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NIGELACTONE, NEW PHTHALIDE GLUCOSIDE FROM THE SEEDS *NIGELLA GLANDULIFERA*

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Abstract – Chemical investigations of the seeds of *Nigella glandulifera* have resulted in the isolation of a new phthalide derivative, nigelactone (**1**), together with two known metabolites. Their structures were established by detailed analysis of their 1D and 2D NMR spectra and mass spectroscopic data. Nigelactone (**1**) possesses a rare structural skeleton found in nature.

Nigella glandulifera FREYN *et* SINT. is an annual erect herbaceous plant, found widely in the southwest and western part of China. Its seeds are commonly eaten in many food preparations by Uigur and are believed to have diuretic, analgesic, spasmolytic, galactagogue and bronchodilator properties.[1] In our previous work, a number of bioactive compounds, such as alkaloids,[2] triterpenoid saponins[3] and flavonol glycosides[4] were isolated from the *N. glandulifera*. Recently we obtained a new phthalide derivative (**1**), named nigelactone, together with two known compounds, glycerol (**2**) and *1*-linoleoyl glycerol (**3**), from the seeds of *N. glandulifera*. This paper reports the isolation and structural elucidation of nigelactone (**1**) (**Figure 1**), which possesses a rare structural skeleton found in nature.

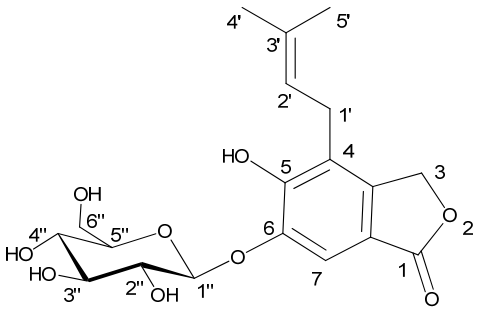

Figure 1. Structure of nigelactone (**1**)

Compound **1** was obtained as a pale yellow solid. Its molecular formula was determined as $C_{19}H_{24}O_9$ according to the $[M+Na]^+$ at 419.1324 (calculated for $C_{19}H_{24}O_9Na$, 419.1318) in the positive HR-ESI-MS.

On acid hydrolysis, **1** gave D-glucose. Strong absorption bands accounting for hydroxyl group (3396 cm^{-1}), α, β-unsaturated γ-lactone group (1730 cm^{-1}) and aromatic group (1614, 1507 cm^{-1}) could be observed in its IR spectrum. Its negative ESI-MS spectrum showed a quasimolecular ion at *m/z* 395.3 $[M-H]^-$. The resulting MS-MS spectrum showed fragment ion at *m/z* 233.2 $[(M-H)-162]^-$, derived from losses of terminal glucose. In the positive ESI-MS spectrum its pseudomolecular ion $[M+H]^+$ was observed at *m/z* 397.2. The adducts $[M+Na]^+$ at *m/z* 419.3 and $[M+K]^+$ at *m/z* 435.3 were also observed. In the ESI-MS^{+2} (*m/z* 419.3, $[M+Na]^+$) spectrum **1** showed fragment at *m/z* 256.9 $[M+Na-162]^+$, also corresponding to the losses of the glucose.

The ^{13}C NMR and DEPT spectra of **1** contained 19 carbon resonances, including two resulting from methyl groups, two from sp^2 methines, and five from sp^3 methines, whereas further three signals resulted from methylene groups, and seven resonances were assigned to quaternary carbons. The ^1H NMR spectrum of **1** showed characteristic signals for an aromatic proton at δ_H 6.65 (1H, s) and 3-methyl-2-butenyl group (δ_H 5.12 t, 3.24 d, 1.73 s, 1.68 s). The presence of the glucose moiety was also suggested by the observation of seven protons at δ_H 3.39, 3.45, 3.47, 3.55, 3.70, 3.92, 4.92. Furthermore, β-configuration at the anomeric proton δ_H 4.92 was determined from its relatively large $^3J_{H1''\text{-}H2''}$ coupling constant (8.0 Hz). Additionally, glycosidation position located on the C-6 was derived from the HMBC correlation between δ_H 4.92 (H-1″) and δ_C 155.3 (C-6). The HMBC correlation from δ_H 6.65 (H-7) to the carbonyl carbon δ_C 171.3 (C-1) and the NOESY correlation from δ_H 6.65 (H-7) to the anomeric proton δ_H 4.92 (H-1″) indicated that the carbonyl group and the glucose existed on the two sides of H-7 position; the HMBC correlations from δ_H 5.17 (H-3) to the carbonyl carbon δ_C 171.3 (C-1) and from δ_H 5.17 (H-3) to three aromatic carbons [δ_C 149.7 (C-3a), δ_C 115.8 (C-4) and δ_C 104.1 (C-7a)] revealed that the γ-lactone was connected with the benzene ring in parallel; the NOESY correlation from δ_H 5.17 (H-3) to δ_H 3.24 (H-1′) confirmed that the 3-methyl-2-butenyl group was located close to the γ-lactone moiety. Analysis of the ^1H-^1H DQF-COSY, HSQC, HMBC and NOESY spectra led to the proposed structure **1** and enabled the complete assignment of the ^1H and ^{13}C NMR spectra (**Table 1**). The important NOESY interactions of compound **1** are shown in **Figure 2**. So compound **1** was identified as 5-hydroxy-4-(3-methyl-2-butenyl)-6-*O*-β-D-glucopyranosyl-1(3*H*)-isobenzofuranone, named nigelactone.

