Isolation of 3,5,7,3',4'-Pentahydroxyflavone - 3-O-α-L-rhamnopyranosyl (1"'→ 6")-*β*-D-glucopyranoside, a Flavonol Glycoside from Citrus Sinensis

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Abstract

The roots of *Citrus sinensis* have yielded a flavonol glycoside. The compound was characterized as 3,5,7,3',4'-Pentahydroxyflavone - 3-O-α-L-rhamnopyranosyl (1"'→ 6")-*β*-D-glucopyranoside on the basis of U.V, I.R, N.M.R (^{1}H, ^{13}C) and mass spectral studies.

Keywords: Rutaceae, *Citrus sinensis*, 3,5,7,3',4'-Pentahydroxyflavone-3-*O*-*α*-L-rhamnopyranosyl (1"'→ 6")-*β*-D-glucopyranoside.

Introduction

Citrus sinensis is one of the important medicinal plants which can be found broadly in the district Shahjahanpur. This plant has been used as an anti-diabetic [1], antimicrobial [2], antifungal [3], hypotensive [4], antioxidating agents [5,6]. Every part of the plant shows various medicinal properties. The leaves and the peels of the fruit can be used to kill mosquito larvae and mites [7]. Essential oil of *C. sinensis* showed larvicidal, repellent and fumigant activities against *Aedes aegypti* L. mosquitoes [8]. Leaf extract of *C. sinensis* can be used in folk medicine to treat neurological disorders and to facilitate the digestion of food [9]. Although an assortment of compounds have been isolated from this plant. However, flavonoids exhibit a broad spectrum of pharmacological properties [10]. The most prevalent flavonoids are hesperidin and naringin (flavonone glycosides), tangeretin and nobiletin (flavon glycosides), present in *Citrus sinensis*. Hesperidin was shown to have anti-inflammatory, antihypertensive, diuretic, analgesic and hypolipidemic properties [11]. It also has antioxidant, anti-allergic, vasoprotective and anti-carcinogenic actions [5, 6]. Nobiletin is a novel anti-inflammatory [12] and immunomodulatory drug [13]. Nobiletin was shown to have anti-proliferative and apoptotic effect on cancer cell lines [14]. There is particular interest on Quercetin because of their broad spectrum of biological activities, including antiproliferative [15], antiallergic, anti-inflammatory, anticancer, antiosteoporotic, antispasmodic, and antihepatotoxic [16], as well as having antioxidative activities [17]. It also showed cytotoxic, antiulcer and gastroprotective effects [18, 19]. Quercetin and its 3-*O*-rutinoside showed several antimicrobial properties [10]. Another flavonoid, Naringin showed cytoprotective, antiulcer [20] and antioxidant activities [21]. Survey of the literature showed that rutin, a flavonoid glycoside found in many plants, e.g. *Sophora japonica* (*Fabaceae*), buckwheat (*Fagopyrum esculentum*, family *Polygonaceae*) and rue (*Ruta graveolens*, family *Rutaceae*), is also known as: Globulariacitrin; lixanthin; Myrticalorin; Osyritrin; Paliuroside; Rutoside; quercetin-3-*O*-(6″-rhamnosyl)-glucoside; Phytomelin; Quercetin-3-rhamnoglucoside; 3-Rutinosylquercetin; Sophorin; Tanrutin; Violaquercitrin; 3-O-Rutinoside quercetin, 3, 3', 4', 5, 7-Pentahydroxyflavone-3-*O*-rutinoside [22, 23].

For the continuation of investigation on the family *Rutaceae* of medicinal plants found in district Muradabad, research on *Citrus sinensis* was selected to be carried out. Previous studies revealed that flavonols are common in *Rutaceae*. Hence the results were collaborated in the study.

