Arabian Journal of Chemistry (2011) **4**, 79–81

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Isolation of a flavone glucoside from *Glycosmis mauritiana* (Rutaceae)

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Received 20 June 2010; accepted 20 June 2010
Available online 25 June 2010

KEYWORDS

Rutaceae;
Glycosmis mauritiana;
Flavone glucoside

Abstract From the ethyl acetate extract of the roots of *Glycosmis mauritiana* a flavone glucoside, luteolin 8-C-β-D-glucopyranoside was isolated. The structure was established by UV, IR, NMR and mass spectral studies.

1. Introduction

Glycosmis mauritiana (syn. *Limonia pentaphylla* Auct; *Glycosmis pentaphylla* Auct. pl.; *Limonia mauritiana* Lam.), belongs to the family Rutaceae, commonly known as Ash-sheora, Orange berry, Rum Berry and Gin Berry. According to Rastogi et al. (1980) there is some perplexity in the name of *Glycosmis mauritiana*. *Glycosmis mauritiana* is native to India, Malaysia, China, Sri Lanka, Myanmar, Thailand, Indonesia and Malaya. *Glycosmis mauritiana* is a small tree or shrub. Fruits have spicy taste. Which ove widely described in old traditional Indian medicines (G.R.I.N. Taxonomy for Plants, 2009; TopTropicals.com-rare plants for home and garden, 2009; Rastogi et al., 1980; Stone, 1985).

As part of our investigations on the chemical investigations of medicinal plants of Rutaceae family found in Shahjahanpur district (Intekhab et al., 2008a,b; Intekhab and Aslam, 2008, 2009), here we are reporting the isolation and characterization of flavone glucoside from ethyl acetate extract of the roots of *Glycosmis mauritiana*.

2. Experimental

2.1. General procedures

Ultra violet absorption spectrum was recorded on Perkin–Elmer Lambda Bio 20 UV spectrometer. IR spectroscopy was performed using the KBr disc method 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 (60–120 mesh). Chemicals are of analytical-reagent grade and TLC Silica gel plates (0.25 mm), were purchased from E-Merck (India).

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doi:10.1016/j.arabjc.2010.06.023

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2.2. Plant material

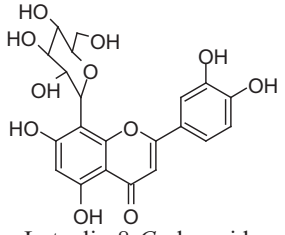
The roots of *Glycosmis mauritiana* were collected from the rural areas of the Shahjahanpur District in the month of September 2008. Authentication was achieved by the comparison with the herbarium specimen deposited in the herbarium of the faculty of botany, G.F. College (Rohilkhand University), Shahjahanpur. Fresh and dried plant material were used as a source for the extraction of secondary plant components. Freshly harvested and dried material is more commonly used since old dried material stored for a period may undergo some qualitative changes.

2.3. Extraction and isolation

Dried, pulverized roots (2.5 kg) of *Glycosmis mauritiana* first defatted with petrol (3 l × 5 times) for the removal of apolar substances and then extracted with chloroform, EtOAc and methanol (3 l × 5 times each). The EtOAc extract was then evaporated under vacuum on rotatory evaporator below 50 °C temperature to yield a brownish mass (68 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). 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 well stirred column. The column was successively eluted with the petrol, chloroform, EtOAc and methanol and their mixtures of increasing polarity. Elution with $CHCl_3$:MeOH (2:8) afforded a yellow powder (1.43 g).

