

# A Methoxy Flavone Glycoside from the Seeds of Citrus Reticulata Blanco

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**Abstract:** A novel flavone glycoside was isolated from the seeds of *Citrus reticulata* Blanco. Its structure has been determined by spectroscopic and degradative methods as 5, 6 – dihydroxy 7, 8, 3' – trimethoxy flavone -4' – O –  $\beta$  – D – xylofuranosyl (1 $\rightarrow$ 4) – O –  $\beta$  – D – glucopyranoside.

**Keywords:** *Citrus reticulata* Blanco, a methoxy flavone glycoside – 5, 6-dihydroxy 7, 8, 3'-trimethoxy flavone - 4' – o –  $\beta$  – D – xylofuranosyl (1 $\rightarrow$ 4) – O –  $\beta$  – D glucopyrano – side.

## I. INTRODUCTION

Citrus (*Citrus reticulata* Blanco) is one of the most commonly consumed and widely distributed fruit in the world, which is possessing extensive bioactivities. Present study aimed to fully understand the flavonoids compositions, antioxidant capacities and in vitro anticancer abilities of different citrus resources.[1] The present paper deals with the isolation and identification of a methoxy flavone glycoside [1] from the seeds of *Citrus reticulata* Blanco.

## II. RESULTS AND DISCUSSION

The EtOAc soluble part of the ethanolic extract obtained from powdered seeds of plant showed two spots on TLC examination, which was subjected to column chromatography over Si-gel. Elution with ---CHCl<sub>3</sub> : MeOH (5 : 2) afforded a yellow crystalline substance [1] which responded Molisch test and all positive tests for flavonoidal glycoside<sup>2,3</sup>, molecular formula C<sub>29</sub> H<sub>34</sub> O<sub>17</sub>, (Found C, 52.20%; H, 5.0%; calculated C, 52.21%; H, 4.9%), m.p. 332<sup>o</sup>C. Its IR spectrum showed absorption maxima at 3324 cm<sup>-1</sup> (free OH), 1170 (O-gly), 2795 (OCH<sub>3</sub>), 2945 (C-H), 795 (aromatic C=C). The UV spectrum showed bands at 240, 290 and 376 nm and its changes in the presence of diagnostic shift reagents<sup>4</sup>, indicated the presence of free OH groups at 5,6-positions and blocked 7,8,3'-hydroxyl groups.

The glycoside [1] on acid hydrolysis with 5% H<sub>2</sub> SO<sub>4</sub> gave an aglycone [2] and sugars which were identified as xylose and glucose (Co-PC and Co-TLC and GLC). The aglycone [2], molecular formula C<sub>18</sub> H<sub>16</sub> O<sub>8</sub> M.P. 235<sup>o</sup>C on alkaline hydrolysis gave two compounds –p-hydroxy-3-methoxy benzoic acid and 2, 5, 6-tri- hydroxy-3, 4-dimethoxy aceto-phenone (mmp and co-TLC). On the basis of alkaline hydrolysis the aglycone [2] was identified as 5, 6, 4'-tri-hydroxy 7, 8, 3'-trimethoxy flavone, which was further confirmed by comparison of m.p., mmp, UV, IR with authentic sample. The glycoside [1] on acetylation with Ac<sub>2</sub>O/Py gave an octacetate derivative, molecular formula C<sub>45</sub> H<sub>50</sub> O<sub>25</sub>, m.p. 200<sup>o</sup>C. The <sup>1</sup>H NMR spectrum of the acetate derivative of [1] showed that the compound [1] is a diglycoside as it showed eight singlet at  $\delta$  2.38, 2.36, 2.01, 2.08, 2.09, 2.05, 2.10 and 2.20 showing the presence of eight acetyl groups in the comp. [1]. The three sharp singlets at  $\delta$  3.75 (3H, s, -OMe), 4.00 (3H, s, OMe): 3.82 (3H, s, -OMe), showed the presence of three methoxyl groups in the glycoside [1]. The <sup>1</sup>H NMR spectrum also exhibited A<sub>2</sub>B<sub>2</sub> pattern of B ring as it showed two double doublets at  $\delta$  7.20 (2H, dd, J = 8.5 Hz and 2.5 Hz, H-2 '6' and  $\delta$  6.65 (2H, dd, J = 8.5 Hz and 2.5 Hz H-3-5'). The H-1 proton of xylose appeared as doublet at 5.69 (1H, d, J = 9 Hz) and H-1 protons of glucose also appeared as a doublet at 5.45 (1H, d, J = 8.8 Hz), from the J value of doublets, it is confirmed that both the sugars are in B configuration. The remaining sugar protons were appeared at  $\delta$  3.95 – 4.42 as a multiplet for eleven protons. <sup>13</sup>C nmr spectrum of the [1] revealed the presence of 29 carbon atoms in the compound and confirmed the structure of glycoside as [1]. In mass spectrum of the glycoside, the molecular ion peak is absent as expected. The mass spectrum showed a fragments at m/e 521 and m/e 522 due to removal of disaccharide. The RDA fragments were appeared at m/e

