Flavonol Triglycosides from the Leaves of *Hammada scoparia* (POMEL) ILJIN

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A new flavonol triglycoside, isorhamnetin 3-*O*-β-D-xylopyranosyl-(1‴→3‴)-α-L-rhamnopyranosyl-(1‴→6″)-β-D-galactopyranoside, has been isolated from the leaves of *Hammada scoparia* together with two known compounds, isorhamnetin 3-*O*-β-D-apiofuranosyl-(1‴→2″)[α-L-rhamnopyranosyl-(1⁗→6″)]-β-D-galactopyranoside and isorhamnetin 3-*O*-α-L-rhamnopyranosyl-(1‴→2″)[α-L-rhamnopyranosyl-(1⁗→6″)]-β-D-galactopyranoside. The structures were determined by spectroscopic methods.

Key words *Hammada scoparia*; Chenopodiaceae; flavonol triglycoside; isorhamnetin

Hammada scoparia (POMEL) ILJIN (=*Arthrophytum scoparium* (POMEL) ILJIN=*Haloxylon articulatum* subsp. *scoparium* (POMEL) BATT.=*Haloxylon scoparium* POMEL) (Chenopodiaceae) is a small, highly-branched halophytic shrub distributed in south–east Spain, North Africa and parts of Iran, Turkey, Iraq and Syria (Irano-Turanian region).[1—3] In Tunisia, it is known locally as 'rimth'[4] and used in traditional medicine to treat eye disorders.[5,6] Aqueous extracts of this plant have also been reported to show anti-cancer,[7] antiplasmodial[7] and larvicidal activity,[8] but relatively little has been published on its phytochemistry. This paper describes the isolation and identification of a new flavonol triglycoside found in the leaves of *H. scoparia*, isorhamnetin 3-*O*-β-D-xylopyranosyl-(1⁗→3‴)-α-L-rhamnopyranosyl-(1‴→6″)-β-D-galactopyranoside (**1**), and two known flavonol triglycosides, isorhamnetin 3-*O*-β-D-apiofuranosyl-(1‴→2″)[α-L-rhamnopyranosyl-(1⁗→6″)]-β-D-galactopyranoside (**2**) and isorhamnetin 3-*O*-α-L-rhamnopyranosyl-(1‴→2″)[α-L-rhamnopyranosyl-(1⁗→6″)]-β-D-galactopyranoside (**3**).

Fractionation of an Me_2CO–H_2O (1 : 1) extract of leaves of *H. scoparia* by column chromatography, preparative paper chromatography and semi-preparative HPLC yielded compounds **1**—**3** as yellow powders. Each of these afforded isorhamnetin (3,5,7,4′-tetrahydroxy-3′-methoxyflavone) and sugars (**1**, galactose, rhamnose and xylose; **2**, apiose, galactose and rhamnose; **3**, galactose and rhamnose) on acid hydrolysis (2 M HCl). The UV spectra of **1**—**3** recorded in MeOH were similar to those of isorhamnetin 3-*O*-glycosides,[9] with distinctive maxima at 254 and 354 nm. Use of UV shift reagents[9,10] confirmed that the hydroxyl groups at C-5, C-7 and C-4′ were free and that the hydroxyl group at C-3 was blocked. Atmospheric pressure chemical ionization (APCI)-MS (positive mode) of both **1** and **2** gave a protonated molecule at *m*/*z* 757 and common fragment ions at *m*/*z* 625, 479 and 317, corresponding to loss of pentose $[(M+H)-132]^+$, deoxyhexose $[(M+H)-(132+146)]^+$ and hexose $[(M+H)-(132+146+162)]^+$, respectively. Likewise APCI-MS (positive mode) of **3** gave a protonated molecule at *m*/*z* 771 and fragment ions at *m*/*z* 625, 479 and 317 corresponding to loss of deoxyhexose $[(M+H)-146]^+$, deoxyhexose $[(M+H)-(146+146)]^+$ and hexose $[(M+H)-(146+146+162)]^+$, respectively. These preliminary data indicated that compounds **1**—**3** were isorhamnetin 3-*O*-triglycosides.

