



5,7,3'4'-TETRAHYDROXY FLAVONE -3-O-β-D-GALACTOPYRANOSIDE FROM THE FLOWERS OF *STRYCHNOS POTATORUM LINN*

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ABSTRACT

The methanol soluble part of the concentrated ethanolic extract of the flowers of *Strychnos potatorum Linn* gave a flavonoidal glycoside AS-2 having molecular formula $C_{21}H_{20}O_{12}$, m.p. 262-263⁰C and $(\alpha)_D^{16} \div 18.6^0$ (in $CHCl_3$), $M^+ = 464$ (By mass spectroscopy). By chemical reactions and spectral analysis, it was identified as; 5,7,3'4'-tetrahydroxy flavone -3-O-β-D-Galactopyranoside.

Key words: *Strychnos potatorum Linn*, Flowers-flavonoidal glycoside 5,7,3'4'-tetrahydroxy flavone-3-O-β-D-galactopyranoside.

INTRODUCTION

The plant *Strychnos potatorum Linn*¹ is known as Nirmali in Hindi and also known as; Tetankottai Katakamu and katakam. It occurs in various region of Deccan, along with Madhya Bharat region of India. This plant is used as a local application in the treatment of lachrymation or copious watering of eyes, in dysentery and diabetes diseases.

EXPERIMENTAL

Isolation of the flavonoidal glycoside AS-2

The methanol soluble part of the concentrated ethanolic extract of the flowers of *Strychnos potatorum Linn*. On removal of the solvent, it gave an amorphous mass which on crystallization from chloroform gave, a crystalline substance which responded a positive test of flavonoidal glycoside and showed single spot on TLC examination thus showing its homogeneous nature.

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The study of the flavonoidal glycoside AS-2

The flavonoidal glycoside analysed for molecular formula $C_{21}H_{20}O_{12}$, m.p. 262-263⁰C and $(\infty)_D^{16} \div 18.6^0$ (in $CHCl_3$), $M^+ = 464$ (By mass spectroscopy).

AS-2 gave positive Molish test and also responded to all characteristic colour reactions of flavonoidal glycoside.

UV spectrum of the glycoside

The wavelengths of maximum absorbance in the UV spectrum of the glycoside were as follows:

MeOH: λ_{max} 257, 279, 300 nm, ($AlCl_3$) 275, 295, 331, 438 nm, ($AlCl_3 + HCl$) 268, 299, 366, 405 nm, ($NaOAc$) 275, 324, 380 nm, ($NaOAc/H_3BO_3$) 262, 298, 377 nm.

IR spectrum of the glycoside

The significant bands obtained in the IR spectrum of the glycoside AS-2 were as follows and the structural units inferred with the help of available literature²⁻³:

3330 cm^{-1} (OH), 2905 cm^{-1} (-C-H stretching), 1678 cm^{-1} (> C=O -Chelated), 1600, 1535, 1450 cm^{-1} (ring system, -CH₃), 1280 cm^{-1} (C-O-C vibrations), 780 cm^{-1} (C-C-H in CH₂)⁴.

A characteristic band at ν_{KBr} 3330 cm^{-1} in the IR spectrum of the glycoside showed the presence of -OH group (s). The glycoside was acetylated with Ac_2O /pyridine and the percentage of acetyl group (s) in the acetylated product (m.p. 340⁰-42⁰C, molecular formula $C_{47}H_{50}O_{26}$ was estimated by the method Wiesenberger as described by Belcher and Godbert (48.3%), which indicated the presence of 10 OH-groups in the glycoside.

The position of the -OH groups and the structure of the glycoside was established by its acidic hydrolysis and studying the products of hydrolysis separately.

The glycoside was therefore hydrolysed with 2 N H_2SO_4 , when sugar (s) and aglycone m.p. 312-14⁰C were obtained.

Study of the aglycone

The aglycone analysed for molecular formula $C_{15}H_{10}O_7$ m.p. 311-12⁰C, $M^+ = 302$ and it was found to be soluble in ethyl acetate, and acetone. It responded to various characteristic reactions of flavonoids.

The flavonoidal nature of the aglycone was suggested on the basis of following colour reactions.

