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ORIGINAL PAPER

A flavone glycoside from *Glycosmis mauritiana*

Javed Intekhab · Mohammad Aslam

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Abstract A flavone glycoside, 5,7,4′-trihydroxy-3′,5′-dimethoxyflavone-6-C-α-L-arabinopyranoside-8-C-β-D-glucopyranosyl(1 → 2″)-glucopyranoside was isolated from the aerial parts of *Glycosmis mauritiana*. The structure was determined based on ultraviolet (UV), infrared (IR), and ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectral data.

Keywords *Glycosmis mauritiana* · Flavonoid · Flavone glycoside

Introduction

Glycosmis mauritiana (syn. *Limonia pentaphylla* Auct., *Glycosmis pentaphylla* Auct., *Limonia mauritiana* Lam.), commonly known as ash-sheora, orange berry, rum berry, and gin berry, is native to India, Malaysia, China, Sri Lanka, Myanmar, Thailand, and Indonesia. *Glycosmis mauritiana* is a small tree or shrub, widely used in Hindu medicine [1–3]. Plants of this genus are used as a traditional medicine for treatment of various diseases [4]. The genus *Glycosmis* (family Rutaceae) is a rich source of quinolone, quinazoline, furoquinoline, carbazole, acridone types of alkaloids, and also sulfur-containing amides, coumarins, and flavonoids [4–7].

As part of our investigations on the chemical constituents of medicinal plants of the Rutaceae family found in the Shahjahanpur District [8–10], we report herein the isolation and characterization of a flavone glycoside from the ethyl acetate extract of the aerial parts of *Glycosmis mauritiana*. During the course on an investigation of the chemical constituents of *Glycosmis mauritiana*, four flavone glycosides were isolated from the ethyl acetate extract of the aerial parts of the title plant.

J. Intekhab (✉) · M. Aslam
Natural Products Research Division,
Post Graduate Department of Chemistry,
G. F. College (Rohilkhand University),
Shahjahanpur 242001, UP, India
e-mail: Javed786khan_khan@yahoo.com

Results and discussion

The compound was obtained as yellow crystals from the ethyl acetate extract by eluting the column with benzene:chloroform (3:14). The compound gave a positive Shinoda test [11], and an alcoholic solution of the compound gave green color with ferric chloride, indicative that the compound was a flavonoid with a free hydroxyl function at C-5 [12]. Its molecular ion peak obtained in its mass spectra at $m/z = 786$ corresponds to the molecular formula $C_{34}H_{42}O_{21}$. The UV spectrum of the compound exhibited absorption maxima at 272 and 333 nm, characteristic of flavonoids [11–13, 15]. Band I (300–380 nm) in MeOH of the compound appears at 333 nm, indicating that the compound belongs to the flavone family unsubstituted at 3-position [11–15]. Addition of NaOMe to a methanol solution produced a bathochromic shift of band I by 68 nm (from 333 to 401 nm), with an increase in absorption intensity, confirming the presence of a free 4′-hydroxy group in the compound [11–15]. The UV spectra of the compound showed a single peak at 272 nm, indicating that the B ring contains only 4′-OH group. The NaOMe spectrum of the compound was stable for 5 min, confirming the absence of a free 3-OH group. The addition of NaOAc to a MeOH solution of the compound produced an increase in absorption intensity, indicating the presence of a free 7-hydroxy group. The disappearance of band I (300–380 nm)

in the $NaOAc/H_3BO_3$ spectra of the compound indicated the absence of *ortho*-dihydroxy groups in both rings. The $AlCl_3$ and $AlCl_3$/HCl test produced no significant shift, in agreement with the absence of *ortho*-dihydroxy groups in the compound [11, 12]. The IR spectrum showed the presence of a carbonyl group at 1,654 cm^{-1} and hydroxy groups at 3,448 cm^{-1}.

