Pergamon

PII: S0031-9422(97)00296-3

Phytochemistry, Vol. 46, No. 2, pp. 359–363, 1997

Printed in Great Britain
0031-9422/97 $17.00+0.00

FLAVONOL TRIGLYCOSIDES FROM SEEDS OF *NIGELLA SATIVA*

I. MERFORT,* V. WRAY,† H. H. BARAKAT,‡ S. A. M. HUSSEIN,‡ M. A. M. NAWWAR‡ and G. WILLUHN§

Institut für Pharmazeutische Biologie, Albert-Ludwigs-Universität Freiburg, Schänzlestr. 1, D-79104 Freiburg, Germany; † GBF-Gesellschaft für Biotechnologische Forschung, Mascheroder Weg 1, D-38124 Braunschweig, Germany; ‡ National Research Centre, El-Dokki, Cairo, Egypt; § Institut für Pharmazeutische Biologie, Heinrich-Heine-Universität Düsseldorf, Universitätsstr. 1, D-40225 Düsseldorf, Germany.

(*Received in revised form* 20 *January* 1997)

Key Word Index—*Nigella sativa*; Ranunculaceae; seeds; flavonol triglycosides; quercetin and kaempferol 3-glucosyl(1 → 2)galactosyl(1 → 2)glucoside, quercetin 3-(6-feruloylglucosyl)(1 → 2)galactosyl(1 → 2)glucoside; flavonol glycosides.

Abstract—Three new flavonoid glycosides quercetin and kaempferol 3-glucosyl(1 → 2)galactosyl(1 → 2)glucoside and quercetin 3-(6-feruloylglucosyl)(1 → 2)galactosyl(1 → 2)glucoside were isolated and identified from seeds of *Nigella sativa*. The structures have been elucidated by enzymic and acid hydrolysis and spectral data (UV, MS, ^{1}H and ^{13}C NMR) and confirmed by 2-D NMR. © 1997 Elsevier Science Ltd

INTRODUCTION

The seeds of *Nigella sativa* L. (Ranunculaceae) are used in Middle Eastern folk medicine as a diuretic, a carminative and for the treatment of bronchial asthma and eczema [1, 2]. Recently its analgesic activity in mice and anti-inflammatory effects have been shown [3–5]. Whereas the alkaloids, saponins and fatty acids have been intensively investigated [6–9], nothing is known about its phenolics.

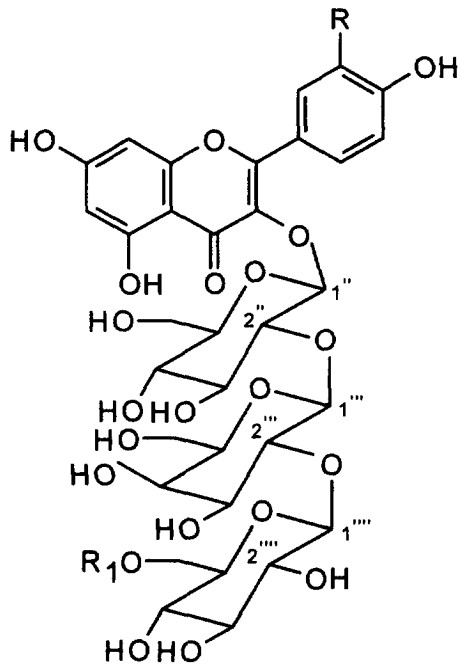
In continuation of our research on polyphenolics in medicinal plants we describe the isolation and structural elucidation of three new flavonol triglycosides (**1–3**) as well as the known flavonoids (**4–6**) in the present paper.

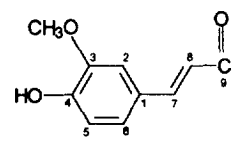
RESULTS AND DISCUSSION

Compound **1** was isolated as an amorphous yellow powder, which appeared to be a quercetin derivative with a substituted 3-hydroxyl group from its UV spectral analysis with diagnostic reagents [10]. Acid hydrolysis on a TLC plate [11] gave glucose and galactose, with glucose as the dominant spot. This result was confirmed by enzymic hydrolysis with β-glucosidase. In the positive ion DCI (NH_3) mass spectra no molecular ion or sequential loss of the sugar moieties was observed. However, fragment ions at *m/z* 303 and 320, due to the protonated aglycone quercetin and $[\text{quercetin}+NH_4]^+$, and fragment ions resulting from the sugar chain at *m/z* 504 $[(3\times162)+NH_4]^+$, 342 $[(2\times162)+NH_4]^+$ and 180 $[162+NH_4]^+$ were found, thus identifying the 3-substituent as a trisaccharide.

