Flavonol Glycosides from *Cadaba glandulosa*

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A new flavonol triglycoside, *rhamnocitrin-3-O-neohesperoside-4′-O-glucoside* was isolated from the ethanol extract of *Cadaba glandulosa* together with two known diglycosides *rhamnocitrin-3-O-neohesperoside* and *rhamnetin-3-neohesperoside.* Characterization of the three compounds was achieved by various spectroscopic methods.

Introduction

The family Capparidaceae (Capparaceae), in Egypt, encompasses 4 genera; *Capparis, Boscia, Cadaba* and *Maerua* (Tackholm, 1974). The family was reported to contain glucosinolates, cyanogenic glycosides, alkaloids, saponins and lupeol triterpenoids (Gibbs, 1974). Different *Cadaba* species were reported to contain alkaloids and sesquiterpene lactones. Cadabicine and cadabicine diacetate (spermidine alkaloids) were isolated from the stem bark of *Cadaba farinosa* (Viqar Uddin *et al.*, 1985, 1987), Stachydrine and 3-hydroxystachydrine from the stem and roots of *C. farinosa, C. fruticosa* and *C rotundifolia* (Viqar Uddin *et al.*, 1975; Yousif *et al.*, 1984) and a betaine-type base called cadabine from the leaves of *C. fruticosa* (Viqar Uddin and Anwar, 1971; Viqar Uddin *et al.*, 1975). Cadabicilone, an eudesmanolide sesquiterpene lactone was isolated from *C. farinosa* (Viqar Uddin *et al.*, 1990).

Some *Cadaba* species were reported as toxic plants, while others were reputed for some medicinal values. The clinical signs in goats fed with *C. rotundifolia* are pronounced depression, diarrhea, frothing at the mouth, dyspnea, ataxia, loss of condition and recumbency (El Dirdiri *et al.,* 1987). *C. juncea* is toxic to the sheep. *C termitaria* caused death in murder trials. On the other hand, *C. farinosa* was reported to relieve general body pain, antidote against poisoning, stimulant, antiscorbutic, purgative , antiphlogistic, anthelmintic, and also for treatment of cough, fever, dysentery and anthrax (Watt and Breyer-Brandwijk, 1962).

Cadaba glandulosa Forssk, (Capparidaceae) is a highly viscid low shrub with small inconspicuous flowers and closely packed glandular-hispid, round leaves. In Egypt, it grows in the Red Sea coastal reign, Gebel Elba and Qena-Qosseir road (Tackholm, 1974). In the Kingdom of Saudi Arabia, it grows in the Red Sea coastal region, Abha, Bisha, Nagran and south Jedda to Madina area as well as between Dahna and Arabian Gulf (Migahid and Hammouda, 1974). *C. glandulosa* was not previously investigated. This prompted to study its constituents.

Results and Discussion

TLC of the chloroform and aqueous fraction of the alcohol extract using **S1** and **S2** revealed the main flavonoidal constituents in the latter as well as two Dragendorff′s positive spots. Trials to isolate Dragendorff′s positive components were unsuccessful. Chromatographic fractionation of this fraction afforded three flavonoids F1, F2 and F3, R_f (**S3**) 0.34, 0.4 and 0.56 and (**S2**) 0.62, 0.53 and 0.27 respectively.

The IR spectrum of **F3** showed strong absorption bands at 3420 (OH), 2970 (C-H), 1650 (C=C, aromatic), and 1620 cm^{-1} (C=O), its reaction (fluorescent yellow in UV with $AlCl_3$) and UV spectral data with diagnostic shift reagents (Mabry *et.al.,*1970) suggested the likely presence of a 3, 7, 4′- trisubstituted flavonol glycoside with free hydroxyl group at 5 position. Two intermediate spots were detected upon mild acid hydrolysis before yielding the aglycone (**S5**) suggesting the probable presence of three sugar moieties. Glucose and rhamnose were detected in the aqueous hydrolysates by paper chromatography; PC (**S4**) and GLC. The mass spectrum (FAB^+) of **F3**

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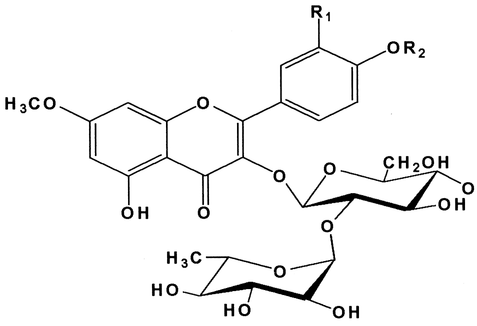

