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The flavonoid Quercetin-3-O-glucuronide from Nelumbo nucifera

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ABSTRACT *The fresh florets of Nelumbo nucifera is examined to contain the flavonoid Quercetin-3-O-glucuronide. Modern physical methods like UV, NMR and chemical reactions, PC and hydrolytic studies were used to ascertain the structure.*

INTRODUCTION

Nelumbo nucifera belonging to Nymphaeceae family is a handsome aquatic herb with stout, creeping rhizome found throughout India. It is a native of China, Japan and possibly India. Nelumbo carpels are considered superior to cereals in nutritive value.1

One tablespoon full of dry fruit powder of N. nucifera is mixed with honey as a tonic. Root and flower powder is taken with warm water daily for a month as blood purifier.2 N nucifera flower powder is prescribed in case of fever, diarrhea, cholera and liver disorders, while a decoction is used in cough, menorrhagia and bleeding piles. Root paste is locally applied in ringworm and other skin. Leaf, pedicle and embryo contain the alkaloid, nelumbine.3 In ayurveda, it is said that the whole plant is sweet, cool and slightly bitter. The flowers of N. nucifera are used for ornament and as offering in temples. The present day lotus perfume is a blend of patchouli, benzoin and storax with phenyl ethyl and cinnamic alcohols. Leaves and flowers possess bacteriostatic action against Gram positive and Gram negative bacteria.4

EXPERIMENTAL

Extraction and fractionation:
Fresh corolla of Nelumbo nucifera (2kg) collected from Kumbakonam of Tanjore district was extracted with 85% methanol (5x500ml). The combined alcoholic extract was concentrated in vacuo. The aq. concentrate was fractionated successively with light petrol (60-80oC) (3x250ml), peroxide free Et2O (4x250ml) and EtOAc (8x250ml). The EtOAc fraction alone was taken up for the study.

EtOAc fraction: (Flavonol glycoside: Quercetin-3-O-glucuronide):
The EtOAc fraction was concentrated in vacuo and left in an ice chest for few days. A yellow solid that separated when subjected to PC revealed the presence of a single glycoside. A yellow solid on recrystallization from methanol was obtained. It developed a green colour with alc.Fe3+ and orange red colour with Mg-HCl, yellow colour with NaOH and appeared as purple spot under UV turning yellow on exposure to NH3. It responded to Wilson's boric acid and Gibb's test but did not answer the Horhammer-Hansel test. It responded to Molisch's test showing that it could be a flavones glycoside. It had λmax MeOH 257, 266sh 330sh, 356; +NaOMe 271sh, 332sh, 412; +AlCl3 273, 303sh, 433; +AlCl3-HCl 268, 303sh, 364, 401 and +NaOAc 271, 334sh, 401; +NaOAc-H3BO3 260, 298sh, 377nm.

Acid hydrolysis of the glycoside:
The glucuronide (50mg) dissolved in hot aq.MeOH was hydrolyzed with 2M HCl at 100oC for about 2h. The excess alcohol was distilled off in vacuo and the resulting aq. solution was extracted with Et2O. The residue from Et2O fraction was studied as presented below.

Identification of the aglycone (Flavonol -Quercetin)
The aglycone recovered from the hydrolytic fraction of the glycoside on recrystallization gave yellow needles (m.p. 305-306°C). It was soluble in organic solvents and sparingly soluble in hot water. It gave a red colour with Mg-HCl; olive green with alc.Fe3+, golden yellow colour with NH3 and NaOH and appeared yellow under UV and UV/NH3. It had λmax MeOH 256, 271sh, 300sh, 370; +NaOMe 245sh, 321, 412(dec); +A1Cl3 272, 304sh, 332, 457; +AlCl3-HCl 265, 301sh, 328, 427; +NaOAc 272, 330, 389 and +NaOAc-H3BO3, 260, 302sh, 338nm.

Identification of sugar moiety:
The aqueous solution from the above was neutralized with PbCO3 and filtered. The concentrated filtrate on PC when examined by paper chromatography agreed with those of glucuronic acid. Thus, the sugar moiety was identified as glucuronic acid.

Isolation of the galactosyl glucoside:
The eluate from the lower band was concentrated and treated with acetone when the mixture was left in an ice chest for a week, a yellow solid yield (0.15%) separated on recrystallization from MeOH. It developed green colour with alc.Fe3+ pink colour with Mg-HCl and yellow colour with NaOH. It responded to Wilson's boric acid test and answered Gibb's test and Molisch's tests. It did not answer Horhammer-Hansel test. It had λmax MeOH 255, 266sh, 300sh, 356; +NaOMe 271, 332sh, 412; +A1Cl3 273, 303sh, 433; +AlCl3-HCl 268, 303sh, 364, 401 and +NaOAc 271, 334sh, 401; NaOAc-H3BO3, 260, 298sh, 377nm.

Hydrolysis of the glycoside:
The glycoside (50mg) was dissolved in hot aq.MeOH (5ml; 50%) and an equal volume of H2SO4 (7%) was added to it. The reaction mixture was then refluxed at 100°C for about 2h.The excess alcohol was distilled off in vacuo. The resulting aqueous solution was extracted with Et2O and the residue in the ether fraction was studied as described below.

