

ISOLATION AND CHARACTERIZATION OF A NEW COMPOUND (6-C- β -D-GLUCOPYRANOSYL RETUSIN) FROM THE BARK OF *Pterocarpus Marsupium*

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ABSTRACT

Natural products are a major source of active ingredients having medicinal properties and also a source of lead compounds that can be used for synthesizing drugs. *Pterocarpus marsupium* is one of the most important plants which have many pharmacologically active ingredients. Extraction, Isolation, purification and identification of two compounds have been carried out from the bark of the Indian medicinal plant "*Pterocarpus marsupium*." The ethyl acetate extract was concentrated and chromatographed on a silica gel column with different solvents. The isolated compounds are 5, 7, 4'-trihydroxy flavanone and 6-C- β -D-glucopyranosyl retusin isoflavone, which has been isolated for the first time. The structural formula of these isolated, purified compounds has been confirmed by UV, ¹H NMR, MS, ¹³C NMR, and IR. No single spectroscopic study can be used for the complete characterization of flavonoids.

Keywords: Acetone, Acetonitrile, 6-C- β -D-glucopyranosyl retusin, Flavone and Isoflavone

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INTRODUCTION

The chemical investigation of the bark of *Pterocarpus marsupium* includes isolation, purification and structural elucidation of different chemical constituents. This research work is focused on the characterization of polyphenol compounds e.g. Flavanone and isoflavone from *Pterocarpus marsupium* Roxb. (Fabaceae), popularly known as Malabar Kino Tree.^{1,2} It is known for its medicinal bark and timber. The plant material is used for the treatment of inflammations headache and also as anti-helminthic, aphrodisiac, antipyretic, alexiteric agent.³

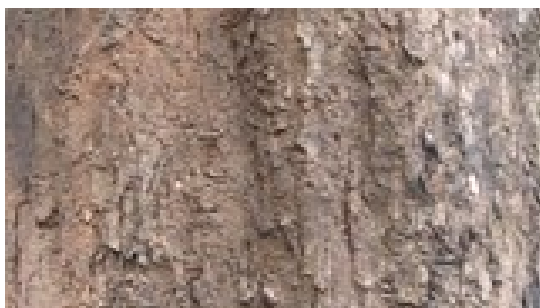


Fig.-1: Bark of *Pterocarpus marsupium*

Various parts of plants have been used in India since ancient times to treat many health problems. Due to its well-known pharmacological activities, efforts are being made to blend modern techniques eg. using chitosan nanoparticles to deliver active constituents of P marsupium more effectively to various parts of the body.⁴

Flavonoids are known as a large subgroup of the phenolic class of plant specialized metabolites. They are widely distributed throughout the plant kingdom⁵. The basic flavone skeleton that forms all flavonoids is

a 15-carbon phenylpropanoid core, which is arranged into two aromatic rings (A and B) linked by a heterocyclic pyran ring C (C6-C3-C6 Skelton), Fig.-2.⁶

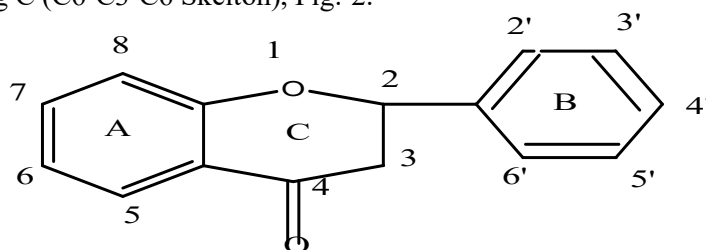


Fig.-2: Molecular Structure of the Flavone Backbone

Flavonoids are divided into several groups, like flavones, isoflavones, flavanones, flavonols, anthocyanins and 3-deoxy flavonoids. They contain various medicinal properties like anti-diabetic⁷ anti-inflammatory⁸, anti-cancer⁹ and anti-oxidant. PTKs (protein tyrosine kinase) are known to be highly expressed in breast, prostate, and stomach cancers and several brain regions, such as hippocampus. High doses of isoflavones suppress the action of PTK (protein tyrosine kinase), which is highly expressed in breast, prostate and stomach cancers, resulting in reducing the carcinogenesis and neuronal degeneration in these tissues.¹⁰ Molecular docking studies of Antidepressant flavonoids show the possibility of designing lead compounds for developing new drugs.¹¹

