Pergamon 0031-9422(95)00167-0 *Phytochemistry*, Vol. 40, No. 1, pp. 349–351, 1995

0031-9422/95 $9.50 + 0.00

FLAVONOL GLYCOSIDES FROM *NITRARIA RETUSA*

AHMED F. HALIM, HASSAN-ELRADY A. SAAD* and NADIA E. HASHISH

Department of Pharmacognosy, Faculty of Pharmacy, University of Mansoura, El-Mansoura-35516, Egypt

(*Received* 21 *December* 1994)

Key Word Index—*Nitraria retusa*; Nitrariaceae; flavonol glycosides; isorhamnetin glycosides; isorhamnetin 3-*O*-4^{Rham}-galactosylrobinobioside.

Abstract—The new flavonol trioside, isorhamnetin 3-*O*-4^{Rham}-galactosylrobinobioside and five known flavonol glycosides, isorhamnetin 3-robinobioside, isorhamnetin 3-rutinoside, isorhamnetin 3-galactoside, isorhamnetin 3-glucoside and free isorhamnetin were isolated from the leaves and young stems of *Nitraria retusa* and characterized by UV and NMR spectroscopy. Isorhamnetin 3-xylosylrobinobioside was also tentatively identified.

INTRODUCTION

The tribe Nitrarioideae was recently separated, on macromorphological grounds from the Zygophyllaceae and treated as a distinct family, the Nitrariaceae. This monogeneric family comprises about six species distributed in temperate and subtropical areas. Its sole representative in Egypt is *N. retusa*, one of the most common shrubs in the halophytic vegetation of the saline deserts. It is known locally as Ghardaq and used by the Bedouins as a source of fuel and the sweet drupes are edible [1–4].

In a previous chemosystematic investigation of the Zygophyllaceae, the leaf flavonoids of *N. retusa* were studied and five isorhamnetin glycosides tentatively identified by means of acid hydrolysis to aglycone and sugars and UV spectral analysis with shift reagents [5]. In the present study, the leaf and young stem flavonoids were isolated and fully characterized.

RESULTS AND DISCUSSION

From the leaves and young stems of *N. retusa* the new flavonol trioside, isorhamnetin 3-*O*-4^{Rham}-galactosylrobinobioside (**1**) and five known flavonol constituents, isorhamnetin (**2**) [6], isorhamnetin 3-robinobioside (**3**) [7, 8], and its 3-rutinoside (**4**) [9–12], 3-galactoside (**5**) [10, 12], and 3-glucoside (**6**) [10] were isolated and fully characterized. In addition, isorhamnetin 3-xylosylrobinobioside (**7**) was tentatively identified. The structural elucidation of the new compound (**1**) is described in the present study.

Isorhamnetin 3-*O*-4^{Rham}-galactosylrobinobioside (**1**) was isolated from the n-butanol and ethyl acetate leaf extracts. The UV spectra [13] showed **1** to be a 3-substituted flavonol with free hydroxyl groups at C-7, C-5 and C-4′. The ^{1}H NMR data showed it to be an isorhamnetin trioside. The sugar moiety showed three anomeric one-proton doublets at δ5.46 and 5.18 with diaxial coupling (J = 7.5 Hz) and at δ4.45 with diequatorial coupling (J = 2 Hz). A three-proton doublet was also observed at δ1.05 (J = 6.2 Hz) indicating that rhamnose is one of the sugars. Like isorhamnetin 3-robinobioside (**3**), galactose and rhamnose were the monosaccharides detected in its acidic hydrolysate. However, the FAB-mass spectrum displayed $[M + H]^+$ at m/z 787 consistent with the presence of an additional galactosyl moiety. It also showed significant fragment ions at m/z 625 $[M + H - 162]^+$, 479 $[M + H - 308]^+$ and 317 $[M + H - 470]^+$ corresponding to the successive loss of a galactosyl, a rhamnosyl and then a galactosyl moiety and proved that rhamnose is the middle sugar. The ^{13}C NMR spectrum confirmed the triose nature of the sugar residue by three anomeric carbon signals at δ101.8, 100.1 and 100 accompanied by a methyl carbon at δ17.9. The glycosylation site at C-3 hydroxyl was confirmed through the downfield resonance of C-2 at δ156.5 and the upfield signal of C-3 at δ133.5 [10, 14]. Comparison of the ^{13}C NMR data of **1** with those of isorhamnetin 3-robinobioside (**3**) (Table 1) revealed that the C-4‴ signal in **3** (δ71.9) was shifted downfield by 4.7 ppm in **1** (δ76.6), whereas C-3‴ and C-5‴ appeared upfield by 2.4 and 2.2 ppm, respectively. Thus, the terminal galactosyl moiety should be attached to the middle rhamnosyl at C-4‴ through a (1 → 4)-β-linkage. The remaining sugar carbon signals are in full agreement with the attachment of the middle rhamnosyl moiety to the 3-*O*-galactosyl through a (1 → 6)-α-linkage as in **3**. Therefore, **1** was identified as isorhamnetin 3-*O*-β-D-galactopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 6)-β-D-galactopyranoside (isorhamnetin 3-*O*-4^{Rham}-galactosylrobinobioside).