Structural elucidation of **1** was achieved by ^{1}H and ^{13}C NMR spectroscopy, including ^{1}H-^{1}H COSY, HMQC and HMBC spectroscopy, which allowed the full assignment of all carbon and proton resonances. The ^{1}H and ^{13}C NMR spectra (Table 1) unambiguously identified **1** as a quercetin derivative with a 3-*O*-substituent [12]. The number and characteristic shifts of the ^{13}C glycosidic signals indicated the presence of three hexose systems in the pyranose form. The assignment of their ^{1}H and ^{13}C signals followed directly from the COSY and HMQC spectra. In all cases the ^{13}C shifts of their C-6 and C-4 signals indicated that these positions were unsubstituted. All sugars had β-glycosidic linkages from the magnitude of the vicinal proton couplings of the anomeric protons in the ^{1}H spectrum. The hexose linked to the hydroxyl group at C-3 of the aglycone was readily identified from the low field shift of its anomeric proton (H-1″, 5.35 ppm) and unambiguously confirmed by a long-range correlation between this proton and C-3 in the HMBC spectrum. This latter spectrum also allowed the interconnectivity of the three sugar units to be unambiguously determined. The anomeric proton H-1‴ (δ 4.78) showed a correlation with C-2″, whose shift of 85.4 ppm was characteristic of a glucose rather than a galactose moiety (expected *ca* 80–83

* Author to whom correspondence should be addressed.



	R	R_1
1	OH	H
2	H	H
3	OH	

Structures of the isolated flavonol triglycosides

ppm [13]), and the reverse correlation between H-2″ (δ 3.70) and C-1‴ (δ 105.1) was also present. Similarly for the third sugar moiety a long-range correlation between H-1⁗ (δ 4.70) and C-2‴ (δ 83.8) and between H-2‴ (δ 3.92) and C-1⁗ (δ 106.6) were observed. The shift of C-2‴ was compatible with the second sugar being galactose (see above). This was indirectly confirmed as the ^{13}C shifts of the terminal sugar were only compatible with those of a β-glucopyranose moiety. Hence, the structure of **1** was determined as quercetin 3-*O*-β-glucopyranosyl(1 → 2)-*O*-β-galactopyranosyl (1 → 2)-*O*-β-glucopyranoside.

UV spectral analysis of **2** indicated that it was a kaempferol 3-*O*-glycoside. The positive DCI (NH_3) mass spectrum showed, in addition to an ion at *m/z* 287 resulting from the protonated aglycone and at *m/z* 304 [aglycone + NH_4]$^+$, the same fragment ions as in the spectra of **1**. Enzymic and acid hydrolysis indicated glucose and galactose as the sugar moieties. The ^1H (1D and 2D COSY) and ^{13}C NMR spectra confirmed the presence of a kaempferol 3-triglycoside, in which the sugars had β-glycosidic linkages. The resonances of the anomeric protons and those of the respective coupled protons at C-2, determined from the COSY spectrum, were similar to those of **1**. The full assignment of all proton resonances by HMBC and HMQC analysis was not possible, because of the small amount of **2**. However, all ^{13}C NMR shifts from the sugar moiety agreed with those of **1**, therefore **2** was identified as kaempferol 3-*O*-β-glucopyranosyl (1 → 2)-*O*-β-galactopyranosyl(1 → 2)-*O*-β-gluco-pyranoside.