EXPERIMENTAL

The plant material was collected from the forest of M.P. (central Province of India) and was identified by the botanical survey of India, Central zone Allahabad. The Bark shaving 1.5 Kg of *P. marsupium* was extracted in ethanol (4 x 2 liters) at reflux temperature. The ethanol extract was concentrated under pressure and the concentrated extract (150 ml) was subject to continuous liquid-liquid extraction employing petroleum ether, hexane, benzene, ethyl acetate and acetone as solvents. The dark brown colored ethyl acetate extract was concentrated and chromatographed on a silica gel column with different solvents and their mixtures to yield the following compounds:

1. Compound A (Benzene: Methanol, 7.5 : 1.5, V/V)
2. Compound B (Benzene: Methanol, 8.5 : 1.5, V/V)

The purity of the compound was checked by PC and TLC. Analytical TLC was carried out on silica gel G Merck 7731 with solvent systems unless and otherwise specified as follows:

1. B: A: W :: 4: 1: 5, V/V
2. CHCl₃: MeOH :: 9: 1, V/V
3. EtOH: Pyridine: Water :: 4: 1: 5, V/V
4. Benzene : Methanol :: 8: 2, V/V

Column chromatography was done on silica gel 60 Merck 7734. UV spectra were recorded in EtOH on Hitachi 2205 spectrophotometer. IR spectra were run as KBr disk on Perkin-Elmer 577. ¹H NMR spectra were recorded at 90 MHz in CDCl₃ solution on varian CFT 20 unless and otherwise specified using TMS as internal Standard. ¹³C NMR were recorded at 25.05 MHz in C₅D₅N solution with TMS employing the FT mode.

RESULTS AND DISCUSSION

Compound A

Its solid crystalline compound, melting point 228.2 °C, soluble in methanol but sparingly soluble in benzene analyzed for C₁₅H₁₂O₅.

A negative test with Molisch¹² and AHP reagent¹³ indicated it to be non-glycosidic.

The aglycone gave brown-violet ferric reaction¹⁴ confirming phenolic OH and red solution both with magnesium / hydrochloric acid¹⁵ and sodium amalgam¹⁶ when followed by acidification.

The aglycone gave characteristic color reactions flavanones¹⁷⁻¹⁹ indicating the presence of a flavanone nucleus supported by its absorption maxima of high intensity at 288 nm (Band II) and 325 nm (Band I, sh), characteristic of flavanones which absorb in the region of 270-395 nm.²⁰

The further structure was confirmed based on color tests and spectral studies of aglycone.

1. It gave bright yellow color with boric acid in the presence of citric acid²¹ and yellow color with zirconium¹⁷oxychloride, indicating the presence of a free OH at position-5 of ring-A.
2. Pink color with vanillin / hydrochloric acid indicated the presence of 1, 3 dihydroxy system in the aglycone.

On acetylation, the aglycone formed triacetate. The above reaction confirmed the presence of three hydroxyl groups in the aglycone. No signals for other groups were observed in the NMR spectrum. The aglycone can, therefore, be represented as (Fig.-3):

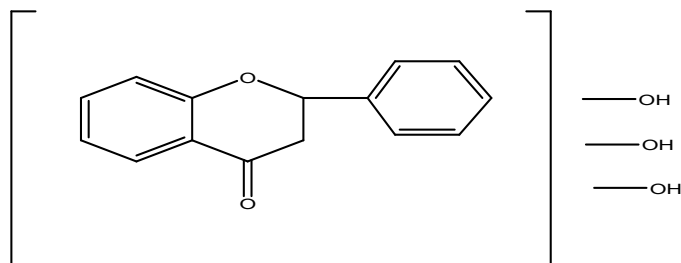


Fig.-3: Aglycone

The elemental analysis of the aglycone corresponded with the above structure. Only the relative positions of hydroxyl group remained to be ascertained. This was done based on a combination of UV Shifts reagents.^{20,22,23} Complete characterization was done with the support of spectral studies of IR, NMR²⁴, Mass spectrum techniques^{25,26} and ¹³C NMR.^{27,28}

In the presence of methanolic NaOMe the absorption maximum underwent a pronounced bathochromic shift of 33 nm, which fixed the positions of free hydroxyl groups at C-5 and -7 of ring-A.

