5-HYDROXY-8, 4’-DIMETHOXYFLAVANONE-7-O-β-D-GLUCOPYRANOSIDE FROM THE FLOWERS OF STRYCHNOS POTATORUM LINN
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ABSTRACT

A flavanone glycoside 5-hydroxy-8,4’ dimethoxyflavanone-7-O- β-D-glucopyranoside has been isolated from the ethyl acetate soluble fraction of the ethanol soluble extract of flower of Strychnos potatorum Linn and has been identified by its chemical and spectral analysis.

Key words: Strychnos potatorum Linn, Flower, Flavanone glycoside, 5-hydroxy-8, 4’ dimethoxyflavanone-7-O-β-D-glucopyranoside.

INTRODUCTION

Strychnos potatorum Linn1 belong to natural order strychnaceae and is known as Nirmali in Hindi. It is also known by names like nut tree, tatankottai. Katakam and katakam. It occurs in various region of Deccan, including peninsular region.

The different parts of this plant are used in the treatment of lachrymation, dysentery, diabetes and skin infection. The seeds are useful for treating to lachrymation or copious watering of eyes, in dysentery and diabetes, where as the roots are useful in leucoderma and the flowers are useful for diabetes mellitus and as folklore medicine.

In controls cracks, ulcers, boils and pimples of human being and also provides nutritions and soothing effect on the circulatory system.

EXPERIMENTAL

Isolation of the glycoside AS-1

The ethyl acetate soluble fraction of the concentrated ethanolic extract of the flowers
of *strychnos potatorum* Linn when worked up by column chromatography yielded a compound (AS-1).

It was found to be homogenous on TLC examination using benzene: methanol: water (7 : 2 : 8) as solvent system and iodine vapors as visualizing agent.

**Study of the glycoside AS-1**

The glycosidic compound (AS-1) (0.076%), analysed for molecular formula C_{23}H_{26}O_{11}. It was found be homogenous on TLC examination. It gave positive response to Shinoda test, Molisch test and specific colour reaction for flavanone glycoside changing from brown to yellow in UV on addition of NH3.

The structure of the glycoside (AS-1) was established by it’s acid hydrolysis with 10% HCl, when it gave an aglycone AS-1(A) and D-glucose as sugar moiety.

**Acid hydrolysis of glycoside AS-1**

The glycoside (AS-1) on its acid hydrolysis with 10% HCl, yielded an aglycone AS-1(A) as a solid residue and glucose as sugar moiety, in the filtrate. The aglycone AS-1(A) and sugar were separated by filtration and studied separately for established the structure of AS-1.

**Study of aglycone AS-1(A)**

TLC examination of the aglycone showed its homogenous nature using benzene-methanol (2 : 1) as the solvent system. The aglycone AS-1(A) crystallizes from methanol to give light yellow coloured compound. It analysed for molecular formula C_{17}H_{16}O_{6}, had m.p. 171-172°C and mass (M)^+ 316 (CIMS). AS-1(A) gave to all the characteristic colour reactions of flavonoids.

**UV-spectrum of aglycone AS-1(A)**

The aglycone in methanol exhibited wave length of maxium absorbance at 294 and 332 nm. It exhibited a bathochromic shift of 30 nm on addition of AlCl_3 and therefore, confirmed it to be a flavanone compound having a free hydroxyl group at C-5.

**Presence of hydroxyl group (-OH group) in AS-1(A)**

A band at 3260 cm\(^{-1}\) in the IR spectrum of the compound AS-1(A) suggested the presence of free –OH group(s) in it. It’s UV-spectrum in presence of NaOMe and NaOAc showed shifts characteristic of 5, 7-dihydroxy flavanone. AS-1(A) on acetylation with
(Ac₂O/pyridine), gave diacetyl derivative having molecular formula C₂₁H₂₀O₈, m.p. 102-103°C, (M)⁺ 400. The percentage of acetyl group (29.89%) was determined by Weisenberger⁵ method as given by Belcher and Godbert⁶, which confirmed the presence of two –OH groups in AS-1(A).

