Research Paper Chemistry

Phytochemical Studies on Antigonan leptopus

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ABSTRACT

The fresh flowers of Antigonon leptopus is found to contain the flavonol quercetin and its glycoside quercitrin. The structures have been characterized by means of modern physical methods like UV, H-1 NMR , C-13 NMR, Rf values, chemical reactions and hydrolytic studies.The in vitro studies showed that the isolated glycoside is capable of stabilizing the HRBC membrane stabilization

Keywords: Antigonon leptopus, quercetin, quercitrin, HRBC membrane stabilization.

Introduction

Antigonon leptopus is popularly known as kodi roja in Tamil. It is also known as Coral vine, Honolulu creeper, Mexican creeper, Bride's tears, Chain-of-love, Hearts on a chain, Love-vine. It belongs to Polygonaceae family. Coral Vine, is a native of Mexico. It is a fast growing, evergreen vine, climbing with tendrils that will reach 40 feet. Leaves are dark green heart-shaped to arrowhead-shaped to 5 inches long. Probably the heart shaped leaves and the delicate pink flowers led to its Mexican name *cadena de amor* or "chain of love". It produces edible tubers. The actual flowers are tiny but the sepals are larger and provide the brilliant colors that range from white to rose-pink to deep coral flowered varieties.

Antigonon leptopus was prepared for consumption by the aboriginal inhabitants of Baja California in a way reminiscent of popcorn. The seeds were toasted by placing them in a flat basket made of flexible twigs torn into several strips and woven to make a solid surface. On top of the seeds they would put live coals, and with both hands they would shake the basket so that the coals come up against the seeds, toasting them but not burning the basket. When the toasting is finished the burned out coals are removed and a major portion of the seeds are burst open exposing a white meal. Afterwards the seeds are separated from the husks from which they have come by dextrously tossing them into the air with the basket, just as wheat is winnowed in Spain. Thus cleaned they would grind and eat the prepared meal. They would also boil it and make fried cakes.[1]

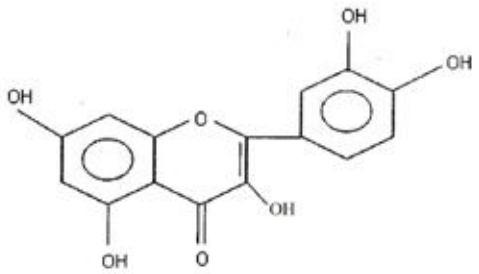
EXPERIMENTAL

Extraction and Fractionation

Fresh flowers (1 kg) of *Antigonon leptopus* collected from in and around Kumbakonam, Thanjavur District, during May-June were extracted with 85% methanol (5 X 500 ml) under reflux. The alcoholic extract was concentrated **in vacuo** and the aqueous concentrate was successively fractionated with petroleum ether (60-80°C) (4 X 250 ml) peroxide - free Et_2O (3 X 250 ml) and EtOAc (4 X 250).The petrol fraction did not yield any crystalline solid and could not be studied further.

Et_2O Fraction (flavonol: quercetin)

The Et_2O fraction was concentrated **in vacuo** and left in and ice-chest for a week. A yellow solid that separated was filtered and studied. On crystallization from MeOH, pale yellow needles were obtained [Gl, m.p. 313-15°C, yield – 0.028]. It was readily soluble in organic solvents and sparingly in hot water. It gave a red color with Mg-HCl, Olive- green color with alc. Fe^{3+}, golden-yellow color with NH_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 reduced ammonical $AgNO_3$ in the cold and Fehling's solution on heating. It answered the Horhammer-Hansel [2], Wilson's boric acid[3]and Gibb's[4] tests. It gave a pentaacetate, m.p. 200-01°C and a pentabenzoate m.p 188-90°C. It had λ^{MeOH} 255, 269 sh, 370: NaOMe 262 sh, 322, 420 (dec.) + $AlCl_3$ 267, 303, 458; + ($AlCl_3$ - HCl) 267,303,351, 428; + NaOAc 275,328,390



Quercetin

and + (NaOAc-H_3BO_3) 262,303 sh, 386 nm and had R_f values as depicted in Table I-1. The ^{1}H-NMR of the flavonol is appended. It was identified as quercetin and the identity as confirmed by CO- mixed-PC and m.m.p with an authentic sample of quercetin from **Physalis minima** [5].

<u>EtOAc fraction:</u> (Quercetin - 3- O- Rhamnoside ; quercitrin)

The EtOAc fraction was concentrated in vacuo and left in an ice – chest for a few days. A yellow solid that separated was filtered and studied. It came out as pale yellow leaflets, m.pt. 182 - 85°c (yield 0.05%) on recrystallisation from MeOH. It was soluble in EtOH and EtOAc but insoluble in cold water. It developed a green colour with alc. Fe^{2+}, pink colour with Mg-HCl and a yellow precipitate with aq. (basic) lead acetate. It reduced ammonical silver nitrate solution but not Fehling's solution. It appeared deep purple under UV that turned yellowish green on exposure to NH_3. It responded to Wilson's boric acid, Molisch's and Gibb's tests but did not answer the Horhammer – Hansel test. It had 256, 266sh, 300sh, 350; + NaOMe 270, 326,393; + $AlCl_3$ 276, 303sh, 353, 400; +NaOAc 272, 320sh, 370 and + (NaOAc - H_3BO_3) 259, 301sh, 368.

