Isolation of Isorhamnetin-3-O-α-rhamnopyranosyl (1"'→ 6")-β-glucopyranoside from Calotropis gigantean

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Abstract

Isorhamnetin-3-O-*α*-rhamnopyranosyl (1"'→ 6")-*β*-glucopyranoside, a flavonoid glycoside was isolated from latex of *Calotropis gigantean*. The compound was characterized on the basis of U.V, I.R and N.M.R (^{1}H, ^{13}C) spectral studies.

Key words: Calotropis gigantean, Isorhamnetin - 3-O-*α*-rhamnopyranosyl (1"'→ 6")-*β*-glucopyranoside

Introduction

Calotropis gigantea (Linn.,), commonly known as *Alarka, Raajaarka, Shvetaarka, Vasuka, Mandaar, Bhaasvanmuula, Dinesh, Prabhaakara, Ravi, Bhaanu, Tapana. Madaar, Aak*, which is the native of India[1]. Every part of the plant can be commonly used for the treatment of variety of diseases such as leprosy, ulcers, tumors and piles[2]. The roots and leaves of *Calotropis Gigantea* are used traditionally for the treatment of abdominal tumours, syphilis, leprosy, skin diseases, piles, wounds, rheumatism, insect-bites, ulceration and elephantiasis[3]. Aerial parts of the plant have been reported to possess anti-diarrheal properties[4] and its flowers show stomachic, digestive and analgesic properties[2, 5]. The roots of the plant have activities on central nervous systems[6] in addition to have pregnancy interceptive properties[7]. The chemical constituents of *Calotropis gigantea* have been extensively investigated which include cardenolides[8-11], flavonoids[12], terpenes[13-15], pregnanes[16-17], non-protein amino acid[18] and 19-nor- and 18,20-epoxy-cardenolides[8-22].

Results and Discussion

Compound was isolated as yellow crystals from butanol extract by eluting the column with $CHCl_3$: MeOH (7:1). The compound gave a purple colored spot on TLC when examined under UV light, which can be characterized as flavonoid. The compound had positive results on test for sugar and flavonoid moiety which suggested that the compound maybe a flavonoid glycoside. Compound gave a yellow-orange color with $AlCl_3$ and green color with $FeCl_3$. Furthermore, a yellow color produced when the compound was mixed with $ZrOCl_2$ suggested that the compound was C_3-O- and C_5-OH substituted. In addition, an orange color with lead acetate suggested that C_5-OH is peri to C_4- carbonyl[23]. The MS of this compound showed $[M+H]^+$ peak at m/z 624 corresponding to the molecular formula $C_{28}H_{32}O_{16}$. In the MS of CG-6, the aglycone peak showed at m/z 316. The characteristic fragment ion peaks at m/z 153 and 121 showed the retro Diels Alder fragmentation of flavonoids[24].

UV spectrum of this compound showed absorption bands I and II at 351 and 255 nm, respectively, which were typical signal for a 3-O-flavonol and indicated the attachment of the sugar residue at C-3 position of the aglycone[25]. IR spectrum exhibited absorption bands at 3404 cm^{-1} suggesting the presence of hydroxyl groups in the molecule, between 1601 and 1429 cm^{-1} were verified and attributed to C=C of aromatic ring and a signal at 1655 cm^{-1} characteristic of chelated ketone carbonyl functionalities.

The ^{1}H-NMR spectra displayed signals typical for flavonoid. The ^{1}H NMR spectrum of the compound exhibited signal at δ 12.68 (1H, s) attributed a chelated hydroxyl group (5-OH). Moreover two singlets were observed at δ 9.39 and 10.86 (2H, s) assigned to three hydroxyl groups at C-4' and C-7. The ^{1}H NMR spectrum of the compound showed two meta-coupled doublets at δ 6.41 and 6.20, each integrating for one proton, were assigned to H-8 and H-6, respectively of ring A of 5, 7-dihydroxyflavonoids[26]. In the ^{1}H NMR spectrum of the compound three ABX type signals at δ 7.94 (1H, d, J = 2.0 Hz, H-2'), 7.49 (1H, dd, J = 8.4, 2.0 Hz, H-6'), and 6.91 (1H, d, J = 8.4 Hz, H-5') due to ring B were observed. The presence of the methoxy group at C-3' was supported by δ 3.94 (3H, s) [27].

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[28].

