

TWO NEW PYRROLOQUINAZOLINOQUINOLINE ALKALOIDS FROM
PEGANUM NIGELLASTRUM †Zhong-Ze Ma,^a Yoshio Hano,^a Taro Nomura,^{*a} and Ying-Jie Chen^b^aFaculty of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274, Japan and ^bShenyang Pharmaceutical University, 103 Whenhua Road, Shenyang 110015, P. R. China

Abstract - Two new alkaloids, luotonins A and B, were isolated from the aerial parts of *Peganum nigellastrum* along with four known quinazoline type alkaloids. The structures of luotonins A and B were determined to be **1** and **2**, respectively, by NMR spectroscopic evidence. Both new alkaloids have a unique structure comprising a quinoline and a quinazoline skeletons.

Peganum nigellastrum Bunge (Zygophyllaceae) is distributed over the Asia and commonly found in the northwest region of China (Chinese name "Luo-Tuo-Hao").¹ The plant has been used as a Chinese traditional medicine for a rheumatism, an abscess, an inflammation and so on.¹ On the other hand, it has been reported that the basic fraction of the *P. nigellastrum* exhibited anti-tumor activity through an immune system.² Six alkaloids, β -carboline type and quinazoline type alkaloids, have been isolated from the plant.^{3,4} In our survey for a development of Chinese medicinal resources, the chemical components of the aerial parts of *P. nigellastrum* were examined. This paper describes the structures of two new alkaloids, luotonins A (**1**) and B (**2**), and the cytotoxic activity against mouse leukemia P-388 cells.

The ethanol extract of the dried aerial parts of *P. nigellastrum* (5 kg) was divided into *n*-hexane, benzene, chloroform, acetone, and methanol soluble parts. The *n*-hexane soluble part was purified by repeated silica gel column chromatography and preparative TLC to give two new alkaloids, luotonins A (**1**) and B (**2**). Analogous purification procedures of the benzene and chloroform parts gave **1** and four known alkaloids, ($\pm$)-vasicine (**3**, = peganine),^{5,6} deoxyvasicine (**4**, = deoxypeganine),⁶ ($\pm$)-vasicinone (**5**),⁷ and deoxyvasicinone (**6**)⁸ (Figure 1). The known compounds were identified by comparing the physical data with the published data as well as by the NMR spectroscopic evidence.

Luotonin A (**1**), pale yellow needles, mp 252° (decomp), showed a positive reaction to Dragendorff test. The molecular formula C₁₈H₁₁N₃O was determined by the high-resolution FABMS spectrum. The IR spectrum of **1** disclosed the absorption bands due to aromatic and conjugated carbonyl groups and the UV spectrum exhibited absorption maxima at 212, 248, 298, 326sh, 341, and 357 nm. The ¹H NMR and the two-dimensional ¹H-¹H COSY spectra of **1** revealed the presence of two sets of *o*-disubstituted benzene rings [δ 7.67, 7.84 (each 1H, dt, *J* = 1.5 and 8.5 Hz), 7.93, 8.46 (each 1H, dd, *J* = 1.5 and 8.5 Hz); δ