Materials and Methods

Plant Material

The roots and leaves of *Citrus sinensis* were collected from the rural areas of Shahjahanpur district in the month of July, 2008, from District Shahjahanpur, India from District Shahjahanpur, India (27.54 L, 79.57 E). 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 material can be used as source for the extraction of secondary plant components. Freshly harvested and dried material are commonly used since dried material are more commonly used since old dried material stored for a period of time may undergo some qualitative changes. Roots and leaves were carefully examined and 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 material (about 1.5 Kg) was

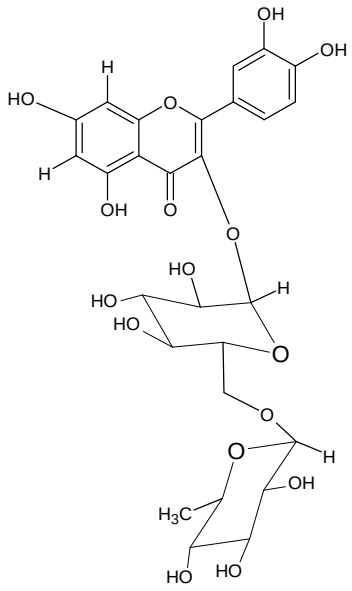
grounded into fine powder and extracted successively with petrol, chloroform, ethyl acetate and methanol.

Instrumentation

Ultra violet 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 MHz). 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

Dried and pulverized roots and leaves (1.5 Kg) of *Citrus sinensis* were first defatted with petrol (3 l x 5 times) and then extracted with chloroform, ethyl acetate and methanol (3 l x 5 times each). The EtOAc extract was then evaporated under vacuum on rotatory evaporator below 50 ^{0}C temperature to yield a brownish mass (53 g). The mass was then subjected to column chromatography. A well-stirred suspension of silica gel (100 -150 g in pet-ether 60-80^0) was poured into column (150 cm x 50 mm, i.d.). When the absorbent was well settled, the excess of petrol was allowed to pass through column. Slurry was made to this mass with silica gel in pet-ether and was digested to well stirred column. The column was successively eluted with the hexane, chloroform, EtOAc and methanol and their mixtures of increasing polarity. Elution with EtOAC: MeOH (11: 3) afforded a yellow powder (0.89 g).



3, 5, 7, 3', 4'-Pentahydroxyflavone- 3-*O*-*α*-rhamnopyranosyl (1"'→ 6")-*β*-glucopyranoside

Compounds

UV λ_{max}: (MeOH) 354, 291, 264 sh, 255, nm, (NaOMe) 412, 328, 330, 272 nm, ($AlCl_3$) 429, 337 sh, 272 nm, ($AlCl_3$/HCl) 401, 354, 301, 267 nm; (NaOAc) 398, 264 nm, (NaOAc/H_3BO_3) 364, 258; IR (KBr) ν_{max}: 3365 and 1652 cm^{-1} ; ^{1}H NMR (300 Hz, $CDCl_3$) δ: 6.26 (1H, d, J=2.1 Hz, H-6), 6.49 (1H, d, J=2.1 Hz, H-8), 7.74 (1H, d, J=2.3 Hz , H-2'), 6.83 (1H, d J= 8.8 Hz, H-5'),7.55 (1H, dd, J=8.8,2.3 Hz, H-6'), 9.71 (1H, s, 4'-OH), 9.39 (1H, s, 3'-OH), 12.68 (1H, s, 5-OH), 10.86 (1H, s, 7-OH), 5.13 (1H, *d*, *J* = 7.6 Hz, H-1″), 4.59 (1H, *d; J* = 1.8 Hz, H-1‴), 3.21-3.74 (4H, *m*, H-2″, H-3″, H-4″, H-5″), 3.20-3.70 (4H, *m*, H-2‴, H-3‴, H-4‴, H-5‴), 3.71 (1H, *dd*, *J* = 10.9 and 4.2 Hz, H-6″ *a*), 3.79 (1H, *dd*, *J* = 10.9 and 4.2 Hz, H-6″ *b*), 1.08 (3H, *d*, *J* = 6.4 Hz, CH_3-6‴); ^{13}C NMR: (300 MHz, $CDCl_3$) 158.2 (C-2), 134.8 (C-3), 178.4 (C-4), 161.3 (C-5), 99.2 (C-6), 166.5 (C-7), 94.7 (C-8), 160.2 (C-9), 105.1 (C-10), 122.4 (C-1'), 116.1(C-2'), 145.7 (C-3'), 148.2 (C-4'), 117.1 (C-5'), 122.2 (C-6'), 102.1 (C-1"), 75.9 (C-2"), 77.4 (C-3"), 71.9 (C-4"), 78.2 (C-5"), 68.8 (C-6"), 102.5 (C-1'"), 72.1 (C-2'"), 72.3 (C-3'"), 73.9 (C-4'"), 71.6 (C-5'"), 18.0 (6'"-CH_3) [rhamnose and glucose moieties, respectively]; MS, m/z (%): 610 $[M+H]^+$ (100), 301 $[A+H]^+$ (40) ; TLC R_F 0.93 in EtOAc: HCO_2H: glacial CH_3CO_2H: H_2O (20:2:2:5).