	R_1	R_2
F_1	H	H
F_2	OH	H
F_3	H	Glc.

showed fragments having *m/z* 809 $(M+K)^+$, 793 $(M+Na)^+$, 771 $(M+1)^+$ for $C_{34}H_{42}O_{20}$; FAB^- MS: 607 (loss of glc), 461 (loss of glc and rha), 299 (loss of 2 glc and rha). Fragment *m/z* 167 for $C_8H_7O_4$ in the MS spectrum indicated 5-hydroxy, 7-methoxy substitution in ring A (Crow *et al.*, 1986). NMR experiments further confirmed the previous conclusions. Two anomeric protons at δ 5.70 (1H, d, *J* 7.3 Hz, H-1″) and δ 5.00 (1H, d, *J* 7.2 Hz, H-1‴′) for two glucose units and the signals at δ 5.20 (1H, H-1‴) and δ 0.80 (3H, d, *J* 6.7 Hz, H-6‴) proved the rhamnose moiety. This was further confirmed from **C-13** data (refer to experiment). HMBC correlation of the anomeric proton at δ 5.00 with C-4′ at δ 158.4, proved that this glucose moiety is attached to 4′ position. The other glucose proton at δ 5.70 was correlated with C-3 at δ 135.2. Rhamnose was concluded to be attached to C-2″ of glucose at position-3 from H-H COSY experiment, downfield shift of C-2″ at δ 80.1 and relative upfield shift of C-1″ at δ 100.4. Moreover the chemical shift of carbon atoms of glucose at position-3 and those of rhamnose moiety are comparable with the reported values for neohesperoside (Agrawal and Bansal, 1989). The proton signal at δ 3.80 (3H, OCH_3) was correlated, in HMBC experiment, with C-7 at δ 167.2 confirmed location of the OCH_3 group at that position. The identity of the aglycone was confirmed by co-chromatography against reference sample using (**S1**), mp. 225–226°, and comparison of its UV data with the reported (Lin *et al.*, 1991). Thus, **F3** was concluded to be *rhamnocitrin-3–O-neohesperoside-4′-O-glucoside.* This compound is reported here for the first time.

By mild acid hydrolysis, **F1** produced an aglycone through one intermediate (**S5**) indicating the presence of a glycoside with two sugar moieties. The sugar moieties were proved to be glucose and rhamnose by PC (**S4**) and GLC. The spectral data of this compound displayed close similarity to **F3.** FAB^+-MS gave *m/z* 609 $(M+1)^+$calculated for $C_{28}H_{32}O_{15}$ [the same with FAB^- experiment (M-1) 607]. This indicates that **F1** should **F3** but lack one hexose moiety. A free hydroxyl group at position-4′ was deduced from **UV** data, a bathochromic shift with increased intensity in band **I** with $NaOCH_3$. Moreover a downfield shift of C4′ in C-13 NMR at δ 161.8 confirmed this result. Thus, the identity of compound **F3** was concluded to be *rhamnocitrin-3-O-neohesperoside.* These data are also comparable with those reported for *rhamnocitrin-3-O-neohesperoside* (Walter and Sequin, 1990) which was previously reported in the leaves of *Boscia salicifolia* (Capparidaceae).

Compound **F2**, on mild acid hydrolysis, gave an aglycone through one intermediate (**S5**) indicating a glycoside with two sugar moieties. UV spectral data with diagnostic shift reagents (Mabry *et al.,* 1970) suggested the likely presence of 3, 7-disubstituted flavonol glycoside with free hydroxyl groups at 5, 3′ and 4′-positions. FAB^+ MS displayed *m/z* 625 $(M+1)^+$ calculated for $C_{28}H_{32}O_{16}$. Fragment *m/z* 167 ($C_8H_7O_4$), in the MS spectrum, indicated 5-hydroxy, 7-methoxy substitution in ring A (Crow *et al.*, 1986). Different NMR experiments confirmed this skeleton. The proton signal at δ 3.70 (3H, OCH_3) was correlated with C-7 at δ 164.9 in the HMBC experiment. This again confirmed location of the OCH_3 group at position-7. Glycosidation at position-3 was also concluded from the HMBC correlation between the anomeric proton of glucose at δ 5.70 and C-3 at δ 132.6. Rhamnose was concluded to be attached to C-2″ from H-H COSY experiment, downfield shift of C2″ at δ 77.3 and relative upfield shift of C1″ at δ 97.3, moreover the chemical shifts of carbon atoms of both sugars are comparable with that of neohesperoside (Agrawal and Bansal, 1989). Glucose and rhamnose were detected in the aqueous hydrolysates by PC (**S4**) and GLC and rhamnetin was proved to be the aglycone, co-chromatography against reference sample **S1** and UV data (Nawwar *et al.*, 1980). Thus, the identity of compound **F1** was confirmed as *rhamnetin-3-O-neo-*

