PERGAMON

Phytochemistry 52 (1999) 943–946

PHYTOCHEMISTRY

Polyphenolic metabolites of *Rhamnus disperma*

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Received 20 January 1999; received in revised form 27 April 1999; accepted 27 April 1999

Abstract

A new quercetin dirhamnoside has been isolated and identified as quercetin 3,4′-di-*O*-α-L-rhamnopyranoside together with 24 structurally known phenolic metabolites from the fruits and aerial parts of *Rhamnus disperma* for the first time. The known compounds have been characterized as kaempferol 3-*O*-robinoside, 3-*O*-rhamninoside, 4′-*O*-rhamninoside, rhamnocitrin 3-*O*-rhamninoside, 4′-*O*-rhamninoside, quercetin 3-*O*-rhamnoside, 3-*O*-galactoside, 7-*O*-galactoside, 3-*O*-methyl 7-*O*-galactoside, 3-*O*-robinoside, 3-*O*-rhamninoside, rhamnetin 3-*O*-rhamninoside, rhamnazin 3-*O*-robinoside, 3-*O*-rhamninoside, two phenolic acids 2,5-dihydroxybenzoic and protocatechuic acids, a coumarin isofraxetin, three aglycones identified as quercetin 3-methyl ether, eriodictyol and taxifolin, together with five flavonols kaempferol, quercetin, rhamnocitrin, rhamnetin and rhamnazin. The structures of the isolated compounds have been established on the basis of chemical and NMR spectroscopic evidence as well as negative ESI-MS in some cases. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Rhamnus disperma; Rhamnaceae; Aerial parts; Fruits; Flavonol rhamninosides; Eriodictyol; Taxifolin; Isofraxetin; ESI-MS; NMR

1. Introduction

Rhamnaceae is a family of 59 genera (about 900 species), including *Rhamnus* which comprises 110 spp. (Trease & Evans, 1989). *Rhamnus* species are known to be rich with anthraquinones (Abegaz & Peter, 1995), flavonol triglycosides (Satake et al., 1993) together with anthracenes (Coskun, 1989), anthrones (Manitto, Monti & Speranza, 1995) or naphthalenes (Lin & Lu, 1995) which were also isolated from some of *Rhamnus* species.

Herein, we report the isolation and structural elucidation of a novel flavonol diglycoside and 24 known phenolic metabolites from an Egyptian *R.* species, namely *R. disperma* Ehrenb. (Täckholm, 1974) (its Arabic name: Za'roor, Areen, Ara or Ern), which had not been examined chemically before.

2. Results and discussion

Through this study, the aqueous ethanol extracts of the fruits and aerial parts were found to contain 25 phenolic metabolites (Table 1).

Each of the dry ground fruits and aerial parts of *R. disperma* were extracted by hot 80% aqueous ethanol under reflux. Both of the extracts were shown by 2D-PC screening to contain a complicated polyphenolic metabolites mixture especially flavonoid glycosides (colours and their changing with ammonia under UV light and their responses towards different spraying reagents, e.g. $FeCl_3$, $AlCl_3$ and naturstoff solns). The fruits extract was fractionated on a polyamide column using H_2O and H_2O–EtOH mixtures with decreasing polarity and then the obtained fractions were subjected to a combination of repeated column chromatography on microcrystalline cellulose and Sephadex LH-20 with aqueous ethanol and/or *n*-BuOH saturated with H_2O

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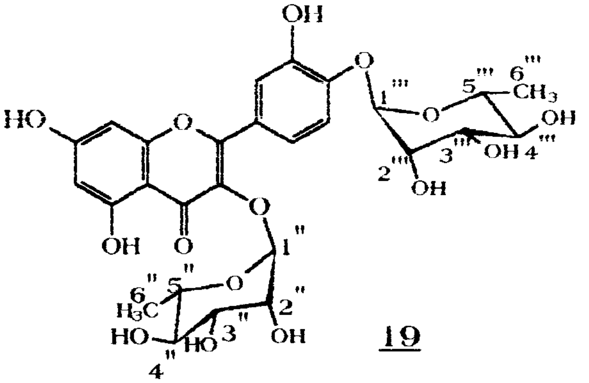
PII: S0031-9422(99)00262-9

Table 1
Phenolic constituents of *R. disperma*[a]