Isorhamnetin 3-*O*-xylosylrobinobioside (**7**) was isolated as a fine yellow powder from the n-butanol leaf

*Author to whom correspondence should be addressed.

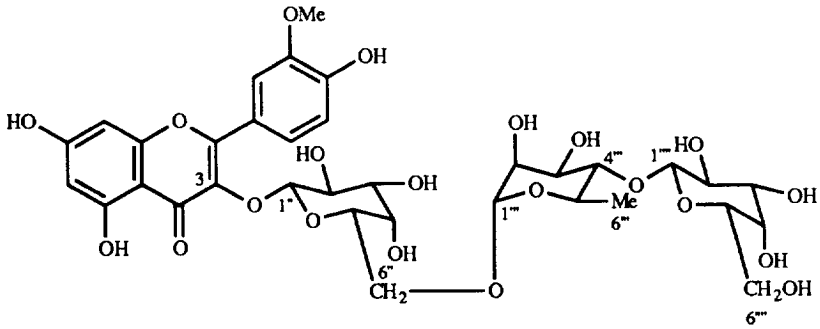

Table 1. ^{13}C NMR spectral data (90 MHz, DMSO-d_6) for compounds **1** and **3***

C	3	1
Aglycone moiety		
2	156.5[a] *s*	156.5[a] *s*
3	133.1 *s*	133.2 *s*
4	177.4 *s*	177.5 *s*
5	161.2 *s*	161.2 *s*
6	98.8 *d*	98.8 *d*
7	164.4 *s*	164.4 *s*
8	93.8 *d*	93.8 *d*
9	156.5[a] *s*	156.3[a] *s*
10	104.0 *s*	104.0 *s*
1′	121.1 *s*	121.1 *s*
2′	113.5[b] *d*	113.7[b] *d*
3′	149.5 *s*	149.3 *s*
4′	147.0 *s*	147.0 *s*
5′	115.2[b] *d*	115.3[b] *d*
6′	122.0 *d*	122.1 *d*
OMe-3′	55.9 *q*	55.9 *q*
Sugar residue		
3-*O*-galactose		
1″	101.8 *d*	101.8 *d*
2″	71.1 *d*	71.1 *d*
3″	73.0 *d*	73.0 *d*
4″	68.0 *d*	68.0 *d*
5″	73.6 *d*	73.6 *d*
6″	65.2 *t*	65.3 *t*
Rhamnose (6 → galactose)		
1‴	100.1 *d*	100.1 *d*
2‴	70.4 *d*	70.8 *d*
3‴	70.6 *d*	68.2 *d*
4‴	71.9 *d*	76.6 *d*
5‴	68.3 *d*	66.1 *d*
6‴	17.9 *q*	17.9 *q*
Galactose (4 → rhamnose)		
1⁗	—	100.0 *d*
2⁗	—	71.6 *d*
3⁗	—	73.3 *d*
4⁗	—	68.2 *d*
5⁗	—	75.9 *d*
6⁗	—	60.9 *t*

*^{13}C Multiplicities were determined by DEPT pulse sequence.

