www.hortflorajournal.com
HortFlora Research Spectrum, 4(1): 89-91 (March 2015) ISSN : 2250-2823

Research Note :

CONFIRMATION OF FLAVONES AND RHAMNOPYRANOSIDE IN *Strychnos potatorum* L. FLOWERS

Manika Barar*, Shilpi Saxena and Arpita Kulshreshtha

Department of Chemistry, C.C.S.University, Meerut.

**E-mail: dr.manikabr@gmail.com*

ABSTRACT : The glycoside on oxidation with sodium meta periodate consumed 3.04 moles of periodate with the liberation of 1.02 moles of formic acid for one mole of the glycoside. It consumed 3.12 moles of periodate and liberated 1.36 moles of formic acid per equivalent for each anhydrohexose sugar unit of the polymer after 60 h. Presence of (1→4)-0-α- type and (1→6)-β- type linkage obtained after methylation was also confirmed by the periodate oxidation results. A flavones glycoside 6,7,3',7' teyramethoxy flavones 5-0-β-D- glucopyranosyl -(1→4)-0-α-L-Rhamnopyranoside has been isolated from the methanolic extract solubile fraction of the rectified spirit soluble extract of the flowers of *Strychnos potatorum* L. and has been identified by its chemical and spectral analysis.

Keywords : *Strychnos potatorum, periodate oxidation and consumption, formic acid, flavones,*

Strychnos potatorum L., belonging to family Loganiaceae, commonly referred as 'clearing nut tree' or Nirmali, is a medium sized glabrous tree having height of 6-18 meters (Peter, 8). It has been reported in *Ayurveda, Siddha,* and *Unani* systems of medicine. Some of the chief constituents found in the plant are Strychine, Diaboline, Isomotiol and Stigmasterol (Chopra et al., 2). Many volatile fragrant compounds have also been analyzed from *Strychnos flowers* (Barar, 1).The seeds of tree are commonly used in traditional medicine as well as purifying water in India. Flowers are white, small, borne on compound inflorescences in the axils of the upper leaves.

Periodate oxidation reaction in the carbohydrate chemistry was first determined by Malaparade (6) and Fluery and Lange (3) had given the periodic acid for the oxidation of glycol group, while Perlin (7) given Lead tetra acetate and periodic acid showed that the glycol group undergo cyclic ester formation with oxidation and reaction is to be a dialdehyde type (Singh,9). Periodate oxidation studies of the seeds polysaccharides of *Nyctanthes arbor- tristis* L., *Pongamia pinnata* L., *Abrus precatorius* L. and *Withania somnifera* Dunal. have already been studied by various scientists for the confirmation of polysaccharide structure which was obtained after methylation studies of polysaccharide (Gringauz, 4; Perlin, 7; Singh, 9).

The methanolic soluble fraction of the concentrated rectified spirit extract of the flowers of *Strychnos potatorum* L. on column chromatography showed the presence of single spot. On crystallization from methanol, light golden yellow coloured needle shaped structure crystals obtained. It gave the tests for glycoside and respond to all the colour reactions of flavones. For periodate consumption, the reaction mixture (5 ml) was taken in a conical flask then added sodium bicarbonate solution (2ml), sodium arsenate solution (0.01 N, 25 ml) and potassium iodide solution (20%, 2ml). Reaction mixture was shaken for 1 hr and added iodine solution (0.01 N,5ml), using starch solution as an indicator. The excess iodine was titrated against sodium thiosulphate solution (0.01N). A blank titration was also carried out in a similar way. The difference between blank and experiment gives the periodate consumption of 3.12 moles of periodate after 60 hrs (Table 1.)

Formic acid liberation was determined by taking the aliquot (5ml) in a conical flask then added ethylene

Table 1: Periodate oxidation of *Strychnos potatorum* flowers' polysaccharide.

Sugar present	Time (hrs)						
	10	20	30	40	50	55	60
Periodate consumption of anhydrohexose sugar unit (moles/ mole)	1.16	1.74	2.12	2.56	2.84	3.12	3.12
Formic acid liberation of anhydrohexose sugar unit(moles/mole)	0.32	0.92	1.08	1.12	1.24	1.32	1.32

Received:03-07-14; Revised:20-12-14; Accepted : 29-01-2015

glycol (10ml) to destroy the excess of periodate ions present in the reaction mixture for 30 min. the formic acid liberation was estimated by titration against sodium hydroxide solution (0.12 N) , using methyl red dye as an indicator. A blank titration was also carried out in a similar way for the estimation of formic acid. It liberated 1.32 moles of formic acid per mole of anhydrohexose sugar units after 60 hrs. Results of periodate consumption and formic liberation of *Strychnos potatorum* L. are given in Table 1.

Study of the glycosides TS-1: The glycoside on elemental analysis gave its molecular formula $C_{18}H_{36}O_{16}$. It gave positive Shinoda and Molish test. It also gave the colour reactions of flavones glycoside. The glycoside showed strong absorption bands at 282 and 335nm. The glycoside on hydrolysis with ethanolis sulphuric acid gave yellow crystalline aglycone TS 1 and D- glucose and L- Rhamnose as a sugar moieties.

