



## **FLAVANONE AND FLAVONE GLYCOSIDE FROM *TAXUS BACCATA***

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### **ABSTRACT**

A substituted flavanone and a flavone glycoside were isolated from the ethanolic extract of *Taxus baccata* (stem bark). They were designated as 7,4'-dihydroxy 3',5'-dimethoxy flavanone (**1**) and flavone 3',4'-dihydroxy-6-methoxy-7-O- $\alpha$ -L-rhamnopyranoside (**2**). Their structure were established by using NMR and FA-BMS techniques.

**Key words:** *Taxus baccata*, Ethanolic extract, Flavanone, Flavone glycoside.

### **INTRODUCTION**

*Taxus baccata* is a small size evergreen tree distributed in temperate Himalaya at 1800-3300 m from Kashmir to Bhutan. Locally this plant is known as Thuner<sup>1</sup>. Medicinally, this plant is used as anticephalalgic, sedative and antispasmodic<sup>2</sup>. Different workers have worked on the hardwood and needles of this plant<sup>3-5</sup> but no work has been reported on the stem bark of the title plant. This paper describes the isolation and characterization of a substituted flavanone and flavone glycoside from the ethanolic extract of stem bark.

### **EXPERIMENTAL**

The melting points are uncorrected. The UV spectrums were measured on Hitachi 320. Perkin Elmer model 202 automatic recording spectrophotometer and Toshinwal manual spectrophotometer. The IR spectra were recorded KBr pellets on Perkin model 577 and KBr discs (JASCO-IR-810 spectrometer). The <sup>1</sup>H-NMR were recorded on UNM-G x 400 JEOL spectrometer at 400 MHz and <sup>13</sup>C-NMR spectra were recorded on same instrument at 100.533 MHz using TMS as internal standard. FA-BMS spectra were recorded on JMS-DX300(JEOL) with direct inlet at 70ev. Column chromatography on silica gel (Merck, 60-120 mesh) TLC on kieselgel (Merck). The spots on TLC were visualized by spraying with FeCl<sub>3</sub>. PC was carried out on Whatman No. 1 paper using the descending mode and hydrogen aniline phthalate as developer.

#### **Plant material**

The plant material was collected from Kanatal, Tehri Garhwal Utrtrakhand (India) in the month of March 2009. The identification of plant was made at the Department of Botany, HNB Garhwal University

Campus Srinagar, Tehri Garhwal, U. K., India. A voucher specimen is available on the herbarium of Botany Department.

### Extraction and isolation

The stem bark (2.5 Kg) was air dried and defatted with light petroleum. The solvent free stem bark was exhaustively extracted with 90% ethanol. The ethanolic extract was concentrated under reduced pressure to afford a yellowish mass. This solid mass was chromatographed on silica gel (CHCl<sub>3</sub> -MeOH as eluents) to afford '1' (500 mg) and '2' (1.5 g).

**Compound-1:** Molecular formula C<sub>17</sub>H<sub>14</sub>O<sub>6</sub>, m.p. 244-246°C, FT-IR  $\nu_{\max}^{\text{kBr}}$  cm<sup>-1</sup> 3340 (OH-Stretching), 1640 (C=O Stretching), UV  $\lambda_{\max}^{\text{MeOH}}$  nm: 360, 295, 265, 256.

<sup>1</sup>H-NMR (DMSO,  $\delta$  ppm): 8.02 (dd, J = 5.2 Hz, H -2'), 6.02 (d, J = 2.5 Hz, H-6), 7.0 (d, J = 2.4 Hz, H-8), 7.14 (d, J = 2.5 Hz, H-5), 2.52 (OCH<sub>3</sub>), 2.53 (OCH<sub>3</sub>), 8.0 (brs, OH), 9.0 (brs, OH).

