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ORIGINAL PAPER

Flavonol glycosides, nigelflavonosides A–F from the whole plant of *Nigella glandulifera* (Ranunculaceae)

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Abstract Six new flavonol glycosides, nigelflavonosides A–F (**1–6**), together with a known compound (**7**) were isolated from the whole plant of *Nigella glandulifera* Freyn et Sint (Ranunculaceae). Structure elucidation, especially the localization of the glycosyl or acetyl groups, and complete ^{1}H- and ^{13}C-NMR assignments of these compounds were carried out using one- and two-dimensional NMR measurements, including ^{1}H- and ^{13}C-NMR, ^{1}H–^{1}H COSY, TOCSY, HMQC, HMBC and NOESY, in addition to HRESI–TOF–MS experiments.

Keywords *Nigella glandulifera* · Ranunculaceae · Flavonol glycoside · Nigelflavonoside

Introduction

The genus *Nigella* (Ranunculaceae) consists of about 20 species, three of which, *N. glandulifera* Freyn et Sint, *N. sativa* and *N. damascena* L. are used in traditional medicine. Triterpenoids, alkaloids and flavonol glycosides are the main constituents in the seeds of *Nigella* genus [1–11]. *N. glandulifera* is an annual herbaceous plant that grows widely in the southwest and western part of China such as Xinjiang autonomous region. The seeds can reinforce the kidney and brain, stimulate menstrual flow, and are used to treat dieresis, tinnitus, memory loss, and amenorrhea, etc. [12]. The chemical constituents of the seed have been reported as essential oils, fatty acids, steroids, flavonol glycoside, oligosaccharides, saponins, and alkaloids [3, 7, 8, 10, 11]. The whole plant of *N. glandulifera* has been used as a folk remedy for treatment of cold, cough and insomnia [13], but the chemical constituents remain unclear. In this paper, we report six new flavonol glycosides, nigelflavonosides A–F (**1–6**) and a known compound, kaempferol 3-*O*-α-L-rhamnopyranosyl(1 → 6)-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 2)]-*O*-β-D-glucopyranoside (**7**), isolated from whole plants of *N. glandulifera*.

Results and discussion

Whole plants of *N. glandulifera* were extracted with 70% EtOH three times, and this 70% EtOH extract (26.67%) was partitioned with solvents $CHCl_3$ and H_2O. The H_2O layer (21.67% from the whole plant) was subjected to D101 column chromatography (H_2O → 70% EtOH → 95% EtOH) to give the water-, 70% EtOH- and 95% EtOH-eluted fractions (10.31, 7.94, and 0.37%, respectively). The 70% EtOH-eluted fraction was subjected to normal-, reversed-phase silica gel and Sephadex LH-20 column chromatographies, and finally HPLC to afford six new compounds (**1–6**), together with a known compound, kaempferol 3-*O*-α-L-rhamnopyranosyl(1 → 6)-*O*-[β-D-glucopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 2)]-*O*-β-D-glucopyranoside (**7**) [11] (Fig. 1).

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Nigelflavonoside A (**1**)

$[\alpha]_D^{25}$ (−35.2° in MeOH), was isolated as a yellow amorphous powder and its molecular formula was determined by HRESI–TOF–MS to be $C_{43}H_{48}O_{24}$. The IR spectrum showed absorption bands ascribable to hydroxyl (3383 cm^{-1}), unsaturated ester carbonyl (1695 cm^{-1}), unsaturated ketone (1655 cm^{-1}) functions, and aromatic ring (1605, 1514, 1451 cm^{-1}). The UV spectrum indicated the presence of a flavonol glycoside acylated with a hydroxycinnamic acid (330, 265 nm). Acid hydrolysis of **1** with 5% aqueous H_2SO_4–1,4-dioxane (1:1, v/v) afforded D-glucose and D-galactose, whose absolute configurations (D-, L-) were determined by GC analysis of their trimethylsilyl thiazolidine derivatives, with a ratio of about 2:1 [14]. The ^{1}H- (DMSO-d_6) and ^{13}C-NMR (DMSO-d_6, Table 1) spectra of **1**, which were assigned by various NMR experiments including ^{1}H–^{1}H COSY, TOCSY, HMQC, HMBC, and NOESY spectra, showed signals assignable to a kaempferol part [δ 12.58 (1H, br. s, 5-OH), 8.02, 6.90 (2H each, both d, J = 8.8 Hz, H-2′,6′, 3′,5′), 6.29, 6.08 (1H each, both br. s, H-8, 6)], two β-D-glucopyranosyl and a β-D-galactopyranosyl moieties [δ 5.75 (1H, d, J = 7.6 Hz, H-1″), 4.70 (1H, d, J = 7.6 Hz, H-1‴), 4.59 (1H, d, J = 8.0 Hz, H-1⁗)], together with a feruloyl group [δ 7.55, 6.47 (1H each, both d, J = 16.0 Hz, H-7⁗″, 8⁗″), 7.27 (1H, d, J = 1.6 Hz, H-2⁗″), 7.09 (1H, dd, J = 8.0, 1.6 Hz, H-6⁗″), 6.73 (1H, d, J = 8.0 Hz, H-5⁗″), 3.78 (3H, CH_3O-3⁗″)]. Furthermore, in HMBC analysis of **1**, long-range correlations were observed between H-1″ [δ_H 5.75 (1H, d, J = 7.6 Hz)] and C-3 (δ_C 132.4), H-1‴ [δ_H 4.70 (1H, d, J = 7.6 Hz)] and C-2″ (δ_C 82.9), H-1⁗ [δ_H 4.59 (1H, d, J = 8.0 Hz)] and C-2‴ (δ_C 81.2), H-6⁗a and 6⁗b [δ_H 4.21 (1H, dd, J = 12.0, 5.2 Hz), 4.46 (1H, br. d, ca. J = 12 Hz)] and the ester carbonyl carbon (δ_C 166.7, C-9⁗″). Finally, the position of the methoxyl group in **1** was clarified by NOESY, which revealed an NOE correlation between the methoxyl and H-2⁗″. On the basis of the above mentioned evidence, the structure of nigelflavonoside A was determined to be kaempferol 3-*O*-(6-*O*-feruloyl)-β-D-glucopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 2)-β-D-glucopyranoside (**1**) (Fig. 2).