The ^{1}H-NMR spectrum of **1** in DMSO-d_6 contained distinctive resonances for one methoxy group and three anomeric protons. Additional glycosidic and aromatic proton resonances were also observed (Table 1). The chemical shift and coupling constant data for the aromatic and methoxy protons together with their corresponding ^{13}C-NMR chemical shifts obtained by heteronuclear single quantum coherence spectroscopy (HSQC) confirmed the identity of the aglycone as isorhamnetin (Table 1). The ^{1}H and ^{13}C resonances of each sugar residue were assigned from double quantum filtered correlation spectroscopy (DQF-COSY), total correlation spectroscopy (TOCSY), HSQC and heteronuclear multiple bond correlation (HMBC) data using the anomeric proton resonances at δ 5.40 (d, J=7.7 Hz), 4.44 (d, J=1.3 Hz) and 4.24 (d, J=7.4 Hz) as starting points (Table 1). These data allowed the glycosidic units to be identified as β-galactopyranose, α-rhamnopyranose and β-xylopyranose, respectively.[11] The interglycosidic linkages and the site of attachment between the glycoside and the aglycone were determined from HMBC data and rotating frame Overhauser enhancement (ROE) connectivities detected in 1D XSROESY experiments.[12] A ^3J(^{1}H, ^{13}C) long-range connectivity (HMBC) between the anomeric proton resonance of β-Gal (δ 5.40) and C-3 (δ 133.0) of the aglycone confirmed the identity of the primary sugar as β-Gal and the site of attachment of the triglycoside as C-3. This was also suggested by the ROE connectivity detected between Gal H-1″ (δ 5.40) and H-2′ of the aglycone (δ 7.98). The ROE connectivies detected between

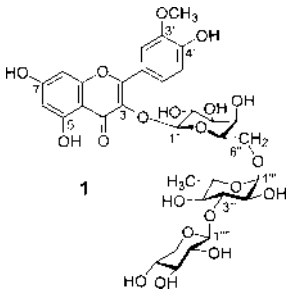

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Table 1. ^{1}H- and ^{13}C-NMR Resonance Assignments for Compound **1** in DMSO-d_6

		δ ^{1}H	δ ^{13}C
	2		156.3
	3		133.0
	4		177.3
	5		161.2
	6	6.16 d (1.7)	98.7
	7		165.1
	8	6.37 d (1.7)	93.7
	9		156.4
	10		103.9
	1′		121.0
	2′	7.98 d (1.8)	113.4
	3′		146.9
	4′		149.4
	5′	6.89 d (8.4)	115.1
	6′	7.50 dd (8.4, 1.8)	121.9
	OCH_3	3.84 s	55.9
Gal	1″	5.40 d (7.7)	101.8
	2″	3.60 m	71.1
	3″	3.45 m	72.9
	4″	3.64 m	67.9
	5″	3.63 m	73.4
	6″	3.63 m	65.2
		3.38 m	
Rha	1‴	4.44 d (1.3)	100.1
	2‴	3.58 m	69.8
	3‴	3.40 dd (9.3, 3.1)	81.0
	4‴	3.32 m	70.8
	5‴	3.45 dd (9.0, 6.1)	67.9
	6‴	1.06 d (6.1)	17.7
Xyl	1⁗	4.24 d (7.4)	105.4
	2⁗	3.03 m	73.8
	3⁗	3.11 m	76.0
	4⁗	3.26 m	69.4
	5⁗	3.63 m	65.5
		2.99 m	

Rha H-1‴ (δ 4.44) and Gal 6″-CH_2 (δ 3.63 and 3.38) characterised the interglycosidic linkage between these sugars as (1‴→6″), a conclusion supported by the downfield shift of Gal C-6″ to δ 65.2 and a ^{3}J(^{1}H, ^{13}C) long-range HMBC connectivity between Rha H-1‴ (δ 4.44) and Gal C-6″ (δ 65.2). An HMBC connectivity observed between the anomeric proton resonance of β-Xyl (δ 4.24) and the downfield-shifted Rha C-3‴ resonance at 81.0 ppm characterised a (1⁗→3‴) linkage between these sugars, as was also confirmed by an ROE connectivity between Xyl H-1⁗ and Rha H-3‴. Further ROE connectivities detected between Xyl H-1⁗ and both Xyl H-3⁗ and Xyl H-5⁗ were as expected for the β-pyranose form of this sugar. The absolute configurations of D for β-Gal and β-Xyl, and L for α-Rha were assumed as those naturally occuring in flavonoid glycosides. Compound **1** was therefore identified as isorhamnetin 3-*O*-β-D-xylopyranosyl-(1⁗→3‴)-α-L-rhamnopyranosyl-(1‴→6″)-β-D-galactopyranoside, a new linear flavonol triglycoside. The molecular formula of $C_{33}H_{40}O_{20}$ determined for **1** by high resolution MS was consistent with this structure. A flavonol triglycoside tentatively identified as isorhamnetin 3-*O*-xylosylrobinobioside was found previously in the leaves of another halophytic shrub, *Nitraria retusa* (Forssk.) Asch. (Zygophyllaceae), by Halim *et al.*[13] However, their preliminary characterisation using only chemical methods did not allow the important interglycosidic linkage between the xylosyl and robinobiosyl (= α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranosyl) units to be determined and the full identity of the compound from this source remains uncertain.