[a,b]Assignments bearing the same superscript in any column are interchangeable.

Isorhamnetin 3-*O*-xylosylrobinobioside (**7**) was isolated as a fine yellow powder from the n-butanol leaf extract. Preliminary studies revealed identical UV spectral data to **1**. D-Xylose, D-galactose and L-rhamnose were detected in its acidic hydrolysate (4% HCl) whereas isorhamnetin 3-robinobioside (**3**) and isorhamnetin 3-galactoside (**5**) were detected as liberated intermediates (1% HCl) by TLC on cellulose using 15% acetic acid. The obtained data support the proposed structure for **7**.

It is noteworthy that isorhamnetin 3-galactoside, the biose robinobiose and the trioses 4^{Rham}-galactosylrobinobiose and xylosylrobinobiose are reported here for the first time in the Nitrariaceae. *Nitraria retusa* appears to have a distinct flavonoid profile, which differs from the other members of the Zygophyllaceae. The presence of isorhamnetin 3-robinobioside as the major flavonol glycoside instead of the common rutinoside in *Zygophyllum* [5] and *Balanites* [12, 15] and of the gentiobioside in *Tribulus* [16] confirms that glycosylation patterns play an important role in the chemosystematics of the Zygophyllaceae and substantiates the taxonomic separation of the subfamily Nitrarioideae from the Zygophyllaceae and its treatment as a distinct family, the Nitrariaceae [1, 2].

EXPERIMENTAL

General. Mps: uncorr. ^{1}H and ^{13}C NMR in DMSO-d_6. CC: polyamide and silica gel 70–230 mesh. Acid hydrolysis and UV spectral analyses with shift reagents were carried out according to standard procedures [13]. Flavonoids detection: UV, 366 nm and $AlCl_3$ reagent. Sugar detection: aniline hydrogen phthalate followed by heating at 100° for 5 min.

Plant material. The leaves and young stems of *Nitraria retusa* (Forssk.) Asch. were collected, in May 1987, from flowering and fruiting plants growing wild in the Mediterranean Coastal strip, 70 km west of Alexandria. The plant identity was kindly verified by Dr I. Mashaly (Department of Botany, Faculty of Science, University of Mansoura) and a voucher specimen is deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Mansoura.

Extraction and isolation. Air-dried, powdered leaves and stems (2 kg each) of *N. retusa* were extracted with EtOH. The concd extracts were separately diluted with H_2O, defatted with petrol and then successively extracted with Et_2O and EtOAc to afford the extracts A & C (leaves) and B & D (stems). The aq. mother liquor from the leaf extracts was further partitioned with n-BuOH satd with H_2O to afford extract E. Evapn of the solvents left the crude extracts A–E (1.0, 0.89, 3.56, 1.2, 10.3 g), respectively. CC of the Et_2O leaf extract (A) and stem extract (B) on polyamide columns eluted with a MeOH–H_2O gradient both gave **2** (18 mg). CC of the EtOAc leaf extract (C) on polyamide eluted with a MeOH–H_2O gradient followed by prep. TLC on silica gel in $CHCl_3$–MeOH–H_2O (80:25:1) and repeated prep. PC in H_2O and 15% HOAc afforded **3** (118 mg), **4** (28 mg), **5** (33 mg), **6** (7 mg) and **1** (8 mg). Additional quantities of **1** (6 mg), **3** (28 mg) and **4** (7 mg) were isolated from the EtOAc stem extract (D) using the same procedures as above. The n-BuOH leaf extract (E) was fractionated on a silica column and the fraction eluted with $CHCl_3$–MeOH–H_2O (70:30:3) was purified first on polyamide column. The resulting yellow band was eluted with 15% aq. MeOH. The concd eluate was further purified by repeated prep. PC in 15% HOAc to afford **7** (15 mg) and an additional quantity of **1** (37 mg).