Nigelflavonoside B (**2**)

$[\alpha]_d^{25}$ (−19.5° in MeOH), was isolated as a yellow amorphous powder and its molecular formula was determined by HRESI–TOF–MS to be $C_{39}H_{50}O_{26}$. The ^{1}H- and ^{13}C-NMR spectra data indicated that **2** had a quercetin skeleton [δ 12.68 (1H, br. s, 5-OH), 7.58 (2H, m, H-2′ and 6′), 6.89 (1H, d, J = 8.8 Hz, H-5′), 6.38, 6.19 (1H each, both br. s, H-8, 6)]. Treatment of **2** with 5% aqueous H_2SO_4–1,4-dioxane (1:1, v/v), trimethylsilyl thiazolidine derivate, followed by GC analysis identified D-glucose, D-galactose, and L-rhamnose [14]. In the HMBC experiment, long-range correlations were observed between H-1″ [δ_H 5.65 (1H, d, J = 7.6 Hz)] and C-3 (δ_C 132.6), H-1‴ [δ_H 4.66 (1H, d, J = 7.6 Hz)] and C-2″ (δ_C 83.3), H-1⁗ [δ_H 4.52 (1H, d, J = 7.6 Hz)] and C-2‴ (δ_C 81.6), H-1⁗″ [δ_H 4.35 (1H, br. s)] and C-6″ (δ_C 66.2). Consequently, the structure of **2** was determined as quercetin 3-*O*-β-D-glucopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 2)[α-L-rhamnopyranosyl(1 → 6)]-β-D-glucopyranoside (**2**) (Fig. 3).

Nigelflavonoside C (**3**)

$[\alpha]_D^{25}$ (−4.0° in MeOH), was isolated as a yellow amorphous powder, and its molecular formula was determined by HRESI–TOF–MS to be $C_{48}H_{56}O_{29}$. Treatment of **3** with 5% aqueous H_2SO_4-1,4-dioxane (1:1, v/v) afforded D-glucose, D-galactose and L-rhamnose, which were identified by GC analysis of their trimethylsilyl thiazolidine derivatives [14]. Similar to **2**, there were a quercetin part [δ 12.68 (1H, br. s, 5-OH), 7.56 (1H, dd, J = 8.4, 2.4 Hz, H-6′), 7.55 (1H, d, J = 2.4, H-2′), 6.85 (1H, d, J = 8.4 Hz, H-5′), 6.33, 6.14 (1H each, both br. s, H-8, 6)], together with two β-D-glucopyranosyl, a β-D-galactopyranosyl and a α-L-rhamnopyranosyl moieties [δ 5.65 (1H, d, J = 7.2 Hz, H-1″), 4.68 (1H, d, J = 7.6 Hz, H-1‴), 4.59 (1H, d, J = 7.6 Hz, H-1⁗)], as indicated by the ^{1}H-NMR spectrum. Compared with **2**, **3** had a caffeoyl group [15] [δ 7.48 (1H, d, J = 16.0 Hz, H-7⁗″), 7.04 (1H, br. s, H-2⁗″), 7.01 (1H, br. d, J = ca. 8 Hz, H-6⁗″), 6.70 (1H, d, J = 8.0 Hz, H-5⁗″), 6.30 (1H, d, J = 16.0 Hz, H-8⁗″)]. The above mentioned evidence, together with the ^{1}H–^{1}H COSY and TOCSY spectra suggested that the structure of **3** was quercetin 3-*O*-(6-*O*-caffeoyl)-β-D-glucopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 2)[α-L-rhamnopyranosyl(1 → 6)]-β-D-glucopyranoside (Fig. 4).

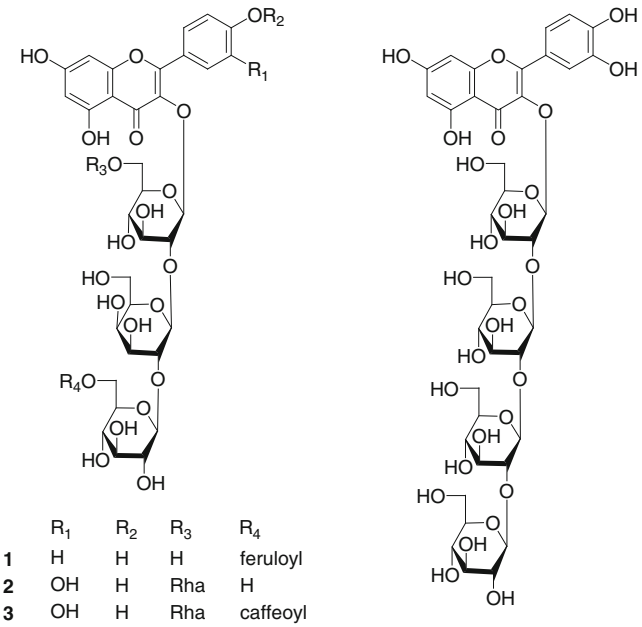
Nigelflavonoside D (**4**)

$[\alpha]_D^{25}$ (−11.2° in MeOH) was isolated as a yellow amorphous powder and its molecular formula was determined by HRESI–TOF–MS to be $C_{44}H_{50}O_{26}$. The glycosyl groups in **4** were determined as D-glucose and D-galactose with the same method as the above mentioned compounds. The ^{1}H-(DMSO-d_6) and ^{13}C-NMR (Table 1) spectra of **4** showed signals assignable to a quercetin part [δ 12.65 (1H, br. s, 5-OH), 7.60 (1H, dd, J = 8.4, 2.0 Hz, H-6′), 7.55 (1H, d, J = 2.0 Hz, H-2′), 6.85 (1H, d, J = 8.4 Hz, H-5′), 6.33 (1H, br. s, H-8), 6.13 (1H, br. s, H-6)], and two β-D-glucopyranosyl and a β-D-galactopyranosyl moieties [δ 5.77 (1H, d, J = 7.2 Hz, H-1″), 4.69 (1H, d, J = 7.2 Hz, H-1‴), 4.60 (1H, d, J = 8.0 Hz, H-1⁗)], together with a sinapoyl group [16] [δ 7.56, 6.54 (1H each, both d, J = 16.0 Hz,

