SRBC Membrane Stabilization Studies on Cupressus Goveniana VAR Abramsiana

**KEYWORDS**

Cupressus goveniana var. Abramsiana (C.B.Wolf) Little, Quercitrin, srbc membrane stabilization

**ABSTRACT**

Fresh leaves of Cupressus goveniana var. Abramsiana (C.B.Wolf) Little have been found to contain the flavonol glycoside quercitrin. The structure of the compound and has been ascertained by modern physical methods like UV, H-1 NMR, C-13 NMR studies, chemical reactions, chromatographic techniques and hydrolytic studies. During SRBC membrane stabilization studies it showed relatively low value of haemolysis at 10 μg of the drug. A higher concentration only hypotonicity-induced haemolysis was observed.

**EXPERIMENTAL**

**EXTRACTION AND FRACTIONATION**

Fresh leaves of Cupressus goveniana var. Abramsiana (C.B.Wolf) Little collected from Kodaikanal hills of Tamilnadu during March were extracted with 80%MeOH(4x500 ml) under reflux. The alcoholic extract was concentrated in vacuo and the aqueous concentrate successively fractionated with benzene(3x250 ml), peroxide free diethyl ether and ethyl acetate(4x250 ml). The benzene and diethyl ether fractions did not yield any isolable material.

**RESULTS AND DISCUSSION**

The EtOAc fraction was concentrated in vacuo and left in an ice chest for 2 days. A yellow solid that separated was filtered and studied. It was recrystallized from MeOH when it afforded yellowcrystals, m.p. 229-30  O yield 0.1%. It was freely soluble in EtOAc and MeOH and sparingly soluble in water. It gave an olive green colour with alc.,FeCl₃, deep pink colour with Mg-HCl, yellow colour with NaOH and appeared deep purple under UV that turned yellow on exposure to NH₃. It did not answered the Horhammer-Hansel test 1 but responded to Willson’s boric acid 3, Gibb’s 4 and Molisch’s test. The pigment had RF as indicated in Table  - and had λmax nm255,269 sh,301 sh,370;(+NaOAc)274 sh,3 21(dec);(+AlCl³) 272,304 sh,333,458;(+AlCl³/ HCl) 265,301 sh,359,428;(+NaOAc)257 sh, 274,329,390(+NaOAc/H₂BO₂) 262,304 sh,388. It was identified as quercetin and the same was confirmed by co-and mixed-PC and m.m.p with authentic sample of quercetin from Physalis minima 5.

Identification of the aglycone:

The EtOAc fraction from the hydrolysate was concentrated in vacuo and left in an ice chest for about a week. A yellow solid that separated was filtered and studied. It came out as pale yellow needles m.p. 316-18  O on recrystallisation from MeOH. It was soluble in organic solvents and sparingly in hot water. It gave a red colour with Mg-HCl, olive green with NH₃ and NaOH, yellow solution with a pale green fluorescence with conc.H₂SO₄ and appeared yellow under UV and V/HN₄. It answered Wilson’s boric acid, Horhammer-Hansel and Gibb’s test but did not respond to Molisch’s test.

Identification of the sugar:(glucose)

The aq. solution from the above hydrolysate was neutralized with BaCO₃ and filtered. The concentrated filtrate on chromatographic examination(PC) gave RF values corresponding to those of glucose. The running properties of the glycoside were in favour of a monoside. The identity of the sugar was also confirmed by direct comparison with an authentic sample of glucose.

**REFERENCES**

1. Horhammer, Hansel and Gibb’s test.
2. Willson’s boric acid test.
3. Gibb’s test.
4. Molisch’s test.
5. Physalis minima.
MHz, DMSO-d$_6$, TMS) of the glycoside, the protons at C-6 and C-8 appear at $\delta$ 6.18 and 6.42 ppm respectively. The C-5' proton appears as a doublet at $\delta$ 6.81 ppm. The 5-OH proton resonates at $\delta$ 12.64 ppm as distinct singlet. The OH protons at C-7, C-3' and C-4' show up to $\delta$ 9.7, 9.45 and 9.22 ppm respectively. The H-1' signal of the flavonol-3-O-rhamnoside is found at $\delta$ 5.45 ppm. The remaining glycosyl protons appear in the range $\delta$ 3.4 to 3.8 ppm.

Supporting evidence for the structure of the glycoside was provided by the analysis of 13 C-NMR(100 MHz, DMSO-d$_6$, TMS) data. Due to glycosylation at 3-position, C-2 and C-4 carbons absorb at $\delta$ 156.3 and 177.2 ppm respectively. C-1', absorbs at $\delta$ 100.9 ppm. The rest of the carbons of the sugar unit appear between $\delta$ 69.9 ppm and 77.6 ppm. Based on this the glycoside have been characterized as quercitrin (quercetin-3-O-rhamnoside).

**SRBC MEMBRANE STABILIZATION STUDIES**

Quercitin isolated from EtOAc fraction was tested for its SRBC membrane stabilization in vitro studies. It showed relatively low value of haemolysis at 10 $\mu$g of the drug, while a plot drawn with concentrations in abscissae and transmittance in ordinates, read at 560 nm in a photoelectric colorimeter. The curve reached a maximum at 50 $\mu$g. As the concentration increases, only hypotonicity-induced haemolysis was observed.

| S.No. | Glycoside concentration in $\mu$g | Percentage of Haemolysis |
|-------|---------------------------------|--------------------------|
| 1     | 10                              | 0.55                     |
| 2     | 20                              | 0.95                     |
| 3     | 30                              | 1.40                     |
| 4     | 50                              | 1.73                     |
| 5     | 100                             | 1.57                     |
| 6     | 150                             | 1.65                     |
| 7     | 200                             | 1.74                     |
| 8     | 250                             | 1.81                     |

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