linn*

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Abstract

Spondias mombin linn is one of the traditional medicinal plants with a lot of potential, valuable and untapped resource of active drugs for treating diseases. It is used in Nigeria for the treatment of cough, wounds, inflammations, diarrhoea, dysentery, haemorrhoids and gonorrhoea. A chemical investigation of the bioactive constituents of the leaves of *Spondias mombin linn* resulted in the isolation of a new steroid, Estra-17-propoxy, 3', 4' – dimethoxy – 3yl benzoate. The structure was elucidated using a two Dimensional spectroscopy, NMR (¹H, ¹³C), IR and MS spectroscopy data.

Keywords: *Spondias mombin*, diuretic, gonorrhoea

1.0 Introduction

For ages medicinal plants have been used for the treatment of man and animal diseases. The survival and continued existence of man depends on the efficiency with which man, with all the resources and technology available to him, harnesses, develops and utilizes plants and plant products (Ayoka *et al*, 2008). *Spondias mombin* is a medicinal plant with a lot of potential, valuable and untapped resource of active drugs for treating diseases.

Spondias mombin linn, a medicinally important plant of Anacardiaceae family, is a small tree that grows up to 20 m (60 ft.) high and 1.5 m (5 ft.) in girth. It flowers between January - May and fruits between July-September (Irvine,1961). The fruits have a sharp, acid taste and are edible. The fruit pulp is either eaten fresh, or made into juice, concentrate, jellies, and sherbets. The fruit-juice is used as a febrifuge and diuretic. The roots are also well-known febrifuge in Ivory Coast. The bark is used as a purgative and in local applications for leprosy. The bark decoction is used for severe cough, causing relief through vomiting, serves as an emetic, a remedy for diarrhea, dysentery, haemorrhoids and a treatment for gonorrhoea and leucorrhoea (Ayoka *et al*, 2008). In Mexico, the decoction of the astringent bark is believed to expel calcifications from the bladder. The bark is reported to contain a certain amount of tannin and this is the reason the dry pulverized bark is used to treat wound. In Belize, a decoction of the young leaves is a remedy for diarrhea and dysentery. The juice of crushed leaves and the powder of dried leaves are also used for the treatment of wounds and inflammations. The gum is employed as an expectorant and to expel tapeworms (Rodrignes and Hesse, 2000; Rodrigne and Samuels, 1999). A decoction of the mashed leaves is used by the Igbos (Nigeria) for washing a swollen face. A leaf infusion is a common cough remedy or used as a laxative for fever with constipation. A leaf decoction is used in treatment of gonorrhoea. A decoction of pounded leaves of *S. mombin* is used as an eye lotion and the juice pressed from young, warm leaves is given to children for stomach troubles. The extract has shown anti-inflammatory activity in Wistar rats (Nworu *et al*, 2011). A tea made from the flowers and leaves is taken to relieve stomach ache, biliousness, urethritis, cystitis and eye and throat inflammations (Villegas *et al*, 1997; Nworu *et al*, 2011). A decoction of the root is used as purgative.

In spite of the various uses of *Spondias mombin* in traditional medicine, the bioactive compounds of not been fully documented.

Herein we report for the first time the isolation and characterization of Estra-17-propoxy, 3', 4' – dimethoxy – 3yl benzoate from the leaves of *Spondias mombin linn*.

2.0 Materials and Method:

2.1 Plant material : The leaves of *Spondias mombin* were harvested from Ovom 1 in Obi ngwa local government area, Abia state, Nigeria. Authentication of plant materials was done by Ibe, Ndukwe of Taxonomy section, Forestry Department, Micheal Okpara University of Agriculture, Umudike, Nigeria.

2.2 Extraction and isolation of plant material : The leaves of *Spondias mombin* were washed and allowed to dry in the laboratory bench. The dried leaves 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°C to a dark brown crude extract (51.4g). The crude extract was partitioned between CHCl₃ and water and a CHCl₃ - soluble fraction (10.5g) was obtained. 8.0g of the CHCl₃ fraction was then partitioned between petroleum ether (60 – 80°C) and aqueous methanol. 4.0g of the CHCl₃ fraction was then subjected to column chromatography over silica gel (200 mesh) and eluted gradually with 100ml petroleum ether, then petroleum ether : CHCl₃ (90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90;), and 100ml CHCl₃; then CHCl₃ : 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 Chromatographic (partition chromatography, column chromatography, and TLC) and spectroscopic (IR, ¹HNMR, ¹³CNMR, COSY, DEPT and MS) techniques were employed to isolate, characterize and identify active constituents from CHCl₃ extracts of the leaves.

Results and Discussion

Compound [1] was eluted with chloroform and petroleum ether at the ratio of 80: 20. Thin Layer chromatography carried on compound [1] showed one spot (R_f 0.56).