Study of aglycone TS-1 : The aglycone on elemental analysis suggested its molecular formula to be $C_{10}H_{18}O_7$, M.P189-190°C. it gave all the characteristics colour tests of flavones, which confirmed the nature of aglycone to be flavanoid.

Position of methoxy group in aglycone TS-1 : Estimation of methoxy group (OCH_3) by Ziesel's Method4 indicated the presence of four methoxy groups in aglycone TS-1.

The position of four methoxy groups were established by the alkaline degradation of the the aglycone which gave two products 2,4 dihydroxy 5,6 dimethoxy benzene (I) and 3,4 dimethoxy benzoic acid (II) thereby indicating the presence of methoxy groups at position 6,7,3',4' in the Aglycone. A survey of literature revealed that the aglycone is identical with 5 hydroxy -6,7,3',4' tetramethoxy flavones (III). The identity of the aglycone was further confirmed by m.p .

Study of sugar moieties : The aqueous hydrolysate obtained by the hydrolysis of the glycoside gave brown colour with aniline hydrogen phthalate and reduced Fehling solution confirming the presence of sugar moieties. On paper chromatographic analysis the aqueous hydrolysate revealed the presence of D-glucose and L – rhamnose.

Quantitative analysis of the sugars : The quantitative analysis of both the sugars in the glycoside was achieved by the procedure of Mishra and Rao and revealed that both the sugars are present in 1:1 ratio. Thus one molecule of the aglycone is attached with one molecule of D- glucose and L-rhamnose in the glycoside.

Periodate oxidation of the glycoside : The glycoside on oxidation with sodium meta periodate consumed 3.04 moles of periodate with the liberation of 1.02 moles of formic acid for one mole of the glycoside. This indicate the presence of a disaccharides and also that both the sugars moieties are in pyranose from which shows that C_1-OH group of glucose is attached to C_4-OH group of rhamnose and also confirm that three vicinal hydrogen atoms are present in glucose and two vicinal hydroxyl groups are present in rhamnose.

Position of attachment of sugar in the aglycone- The sugar linkage in the glycoside was established by comparing the properties of the glycoside with that of the aglycone. The aglycone produced red colour when spotted on filter paper and sprayed with p- toluene sulphonic acid and on subsequent heating whereas the glycoside did not , thereby showing the presence of free hydroxyl group at position C-5.

The hydrolysis of the glycoside with enzyme showed the appearance of one spot corresponding to D- glucose indicating that D glucose was attached to L-rhamnose through α-linkage and which could be hydrolyzed by an enzyme emulsion.Periodate oxidation confirm the presence of glucose and rhamnose. *S.potatorum* Linn seeds yielded a water soluble polysaccharides as D- glucose and Rhamnose in the molar ratio 1:3 moles by Gas, TLC, Column, and Paper chromatographic analysis. For periodate oxidation the purified seeds polysaccharide was oxidized with sodium metaperiodate with the usual manner. It consumed 3.12 moles of periodate and liberate 1.36 moles of formic acid per equivalent for each anhydrohexose sugar unit of the polymer after 60 h. Presence of (1$\rightarrow$4)-0-α- type and (1$\rightarrow$6)-β- type linkage obtained after methylation results are also confirmed by the periodate oxidation results. Polysaccharides were containing free hydroxyl groups resulting in the consumption of Periodate oxidation reaction (Harold, 5)

REFERENCES

1. Barar, M. (2014). Chromatographic analysis of volatile fragrant compounds from flowers of *Strychnos potatorum* L. *HortFlora Res. Spectrum,* **3** (1): 106-107.
2. Chopra, R.N., Nayar, S.L and Chopra, I.C. (1980).*Glossary of Indian Medicinal Plants.* CSIR Publication, page 236, reprinted (1980).

3. Fluery, P. and Lange, J. (1933). *J. Pharma Chem.,* **17** : 107.
4. Gringauz, A. (1992). *Introduction to Medicinal Chemistry*. New Age International Publishers.
5. Harold, H. Strain (1945). *Chromatographic Adsorption Analysis.* Wiley InterSciene Publication, New York (1945).
6. Malaparade, L. (1928). Bulletin. Soc. Chem., **143** : 683.
7. Perlin, A. S. 1976). *Advances in Carbohydrate Chem,* **14-B** : 9
8. Peter, P.I (2008). *Herbs and Trees for Wellness*. World Wellness Open University, Revised Edition, Chennai, 2008 , 89-90.
9. Singh, R.B. (2013). Periodate oxidation method used for the confirmation of seeds polysaccharide structure of *Withania somnifera* Dunal plant. *Arch. Appl. Sci. Res.,* **5** (5):8-10.

❑

Citation : Barar M., Saxena S. and Kulshreshtha A. (2015). Confirmation of flavones and rhamnopyranoside *in Strychnos potatorum* L. flowers. *HortFlora Res. Spectrum,* **4**(1) : 89-91.