3,5,7,3',4'-Pentahydroxyflavone: $C_{15}H_{10}O_7$; UV λ_{max}: (MeOH) 371, 257 nm, (NaOMe) 424, 325 decompose., 287 nm, ($AlCl_3$) 445, 362, 272, 252 nm, ($AlCl_3$/HCl) 428, 340, 271 nm, (NaOAc) 388, 310 nm, (NaOAc/H_3BO_3) 386, 326, 262 nm; MS, *mlz* (%): 302 $[M]^+$ (100), 273 (10), 153 (13), 137 (18); ^{1}H NMR (300 Hz, $CDCl_3$) δ: 9.32 (s, 3-OH), 12.46 (s, 5-OH), 10.73 (s, 7-OH), 9.34 (s, 3'-OH), 9.54 (s, 4'-OH), 6.21 (1H, d, J = 2.3 Hz, H-6), 6.37 (1H, d, J = 2.3 Hz, H-8), 6.87 (1H, d, J = 8.2 Hz, H-5'), 7.57 (1H, dd, J = 2.5, 8.2 Hz, H-6'), 7.71 (1H, d, J = 2.5 Hz, H-2'); ^{13}C NMR (300 Hz, $CDCl_3$): 147.9 (C-2) 136.2 (C-3), 176.3 (C-4), 161.5 (C-5), 98.3 (C-6), 164.7 (C-7), 94.4 (C-8), 157.2 (C-9), 103.4 (C-10), 121.1 (C-1'), 116.0 (C-2'), 146.2(C-3'), 148.7 (C-4'), 116.2 (C-5'), 120.6 (C-6')

Results and Discussion

The compound was obtained as yellow powder from the ethyl acetate extract. The compound gave a purple colored spot on TLC, when examined under UV light, characteristic of the flavanoid. The compound showed positive test for sugar and flavonoid moiety suggested that the compound may be a flavanoid glycoside. Compound gave yellow-orange color with $AlCl_3$ and green with $FeCl_3$ suggested that there is a hydroxyl group in the flavonoid at C-5. Further a yellow color produced with $ZrOCl_2$ also suggested that C_3-O- and C_5-OH substituted. In addition to these colors an orange color with lead acetate suggested that C_5-OH is peri to C_4- carbonyl

[24]

The UV spectrum is an impotent tool to distinguish between flavones and flavonols (3-hydroxyflavones). Band I of flavones occurs in the range 304-350 nm whereas band I of flavonols appears at a longer wavelength (352-385 nm). Since the UV spectrum in methanolic solution of our compound exhibited two major absorption bands at 255 and 354 nm, which confirmed the flavonol structure [25]. However, in flavonols with a substituted 3-hydroxy group (methylated or glycosylated), band I appears in the range 328-357 nm, confirmed that C_3-O- is substituted. In 3´, 4´- or 3´, 4´, 5´- oxygenated flavonol usually exhibited two absorption peaks (or one maximum with a shoulder) between 250-275 nm, while the 4´-oxygenated equivalents have only one peak in this range. In UV spectra of the compound a shoulder appeared in band II at 264 nm was indicative of 3', 4'-dihydroxy goups. The free hydroxyl groups at position 5, 7 and 4' were deduced from the bathochromic shift of band I with $AlCl_3$/HCl, of band II with NaOAc and band I with NaOH [26, 27].