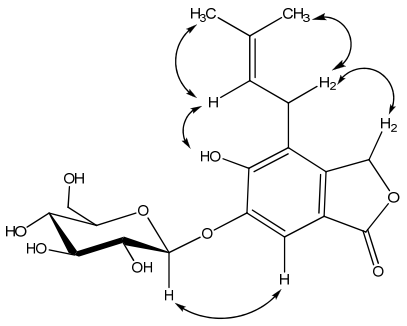

Figure 2. The Key NOESY correlations of compound **1**

Table 1. NMR data for compound **1** in methanol-d_4 (δ in ppm)

Position	δ_H	δ_C	HMBC(δ_H to δ_C)
1		171.3	
3	5.17 (2H, s)	68.6	C1, C3a, C4, C7a
3a		149.7	
4		115.8	
5		162.5	
6		155.3	
7	6.65 (1H, s)	102.4	C1, C5, C6, C7a
7a		104.1	
1′	3.24 (2H, d, 6.8)	23.8	C4, C4a, C5, C2′, C3′
2′	5.12 (1H, t, 6.8)	120.7	C4, C4′, C5′
3′		132.3	
4′	1.68 (3H, s)	24.4	C2′, C3′, C5′
5′	1.73 (3H, s)	16.4	C2′, C3′, C4′
1″	4.92 (1H, d, 8.0)	100.7	C5″, C6
2″	3.55 (1H, dd, 8.7, 8.0)	72.8	C1″, C3″
3″	3.47 (1H, dd, 8.5, 9.2)	76.1	C2″, C4″
4″	3.39 (1H, dd, 9.2, 8.8)	69.9	C3″, C5″, C6″
5″	3.45 (1H, m)	77.0	C4″
6″	3.92 (1H, d, 12.0)	61.1	C4″, C5″
	3.70 (1H, dd, 12.0, 5.6)		C4″, C5″

Nigelactone (**1**), a prenylated phthalide glycoside, is one of the acyclic hemiterpenoid phthalides. To the best of our knowledge, only six compounds of the acyclic hemiterpenoid phthalides (*O*-prenylated form not included) were found in nature,[5-8] and their carbonyl groups are all positioned *peri* to the aromatic methoxy or hydroxy moiety. So nigelactone (**1**) is the first acyclic hemiterpenoid phthalide in nature, which carbonyl group is placed *peri* to the aromatic hydrogen. Since phthalide derivatives are compounds of the polyketide metabolism,[9] this type of phthalides such as in nigelactone (**1**) could be formed owing to distinct ring-closure position in polyketides, and then this type of phthalides might differ significantly from the other in terms of its biosynthesis.[9]

EXPERIMENTAL

General. Optical rotation was measured on a Rudolph Autopol IV polarimeter. UV spectrum was taken in MeOH using a Hitachi U-3310 spectrophotometer. IR spectrum was recorded in KBr discs on a Nicolet FT-IR AVATAR 370 spectrophotometer. ESI-MS and HR-ESI-MS were obtained on a Finnigan Surveyor LC-LCQ Advantage Max and a Bruker micrOTOF-Q II spectrometers, respectively. ^{1}H- and ^{13}C-NMR spectra were acquired on a Bruker Avance III 400 spectrometer with the residual solvent signals as an internal standard (CD_3OD δ_H 3.30 ppm, δ_C 47.6 ppm). ^{1}H-^{1}H DQF-COSY, NOESY, HSQC and HMBC

spectra were recorded using conventional pulse sequences. Silica gel 60H (400-500 mesh) and Silica gel GF_{254} sheets (0.20-0.25 mm) (both from Qingdao Haiyang Chemical Group Co., China) were used for column chromatography and TLC, respectively.

Plant material. The seeds of *Nigella glandulifera* FREYN *et* SINT. were collected from Ürümuqi in Xinjiang Uigur autonomy, China, in February 2011, and identified by Prof. Qing-Hua Liu, Xinjiang Institute of Materia Medica.