A greenish colour with ethanolic FeCl_3 , a deep yellow colour with liquid ammonia, showing fluorescence under UV light, a red colour with and hydrochloric acid, a yellowish orange colour with characteristic fluorescence with conc. H_2SO_4 .

UV spectrum of the aglycone

The wave lengths of maximum absorbance in the UV spectrum of the aglycone were at; MeOH: λ_{max} 395, 348 and 295 nm, (NaOMe) 394, 377, 271 nm.

IR Spectrum of the aglycone

The significant peaks obtained in the IR spectrum of the aglycone and the structural units inferred with the help of available literature.

3305 cm^{-1} (-OH), 2894 cm^{-1} (-C-H-stretching), 1686 cm^{-1} (> C = O-Chelated), 1600, 1535 cm^{-1} (ring system), 1280 cm^{-1} (-C-O-C vibrations C-O-H), 765 cm^{-1} (-C-H in CH_2).

OH group (s)

A band at 3305 cm^{-1} in the IR spectrum of the aglycone indicated the presence of OH group (s) in it. The number of -OH group (s) in it were estimated by the acetylation with Ac_2O /pyridine (acetylated product, m.p. 198 $^{\circ}\text{C}$, molecular formula, $\text{C}_{25}\text{H}_{20}\text{O}_{12}$), and percentage of the acetyl group (40.85) in the acetylated product was determined by the method of Wiesenberger as described by Belcher and Godbert, thereby showing the presence of 5-OH groups in the aglycone.

The aglycone on fusion with 50% ethanolic KOH gave two compounds identified as protocatechuic acid (III) (by mmp, Co-TLC, Co-PC) molecular formula $\text{C}_7\text{H}_6\text{O}_4$, m.p. 198-99 $^{\circ}\text{C}$, M^+ = 154 and phloroglucinol (II) (by mmp, Co-TLC and Co-PC) molecular formula $\text{C}_6\text{H}_6\text{O}_3$, m.p. 117-18 $^{\circ}\text{C}$, M^+ = 126.

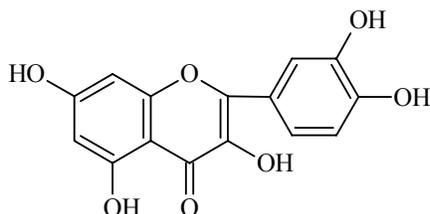
The positions of -OH groups were established by studying the degradation products of aglycone and various UV shifts.

Formation of 4:5 dihydroxy benzoic acid (Protocatechuic acid) on alkaline degradation confirmed the presence of -OH groups at C-3' and C-4' in the aglycone.

Formation of phloroglucinol showed the presence of two -OH groups at C-5 and C-7, respectively.

Remaining fifth –OH group was shown to be present at C-3 on the basis of, a characteristic color reaction with Zn/HCl zirconiumoxy chloride in citric acid, further suggested the presence of OH groups at C-3.

Keeping all facts together, the structure to the aglycone was assigned as; 3,5,7,3',4' pentahydroxy flavone⁵⁻⁶.



Study of the glycoside hydrolysate

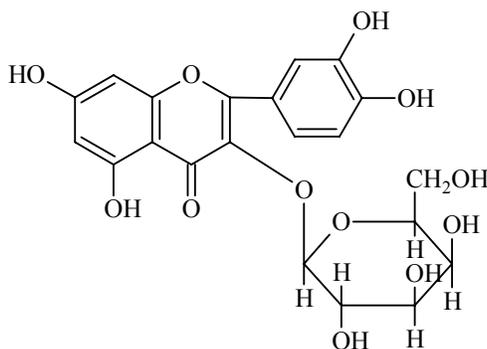
The aqueous hydrolysate obtained by the hydrolysis of the glycoside AS-2 was neutralized with BaCO₃ and barium sulphate was filtered off. The filtrate was concentrated to a yellow mass and was found to give brown colour with aniline hydrogen phthalate and also reduced Fehling's solution and indicated the presence of D-galactose. (by Co-PC and Co-TLC).

Periodate oxidation of the glycoside

The sodium metaperiodate oxidation of the glycoside indicated that the sugar was present in the pyranose form.

RESULTS AND DISCUSSION

Thus, the structure of the glycoside AS-2 was assigned as; 5,7,3',4' – tetrahydroxy flavone -3-O-β-D-galactopyranoside.



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