The IR spectrum also showed two strong absorption bands at 3365 and 1652 cm^{-1} indicating the presence of hydroxyl and conjugated carbonyl groups, respectively [28].

The ^{1}H NMR spectrum of the compound exhibited signal at δ 12.68 (1H, s) attributed a chelated hydroxyl group (5-OH). Moreover three singlets were observed in the range δ 9.39 - 10.86 (3H, s) assigned to three hydroxyl groups at C-3', 4' and C-7. The ^{1}H NMR spectrum of the compound showed two meta-coupled doublets at δ 6.49 and 6.26, each integrating for one proton, were assigned to H-8 and H-6, respectively of ring A of 5, 7-dihydroxyflavonoids [28]. The ^{1}H NMR also demonstrated a one proton doublet at 7.74 (1H, d, *J* = 2.3 Hz) assignable to H-2', whereas, two protons signals appeared at δ 6.83 (1H, d, *J* = 8.3 Hz) and 7.55 (1H, dd, *J* = 2.3, 8.3 Hz) were assignable to H-5' and H-6', respectively. The appearance of two doublets and one double doublet and their coupling constant values are further in agreement with the hydroxyl groups at C-3'/C-4' [29-31].

The ^{1}H NMR spectra of the compound exhibited signals at δ 5.13 (1H, d, *J*= 7.6 Hz) and 4.59 (1H, d, *J*= 1.8 Hz) applicable for two sugar anomeric protons suggesting the presence of rhamnoglucoside linkage. Usually the anomeric resonances of *α*-glycosides resonate at a downfield position by 0.3-0.5 ppm compared with that of the corresponding *β*-glycosides. Thus, resonances at the lowest yield (4.5-5.5 ppm), which are doublets with $^3J_{1,2}$ in the range 1-4 Hz, are of α-anomeric protons, whereas β-anomeric protons appear as doublets between 4.0 and 4.8 ppm with $^3J_{1,2}$ in the range 6-8 Hz in monosaccharides stereochemistry [29-31].

Signals of other sugar protons appeared at δ 3.20-3.74. The signal at δ 1.08 was assignable to a methyl group of rhamnose. Acid hydrolysis of this compound under reflux condition provided quercetin. The structure of quercetin is proved by the direct comparison of spectroscopic data with that of reported in literature. Sugar was identified as *α*-rhamnose and *β*-glucose moieties, respectively when compared with authentic sample by Co-PC. The anomeric proton signals were consistent with the *β*-configuration of glucose, and α-configuration of a rhamnose (i.e., rutinoside). Thus structure of the compound is characterized as quercetin- 3-O-rutinoside.

This structure was further confirmed by ^{13}C NMR spectral studies [32]. The ^{13}C NMR spectrum of the compound showed a total of twenty seven signals for the carbon atoms. A signal was observed at δ 178.4 was allocated to C-4. Signals observed at δ 166.5, 161.3, 145.7, 148.2 were ascribed for four hydroxyl groups at C-7, C-5, C-3' and C-4' respectively [32]. In ^{13}C NMR two signals observed at δ 99.2 and 94.7 assignable for C-6 and C-8 as in 5,7-dihydroxy flavonols, C-6 resonates at ~5 ppm lower field relative to C-8. The connection (1'''→ 6'') of sugar moiety was confirmed by the chemical shift of the CH_2-6'' (δ 68.8). On the basis of these spectral data the compound was identified as 3, 5, 7, 3', 4'-Pentahydroxyflavone - 3-*O*-α-rhamnopyranosyl (1'''→ 6'')-*β*-glucopyranoside. All these spectral data were in good concurrence with those reported in literature [33] for rutin.

Conclusion

The presence of 3, 5, 7, 3', 4'-Pentahydroxyflavone-3-*O*-*α*-rhamnopyranosyl (1'''→ 6'')-*β*-glucopyranoside from *Citrus sinensi* has not been reported previously and therefore this is the first report of its occurrence in this plant.

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