†Dedicated to the memory of the late Professor Shun-ichi Yamada

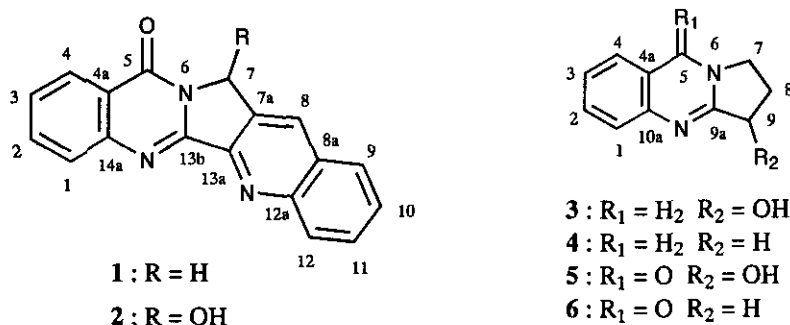
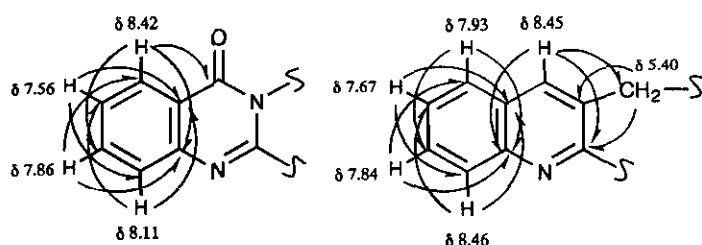
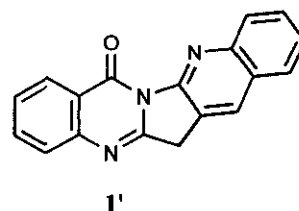
Figure 1 Quinazoline type alkaloids from the aerial parts of *Peganum nigellastrum*Figure 2 ¹³C-¹H long-range correlations in the HMBC spectrum ($J_{\text{CCH}} = 6$ Hz) of 1

Figure 3 The other possible formula (1') for the structure of luotonin A

Table 1 ¹³C NMR chemical shifts (ppm) of 1, 2 and 5

C No.	1	C-H connectivity	2	C-H connectivity	5 [#]
C-1	128.8	8.11 (dd, J = 1.5, 8.0)	129.2	8.11 (dd, J = 1.5, 8.0)	126.5
C-2	134.6	7.86 (dt, J = 1.5, 8.0)	135.1	7.87 (dt, J = 1.5, 8.0)	134.6
C-3	127.5	7.56 (dt, J = 1.5, 8.0)	127.9	7.60 (dt, J = 1.5, 8.0)	127.1
C-4	126.5	8.42 (dd, J = 1.5, 8.0)	126.5	8.44 (dd, J = 1.5, 8.0)	126.7
C-4a	121.3		121.9		120.9
C-5	160.7		161.6		160.6
C-7	47.3	5.40 (2H, br s)	80.9	7.14 (s)	(C-7) 43.7
C-7a	129.5		131.3		(C-8) 29.3
C-8	131.6	8.45 (s)	133.7	8.58 (s)	(C-9) 71.9
C-8a	128.8		128.9		(C-9a) 160.4
C-9	128.0	7.93 (dd, J = 1.5, 8.5)	128.6	8.00 (dd, J = 1.5, 8.0)	(C-10a) 148.1
C-10	128.6	7.67 (dt, J = 1.5, 8.5)	128.8	7.73 (dt, J = 1.5, 8.0)	
C-11	130.7	7.84 (dt, J = 1.5, 8.5)	131.4	7.89 (dt, J = 1.5, 8.0)	
C-12	130.7	8.46 (dd, J = 1.5, 8.5)	130.9	8.48 (dd, J = 1.5, 8.0)	
C-12a	149.4		150.3		
C-13a	151.6		150.4		
C-13b	152.6		150.9		
C-14a	149.3		149.5		

measured in CDCl₃ # Our data

7.56, 7.86 (each 1H, dt, $J = 1.5$ and 8.0 Hz), 8.11, 8.42 (each 1H, dd, $J = 1.5$ and 8.0 Hz)], an olefinic proton [δ 8.45 (1H, s)], and an isolated methylene protons [δ 5.40 (2H, brs)] (Table 1). These protons were correlated with the ^{13}C signals by the ^{13}C - ^1H COSY spectrum (Table 1). Analysis of the HMBC spectrum, considering the molecular formula, gave two alkaloid parts, a quinoline and a quinazoline (Figure 2). The presence of a quinazoline skeleton was also supported by comparison of the ^{13}C chemical shift values of the part with the relevant carbons of vasicinone (**5**) (Table 1). From the above results, luotonin A is a pyrroloquinazolinoquinoline derivative and two possible structures (**1**) and (**1'**) were proposed for the compound (Figure 3). The chemical shift values of the methylene protons (δ 5.40) and the methylene carbon (δ 47.3) at the C-7 position preferably indicated the formula (**1**).⁹ Thus the structure of new quinazolinoquinoline derivative, luotonin A, was represented by the formula (**1**).