213, and 164 due to  $[A_1 + H^+]$  and  $B_1$  fragment. The formation of  $[A_1 + H^+]^+$  and  $[B_1]^+$  fragment confirmed the presence of two methoxyl and two hydroxyl groups in A ring and one methoxyl group in B ring.

Enzymatic hydrolysis of the glycoside with almond emulsin liberated D-xylose and D- glucose, indicating that the D-xylose was attached to D- glucose through a  $\beta$ -linkage and further confirmed that d- glucose and attached to the aglycone by  $\beta$ -linkage.

Pyranose form of sugars was confirmed by periodate oxidation<sup>5</sup>. Acid hydrolysis of the permethylated glycoside gave 2, 3, 5 – tri-O-methyl xylose and 2, 3, 4, 6 – tetra-O-methyl glucose which indicated that xylose was attached to glucose by (1 $\rightarrow$ 4) linkage and that the glucose moiety was attached to the aglycone [2] by  $C_1$ -OH. Thus the compound [1] was identified as 5, 6-dihydroxy – 7, 8, 3' -tri-methoxy-4'-O- $\beta$ -D-xylofuranosyl (1 $\rightarrow$ 4) O-  $\beta$ -D-glucopyranose side.

### III. EXPERIMENTAL

#### A. General Experimental Methods

Melting points were determined, are uncorrected. The uv spectra was taken on a Hitachi – 320 spectrophotometer and MS were obtained on a Jeol – D – 300 (EI/CI) operating at 65 eV. The  $^1H$  –nmr spectra were taken on a perkin Elmer R -32 (90 MHz) instrument and  $^{13}C$ -nmr spectra were obtained on a Bruker WM- 400 (400 MHz) were given in value (PPM) with as an internal standard and  $CDCl_3$  as solvent. IR spectra were recorded for KBr discs on a Perkin Elmer 881 spectrophotometer.

#### B. Isolation

Air dried seeds (2 kg) were extracted with 95% EtOH and the extract was concentrated under reduced pressure to a yellow viscous mass. The EtOAc soluble part of EtOH extract showed one spot on tlc (EtOAc – Acetone -  $H_2O$ 10:3:1:1), and subjected to CC over si-gel. On elution with  $CHCl_3$  - MeOH (8:2), compound [1] was obtained as yellow crystals (0.065%) molecular formula,  $C_{29}H_{34}O_{17}$ ; M.P. 336°C,  $[M]^+ 654$ , (found C, 53.20% H 5.0%, calculated C, 53.21% H 5.1%). UV spectrum of [1] showed characteristic peaks at  $\lambda$

MeOH max 250, 287 and 362,  $\lambda$  max (NaOMe) 272, 391,  $\lambda$ max (Al  $Cl_3$  - HCl) 245 sh, 260 sh, 380;  $\lambda$  max (NaOAc) 299, 309, 342, 395;  $\lambda$  max (NaOAc- $H_3BO_3$ ) 297 sh and IR  $\mu$  KBr max  $cm^{-1}$  3345, 1110, 2872, 2950, 825 1660, 1600, 1585. Compound 1 (75 mg) was mixed with  $Ac_2O$ /py (10 ml.). It formed octacetate derivative, molecular formula  $C_{45}H_{50}O_{25}$ ; M.P. 98°C, acetyl (34.7%)  $[M]^+ 990$ , suggested eight acetylisable – OH groups.

The  $^1H$  NMR data (90 MHz,  $CDCl_3$ , PPM),  $\delta$  3.75 (3H, s, - OMe), 4.00 (3H, s, OMe); 3.82 (3H, s, -OMe); 7.20 (2H, dd,  $J = 8.5$  Hz and 2.5 Hz H-2'-6'); 6.65 (2H, dd  $J = 8.5$  Hz and 2.5 Hz H-3-5'); 2.48 (s, 3H-5-OAc), 2.36 (s, 3H, 6-OAc), 5.45 (1H, D,  $J = 8.8$  Hz, 1''- anomeric proton), 2.02 (3H, s, 2''-OAc), 2.08 (3H, s, -3''-OAc), 3.09 (3H, s, 6''-OAc), 5.69 (1H, d,  $J = 9.0$  Hz, 1'''-anomeric proton) 2.05 (3H, s, 2'''-OAc), 2.10 (3H, s, -3'''-OAc), 2.15 (3H, s, 5'''-OAc), 3.95 – 4.42 (11H, m, protons of sugar residue. EIMS data of [1]:  $[M]^+$  absent, m/z 521, 522, 360, 332, 331, 213, 212, 184, 164.