hesperoside. This compound was previously isolated from *Derris trifoliata*, *Cassia occidentalis*; Leguminosae (Singh and Singh, 1985; Nair *et al.*, 1986) and *Boscia salicifolia*; Capparidaceae (Harborne and Baxter, 1999).

Experimental

General

Mps uncorr., UV spectra were run in MeOH (Unicam SP 1800 Ultraviolet Spectrophotometer) and IR spectra in KBr discs (Unicam SP 1000 Infrared Spectrophotometer). NMR spectra were run at 600 or 400 MHz (^{1}H) and 150 or 100 MHz (^{13}C) in DMSO-D_6 or CD_3OD using TMS as internal standard. MS was obtained by FAB$^+$ and FAB$^-$ (Jeol JMS MS-700). TlC was done using silica gel chromatoplates; Kieselgel 60 F_{254} (Merck). Solvent mixtures **S1**: CH_2Cl_2 -CH_3OH (9.5 : 0.5 v/v); **S2**: EtOAc-CH_3OH-H_2O (100: 20:10 v/v); silica gel RP-C18, **S3**: H_2O-CH_3OH (4:6 v/v)**,** were used as developers**,** $AlCl_3$ spray. PC using Whatman filter paper No. 1 and solvent mixtures consisted of butanol- benzene- pyridine- water (4:1:3:3 v/v) **S4**, aniline phthalate spray and 15% AcOH solvent, $AlCl_3$ spray **S5**.

Plant material

Aerial parts of *C. glandulosa* were collected from Kolais area, 40 m above sea level, between Jedda and Mekka, Kingdom of Saudi Arabia, 1995 by Prof. Dr. Mohammed A. Al-Yahya. Dr. Sultan Ul-Abidin, taxonomist of the faculty of Pharmacy King Saud University confirmed the identity of the plant. A voucher specimen has been deposited at the herbarium of the department of Pharmacognosy, KSU.

Extraction

700-g powdered aerial parts were homogenized with 5-l ethanol (90%). The filtered extract was evaporated in vacuum to yield 23.5 g residue, which was partitioned between H_2O and $CHCl_3$ (400 ml, each), to yield 6 g and 17 g for the $CHCl_3$ and H_2O fraction, respectively.

Isolation of the flavonoids

The aqueous fraction (17 g) was loaded on a silica gel column (200 g). Elution was started with CH_2Cl_2 (4 × 450 ml fractions were collected), 10% methanol mixture (16 × 60 ml), followed by $CHCl_3$ – CH_3OH – H_2O (350:115:35v/v/v) and 25 × 20 ml fractions were collected. Fractions 2–8 (127 mg), of the last solvent mixture, contained a single major flavonoid **F1**, Fractions 12–13 (85 mg) contained the major flavonoid **F2** and fractions 15–23 (72 mg) contained the major flavonoid **F3**. Preparative TLC using S**2** and recrystallization from methanol afforded **F1** (35 mg), **F2** (21 mg) and **F3** (25 mg).

