



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' dimethoxy-flavanone-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

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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 to be homogenous on TLC examination. It gave positive response to Shinoda test, Molisch test and specific colour reaction for flavanone glycoside²⁻⁴ changing from brown to yellow in UV on addition of NH_3 .

The structure of the glycoside (**AS-1**) was established by its 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 maximum 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.

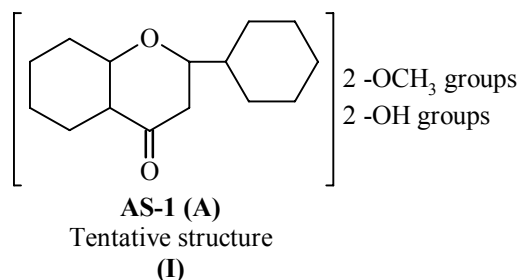
Presence of hydroxyl group (-OH group) in AS-1(A)

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. Its 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)**.

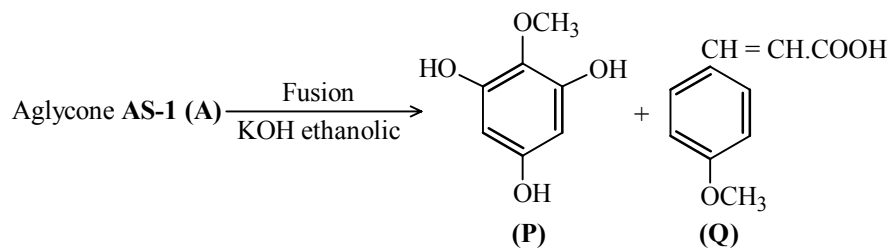
Presence of methoxyl group in AS-1

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 -



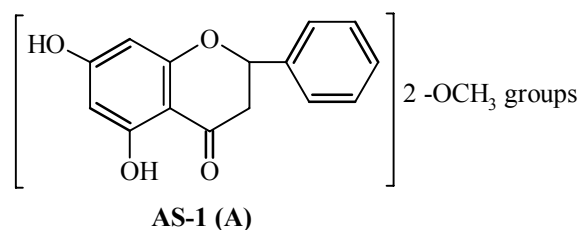
Alkaline degradation of AS-1(A)

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.



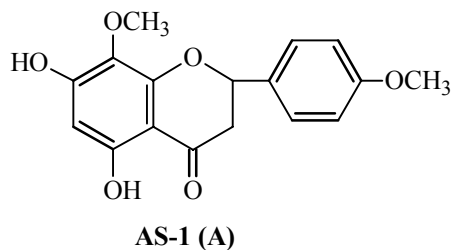
The aglycone **AS-1(A)** showed bathochromic shift of 47 nm in band I with AlCl₃ confirming the presence of –OH groups at C-5 and of 15 nm in band II with NaOAc, which confirmed the position of another –OH group at C-7⁸. The formation of p-methoxy phloroglucinol as one of the degradation product confirmed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



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 presence⁹ 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 –



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 pressure, to get a golden yellow coloured semisolid residue. This residue was examined by PC and revealed the presence of D-glucose (R_f = 0.18), as confirmed by Co-PC and Co-TLC¹⁰ with authentic sample of D-glucose.

Quantitative estimation of sugar

The quantitative estimation of sugar was done by the procedure of Mishra and Rao Mohan¹¹, 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**¹².

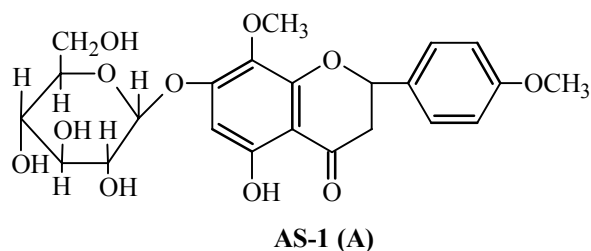
Position of attachment of sugar to the aglycone AS-1(A) in AS-1

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 AlCl₃ and CH₃ 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 NaOCH₃ and the lack of a band II peak with CH₃ 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-β-D-Glucose (confirmed by Co-TLC) confirming that C₁-OH of glucose was involved in glycosylation in the glucoside. The glucoside (**AS-1**) on enzymatic hydrolysis¹³ with β-glucosidase released the glucose sugar and aglycone, **AS-1(A)** thereby, indicating β- linkage between aglycone and glucose. Thus, the glucoside was assigned the structure as 5-hydroxy-8,4'-dimethoxy flavanone-7-O-β-D-glucopyranoside (II) by keeping together all the above facts and considering all reactions as –



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