#### C. Acid Hydrolysis

Compound [1] (400 mg) was hydrolysed with 7%  $H_2SO_4$  by refluxing for about (8 hrs) yielded alycone [2]. The hydrolysate was neutralized with  $BaCO_3$  and  $BaSO_4$  was filtered off and the filtrate was concentrated to a yellow viscous mass and was found to contain two sugars xylose (Rf 0.29 and glucose (Rf 0.17) (Co-PC and Co-TLC).

#### D. Identification of the Aglycone [2]

Yellow crystalline solid, (0.22%) m.p. 235°C,  $[M]^+ 360$  molecular formula  $C_{18}H_{16}O_8$  (found C-60.0%, H4.4%, Calculated C 60.02%, H 4.3%), UV  $\lambda$  MeOH max nm 249 sh, 293, 344;  $\lambda$  (NaOMe) 260,  $\lambda$  Al  $Cl_3$  242 sh, 262, 307, 381;  $\lambda$  (Al  $Cl_3$ - HCl) 241sh, 389;  $\lambda$  (NaOAc), 295, 309, 342, 393;  $\lambda$  (NaOAc-  $H_3BO_3$ ) 297 sh, IR  $\mu$  KBr max  $cm^{-1}$  3421, 2844, 1661, 1650, 1586, 822.  $^1H$  NMR of the acetylated derivative of [2] (90 MHz DMSO- $d_6$ )  $\delta$  3.90 (3H, s, - OMe), 4.05 (3H, s, -OMe), 3.65 (3H, s, -OMe), 7.53 (2H, dd,  $J = 8.6, 2.5$ , H-3-5'), 6.56 (2H, dd,  $J = 8.6, 2.5$ ; H-2'-6'), 2.47 (3H, s, 5-OAc), 2.32 (3H, s, 6-OAc). EIMS data  $[M]^+$ : 360, 332, 331, 213, 212, 184, 164.

### E. Permethylation of [1] followed by Acid Hydrolysis

(1) On per methylation followed by acid hydrolysis gave methylated sugars which were identified as 2, 3, 5-tri-O-methyl xylose and 2, 3, 4, 6-tetra-O-methyl glucose, thereby confirming that C<sub>4</sub>-OH of D-glucose was linked with C<sub>1</sub>-OH of D-xylose.

### G. Quantitative Estimation Of The Sugars

Quantitative estimation of the sugars in the glycoside was carried out by the procedure of Mishra and Rao<sup>6</sup> which revealed that two sugars were present in equimolar ratio 1:1.

### H. Periodate Oxidation of the Glycoside

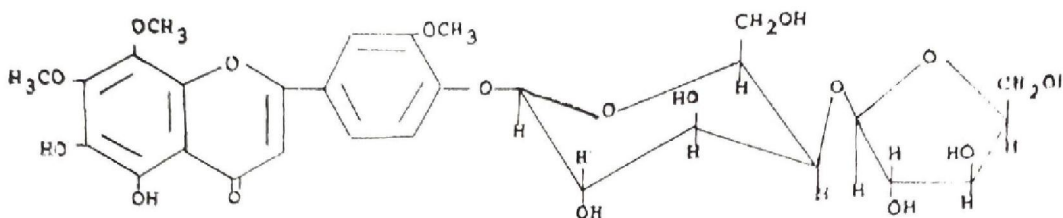
The sodium meta per iodate oxidation of the glycoside (100 mg) consumed 3.01 moles of per iodate and liberated 1.15 moles of formic acid there by confirming the presence of two moles of sugar attached to the Alcyone and also confirmed that D-glucose was present in pyranose form and D-xylose was in furanose form.

## IV. ACKNOWLEDGEMENT

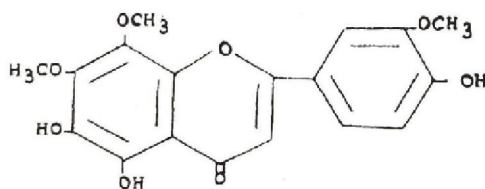
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(i)



(ii)