Rhamnocitrin-3-O-neohesperoside-4′-O-glucoside **F3**: Yellow powder; FAB$^+$-MS: *m/z* 809 $(M+K)^+$, 793 $(M+Na)^+$, 771 $(M+1)^+$, $C_{34}H_{42}O_{20}$; FAB$^-$: 607 (loss of glc), 461 (loss of glc and rha), 299 (loss of 2 glc and rha), 167 ($C_8H_7O_4$). UV λ_{max}: CH_3OH 341, 316^{sh}, 267; +$NaOCH_3$ 348, 282; +$AlCl_3$ 369, 303^{sh}, 277, 230^{sh}; +HCl 389, 303^{sh}, 277, 230^{sh}, + NaOAc 348, 267, +H_3BO_3 348, 267. IR ν_{max} KBr cm^{-1}: 3420 (br), 2970, 2940, 1650, 1620, 1450, 1300, 1270, 1167, 1075, 880. ^{1}H-NMR 400 MHz (CD_3OD): δ 8.10 (2H, d, *J* 9.0 Hz, H-2′, 6′), δ 7.10 (2H d, *J* 9.6 Hz, H-3′, 5′), δ 6.30 (d, *J* 2.1 Hz, H-6), δ 6.60 (d, *J* 2.1 Hz, H-8), δ 3.80 (3H, OCH_3), δ 5.70 (d, *J* 7.3 Hz, H-1″), δ 3.09 H-2″, δ 3.28 H-3″, δ 3.12 H-4″, δ 3.39 H-5″, δ 3.48, 3.54 (2H, H-6″), δ 5.20 H-1‴, δ 3.20 H-2‴, δ 3.46 H-3‴, δ 3.08 H-4‴, δ 3.73 H-5‴, δ 0.80 (3H, d, *J* 6.7 Hz H-6‴), δ 5.00 (d, *J* 7.2 Hz, H-1‴′), δ 3.28 H-2‴′, δ 3.38 H-3‴′, δ 3.12 H-4‴′, 3.42 H-5‴′, δ 3.68, 3.72 (2H, H-6‴′). ^{13}C-NMR 100 MHz (CD_3OD): δ 158.2 C-2, δ 135.2 C-3, δ 179.5 C-4, δ 162.9 C-5, δ 99.0 C-6, δ 167.2 C-7, δ 93.1 C-8, δ 161.1 C-9, δ 106.9 C-10, δ 125.9 C-1′, δ 131.9 C-2′, 6′, δ 117.2 C-3′, 5′, δ 158.4 C-4′, δ 56.4 OCH_3, δ 100.4 C-1″, δ 80.1 C-2″, δ 77.9 C-3″, δ 71.8 C-4″, δ 78.9 C-5″, δ 62.6 C-6″, δ 101.8 C-1‴, δ 69.9 C-2‴, δ 72.4 C-3‴, δ 74.0 C-4‴, δ 69.9 C-5‴, δ 17.6 C-6‴, δ 100.7 C-1‴′, δ 74.8 C-2‴′, δ 78.2 C-3‴′, δ 71.3 C-4‴′, δ 78.4 C-5‴′, δ 62.5 C-6‴′. *Rhamnocitrin:* UV λ_{max}: CH_3OH 371, 258; + $NaOCH_3$ 401,265; + $AlCl_3$ 412, 355^{sh}, 303^{sh}, 275; +HCl 412, 356^{sh}, 300^{sh}, 273; + NaOAc, 375, 260, 218; + H_3BO_3 373, 262, 220.

Rhamnocitrin 3-O-neohesperoside **F1**: Yellow powder; Mp 162°; FAB$^+$ MS: *m/z* 631 $(M+Na)^+$, 609 $(M+1)^+$, 463 (loss of rha), 301 (loss of rha and glc), 167 ($C_8H_7O_4$); FAB$^-$ MS: *m/z* 699 (M+glycerol), 607 (M-1), 299 (loss of rha and glc). UV λ_{max}: CH_3OH 347, 267, 205; +$NaOCH_3$ 400, 353^{sh}, 302^{sh}, 275; +$AlCl_3$ 400, 348, 303^{sh}, 270, 234^{sh};

+HCl 400, 347, 302^{sh}, 274, 235^{sh}; + NaOAc 366, 348, 267, 214; +H_3BO_3, 348, 267, 217. IR ν_{max} KBr cm^{-1}: 3400 (br), 2970, 2940, 1650, 1600,1450, 1300,1270,1167, 1075,880. ^{1}H-NMR 600 MHz (DMSO-d_6): δ 8.00 (2H, d, *J* 9.6 Hz, H-2′, 6′), δ 6.80 (2H, d, *J* 9.6 Hz, H-3′, 5′), δ 6.30 (H-6), δ 6.70 (H-8), δ 3.70 (3H, s, OCH_3), δ 5.60 d, *J* 7.8 Hz, H-1″, 3.40 H-2″, δ 3.10 H-3″, δ 3.60 H-4″, δ 3.10 H-5″, 3.20–3.50 (2H, H-6″), δ 5.00 H-1‴, δ 3.66 H-2‴, δ 3.70 H-3‴, δ 3.40 H-4‴, δ 3.25 H-5‴, δ 0.70 d, *J* 6.0 (3H, H-6‴). ^{13}C-NMR 150 MHz (DMSO-D_6): δ 156.2 C-2, δ 132.6 C-3, δ 177.2 C-4, δ 164.0 C-5, δ 97.3 C-6, δ 164.9 C-7, δ 93.8 C-8, δ 158.4 C-9, δ 105.6 C-10, δ 122.8 C-1′, δ 130.7 C-2′, 6′, δ 115.2 C-3′, 5′, δ 161,8 C-4′, δ 54.8 OCH_3, δ 97.3 C-1″, δ 77.3 C-2″, δ 76.2 C-3″, δ 70.5 C-4″, δ 76.8 C-5″, δ 60.7 C-6″, δ 100.2 C-1‴, δ 70.0 C-2‴, δ 70.2 C-3‴, δ 70.4 C-4‴, δ 68.2 C-5‴, δ 17.2 C-6‴.