Luotonin B (**2**), a pale yellow powder, showed a positive reaction to Dragendorff test. The molecular formula $\text{C}_{18}\text{H}_{11}\text{N}_3\text{O}_2$ was determined by the high-resolution FABMS spectrum. The IR spectrum of **2** disclosed absorption bands due to aromatic and conjugated carbonyl groups and the UV spectrum closely similar to that of **1**, suggesting **2** to be a congener of **1**. The ^1H NMR and the ^1H - ^1H COSY spectra of **2** revealed the presence of two sets of *o*-disubstituted benzene rings, an olefinic proton, and an oxymethine proton (Table 1). These protons were correlated with the ^{13}C signals by the ^{13}C - ^1H COSY spectrum (Table 1). Comparison of the ^1H NMR and ^{13}C NMR spectra of **2** with those of **1** led to 7-hydroxyluotonin A as the structure of **2** (Table 1). The NOESY spectrum showing a significant correlation between the olefinic proton at δ 8.58 and the oxymethine proton at δ 7.14 supported the structure (Figure 4). The appearance of the oxymethine proton at the extremely lower-field could be due to an anisotropic

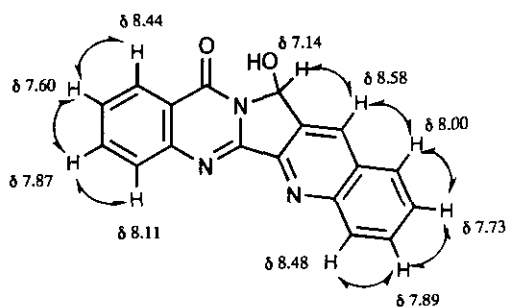


Figure 4 NOESY spectrum of **2**

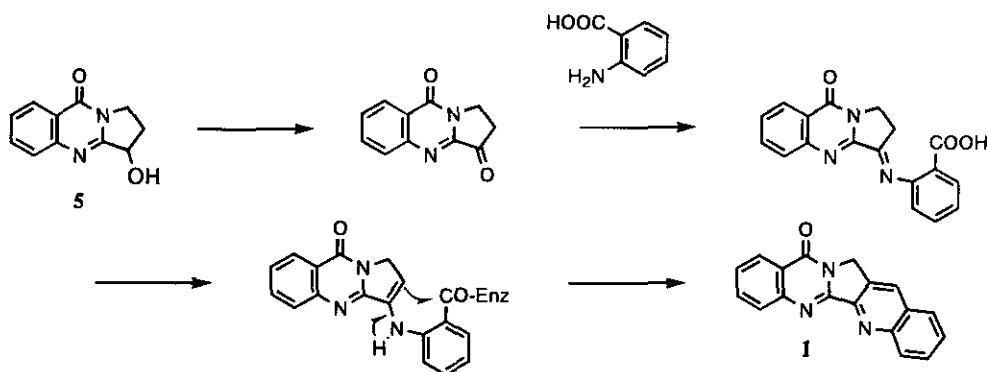


Figure 5 Hypothesis of the biosynthetic route to luotonin A (**1**) from vasicinone (**5**)

effect of the carbonyl group at the C-5. This fact also indicated the validity of the structure of luotonin B. Furthermore, an exposure of a chloroform solution of **1** to sunlight for two weeks gave **2**.^{10,11} The structure of luotonin B has thus been confirmed to be the formula (**2**).