Table 1 ^{13}C-NMR spectra data for compounds **1**, **2**, and **4**–**6** in DMSO-d_6

	Compound						Compound				
Position	**1**	**2**	**4**	**5**	**6**	Position	**1**	**2**	**4**	**5**	**6**
C-2	154.8	155.8	155.1	154.5	155.3	α-L-Rha					
C-3	132.4	132.6	132.6	133.3	132.7	1′′′′		100.3			
C-4	176.9	177.2	177.1	177.4	177.0	2′′′′		70.2			
C-5	161.1	161.1	161.1	161.1	161.0	3′′′′		70.4			
C-6	99.3	98.6	98.7	98.7	98.9	4′′′′		71.7			
C-7	163.8	164.0	164.7	164.4	163.7	5′′′′		68.1			
C-8	93.8	93.5	93.3	93.6	93.6	6′′′′		17.5			
C-9	156.4	156.2	156.1	156.2	156.2	β-D-Glc					
C-10	102.9	103.7	103.5	103.8	103.1	1′′′′				101.2	103.8
C-1′	120.9	121.1	121.0	124.3	120.8	2′′′′				73.1	74.5
C-2′	130.6	116.0	115.8	116.1	115.6	3′′′′				75.7	75.9
C-3′	115.2	144.7	144.8	146.0	144.8	4′′′′				69.7	69.5
C-4′	159.9	148.3	148.6	147.4	148.7	5′′′′				77.1	77.2
C-5′	115.2	115.3	115.3	115.4	115.2	6′′′′				60.6	60.7
C-6′	130.6	121.6	121.6	121.3	121.5	Feruloyl					
β-D-Glc						1′′′′	125.3				
1′′	97.9	98.1	97.8	97.8	98.1	2′′′′	111.0				
2′′	82.9	83.3	83.2	83.7	81.9	3′′′′	147.8				
3′′	76.2	75.8	76.1	75.9	76.0	4′′′′	145.2				
4′′	68.8	69.0	68.9	68.9	68.9	5′′′′	115.4				
5′′	77.1	75.7	77.2	77.5	76.3	6′′′′	123.1				
6′′	60.2	66.2	60.5	60.5	60.6	7′′′′	149.4				
β-D-Gal						8′′′′	114.0				
1′′′	102.5	102.9	102.6	103.1		9′′′′	166.7				
2′′′	81.2	81.6	80.9	81.8		OCH_3	55.5				
3′′′	73.0	73.1	73.1	73.0		Sinapoyl					
4′′′	67.0	67.0	67.0	67.0					124.2		
5′′′	74.5	74.4	74.4	74.4					106.1		
6′′′	59.2	59.3	59.1	59.1					147.8		
β-D-Glc									138.1		
1′′′					101.6				147.8		
2′′′					83.8				106.1		
3′′′					75.8				145.5		
4′′′					69.2				114.6		
5′′′					76.6				166.7		
6′′′					60.6				55.9		
β-D-Glc											
1′′′′	104.1	104.4	104.1	104.5	102.0						
2′′′′	74.6	74.7	74.5	74.8	82.8						
3′′′′	75.8	75.8	75.8	75.8	75.8						
4′′′′	69.4	69.5	69.4	69.5	69.2						
5′′′′	74.2	77.3	74.2	77.3	77.2						
6′′′′	63.1	60.8	63.1	60.8	60.5						

H-8′′′′, 7′′′′), 6.99 (2H, s, H-2′′′′,6′′′′), 3.77 (6H, s, CH_3O-3′′′′, 5′′′′)]. Moreover, in HMBC analysis of **4**, long-range correlations were observed between H-1′′ [δ_H 5.77 (1H, d, $J = 7.6$ Hz)] and C-3 (δ_C132.6), H-1′′′ [δ_H 4.69 (1H, d, $J = 7.2$ Hz)] and C-2′′ (δ_C 83.2), H-1′′′′ [δ_H 4.60 (1H, d, $J = 8.0$ Hz)] and C-2′′′ (δ_C 80.9), H-6′′′′b and 6′′′′a [δ_H



	R_1	R_2	R_3	R_4
1	H	H	H	feruloyl
2	OH	H	Rha	H
3	OH	H	Rha	caffeoyl
4	OH	H	H	sinapoyl
5	OH	Glc	H	H
7	H	H	Rha	H

Fig. 1 Compounds isolated from the whole plant of *Nigella glandulifera* Freyn et Sint

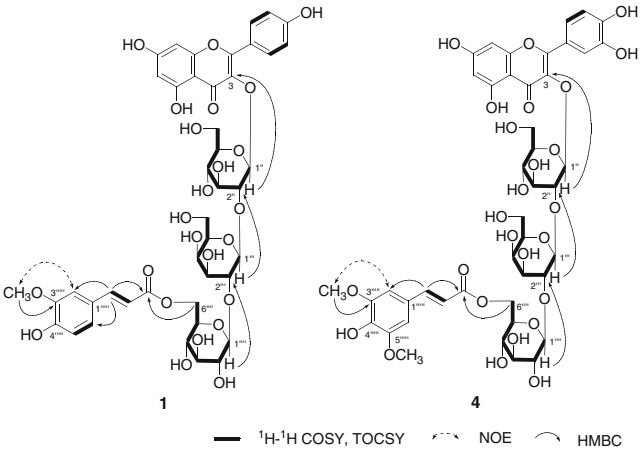


Fig. 2 Key ^{1}H–^{1}H COSY, TOCSY, HMBC and part of NOE correlations of **1** and **4**

4.47 (1H, br. d, J = ca. 12 Hz), 4.24 (1H, dd, J = 12.0, 5.2 Hz)] and the ester carbonyl carbon (δ_C 166.7, C-9′′′′′). The positions of the methoxyl groups in **4** were clarified by NOESY experiment, which showed NOE correlation between the methoxyl protons [δ 3.77 (6H, s, CH_3O-3′′′′′, 5′′′′′)] and protons at positions 2′′′′′, 6′′′′′ (δ 6.99). In summary, the structure of nigelflavonoside D was determined to be quercetin 3-*O*-(6-*O*-sinapoyl)-β-D-glucopyranosyl(1 → 2)-β-D-galactopyranosyl(1 → 2)-β-D-glucopyranoside (**4**).