Rhamnetin 3-O-neohesperoside **F2**: yellow powder; Mp. 210–211°; FAB$^+$-MS: *m/z* 663 (M+K)$^+$, 647 (M+Na)$^+$, 625 (M+1)$^+$, $C_{28}H_{32}O_{16}$; 477 (loss of rha), 317 (loss of rha and glc); UV λ_{max}: CH_3OH 357, 307, 256; +$NaOCH_3$ 403, 271; +$AlCl_3$ 427, 335, 272; +HCl 400, 357, 270; + NaOAc 372, 260; +H_3BO_3 371, 261. IR ν_{max} KBr cm^{-1} 3400 (br), 2950, 2922, 1652, 1600, 1456, 1300, 1210, 1147, 1067, 1050, 878 and 800. ^{1}H-NMR 400 MHz (CD_3OD): δ 6.30 (1H, d, *J* 2.1 Hz, H-6), δ 6.50 (1H d, *J* 2.1 Hz, H-8), δ 6.80 (1H, d, *J* 0.8 Hz, H-2′), δ 7.60 (1H, d, *J* 10 Hz, H-5′), δ 7.60 (1H, dd, *J* 10.0, 0.8 Hz, H-6′), δ 3.9 (3H, OCH_3), δ 5.70 (1H, d, *J* 7.6, Hz, H1″), δ 3.40 H-2″, δ 3.10 H-3″, δ 3.50 H-4″, δ 3.10 H-5″, δ 3.20–3.50 (2H, H-6″), δ 5.20 H-1‴, δ 3.60 H-2‴, δ 3.70 H-3‴, δ 3.40 H-4‴, δ 3.20 H-5‴, δ 0.90 d, *J* 6.0 (3H, H-6‴). ^{13}C-NMR: 100 MHz (HD_3OD): δ 158.6 C-2, δ 135.1 C-3, δ 179.8 C-4, δ 163.3 C-5, δ 99.2 C-6, δ 167.4 C-7, δ 93.3 C-8, δ 159.2 C-9, δ 107.2 C-10, δ 123.6 C-1′, δ 116,4 C-2′, δ 146.4 C-3′, δ 150.1 C-4′, δ 117.6 C-5′, δ 123.6 C-6′, δ 56.8 OCH_3, δ 100.7 C-1″, δ 80.5 C-2″, δ 78.7 C-3″, δ 72.0 C-4″, δ 79.3 C-5″, δ 62.9 C-6″, δ 103.1 C-1‴, δ 72.7 C-2‴, δ 74.3 C-3‴, δ 72.7 C-4‴, δ 70.4 C-5‴, δ 17.8 C-6‴. *Rhamnetin:* UV λ_{max}: CH_3OH 370, 271^{sh}, 258; + $NaOCH_3$ 430,332,285,240; + $AlCl_3$ 450, 332^{sh}, 303^{sh}, 275; +HCl 423, 361^{sh}, 300^{sh}, 265; + NaOAc 420^{sh}, 387, 290, 258; + H_3BO_3 390, 260.

Acid hydrolysis

An alcoholic solution (10 mg), of each glycoside, was refluxed in boiling water bath with an equal volume of 1 N HCl. The solution was monitored by PC (**S5**) at time intervals of 5 min for 1 h. Excess acid was precipitated with Ag_2O, the alcohol evaporated and the aglycone extracted with EtOAc. The sugars in the aqueous solution were examined by PC (**S4**) and GLC and the aglycones were subjected to TLC (**S1**) and UV analysis.

GLC analysis of sugars

The neutral aqueous hydrolysates were silylated with BSFTA/TMS for 15 min at room temperature in pyridine. Silylated sugars were subjected to GLC analysis: column BP5–25 m, 0.25 mm i. d; column temperature 200–300 C°; 5 °C/min; 20 min; detector temperature 300 °C (Fid); helium.

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