On the other hand, quinazoline alkaloids including vasicine (**3**) has been found to be biosynthesized from anthranilic acid as a key intermediate.^{12,13} As luotonins A (**1**) and B (**2**) coexist quinazoline alkaloids, besides they have a vasicinone structure in the molecule, they seem to be biosynthesized from vasicinone (**5**) and anthranilic acid as shown in Figure 5. Luotonins A (**1**) and B (**2**) were the first report of the pyrroloquinazolinoquinoline derivative.

Cytotoxicity of luotonin A (**1**) and the other quinazoline alkaloids (**3**, **5**, and **6**) against mouse leukemia P-388 cells were tested and the results are summarized in Table 2. Among them, luotonin A (**1**) showed the cytotoxic activity at a low concentration.

Table 2 Cytotoxic activity against P-388 cells

compound	IC ₅₀ values (μg/ml)
luotonin A (1)	1.8
vasicine (3)	> 100
vasicinone (5)	> 100
deoxyvasicinone (6)	79

EXPERIMENTAL

Abbreviations: s = singlet, d = doublet, dd = double doublet, t = triplet, br = broad, sh = shoulder.

Plant material: The aerial parts of *P. nigellastrum* Bunge was collected in Beishan area, the suburbs of Wuhai city, Inner Mongolia, China, in August 1994, and identified by Prof. Shi-rui Xing, Ningxia Institute for Drug Control. The specimens were deposited in Shenyang Pharmaceutical University.

Melting points were determined by Yanaco micro-melting point apparatus MP-500V and are uncorrected. IR spectra were recorded on a JASCO FTIR 300 spectrophotometer and UV spectra were recorded on a Shimadzu 265 UV spectrophotometer. NMR spectra were recorded on a JEOL JNM EX-400 FTNMR spectrometer. In which the chemical shifts are given by δ (ppm) with tetramethylsilane (TMS) as an internal standard and coupling constants (J) are in Hz. MS spectra were recorded on a JEOL JMS DX-303 spectrometer. Wakogel C-200 and B-5 FM (Silica gel, Wako Pure Chemical Co., Ltd, Osaka, Japan) were used for column chromatography and TLC, respectively. HPLC was carried out on a SSC Flow System E-3100 (Senshu Scientific Co., Ltd., Tokyo, Japan) equipped with a SSC-3000B UV monitor. SSC Senshu Pack Silica 4251-N (10 ϕ x 250 mm) and Aquasil SS-762N (20 ϕ x 250 mm) were used for HPLC at a flow rate of 2 mL/min.

Isolation of luotonins A (**1**) and B (**2**)

The dried aerial parts of *P. nigellastrum* (5 kg) was extracted with ethanol (8 L) under the reflux for 2 h. This procedure was repeated three times. The ethanol solution was evaporated in vacuo to give a residue (700 g). The ethanol extract (350 g) was successively fractionated over Amberlite XAD-2 by elution with *n*-hexane, benzene, chloroform, acetone, and methanol to prepare each soluble parts. The *n*-hexane soluble part (17 g) was chromatographed over silica gel (250 g) using *n*-hexane increasing amount of ethyl acetate as an eluent to give frs. 1 - 72 (200 mL/fr.). The combined fraction (frs. 53 - 58, 320 mg) eluted with *n*-hexane - ethyl acetate (2 : 1) was purified by preparative TLC [solvent system, chloroform - methanol (8 : 1), chloroform - acetone (5 : 1)] to give luotonins A (**1**, 10 mg) and B (**2**, 1.5 mg). The benzene part (15 g) was subjected to silica gel (250 g) column chromatography using *n*-hexane increasing amount of ethyl acetate as an eluent to give frs. 1 - 300 (300 mL/fr.). The combined fraction (frs. 178 - 182, 120 mg) eluted with *n*-hexane - ethyl acetate (3 : 1) was fractionated by preparative TLC [solvent system, chloroform - methanol (8 : 1), chloroform - acetone (5 : 1)] followed by HPLC [solvent system, chloroform - ethyl acetate (1 : 1)] to give luotonins A (**1**, 6 mg). The combined fraction (frs. 193 - 203, 650 mg) eluted with *n*-hexane - ethyl acetate (2 : 1) was purified by repeated preparative TLC [solvent system, chloroform - acetone (2 : 1 and 1 : 1)] followed by HPLC [solvent system, chloroform - ethyl acetate (1 : 4)] to yield deoxyvasicinone (**6**, 46 mg) and vasicinone (**5**, 150 mg). The chloroform part (30 g) was chromatographed over silica gel (300 g) using benzene increasing amount of acetone as an eluent to prepare frs. 1 - 315 (250 mL/fr.). Recrystallization of the combined fraction (frs. 121 - 310, 3.8 g) eluted with benzene - acetone (2 : 1) from chloroform yielded vasicine (**3**, 3 g). The combined fraction (frs. 317 - 325, 5.2 g) eluted with benzene - acetone (1 : 1) was fractionated over silica gel (200 g) column chromatography (solvent system, chloroform - methanol) to prepare frs. 1' - 35' (200 mL/fr.). The combined fraction (frs. 19' - 25', 1.4 g) was purified by preparative TLC [solvent system, chloroform - methanol (2 : 1)] to give deoxyvasicine (**4**, 1 g).