2.4. Compound

Orientin (Luteolin-8-*C*-glucoside; Lutexin) $C_{21}H_{20}O_{11}$: yellow powder, UV $\lambda_{nm:}$ (MeOH) 350, 269, 257, (MeOH–NaOMe) 268, 277, 405, (MeOH–NaOAc) 272, 277, 327, 393), (MeOH–NaOAc–H_3BO_3) 267, 303, 375, 422, (MeOH–$AlCl_3$) 274, 333, 418, (MeOH–$AlCl_3$–HCl) 267, 276, 313, 357, 384; IR (KBr) ν_{max}: 3410 (–OH), 1655 (α, β-unsaturated carbonyl group), 1613 (aromatic C═C) cm^{-1}; ESIMS: 448 $[M]^+$, 430 $[M\text{-}H_2O]^+$, 314 $[M\text{-}C_8H_6O_2]^+$, 299 $[M\text{-aglycone-}CH_2]^+$, 286 $[M\text{-}162]^+$, 134 $[M\text{-}314]^+$, 69 $[M\text{-}379]^+$; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 13.15 (1H, s, 5-OH), 10.82-9.15 (3H, s, 3′, 4′, 7′-OH), 7.52 (1H, dd, J = 2.2, 8.3 Hz, H-6′), 7.45 (1H, d, J = 2.2 Hz, H-2′), 6.90 (1H, d, J = 8.3 Hz, H-5′), 6.65 (1H, s, H-3), 6.56 (1H, s, H-6), 5.05 (1H, d, J = 7.0 Hz), 3.30–3.90 (sugar protons, m). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 164.16 (C-2), 102.41 (C-3), 182.03 (C-4), 160.47 (C-5), 98.33 (C-6), 162.80 (C-7), 104.65 (C-8), 156.00 (C-9), 104.00 (C-10), 121.97 (C-1′), 114.07 (C-2′), 145.95 (C-3′),149.90 (C-4′), 115.78 (C-5′), 119.45 (C-6′), 73.50 (C-1″), 70.90 (C-2″), 78.88 (C-3″), 70.83 (C-4″), 82.04 (C-5″), 61.76 (C-6″); *Rf*: 0.58: EtOAc–HOAc–HCO_2H–H_2O (10:1:1:2.5), 0.67: EtOAc–butanone–HCO_2H–H_2O (5:3:1:1); elemental analysis: Calcd. %C = 54.82, %H = 4.55, %O = 43.64. Found: %C = 54.96, %H = 4.48, %O = 43.75.



Luteolin-8-*C*-glucoside

3. Results and discussion

Compound, a yellow powder, exhibited a molecular ion peak at m/z 448 $[M + H]^+$ in its electro spray mass spectrum corresponded to the molecular formula $C_{21}H_{20}O_{11}$. The compound showed positive ferric chloride and Shinoda tests for flavonoids, suggested that the compound may be a flavonoid (Geissman, 1962; Markham, 1982). These results also suggested that compound is a flavonoid derivative with a free hydroxyl group at C-5 Geissman, 1962.

The UV spectrum in MeOH gave maxima at 269 and 350 nm, respectively, indicating that the compound belongs to the flavone groups. 3′, 4′- or 3′, 4′, 5′-oxygenated flavones usually exhibit two absorption peaks (or one maximum with a shoulder) between 250 and 275 nm, while the 4′-oxygenated equivalents have only one peak in this range (Markham and Mabry, 1994). The methanol UV spectra of this compound showed two peaks at 257 and 269 nm, respectively, indicating that the B-ring contain 3′, 4′- or 3′, 4′, 5′-OH groups. The NaOMe spectrum of the compound was stable for 5 min confirming the absence of 3-OH group. The NaOAc/H_3BO_3 spectrum also indicated for the presence or absence of an ortho-dihydroxy group at all locations of the flavonoid nucleus (Markham and Mabry, 1975; Harborne and Baxter, 1999; Porter, 1988; Mabry and Markham, 1975).

Flavones containing ortho-dihydroxy group in ring – B shows a consistent 12–30 nm bathochromic shift of band I in the presence of NaOAc/H_3BO_3, diagnostic for the presence of an ortho-dihydroxy group (at C-6, 7 or C-7, 8) in the A-ring. Since this compound produced a significant bathochromic shift (350–375 nm) is diagnostic for the presence of an ortho-dihydroxy group (at C-3′, 4′ or C-4′, 5′) in the B-ring.

