

Novel Source of Bioactive (+) Pinitol – *Senna hirsuta* Irwin and Barneby

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Abstract

Phytochemical investigation of ethanol extract of *Senna hirsuta* yielded 5-Acetyl-7-hydroxy-2-methylchromone and 1-O-methyl-D-chiro-inositol also known as (+) Pinitol. The structure and relative configuration of these compounds were elucidated on the basis of their spectroscopic data, especially MS and NMR techniques. It is worth mentioning that these compounds are isolated from *Senna hirsuta* for the first time. This plant could be an alternative source of (+) Pinitol, a commercialized anti-diabetic and anti-inflammatory agent.

Key words: *Senna hirsuta*, Phytochemical investigation, Spectroscopic techniques, (+) Pinitol.

Introduction

Senna hirsuta (also called *Cassia hirsuta*) belongs to the family *Fabaceae*. The legume family *Fabaceae* is the third largest family of flowering plants with more than 18,000 described species. *Fabaceae* comprises of three sub-families namely: *Papilionoideae*, *Caesalpinioideae* and *Mimosoideae*. *Senna hirsuta* belongs to the sub-family *Caesalpinioideae*.

Senna is the major genus that possesses about 600 species comprising trees, shrubs, vines and herbs with numerous species growing in the Southeast Asia, Africa, Northern Australia and Latin America [1-2]. *Senna* species have been of keen interest in phytochemical and pharmacological research due to their medicinal values. They are well known in folk medicine to be useful in the treatment of gastro-intestinal disorders like ulcers, jaundice and venereal diseases, arthritis, dermatitis, rheumatism and tuberculosis [3-5]. They have been found to exhibit antimicrobial, anticancer and anti-mutagenic activities [6-9]. The effectiveness of these plants extends to the management

of sickle cell anaemia [10]. Compounds such as fistucacidin, (-) epiafzelechin-3-O-glucoside, procyanidin B, (-) epiafzelechin, sennoside A and B, Kaempferol, oxyanthraquinone, dihydroxyanthraquinone, emodin and chrysophanol have been isolated from some *Senna* species [11-13]. Aqueous and ethanol extracts of the leaves, stem barks and roots of *Senna hirsuta* have been investigated for their phytochemical and antibacterial activities [12]. However, there is no report on the isolation of the chemical constituents. Therefore, this paper reports the isolation and characterization of two compounds from *Senna hirsuta*.

Experimental

Plant Collection and Identification

The leaves of *Senna hirsuta* were collected along Arulogun road, Ojoo, Ibadan in October, 2010. The plant sample was authenticated at the Herbarium of the Forestry Research Institute, Ibadan, and voucher specimen (FHI 109488) of the plant was deposited at the herbarium for further reference.

Plant Preparation and Extraction

The air-dried, ground plant samples (583.7 g) of *S. hirsuta* were soaked in ethanol for 3 days. The extract was concentrated under reduced pressure at 40°C. The crude ethanol extract was partitioned in hexane to obtain a defatted ethanol extract of *S. hirsuta* (25 g).

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The yield of the defatted ethanol extracts of *S. hirsuta* was 4.3%.

General Experimental Procedure

Optical rotations were measured using a Rudolph research Autopo III automatic polarimeter. Melting points obtained on a Fisher-Johns micro melting point apparatus and are uncorrected. The IR spectra data were recorded on a FT-IR spectrometer, Perkin Elmer instruments. High-resolution ESI mass spectra were recorded using the Micromass Autospec instrument. The NMR spectra (^1H , ^{13}C) were determined on DRX-400 spectrometer data (400 MHz for ^1H and 100 MHz for ^{13}C) in deuterodimethylsulphoxide and deuteromethanol. Silica gel Thin Layer Chromatographic (TLC) separations were carried out using precoated plastic sheet (Alltech, 0.25 mm thickness silica gel with fluorescent indicator, 40×80 mm). TLC spots were viewed by exposure to UV light at 254 nm, iodine vapour or phosphomolybdic acid with heating. Glass column of varying sizes (depending on weight of extract) and silica gel (70-230 mesh) were used for open column chromatography. Polyamide adsorbent (Discovery DPA-6S adsorbent, Supleco) was used for tannins removal.