Compound **3** was only obtained together with a trace of another flavonoid glycoside. Acid hydrolysis gave glucose and galactose. The positive DCI (NH_3) mass spectrum showed the same fragment ions as that of **1**, together with an ion at *m/z* 680, due to an ammoniated trisaccharide esterified with an acyl residue of 176. The mass spectrum taken in the ESI negative mode, in DMSO-d_6, gave an ion at *m/z* 964 $[M+D-H]^-$ together with further fragment ions at *m/z* 788 $[M+D-H-176]^-$ and 301 $[aglycone-H]^-$ thus indicating that **3** was composed of quercetin and three hexose moieties, one of which was acylated with an aromatic acid. The ^1H and ^{13}C NMR data correlated well with those of **1**, except for some signals from one of the two glucose moieties (^{13}C NMR spectrum: C-5 and C-6, ^1H NMR spectrum: H-6a and H-6b). Additionally signals for *trans*-ferulic acid were present. The feruloyl residue must be attached to the terminal glucose, as this sugar was resistant to enzymic hydrolysis with β-glucosidase. The downfield shift of the methylenes H-6a⁗ and H-6b⁗ together with the downfield shift of C-6⁗ and the slight upfield shift of C-5⁗ indicated that this acyl moiety was bound to C-6⁗ -OH of glucose [14]. Thus, **3** is identified as quercetin 3-*O*-(6-feruloyl-β-glucopyranosyl) (1 → 2)-*O*-β-galactopyranosyl(1 → 2)-*O*-β-gluco-pyranoside.

On addition to these triglycosides quercetin 3-glucoside (**4**), kaempferol 3-glucoside (**5**) and rutin (**6**) were isolated and identified by acid hydrolysis, DC-MS, UV and ^1H NMR data and comparative TLC. The three triglycosides (**1**–**3**) have been isolated for the first time in nature, and the trisaccharide has not been found previously in combination with a flavonoid.

EXPERIMENTAL

Plant material. Seeds from *Nigella sativa* L. were collected from plants cultivated near El-Fayuom, 70 km south of Cairo in May 1994. The plant was identified by Prof. L. Boulos, National Research Centre (NRC), Cairo. A voucher specimen is deposited at the Herbarium of the NRC.

Extraction and isolation. The crushed seeds were exhaustively extracted with *n*-hexane followed by 70% EtOH. 50 g of the concd EtOH extract were dissolved

Table 1. ^{13}C NMR data for compounds **1–3** (100.6 MHz, CD_3OD, TMS as int. standard)*

C	**1** δ (ppm)†	Unambiguous long range ^1H-^{13}C correlations	**2** δ (ppm)	**3** δ (ppm)
Aglyc.				
2	158.5 *s*	H-2′, H-5′‡, H-6′	158.7 *s*	158.4 *s*
3	135.1 *s*	H-1″	134.8 *s*	135.1 *s*
4	179.9 *s*	H-6‡, H-8‡	179.9 *s*	179.9 *s*
5	163.2 *s*	H-6	161.5 *s*	162.9 *s*
6	99.8 *s*	H-8	100.0 *s*	100.0 *s*
7	165.8 *d*	H-6, H-8	167.6 *d*	166.3 *d*
8	94.7 *s*	H-6	95.1 *s*	94.8 *s*
9	158.6 *d*	H-8	158.8 *d*	158.4 *d*
10	105.8 *s*	H-6, H-8	105.9 *s*	105.7 *s*
1′	123.0 *s*	H-5′	122.8 *s*	123.0 *s*
2′	117.8 *d*	H-5′, H-6′	132.2 *d*	117.8 *d*
3′	145.9 *s*	H-2′, H-5′, H-6′	116.3 *d*	145.8 *s*
4′	149.8 *s*	H-2′, H-5′, H-6′	161.5 *s*	149.8 *s*
5′	116.3 *d*	H-2′‡, H-6′	116.3 *d*	116.3 *d*
6′	123.2 *d*	H-2′	132.2 *d*	123.2 *d*
Glc				
1″	101.1 *d*	H-2″, H-3″, H-5″	100.9 *d*	101.2 *d*
2″	85.4 *d*	H-1‴	85.2 *d*	84.8 *d*
3″	78.9 *d*		78.8 *d*	77.9 *d*
4″	70.3 *d*		70.4 *d*	70.2 *d*
5″	78.4 *d*	H-1″, H-3″	78.4 *d*	78.2 *d*
6″	62.4 *t*		62.4 *t*	62.2 *t*
Gal				
1‴	105.1 *d*	H-2″, H-2‴, H-5‴	104.9 *d*	104.6 *d*
2‴	83.8 *d*	H-1‴, H-1⁗	83.5 *d*	83.7 *d*
3‴	75.1 *d*		75.0 *d*	75.0 *d*
4‴	69.8 *d*		69.8 *d*	69.9 *d*
5‴	76.2 *d*	H-1‴	76.3 *d*	76.1 *d*
6‴	61.9 *t*		61.9 *t*	61.9 *t*
Glc				
1⁗	106.6 *d*	H-2‴, H-2⁗, H-3⁗	106.4 *d*	106.5 *d*
2⁗	76.4 *d*		76.3 *d*	76.3 *d*
3⁗	77.6 *d*		77.6 *d*	77.6 *d*
4⁗	71.0 *d*		71.1 *d*	71.3 *d*
5⁗	77.4 *d*		77.4 *d*	76.1 *d*
6⁗	62.4 *t*		62.4 *t*	64.5 *t*
ferul.				
1				127.7 *s*
2				111.8 *d*
3				149.3 *s*
4				150.6 *s*
5				116.4 *d*
6				124.3 *d*
7				147.3 *d*
8				115.3 *d*
9				169.3 *s*
3-OMe				56.5 *q*