The structures of compounds **2** and **3** were determined independently by the procedures outlined above to be the branched isorhamnetin triglycosides, isorhamnetin 3-*O*-β-D-apiofuranosyl-(1‴→2″)[α-L-rhamnopyranosyl-(1⁗→6″)]-β-D-galactopyranoside and isorhamnetin 3-*O*-α-L-rhamnopyranosyl-(1‴→2″)[α-L-rhamnopyranosyl-(1⁗→6″)]-β-D-galactopyranoside, respectively. Compound **2** was identified recently as a constituent of the aerial parts of *Astragalus vulneraria* DC. (Leguminosae)[14] but has not been reported from any other source. A complete set of ^{1}H- and ^{13}C-NMR resonance assignments for this compound in DMSO-d_6 is given for reference in the Experimental section (the original data were obtained in CD_3OD). Compound **3** was first described from *Primula veris* (Primulaceae),[15] but is present in several other taxa, including *Lysimachia fortunei* (Primulaceae),[16] *Alangium premnifolium* (Alangiaceae),[17] and *Chenopodium pallidicaule*.[18] All three isorhamnetin triglycosides described in the present study are new to *Hammada scoparia*, from which only isorhamnetin 3-*O*-α-L-rhamnopyranosyl-(1‴→6″)-β-D-galactopyranoside (isorhamnetin 3-*O*-robinobioside) had been reported previously.[19]

Experimental

UV spectra were recorded on a Shimadzu 1601 UV–visible spectrophotometer. Spectra were recorded both in MeOH and with the addition of shift reagents.[9,10] ^{1}H- and ^{13}C-NMR spectra were recorded in DMSO-d_6 on Bruker 400 MHz instruments. Standard pulse sequences and parameters were used for the experiments. Chemical shift references were obtained from the solvent resonances of DMSO-d_6 at δ_H 2.50 and δ_C 39.5, relative to tetramethylsilane (TMS). High resolution electrospray ionization (ESI)-MS (positive mode) were obtained on a Bruker Apex II instrument with an internal calibrant. Positive ion APCI-MS were obtained using a quadrupole ion-trap instrument (Finnigan LCQ) as described previously.[20] Analytical and semi-preparative HPLC were carried out using a Waters LC600 pump and a 996 photodiode array detector. A Merck LiChrospher 100RP-18 (250×4.0 mm i.d.; 5 μm particle size) column with a 20 min linear gradient of 25—100% MeOH : HOAc : H_2O (18 : 1 : 1) in 2% aq. HOAc at 1 ml/min was used for analytical HPLC. An identical LiChrospher column but with 10 mm i.d. was used for semi-preparative HPLC with a flow rate of 4.5 ml/min. The column temperature was maintained at 30 °C in both cases.

Plant Material *Hammada scoparia* (Pomel) Iljin was collected in November 1999 at Sfax, Tunisia. The leaves were carefully detached from the fresh plants and air-dried. Voucher specimens (No. LCSN101) have been deposited at the Laboratoire de Chimie des Substances Naturelles, Faculty of Sciences, University of Sfax, Tunisia, and the Royal Botanic Gardens, Kew.

Extraction and Isolation Air-dried leaves of *Hammada scoparia* (2.4 kg) were extracted at room temperature in Me_2CO–H_2O (1 : 1). The filtrate was concentrated under reduced pressure, made alkaline with aqueous ammonia and partitioned with $CHCl_3$ and *n*-BuOH successively, to give extracts A and B. The remaining aqueous layer was acidified to pH 2 with HCl and partitioned with EtOAc and *n*-BuOH successively, giving extracts C and D. Column chromatography of D (35 g) on Sephadex LH-20 (H_2O–MeOH gradient) gave 11 fractions (D.1—D.11). Fraction D.3 (1.6 g) was purified further by preparative paper chromatography (Whatman 3MM) to remove tannins and phenolic acids; first using BAW (*n*-BuOH, HOAc, H_2O, 4 : 1 : 5, v/v, upper layer) and then $CHCl_3$–HOAc–H_2O (15 : 15 : 2). Final purification of the yellow band was achieved by semi-preparative HPLC with a gradient method (solvent A=MeOH, solvent B=H_2O; A=25%, B=75% at t=0 min; A=100% at t=20 min; A=100% at t=25 min and A=25%, B=75% at t=26 min) to give **1** (7 mg) and **3** (4 mg). Fraction D.4 (100 mg) was purified directly by semi-preparative HPLC using the same gradient method to yield **2** (8 mg).