Signals of other sugar protons appeared at δ 3.20 – 3.90. The signal at δ 1.08 was assignable to a methyl group of rhamnose. Acid hydrolysis of this compound under reflux condition provided isorhamnetin. The structure of isorhamnetin is proved by the direct comparison of spectroscopic data with that of reported in literature[29]. 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 isorhamnetin- 3-O-rutinoside. This structure was further confirmed by ^{13}C NMR spectral studies. 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 179.4 which was allocated[30] to C-4. Signals observed at δ 166.5, 162.3, 135.1 were ascribed for three hydroxyl groups at C-7, C-5 and C-4' respectively[30]. 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[31] to C-8. The connection (1"'→ 6") of sugar moiety was confirmed by the chemical shift of the CH_2-6" (δ 68.8). The glycosylation site at C-3 hydroxyl was confirmed through the downfield resonance of C-2 at δ_C 156.5 and the upfield signal[32, 33] of C-3 at δ_C ~ 133.5. On the basis of these spectral data the compound was identified as isorhamnetin-3-O-*α*-rhamnopyranosyl (1"'→ 6")-*β*-glucopyranoside (Figure 1)[27-33].

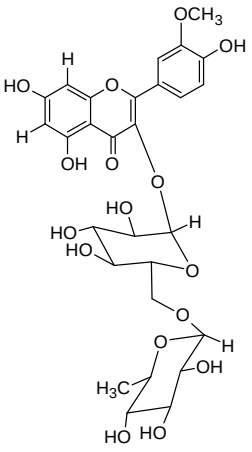

Figure 1 Chemical structure of isorhamnetin-3-O-*α*-rhamnopyranosyl (1"'→ 6")-*β*-glucopyranoside

Experiment

Plant material

Latex of *Calotropis gigantea* were collected from the rural areas of district Rewa and Bhopal at May 2009 and a specimen sample was preserved in the department of research, Jawahar Lal Nehru Cancer Hospital and Research, Bhopal.

Extraction

The dried latex of plant (1.6 kg) was soaked in ethanol at room temperature for two weeks. The filtrate was evaporated under reduced pressure to obtain a dark brown gummy mixture (900 g). The mixture was partitioned with n-hexane, chloroform, acetone, ethyl acetate and n-butanol accordingly to obtain petrol-ether fraction (35 g), chloroform fraction (95 g), ethyl acetate fraction (20 g) and n-butanol fraction (11 g). The alcoholic extracts obtained were decanted in 500 ml distilled water to form water soluble and water insoluble fractions. The water insoluble part (ppt.) after partitioned with benzene was dissolved in methanol to provide 15 g of methanolic extract. Slurry was mixed to methanolic extract with 5 g of silica gel in pet-ether and was added to a well-settled column. The column was successively eluted with the solvents and solvent mixtures of increasing polarity. Elution with $CHCl_3$: MeOH (7:1) afforded yellow crystals.

Compound

Yellow crystals; IR ($CHCl_3$) $\lambda_{max:}$ 3404, 1601, 1429, 1655 cm^{-1}; Mass spectra m/z 624; UV λ_{max}(MeOH) 351, 264 sh, 255, nm; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H, ppm: 12.68 (1H, s, 5-OH), 10.86 (1H, s, 4'-OH), 9.39 (1H, s, 7-OH), 6.41 (1H, s, H-8), 6.20 (1H, s, H-6), 7.94 (1H, d, J = 2.0 Hz, H-2'), 7.49 (1H, dd, J = 8.4, 2.0 Hz, H-6'), 6.91 (1H, d, J = 8.4 Hz, H-5'), 5.13 (1H, d, *J*= 7.6 Hz, H-1"), 4.59 (1H, d, *J*= 1.8 Hz, H-1"'), 3.20 – 3.90 (14 H, m sugar protons), 3.94 (3H, s, 3'-OCH_3),1.08 (3H, d, J = 6.0 Hz, 6"'-CH_3), [rhamnose]; ^{13}C-NMR ($CDC1_3$, 100 MHz): 156.4 (C-2), 133.4 (C-3), 179.4 (C-4), 164.5 (C-5), 99.2 (C-6), 162.3 (C-7), 94.7 (C-8), 157.4 (C-9), 106.3 (C-10), 121.9 (C-1′), 112.4 (C-2′), 149.5 (C-3′), 135.1 (C-4′), 114.5 (C-5′), 124.0 (C-6′), 104.9 (C-1″), 76.2 (C-2″), 77.7 (C-3″), 71.5 (C-4″), 78.5 (C-5″), 68.5 (C-6″), 102.6 (C-1‴), 72.2 (C-2‴), 72.3 (C-3‴), 73.8 (C-4‴), 69.8 (C-5‴), 17.9 (C-6‴), 55.6 (3′-OMe), which was characterized as isorhamnetin-3-O-*α*-rhamnopyranosyl (1"'→ 6")-*β*-glucopyranoside

Conclusion

A flavonoid glycoside was isolated from latex of *Calotropis gigantean* and was characterized as isorhamnetin-3-O-*α*-rhamnopyranosyl (1"'→ 6")-*β*-glucopyranoside.

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