* Multiplicities by DEPT.
† Assignments by HMBC and HMQC.
‡ Low intensity correlations.

in 100 ml EtOH and applied to a polyamide column (Macherey and Nagel). Sepn was initiated with H_2O–EtOH (9:1) and the EtOH content gradually increased in 10% steps. The flavonoid containing fr. was eluted with H_2O–EtOH (3:7), yielding **4** (22 mg) and **5** (10 mg) and a mixt. of **1–3** and **6**. Further sepn was performed by CC on Sephadex LH-10 with MeOH followed by MPLC on LiChropep RP 18 (25–40 μm, Fa. Merck) using increasingly polar mixts of H_2O–MeOH (20–50% or 0–40%, within 60 min) at a flow rate of 5

ml min^{-1}. Isolated amounts: **1** 69 mg, **2** 5 mg, **3** 12 mg, **6** 10 mg.

Enzymic hydrolysis. Enzymic hydrolysis was carried out with β-glucosidase at pH 5.0 for 24 hr at 37° followed by TLC analysis of the product in EtOAc–MeOH–HOAc–H_2O (12:3:3:2) and detection with thymol–anisaldehyde–H_2SO_4 [11].

Instrumentation. NMR: Bruker ARX-400 and DMX-600 NMR spectrometers, 400/600 MHz (^1H NMR) and 100.6/150.8 MHz (^{13}C NMR), respectively, in CD_3OD, TMS as int. standard; MS: DCI, Finnigan INCOS 50, NH_3 as reactant gas (emitter heating rate 10 mA s^{-1}, calibration with FC43); ESI-MS, negative mode, direct flow injection technique, sample in MeOH–DMSO-d_6 was introduced (1.25 ml min^{-1}) together with MeOH sheath-gas into the ESI ion source of a Finnigan MAT 4600 spectrometer; MPLC: Two Knauer HPLC Pumps 64, Knauer HPLC Programmer 50, Du Pont Spectrophotometer 860.

