Two New Alkaloids from the Aerial Part of *Peganum nigellastrum*

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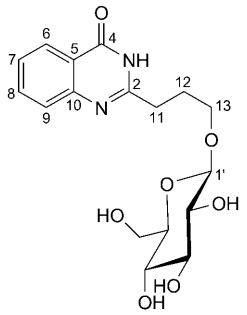
Two new alkaloids, pegamine β-D-glucopyranoside (**1**) and 2-deoxypeganylacetic acid (**2**), together with the novel 3,4-dihydro-4-hydroxynaphthalene-2-carboxylic acid (**3**), were isolated from the aerial part of *Peganum nigellastrum*. The structures of these compounds were elucidated on the basis of spectroscopic analyses, including 1D- and 2D-NMR, and ESI-MS/MS.

Introduction. – *Peganum nigellastrum* BUNGE, family Zygophyllaceae, is widely distributed over Asia, and commonly occurs in Xinjiang, Gansu, Inner Mongolia, and Shanxi, P. R. China. The whole plants or seeds of *P. nigellastrum* have been used for a long time in the treatment of rheumatism, irregular menstruation, cough, asthma *etc.* [1]. Previous investigations on *P. nigellastrum* led to the isolation of a series of luotonin alkaloids [2], and some of them showed good cytotoxic activity against mouse leukemia cells (P-388), and inhibitory activity against topoisomerase I and II [3]. To find further potentially bioactive alkaloids, we studied the EtOH extract of the aerial part of *P. nigellastrum*, and obtained two new alkaloids, **1** and **2**, and one new dihydronaphthalene-carboxylic acid, **3**. Here, we describe the isolation and structure elucidation of these three new compounds.



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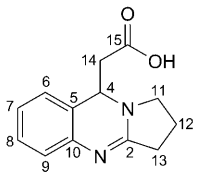

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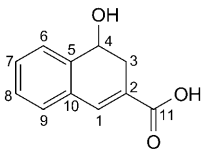

3[1])

Results and Discussion. – Compound **1** was obtained as a colorless, amorphous solid, and showed a positive reaction in *Dragendorff*'s test. The ESI-MS/MS peaks at *m/z* 367 ($[M+H]^+$), 365 ($[M-H]^-$), 205 ($[M+H-\text{glucose}]^+$) indicated that the

[1]) Arbitrary numbering. For systematic names, see *Exper. Part.*

structure contained one hexose unit, and an even number of N-atoms. The ^{1}H-NMR spectra displayed typical signals for an *ortho*-disubstituted benzene ring at δ(H) 8.18 (*dd*, J = 8.1, 1.1, 1 H), 7.48 (*dt*, J = 8.1, 1.1, 1 H), 7.79 (*dt*, J = 8.1, 1.5, 1 H), and 7.64 (*d*, J = 8.1, 1 H), one anomeric H-atom of a sugar moiety at δ(H) 4.28 (*d*, J = 7.8), and two CH_2 groups at δ(H) 2.80–2.87 (CH_2(11)) and 2.09–2.14 (CH_2(12)). In the region between δ(H) 3.1–4.0, except the signals of six non-anomeric H-atoms, two signals at δ(H) 3.68–3.71 (*m*, 1 H) and 3.96–4.01 (*m*, 1 H) were assigned to the two H-atoms of a CH_2O group *via* their HSQC correlations with δ(C) 69.5 (*t*, CH_2(13)) (*Table 1*).

Table 1. *^{1}H- and ^{13}C-NMR Data of Compound* **1**[1]). δ in ppm, J in Hz. Arbitrary C-atom numbering as indicated in the formula.