<sup>13</sup>C-NMR (DMSO,  $\delta$  ppm): 83.8 (C-2), 30.4 (C-3), 179.8 (C-4), 122.7(C-5), 116.0 (C-6), 163.6 (C-7), 94.2 (C-8), 148.8 (C-9), 105.5 (C-10), 123.8 (C-1'), 121.6 (C-2'), 158.0 (C-3'), 145.2 (C-4'), 159.0 (C-5'), 122.8 (C-6'), 51.0 (OCH<sub>3</sub>), 59.2 (OCH<sub>3</sub>)

EI-MS(m/z): 314 (M)<sup>+</sup>, 298 (M-OH), 263 (M-OH + OCH<sub>3</sub>), 246 [M-2xOH + OCH<sub>3</sub>]

**Compound-2:** Yellow needles shapes. M.P. 240-242°C, Molecular formula : C<sub>22</sub>H<sub>26</sub>O<sub>10</sub>

UV  $\lambda_{\max}^{\text{MeOH}}$  nm : 270, 276, 307, 428

IR  $\nu_{\max}^{\text{kBr}}$  cm<sup>-1</sup> : 3410, 1650, 1600, 1525, 1430

<sup>1</sup>H-NMR (C<sub>5</sub>D<sub>5</sub>N)  $\delta$  (ppm) : 6.06 (s, H -3), 7.1 (d, J = 7.2 Hz, H - 5'), 6.9 (d, J = 6.3 Hz, H - 8), 7.3 (s, H-5), 6.38 (s, H-2'), 1.271 (rham, 3H), 2.16 (3H, OCH<sub>3</sub>), 3.32-3.43 (5H, larger protons), 4.2 (s, anomeric proton).

<sup>13</sup>C-NMR (CD<sub>3</sub>OD)  $\delta$ (ppm): 146 (C-2), 106.2 (C-3), 179.8 (C-4), 122 (C-5), 163.4 (C-6), 165.8 (C-7), 94.0 (C-8), 140.8 (C-9), 105.4 (C-10), 121 (C-1'), 116 (C-2'), 158 (C-3'), 159 (C-4'), 116.3 (C-5'), 116.9 (C-6'), 53.7 (OCH<sub>3</sub>) rhamnose 103 (C-1''), 72.0 (C-2''), 71.9 (C-3''), 73.2 (C-4''), 70.1 (C-5''), 17.6 (C-6'', CH<sub>3</sub>).

FAB – MS (m/z): 489 (M+K)<sup>+</sup>, 456 [M]<sup>+</sup>, 307 [M+3H -146]<sup>+</sup>, 289 [M + 3H - 146 + H<sub>2</sub>O], 273 [M + 3H -146+2H<sub>2</sub>O], 242 [M-146 + 20H + OCH<sub>3</sub>]

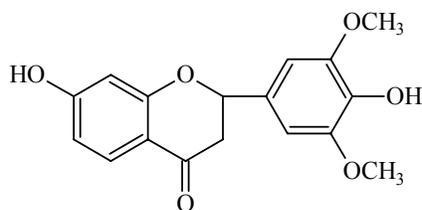
Compound '2' was hydrolysed with 7% methanolic HCl. It was refluxed for 4 hours and furnished aglycone, which was identified as 3',4',-7 trihydroxy-6-methoxy flavanone by direct comparison (mmp, cotic and super imposable IR) with an authentic sample. The neutralized and concentrated aqueous hydrolysate showed the presence of L-rhamnose (PC, solvent EtOAc, C<sub>5</sub>H<sub>5</sub>N-H<sub>2</sub>O; 10:4:3), R<sub>f</sub> value 0.42.

## RESULTS AND DISCUSSION

**Compound '1'** m.p. 244-246°C, homogeneous in TLC was found to be flavanone as it gave brown colour with FeCl<sub>3</sub> and positive Shinoda's test<sup>6</sup>. On the basis of elemental analysis and molecular weight

determination ( $M^+$  314), its molecular formula was established as  $C_{17}H_{14}O_6$ . The presence of two methoxyl groups were determined with the help of Zeisel's method<sup>7</sup>. Presence of two hydroxyl group was confirmed as it forms diacetate on acetylation ( $AC_2O$ /Pyridine).

