Research Paper

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Bioflavonoids of Ipomoea Aquatica Fork

Chemistry

KEYWORDS : Ipomoea aquatica, Convolvulaceae, kaempferol, kaempferol 3-O-sophoroside

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ABSTRACT

Fresh pinkish flowers of Ipomoea aquatic of Convolvulaceae have been chosen for analysing the presence of the yellow pigments. The flavonol, kaempferol and its glycoside, kaempferol 3-O-sophoroside have been identified. The structure of isolated polyphenols were characterised by means of modern physical methods like UV, H-1 nmr, C-13 nmr , chemical reactions, chromatographic techniques and hydrolytic studies

INTRODUCTION

Ipomoea aquatica Fork (syn.) *I. Reptans* (Linn) of Convolvulaceae is an annual trailing on mud or floating on water. It has a thick hollow rooting at the nodes. The limb of the flowers is pale purple while the throat and tube purple. It is distributed throughout India, Srilanka, tropical Asia, Africa and Australlia. It is cultivated throughout Southeast Asia and is widely consumed vegetable in the region. It is supposed to possess an insulin-like activity according to indigenous medicine in srilanka [(1)]. It showed antimicrobial, analgesic, spasmolytic, spasmogenic, hypotensive, psychotomimetic, diabetic, scorpion venom antidote, emetic, diuretic, purgative, to treating debility, liver complaints, ringworm, leucoderma, leprosy, fever, against nosebleed and high blood pressure and anticancer activities [(2)].

Quercetin[(3)] and 3α , 7β – O – D- diglycopyranosyl- dihydroquercetin [(4)] have been reported from leaves of *I. aquatica*. With a view to locating additional flavanoids, the flowers of *I. aquatica* have been investigated and the results are presented hereunder.

EXPERIMENTAL

Extraction and fractionation:

Fresh flowers (800g) of *I. aquatica* collected from stagnant waters in and around Tranquebar in Nagapattinam district during October, were extracted with 90% MeOH (4x500ml) under reflux. The specimen for the *I. aquatica* is kept at Rapinat Herbarium and Centre for Molecular Systematics, St. Josheph 's college (Campus), Tiruchirappalli- 620 002, the specimen number being SA 015. The alc. extract was concentrated *in vacuo* and the aq. concentrate successively fractionated with petrol (b.p. 60 - 80^0 C) (3x250ml), peroxide –free Et_2O (4x250ml). The petrol fraction did not yield any isolable material.

Et_2O fraction: (flavonol : Kaempferol)

The residue from the Et_2O fraction was taken up in Me_2CO and left in an ice – chest for a few days, when yellow needles (MeOH) m.p 278 - 80^{0}C (yield 0.02%) were separated. It had nm 253sh, 266, 294sh, 322sh, 367; +NaOMe 278, 316, 416(dec.); (+$AlCl_3$) 260sh, 268, 303sh, 350, 424; (+$AlCl_3$ - HCl) 256sh, 269, 303sh, 348, 424; +NaOAc, 274, 303, 387, and +(NaOAc +H_3BO_3) 267, 297sh, 320sh, 372. It was soluble in organic solvents but insoluble in water. It developed a reddish orange colour with Mg – HCl and yellow colour with NaOH. It appeared pale yellow under UV with or without NH_3. It responded to Wilson's boric acid, Horhammer Hansel and Gibb's tests but did not answer the Molisch's test. The structure has been ascertained by comparing the sample with an authentic sample isolated from *Bauhunia acuminata* [(5)].

EtOAc fraction : (flavonol glycoside : Kaempferol -3-O-Sophoroside)

The EtOAc fraction was concentrated *in vacuo* and left in an ice chest for a week. A yellow solid that separated was filtered and studied. When crystallised from MeOH, it came out as yellow long needles, m.p. 194 - 98^{0}C (Yield 0.01%) on recrystallisation from MeOH. It is soluble in water and sparingly solutble in EtOH. It is insoluble in non polar solvents. It answered the Wilson's boric acid and Molisch's and Gibb's tests but did not respond to Horhammer – Hansel test. It developed a green colour with Mg – HCl . It appeared dark purple under UV that turned dark greenish yellow on exposure to NH3. It had nm 266, 350; +NaOMe 275, 325, 397; +AlCl3 274, 304, 352, 397; +$AlCl_3$ / HCl 275, 302, 346, 393; + NaOAc 274, 308, 386; +NaOAc / H_3BO_3 266, 353.

Hydrolysis of the glycoside:

The glycoside (0.05g) dissolved in hot aq. MeOH (2ml, 50%) was hydrolysed with H_2SO_4 (5%) at 100^0 C for about 2h and the hydrolytic products identified as described below.

Identification of aglycone: (flavonol : kaempferol)

The (Yellow) aglycone on recrystallisation from MeOH afforded a yellow crystalline solid m.p. 278 - 80^0 C which was identified as kaempferol by colour reactions, behaviour under UV and R_f . It had the same UV spectral values, mentioned under ether fraction.

Identification of sugar : (Sophoroside)

The aq. solution from the above was neutralized with $BaCO_3$ and filtered. An aliquot of this was cautiously neutralized with $NaHCO_3$ and the sugar estimated quantitatively by Folin – Wu micro method [(6)]. The sugar content was in agreement for a bioside. The concentrated filtrate when examined by paper chromatography gave R_f values corresponding to those of sophoroside. The running properties of sugars were also in favour of a bioside. The identity of the sugar was confirmed with authentic sample of sophoroside.

RESULTS AND DISCUSSION:

The fresh pinkish white flowers of *I. aquatica* have been found to contain the aglycone, flavonol kaempferol and its 3 – O- sophoroside.