	δ(H)[a])	δ(C)[b])
C(2)		159.4 (*s*)
C(4)		164.4 (*s*)
C(5)		121.8 (*s*)
H–C(6)	8.18 (*dd*, J = 8.1, 1.1)	127.1 (*d*)
H–C(7)	7.48 (*dt*, J = 8.1, 1.1)	127.6 (*d*)
H–C(8)	7.79 (*dt*, J = 8.1, 1.5)	135.9 (*d*)
H–C(9)	7.64 (*d*, J = 8.1)	127.4 (*d*)
C(10)		150.0 (*s*)
CH_2(11)	2.80–2.87 (*m*)	33.0 (*t*)
CH_2(12)	2.09–2.14 (*m*)	28.9 (*t*)
H_a–C(13)	3.68–3.71 (*m*)	69.5 (*t*)
H_b–C(13)	3.96–4.01 (*m*)	
H–C(1′)	4.28 (*d*, J = 7.8)	104.4 (*d*)
H–C(2′)	3.15 (*dd*, J = 7.8, 9.1)	75.1 (*d*)
H–C(3′)	3.34–3.37 (*m*)	78.0 (*d*)
H–C(4′)	3.26–3.27 (*m*)	71.6 (*d*)
H–C(5′)	3.27–3.28 (*m*)	78.1 (*d*)
H_a–C(6′)	3.66–3.67 (*m*)	62.7 (*t*)
H_b–C(6′)	3.86 (*dd*, J = 12.0, 1.5)	

[a]) Recorded at 500 MHz in CD_3OD. [b]) Recorded at 125 MHz in CD_3OD.

The ^{13}C-NMR spectra showed 15 C-atom signals directly, and another two at δ(C) 164.4, 150.0 could be detected by the HMBC spectrum. Of these 17 C-atom signals, those at δ(C) 104.4 (*d*, C(1′)), 78.1 (*d*, C(5′)), 78.0 (*d*, C(3′)), 75.1 (*d*, C(2′)), 71.6 (*d*, C(4′)), and 62.7 (*t*, C(6′)) indicated that the hexose was glucose [4]. The characteristic signals at δ(C) 164.4 (*s*, C(4)), 159.4 (*s*, C(2)), 150.0 (*s*, C(10)), 135.9 (*d*, C(8)), 127.6 (*d*, C(7)), 127.4 (*d*, C(9)), 127.1 (*d*, C(6)), 121.8 (*s*, C(5)) indicated the presence of a quinazolone system, containing a vicinally disubstituted benzene ring.

In the HMBC spectrum (*Fig. 1*), the correlations H–C(11)/C(2) and C(13), H–C(12)/C(2) and C(13), and H–C(6)/C(4) confirmed the aglycone moiety of compound **1** to be pegamine [5] (obtained from the *P. nigellastrum* before). The correlation H–C(1′)/C(13) suggested that the glucose is connected to C(13) directly, and that it has a β-D-glycoside linkage according to the coupling constant of the anomeric H-atom (J = 7.8 Hz) and the ^{13}C-shift of the anomeric C-atom (δ(C) 104.4).

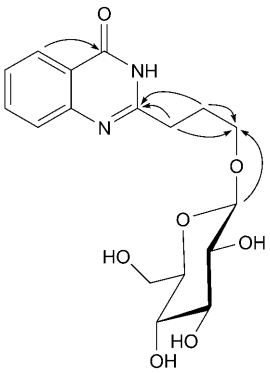

Fig. 1. *Key HMBC correlations of* **1**

Therefore, the structure of compound **1** was identified as pegamine β-D-glucopyranoside.

Compound **2** was obtained as white powder, and showed a positive reaction in the *Dragendorff* test. On the basis of ESI-MS (231 ($[M + H]^+$), 253 ($[M + Na]^+$), 229 ($[M - H]^-$)), and ^{1}H- and ^{13}C-NMR spectra, the molecular formula was determined as $C_{13}H_{14}N_2O_2$.

In the ^{1}H-NMR spectra, the signals of three couples of H-atoms were observed at δ(H) 3.67–3.72 and 4.03–4.09 (CH_2(11)), 2.18–2.26 and 2.27–2.31 (CH_2(12)), and 2.92–2.96 and 3.00–3.08 (CH_2(13)), indicating the presence of a –CH_2–CH_2–CH_2– group. The coupling correlations of H-atoms at δ(H) 5.30 (*t*, *J* = 5.1, 1 H) and 2.72 (*d*, *J* = 5.1, 2 H) suggested a –CH–CH_2–COOH group.