Analysis of IR is shown in Table 1. The IR revealed V_{max} 2910cm⁻¹, 1700cm⁻¹, 1250cm⁻¹ and 1600cm⁻¹ for aliphatic, carbonyl, ether and aromatic bands.

Analysis of ¹H NMR is shown in Table 2. The ¹H NMR Spectrum depicted presence of methyl protons at δH 0.400, δH 0.413, δH 0.495 and δH 0.524. The spectrum revealed presence of two methoxy groups at δH 3.879 and δH 3.942. Chemical shifts for aromatic protons were observed at δH 5.034 - δH 6.117.

¹³C NMR spectrum was analyzed in Table 4.4 The spectrum revealed the presence of twelve aromatic carbons at δC 116.34, δC 117.42, δC 119.95, δC 121.59, δC 123.38, δC 124.00, δC 126.09, δC 129.00, δC 129.21, δC 129.89, δC 130.94 and δC 131.94. Methyl carbons showed their absorbance at δC 10.26, δC 10.96, δC 11.20 and δC 13.24. Methylene carbons chemical shifts were seen at δC 18.00 - δC 24.60 while the methane carbons peaks were observed at δC 25.37, δC 26.07, δC 29.02 and δC 33.39.

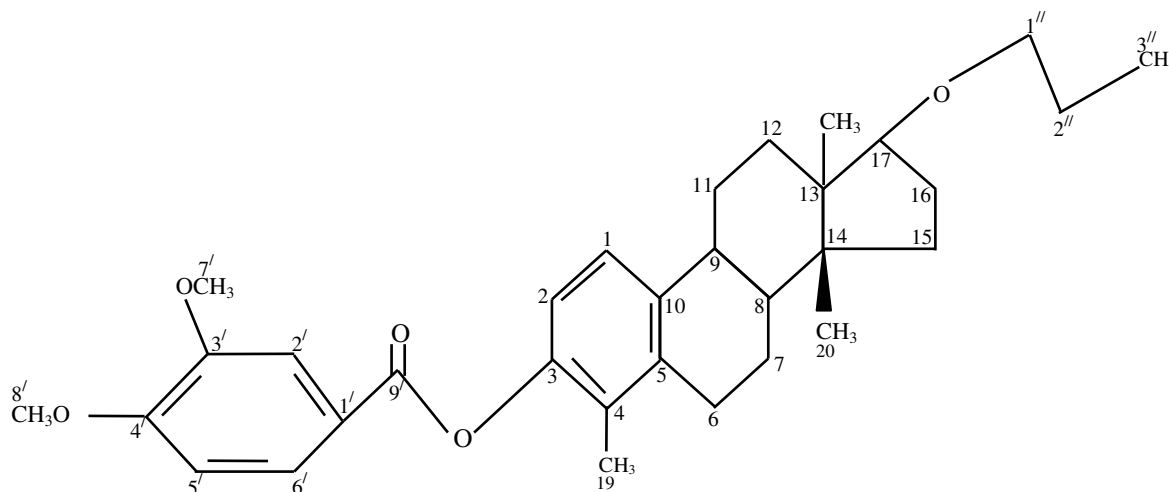


Fig 1: Estra - 17 - propoxy, 3', 4' - dimethoxy-3yl benzoate

Based on the chromatographic data, HREIMS, NMR, IR, COSY and DEPT, compound [1] was proposed as Estra - 17 - propoxy, 3', 4' - dimethoxy benzoate with molecular formula C₃₂H₄₂O₅ m/z 506 calculated.

Fragmentation pattern of compound [1] is shown in fig [2]. Detachment of the benzoate with two methoxy groups attached afforded the base peak m/z 180.7 calculated for m/z 181 (C₉H₉O₄). Detachment of C₂₀H₂₅ ion from the compound gave a peak at m/z 265.

The isolated compound is a steroid. It has the skeleton of Estradiol, a sex hormone. The protons in the hydroxyl groups attached at C₃ and C₁₇ were replaced with benzoate and propane respectively. Estradiol protects against brain injury, neurodegeneration and cognitive decline. It protects against stroke injury (Dubal *et al*, 2000, Uchegbu and Echeme, 2013). Estradiol has been found to reduce anxiety and depression – like behavior of aged female mice (Walf and Frye, 2005). A related compound, Estra-2''-en-17-ol- 3-yl benzoate was isolated from a medicinal plant, *Mucuna pruriens* (utilis) which suggested the reason *Mucuna pruriens* seeds are used by the herbalists as anti- depressant, in the treatment of disorders of the male and female reproductive systems, parkinson disease and increase libido in both men and women (Uchegbu and Echeme, 2013). Steroids have been found to be important hormone regulator which possesses oxytocic, anti-inflammatory, antioxidant, anti-asthmatic, bronchodilator and anti-spasmodic properties (McMurry,1998; Solomons,1998 ; Okwu and Ohenhen, 2012). However the presence of this compound in *Spondis mombin* indicates that the plant has biological and physiological activities. It suggests that the plant can be used as an anti- depressant and in the treatment of parkinson disease. The presence of steroid in *Spondis mombin* also suggests that the plant can be used as a herb in the treatment of asthma, bronchitis, bile regulation, hormone regulation, lactation stimulation and birth control in phytomedicine. Thus this authenticates the use of this plant in the treatment of diseases in Nigeria.