Identification of aglycone (Flavonol-quercetin):
The aglycone recovered from the hydrolytic fraction of the glycoside on recrystallisation gave yellow needles, m.p. 305-306°C (yield 0.02%). It was soluble in organic solvents and sparingly soluble in hot water. It gave a red colour with Mg-HCl, olive green with alc.Fe3+, golden yellow colour with NH3 and NaOH. It appeared yellow under UV and UV/NH3 and it answered Horhammer-Hansel test and Wilson's boric acid tests. It had λmax MeOH values of 254, 271sh, 300sh, 368; +NaOMe 245sh, 321, 412(dec); +A1Cl3 272, 304sh, 332, 457; +AlCl3-HCl 265, 301sh, 328, 427; +NaOAc 272, 330, 389(dec); and +NaOAc-H3BO3, 260, 302sh, 338nm. Aglycone part of the compound was identified as Quercetin.

Identification of sugar moiety:
The aqueous solution from the above was neutralized with

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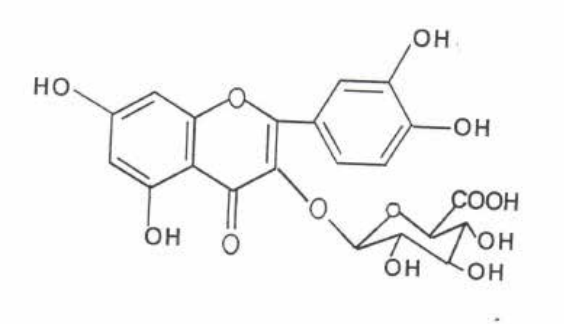
BaCO3 and filtered. An aliquot of this was cautiously neutralized with NaHCO3 and the concentrated filtrate indicated the presence of galactose and glucose on PC using aniline hydrogen phthalate as spray reagent. The identity of sugars was confirmed by CO and PC with authentic samples of galactose and glucose.

RESULTS AND DISCUSSION

The fresh corolla of N. nucifera has been found to contain quercetin-3-O-glucuronide. The UV spectral values of the glycoside and the aglycone are respectively 359nm (band I) and 1257nm (band II) and 370nm (band I) and 256nm (band II) respectively, indicating the presence of flavonol skeleton in both and also revealing the presence of glycosylation at C-3. This is further evidenced by the aglycone responding to the Horharnmer-Hansel test, while the glycoside did not. A bathochromic shift of 56nm in band I observed in its NaOMe spectrum indicated the presence of free -OH at C-4'. A bathochromic shift of 45nm in the glycoside and 57nm for aglycone in AlCl3-HCl spectra was noticed. This confirms the presence of free OH at C-5. The NaOAc spectra of both glycoside and aglycone indicated the presence of free OH at C-7, as evidenced by the bathochromic shift of 14nm and 16nm, respectively. A bathochromic shift of 18nm in band I for glycoside and aglycone on the addition of H3BO3 indicated the presence of an O-dihydroxyl grouping in the B-ring (at C-3' and C-4') in both, a shift of 32nm in the glycoside and 30nm in the aglycone (band I) with respect to AlCl3-HCl spectra. In the 1H NMR spectrum (400MHz, DMSOd6,TMS) of the glycoside quercetin 3-O-glucuronide, the signal appearing at δ12.3ppm corresponds to the -OH at C-5. The signal at δ9.8-10.5ppm is due to the hydroxyl proton at C-7. The doublet appearing in the region of δ7.4ppm (d, J=8Hz) and δ7.lppm corresponds to the proton at C-2' and C-6', while the proton of C-3' appears at δ8.05ppm. The signal appearing at δ6.8ppm (d, J=8Hz) corresponds to C-5' proton. C-8 proton due to meta coupling with C-6 proton appears as a doublet at δ6.6ppm (d, J=2.2Hz). C-6 proton, due to meta coupling with C-8 proton appears as a supporting evidence for the structure of the flavonol glycoside quercetin 3-O-glucuronide is provided by the 13C-NMR (100MHz, DMSO –d6,TMS) spectral data. The 13C-NMR spectral data for the corresponding aglycone taken out from the literature are also tested for easy comparison. Due to glycosylation, the signal of C-3 is shifted upfield by δ2.6ppm. The down field shift of 'ortho related' C-2 signal by δ12.54ppm also confirms this. The large shift in C-2 resonance also reflects the semi-olefinic character of the flavonol C-2, C-3 double bond. The signal at δ0.82ppm of C-10 is less intense due to the longer relaxation time of the quaternary carbon. The signal C-6" at δ9.7ppm confirms the glycoside as 3-O-glucuronide and the identity was confirmed by CO and PC with an authentic sample of quercetin-3-O-glucuronide from Frankenialyerulenta.5

The structure of the glycoside quercetin-3-O-glucuronide was further evidenced by mass spectrum The mass spectrum of the glycoside quercetin-3-O-glucuronide shows prominent peaks at m\z 479 is in agreement with the identification of the glycoside as quercetin-3-O-glucuronide. The fragments at m\z 154 and m\z 136 illustrate the substitution pattern in A and B-rings. Other peaks are quercetin-3-O-glucuronide also in favour of the structure of the compound. Based on the above evidences, the glycoside quercetin-3-O-glucuronide has been characterized as quercetin-3-O-glucuronide. (Misquelianin).



CONCLUSION

The air-dried leaves of Nelumbo nucifera were found to contain Quercetin-3-O-glucuronide. The structure of the compound have been ascertained by chemical reactions, chromatographic and spectroscopic techniques.

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