The presence of a hydroxyl group at position-7 was confirmed by bathochromic shift of 34 nm with fused sodium acetate. Bathochromic shift of 23 nm with AlCl₃ indicating the presence of free hydroxyl group at position-5 or orthodihydroxy group in ring-A. No change in position of band II. On addition of hydrochloric acid to aluminum chloride reagent confirmed the presence of free hydroxyl at position-5 of ring-A and the absence of an orthodihydroxy system in the aglycone supported by no shift with sodium acetate / boric acid reagent.

NMR spectrum of aglycone showed quartets at δ 5.2 and 2.6-2.8 characteristic for flavanone Skelton. The presence of free protons at position-6 and 8 were confirmed by meta-coupled doublet at δ 5.9 and 6.0, respectively. The NMR spectrum also confirmed A₂B₂ system in ring-B when it showed ortho coupled doublet at δ 6.7 and 7.1 for protons at positions 3' (5') and 2' (6') respectively. It also confirmed that only position 4' of ring-B is substituted.

The above conclusion was supported by mp and co-chromatography of p-hydroxy benzoic acid, one of the alkaline degradation products of the aglycone, with an authentic sample. The aglycone, can, therefore, be represented as 5, 7, 4'-trihydroxy flavanone (Fig.-4).

This aglycone has been reported earlier from other plants and is known as Naringenin(NAR).Recent studies showing the potential anti-inflammatory role of Naringenin in COVID-19 associated risk factors and SARS-CoV-2 infection have once again proved the medicinal importance of polyphenols.^{29,30}

Chromatography

TLC of the compound on silica gel 'G' plates gave R_f 0.89 (solvent system: n-butanol: Acetic Acid: Water: 4: 1: 5, v/v: upper layer, spray: NaBH₄).

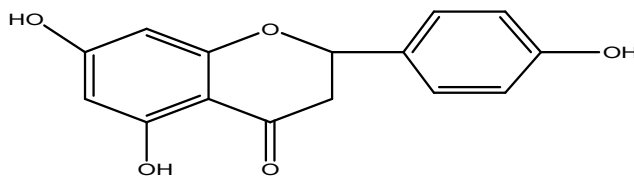


Fig.-4:5, 7, 4'-trihydroxy flavanone

Spectral Studies**UV Spectrum**

UV spectrum data of compound A is given in Table-1.

Table-1: Values of Band II, Band I and Shift of Compound 'A'

S. No.	Solvent / Shift Reagent	λ max' nm		Shift, nm
		Band II	Band I	
Aglcone				
1	Methanol	288	325 (sh)	--
2	MeOH / NaOMe		321	33 (band II)
3	MeOH / NaOAc		322	34 (band II)
4	MeOH / AlCl ₃		311	23 (band II)
5	MeOH / AlCl ₃ -HCl		311	23 (band II)
6	MeOH / NaOAc-H ₃ BO ₃		288	Nil

NMR Spectrum (90 MHz, CCl₄ in δ)

5.9 (d, $J = 2.5$ Hz, 1H, C-6), 6.0 (d, $J = 2.5$ Hz, 1H, C-8), 5.2 (quartets overlapping, $J_{cis} = 5.1$ Hz, and $J_{trans} = 11$ Hz, C-2), 2.8 (quartet, $J = 17$ Hz, C-3), 6.7 (d, $J = 9$ Hz, 2H, C-2', 6') 7.1 (d, $J = 9$ Hz, 2H, C-3', 5').

¹³C NMR

115.85912, 115.85912, 43.39357, 165.39999, 103.15, 96.08, 164.8, 130.66333, 97.77164, 128.59833, 128.59833, 164.60408, 78.595, 197.7, 157.82517 ppm.

MS(m / z)

517, 502, 486, 470, 352, 209, 192, 177, 161, 151, 133, 119, 103, 99, 89.

IR (cm⁻¹)

3250, 3100, 2900, 2850, 2700, 2550, 1600, 1650, 1500, 1450, 1400, 1300, 1250, 1150, 1050, 1800, 550.