Fruits	Aerial parts
Kaempferol 3-*O*-β-D-robinoside (**1**)	protocatechuic acid (**13**)
Kaempferol 3-*O*-β-D-rhamninoside (**2**)	2,5-dihydroxybenzoic acid (**14**)
Kaempferol 4′-*O*-β-D-rhamninoside (**3**)	isofraxetin (**15**)
Rhamnocitrin 3-*O*-β-D-rhamninoside (**4**)	quercetin 3-*O*-β-D-galactopyranoside (**16**)
Rhamnocitrin 4′-*O*-β-D-rhamninoside (**5**)	quercetin 7-*O*-β-D-galactopyranoside (**17**)
Quercetin 3-*O*-α-L-rhamnopyranoside (**6**)	quercetin 3-methyl ether 7-*O*-β-D-galactopyranoside (**18**)
Quercetin 3-*O*-β-D-robinoside (**7**)	quercetin 3,4′-di-*O*-α-L-rhamnopyranoside (**19**)
Quercetin 3-*O*-β-D-rhamninoside (**8**)	rhamnazin 3-*O*-β-D-robinoside (**20**)
Rhamnetin 3-*O*-β-D-rhamninoside (**9**)	rhamnazin 3-*O*-β-D-rhamninoside (**21**)
Kaempferol (**10**)	quercetin 3-methyl ether (**22**)
Quercetin (**11**)	eriodictyol (**23**), taxifolin (**24**)
Rhamnetin (**12**)	rhamnazin (**25**), (**2**), (**4**), (**8**), (**9**), (**11**), (**12**)

[a] Robinoside = α-L-rhamnopyranosyl-(1 → 6)-*O*-β-D-galactopyranoside, rhamninoside = α-L-rhamnopyranosyl-(1 → 3)-*O*-α-L-rhamnopyranosyl-(1 → 6)-*O*-β-D-galactopyranoside, rhamnocitrin = kaempferol 7-methyl ether, rhamnetin = quercetin 7-methyl ether, rhamnazin = quercetin 7,3′-dimethyl ether.

solvent systems to afford 12 known flavonoids (**1–12**). The aerial parts extract was treated exactly as the fruits extract to give 19 polyphenolic metabolites identified as given in Table 1 among which the novel natural flavonol diglycoside **19**. The structures of the known compounds have been established by conventional methods of analysis and confirmed by negative ESI-MS with in-source CID in some cases, ^1H and ^{13}C NMR spectroscopy.



19

Direct flow injection −ve ESI-MS was used to obtain more information about the structures of the flavonol triosides. Even though in general only the molecular ion is formed, the technically simple induction of fragmentation by the variation of in-source collision-induced dissociations (CID) voltages from −50 to −120 V allows the aglycone and the content of sugar units to be determined very efficiently. During the analysis, $[M-H]^-$ ions were detected as base peaks often without any further fragment for all triosides. However, adducts with chloride $[M+Cl]^-$ and doubly charged $[M-2H]^{2-}$ ions were also observed to detect M_r of these metabolites. Which of these ions appear together with the common $[M-H]^-$ ion depends on the concentrations, the desolvation potential and the concentration of Cl^- in the system (Nawwar, Marzouk, Nigge & Linsheid, 1997). Upon CID, the fragment ions [M-rhamnosyl]$^-$, [M-2 rhamnosyl]$^-$, [M-rhamninosyl]$^-$ (=[aglycone–H]$^-$) and its oxidised ion [M-rhamninosyl-H]$^-$ were recorded, confirming both of the rhamninose and aglycone identities. As examples, ESI-MS results were given in Table 2 for the triosides **2**, **4**, **8** and **9**.

Compound **19**, was obtained as a yellow amorphous powder exhibiting chromatographic properties and a purple fluorescence under UV light, changing to a yellow colour with ammonia suggesting a flavonoid glycoside. On complete acid hydrolysis with 2 N HCl followed by CoPC with the authentic samples, quercetin and L-rhamnose were obtained. Moreover, on UV spectral analysis, this compound gave a typical MeOH spectrum of a quercetin derivative and a bathochromic shift of band II in the NaOAc and $AlCl_3$/HCl spectra indicated the presence of free 7- and 5-OH groups, respectively (Mabry, Markham & Thomas, 1970). The disappearance of the bathochromic shift of band I in the NaOAc spectrum on addition of H_3BO_3 means that **19** contains only one free OH group at the B-ring. On account of the given above data and properties,

Table 2
Negative ESI-MS with in-source CID data of **2**, **4**, **8** and **9**[a]

Compound No.	I	II	III	IV	V	VI	VII	M_r
2	739	775	369	593	447	285	284	740
4	753	789	376	607	461	299	298	754
8	755	791	377	609	463	301	300	756
9	769	805	384	623	477	315	314	770

[a] I = $[M-H]^-$, II = $[M+Cl]^-$, III = $[M-2H]^{2-}$, IV = [M-rhamnosyl]$^-$, V = [M-2 rhamnosyl]$^-$, VI = [M-rhamninosyl]$^-$ and VII = [M-rhamninosyl-H]$^-$ amu.

the structure of **19** was suggested to be quercetin 3,4′-di-*O*-rhamnoside, which was confirmed by ^{1}H NMR.