Hydrolysis of the glycoside:
The glycoside (0.05g, 0.1m mole) dissolved in hot aq. MeOH (2ml, 50%) was hydrolysed with H_2SO_4(5%) at 100□c for about 2hr and the hydrolytic products identified as described below.

Identifcation of aglycone: (flavonol: quercetin)
The aglycone on crystallisation (Me_2OH), gave yellow needles, m.p. 316 - 18□c. The aglycone that resulted was characterised as quercetin.

Identification of sugar: (rhamnose)
The concentrated filtrate from the neutralised aq. Hydrolysate when examined by PC gave R_f values corresponding to those of rhamnose . The identify of the sugar was also confirmed by direct comparison with an authentic sample of rhamnose. A quantitative hydrolysis revealed the aglycone : sugar ratio to be 1:1 confirming the presence of a monoside. The glycoside was therefore identified as quercetin 3 rhamnoside and confirmed by co – and mixed – PC with an authentic sample of quercetin 3- rhamnoside.

RESULTS AND DISCUSSION
The fresh flowers of Antigonan leptopus have been found to contain quercetin and quercetin 3 – rhamnoside.

The UV spectrum of the glycoside exhibited 2 major absorption peaks at 350 nm (band I) and 256 nm (band II). The band I absorption of the glycoside is reminiscent of a flavonol skeleton. A comparison of band I absorption of the glycoside and that of the aglycone revealed that there may be 3-glycosylation in the flavonol.

A bathochromic shift of 43nm (band I) in NaOMe confirmed the presence of a free –OH at C- 4'. The $AlCl_3$ spectra (with and without HCl) showed four absorption peaks to reveal the presence of a free 5-OH group. It was confirmed by the bathochromic shift of 50 nm on the addition of ($AlCl_3$ – HCl) in the glycoside. The presence of a free –OH at C-7 was evident from the +16nm (band II) shift on the addition of NaOAc. The band I absorption in $AlCl_3$ spectrum is 30nm more than that noticed on addition of $AlCl_3$ – HCl. This is indicative of the existence of an o-dihydroxyl group in the B-ring.

In the ^{1}H NMR spectrum (270 MHz, DMSO-d_6. TMS the A-ring protons at C-6 and C-8 appear as doublets at δ6.8 and δ6.4 ppm respectively. The 5 –OH proton resonates at δ6.8ppm as doublet. The protons at C-2' and C-6' appear at δ7.3 and 7.5ppm. The methyl protons of rhamnose moiety can be located as a doublet at δ0.9ppm. The H-1^1 of the rhamnoside resonates at δ5.2ppm. The remaining sugar protons appear in the range δ3.2 – 3.4 ppm.

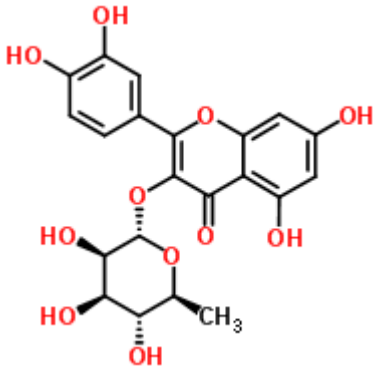
Supporting evidence for the structure of the glycoside was provided by the analysis of 13C-NMR(67.89MHz, DMSO –d_6 , TMS) data and a complete assignment is given in Table I- II. Due to glycosylation at 3- position , C-2 and C-4 carbons absorb at δ156.5 and 177.4 ppm respectively. C-1" absorbs at δ101.8 ppm. The rest of the carbons of the rhamnosyl unit except C-6" appear between δ70 and 71.2ppm. C-6" appear at δ17.4ppm.

Based on this the glycoside has been characterised as quercitrin (quercetin 3-O- rhamnoside)

TABLE I –II
^{13}C-NMR DATA AND THEIR ASSIGNMENT FOR THE GLYCOSIDE FROM THE FLOWERS OF *Antigonan leptopus*

Compound	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Glycoside (δ ppm)	156.5	134.2	177.7	161.2	98.7	164.2	93.7	157.3	104.1

Compound	C1'	C2'	C3'	C4'	C5'	C6'	C1"	C2"	C3"	C4"	C5" C6"
Glycoside	121.2	115.5	145.1	148.4	115.8	120.8	101.8	70.4	70.5	71.2	70.0 17.4



Quercitrin

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