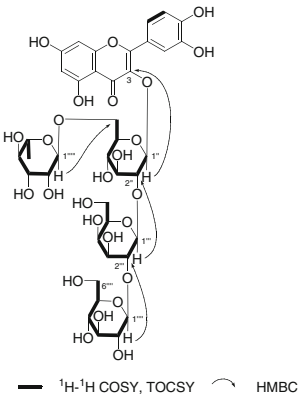


Fig. 3 Key ^{1}H–^{1}H COSY, TOCSY, HMBC correlations of **2**

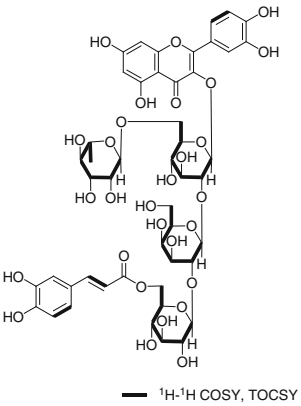
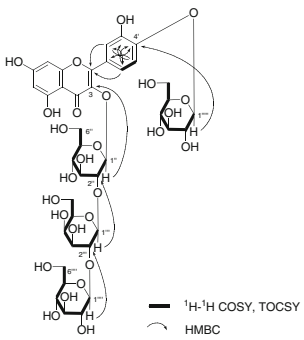


Fig. 4 ^{1}H–^{1}H COSY, TOCSY correlations of **3**

Nigelflavonoside E (**5**)

$[\alpha]_D^{25}$ (−39.9° in MeOH), was also isolated as a yellow amorphous powder, the molecular formula of which was determined by HRESI–TOF–MS to be $C_{39}H_{50}O_{27}$. Treatment of **5** with 5% aqueous H_2SO_4–1,4-dioxane (1:1, v/v) yielded D-glucose and D-galactose, which were identified by GC analysis of their trimethylsilyl thiazolidine derivatives [14]. Comparing the NMR data with those of compounds **2**–**4**, **5** also had a quercetin part [δ 12.63 (1H, br. s, 5-OH), 7.74 (1H, dd, J = 8.8, 2.0 Hz, H-6′), 7.69 (1H, d, J = 2.0 Hz, H-2′), 7.28 (1H, d, J = 8.8 Hz, H-5′), 6.46, 6.23 (1H each, both br. s, H-8, 6)]. Furthermore, the ^{1}H-NMR chemical shifts of 2′-, 5′-, 6′-positions in **5** were downfield compared with **2**–**4** [e.g., **3**: 7.56 (1H, dd, J = 8.4, 2.4 Hz, H-6′), 7.55 (1H, d, J = 2.4, H-2′), 6.85 (1H, d, J = 8.4 Hz, H-5′)], which indicated that there was a sugar substitute in the B ring. Indeed, the long-range correlation between δ_H 4.91 (H-1′′′′′) and δ_C 147.4 (C-4′) observed in the HMBC spectra corroborated the above mentioned deduction. Other sugar links were assigned by various NMR experiments including ^{1}H–^{1}H COSY, TOCSY, NOESY, HMQC, and HMBC spectra. Finally, the structure of nigelflavonoside E was determined to be

Fig. 5 Key ^{1}H–^{1}H COSY, TOCSY, HMBC correlations of **5**



quercetin 3-*O*-*β*-D-glucopyranosyl(1 → 2)-*β*-D-galactopyranosyl(1 → 2)-*β*-D-glucopyranosyl-4′-*O*-*β*-D-glucopyranoside (**5**) (Fig. 5).

Nigelflavonoside F (**6**)

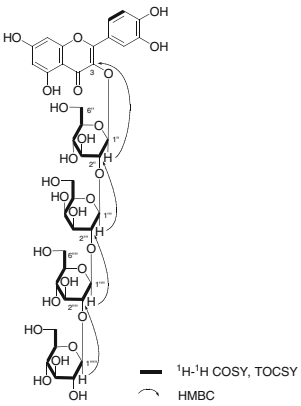
$[\alpha]_D^{25}$ (−22.7° in MeOH), was isolated as a yellow amorphous powder with the molecular formula $C_{39}H_{50}O_{27}$ as determined by HRESI–TOF–MS. Following acid hydrolysis of **6** with 5% aqueous H_2SO_4–1,4-dioxane (1:1, v/v), and serial dilution, only D-glucose was detected [14]. The ^{1}H- (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra of **6**, which were assigned by various NMR experiments including ^{1}H–^{1}H COSY, TOCSY, NOESY, HMQC, and HMBC spectra, showed signals assignable to a quercetin part [δ 12.65 (1H, br. s, 5-OH), 7.58 (1H, br. d, J = ca. 8 Hz, H-6′), 7.55 (1H, br. s, H-2′), 6.83 (1H, d, J = 8.0 Hz, H-5′), 6.28, 6.08 (1H each, both br. s, H-8, 6)], together with four *β*-D-glucopyranosyl moieties [δ 5.70 (1H, d, J = 7.2 Hz, H-1′'), 4.79 (1H, d, J = 7.6 Hz, H-1‴), 4.74 (1H, d, J = 7.6 Hz, H-1⁗), 4.58 (1H, d, J = 7.6 Hz, H-1⁗′)]. Furthermore, in HMBC analysis of **6**, long-range correlations were observed between H-1′' [δ_H 5.70 (1H, d, J = 7.2 Hz)] and C-3 (δ_C132.7), H-1‴ [δ_H 4.79 (1H, d, J = 7.6 Hz)] and C-2″ (δ_C 81.9), H-1⁗ [δ_H 4.74 (1H, d, J = 7.6 Hz)] and C-2‴ (δ_C 83.8), H-1⁗′ [δ_H 4.58 (1H, d, J = 7.6 Hz)] and C-2⁗′ (δ_C 82.8). Consequently, the structure of nigelflavonoside F was determined to be quercetin 3-*O*-*β*-D-glucopyranosyl(1 → 2)-*β*-D-glucopyranosyl(1 → 2)-*β*-D-glucopyranosyl(1 → 2)-*β*-D-glucopyranoside (**6**) (Fig. 6).

