Isolation of 5,6,7,8,3',5'-hexamethoxy flavanone-4'-α-L-rhamnopyranoside, a Flavanone Glucoside from Feronia Limonia

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Abstract

A flavanone glucoside was isolated from *Feronia limonia*,. The compound was characterized as 5,6,7,8,3',5'-hexamethoxy flavanone-4'-*α*-L-rhamnopyranoside on the basis of U.V, I.R, N.M.R (^{1}H, ^{13}C) and mass spectral studies.

Keywords: Feronia limonia, Rutaceae, flavanone glucoside, 5, 6, 7, 8, 3', 5'-hexamethoxy flavanone-4'-*α*-L-rhamnopyranoside

Introduction

Feronia limonia Swingle [*Feronia elephantum* Correa / *Limonia acidissima* L. / *Schinus limonia* L.] belongs to the family *Rutaceae*. It also calls wood apple, elephant apple, monkey fruit, curd fruit, kath bel or other dialectal names in India [1]. It is one of the most important medicinal plants commonly found in Shahjahanpur district. This plant is prescribed as a traditional medicine for the treatment of various ailments [1]. *Feronia elephantum* has a wide range of biological activities such as adaptogenic activity. It can be used for treatment of blood impurities, leucorrhoea, dyspepsia, jaundice [2]. It also acts as hepato-protectant [3, 4]. All parts of the plants can be prescribed in indigenous system of medicine for the treatment of various ailments. Leaves, barks, roots and fruit pulps can be used to treat snakebite [5, 6]. The bark can be chewed with *Barringtonia* which can be applied on venomous wounds. In India, the fruit is used as a hepatic and cardiac tonic agent, curing diarrhea and dysentery [6], it has effective treatment for hiccough, sore throat and diseases of gums. The pulp poultice can be applied onto bites and stings of venomous insects. Young leaves can be mixed with juice, milk and candy for the treatment of biliousness and intestinal troubles [1, 2]. Fruits, leaves and stem bark of *F. limonia* were studied to have anti-tumor [7], larvicidal [8] and antimicrobial activities [9-11]. Fruit pulp showed anti-inflammatory, antipyretic and analgesic activities [12]. Leaves of the *F. limonia* showed anthelmintic activity [13].

Different parts of the plant were investigated and they were found to have coumarins, furanocoumarins, lignans, alkaloids, steroids and flavonoids. The unripe fruits contain stigmasterol. Root bark yielded osthol, geranyl umbelliferone, marmin, marmesin, aurapten, bergapten, isopimpinellin, fernoil [14-16]. The heartwood contains ursolic acid and a flavanone glycoside, 7-methylporiol-*β*-D-xylopyranosyl-D-glucopyranoside [17]. The stem bark of *Feronia limonia* yielded flavanone, an alkaloid, coumarins, flavanone, lignan, sterols and triterpene (Rahman and Gray, 2002). Psoralen, bergapten, orientin, vitexin and saponarin had been isolated from leaves.

Materials and Methods

Plant Material

The roots and leaves of *Feronia limonia* were collected from the rural areas of Shahjahanpur district in the month of January. The plant was identified by the Department of Botany of G. F. College (Rohilkhand University) Shahjahanpur, where a voucher specimen has been deposited. Fresh or dried plant materials were used as source for the extraction of secondary plant components. Freshly harvested and dried materials were used because old dried material stored for a period of time would have some qualitative changes. Roots and leaves were carefully examined. Old, insect-damaged and fungus-infested roots and leaves were removed. Healthy roots and leaves were spread out and dried in the laboratory at room temperature until they can be broken easily by hand. Air dried plant

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material (about 1 kg) was grounded into fine powder and extracted successively with hexane, chloroform, ethyl acetate and methanol.

Instrumentation

Ultraviolet absorption spectrum was recorded on Perkin-Elmer Lambda Bio 20 UV spectrometer. IR spectroscopy was performed on Perkin-Elmer 1710 infrared Fourier transformation spectrometer. NMR spectra were recorded on Bruker AVANCE DRX- 300 (300, 100 Hz). Chemical shifts are shown in δ values (ppm.) with tetramethylsilane (TMS) as an internal reference. FEBMS was recorded on JEOLSX 1021/DA-6000 mass spectrometer. Column chromatography was carried using silica gel (Merk 7749).

Extraction and Isolation

Air roots and leaves (1.9 kg) of *Feronia limonia* were first defatted with hexane (3L x 5 times) and then soxheleted with chloroform, ethyl acetate and methanol (3L x 5 times each). The EtOAc extract was then evaporated under vacuum on rotatory evaporator below 50 ^{o}C temperature to yield a brownish mass (54 g). A well-stirred suspension of silica gel (100–150 g in petrol-ether at 60–80 ^{o}C) was poured into column (150 cm long and 50 mm in diameter). When the absorbent was well settled, the excess of petrol-ether was allowed to pass through column. Slurry was made to this mass with silica gel in petrol-ether and was digested to a well stirred column. The column was successively eluted with the hexane, benzene, chloroform, acetone, EtOAc and methanol and their mixtures of increasing polarity. Elution with benzene: acetone (6:4) afforded a yellow powder (0.85 g). Compound gave positive Shinoda test.

