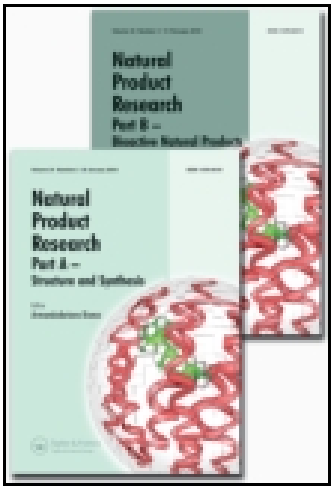

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A new flavonol glycoside from the seeds of Nigella glandulifera

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A new flavonol glycoside from the seeds of *Nigella glandulifera*

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A new flavonol glycoside, kaempferol 3-*O*-α-L-rhamnopyranosyl (1 → 6)-*O*-[β-D-glucopyranosyl (1 → 2)-*O*-β-D-galactopyranosyl (1→2)]-*O*-β-D-glucopyranoside (**1**), together with a known compound, kaempferol 3-*O*-β-D-glucopyranosyl (1 → 2)-*O*-β-D-galactopyranosyl (1 → 2)-*O*-β-D-glucopyranoside (**2**) was isolated from the seeds of *Nigella glandulifera*. Their structures were elucidated on the basis of spectral analysis, including ESI–MS, ESI–MS/MS, HR-ESI–MS, DQF–COSY, TOCSY, HSQC and HMBC techniques.

Keywords: *Nigella glandulifera*; kaempferol tetraglycoside; flavonol glycoside

1. Introduction

Nigella glandulifera FREYN *et* SINT. (Ranunculaceae) is an annual erect herbaceous plant, found widely in the southwest and western part of China. The whole herb has been used as a folk remedy for the treatment of colds, cough and insomnia. *Nigella glandulifera* seeds are commonly eaten in the form of many food preparations by Uigur. The seeds are believed to have diuretic, analgesic, spasmolytic, galactagogue and bronchodilator properties, and they can cure edema, urinary calculus and bronchial asthma (State Administration of Traditional Chinese Medicine, 1999). Our previous phytochemical study on the seeds of this plant revealed the presence of 11 compounds (Y. Liu, Yang, & Q. Liu, 2004, 2005a). In this study, we have obtained a new flavonol glycoside (**1**), together with a known compound (**2**) (Merfort et al., 1997) from the seeds of *N. glandulifera*. This article reports the isolation and structural elucidation of compounds **1–2** (Figure 1).

2. Results and discussion

Compound **1** was obtained as a yellowish solid, and its HCl/Mg reaction and Molish reaction are positive. Its molecular formula was determined as $C_{39}H_{50}O_{25}$ according to the $[M+H]^+$ at 919.2702 (calculated for $C_{39}H_{51}O_{25}$, 919.2720) in the positive HR-ESI–MS. On acid hydrolysis, **1** gave kaempferol as an aglycone along with glucose,

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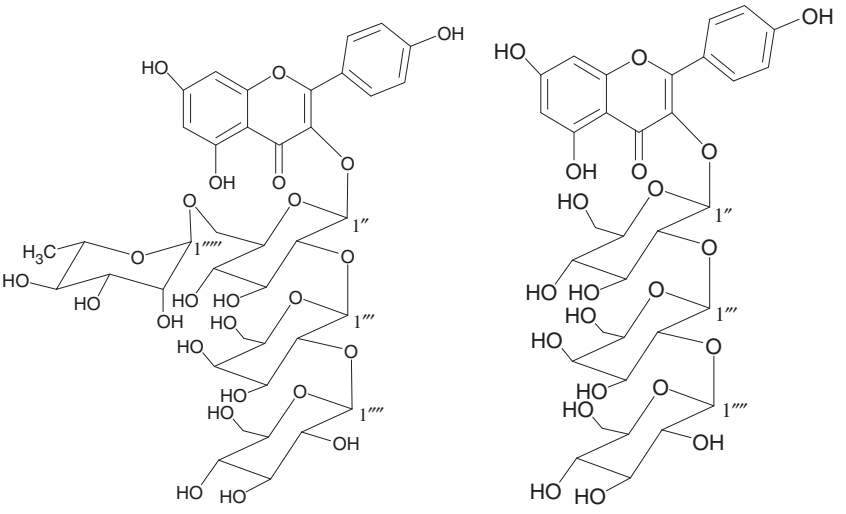

1 **2**

Figure 1. Structures of the compounds **1** and **2**.

