Isolation of 5, 7, 4'-trihydroxy flavone-8-C-β-D-glucopyranoside, a Flavone Glycoside from the Roots of Bauhania Retusa

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Abstract

Roots of *Bauhania retusa* Roxb. yielded a new flavone glycoside. The isolated flavone glycoside is reported for the first time from this plant. The compound was characterized as 5, 7, 4'-trihydroxy flavone-8-C-*β*-D-glucopyranoside on the basis of U.V, I.R and N.M.R (^{1}H, ^{13}C) spectral studies.

Key words: Caesalpiniaceae, Bauhania retusa, 5, 7, 4'-trihydroxy flavone-8-C-β-D-glucopyranoside

Introduction

Bauhania retusa Roxb., (syn. B. semla Wunderlin., Family: *Caesalpiniaceae*.) is an India medicinal plant. This plant is prescribed in indigenous system of medicine for the treatment of various ailments such as diabetes, inflammation, snake bite, dysentery, sores, liver disorders, ulcers, piles and skin diseases. It also showed hypoglycaemic, hypocholesterolaemic and diuretic activities [1-3].

Previous studies on the chemical constituents of *Bauhania retusa* have revealed the occurrence of various flavonoids and their glycosides [4-7]. Flavonoids were exhibited in many ranges of activities including anti-allergic, anti-inflammatory, anticancer, antioxidant, antispasmodic, antibiotics, anti-viral and hepatoprotective, anti-thrombotic, and vasodilatory activities.

The investigation of the roots had led to the isolation of a new flavone glycoside. The isolation and characterization of a flavone from methanol extract of the roots of *Bauhania retusa*.were carried out.

Results and Discussion

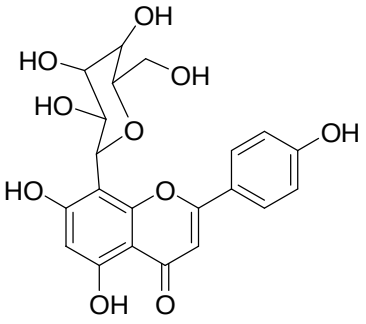
The compound was isolated as yellow amorphous powder (m.p 272-274 ^{o}C, dec.) from methanol extract by eluting column with chloroform: EtOAc (7:3). Compound was first identified as a flavonoid based on its carroty spot on TLC plate visualized with NP-PEG reagent. The compound showed positive Feigel test for sugar moiety and a positive Shinoda test [12] for flavonoids, suggested that the compound may be a flavonoid glycoside. The molecular ion peak at m/z 433 $[M+H]^{+}$ in its electro spray mass spectrum corresponded to the molecular formula $C_{21}H_{20}O_{10}$.

The IR spectra showed absorption bands at 3410 (-OH), 1655 (*α, β*- unsaturated carbonyl group), 1613 (aromatic C=C) cm^{-1} functionalities. The UV spectrum of the compound exhibited the absorption maxima at 271, 334 nm, characteristic of flavonoids, suggesting that the compound belongs to the flavone family unsubstituted at 3-position [13]. In flavonoids two major absorption peaks occurred in the region 240-400 nm. The peak above 300 nm is associated with the absorption of cinnamoyl ring (ring B) and that of below 300 nm for benzoyl ring (ring A). The cinnamoyl ring absorption pattern is also important in providing the information regarding the type of flavonoids, its oxidation pattern and also helpful in distinguishing between flavones and flavonols. The absorption peaks up to 350 nm occurs for flavones, where as beyond 350 nm for flavonols. We observed absorption maxima at 334 nm in cinnamoyl ring region. Therefore the compound belongs to flavones. The oxidation pattern of the cinnamoyl ring can also be identified by the nature of the peak. In 3', 4' di or 3', 4', 5' tri oxygenated flavones UV spectra exhibits usually two absorption peaks or some times one maximum with a shoulder between 250-280 nm, while in 4' -oxygenated flavones only one peak occurs in the same region. Since the UV spectra of our compound exhibited merely one peak in this region, thus it is regarded as 4' -hydroxy flavone [14-19]. The addition of NaOMe to MeOH solution of the compound produced bathochromic shift with an increase in the intensity of absorption, confirmed the presence of free 4' -hydroxyl

group. The disappearance of band in the range 300-380 nm, in NaOAc/ H_3BO_3 spectra of the compound indicated the absence of ortho-dihydroxy groups in both rings. The $AlCl_3$ and $AlCl_3$/ HCl spectra showed no significant hypsachromic shift also indicating that there are no ortho dihydroxy groups in the compound [14-18]. No sugar was released when compound heated prolong with acid, confirmed the presence of C-glycosylation. The $[M-18]^+$ peaks in mass spectrum also supported the presence of C-glycosylation. A peak $[M]^+$ at 433 established the structure as flavone glucopyranoside bearing three hydroxyl groups.

The ^1H NMR spectrum of the compound showed a one-proton singlet at δ 6.76 characteristic of H-3 proton. The spectrum also demonstrated two doublets at δ 6.92 and δ 7.98 (J=9.2 Hz) assignable to H-3′/H-5′ and H-2′/ H-6′ proton. The appearance of two doublets and their coupling constant value are further in agreement with the hydroxyl group at 4′ position [17]. The anomeric proton of sugar appeared at δ 4.68. The acid stable bathochromic shift with $AlCl_3$ in the UV spectrum and a singlet at δ 12.98 in the ^1H NMR spectra confirmed the presence of free OH group at C-5. This in turn fixed the position of C-rhamnosyl at C-8. The position of two hydroxyl groups were assigned as C-5 and C-7, which is according to UV shift pattern with diagnostic NaOAc reagent [15-21].