The ^{13}C-NMR spectra showed the characteristic signals of quinazoline at δ(C) 164.4 (C(2)), 56.5 (C(4)), 117.9 (C(5)), 128.1 (C(6)), 128.0 (C(7)), 130.0 (C(8)), 127.8 (C(9)), and 143.7 (C(10)), and the signals of four H-atoms at δ(H) 7.28–7.30 (*m*, 1 H), 7.25–7.27 (*m*, 1 H), 7.21 (*t*, *J* = 7.5, 1 H), and 6.96–6.98 (*m*, 1 H) confirmed the quinazoline skeleton (*Table 2*). These evidences suggested that compound **2** is a deoxypeganine [6] (obtained from *P. nigellastrum* before) that was substituted by –CH–CH_2–COOH at C(4). We named it 2-deoxypeganylacetic acid.

Compound **3** was obtained as white powder. The ^{1}H-NMR spectra exhibited the typical signals of vicinally disubstituted benzene ring at δ(H) 7.69 (*d*, *J* = 7.0, 1 H), 7.35 (*d*, *J* = 7.0, 1 H), 7.11 (*dt*, *J* = 7.0, 1.0, 1 H), and 7.04 (*dt*, *J* = 7.0, 1 H). The couplings of H-atoms with the signals at δ(H) 3.85 (*dd*, *J* = 9.0, 4.0, 1 H) (H–C(4)), 3.51 (*dd*, *J* = 15.0, 4.0, 1 H), and 3.14 (*dd*, *J* = 15.0, 9.0, 1 H) (CH_2(3)), and their HSQC correlations (*Table 3*) indicated the presence of a –CH_2–CH(OH)– group. In the ^{13}C-NMR spectrum, δ(C) 174.4 (*s*, C(11)) corresponds to a COOH group; the signals at δ(C) 125.1 (*d*, C(1)) and 109.6 (*s*, C(2)), and the HSQC correlations between δ(C) 125.1 and δ(H) 7.19 (*s*, 1 H) suggested a –C=CH– group.

The HMBC correlations (*Fig. 2*) H–C(1)/C(3) and C(10), H–C(3)/C(11), and H–C(4)/C(2) indicated a dihydronaphthalene for the structure of compound **3** [7], substituted at C(2) by a COOH group and at C(4) by a OH group. This assignment was confirmed by the NOESY correlations H–C(5)/H–C(6), H–C(4), and H–C(3); and H–C(1)/H–C(3) and H–C(4) (*Fig. 2*). Thus, the structure of compound **3** was unequivocally determined as 3,4-dihydro-4-hydroxynaphthalene-2-carboxylic acid.

Table 2. *^{1}H- and ^{13}C-NMR Data of Compound* **2**[1]). δ in ppm, J in Hz. Arbitrary C-atom numbering as indicated in the formula.

	δ(H)[a])	δ(C)[b])
C(2)		164.4
H–C(4)	5.30 (*t*, *J* = 5.1)	56.5
C(5)		117.9
H–C(6)	7.25–7.27 (*m*)	128.1
H–C(7)	7.21 (*t*, *J* = 7.5)	128.0
H–C(8)	7.28–7.30 (*m*)	130.0
H–C(9)	6.96–6.98 (*m*)	127.8
C(10)		143.7
H_a–C(11)	3.67–3.72 (*m*)	44.6
H_b–C(11)	4.03–4.09 (*m*)	
H_a–C(12)	2.18–2.26 (*m*)	19.7
H_b–C(12)	2.27–2.31 (*m*)	
H_a–C(13)	2.92–2.96 (*m*)	31.4
H_b–C(13)	3.00–3.08 (*m*)	
CH_2(14)	2.72 (*d*, *J* = 5.1)	53.1
C(15)		176.9

[a]) Recorded at 500 MHz in CD_3OD. [b]) Recorded at 75 MHz in CD_3OD.

Table 3. *^{1}H- and ^{13}C-NMR Data of Compound* **3**[1]). δ in ppm, J in Hz. Arbitrary C-atom numbering as indicated in formula.

