Dihydroflavonol glycoside from *Citrus sinensis* roots

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Summary

The roots of *Citrus sinensis* have yielded a flavanoid glycoside. The compound was characterized as dihydrokaempferol 7,4'-dimethyl ether-3-O-α-rhamnopyranosyl(1'''→ 6'')-β-glucopyranoside on the basis of U.V, I.R and N.M.R (^{1}H, ^{13}C).

Key words: *Rutaceae, Citrus sinensis, dihydrokaempferol*

INTRODUCTION

Several species of the genus *Citrus* which belongs to the plant family *Rutaceae*, exhibited cytotoxic, analgesic and wound-healing activities. A large number of chemical constituents have been identified from this genus, mainly flavonoids, lignans, coumarins, sitosterol and acridones [1, 9]. Three types of flavonoids have been found to occur in *Citrus* species: flavanones, flavones and flavonols [2]. The flavonoids in *Citrus* usually occur as glycosides, the permethoxylated flavones are exception which occur as free aglycones [2].

Flavonoid glycosides are widely distributed in the plant kingdom. These are of particular interest due to their antioxidant activity through scavenging oxygen radicals, their role in cardiovascular disease, aging, cancer and inflammatory disorders [3].

Citrus sinensis is one of important medicinal plants found broadly in the Shahjahanpur district. This plant is prescribed in indigenous system of medicine for the treatment of various ailments. It has been used as an antidiabetic, antimicrobial

[4], antifungal [5], hypotensive [6], antioxidant [7, 8], carminative, insect repellent, antibacterial, larvicidal, antiviral, uricosuric and antihepatotoxic agent [9]. The leaves and the peel of the fruit have been used to kill mosquito larvae and mites [10]. There are strong evidences that essential oil of *C. sinensis* showed larvicidal, repellent and fumigant activities against *Aedes aegypti* L. mosquitoes [11]. Leaf extracts of *C. sinensis* have been used in folk medicine to treat neurological disorders and to facilitate the digestion of food [12].

Although several compounds obtained from *Citrus sinensis* have been reported [1, 9], among them flavonoids exhibit a broad spectrum of pharmacological properties [13]. The most prevalent flavanones are hesperidin, naringin, tangeretin and nobiletin present in *Citrus sinensis*. Hesperidin was shown to have anti-inflammatory, antihypertensive, diuretic, analgesic and hypolipidemic properties [14]. It also has antioxidant, anti-allergic, vasoprotective and anti-carcinogenic actions [7, 8]. Numerous literatures clearly indicated other pharmacological properties of flavonoids [15-17]. In continuation of our investigation [18-22] on Rutaceous plants found in Shahjahanpur district, phytochemical investigation on *Citrus sinensis* was carried out.

MATERIALS AND METHODS

Plant material

The roots and leaves of *Citrus sinensis* were collected from rural areas of Shahjahanpur district in April 2008. The plant was identified by the Department of Botany of G. F. College (Rohilkhand University), Shahjahanpur, where a voucher specimen has been deposited. Fresh and dried plant material was used as source for the extraction of secondary plant components. 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 a room temperature until they could been easily broken by hand.

Instrumentation

Ultra violet absorption spectrum was recorded on Perkin-Elmer Lambda Bio 20 UV spectrometer. The IR spectroscopy was performed on Perkin-Elmer 1710 infrared fourier transformation spectrometer. The 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. Column chromatography was carried out using silica gel (Merck 7749).

Extraction and isolation

Air dried roots of *Citrus sinensis* (about 1 kg) were at first defatted with hexane (3 lt x 5 times) and then soxheleted successively with chloroform, EtOAc and methanol (3 lt x 5 times each). The EtOAc extract was then evaporated under vacuum on rotatory evaporator below 50°C temperature to yield a brownish mass (60 g). A well-stirred suspension of silica gel (100–150 g in petrol-ether 60–80°) was poured into column (150 cm long and 50 mm in diameter). Slurry of brownish mass was made with silica gel in petrol-ether and 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 $CHCl_3$: MeOH (7:1) afforded a yellow powder (0.23 g). Compound gave positive Shinoda test and alcoholic solution $FeCl_3$ (alc.) gave green color.

Compound

Dihydrokaempferol 7,4'-dimethyl ether-3-O-α-rhamnopyranosyl(1'''→ 6'')-β-glucopyranoside; UV (MeOH) λ_{max}/nm: 336, 309, 289; IR (KBr) ν_{max}/cm^{-1}: 3421 (-OH), 2925 (C-H), 1654(α, β-unsaturated C=O), 1602(C=C), 1493 (C=O, aromatic), and 1108-1018 (glycosidic nature) cm^{-1} ; ^{1}H-NMR (300 MHz, DMSO-d6, δ / ppm): 12.65 (1H, s, 5-OH), 8.06 (2H, *d*, *J* = 8.5 Hz, H-2'/6'), 6.88 (2H, *d*, *J* = 8.5 Hz, H-3'/5'), 6.38 (1H, *d*, *J* = 2.4 Hz, H-8), 6.26 (1H, *d*, *J* = 2.4 Hz, H-6), 5.31 (1H, *d*, *J* = 13.0 Hz, H-2), 5.19 (1H, *d*, *J* = 7.7 Hz, H-1''), 4.58 (d, lH, J= 6. 0 Hz, H-l'''), 4.19 (1H, *d*, *J* = 12.7 Hz, H-3), 3.86 (3H, *s*, 7-OMe), 3.84 (3H, s, 4'-OMe), 1.08 (d, 3H, J = 6.1 Hz, Rham-CH_3); δ 3.22-3.43 (m, rhamno glucosyl protons); ^{13}C NMR (100 MHz DMSO-d_6, δ / ppm): 82.5 (C-2), 69.9 (C-3), 198.4 (C-4), 163.1 (C-5), 97.5 (C-6), 168.3 (C-7), 92.2 (C-8), 161.7 (C-9), 103.8 (C-10), 123.0 (C-l'), 131.6 (C-2' ,6'), 115.4 (C- 3' ,5'), 160.6 (C-4'), 101.6 (C-1''), 74.3 (C- 2''), 75.5 (C-3''), 71.1 (C-4''), 76.0 (C-5''), 68.1 (C-6''), 102.7 (C-l'''), 70.9 (C-2'''), 71.6 (C-3'''), 72.6 (C-4'''), 69.1 (C-5'''), 19.2 (C-6'''), 56.20 (4'-OCH_3), 55.90 (7-OCH_3)