Removal of Tannins

The defatted ethanol extract of *S. hirsuta* (10.0 g) was dissolved in water/ethanol (1:1). The solution was pipetted in column (length = 82 cm, id = 3.0 cm) packed with 150 g of polyamide adsorbent. The columns were preconditioned and eluted with water/ethanol (1:1) solvent mixture. The eluted fractions were collected and made up with equal volume of acetonitrile and left to dry under streams of air in the fume chamber. The tannins-free aqueous-ethanol extract (7 g) was subjected to open column chromatography.

Fractionation

The obtained extract (7.0 g) was pre-adsorbed on silica gel and loaded on column (length = 87 cm, id = 3.0 cm) packed with 210 g silica gel. The column was first eluted with hexane

and increasing concentration of ethyl acetate and methanol. A total of ninety 100 mL fractions were collected. Fractions were spotted on analytical pre-coated TLC plate and similar fractions were pooled resulting in eight combined fractions. Fractions 19-20 eluted with 90% ethyl acetate in methanol gave **1** and fractions 27-28 eluted with 95 % ethyl acetate in methanol afforded **2**.

5-Acetyl-7-hydroxy-2-methylchromone (**1**):

White solid (48.8 mg)

Decomp: 218-220 °C.

R_f: 0.6 (EtoAc-MeOH, 4:1).

IR (MeOH): 3174, 1641, 1584 cm⁻¹.

UV/Vis_{max} (MeOH) nm (log ε): 250(3.42), 291(3.22).

^1H NMR: Table 1

^{13}C NMR: Table 1

HRESIMS m/z [M-1]⁻ calcd 231.0663 for C₁₃H₁₂O₄; found 231.0659.

(+) Pinitol (**2**):

White solid (70.3 mg),

R_f = 0.56 in 4:1 EtOAc: MeOH; mp 185 - 187 °C;

[α]_D²³ = +59.4 (c 1.9 × 10⁻³, MeOH)

IR (MeOH) 3400, 2906 cm⁻¹

^1H NMR: Table 2

^{13}C NMR: Table 2

HRESIMS m/z [M+Na]⁺ calcd 217.0749 for C₇H₁₄O₆Na; found 217.0679.

Results and Discussion

The structure of **1** was determined through analysis of its ^1H NMR, ^{13}C NMR, HMBC, COSY, IR, UV and HRESIMS. The negative ion HRESIMS of **1** showed the molecular ion at m/z 231.0659 [M-H]⁻ which gave molecular formula C₁₃H₁₂O₄ (DBE=8). The unsaturation was accounted for as two fused rings, four olefinic and two carbonyl groups. The proton NMR spectrum displayed two doublets at meta positions to each other with a small coupling constant (J=2.3 Hz) at 6.58 and 6.72 and a singlet peak 5.95. Signals observed at 2.19 and 4.10 ppm are indicative of methyl and methylene protons on a carbonyl carbon while the peak at 2.28 is

characteristic of methyl group attached to an oxygenated olefinic carbon. The proton NMR spectrum was in full agreement with a 5, 7-substituted chromone derivative [13]. The ^{13}C NMR and DEPT spectra revealed the presence of thirteen carbon atoms, seven of which are quaternary, three methine, one methylene and two methyl carbons. Six aromatic carbons resonated between 102.9 – 162.6 (two of which are oxygenated), two olefinic carbons at 111.8 and 165.9, two carbonyl at 179.2 and 205.9, two methyl carbons at 21.1 and 31.0 and methylene carbon at 50.4.

Strong correlations were observed in the HMBC spectrum between H-3 and C-2, 10,

14; H-8 and C-6, 9; H-6 and C- 8, 10, 11; H-13 and C-11, 12. These correlations further established chromone ring. The IR spectrum displayed absorption peak at 1641 cm^{-1} due to the presence of $\text{C}=\text{C}$ - unsaturated carbonyl, peak at 1720 cm^{-1} confirmed the presence of a saturated carbonyl while 1584 cm^{-1} and 3174 cm^{-1} were the vibrational frequency for conjugated carbon-carbon double bond and hydroxyl groups respectively. The spectral data of “1” were in perfect agreement with the published spectral data of 5-Acetyl-7-hydroxyl-2-methylchromone [14] hence, 1 is 5-Acetyl-7-hydroxyl-2-methylchromone.

