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SRBC Membrane Stabilization Studies on Cupressus Goveniana VAR Abramsiana

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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 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 concentrationst only hypotonicity-induced heamolysis was observed.*

Introduction

Cupressus goveniana var. *abramsiana (C.B.Wolf) Little*[1] of Cupressaceae is distributed throughout USA: California, Santa Cruz Mountains, at 490-760 m .It is grown as an ornamental tree in hill resorts of India. It can be distinguished from the other varieties only by its large cones. In the absence of any pharmacological work, fresh leaves of **Cupressus goveniana var. abramsiana *(C.B.Wolf) Little*** have been chosen for investigating their flavonoid content and membrane stabilization properties.

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.

EtoAc fraction:(flavonol glycoside:Quercitrin)

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 ^{0}C(yield 0.1%). It was freely soluble in EtOAc and MeOH and sparingly soluble in water. It gave an olive green colour with alc.$FeCl_3$,deep pink colour with Mg-HCl,yellow colour with NaOH and appered deep purple under UV that turned yellow on exposure to NH_3 .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 λ^{MeOH}_{max} nm257,269 sh,299 sh,362;(+NaOMe)272,327,409;(+$AlCl_3$) 275,303 sh,333,430; (+ $AlCl_3$/HCl) 274, 303 sh,353,401; (+NaOAC)271,320 sh,372;(+NaOAC/H_3BO_3) 265,300 sh, 372. The ^{1}H- and 13 C-NMR of the glycoside are appended(Figs ---------- It can be distinguished from the other varieties only by its large cones and). The identity of the glycoside was confirmed by direct comparison with an authentic sample of the same from the seeds of **Bauhunia acuminate** [11] .

Hydrolysis of the glycoside:(flavonol:quercetin)

To a solution of the glycoside(0.1 g,0.2 mmole) in hot aq. MeOH (10 ml,50%) an equal volume of H_2SO_4 (10%) was added and the mixture refluxed at 100 ^{0} C for 2h and the hydrolytic products identified as described below.

Identification of the aglycone:

The Et_2O fraction from the hydrosylate 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 ^{0} C 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 withNH_3 and NaOH,yellow solution with a pale green fluorescence with conc.H_2SO_4 and appeared yellow under UV and UV/NH_3.It answered Wilson's boric acid,Horhammer-Hansel and Gibb's testsbut did not respond to Molisch'stest. It had λ^{MeOH}_{max} nm 255,269 sh,301 sh,370;(+NaOMe)247sh,321(dec);(+$AlCl_3$)272,304 sh,333,458;(+AlCl 3 /HCl) 265,301 sh,359,428;(+NaOAc)257 sh,274,329,390;(+NaOAc/H_3BO_3)262,304 sh,388. It was identified as quercetrin and the same was confirmed by co-and mixed-PC and m.m.p with authentic sample of quercetrin from **Physalis minima** [5] .

Identification of the sugar:(glucose)

The aq.solution from the above hydrosylate was neutralized with $BaCO_3$ 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.

RESULTS AND DISCUSSION

The fresh leaves of *Cupressus goveniana* var. *abramsiana (C.B.Wolf) Little* have been found to contain quercetin-3-O-rhaminoside. The UV spectrum of the glycoside showed two major absorption peaks at 362nm (band-I) and 257 nm (band-II) showing a flavonol skeleton. A bathochromic shift of 47nm in band –I observed in its NaOMe spectrum indicated the prescence of a free 4'-OH group. The $AlCl_3$ –HCl spectra of the glycoside as well as its aglycone showed 3 absorption peaks and a shoulder indicating a free 5-OH group in both. The glycoside as well as its aglycone did not exhibit any intense UV fluorescence ascertaining the prescence of a free hydroxyl group at C-5 in both. A bathochromic shift of 39 nm and 58 nm respectively in $AlCl_3$

-HCl spectra was yet another evidence for the same. The prescence of a ortho di hydroxyl group in the B-ring could be interfered from a shift of +10nm noticed in the glycoside and +18nm noticed in case of the aglycone on the additionof H_3BO_3. In the $AlCl_3$ spectrum, an absorption peak was noticed at 430nm (band-I) which on addition of HCl reduced by 29nm. This is anotherevidence for the presence of a catechol type di-OH group in the B-ring. In the ^{1}H-NMR spectrum(400

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MHz,DMSO-d_6 ,TMS)of the glycoside,the protons at C-6 and C-8 appear at δ6.18 and6.42 ppm respectively. The C-5' proton appears as a doublet at δ 6.81ppm. The 5-OH proton resonates at δ12.64 ppm as distinct singlet. The OH protons at C-7,C-3'and C-4' show upto δ 9.7,9.45 and 9.22ppm respectively. The H-1"signal of the flavonol-3-O-rhaminoside is found at δ5.45ppm. The remaining glycosyl protons appear in the range δ3.4 to 3.8ppm.

Supporting evidence for the structure of the glycoside was provided by the analysis of 13 C-NMR(100MHz,DMSO-d 6 ,TMS) data. Due to glycosylation at 3-position,C-2 and C-4 carbons absorb at δ156.3 and 177.2ppm respectively. C-1",absorbs at δ100.9 ppm.The rest of the carbons of the sugar unit appear between δ69.9 ppm and 77.6 ppm. Based on this the glycoside have been characterized as quercitrin (quercetrin-3-O-rhaminoside).

SRBC MEMBRANE STABILIZATION STUDIES

Quercitrin isolated from EtOAc fraction was tested for its SRBC membrane stabilization[5] *in vitro* studies. It showed relatively low value of haemolysis at 10 µg 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 µg . As the concentration increases, only hypotonicity-induced heamolysis was observed.

S.No.	Glycoside concentration in µg	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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REFERENCE 1.http://www.botanik, Uni-bonn. De/conifers/cu/cup/abramsiana.htm 2. L.Horhammer and R.Hansel, Arch. Pharm. Berl., 1955,288,315. 3.C.W.Wilson, J. Amer.Chem. Soc., 1939,61,2303. 4.F.E.King, T.J. King and L.C. Manning, J. Chem. Soc., 1957,563. 5.V.Sethuraman and N.Sulochana, Fitoterapia, 1988, 59,335.