RESULTS AND DISUSSION

The compound was isolated as a yellow powder from ethyl acetate extract. This compound gave a purple spot on TLC, when examined under UV light, showed positive test for sugar and flavonoid moiety suggested that the compound may be a flavanoid glycoside [23].

In IR spectrum of the compound absorption bands were found at 3421 (O–H), 2925 (C–H), 1654 (α, β-unsaturated C=O), 1602 (C=C), 1493 (C=O, aromatic), and 1108—1018 cm^{-1} (glycosidic nature) functionalities. The UV spectrum of the compound showed absorption maxima at 336, 309(sh) and 289 nm characteristic of dihydrokaempferol-3-*O*-substituent [23-25].

In the ^{1}H NMR spectra a one proton singlet observed at δ 12.65 assignable for 5-OH peri to the carbonyl group C-4. The ^{1}H NMR of this compound displayed signals at δ 8.06 (2H, d, J = 8.5 Hz, H-2', 6'), and δ 6.88 (2H, d, J = 8.5 Hz, H-3', 5'), both of which integrated for two protons, assignable to *p*-disubstituted aromatic protons [23-25]. The appearance of two doublets and their coupling constant values are in agreement C-4' was substituted. Furthermore two doublets corresponding to one proton each appeared at δ 6.26 and δ 6.38 were assigned to the *m*-coupled aromatic protons H-6 and H-8. The presence of two methoxy groups at C-4' and C-7 was supported by δ_H 3.86 and δ_H 3.84, δ_c 56.20 and δ_c 55.90 signals in their NMR spectra. In ^{1}H NMR a one-proton doublet appeared at δ 4.19 was assigned to one H-3 proton of pyron ring. Another one-proton doublet observed at δ 5.31 was assigned to H-2 proton of the pyron ring. 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 $^{3}J_{1,2}$ 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 $^{3}J_{1,2}$ in the range 6-8 Hz in monosaccharides stereochemistry [26].

The ^{1}H NMR spectrum of the compound exhibited signals at δ 5. 19 (1H, d, J = 7.7 Hz, H-1") and 4.58 (1H, d, J = 1.8 Hz, H-l'"), assignable to two sugar anomeric protons suggesting the presence of rhamnoglucoside linkage at C-3. The anomeric proton signals were consistent with the β-configuration of glucose, and α-configuration of a rhamnose. In ^{1}H NMR spectra signals observed at δ 1.08 assignable to a methyl group of rhamnose and at δ 3.22-3.43 appeared for other sugar protons of rutinoside. These data suggested the structure of the compound as dihydrokaempferol-3-O-rutinoside.

The ^{13}C NMR data of the compound further supported the presence of a dihydrokaempferol skeleton and the two sugar moieties. The low field signal at δ 198.4 was due to the carbonyl group of C-4. A high field signal at δ 19.2 was due to the methyl group of the rhamnose unit [27]. Two anomeric carbons of glucose and rhamnose appeared at δ 101.6 and 102.7, respectively. A signal at δ 68.1 was due to the methylene group of the glucose unit C-6". Other signals due to the sugar residue occurred at δ 69.1–76.0. From the spectroscopic data and also from the comparison with previously published data for Dihydrokaempferol 7, 4'-dimethyl ether 3-O-rutinoside [24, 26], the connection ((1'"→ 6") of sugar moiety was confirmed by the chemical shift of the CH_2-6" (δ 68.1). On the basis of these spectral data the compound was identified as dihydrokaempferol 7, 4'-dimethyl ether-3-O-α-rhamnopyranosyl(1'"→ 6")-β-glucopyranoside (fig. 1) [26, 27].

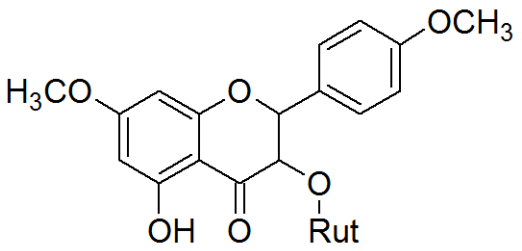

Figure 1. Dihydrokaempferol 7, 4'-dimethyl ether -3-O-α-rhamnopyranosyl (1'''→ 6")-β-glucopyranoside

CONCLUSION

From the survey of the literature [28] it was found that there is only one report for the isolation of dihydrokaempferol from *Citrus sinensis* but its glycoside is a new report according to that species.

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GLIKOZYD DIHYDROFLAWONOLOWY OTRZYMANY Z KORZENI *CITRUS SINENSIS*

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S t r e s z c z e n i e

W korzeniach *Citrus sinensis* znaleziono glikozyd flawonoidowy. Na podstawie badań UV, IR i NMR (^{1}H, ^{13}C) składnik ten zidentyfikowano jako dihydrokamferol 7, 4'-dimetyl eter -3-O-α-ramnopyranozyl (1'''→ 6'')-β-glukopyranozyd.

Słowa kluczowe: *Rutaceae, Citrus sinensis, dihydrokamferol*