galactose and rhamnose. The ultraviolet (UV) spectrum showed absorption maxima at 225, 267 and 348 nm. Its infrared (IR) spectrum exhibited absorption bands at 3400 cm^{-1} (–OH), 1655 cm^{-1} (C = O), 1610, 1508 cm^{-1} (–Ph) and 1070 cm^{-1} (C–O). Its negative ESI–MS spectrum, showed a quasimolecular ion at m/z 917.5 $[M–H]^-$. This parent ion was selected using the quadrupole mass analyser and collision-induced decomposition gave daughter ions. The resulting MS–MS spectrum showed fragment ions at m/z 755.2$[(M-H)–162]^-$ and at m/z 771.2$[(M-H)–146]^-$, derived from independent losses of terminal hexose and deoxyhexose units, and at m/z 593.1$[(M-H)$-162-162$]^-$, indicative of the subsequent losses of hexose moieties. In the positive ESI–MS spectrum we observed the pseudomolecular ion $[M+H]^+$ at m/z 919.3. The adducts $[M+Na]^+$ at m/z 941.3 and $[M+K]^+$ at m/z 957.3 were also observed. In the ESI–MS^{+2} (m/z 941.3, $[M+Na]^+$) spectrum, **1** showed fragment at m/z 655.2 $[M+Na-286$ (aglycone)$]^+$. In the ESI–MS^{+3} (m/z 655.2, $[M+Na-286]^+$) spectrum **1**, showed fragment at m/z 509.1 $[M+Na$-286-146$]^+$ and at m/z 493.1 $[M+Na$-286-162$]^+$, also due to independent losses of terminal deoxyhexose and hexose units, at m/z 347.1 $[M+Na$-286-162-162$]^+$ corresponding to the losses of the two hexoses.

The NMR spectral pattern was similar to that of **2** except for more signals due to the rhamnose moiety. The ^{13}C-NMR shifts of aglycon part of **1** corresponded well with the shifts for kaempferol (Y. Liu, Yang, & Q. Liu, 2005b), the significant difference being those referred to C-2 (+12.4 ppm) and C-4 (+3.3 ppm). These shifts are analogous to **2** when the 3-hydroxy group is glycosylated in a flavonol glycoside. Four anomeric protons were easily identified in the spectra of **1**. They resonated at δ 5.34 (d, $J = 8.0$ Hz), 4.71 (d, $J = 7.6$ Hz), 4.65 (d, $J = 7.2$ Hz) and 4.49 (br. s) and correlated to carbons at δ 100.6, 104.4, 105.6 and 101.9, respectively, in HSQC spectrum. From the assigned aglycon and sugar values, it was apparent that a tetrasaccharide unit was attached to C-3 of the aglycon. The structure of the

tetrasaccharide chain has been assigned by a combination of ^{1}H-^{1}H DQF-COSY, TOCSY, HSQC and HMBC experiments.

Information about the sequence of the tetrasaccharide chain was deduced from HMBC experiments. Key correlation peaks were observed between the anomeric proton of the outer glucose (δ 4.65) and the C-2 of the galactose (δ 82.5), between the anomeric proton of the galactose (δ 4.71) and the C-2 of the inner glucose (δ 84.3) and between the anomeric proton of the rhamnose (δ 4.49) and the C-6 of the inner glucose (δ 68.0). The absence of any ^{13}C-NMR glycosidation shifts for the rhamnopyranosyl residue suggested that the rhamnose a was terminal unit.

The β-configurations at the anomeric positions for the galactopyranosyl and the glucopyranosyl units were easily determined from their relatively large $^{3}J_{H1-H2}$ coupling constants (7.2–8.0 Hz). The α-configuration in the rhamnose residue was clear from its H-1 nonsplitting pattern and their distinct C-3 and C-5 chemical shift differences from that of methyl β-L-rhamnopyranoside. From these considerations and by comparison with the data of compound **2**, the structure of compound **1** was established as kaempferol 3-*O*-α-L-rhamnopyranosyl (1→6)-*O*-[β-D-glucopyranosyl (1→2)-*O*-β-D-galactopyranosyl (1→2)]-*O*-β-D-glucopyranoside.

3. Experimental

3.1. *General*

Optical rotations were measured on a Perkin-Elmer 241 polarimeter. The UV spectra were taken in MeOH using a spectrophotometer. The IR spectrum was recorded in KBr discs on a Nicolet FT-IR IMPACT 400 spectrophotometer. FAB-MS, ESI–MS and HR-ESI–MS were obtained on an Autospec-Ultima ETOF spectrometer, a Finnigan Surveyor LC-LCQ Advantage Max and an Agilent 6520 Accurate-Mass Q-TOF LC/MS, respectively. ^{1}H- and ^{13}C-NMR spectra were acquired on a Bruker Avance III 400 spectrometer in CD_3OD. ^{1}H-^{1}H DQF-COSY, TOCSY, HSQC and HMBC spectra were recorded using conventional pulse sequences. Silica gel H (400–500 mesh, from Qingdao Haiyang Chemical Group Co., China) and sephadex LH-20 (Amersham Pharmacia Biotech Co., Sweden) were used for column chromatography.