From the ^{1}H NMR spectrum, a 5,7-dihydroxy A-ring was evidenced by the two *meta*-doublets at δ ppm 6.18 (J = 2.1 Hz) and 6.37 (J = 2.1 Hz) for H-6 and H-8, respectively. In addition, a 3′,4′-dioxygenated B-ring was evidenced from three aromatic resonances located at 7.16 (1H, d, J = 8.5 Hz), 7.29 (1H, dd, J = 2.1 and 8.5 Hz) and 7.36 (1H, d, J = 2.1 Hz), assigned to H-5′, H-6′ and H-2′, respectively. The downfield shift of H-5′ in correspondence with quercetin itself confirms the *O*-glycosylation at 4′-position of the B-ring. The attachment of the two *O*-α-rhamnosyl moieties at C-3 and C-4′ of the aglycone were deduced from the two anomeric proton resonances at 5.38 (1H, d, J = 1.5 Hz) and 5.16 (1H, d, J = 1.5 Hz) which were assigned to H-1″ and H-1‴, respectively. Finally, the *O*-α-configuration and $^1C_4$-pyranose conformation of the two rhamnoside moieties were proved by the two resonances at 4.0 (1H, dd, J = 1.5 and 3.3 Hz, H-2″) and 3.92 (1H, dd, J = 1.5 and 3.3 Hz, H-2‴) together with a dd resonance at 3.75 (J = 9 and 3.3 Hz) identified as H-3″ and the two most upfield resonances at δ 1.12 (3H, d, J = 5.98 Hz) and δ 0.8 (3H, d, J = 5.98 Hz) assignable to the two methyl groups of the two rhamnoside moieties at C-3 and C-4′, respectively. On the basis of this data, **19** was identified as quercetin 3,4′-di-*O*-α-L-$^1C_4$-rhamnopyranoside, which has not been previously reported in nature.

In the present report, it was concluded that both fruits and aerial parts of *R. disperma* elaborate different flavonol glycosides especially those of quercetin (Table 1). On the other hand, fruits are distinguished from the aerial parts by the presence of kaempferol 3- and 4′-glycoside derivatives.

3. Experimental

3.1. Plant material

Fresh aerial parts and fruits of *R. disperma* Ehrenb. were collected from Southern Sinai mountains, Egypt, during September 1996 and authenticated by Dr. L. Boulos, Professor of Botany, NRC, Cairo, Egypt. A voucher specimen is deposited at the herbarium of the NRC.

3.2. Isolation and identification

Fresh aerial parts and fruits of *R. disperma*, dried in the shadow in an air-draft were separately comminuted to powder and exhaustively extracted with hot EtOH–H_2O (4:1). Each of the two concd. extracts was chromatographed on a Polyamide 6S CC (Riedel-De Häen AG, Seelze Hannover, Germany) and eluted by H_2O followed by H_2O–EtOH mixts of decreasing polarity. The two columns were followed by using the long UV light and 2D-PC to give 8 major flavonoid frs in the case of the fruits extract and 12 frs from the aerial parts extract. The obtained major flavonoid frs from the two extracts were subjected to a combination of repeated column chromatography on microcrystalline cellulose for CC (E. Merck, Darmstadt, Germany) and Sephadex LH-20 with 80% aq. EtOH and/or *n*-BuOH saturated with H_2O solvent systems to afford 12 pure known flavonoids (**1**–**12**) and 19 polyphenolic metabolites (**13**–**25** and **2**, **4**, **8**, **9**, **11** and **12**), respectively.

3.3. UV analysis

Chromatographically pure materials dissolved in analytically pure MeOH were subjected to UV spectroscopy in a 4 ml quartz cell (1 cm optical pathway) using a shimadzu UV-240 spectrophotometer. In the case of flavonoids, all shift reagent spectra were measured by adding the reagents separately to the methanolic soln of the investigated material (Harborne, Mabry & Mabry, 1975).