Experimental

General experimental procedures

Optical rotations were measured on a Rudolph Autopol® IV automatic polarimeter (l = 50 mm), whereas IR were recorded on a Varian 640-IR FT-IR spectrophotometer and

Fig. 6 Key ^{1}H–^{1}H COSY, TOCSY, HMBC correlations of **6**



UV spectra on a Varian Cary 50 UV–Vis spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were determined on a Varian 400MR spectrometer at 400 MHz for ^{1}H- and 100 MHz for ^{13}C-NMR, with tetramethylsilane (TMS) as an internal standard. Positive- and negative-ion HRESI–TOF–MS were recorded on an Agilent Technologies 6520 Accurate-Mass Q-Tof LC/MS spectrometer.

A highly porous synthetic resin (D101) was purchased from Haiguang Chemical (Tianjin, China). Silica gel CC were obtained from Qingdao Haiyang Chemical (48–75 μm, Qingdao, China). Sephadex LH-20 (GE Healthcare Life Sciences, Glattbrugg, Switzerland) were used to purify the total flavonoids from the whole residue. HPLC was performed on ODS (Cosmosil 5C18-MS-II, Tokyo, Japan; Φ = 20 mm, L = 250 mm, flow rate: 9.0 ml/min), and the eluate was monitored with a UV detector (Shimadzu RID-10A UV–VIS, Japan). TLC plates pre-coated with Silica gel GF_{254} (Tianjin Silida Technology, Tianjin, China) were used to detect the purity of isolate achieved by spraying with 10% aqueous H_2SO_4–EtOH, following by heating.

Plant material

N. glandulifera Freyn et Sint. was cultivated at the planting base of West Tianshan Herbs Co., Ltd., Xinjiang Pharmaceutical Group Co., and plant material was identified by Professor Tianxiang Li. A voucher specimen (2009.10.10) of this plant is on file in our laboratory.

Extraction and isolation

Dried whole plants of *N. glandulifera* Freyn et Sint. (2.0 kg) were extracted with 70% EtOH under reflux three times to give the 70% EtOH extract (533 g). The 70% EtOH extract (266 g) was suspended in H_2O, and then partitioned with $CHCl_3$, to give a $CHCl_3$ soluble fraction

(50 g) and an aqueous soluble fraction (216 g), respectively. The latter (194 g) was subjected to D101 resin, eluted with H_2O, 70% EtOH and 95% EtOH, successively, to give the correspondent elutes: 92.8, 71.5, 3.3 g, respectively. The 70% EtOH elute (20.0 g) was subjected to silica gel column chromatography [300 g, $CHCl_3$–MeOH–H_2O (10:3:1 → 7:3:1 → 6:4:1, v/v/v, the lower layer) → MeOH] to give 13 fractions [Fr. 1 (0.1 g), Fr. 2 (0.3 g), Fr. 3 (1.5 g), Fr. 4 (0.7 g), Fr. 5 (0.4 g), Fr. 6 (0.4 g), Fr. 7 (0.4 g), Fr. 8 (0.4 g), Fr. 9 (0.7 g), Fr. 10 (1.9 g), Fr. 11 (4.5 g), Fr. 12 (3.5 g), Fr. 13 (3.5 g)]. Fr. 9 (0.7 g) was separated by Sephadex LH-20, eluted with $CHCl_3$–MeOH (1:1, v/v), and finally purified by HPLC [CH_3CN–H_2O (18:82, v/v) + 1% HAc], to afford **1** (13.5 mg, 0.0054%) and **4** (11.8 mg, 0.0047%). Fr. 13 (3.5 g) was subjected to Sephadex LH-20, eluted with MeOH–H_2O (1:1, v/v), and finally separated by HPLC [CH_3CN-H_2O (13:87, v/v) + 1% HAc], to yield **7** (17.6 mg, 0.0070%), **2** (81.2 mg, 0.032%), **3** (5.4 mg, 0.0022%), **5** (12.5 mg, 0.0050%), together with **6** (18.1 mg, 0.0072%).