3.2. *Plant material*

The seeds of *N. glandulifera* FREYN *et* SINT were collected from Ürümuqi in Xinjiang Uigur autonomy, China, in February 2002, and identified by Professor Yong-min Liu, Xinjiang Institute for the Control of Pharmaceutical Products. A voucher specimen (HB-02-0153) was deposited at the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

3.3. *Extraction and isolation*

The oil-free seeds (18 kg) of *N. glandulifera* were extracted four times with 95% EtOH for 2 h under reflux and then extracted four times with 50% EtOH for 2 h under reflux. After combination and removal of the solvent *in vacuo*, the EtOH

extract was then suspended in distilled water and partitioned successively with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH. The *n*-BuOH-soluble (250 g) fraction was chromatographed over silica gel and eluted with $CHCl_3$–MeOH gradient solvent (5:1 ~ 0:5). Combination of similar fractions on the basis of thin-layer chromatography analysis afforded five fractions. Fraction 3 was subjected to polyamide chromatography and eluted with H_2O–EtOH (3:1), and then to sephadex LH-20 by elution with MeOH–H_2O (1:9) to yield **1** (22 mg) and **2** (35 mg).

Compound **1**. yellowish solid, its HCl/Mg and Molish reaction are positive, m.p. 172–174°C. $[\alpha]_D^{20} - 10.7°$ ($c = 0.056$, H_2O). UV λ_{max} (MeOH) nm: 225, 267 and 348. IR (KBr) ν_{max} cm^{-1}: 3400, 1655, 1610, 1508, 1448, 1363, 1178, 1076 and 841. ESI–MS, *m*/*z* (negative mode): 917.5 $[M–H]^-$ and 953.2 $[M+Cl]^-$; ESI–MS^{-2}, *m*/*z* (M′ = 917.5[M–H]$^-$): 755.2 [(M–H)−162]$^-$, 771.2 [(M–H) –146]$^-$, 593.1 [(M–H)–162-162]$^-$, 575.2 [(M – H)–162-162-H_2O]$^-$ and 285 [aglycone-H]$^-$. ESI–MS, *m*/*z* (positive mode): 919.3 $[M+H]^+$, 941.3 $[M+Na]^+$ and 957.3 $[M+K]^+$; ESI–MS^{+2} *m*/*z* (M′ = 941.3 $[M+Na]^+$): 655.2 [M+Na – 286 (aglycone)]$^+$; ESI–MS^{+3} *m*/*z* (M″ = 655.2 [M+Na – 286]$^+$): 509.1 [M+Na – 286 – 146]$^+$, 493.1 [M+Na-286-162]$^+$ and 347.1 [M+Na – 286-162-162]$^+$. FAB-MS: *m*/*z* 941.2 $[M+Na]^+$ and 963.3 [M–H+2Na]$^+$. HR-ESI–MS, *m*/*z*: 919.2702 $[M+H]^+$ (calculated for $C_{39}H_{51}O_{25}$, 919.2720). ^{1}H-NMR (CD_3OD) δ 8.02 (2H, d, $J = 8.4$ Hz, H-2′, 6′), 6.91 (2H, d, $J = 8.4$ Hz, H-3′, 5′), 6.31 (1H, br.s, H-8), 6.13 (1H, br.s, H-6), glucose: δ 5.34 (1H, d, $J = 8.0$ Hz, H-1″), 3.61 (1H, H-2″), 3.69 (1H, H-3″), 3.32 (1H, H-4″), 3.57 (1H, H-5″), 3.73 (1H, H-6a″) and 3.34 (1H, H-6b″); galactose: δ 4.71 (1H, d, $J = 7.6$ Hz, H-1‴), 3.83 (1H, H-2‴), 3.73 (1H, H-3‴), 3.88 (1H, H-4‴), 3.51 (1H, H-5‴) and 3.64 – 3.60 (2H, H-6a‴ and H-6b‴); glucose: δ 4.65 (1H, d, $J = 7.2$ Hz, H-1⁗), 3.29 (1H, H-2⁗), 3.35 (1H, H-3⁗), 3.31 (1H, H-4⁗), 3.42 (1H, H-5⁗), 3.85 (1H, H-6a⁗) and 3.70 (1H, H-6b⁗); rhamnose: δ 4.49 (1H, br.s, H-1⁗), 3.57 (1H, H-2⁗), 3.44 (1H, H-3⁗), 3.25 (1H, H-4⁗), 3.37 (1H, H-5⁗) and 1.09 (3H, d, $J = 6.0$ Hz, H-6⁗). ^{13}C-NMR (CD_3OD) δ 179.1 (C-4), 165.1 (C-7), 162.1 (C-5), 160.8 (C-4′), 159.0 (C-9), 158.5 (C-2), 134.2 (C-3), 132.3 (C-2′, 6′), 122.9 (C-1′), 116.4 (C-3′, 5′), 104.7 (C-10), 99.8 (C-6) and 96.0 (C-8); glucose: δ 100.6 (C-1″), 84.3 (C-2″), 77.1 (C-3″), 70.8 (C-4″), 71.7 (C-5″) and 68.0 (C-6″); galactose: δ 104.4 (C-1‴), 82.5 (C-2‴), 74.6 (C-3‴), 69.6 (C-4‴), 76.1 (C-5‴) and 61.6 (C-6‴); glucose: δ 105.6 (C-1⁗), 75.9 (C-2⁗), 78.3 (C-3⁗), 70.8 (C-4⁗), 77.1 (C-5⁗) and 62.1 (C-6⁗); rhamnose: δ 101.9 (C-1⁗⁗), 71.1 (C-2⁗⁗), 72.0 (C-3⁗⁗), 73.5 (C-4⁗⁗), 69.7 (C-5⁗⁗) and 17.7 (C-6⁗⁗).