**Presence of methoxyl group in AS-1**

The glycoside AS-1 gave IR band at 2868 cm⁻¹ indicating the presence of –OCH₃ group(s) in the aglycone AS-1(A). Estimation of OCH₃ group (15.02%) by Ziesel’s⁷ method indicated the presence of two methoxy group in AS-1(A). As such on the basis of above facts, a tentative structure to the flavanone AS-1(A) was assigned as (I), which is -

![Tentative structure](image)

**Alkaline degradation of AS-1(A)**

Aglycone AS-1(A) was fused with 50% ethanolic KOH, when it yielded two compounds P and Q, which were separated by column chromatography and were identified as- p-methoxy phloroglucinol (P), molecular formula C₇H₈O₄, m.p. 184°C and M⁺ 156 (CIMS) and p-methoxycinnamic acid (Q), molecular formula C₁₀H₁₁O₃, m.p. 174°C and M⁺ 179 (CIMS). The degradation products of KOH were identified by m.m.p., Co-PC and Co-TLC with authentic samples.

![Fusion reaction](image)

The aglycone AS-1(A) showed bathochromic shift of 47 nm in band I with AlCl₃ confirming the presence of –OH groups at C-5 and of 15 nm in band II with NaOAc, which confirmed the position of another –OH group at C-7⁸. The formation of p-methoxy phloroglucinol as one of the degradation product confirmed the position of –OH group at C-5.
and C-7 in the aglycone, AS-1(A). Thus, a tentative structure to the aglycone AS-1 (A) was assigned as –

\[
\begin{array}{c}
\text{OH} \\
\text{O} \\
\text{O} \\
\text{OH} \\
\text{AS-1 (A)}
\end{array}
\]

2 -OCH₃ groups

**Position of methoxyl group (-OCH₃ GR) in AS-1(A)**

The identification of p-methoxy cinnamic acid on alkali fusion as one product of the aglycone AS-1(A), indicated the presence of one -OCH₃ group at C-4 in ring-B of AS-1(A) and the second –OCH₃ group at C-8. Two singlets, each of three protons intensity, which appeared at δ 3.88 and δ 3.87 in the ¹H NMR of AS-1(A) thus further confirmed the position of OCH₃ group at C-4; and C-8. Finally on keeping all the above facts together, AS-1(A) was assigned the structure is –

\[
\begin{array}{c}
\text{OH} \\
\text{O} \\
\text{OCH₃} \\
\text{OCH₃} \\
\text{AS-1 (A)}
\end{array}
\]

**Study of sugar moiety**

The aqueous hydrolysate obtained as a result of hydrolysis of the glycoside AS-1, was neutralized with BaCO₃ and BaSO₄ was filtered off. The filtrate was concentrated under reduced presume, to get a golden yellow coloured semisolid residue. This residue was examined by PC and revealed the presence of D-glucose (Rₜ = 0.18), as confirmed by Co-PC and Co-TLC with authentic sample of D-glucose.

**Quantitative estimation of sugar**

The quantitative estimation of sugar was done by the procedure of Mishra and Rao Mohan, which showed that glycoside contained aglycone and glucose sugar in an equimolar ratio of one molecule each in AS-1.
Periodate oxidation of the glycoside AS-1

The glycoside AS-1 on treatment with sodium metaperiodate consumed 2.04 moles of periodate and liberated 1.05 moles of formic acid, revealing the presence of one molecule of D-glucose attached to aglycone and also indicated that the sugar was in the pyranose form in the glycoside AS-1\textsuperscript{12}.

Position of attachment of sugar to the aglycone AS-1(A) in AS-1

Glucose was attached with the aglycone AS-1(A) at C-7 position because the aglycone gave red colour with p-toluene sulphonic acid, which is characteristic reaction for C-7 –OH group.

Specifically a bathochromic shift with AlCl\textsubscript{3} and CH\textsubscript{3} COONa indicated the presence of OH groups at C-5 and C-7 in the aglycone. The absences of a band III peak with the NaOCH\textsubscript{3} and the lack of a band II peak with CH\textsubscript{3} COONa indicated the presence of C-7 –OH group in the aglycone and its absence in the glycoside, AS-1, thereby confirming that C-7 OH of AS-1 was involved in glycosidic linkage in it.

RESULTS AND DISCUSSION

The acid hydrolysis of permethylated glucoside obtained by Kuhn’s procedure gave 2,3,4,6-tetra-O-methyl-\(\beta\)-D-Glucose (confirmed by Co-TLC) confirming that C\textsubscript{1}-OH of glucose was involved in glycosylation in the glucoside. The glucoside (AS-1) on enzymatic hydrolysis\textsuperscript{13} with \(\beta\)-glucosidase released the glucose sugar and aglycone, AS-1(A) thereby, indicating \(\beta\)- linkage between aglycone and glucose. Thus, the glucoside was assigned the structure as 5-hydroxy-8,4’-dimethoxy flavanone-7-O-\(\beta\)-D-glucopyranoside (II) by keeping together all the above facts and considering all reactions as –
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