Isorhamnetin 3-*O*-β-D-Xylopyranosyl-(1⁗→3‴)-α-L-rhamnopyranosyl-(1‴→6″)-β-D-galactopyranoside (**1**): UV λ_{max} (MeOH) nm 254, 355; (MeOH+NaOH) 272, 414; (MeOH+$AlCl_3$) 268, 399; (MeOH+$AlCl_3$+HCl) 268, 400; (MeOH+NaOAc) 275, 371; (MeOH+NaOAc+H_3BO_3) 256,

359. ^{1}H- and ^{13}C-NMR: see Table 1. APCI-MS (positive mode) *m/z*: 757 $[M+H]^+$, 625 $[(M+H)-132]^+$, 479 $[(M+H)-(132+146)]^+$, 317 $[(M+H)-(132+146+162)]^+$. HR-ESI-MS *m/z*: 757.2197 $[M+H]^+$ (Calcd for $C_{33}H_{41}O_{20}$, 757.2186).

Isorhamnetin 3-*O*-*β*-D-Apiofuranosyl-(1‴→2″)[*α*-L-rhamnopyranosyl-(1⁗→6″)]-*β*-D-galactopyranoside (**2**): UV λ_{max} (MeOH) nm 254, 354; (MeOH+NaOH) 270, 408; (MeOH+$AlCl_3$) 269, 404; (MeOH+$AlCl_3$+HCl) 270, 400; (MeOH+NaOAc) 274, 366; (MeOH+NaOAc+H_3BO_3) 254, 355. ^{1}H-NMR (DMSO-d_6) *δ*: 7.97 (1H, d, *J*=2.1 Hz, H-2′), 7.53 (1H, dd, *J*=8.5, 2.1 Hz, H-6′), 6.86 (1H, d, *J*=8.5 Hz, H-5′), 6.36 (1H, d, *J*=2.0 Hz, H-8), 6.13 (1H, d, *J*=2.0 Hz, H-6), 3.85 (3H, s, 3′-OCH_3); *β*-D-Gal: 5.58 (1H, d, *J*=7.7 Hz, H-1″), 3.79 (1H, dd, *J*=9.3, 7.7 Hz, H-2″), 3.62 (1H, m, H-4″), 3.62 (1H, m, H-3″), 3.59 (1H, m, H-5″), 3.58, 3.25 (2×1H, 2×m, 6″-CH_2); *β*-D-Api: 5.28 (1H, d, *J*=1.2 Hz, H-1‴), 3.81 (1H, d, *J*=9.3 Hz, 4‴-CH_2A), 3.79 (1H, m, H-2‴), 3.47 (1H, m, 5‴-CH_2A), 3.45 (1H, d, *J*=9.3 Hz, 4‴-CH_2B), 3.40 (1H, m, 5‴-CH_2B); *α*-L-Rha: 4.39 (1H, d, *J*=1.4 Hz, H-1⁗), 3.38 (1H, m, H-2⁗), 3.36 (1H, m, H-5⁗), 3.30 (1H, m, H-3⁗), 3.10 (1H, m, H-4⁗), 1.05 (3H, d, *J*=6.2 Hz, 6⁗-CH_3); ^{13}C-NMR (DMSO-d_6) *δ*: 177.0 (C-4), 164.5 (C-7), 161.1 (C-5), 156.3 (C-9), 155.9 (C-2), 149.3 (C-4′), 146.9 (C-3′), 132.8 (C-3), 121.9 (C-6′), 121.1 (C-1′), 115.0 (C-5′), 113.3 (C-2′), 103.7 (C-10), 99.2 (C-6), 93.6 (C-8), 55.8 (3′-OCH_3); *β*-D-Gal: 98.7 (C-1″), 74.3 (C-2″), 73.4, 73.2 (C3″/C5″), 68.2 (C-4″), 65.0 (C-6″); *β*-D-Api: 108.7 (C-1‴), 79.2 (C-3‴), 75.9 (C-2‴), 74.0 (C-4‴), 64.5 (C-5‴); *α*-L-Rha: 100.0 (C-1⁗), 71.8 (C-4⁗), 70.5 (C-3⁗), 70.3 (C-2⁗), 68.2 (C-5⁗), 17.8 (C-6⁗). APCI-MS (positive mode) *m/z*: 757 $[M+H]^+$, 625 $[(M+H)-132]^+$, 479 $[(M+H)-(132+146)]^+$, 317 $[(M+H)-(132+146+162)]^+$. HR-ESI-MS *m/z*: 757.2192 $[M+H]^+$ (Calcd for $C_{33}H_{41}O_{20}$, 757.2186).