Compound **2.** yellowish solid, its HCl/Mg reaction and Molish reaction are positive, m.p. 152–154°C; $[\alpha]_D^{20} - 21.5°$ ($c = 0.065$, MeOH). UV λ_{max} (MeOH) nm: 225, 267 and 348. IR (KBr) ν_{max} cm^{-1}: 3386, 1655, 1608, 1506, 1441, 1361, 1178, 1074, and 843. FAB-MS: *m*/*z* 795.1 $[M+Na]^+$ and 817.2 [M–H+2Na]$^+$. ^{1}H-NMR (CD_3OD) δ 8.05 (2H, d, $J = 8.0$ Hz, H-2′, 6′), 6.92 (2H, d, $J = 8.0$ Hz, H-3′, 5′), 6.37 (1H, br.s, H-8), 6.17 (1H, br.s, H-6), 5.40 (1H, d, $J = 7.2$ Hz, H-1″), 4.72 (1H, d, $J = 7.6$ Hz, H-1‴) and 4.64 (1H, d, $J = 7.6$ Hz, H-1⁗). ^{13}C-NMR (CD_3OD) δ 179.8 (C-4), 165.9 (C-7), 163.2 (C-5), 161.6 (C-4′), 158.9 (C-9), 158.5 (C-2), 135.0 (C-3), 132.5 (C-2′, 6′), 122.8 (C-1′), 116.4 (C-3′, 5′), 105.9 (C-10), 99.9 (C-6) and 94.7 (C-8), glucose: δ 101.0 (C-1″), 85.1 (C-2″), 78.9 (C-3″), 70.5 (C-4″), 78.4 (C-5″) and; 62.4 (C-6″); galactose: δ 104.9 (C-1‴), 83.5 (C-2‴), 75.0 (C-3‴), 69.9 (C-4‴), 76.3 (C-5‴) and 62.0 (C-6‴); glucose: δ 106.4 (C-1⁗), 76.3 (C-2⁗), 77.6 (C-3⁗), 71.1 (C-4⁗), 77.4 (C-5⁗) and 62.4 (C-6⁗).

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References

Liu, Y.M., Yang, J.Sh., & Liu, Q.H. (2004). A new alkaloid and its artificial derivative with an indazole ring from *Nigella glandulifera*. *Chemical & Pharmaceutical Bulletin, 52*, 454–455.

Liu, Y.M., Yang, J.Sh., & Liu, Q.H. (2005a). Studies on chemical constituents in the seeds of *Nigella glandulifera*. *China Journal of Chinese Meteria Medica, 30*, 980–983.

Liu, Y.M., Yang, J.Sh., & Liu, Q.H. (2005b). Studies on chemical constituents from the flowers of *Carthamus tinctorius* L. *Journal of Chinese Medicinal Materials, 28*, 288–289.

Merfort, I., Wray, V., Barakat, H., Hussein, S., Nawwar, M., & Willuhn, G. (1997). Flavonol triglycosides from seeds of *Nigella sativa*. *Phytochemistry, 46*, 359–363.

State Administration of Traditional Chinese Medicine (1999). *Chinese medicinal herbs* (Vol. III, pp. 236–237). Shanghai: Shanghai Science and Technology Publishing House.