Table 1: $^1\text{H}^{\text{a}}$ NMR $^{13}\text{C}^{\text{b}}$ NMR and HMBC Assignment of “1” in DMSO

Positions	$^1\text{H}^{\text{a}}$	$^{13}\text{C}^{\text{b}}$	HMBC
2	-	165.9	-
3	5.95(s)	115.4	C- 2, 14
4	-	179.2	-
5	-	139.8	-
6	6.58(d, J = 2.3 Hz)	119.6	C- 8, 11
7	-	162.6	-
8	6.72(d, J = 2.3 Hz)	102.9	C- 6, 7, 9
9	-	160.5	-
10	-	111.8	-
11	2.28(s)	50.4	C- 10
12	-	205.9	-
13	2.19(s)	31.0	C- 11, 12
14	4.10(s)	21.1	C- 3

^a400 MHz; ^b100 MHz

Coupling constants (J in Hz) are given in parentheses

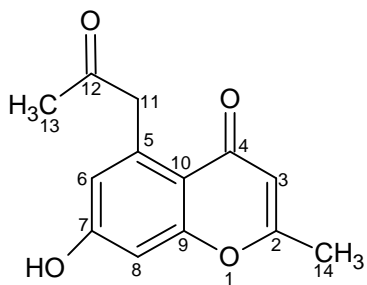


Fig. 1: (Fractions 19-20 eluted with 90% ethyl acetate in methanol).

The ^1H NMR, ^{13}C NMR, IR and HRESIMS spectra were used to determine the structure of 2. The ^{13}C NMR spectrum showed the presence of seven carbon atoms which include six oxygenated methine and one methoxy, carbon atoms between 61.1 to 85.4 ppm.

Signals observed in proton NMR spectrum (3.37- 4.03) were suggestive of sugar protons. The H-1 and H-6 appeared as a 2H doublet at 4.03 (AB system, $J=2.4$ Hz), H-2 and H-5 as double doublets at 3.78 and 3.84 respectively ($J=9.6, 2.4$ Hz), H-3 and H-4 as triplet at 3.37 and 3.67 ($J=9.6$ Hz)

respectively. The homonuclear decoupling experiment showed that protons H-1 and H-6 were directly connected to H-2 and H-5 respectively as the irradiation of the signal centered at 4.03 changed double doublets signal to a doublet. Protons of the methoxy group were singlet at 3.62.

The positive ion HRESIMS depicted a compound with base ion peak at m/z 217.0679 ($M+Na$) which produced molecular formula of $C_7H_{14}O_6Na$. IR spectrum revealed

absorption at 3304 and 3400 cm^{-1} due to the presence of hydroxyl group. Absorptions at 2906 and 2945 cm^{-1} are expected of C-H (sp^3) bond (stretching). The spectral data of **2** were in perfect agreement with that of (+) Pinitol, 1-O-methyl-D-chiro-inositol. (+) Pinitol is known for its antidiabetic, anti-inflammatory and feeding stimulant activities [16-18].

Table 2: $^1H^a$ NMR and $^{13}C^b$ NMR Assignment of **2** in D_2O

Positions	$^1H^a$ (mult, J)	$^{13}C^b$
1	4.03, d (2.4 Hz)	85.4
2	3.78, dd (9.6, 2.4 Hz)	72.5
3	3.37, t (9.6 Hz)	74.0
4	3.67, t (9.6 Hz)	71.8
5	3.84, dd (9.6, 2.4 Hz)	74.1
6	4.03,d (2.4 Hz)	73.5
1'	3.62, s	61.1

^a400 MHz; ^b100 MHz

Coupling constants (J in Hz) are given in parentheses

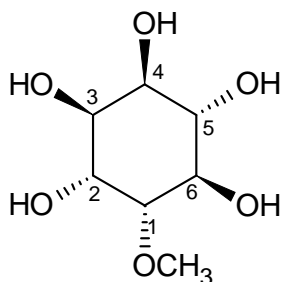


Fig. 2: (Fractions 27-28 eluted with 95% ethyl acetate in methanol).

Conclusion

Two compounds were isolated from *S. hirsuta* namely: 5-Acetyl-7-hydroxyl-2-methylchromone and (+) Pinitol for the first time.

Acknowledgment

OOO is grateful to MacArthur Foundation Grant, University of Ibadan, Nigeria for sponsoring her visit to University of Iowa, Iowa-city, U.S.A for part of her Ph.D benchwork.

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