The UV spectrum of the aglycone exhibited two major peaks at 367nm (band I) and 266nm (band II), to reveal a flavonol skeleton. A bathochromic shift of 49nm, on the addition of NaOMe showed the presence of a free 4' – OH group in the B – ring. A shift of 57nm, on the addition of $AlCl_3$ – HCl showed the presence of a free 5 – OH in the A ring. The presence of a free –OH at C-7 was ascertained by a shift of +8nm (band II), on the addition of NaOAc. The $AlCl_3$ spectrum was exactly same as that of ($AlCl_3$ / HCl) revealing the absence of a catechol type of substitution in the B – ring. The H_3BO_3 spectrum also confirmed it, as there was only +5nm (band I) shift, on the addition of NaOAc / H_3BO_3.

The 400MHz ^{1}H-NMR(DMSO-d_6, TMS) spectrum of the flavonol shows a signal at δ13 ppm, revealing the presence of a free 5 –OH proton. The C-6 proton occurs as a doublet at δ6.2 ppm, higher field than that of C-8 proton which also occurs as doublet at δ6.4 ppm. Protons at C-2',3',5' and 6'(due to free rotation of the B –ring) appear as two pairs of ortho coupled doublets. The H – 3', 5' doublet occurs at δ6.85 ppm upfield from the H – 2',6'

doublet which in turn occurs at δ8 ppm due to the shielding effect of the oxygen substituent. The 7 – OH proton resonates at δ10.0 ppm as a distinct singlet.

Supporting evidence for the structure of the flavonol is provided by the ^{13}C-NMR (100 MHz, DMSO –d_6, TMS) (fig I – 8) spectral data.

The UV I band absorption of the glycoside was at 350 nm which is again indicative of a flavonol skeleton. A comparison of band I absorption of the glycoside and the aglycone revealed that there may be 3- glycosylation in the flavonol. A bathochromic shift of 47nm (band I) ascertained the presence of free –OH at C-4'. The $AlCl_3$ spectra, with or without HCl showed four absorptions peak to reveal, the presence of a free 5 – OH group. It was confirmed by the bathochromic shift of 43 nm, on the addition of $AlCl_3$ /HCl. The presence of a free –OH at C-7 was evident from the +8nm (band II) shift on the addition of NaOAc. The H_3BO_3 spectrum was showed the absence of catechol type MeOH to show the absence of a catechol type of substitution in the B – ring.

The 400MHz , ^{1}H –NMR(DMSO – d_6, TMS) (fig I – 9) spectrum of the flavonol glycoside shows a signal at δ 12.58 ppm, revealing the presence of a free 5 –OH proton. The A – ring proton at C-6 appearss as doublet at δ 6.11 ppm (J = 1.9Hz). The C-8 protom appears as doublet at δ 6.35 ppm (J = 2.0 Hz) . In the B – ring the protons at C-2' and 6',3',5' due to the free rotation of phenyl ring appear as two pairs of ortho coupled doublets at δ 6.83 ppm (2H, d, J = 8.9Hz, H – 3', 5) and δ 7.97 ppm (2H, d, J = 8.8 Hz, H -2', 6'). The H – 3', H – 5' doublet occurs upfield from H – 2', H – 6' doublet due to the shielding effect of the oxygenation at C – 4'. The H- 1" signal of the glucose moiety appears at δ5.62 ppm (H, d, J = 7.1 Hz, 3 – O – glycosyl H – 1) and the H – 1"' signal of the glucose moiety of the sophoroside appears at δ 4.53 ppm (1 –H , d, J = 7.8Hz,(1 2)- O-glycosyl, H – 1 "'). The β linkage of the glucose moiety of the two glucose units of the sophoroside is evident from the large coupling constant of H – 1" and H – 1"'. The C – 6 proton of the A – ring appear as a doublet at δ 6.11 ppm (1H, d, J = 1.9Hz, H – 6). The 7 –OH proton appears as doublet δ10.0 ppm.

Supporting evidence for the structure of the flavonol glycoside is provided by the ^{13}C-NMR (100 MHz, DMSO –d_6, TMS) spectral data.

On this basis the identity of the pigments obtained from Et_2O and EtOAc solubles can be confirmed as kaempferol and its 3 – O – sophoroside respectively.

REFERENCE

1) Malalavidhane, T.S, Wickramsinghe, S.M.D.N and Jansz ,E.R., Oral hypoglycaemic activity of Ipomoea aquatica. Journal of Ethnopharmacology; 2000, 72, 293. | 2) Uawonggul, N., Chaveerach, A., Thammasirirak, S., Arkaravichien,T., Chuachan ,C., Daduang, S., Screening of plants acting against Heterometrus laoticus scorpion venom activity on fibroblast cell lysis. J Ethnopharmacol, 2006, 103, 201. | 3) Mohd. Jishan Khan, Vipin Saini, Varum S. Bhati, Manvendra S. Karchuli, Sanjay B. Kasture, Pelagia Research Library, European Journal Of Experimental Biology, 2011, 1(1), 63. | 4) Marilena Meira, Eliezer Pereira da silva, Jorge M. David, Juceni P. David, Review of the genus Ipomoea: traditional uses, Chemistry and biological activities, Revista Brasileria de Farmacognosia Brazilian Journal of Pharmocognosy, 2012, 22(3), 682. | 5)Barnabas, C.G.G., and Nagarajan, S., J. Madras Univ., 1979, 42B, 51. | 6) Oser. B. L. , Hawk's physiological chemistry, McGraw – Hill, London , 1965, 1052. |