Quercetin 3-O-β-*glucopyranosyl*(1 → 2)-O-β-*galactopyranosyl*(1 → 2)-β-*glucopyranoside* (**1**). UV λ_{max}^{MeOH} nm 255, 265 (sh), 355; +NaOMe 268, 327, 403; +NaOAc 273, 323, 385; +NaOAc–H_3BO_3 262, 300 (sh), 377; +$AlCl_3$ 273, 295 (sh), 330, 430; +$AlCl_3$-HCl 268, 275 (sh), 300 (sh), 360, 398. Positive DCI-MS *m/z* (rel. int.) 504 $[(3\times162)+NH_4]^+$, (14), 342 $[(2\times162)+NH_4]^+$ (26), 320 $[aglycone+NH_4]^+$ (7), 303 $[aglycone+H]^+$ (27, 180 $[hexose+NH_4]^+$ (95); ^1H NMR (400 MHz, CD_3OD, TMS as int. standard, assignments are based on ^1H-^1H COSY, HMBC and HMQC, multiplicities of most sugar resonances were not determined, because of signal overlap): aglycone: δ 7.80 (1H, *d*, $J_{2',6'}$ = 2 Hz, H-2′), 7.58 (1H, *dd*, $J_{5',6'}$ = 8.1 Hz, H-6′), 6.96 (1H, *d*, H-5′), 6.41 (1H, *d*, $J_{6,8}$ = 2 Hz, H-8), 6.23 (1H, *d*, H-6), glucose: δ 5.35 (1H, *d*, $J_{1'',2''}$ = 7 Hz, H-1″), 3.75 (1H, H-6a″), 3.70 (1H, H-2″), 3.6 (1H, H-6b″), 3.47 (1H, H-4″), 3.45 (1H, H-3″), 3.22 (1H, H-5″), galactose: δ 4.78 (1H, *d*, $J_{1''',2'''}$ = 7.8 Hz, H-1‴), 3.95 (1H, H-4‴), 3.92 (1H, H-2‴), 3.76 (1H, H-3‴),. 3.7–3.68 (2H, H-6a‴ and H-6b‴), 3.62 (1H, H-5‴), glucose: δ 4.7 = (1H, $J_{1'''',2''''}$ = 7.5 H, H-1⁗), 3.94 (1H, H-6a⁗), 3.79 (1H, H-6b⁗), 3.70 (1H, H-5⁗), 3.47 (1H, H-3⁗), 3.46 (1H, H-4⁗), 3.36 (1H, H-2⁗).

Kaempferol 3-O-β-*glucopyranosyl*(1 → 2)-O-β-*galactopyranosyl*(1 → 2)-β-*glucopyranoside* (**2**). UV λ_{max}^{MeOH} nm 265, 300 (sh), 348; +NaOMe 273, 324, 395; +NaOAc 273, 310, 385; +NaOAc-H_3BO_3 266, 300 (sh), +355; $AlCl_3$ 272, 302, 348, 392; +$AlCl_3$-HCl 272, 300, 345, 392. Positive DCI-MS *m/z* (rel. int.) 504 $[(3\times162)+NH_4]^+$ (7), 342 $[(2\times162)+NH_4]^+$ (9), 304 $[aglycone+NH_4]^+$ (6), 287 $[aglycone+H]^+$ (12), 180 $[hexose+NH_4]^+$ (100). ^1H NMR (400 MHz, CD_3OD, TMS as int. standard): aglycone: δ 8.09 (2H, *dd*, $J_{2',3'}$ = 8.5 Hz, $J_{5',6'}$ = 8.5 Hz, H-2′ and H-6′), 6.97 (2H, *dd*, H-3′ and H-5′), 6.36 (1H, *s(br)*, H-8), 6.18 (1H, *s(br)*, H-6), 5.40 (1H, *d*, $J_{1'',2''}$ = 7 Hz, H-1″), 4.78 (1H, *d*, $J_{1''',2'''}$ = 7.6 Hz, H-1‴), 4.72 (1H, *d*, $J_{1'''',2''''}$ = 7.5 Hz, H-1⁗), 3.90 (1H, H-2⁗), 3.62 (1H, H-2″), 3.3 (1H, H-2⁗).