Luotonin A (1)

Compound (**1**) was recrystallized from chloroform - acetone to give colorless needles, mp 252 °C (decomp). Dragendorff test: positive (orange). UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 212 (4.61), 248 (4.71), 298 (4.00), 326 (sh, 4.18), 341 (4.27), 358 (4.21). IR $\nu_{\max}$ (KBr) cm^{-1} : 3050, 2924, 2854, 1678, 1629, 1606, 1466, 1097 1033, 769, 691. EIMS m/z (rel. int.): 285 (62, M^+), 257 (8.8), 185 (31), 149 (100). HR-FABMS: m/z 286.0959 [($M+H$) $^+$, $C_{18}H_{12}N_3O$, requires 286.0980]

Luotonin B (2)

Compound (**2**) was obtained as an amorphous powder. Dragendorff test: positive (orange). $[\alpha]_D^{20}$ ($c = 0.01$ in chloroform). UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 212 (4.67), 249 (4.69), 296 (sh, 4.10), 322 (sh, 4.27), 337 (4.32), 352 (4.22). IR $\nu_{\max}$ (KBr) cm^{-1} : 3412, 2924, 2854, 1684, 1635, 1608, 1467, 1384, 1099, 1070, 767, 691. FABMS: m/z 302 ($M+H$) $^+$. HR-FABMS: m/z 302.0927 [($M+H$) $^+$, $C_{18}H_{12}N_3O_2$, requires 302.0930].

Formation of 2 from 1

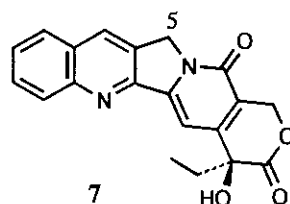
A chloroform solution (5 mL) of luotonin A (**1**, 10mg) was directly exposed to sunlight for two weeks. The product was purified by preparative TLC [chloroform - acetone (8 : 1)] to give compound (**8**, 0.5mg). The compound (**8**) was identified as **2** by direct comparison with **2**, including NMR data.

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9. Compound (**1**) has the similar partial structure to camptothecin (**7**)¹⁴ which has been isolated from *Camptotheca acuminata*. The chemical shift values of the methylene protons (δ 5.27, DMSO-*d*₆) and the methylene carbon (δ 51.22, DMSO-*d*₆) at the C-5 position of **7** supported the formula (**1**) for the structure of luotonin A.
10. Luotonin B (**2**) has no optical activity. The photo-oxidative reaction of **1** to **2** allowed to deduce luotonin B to be an artifact. However, the compound (**2**) was surely recognized in the original *n*-hexane soluble part by a detailed HPLC analysis.
11. The photo-oxidative reaction under an exposure to sunlight was mild condition in this case. The experiment with a 100W high-pressure mercury lamp gave many products.
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