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Table 1: Infra – red analysis of compound [1]

IR Absorption (cm ¹)	Functional Group	Compound Type
2910	CH ₂	Aliphatic
1700	C = O	Carbonyl
1600	C = C	Aromatic
1250	C = O	Ether

Table 2: ¹H NMR and ¹³C NMR chemical shifts of compound [1]

Position	δ C		δ H		Proton
	Chemical Shift (δ)	Carbon	Chemical Shifts (δ)	Multiplicity	
1	116.34	CH	5.034	1Hd	CH
2	117.42	CH	5.186	1Hd	CH
3	119.95	C	-	-	-
4	121.59	C	-	-	-
5	123.38	C	-	-	-
6	18.00	CH ₂	4.012	2Hm	CH ₂
7	18.16	CH ₂	4.053	2Hm	CH ₂
8	25.37	CH	4.874	1Hm	CH
9	26.07	CH	4.893	1Ht	CH ₂
10	124.00	C	-	-	-
11	19.53	CH ₂	4.392	2Hm	CH ₂
12	21.56	CH ₂	4.358	2Ht	CH ₂
13	29.03	C	-	-	-
14	33.00	C	-	-	-
15	22.78	CH ₂	4.392	2Hm	CH ₂
16	23.95	CH ₂	4.394	2Hq	CH ₂
17	33.39	CH	4.919	1Hs	CH ₂
18	10.26	CH ₃	0.400	3Hs	CH ₃
19	10.96	CH ₃	0.413	3Hs	CH ₃
20	11.20	CH ₃	0.495	3Hs	CH ₃
1'	126.09	C	-	-	-
2'	129.00	CH	6.096	1Hs	CH

3'	129.21	C	-	-	-
4'	129.89	C	-	-	-
5'	130.94	CH	6.083	1Hd	CH
6'	131.42	CH	6.117	1Hd	CH
7'	67.85	OCH ₃	3.879	3Hs	OCH ₃
8'	73.50	OCH ₃	3.942	3Hs	OCH ₃
9'	166.75	C = O	-	-	-
1''	24.50	CH ₂	4.498	2Hm	CH ₂
2''	24.50	CH ₂	4.720	2Hm	CH ₂
3''	13.24	CH ₃	0.534	3Hs	CH ₃