The IR spectrum showed a peak at 3340 and 1640  $cm^{-1}$  indicate the presence of hydroxyl and keto groups. The UV absorption bands at 256, 265, 295 and 360 nm confirmed the presence of flavonoid nucleus. The  $^1H$ -NMR spectrum displayed the peaks at  $\delta$  8.02 (dd,  $J = 2.0, 5.3$  Hz), 6.02 (d,  $J = 2.5$  Hz), 7.0 (d,  $J = 2.4$  Hz) and 7.14 (d,  $J = 2.5$  Hz) were assigned for H-2', H-6', H-8 and H-5, respectively. Two doublets of 4.5Hz coupling constant appeared as  $\delta$  1.60 and 2.70 where assigned for C-3 cis and trans hydrogen, which were again supported by  $^{13}C$ -NMR spectra appeared at  $\delta$  2.52 and 2.53 and two brs signals at  $\delta$  8.0 and 9.0 were assigned for OH functional group  $^{13}C$ -NMR spectrum displayed fourteen carbon atoms, of which a downfield peak at  $\delta$  177.4 represents carbonyl carbon. The position of two methoxy groups resonated at  $\delta$  51.0 and 59.2 were found to be attached at C-3' ( $\delta$  158.0) and C-5' (159.0). Whereas the peak position of C-4' appeared at 145.2 confirmed OH group at this position. These substitution were further supported by mass fragmentation pattern present in FAB-MS of 1. The  $^1H$  and  $^{13}C$ -NMR values were assigned with the reported data of dihydroxy flavanone<sup>8</sup>. Thus the structure of **1** was established as 7, 4'-dihydroxy -3', 5'-dimethoxy flavanone.



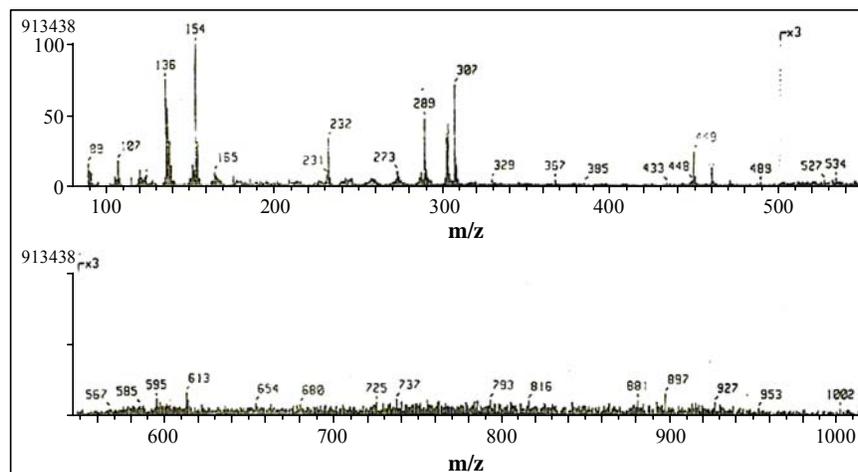
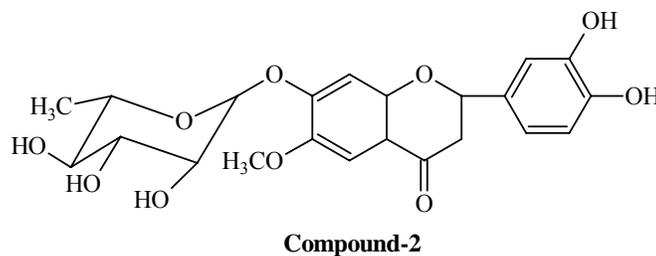
**Compound-1**

**Compound '2'** homogeneous in TLC and crystallized as yellow crystals from methanol (m.p. 240-242 $^{\circ}$ C). It was found to be a flavone glycoside as it gave positive colour with aqueous NaOH,  $FeCl_3$ ,  $Mg/HCl$  and Molish reagent. On the basis of elemental analysis and molecular weight determination ( $M^+$  450), the molecular formula  $C_{22}H_{26}O_{10}$  was assigned to it. Presence of one methoxyl group was determined by Zeisel's method<sup>7</sup>.