Extraction and isolation. The oil-free seeds (18 kg) of *N. glandulifera* were extracted three times with 95% EtOH for 2 h under reflux and then extracted three 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, EtOAc and *n*-BuOH. The EtOAc fraction (80 g) was chromatographed over silica gel and eluted with $CHCl_3$-MeOH gradient solvent (20:1~1:1). Combination of similar fractions on the basis of TLC analysis afforded 9 fractions. Fraction 5 was subjected to silica gel column chromatography and eluted with $CHCl_3$–MeOH (10:1) to yield **1** (25 mg), **2** (17 mg) and **3** (89 mg).

Nigelactone (1): pale yellow solid, mp 113-115 ˚C; $[\alpha]_D^{20}$ -80.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.05), 261 (2.69), 291 (3.31) nm; IR (KBr) ν_{max} 3396, 2919, 1730, 1614, 1507, 1363, 1237, 1061 cm^{-1}; ^{1}H-NMR (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD) data provided in Table 1; ^{1}H-NMR (400 MHz, DMSO-d_6, standard TMS) δ 1.64 (3H, s, 4′-CH_3), 1.70 (3H, s, 5′-CH_3), 3.16 (2H, d, *J* = 6.8 Hz, H-1′), 3.25 (1H, m, H-4″), 3.29 (1H, m, H-5″), 3.28 (1H, m, H-3″), 3.30 (1H, m, H-2″), 3.53 (1H, dd, *J* = 5.6, 11.6 Hz, H-6_A″), 3.69 (1H, d, *J* = 11.6 Hz, H-6_B″), 4.94 (1H, d, *J* = 6.8 Hz, H-1″), 5.11 (1H, t, *J* = 7.0 Hz, H-2′), 5.16 (2H, s, H-3), 6.70 (1H, s, H-7), 10.68 (1H, s, 5-OH); ESI-MS (negative mode) *m/z* 395.3 $[M-H]^-$; ESI-MS^{-2} (negative mode, M′ = 395.3 $[M-H]^-$) *m/z* 233.2 $[(M-H)-162]^-$; ESI-MS (positive mode) *m/z* 397.2 $[M+H]^+$, 419.3 $[M+Na]^+$, 435.3 $[M+K]^+$; ESI-MS^{+2} (positive mode, M′ = 419.3 $[M+Na]^+$) *m/z* 256.9 $[M+Na-162]^+$; HR-ESI-MS (positive mode) *m/z* 419.1324 $[M+Na]^+$ (calcd 419.1318 for $C_{19}H_{24}O_9Na$).

Acid Hydrolysis of (1): Compound **1** (6 mg) was refluxed with 10% HCl in 75% EtOH (9 mL) for 6 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The H_2O layer was neutralized with Ag_2CO_3 and analyzed by TLC [EtOAc-MeOH-AcOH-H_2O (12:3:3:2)]. The R*f* value (0.43) of the sample was identical to that of standard glucose. Next, the H_2O layer was evaporated to dryness under reduce pressure and the residue was separated on silica gel by CC. Fractions were detected by TLC. The fraction which contained the pure sugar was concertrated, then was measured on the Autopol IV polarimeter, The positive optical rotation value was showed, thus the sugar moiety of **1** was identified as D-glucose.

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REFERENCES

1. State Administration of Traditional Chinese Medicine, *Chinese Medicinal Herbs,* Vol. III. Shanghai Science and Technology Publishing House, Shanghai, 1999, pp. 236-237.
2. Y. M. Liu, J. Sh. Yang, and Q. H. Liu, *Chem. Pharm. Bull.*, 2004, **52**, 454.
3. Z. Tian, Y. M. Liu, S. B. Chen, J. Sh. Yang, P. G. Xiao, L. Wang, and E. Wu, *Molecules*, 2006, **11**, 693.
4. Y. M. Liu, Q. H. Liu, and B. Q. Chen, *Nat. Prod. Res.*, 2011, **25**, 1334.
5. H. Lv, G. Sabir, B. Kungurhan, Y. Q. Liu, and H. A. Aisa, *J. Asian Nat. Prod. Res.*, 2009, **11**, 352.
6. J. Vrkoc, M. Budesinsky, L. Dolejs, and S. Vasickova, *Phytochemistry*, 1975, **14**, 1845.
7. G. H. Li, L. Lei, M. Duan, and K. Q. Zhang, *Chem. Biodiv.*, 2006, **3**, 210.
8. E. Habib, F. Leno, J. D. Bauer, R. A. Hill, P. Carvalho, H. G. Cutler, and S. J. Cutler, *J. Nat. Prod.*, 2008, **71**, 1915.
9. A. Celso, K. Stefan, P. Miguel, and M. K. Gabriele, *Beilstein J. Org. Chem.*, 2011, **7**, 1636.