Characterization data

*Nigelflavonoside A (**1**)*

^{1}H-NMR (DMSO-d_6) δ: 12.58 (1H, br. s, 5-OH), 8.02 (2H, d, J = 8.8 Hz, H-2′,6′), 7.55 (1H, d, J = 16.0 Hz, H-7‴″), 7.27 (1H, d, J = 1.6 Hz, H-2‴″), 7.09 (1H, dd, J = 8.0, 1.6 Hz, H-6‴″), 6.90 (2H, d, J = 8.8 Hz, H-3′,5′), 6.73 (1H, d, J = 8.0 Hz, H-5‴″), 6.47 (1H, d, J = 16.0 Hz, H-8‴″), 6.29 (1H, br. s, H-8), 6.08 (1H, br. s, H-6), 5.75 (1H, d, J = 7.6 Hz, H-1″), 4.70 (1H, d, J = 7.6 Hz, H-1‴), 4.59 (1H, d, J = 8.0 Hz, H-1‴″), [4.46 (1H, br. d, ca. J = 12 Hz), 4.21 (1H, dd, J = 12.0, 5.2 Hz), H_2-6‴″], 3.78 (3H, s, CH_3O-3‴″), 3.73 (1H, br. d, ca. J = 3 Hz, H-4‴), 3.66 (1H, dd, J = 9.6, 7.6 Hz, H-2‴), 3.58 (1H, dd, J = 9.6, 2.8 Hz, H-3‴), 3.55 (1H, m, overlapped, H-3″), 3.55 (1H, m, H-5‴″), [3.54 (1H, m, overlapped), 3.40 (1H, br. d, ca. J = 13 Hz), H_2-6‴], [3.49 (1H, dd, J = 12.0, 2.0 Hz), 3.21 (1H, br. d, ca. J = 12 Hz), H_2-6″], 3.39 (1H, dd, J = 9.6, 7.6 Hz, H-2″), 3.35 (1H, br. d, ca. J = 6 Hz, H-5‴), 3.32 (1H, dd, J = 8.8, 8.0 Hz, H-3‴″), 3.22 (1H, dd, J = 8.0, 8.0 Hz, H-4‴″), 3.14 (1H, dd, J = 8.8, 8.8 Hz, H-4″), 3.11 (1H, dd, J = 8.8, 8.0 Hz, H-2‴″), 3.05 (1H, m, H-5″). ^{13}C-NMR (DMSO-d_6) δ: see Table 1. IR (KBr) cm^{-1}: 3383, 2922, 2857, 1695, 1655, 1605, 1514, 1451, 1361, 1277, 1177, 1076, 890, 842, 817. UV λ_{max} (MeOH) nm (log ε): 330 (4.47), 265 (4.36). HRESI–TOF–MS: positive-ion mode m/z 971.2415 $[M + Na]^+$ (Calcd for $C_{43}H_{48}O_{24}Na$, 971.2428), negative-ion mode m/z 947.2448 $[M–H]^-$ (Calcd for $C_{43}H_{47}O_{24}$, 947.2463). $[\alpha]_D^{25}$ −35.2° (c 0.5, MeOH).

*Nigelflavonoside B (**2**)*

^{1}H-NMR (DMSO-d_6) δ: 12.68 (1H, br. s, 5-OH), 7.58 (1H, dd, J = 8.8, 2.4 Hz, H-6′), 7.56 (1H, d, J = 2.4 Hz, H-2′), 6.89 (1H, d, J = 8.8 Hz, H-5′), 6.38 (1H, br. s, H-8), 6.19 (1H, br. s, H-6), 5.65 (1H, d, J = 7.6 Hz, H-1″), 4.66 (1H, d, J = 7.6 Hz, H-1‴), 4.52 (1H, d, J = 7.6 Hz, H-1‴″), 4.35 (1H, br. s, H-1‴″), [3.78 (1H, br. d, *Ca*. J = 12 Hz), 3.54 (1H, dd, J = 12.0, 4.8 Hz), H_2-6‴″)], 3.74 (1H, br. d, ca. J = 3 Hz, H-4‴), [3.66 (1H, dd, J = 12.0, 2.8 Hz), 3.26 (1H, m, overlapped), H_2-6″)], 3.65 (1H, dd, J = 8.0, 7.6 Hz, H-2‴), 3.62 (1H, dd, J = 9.6, 8.4 Hz, H-3″), 3.60 (1H, dd, J = 9.6, 3.2 Hz, H-3‴), [3.50 (1H, dd, J = 12.0, 6.0 Hz), 3.32 (1H, dd, J = 12.0, 6.0), H_2-6‴], 3.46 (1H, dd, J = 8.4, 7.6 Hz, H-2″), 3.37 (1H, dd, J = 9.6, 6.0 Hz, H-5‴), 3.34 (1H, br. s, H-2‴″), 3.29 (1H, m, overlapped, H-5‴″), 3.26 (1H, m, overlapped, H-5″), 3.25 (1H, m, overlapped, H-3‴″), 3.24 (1H, m, overlapped, H-3‴″), 3.24 (1H, m, overlapped, H-5‴″), 3.15 (1H, dd, J = 9.6, 8.4 Hz, H-4″), 3.13 (1H, dd, J = 9.6, 8.0 Hz, H-4‴″), 3.08 (1H, dd, J = 8.4, 7.6 Hz, H-2‴″), 3.07 (1H, dd, J = 8.8, 8.8 Hz, H-4‴″), 0.98 (3H, d, J = 6.0 Hz, CH_3-6‴″). ^{13}C-NMR (DMSO-d_6) δ: Table 1. IR (KBr) cm^{-1}: 3391, 2925, 1655, 1608, 1506, 1448, 1362, 1303, 1273, 1200, 1169, 1073, 1036, 887, 810. UV λ_{max} (MeOH) nm (log ε): 355 (4.23), 255 (4.34). HRESI–TOF–MS: Positive-ion mode m/z 957.2473 $[M + Na]^+$ (Calcd for $C_{39}H_{50}O_{26}Na$, 957.2483), Negative-ion mode m/z 933.2525 $[M–H]^-$ (Calcd for $C_{39}H_{49}O_{26}$, 933.2518). $[\alpha]_D^{25}$ −19.5° (c 0.7, MeOH).