3.4. NMR analyses

NMR analyses were measured on a JEOL EX-270 MHz spectrometer. ^{1}H NMR resonances were measured relative to TMS and ^{13}C NMR resonances to DMSO-d_6 and converted to TMS scale by adding 39.5. Typical conditions: spectral width = 4000 Hz for ^{1}H and 17500 Hz for ^{13}C, 32 K data points and a flip angle of 45°.

3.5. Negative ESI-MS spectrometry

Negative ESI-MS spectrometry were measured on a double focusing sector field mass spectrometer MAT 95 (Nawwar et al., 1997) (Finnigan, Bremen, Germany) equipped with a Finnigan ESI II ion source (at ISAS-Institut Dortmund, Germany). A soln of the compound was directly injected by a syringe Harvard infusion pump, into the sprayer of the ES source

Table 3
R_f-values and UV spectral data of **19**[a]

R_f (×100)		UV spectral data, λ_{max} (nm)					
solvent-c	d	I	II	III	IV	V	VI
37.5	48.4	205	272	254	203	202	229
		254	373	264	237	235	272
		264		342	270	270	376
		340			310,360	315sh, 360	

[a] I = MeOH, II = I + NaOAc, III = II + H_3BO_3, IV = I + $AlCl_3$, V = IV + HCl and VI = I + NaOMe.

Table 4
^{1}H NMR spectral data of **19** (δppm, in DMSO-d_6, room temp.)[a]

Aglycone moiety		Rhamnoside moieties			
H-6	6.18 d (2.1 Hz)	H-1″	5.38 d (1.5 Hz)	H-1‴	5.16 d (1.5 Hz)
H-8	6.37 d (2.1 Hz)	H-2″	4.0 dd (1.5, 3.3 Hz)	H-2‴	3.92 dd (1.5, 3.3 Hz)
H-5′	7.16 d (8.5 Hz)	H-3″	3.75 dd (9, 3.3 Hz)		
H-6′	7.29 dd (8.5, 2.1 Hz)	H-6″	1.12 d (5.98 Hz)	H-6‴	0.80 d (5.98 Hz)
H-2′	7.36 d (2.1 Hz)	H-3‴, H-4″, H-4‴, H-5″, H-5‴	3.1-3.6 m		

[a] *J* values in Hz are presented in parentheses.

(0.5–1 μl min^{-1}). MeOH as sheath-liquid flow (5 μl min^{-1}) was also fed into the sprayer through an aluminised fused silica capillary (MAT 95, i.d. 0.21 mm) while SF_6 with a flow ca. 9 ml min^{-1}, was fed to the spray chamber sample through the outer capillary, to prevent plasma formation during the production of negative ions. In the ion source the desolvation capillary (i.d.: ca. 0.5 and l = 100 mm) was heated to an appropriate temp. for declustering and desolvation (T = 200–280°C) depending on the nature of the sample. The ESI voltage was 3 kV above the acceleration potential of the spectrometer (ca. 5000 V). The desolvation capillary/first skimmer voltage was about 0–80 V, increased up to 500 V for initiating collision induced dissociations (CID).

3.6. PC-Chromatography

PC-Chromatography was carried out on Whatman No. 1 paper, using solvent systems: (a) H_2O; (b) HOAc–H_2O, 3:47; (c) HOAc–H_2O, 3:17; (d) n-BuOH–HOAc–H_2O, (4:1:5, top layer) and (e) C_6H_6–n-BuOH–H_2O–pyridine (1:5:3:3, top layer) for sugar analysis.

Quercetin 3,4′-di-*O*-α-L-$^1C_4$-rhamnopyranoside (**19**). Yellow amorphous powder (11 mg.), dark purple under UV, dark yellow under UV/NH_3. R_f's and UV spectral data: Table 3. A small amount of **19** (6 mg) was hydrolysed with 2 N HCl–MeOH, under reflux at 100°C for 2 h to yield L-rhamnose (R_f-value on silica gel 0.33, in $CHCl_3$–MeOH–H_2O) and quercetin (CoPC); ^{1}H NMR, in DMSO-d_6 Table 4.

Acknowledgements

The authors express their deep gratitude to the Deutsche Forschungs-gemeinschaft for financial support to M.A.M.N. They are also greatly indebted to W. Nigge (Institut für Spektrochemie, Dortmund, Germany) for help with the CID-ESI-MS measurements.

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