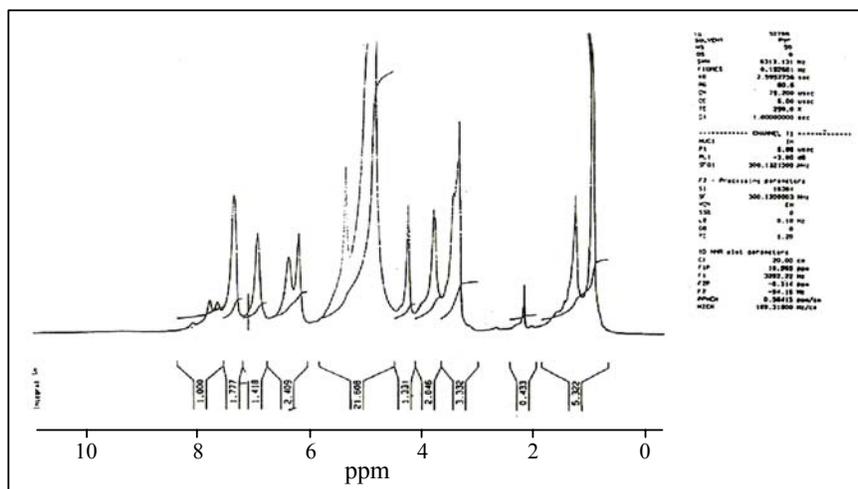
In UV  $\lambda_{max}^{MeOH}$  shifts were observed at 270 and 307 nm, which were shifted to 276 and 428, respectively by addition of  $AlCl_3$ . The IR spectrum exhibited absorption bands at  $\nu_{max}^{kBr}$  3400 (O-H stretching), 2940, 2870 (C-H stretching), 1635 ( $> C=O$  of pyrone system), 1360, 1366 (C-O- $CH_3$ ), 1055 (-C-O-stretching) and 830  $cm^{-1}$ .

The fragmentation peak in FA-BMS of '2' displayed at 307 ( $M-146$ )<sup>+</sup>, 289 [ $M-(146 + OH + 3H)$ ]<sup>+</sup>, 272 [ $M-146 + 2 \times OH$ ]<sup>+</sup> and 241 [ $M - (146 + 20H + OCH_3)$ ]<sup>+</sup> showed the loss of one deoxyhexose, two hydroxyl and one methoxyl groups, respectively.  $^1H$ -NMR spectra of '2' displayed peaks at  $\delta$  6.06 and  $\delta$  7.1 were assigned for H-3 and H-5'. The singlets at  $\delta$  6.3 and  $\delta$  7.3 were assignable for H-2' and H-5. The doublet at  $\delta$  6.9 (d,  $J = 6.3$  Hz) were assigned for H-8. Further the doublet at 1.27 and singlet at  $\delta$  2.16 were assigned for rhamnose  $CH_3$  and aromatic  $OCH_3$  groups of compound. Methoxy carbon function resonated at  $\delta$  53.7 which were assigned at C-6 of the ring. The position of singlet at 4.2 indicate a configuration of rhamnose.

The  $^{13}\text{C}$ -NMR spectra of '2' the downfield peak at  $\delta$  179.8 assigned for C-4 (keto group), 163.4 (C-6), 159.6 (C-4'), 158.0 (C-3') and 165.8 (C-7') support substitution at these position. The downfield value of carbon at C-7 (43 ppm) showed glycosidation at this position. Methoxy carbon function resonated at  $\delta$  53.7 which was assigned at C-6 of the ring '2'. On hydrolysis afford aglycone, which was identified as 3',4',7- trihydroxy-6-methoxy flavones by comparison with  $^1\text{H}$  and  $^{13}\text{C}$ -NMR data of aglycone with the reported data of compound in literature. The glycone was confirmed as rhamnose (pc and rf value). All these values were compared with the reported value of 3',4', dihydroxy-6-methoxy flavones glycoside. Hence '2' was identified as flavone-3',4'-dihydroxy-6-methoxy-7-O- $\alpha$ -L-rhamnopyranoside.



**Fig. 1: FAB-MS of Compound '2'**



**Fig. 2:  $^1\text{H}$  NMR Spectrum of Compound '2'**

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