*Nigelflavonoside C (**3**)*

^{1}H-NMR (DMSO-d_6) δ: 12.68 (1H, br. s, 5-OH), 7.56 (1H, dd, J = 8.4, 2.4 Hz, H-6′), 7.55 (1H, d, J = 2.4 Hz, H-2′), 7.48 (1H, d, J = 16.0 Hz, H-7‴″), 7.04 (1H, br. s, H-2‴″), 7.01 (1H, br. d, ca. J = 8 Hz, H-6‴″), 6.85 (1H, d, J = 8.4 Hz, H-5′), 6.70 (1H, d, J = 8.0 Hz, H-5‴″), 6.33 (1H, br. s, H-8), 6.30 (1H, d, J = 16.0 Hz, H-8‴″), 6.14 (1H, br. s, H-6), 5.65 (1H, d, J = 7.2 Hz, H-1″), 4.68 (1H, d, J = 7.6 Hz, H-1‴), 4.59 (1H, d, J = 7.6 Hz, H-1‴″), [4.46 (1H, br. d, ca. J = 12 Hz), 4.19 (1H, dd, J = 12.0, 5.2 Hz), H_2-6‴″], 4.32 (1H, br. s, H-1‴″), [3.74, 3.52 (each 1H, both m), H_2-6‴], 3.71 (1H, br. d, ca. J = 3 Hz, H-4‴), [3.65 (1H, br. d, J = 13 Hz), 3.25 (1H, m, overlapped), H_2-6″], 3.63 (1H, dd, J = 8.0, 7.6 Hz, H-2‴), 3.61 (1H, m, overlapped, H-3″), 3.60 (1H, m, overlapped, H-3‴), 3.53 (1H, m, H-5‴″), 3.44 (1H, dd, J = 8.0, 7.2 Hz, H-2″), 3.37 (1H, m, overlapped, H-5‴), 3.35 (1H, m, overlapped, H-2‴″), 3.25 (1H, m, overlapped, H-3‴″), 3.24 (1H, m, H-5″), 3.23 (2H, m, overlapped, H-3‴″ and 5‴″), 3.18 (1H, dd, J = 9.2, 8.0 Hz, H-4‴″), 3.14 (1H, dd, J = 9.6, 9.6 Hz, H-4″), 3.10 (1H, dd, J = 8.4, 7.6 Hz, H-2‴″), 3.04 (1H, dd, J = 9.6, 9.2 Hz,

H-4‴″), 0.93 (3H, d, $J = 6.0$ Hz, CH_3-6‴″). IR (KBr) cm^{-1}: 3377, 2922, 2851, 1722, 1656, 1600, 1509, 1453, 1384, 1254, 1205, 1173, 1068, 840; UV λ_{max} (MeOH) nm (log ε): 335 (4.17), 255 (4.31). HRESI–TOF–MS: positive-ion mode *m/z* 1119.2777 $[M + Na]^+$ (Calcd for $C_{48}H_{56}O_{29}Na$, 1119.2799), negative-ion mode *m/z* 1095.2817 $[M–H]^-$ (Calcd for $C_{48}H_{55}O_{29}$, 1095.2834). $[\alpha]_D^{25}$ –4.0° (c 0.1, MeOH).

*Nigelflavonoside D (**4**)*

^{1}H-NMR (DMSO-d_6) δ: 12.65 (1H, br. s, 5-OH), 7.60 (1H, dd, $J = 8.4$, 2.0 Hz, H-6′), 7.56 (1H, d, $J = 16.0$ Hz, H-7‴″), 7.55 (1H, d, $J = 2.0$ Hz, H-2′), 6.99 (2H, s, H-2‴″, 6‴″), 6.85 (1H, d, $J = 8.4$ Hz, H-5′), 6.54 (1H, d, $J = 16.0$ Hz, H-8‴″), 6.33 (1H, br. s, H-8), 6.13 (1H, br. s, H-6), 5.77 (1H, d, $J = 7.6$ Hz, H-1″), 4.69 (1H, d, $J = 7.2$ Hz, H-1‴), 4.60 (1H, d, $J = 8.0$ Hz, H-1‴″), [4.47 (1H, br. d, ca. $J = 12$ Hz), 4.24 (1H, dd, $J = 12.0$, 5.2 Hz), H_2-6‴″], 3.77 (6H, s, CH_3O-3‴″,5‴″), 3.71 (1H, d, $J = 2.8$ Hz, H-4‴), 3.66 (1H, dd, $J = 9.6$, 7.2 Hz, H-2‴), 3.57 (1H, dd, $J = 9.6$, 2.8 Hz, H-3‴), 3.53 (1H, dd, $J = 8.8$, 8.0 Hz, H-3″), 3.51 (1H, m, H-5‴″), [3.46 (1H, dd, $J = 12.8$, 5.6 Hz), 3.24 (1H, m, overlapped), H_2-6″], 3.43 (1H, dd, $J = 8.0$, 7.6 Hz, H-2″), 3.42, 3.28 (1H, both m, H_2-6‴), 3.34 (1H, m, H-5‴), 3.27 (1H, dd, $J = 8.8$, 8.4 Hz, H-3‴″), 3.23 (1H, dd, $J = 8.8$, 8.8 Hz, H-4‴″), 3.13 (1H, dd, $J = 8.8$, 7.6 Hz, H-4″), 3.11 (1H, dd, $J = 8.4$, 8.0 Hz, H-2‴″), 3.07 (1H, m, H-5″). ^{13}C-NMR (DMSO-d_6) δ: see Table 1. IR (KBr) cm^{-1}: 3377, 2924, 2856, 1698, 1655, 1607, 1515, 1457, 1364, 1300, 1202, 1172, 1113, 1074, 1022, 826. UV λ_{max} (MeOH) nm (log ε): 335 (4.06), 255 (4.19). HRESI-TOF–MS: positive-ion mode *m/z* 1017.2481 $[M + Na]^+$ (Calcd for $C_{44}H_{50}O_{26}Na$: 1017.2483), negative-ion mode *m/z* 993.2536 $[M–H]^-$ (Calcd for $C_{44}H_{49}O_{26}$, 993.2518). $[\alpha]_D^{25}$ –11.2° (c 0.5, MeOH).