Compound:

5, 6, 7, 8, 3', 5'-hexamethoxy flavanone-4'-α-L-rhamnopyranoside; Yellow powder; UV (MeOH) λmax (nm): 225, (3.4), 274 (loge 4.0) and 327 (loge 3.7); IR (KBr) cm^{-1}: 1688, and 1613, 1581, 1510, 1102, 815, 802; ^{1}H-NMR (300 MHz, CDCl3, δ): 5.34(J=12.8, 3.0 Hz, H-2), 3.03(J=17.0, 12.8 Hz, H-3ax), 2.77(J=17.0, 3.0 Hz, H-3eq), 7.02 (2H, s, H-2'/6') 1.08 (3H, d, J = 6.0 Hz, 6"-Me), 3.81 (6H, s, 2xOMe), 4.05, 3.90, 3.97, 3.85 (3H, s, 4x OMe), 4.10-3.10 (m, remaining sugar protons), 4.81 (1H, br s, H-1"); ^{13}C-NMR (100 MHz, $CDCl_3$, δ): 189.9 (s, C-4), 153.4 (s, C-7), 152.6 (s, C-5), 150.0 (s, C-9), 145.68 (C-3'/5'), 141.1 (s, C-6), 138.0 (C-8), 135.87(C-4'), 121.71 (C-1'), 111.4 (C-10), 107.16 (C-2'/6'), 102.76 (C-1"), 79.1 (C-2), 71.61 (C-5"), 71.47 (C-2"), 70.35 (C-3"), 69.84(C-4"), 57.4 (6H, 3'/5'-OMe), 45.8 (C-3), 61.7, 61.6, 61.5, 61.4 (4x OMe), 17.94 (6"-CH3); MS m/z: 566 [M]$^{+}$, 419 [M-Rhm], 403, 240, 179, 147

Results and Discussion

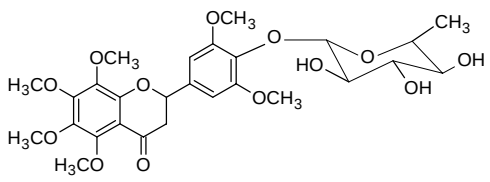
The compound was obtained as yellow amorphous powders. It gave a coloured spot on TLC when examined under UV light and showed positive test for sugar and flavonoid moiety. These suggested that the compound maybe a flavanoid glycoside. The molecular ion peak at m/z 566 [M+H] $^{+}$ in its electro spray mass spectrum corresponded to the molecular formula $C_{27}H_{34}O_{13}$.

Acid hydrolysis of this compound furnished an aglycone, which demonstrated a molecular ion peak at m/z 419 in its mass spectrum, suggesting a flavanone bearing six methoxy groups and a glycoside.

The IR spectrum of this compound showed absorption bands at 1688, and 1613, 1581, 1510, 1102, 815, 802 cm^{-1} assignable to the methoxy, chelated carbonyl, and aromatic ring, while absorption maxima characteristic of the flanvanone structure were observed at 225, (3.4), 274 (loge 4.0) and 327 (loge 3.7) nm in its UV spectrum. Acid hydrolysis of the compound with 1 M HCl furnished L-rhamnose, which was identified by Co-PC with authentic sample.

In the ^{1}H-NMR spectrum, Two double doublet appeared at δ 2.77(J=17.0, 3.0 Hz) and 3.03(J=17.0, 12.8 Hz) were assigned to two H-3 proton of pyron ring. Another one proton double doublet observed at δ 5.34(J=12.8, 3.0 Hz) was assigned to H-2 proton of the pyron ring. These signals are the characteristic flanvanone [18]. In the ^{1}H-NMR spectrum four signals observed at δ 4.05 - 3.85 assigned the position of the four methoxy groups on the A-ring. In the ^{13}C-NMR spectrum, four methoxy signals were observed at lower magnetic field (δ 61.7—61.4). This suggests the presence of substituents at both ortho positions of the four methoxy groups [19], which were assigned to methoxyls on C-5, C-6, C-7 and C-8 of the A-ring. The ^{1}H-NMR of this compound showed six proton singlet at δ 3.87 indicated the presence of two methoxyl groups substituted on an aromatic ring B at C-3' and C-5'[20].

Acid hydrolysis of this compound yielded α-L-rhamnose. The location of the rhamnose moiety at 4'-OH in was concluded through the intrinsic upfield location of H-1" (δ < 5) as a broad singlet at δ 4.81 and 6" -CH3 at δ 1.08 (d, J = 6.0 Hz) [18]. The glycosidation of 4'-OH was confirmed from the alternative α-up/β-downfield effects as upfield of C-4' (135.87, ~ Δ -2 ppm), slight downfield of C-3'/5' (145.68, ~ Δ +1.5 ppm), upfield of C-2'/6' (107.16, ~ Δ -2 ppm) and downfield of C-1' at (120.71, ~ Δ +1.5 ppm) [21].



[5, 6, 7, 8, 3', 5'-hexamethoxy flavanone-4'-α-L-rhamnopyranoside]

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Conclusion

A flavonoid from *Feronia limonia* was isolated. On the basis of spectral data, the compound was identified as 5, 6, 7, 8, 3', 5'-hexamethoxy flavanone-4'-*α*-L-rhamnopyranoside. From literature search, 5, 6, 7, 8, 3', 5'-hexamethoxy flavanone-4'-*α*-L-rhamnopyranoside is the first report from *Feronia limonia*, in which it is a novel compound [18].

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