Quercetin 3-O-(6-*feruloyl*-β-*glucopyranosyl*)(1 → 2)-O-β-*galactopyranosyl*(1 → 2)-β-*glucopyranoside* (**3**). Positive DCI-MS *m/z* (rel. int.) 680 $[(3\times162)+feruloyl+NH_4]^+$ (5), 504 $[(3\times162)+NH_4]^+$ (26), 342 $[(2\times162)+NH_4]^+$ (39), 320 $[aglycone+NH_4]^+$ (39), 303 $[aglycone+H]^+$ (60), 180 $[hexose+NH_4]^+$ (100). ^1H NMR (400 MHz, CD_3OD, TMS as int. standard): aglycone: δ 7.74 (1H, *d*, $J_{2',6'}$ = 2 Hz, H-2′), 7.54 (1H, *dd*, $J_{5',6'}$ = 8 Hz, H-6′), 6.94 (1H, *d*, H-5′), 6.37 (1H, *d*, $J_{6,8}$ = 2, H-8), 6.22 (1H, *d*, H-6), acyl moiety: δ 7.64 (1H, *d*, $J_{7,8}$ = 15.9 Hz, H-7), 6.40 (1H, *d*, H-8), 7.18 (1H, *d*, $J_{2,6}$ = 1.7 Hz, H-2), 7.08 (1H, *dd*, $J_{5,6}$ = 8.2 Hz, H-6), 6.77 (1H, *d*, H-5), 3.88 (3H, *s*, 3-OCH_3), sugar moities: 5.41 (1H, *d*, $J_{1'',2''}$ = 7.2 Hz, H-1″), 4.81 (1H, *d*, $J_{1''',2'''}$ = 7.7 Hz), 4.72 (1H, *d*, $J_{1'''',2''''}$ = 7.9 Hz, H-1⁗), 4.60 (1H, *dd*, $J_{6a'''',5''''}$ = 2, $J_{6a'''',6b''''}$ = (−12.1), H-6a⁗), 4.36 (1H, *dd*, $J_{6b'''',5''''}$ = 5.7, H-6b⁗), 3.90 (1H, 2‴), 3.64 (1H, H-2″), 3.57 (1H, H-5⁗), 3.32 (1H, H-2⁗).

Acknowledgements—We are grateful to E. Müller, Institut für Pharmazeutische Biologie, Heinrich-Heine-Universität Düsseldorf for experimental assistance; to Dr U. Matthiesen, Institut für Klinische Chemie und Laboratoriumsdiagnostik; Heinrich-Heine-Universität Düsseldorf for the DCI mass spectra; Dr M. Linscheid, Institut für Spektrochemie, Dortmund for the ESI-mass spectrum; B. Jaschok-Kentner and C. Kakoschke, GBF, Braunschweig for the NMR spectra and to the Deutsche Forschungsgemeinschaft for financial support (Wi 383/6-1).

REFERENCES

1. Boulos, L., *Medicinal Plants of North Africa*. Reference Publications, Algonac, Michigan, 1983, p. 150.
2. Sayed, M. D., *Journal of Ethnopharmacology*, 1980, **2**, 19.
3. Khanna, T., Zaida, F. A. and Dandiya, P. C., *Fitoterapia*, 1993, **64**, 407.
4. Houghton, P. J., Zarka, R., de las Heras, B. and Hoult, J. R. S., *Planta Medica*, 1995, **61**, 33.
5. Akhtar A. H., Ahmad, K. D., Gilani, S. N. and Nazir, A., *Fitoterapia*, 1996, **67**, 195.
6. Atta-ur-Rahman, Malik, S., He, C. and Clardy, J., *Tetrahedron Letters*, 1985, **26**, 2759.
7. Atta-ur-Rahman, Malik, S., Ahmad, S., Chaudhary, I. and Habib-ur-Rehman, *Heterocycles*, 1985, **23**, 953.
8. Ansari, A. A., Hassan, S., Kenne, L., Atta-ur-Rahman and Wehler, T., *Phytochemistry*, 1988, **27**, 3977.
9. Deshpande, R. S., Adhikary, P. R. and Tipnis, H. P., *Bulletin of Grain Technology*, 1974, **12**, 232.
10. Mabry, T. J., Markham, K. R. and Thomas, M. B., *The Systematic Identification of Flavonoids*. Springer, Heidelberg, 1970.

11. Merfort, I. and Wendisch, D., *Planta Medica*, 1987, **53**, 434.
12. Markham, K. R. and Chari, V. M., in *The Flavonoids: Advances in Research*, ed. J. B. Harborne and T. J. Mabry. Chapman and Hall, London, 1982, Chap. 2.
13. Strack, D., Meurer, B., Wray, V., Grotjahn, L., Austenfeld, F. A. and Wiermanns, R., *Phytochemistry*, 1984, **23**, 2970.
14. Hosokawa, K., Fukunaga, Y., Fukushi, E. and Kawabata, J., *Phytochemistry*, 1995, **40**, 567.