Compound B

Melting point 196°C was analyzed for C₂₂H₂₂O₁₀. It gave all the color reactions characteristics of isoflavone.²⁶ Chromatography properties of the compound indicated it to be glycoside. However, neither sugar nor aglycone was obtained on acid hydrolysis. The compound showed UV spectrum and diagnostic shifts typical for an isoflavone retusin.

The ¹H NMR spectrum and ¹³C NMR spectrum of the compound showed signals like retusin and also those attributed to a hexosyl moiety at δ 3.35 ppm for sugar protons and its anomeric proton at δ 4.59 ppm (d, $J = 9$ Hz). The large coupling constant for anomeric protons of sugar is characteristic for C-C- β -linked glucose.²⁷ The downfield signal that δ 7.7 ppm integrated for one proton at C-5 due to deshielding by keto group at position-4.

These observations confirmed that the sugar was attached to isoflavone nucleus by a C-C linkage.

The C-6 linkage of hexose sugar was evidenced by high intensity of (M⁺-31) ion in the ms of glycoside²⁷ and by the low field position at (δ 124.1 ppm) of C-6 in the ¹³C NMR of Compound E.²⁶

In the ¹³C NMR spectrum of C-glycoside, the chemical shifts of the sugar carbons were found to match closely with those reported for - β -D-glucopyranoside moiety in methyl glycoside of β -D-glucopyranoside.²⁶

The strong peaks at m / z 298, 297, 168 and 132 confirmed that compound B is 6-C- β -D-glucopyranosyl retusin (Fig.-5).

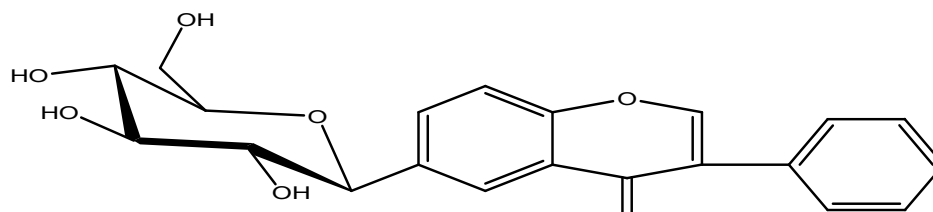


Fig.-5: 6-C- β -D-glucopyranosyl retusin

This retusin was reported first time in nature. It has not been reported earlier from any other plant sources.

Chromatography

TLC of the compound on silica gel 'G' plates gave R_f 0.41 (solvent system: n-butanol: Acetic Acid: Water: 6: 2: 2, v/v: upper layer, spray: NaBH_4).

Spectral Studies, λ_{max} (MeOH) nm

261, 308 (sh); +NaOMe 278, 317; + AlCl_3 281, 322 (sh), 261, 308 (sh); +NaOAc 277, 314 (sh), H_3BO_3 : 269, 310.

^1H NMR (90 MHz, DMSO, $-\text{d}_6$, Values in δ)

7.9 (s, 1H, C-5), 8.1 (s, 1H, C-2), 4.00 (s, 3H, OCH_3), 7.47 (d, $J = 9\text{Hz}$, 2H, C-2', 6'), 3.35 (m, 6H, glucosyl), 4.59 (1H, d, $J = 9\text{Hz}$, H-1 of glucose), 6.96 (d, $J = 9\text{ Hz}$, 2H, C-3'5').

^{13}C NMR

120.35, 129.98, 62.21111, 71.51667, 112.25646, 128.46333, 128.46333, 125.25502, 153.3735, 128.57023, 128.57023, 72.575, 127.69405, 128.92353, 79.45, 155.725, 175.3, 78.84968, 137.39053, 124.069, 82.16667 ppm.

ms(m/z)

446, 428, 410, 392, 298, 297, 168, 132.

IR (cm^{-1})

3445, 3030, 2890, 1900, 1675, 1500, 1100, 970, 880

Further study of the new compound (B) will explore its potential for therapeutic activities.

CONCLUSION

Two Flavonoids compounds from the Indian medicinal plant "*Pterocarpus marsupium*" have been isolated and identified as 5, 7, 4'-trihydroxy flavanone and 6-C- β -D-glucopyranosyl retusin. which is being reported for the first time.

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