The presence of an ortho-dihydroxy group in the B-ring of flavones can be detected by comparison of the spectrum of the flavonoid in the presence of $AlCl_3$ with that obtained in $AlCl_3$/HCl. A hypsochromic shift of 30–40 nm was observed in band I of the $AlCl_3$ spectrum on the addition of acid concluded the presence ortho-dihydroxy group.

The bathochromic shift with $AlCl_3$, i.e. the band I shift from 350 nm, splitting into two bands with peaks at 418 nm 333 nm, indicated the presence of an OH-group in position 5 (Markham and Mabry, 1975; Harborne and Baxter, 1999). After adding the NaOAc, the band shift II by 12 nm (277–269 nm), indicated the presence of OH-group at position 7. After the addition of NaOAc + H_3BO_3 the shift of band I by 25 nm (375–350 nm), indicated the presence of OH-group in positions 3′ and 4′ (Markham and Mabry, 1975; Harborne and Baxter, 1999; Porter, 1988; Porter, 1994).

The IR spectra showed absorption bands at 3410 (–OH), 1655 (α, β-unsaturated carbonyl group), 1613 (aromatic C═C) cm^{-1} functionalities. The ^{1}H NMR spectrum of the compound exhibited signal at δ 13.15 (1H, s) attributed a chelated hydroxyl group. Further four signals observed at δ 10.82–9.15 were due to a phenolic hydroxyl groups. The ^{1}H NMR also demonstrated two one proton doublets at δ 7.45 (1H, d, J = 2.2 Hz) and δ 6.90 (1H, d, J = 8.3 Hz) and one double doublet 7.52 (1H, dd, J = 2.2, 8.3 Hz) assignable to H-2′, H-5′ and H-6′ protons, respectively. The appearance of two doublets and their coupling constant values are further in agreement with the hydroxy groups at C-3′ and C-4′ i.e. a

luteolin moiety as a basic skeleton (Porter, 1988, 1994; Mabry and Markham, 1975; Mabry et al., 1970; Ito et al., 1988).

The ^{1}H NMR displayed one proton singlet at δ 6.65 could be assigned to H-3 proton (Porter, 1994). In addition, the methine carbon signal at δ_c 102.41 was attributed to C-3 in the ^{13}C NMR spectrum, indicating a 5, 7 dihydroxy-flavone. In ^{1}H NMR a one proton singlet appeared at δ 6.56 attributed for H-6 proton. The resonances of the anomeric proton observed in the low-field region in the ^{1}H NMR spectra at δ 5.05 (1H, d, $J = 7.0$ Hz) of the compound, implied that compound was luteolin glucoside.

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 (Mabry et al., 1970).

The structure was further supported by its ^{13}C NMR spectrum (Ito et al., 1988; Agrawal, 1989), which demonstrated a downfield signal at δ 182.03 clearly assigned to carbonyl carbon C-4. The three downfield signals appeared at δ 145. 95, 149.90, 160.47 and 162.80 were assigned to C-3′, 4′, 5 and 7, bearing hydroxyl group. Further, a signal at δ 98.33 assigned to C-6 further supported that hydroxyl group present at C-5 and C-7. The anomeric carbon signaled at δ 73.50 in its ^{13}C NMR spectrum indicating the 8-C-β-D-glucopyranoside structure of the compound (Tomczyk et al., 2002). The position of sugar was concluded to be at C-8-OH based on comparison of ^{1}H and ^{13}C NMR spectra with those reported in the literature of known compound orientin (luteolin 8-C-β-D-glucopyranoside) (Tomczyk et al., 2002).

4. Conclusion

From the survey of the literature to the best of our knowledge luteolin 8-C-β-D-glucopyranoside was previously unknown from *Glycosmis mauritiana* and further examination of the constituents of this plant is currently in progress.

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