	δ(H)[a])	δ(C)[b])
H–C(1)	7.19 (*s*)	125.1 (*d*)
C(2)		109.6 (*s*)
H_a–C(3)	3.14 (*dd*, *J* = 15.0, 9.0)	28.5 (*t*)
H_b–C(3)	3.51 (*dd*, *J* = 15.0, 4.0)	
H–C(4)	3.85 (*dd*, *J* = 9.0, 4.0)	56.7 (*d*)
H–C(5)	7.69 (*d*, *J* = 7.0)	119.3 (*d*)
H–C(6)	7.04 (*dt*, *J* = 7.0, 1.0)	120.1 (*d*)
H–C(7)	7.11 (*dt*, *J* = 7.0, 1.0)	122.7 (*d*)
H–C(8)	7.35 (*d*, *J* = 7.0)	112.4 (*d*)
C(9)		128.5 (*s*)
C(10)		138.4 (*s*)
C(11)		174.4 (*s*)

[a]) Recorded at 500 MHz in CD_3OD. [b]) Recorded at 75 MHz in CD_3OD.

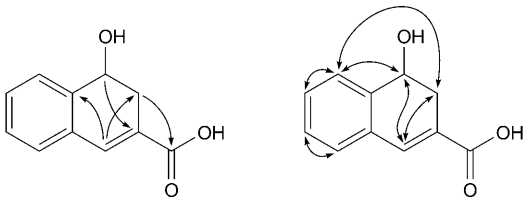

Fig. 2. *Key HMBC* (H → C) *and NOESY* (H ↔ H) *correlations of* **3**

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Experimental Part

General. TLC: precoated SiO_2 GF_{254} plates (*Qingdao Marine Chemical Plant*, Qingdao, P. R. China). Column chromatography (CC): silica gel (SiO_2; 100–200 mesh, 200–300 mesh; *Qingdao Marine Chemical Plant*, Qingdao, P. R. China), *Sephadex LH-20* (*GE Healthcare Bio-Sciences AB, USA*), and *YMC*GEL® ODS-A* (500 mesh, *YMC Co., Ltd.*, Japan). ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *Bruker-AV-500* and *Bruker-AV-300* spectrometers; δ in ppm rel. to Me_4Si, J in Hz. MS: *Agilent-1100-JC/MSD-Trap* (ESI-MS-MS) spectrometer; in m/z.

Plant Material. *P. nigellastrum* aerial parts were collected from Minqin of Gansu Province, P. R. China. A voucher specimen was identified by Prof. *X.-H. Song* (China Pharmaceutical University) and has been deposited with the Herbarium of China Pharmaceutical University, Nanjing, P. R. China (reference No. 200701208).

Extraction and Isolation. The dried and powdered aerial parts (75 kg) of *P. nigellastrum* were percolated three times successively with 95% EtOH at 70–80°. The combined extract was concentrated under reduced pressure to afford a dark brown residue (15 kg). This extract (2.9 kg) was subjected to CC (SiO_2 (100–200 mesh); petroleum ether (PE), AcOEt, AcOEt/MeOH 1:1, and MeOH). The AcOEt/MeOH and MeOH fractions were monitored by TLC, and grouped in four major fractions, *Frs. 1–4*. Compounds **1** (45 mg) and **3** (5 mg) were purified from *Fr. 2* (530 g), by repeated CC (SiO_2 (200–300 mesh), *Sephadex LH-20*, and *ODS-A*). *Fr. 4* was evaporated to dryness under reduced pressure, then acidified with 2% HCl, filtered, and the aq. acid soln. was made alkaline with 25% NH_4OH to pH 10. The alkaline soln. was extracted with CH_2Cl_2 and BuOH successively. The BuOH fraction (50 g) was subjected to repeated CC (SiO_2 (200–300 mesh) and *Sephadex LH-20*) to give compound **2** (46 mg).

*Pegamine β-*D*-Glucopyranoside* (*= 3-(3,4-Dihydro-4-oxoquinazolin-2-yl)propyl β-*D*-Glucopyranoside*; **1**). Colorless, amorphous solid. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS-MS: 367 ($[M+H]^+$), 365 ($[M-H]^-$), 205 ($[M+H-\text{glucose}]^+$).

2-Deoxypeganylacetic Acid (*= (1,2,3,9-Tetrahydropyrrolo[2,1-*b*]quinazolin-9-yl)acetic Acid*; **2**). White powder. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 231 ($[M+H]^+$), 253 ($[M+Na]^+$), 229 ($[M-H]^-$).

3,4-Dihydro-4-hydroxynaphthalene-2-carboxylic Acid (**3**). White powder. ^{1}H- and ^{13}C-NMR: *Table 3*.

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