The ^1H NMR spectra of the compound displayed a signal at $\delta = 12.98$ ppm assignable to a strongly bonded phenolic hydroxyl group [11–16]. A singlet at $\delta = 6.68$ ppm was assigned to the H-3 proton of the pyron ring [12–16]. A singlet signal of two protons at $\delta = 7.26$ ppm was assigned to H-2′ and H-6′ in the 3′,4′,5′-trisubstituted phenyl ring. The ^1H NMR of this compound showed a six-proton singlet at $\delta = 3.81$ ppm, indicating the presence of two methoxy groups substituted on aromatic ring B at C-3′ and C-5′. In the ^1H NMR spectrum of the compound, three anomeric proton signals observed at $\delta = 4.78$ ppm ($J = 9.9$ Hz), 4.08 ppm ($J = 9.2$ Hz), and 4.83 ppm ($J = 9.4$ Hz) clearly indicated the presence of two glucopyranose and one arabinopyranose moieties in the molecule. The NMR data clearly indicated the presence of two hexose sugars linked together. Their coupling constant values clearly indicated the existence of two β-glucopyranosyl and one α-L-arabinopyranosyl moieties [15, 16]. The anomeric carbon signals at $\delta = 71.50$ (C-1″), 104.89 (C-1‴), and 74.2 ppm (C-1⁗) in its ^{13}C NMR spectrum indicated the 8-C-β-D-glucopyranosyl(1 → 2″)-glucopyranoside and 6-C-α-L-arabinopyranoside structure of the compound. The chemical shift of C-4 in α-L-arabinopyranose is in the range of $\delta = 68$–71 ppm, while in β-L-arabinofuranose, C-4 appears at about $\delta = 85$ ppm. In the ^{13}C NMR spectrum the upfield shift at $\delta = 109.1$ and 104.2 ppm revealed that the C-6 and C-8 positions of the flavonoid were glycosylated [15–17]. The ^1H-^{13}C long-range heteronuclear multiple-bond correlations (HMBC) of $\delta_H = 4.83$ ppm (H-1, ara) to $\delta_C = 109.13$ ppm (C-6) and $\delta_H = 4.78$ ppm (H-1, glc I) to $\delta_C = 104.22$ ppm (C-8) also support the attachment of a glucoside moiety to this compound at C-6 and C-8. An additional long-range correlation was noted between $\delta_H = 4.78$ ppm (H-1, Glc I) and $\delta_C = 74.31$ ppm (C-1, Glc II). These observations supported a diglycosidic saccharide structure at C-8 for this compound, having an inner glucose and one arabinose moieties. The spectral analysis, on comparison with literature, confirmed the presence of an interglucosidic linkage (1⁗ → 2‴) in the compound [17–19].

The ^{13}C NMR spectrum demonstrated a downfield signal at 181.50 ppm, clearly assignable to carbonyl carbon C-4 of the pyron ring. Another signal observed at 103.32 ppm was indicative of C-3. The three downfield signals appearing at $\delta = 159.20$, 161.62, and 139.93 ppm were assigned to C-5, C-7, and C-4′ carbon atoms bearing hydroxyl groups [17–19].

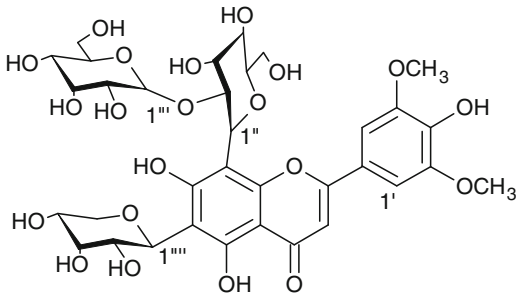

Fig. 1 5,7,4′-Trihydroxy-3′,5′-dimethoxyflavone-6-C-α-L-arabinopyranoside-8-C-β-D-glucopyranosyl(1 → 2″)-glucopyranoside

These data suggested the structure of the compound as 5,7,4′-trihydroxy-3′,5′-dimethoxy-6-C-α-L-arabinopyranoside-8-C-β-D-glucopyranosyl(1 → 2″)-glucopyranoside (Fig. 1). This compound has been isolated for the first time in this genus and, to the best of our knowledge, seems to be a new compound. The structures of three other known compounds were identified by comparison of the spectral data (UV, ^1H, and ^{13}C NMR) with literature as arborinine, 1-hydroxy-10-methylacridone, and 1,3-dimethoxy-10-methylacridin-9-one [20–22].