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

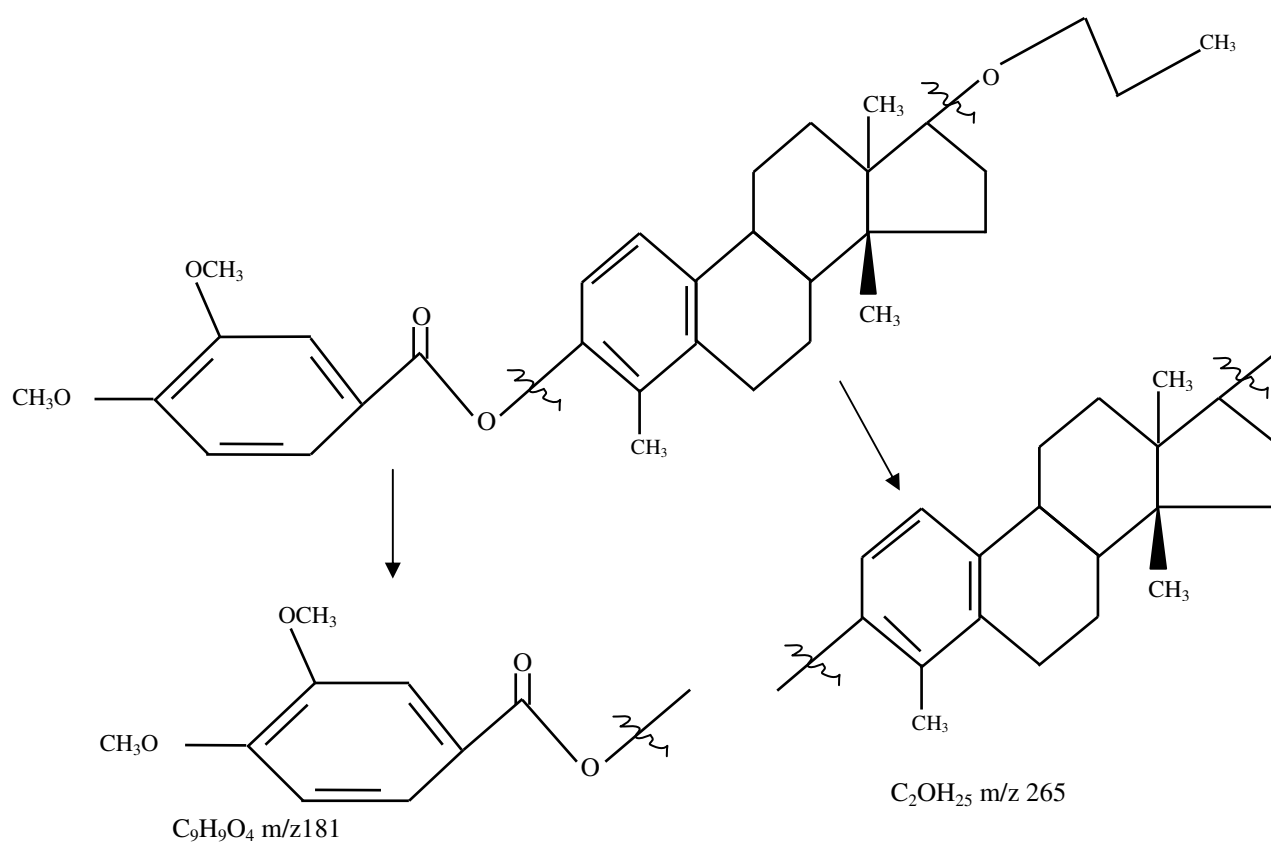


Fig.2: Fragmentation Pattern of Compound [1]

References

- Adepoju, O.T and Oyewole, O.E. (2008). "Nutrient Composition and Acceptability Study of Fortified Jams from *Spondias Mombin* (Hog Plum, *Iyeye* in Yoruba) Fruit Pulp". *Nigerian Journal of Nutritional Science* 29 (02): pp.180–189.
- Ayoka, A.O.; R.O. Akomolafe, O.S. Akinsomisoye, and O.E.Ukponmwan (2008). Medicinal and Economic Value of *Spondias mombin*. *African Journal of Biomedical Research* 11(2) pp.129–136.
- Dubal, D.B, Zhu, H. Yu, J., Rau, S.W., Shughrue, P.J., Merchenthaler, I., Kindy, M.S and Wise, P.M., (2000). "Estrogen receptor α , not β , is a critical link in estradiol-mediated protection against brain injury". *Proc. Natl. Acad. Sci. USA*, 10, 1073.
- Irvine, F.R. (1961). *Woody Plants of Ghana*. Oxford University Press, Great Britain. p. 565-566
- McMurry, J. (1998). *Organic Chemistry*. Books/Cole Publishing Company California. Pp. 1025 – 1031.
- Nworu, C.S, Akah PA, Okoye FB, Toukam DK, Udeh J, Esimone CO (2011)., "The leaf extract of *Spondias mombin* L. displays an anti-inflammatory effect and suppresses inducible formation of tumor necrosis factor- α and nitric oxide (NO)." *J Immunotoxicol.* 8(1):10-6
- Okwu D.E and Ohenhen, O.N (2010). Isolation and characterization of Steroidal Glycosides from the leaves of *Stachytarpheta Jamaicensis* Linn Vahl. *Der Chemica Sinica*, 1 (2): 6-14
- Rodrigues K. F. and Hasse M. (2000): Antimicrobial activities of secondary metabolites produced by endophytic fungi from *Spondias mombin*. *Journal of Basic Microbiology* 40, 261 – 267.
- Rodrigues K. F. and Samuels G. J. (1999): Fungal endophytes of *Spondias mombin* leaves in Brazil. *Journal of Basic Microbiology* 39, 15 – 18.
- Solomons TWG (1998) *Organic Chemistry*. John Wiley and Sons New York Pp. 1053 – 1062.
- Uchegbu and Echeme (2013). Isolation and characterization of Estra – 2'' -en – 17 – ol, 3 yl benzoate from *Mucuna pruriens* (Utilis). 2013. *Journal of Natural Sciences Research*, Vol 3 (11):84 – 87
- Villegas L. F., Fernadz T. D., Maldonado H., Torres R., Zavaleta A., Vaisberg A. J. and Hammond G. B. (1997): Evaluation of wounds healing of selected plants from Peru. *Journal of Ethnopharmacology* 55, 193 – 200.
- Vollhardt KPC, Schore WE (1994) *Organic Chemistry*. WH Freeman and Company. New York Pp. 126 – 128.
- Walf, A.A and Frye, C.A. (2005). Anxiety and anti- depressive behavior produced by Physiological estradiol regimen may be modulated by hypothalamic – pituitary adrenal axis activity. *Neuropsychopharmacology* 30(7): 1288 – 1301.