Isorhamnetin 3-*O*-*α*-L-Rhamnopyranosyl-(1‴→2″)[*α*-L-rhamnopyranosyl-(1⁗→6″)]-*β*-D-galactopyranoside (**3**): UV λ_{max} (MeOH) nm 254, 355; (MeOH+NaOH) 269, 409; (MeOH+$AlCl_3$) 269, 406; (MeOH+$AlCl_3$+HCl) 269, 401; (MeOH+NaOAc) 274, 370; (MeOH+NaOAc+H_3BO_3) 255, 356. ^{1}H- and ^{13}C-NMR: in agreement with published data.[16,18] APCI-MS (positive mode) *m/z*: 771 $[M+H]^+$, 625 $[(M+H)-146]^+$, 479 $[(M+H)-(146+146)]^+$, 317 $[(M+H)-(146+146+162)]^+$. HR-ESI-MS *m/z*: 771.2360 $[M+H]^+$ (Calcd for $C_{34}H_{43}O_{20}$, 771.2342).

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References

1) Maire R., "Flore de l'Afrique du Nord," Vol. 8, Éditions Paul Lechevalier, Paris, 1962, pp. 161—164.
2) Greuter W., Burdet M. H., Long G. (eds.), "MED-CHECKLIST, A Critical Inventory of the Vascular Plants of the Circum-Mediterranean Countries," Vol. 1, Editions des Conservatoire et Jardin Botaniques de la Ville de Genève, Genève, 1984, p. 304.
3) Jafri S. M. H., Rateeb F. B., "Flora of Libya," Vol. 58 Chenopodiaceae, ed. by Jafri S. M. H., El-Gadi A., Al Faateh University, Faculty of Science, Tripoli, 1978, pp. 88—95.
4) Jarraya R., Chaieb M., Damak M., *Pl. Méd. Phytothérap.*, **26**, 177—189 (1993).
5) Boukef M. K., "Les Plantes dans la Médicine Traditionelle Tunisienne," Agence de Coopération Culturelle et Technique, 1986, pp. 82—83.
6) Le Floc'h E., "Contribution à une Etude Ethnobotanique de la Flore Tunisienne," Imprimerie Officielle de la République Tunisienne, Tunis, 1983, pp. 83—85.
7) Sathiyamoorthy P., Lugasi-Evgi H., Schlesinger P., Kedar I., Gopas J., Pollack Y., Golan-Goldhirsh A., *Pharm. Biol.*, **37**, 188—195 (1999).
8) Sathiyamoorthy P., Lugasi-Evgi H., Van Damme P., Abu-Rabia A., Gopas J., Golan-Goldhirsh A., *Int. J. Pharmacognosy*, **35**, 265—273 (1997).
9) Mabry T. J., Markham K. R., Thomas M. B., "The Systematic Identification of Flavonoids," Springer-Verlag, Berlin, 1970.
10) Markham K. R., "Techniques of Flavonoid Identification," Academic Press, London, 1982.
11) Markham K. R., Geiger H., "The Flavonoids. Advances in Research since 1986," ed. by Harborne J. B., Chapman and Hall, London, 1994, pp. 441—497.
12) Gradwell M. J., Kogelberg H., Frenkiel T. A., *J. Magn. Reson.*, **124**, 267—270 (1997).
13) Halim A. F., Saad H.-E. A., Hashish N. E., *Phytochemistry*, **40**, 349—351 (1995).
14) Bedir E., Çalis I., Piacente S., Pizza C., Khan I. A., *Chem. Pharm. Bull.*, **48**, 1994—1995 (2000).
15) Andersen W. K., Omar A. A., Christensen S. B., *Phytochemistry*, **26**, 291—294 (1987).
16) Yasukawa K., Sekine H., Takido M., *Phytochemistry*, **28**, 2215—2216 (1989).
17) Kijima H., Ide T., Otsuka H., *J. Nat. Prod.*, **58**, 1753—1755 (1995).
18) Rastrelli L., Saturnino P., Schettino O., Dini A., *J. Agric. Food Chem.*, **43**, 2020—2024 (1995).
19) Benkrief R., Brum-Bousquet M., Tillequin F., Koch M., *Ann. Pharm. Franç.*, **48**, 219—224 (1990).
20) Grayer R. J., Kite G. C., Abou-Zaid M. M., Archer L. J., *Phytochem. Anal.*, **11**, 257—267 (2000).