Isorhamnetin 3-*O*-4^{Rham}-*galactosylrobinobioside* (**1**). Yellow powder, mp 210–212° (dec.), R_f 0.35 (BAW), 0.73 (15% HOAc). UV λ_{max} nm: 359, 258 (MeOH); 415 (+56), 271 ($NaOCH_3$); 403 (+46), 270 ($AlCl_3$); 403 (+46), 268 ($AlCl_3$/HCl); 402, 274 (+16) (NaOAc); 364 (+5), 257 (NaOAc/H_3BO_4). ^{1}H NMR (360 MHz; DMSO-d_6): δ8.05 (1H, *d*, J = 2.1 Hz, H-2′), 7.55 (1H, *dd*, J = 9, 2.1 Hz, H-6′), 6.91 (1H, *d*, J = 9 Hz, H-5′), 6.44 (1H, *d*, J = 2.1 Hz, H-8), 6.23 (1H, *d*, J = 2.1 Hz, H-6), 3.90 (3H, *s*, OMe-3′), 5.46 (1H, *d*, J = 7.5 Hz, H-1″), 5.18 (1H, *d*, J = 7.5 Hz, H-1‴), 4.45 (1H, *d*, J = 2 Hz, H-1⁗), 5.51–3.0 (others, *m*), 1.05 (3H, *d*, J, = 6.2 Hz, Me-6‴). ^{13}C NMR (90 MHz, DMSO-d_6): See Table 1. FAB-MS (xenon, 3-NOBA): *m/z* 787 $[M + H]^+$, 625 $[M + H - gal]^+$, 479 $[M+H - gal - rham]^+$, 317 $[M + H - 2\ gal - rham]^+$.

REFERENCES

1. Tackholm, V. (1974) *Students Flora of Egypt*, 2nd edn, pp. 31, 313, 868. Cairo University, Cooperative Printing Company, Beirut.
2. Tackholm, V. and Boulos, L. (1972) *Supplementary Notes to Students Flora of Egypt*, 2nd Edn, p. 85, Publication of the Cairo University Herbarium, No. 5.
3. Zohary, M. (1972) *Flora Palaestina*, Part II, p. 257, Israel Academy of Sciences and Humanities.
4. Daoud, H. (1985) *Flora of Kuwait*, Vol. 1, p. 56. KPI Publication in Association with Kuwait University.
5. Saleh, N. A. M. and El-Hadidi, M. N. (1977) *Biochem. Syst. Ecol.* **5**, 121.
6. Harborne, J. B., Mabry, T. J. and Mabry, H., (eds). (1975) *The Flavonoids*. Chapman & Hall, London.
7. Buschi, C. A. and Pomilio, A. B. (1982) *J. Nat. Prod.* **45**, 557.
8. Saleh, N., Mansour, R. and Markham, K. (1990) *Phytochemistry* **29**, 1344.
9. Harborne, J. B. and Mabry, T. J. (1982) *The Flavonoids, Advances in Research*. Chapman & Hall, London.
10. Markham, K. R., Ternai, B., Stanley, R., Geiger, H. and Mabry, T. J. (1978) *Tetrahedron* **34**, 1389.
11. Wenkert, E. and Gottlieb, H. E. (1977) *Phytochemistry* **16**, 1811.
12. Kamel, M. S., Ohtani, K., Kurokawa, T., Assaf, M. H., El-Shanawany, M. A., Ali, A. A., Kasai, R., Ishibashi, S. and Tanaka, O. (1991) *Chem. Pharm. Bull.* **39**, 1229.
13. Mabry, T. J., Markham, K. R. and Thomas, M. P. (1970) *The Systematic Identification of Flavonoids*. Springer, Berlin.
14. Markham, K. R. and Ternai, B. (1976) *Tetrahedron* **32**, 2607.
15. Maksoud, S. A. and El-Hadidi, M. N. (1988) *Plant Syst. Evol.* **106**, 153.
16. Saleh, N., Ahmed, A. and Abdalla, M. (1982) *Phytochemistry* **21**, 1995.