Experimental

The UV absorption spectrum was recorded on a Perkin-Elmer Lambda Bio 20 UV spectrometer. IR spectroscopy was performed on a PerkinElmer 1710 infrared Fourier-transform spectrometer. NMR spectra were recorded on a Bruker Avance DRX-300. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. FEB-MS was recorded on a JEOLSX 1021/DA-6000 mass spectrometer. Column chromatography was carried using silica gel (60–120 mesh). Melting points were determined on a Perfit apparatus. Chemicals of analytical reagent grade were purchased from E-Merck (India).

Plant material

The aerial parts of *Glycosmis mauritiana* were collected from rural areas of the Shahjahanpur District during September. Authentication was achieved by comparison with the herbarium specimen deposited in the herbarium of the Faculty of Botany, G. F. College (Rohilkhand University), Shahjahanpur. Fresh or dried plant material can be 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.

Extraction and isolation

Aerial parts were carefully examined, and old, insect-damaged or fungus-infested parts were removed. The healthy fraction was dried in the laboratory at room temperature in shade. Aerial parts were ground to a fine powder using a mill. The air-dried coarsely powdered mass of the aerial parts of *Glycosmis mauritiana* was soxhleted successively with petroleum ether, ethyl acetate, and finally with methanol. The ethyl acetate extracts were concentrated to obtain a thick mass. A slurry of this mass was made using silica gel in petroleum ether. This slurry was digested over the silica gel column of required weight, well settled in petroleum ether. Each column was eluted with different solvents and their mixtures of increasing polarity. Each fraction of these eluents was scrutinized by thin-layer chromatography (TLC). The similar fractions were pooled together. The compound 5,7,4′-trihydroxy-3′,5′-dimethoxy-6-C-α-L-arabinopyranoside-8-C-β-D-glucopyranosyl (1 → 2″)-glucopyranoside was isolated as yellow crystals from ethyl acetate fraction by eluting the column with benzene:chloroform (3:14).

5,7,4′-Trihydroxy-3′,5′-dimethoxyflavone-6-C-α-L-arabinopyranoside-8-C-β-D-glucopyranosyl(1 → 2″)-glucopyranoside ($C_{34}H_{42}O_{21}$)

Yellow crystals; m.p.: 241–243 °C; UV (MeOH): $\lambda_{max} = 272, 333$ nm; UV (MeOH–NaOMe): $\lambda_{max} = 281.0$, 401.0 nm; UV (MeOH:$AlCl_3$): $\lambda_{max} = 285.3$, 320.1 (sh) nm; UV (MeOH–$AlCl_3$–HCl): $\lambda_{max} = 290.0$, 318.2 (sh), 348.0 nm; UV (MeOH–NaOAc): $\lambda_{max} = 278.1$, 308.6, 382.7 (sh) nm; UV (MeOH–NaOAc–H_3BO_3): $\lambda_{max} = 279.4$, 307.9, 326 (sh) nm; IR (KBr): = 3,448, 1,654 cm^{-1}; ^1H NMR (DMSO-d_6): $\delta = 12.98$ (1H, s, 5-OH), 6.68 (1H, s, H-3), 7.26 (2H, s, H-2′/6′), 3.81 (6H, s, 3′,5′-OMe), 4.78 (1H, d, $J = 9.9$ Hz, H-1″(8-C-glucose)), 4.08 (1H, d, $J = 9.2$ Hz, H-1‴ (2″-*O*-glucose)), 4.83 (1H, d, $J = 9.4$ Hz, 6-C-arabinose), 3.40–4.50 (17H, m, sugar) ppm; ^{13}C NMR (DMSO-d_6): $\delta = 163.43$ (C-2), 103.32 (C-3), 181.50 (C-4), 159.20 (C-5), 109.13 (C-6), 161.62 (C-7), 104.22 (C-8), 155.21 (C-9), 105.30 (C-10), 121.40 (C-1′), 104.84 (C-2′/6′), 149. 98 (C-3′/5′), 139.93 (C-4′), 71.50 (C-1″), 81.15 (C-2″), 78.26 (C-3″), 70.15 (C-4″), 81.65 (C-5″), 60.40 (C-6″), 104.89 (C-1‴), 74.31 (C-2‴), 76.00 (C-3‴), 69.10 (C-4‴), 75.93 (C-5‴), 60.62 (C-6‴), 74.21 (C-1⁗), 68.82 (C-2⁗), 74.90 (C-3⁗), 69.31 (C-4⁗), 70.8 (C-5⁗) ppm; MS: $m/z = 786$.

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