*Nigelflavonoside E (**5**)*

^{1}H-NMR (DMSO-d_6) δ: 12.63 (1H, br. s, 5-OH), 7.74 (1H, dd, $J = 8.8$, 2.0 Hz, H-6′), 7.69 (1H, d, $J = 2.0$ Hz, H-2′), 7.28 (1H, d, $J = 8.8$ Hz, H-5′), 6.46 (1H, br. s, H-8), 6.23 (1H, br. s, H-6), 5.77 (1H, d, $J = 7.6$ Hz, H-1″), 4.91 (1H, d, $J = 7.2$ Hz, H-1‴″), 4.67 (1H, d, $J = 7.6$ Hz, H-1‴), 4.54 (1H, d, $J = 7.6$ Hz, H-1‴″), 3.80 (1H, br. d, ca. $J = 11$ Hz), 3.56 (1H, m, overlapped), H_2-6‴″)], 3.78, 3.54 (each 1H, both m, H_2-6‴″), 3.78 (1H, m, overlapped, H-4‴), 3.68 (1H, dd, $J = 9.2$, 7.6 Hz, H-2‴), 3.64 (1H, dd, $J = 8.0$, 7.6 Hz, H-3″), 3.63 (1H, br. d, ca. $J = 9$ Hz, H-3‴), [3.54 (1H, m, overlapped), 3.38 (1H, dd, $J = 11.6$, 6.0 Hz), H_2-6‴], [3.54 (1H, m, overlapped), 3.31 (1H, dd, $J = 12.0$, 5.6 Hz), H_2-6″], 3.46 (2H, m, H-5‴ and 5‴″), 3.43 (1H, dd, $J = 7.6$, 7.6 Hz, H-2″), 3.39 (1H, d, $J = 8.4$, 8.4 Hz, H-3‴″), 3.36 (1H, dd, $J = 8.4$, 7.2 Hz, H-2‴″), 3.32 (1H, m, overlapped, H-5‴″), 3.27 (1H, dd, $J = 9.2$, 8.4 Hz, H-3‴″), 3.23 (1H, dd, $J = 8.4$, 7.2 Hz, H-4‴″), 3.18 (1H, m, overlapped, H-4‴″), 3.16 (1H, m, overlapped, H-4″), 3.14 (1H, m, overlapped, H-5″), 3.10 (1H, dd, $J = 8.4$, 7.6 Hz, H-2‴″). ^{13}C-NMR (DMSO-d_6) δ: see Table 1. IR (KBr) cm^{-1}: 3382, 2923, 2890, 1655, 1610, 1505, 1441, 1364, 1306, 1251, 1203, 1172, 1074, 1042, 892, 803. UV λ_{max} (MeOH) nm (log ε): 345 (4.23), 265 (4.37). HRESI–TOF–MS: positive-ion mode *m/z* 973.2426 $[M + Na]^+$ (Calcd for $C_{39}H_{50}O_{27}Na$: 973.2432), negative-ion mode *m/z* 949.2464 $[M–H]^-$ (Calcd for $C_{39}H_{49}O_{27}$, 949.2467). $[\alpha]_D^{25}$ –39.9° (c 1.5, MeOH).

*Nigelflavonoside F (**6**)*

^{1}H-NMR (DMSO-d_6) δ: 12.65 (1H, br. s, 5-OH), 7.58 (1H, br. d, ca. $J = 8$ Hz, H-6′), 7.55 (1H, br. s, H-2′), 6.83 (1H, d, $J = 8.0$ Hz, H-5′), 6.28 (1H, br. s, H-8), 6.08 (1H, br. s, H-6), 5.70 (1H, d, $J = 7.2$ Hz, H-1″), 4.79 (1H, d, $J = 7.6$ Hz, H-1‴), 4.74 (1H, d, $J = 7.6$ Hz, H-1‴″), 4.58 (1H, $J = 7.6$ Hz, H-1⁗), [3.72 (1H, br. d, ca. $J = 12$ Hz), 3.56 (1H, m, overlapped), H_2-6⁗], [3.72 (1H, br. d, ca. $J = 12$ Hz), 3.50 (1H, m, overlapped), H_2-6‴″], 3.60 (1H, dd, $J = 8.0$, 8.0 Hz, H-3″), 3.57 (1H, dd, $J = 8.40$, 7.2 Hz, H-2″), 3.56, 3.30 (each 1H, both m, H_2-6‴), 3.50, 3.18 (each 1H, both m, H_2-6″), 3.42 (2H, m, H-3‴ and H-3‴″), 3.29 (1H, dd, $J = 8.4$, 7.6 Hz, H-2‴″), 3.23 (1H, dd, $J = 8.0$, 7.6 Hz, H-2‴), 3.20 (1H, m, overlapped, H-5⁗″), 3.18 (1H, dd, $J = 9.6$, 9.6 Hz, H-4″), 3.16 (1H, m, H-5‴), 3.15 (3H, m, H-3⁗″, 4‴ and 4⁗″), 3.13 (1H, m, H-5⁗″), 3.09 (1H, dd, $J = 9.2$, 9.2 Hz, H-4⁗″), 3.07 (1H, m, H-5″), 3.03 (1H, dd, $J = 8.4$, 7.6 Hz, H-2⁗″). ^{13}C-NMR (DMSO-d_6) δ: see Table 1. IR (KBr) cm^{-1}: 3396, 2925, 2856, 1652, 1608, 1505, 1446, 1362, 1302, 1200, 1171, 1074, 1031, 891, 824. UV λ_{max} (MeOH) nm (log ε): 355 (4.10), 255 (4.47). HRESI–TOF–MS (positive-ion mode) *m/z* 973.2432 $[M + Na]^+$ (Calcd for $C_{39}H_{50}O_{27}Na$: 973.2432), negative-ion mode *m/z* 949.2470 $[M–H]^-$ (Calcd for $C_{39}H_{49}O_{27}$, 949.2467). $[\alpha]_D^{25}$ –22.7° (c 0.3, MeOH).

Acid hydrolysis of **1**–**6**

A solution of nigelflavonoside A–F (**1**–**6**, each 2.0 mg) in 5% aqueous H_2SO_4–1,4-dioxane (0.5 ml) was heated under reflux for 1 h, respectively. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and removed by filtration. After removal of the solution from the filtrate in vacuo, the residue was treated with H_2O and MeOH on ODS column. The H_2O eluate was concentrated and the residue was reacted with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (0.2 ml) at

60°C for 2 h, then added *N*,*O*-bis(trimethylsilyl)acetamide (BSTFA, 0.2 ml) stirred under reflux at 60°C for 2 h. The supernatant was then subjected to GC analysis to identify the derivatives of D-glucose (a), D-galactose (b), and L-rhamnose (c); GC conditions: column, Agilent Technologies INC Catalog 19091J-413 HP-5, 30 m × 0.320 mm (i.d.) capillary column; column temperature, 230°C; carrier gas, N_2; t_R, (a) 19.4 min, (b) 17.9 min, (c) 11.1 min.

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