The structure was further supported by its ^{13}C NMR spectrum, which demonstrated a downfield signal at δ 182.06 clearly assigned to carbonyl carbon C-4. The three downfield signals appeared at δ 160.10, 159.80 and 156.10 were assigned to C-4′, 5 and 7, bearing hydroxyl group. Further, a signal at δ 98.02 assigned to C-6 supported that hydroxyl group present at C-5 and 7. The position of sugar was concluded to be at C-8 based on comparison of ^1H and ^{13}C NMR spectral data [15, 17, 20] with a known compound 5, 7, 4'-trihydroxy flavone-8-C-*β*-D-glucopyranoside.
Thus on the basis of the above spectral evidences the structure of the isolated compound was finally concluded to be 5, 7, 4'-trihydroxy flavone-8-C-*β*-D-glucopyranoside. This compound is the first time to be reported from *Bauhania retusa*.



5, 7, 4'-trihydroxy flavone-8-C-*β*-D-glucopyranoside

Experiment

Ultraviolet absorption spectrum was recorded on Perkin-Elmer Lambda Bio-20 UV spectrometer. I. R spectroscopy was performed on Perkin-Elmer 1710 infrared fourier transformation spectrometer. NMR spectra were recorded on Bruker AVANCE DRX- 300(300 Hz). FEBMS was recorded on JEOLSX 1021/DA-6000 mass spectrometer.

Plant material

Roots of *Bauhania retusa* were collected from the rural areas of district Shahjahanpur in the month of April and identified by the Head Department of Botany of G. F. College.

Extraction

Roots were carefully examined and old, insect damaged and fungus-infested roots were removed. Healthy roots were spread out and dried in the laboratory at room temperature until they can be broken easily by hand. Roots were ground to a fine powder, using a mill. Air-dried roots of *Bauhania retusa* were first defatted with petrol (3L x 5 times) to obtain 35 g of petrol-extract on distillation under reduced pressure. The marc was then extracted with C_2H_5OH (3L x 5 times). The solvent was evaporated under vacuum on rotatory evaporator below 50 ^oC temperature to yield alcoholic extract (27g). The alcoholic extract thus obtained was poured in 500 ml distilled water to get water soluble and insoluble portions. The water insoluble part (ppt.) after partitioned with C_6H_6 was dissolved in MeOH to provide 15 gm of methanolic extract. A well-stirred suspension of silica gel (100 -150 g in pet-ether 60-800) was poured into column (150 cm long and 50 mm in diameter). When the absorbent was well settled, the excess of petrol was allowed to pass through column. Slurry was made to methanolic extract with 5 gm of silica gel in pet-ether and was digested to well settle column. The column was successively eluted with the solvents and solvent mixtures of increasing polarity. Elution with chloroform: EtOAc (7:3) afforded yellow Crystals.

Compound

Yellow Crystals; m.p.: 272-274 ^oC; R_f : 0.78 (CH_2Cl_2: MeOH 40:10) UV λmax(nm): (MeOH) 271, 304 (sh), 334; (MeOH- NaOMe) 281.0, 336, 399.9; (MeOH-NaOAc) 279.1, 392.7(sh); (MeOH-NaOAc-H_3BO_3) 284.4, 320.9, 396(sh); (MeOH: $AlCl_3$) 278.2, 285.3, 305.3, 345.1(sh); (MeOH-$AlCl_3$-HCl) 280.0, 305.2(sh), 344.0, 383; IR (KBr) νmax : 3410 (-OH), 1655 (*α, β*- unsaturated carbonyl group), 1613 (aromatic C=C) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 12.98(1H, s, 5-OH), 10.83 (1H, brs, 7- OH), 10.34 (1H, brs, 4'-OH), 7.98(2H, d, J=9.2 Hz, H-2'/ 6'), 6.92(2H, d, J=9.2 Hz, H-3'/ 5'), 6.76(1H, s, H-3), 6.27(1H, s, H-6), 4. 86 (1H, d, J=9.8 Hz, H-1"), 3.78 (1H, d, J=9.8 Hz, H-2″), 3.24-3.83 (6H, m, Sugar); ^{13}C NMR (DMSO-d_6) δ : 163.91, (C-2), 102.31(C-3),

182.06(C-4), 159.80(C- 5), 98.02(C-6), 156.10(C-7),126.5(C-8), 160.10 (C-9), 104.30(C- 10),122.42(C-1'), 127.50 (C-2', 6'), 115.3 (C-3'), 111.3 (C-5'), 162.9(C-4'), 73.32 (C-1''), 71.12 (C-2''), 78.90(C- 3''), 71.74(C-4''), 79.40(C-5''), 61.8 (C-6''); MS m/z: 433[M+H] $^{+}$, 313[M+H-120] $^{+}$, 307, 271[M+H-162], 257, 242, 225, 176, 254, 136, 106, 89, 77.

Conclusions

From the literature search, 5, 7, 4'-trihydroxy flavone-8-C-*β*-D-glucopyranoside was previously unknown from *Bauhania retusa*. Therefore, this is the first report for this compound to be isolated from the roots of *Bauhania retusa* Roxb.

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