# TWO NEW PYRROLOQUINAZOLINOQUINOLINE ALKALOIDS FROM *PEGANUM NIGELLASTRUM* †

Zhong-Ze Ma,[a] Yoshio Hano,[a] Taro Nomura,*[a] and Ying-Jie Chen[b]

[a]Faculty of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274, Japan and [b]Shenyang 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.

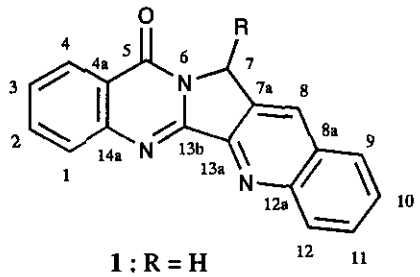
*Peganum nigellastrum* Bunge (Zygophyllaceae) is distributed over the Asia and commonly found in the northwest region of China (Chinese name "Luo-Tuo-Hao").[1] The plant has been used as a Chinese traditional medicine for a rheumatism, an abscess, an inflammation and so on.[1] On the other hand, it has 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, (±)-vasicine (**3**, = peganine),[5,6] deoxyvasicine (**4**, = deoxypeganine),[6] (±)-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 $C_{18}H_{11}N_3O$ 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

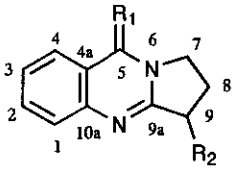
---

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



**1** : R = H

**2** : R = OH



**3** : $R_1 = H_2$ $R_2 = OH$

**4** : $R_1 = H_2$ $R_2 = H$

**5** : $R_1 = O$ $R_2 = OH$

**6** : $R_1 = O$ $R_2 = H$

Figure 1 Quinazoline type alkaloids from the aerial parts of *Peganum nigellastrum*

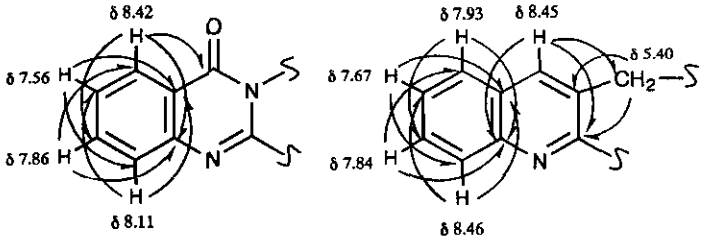
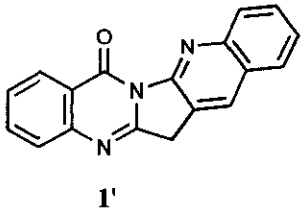


Figure 2 $^{13}C$-$^{1}H$ long-range correlations in the HMBC spectrum ($J_{CCH}$ = 6 Hz) of **1**



**1'**

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

Table 1 $^{13}C$ 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 | | | |

meaured in $CDCl_3$ # 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, br s)] (Table 1). These protons were correlated with the $^{13}C$ signals by the $^{13}C$-$^{1}H$ 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 $C_{18}H_{11}N_3O_2$ 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 $^{1}H$ NMR and the $^{1}H$-$^{1}H$ 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 $^{13}C$-$^{1}H$ COSY spectrum (Table 1). Comaparison of the $^{1}H$ 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

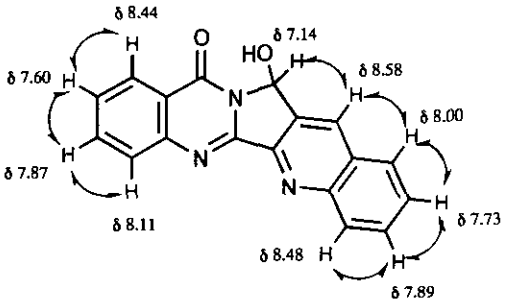



Figure 4 NOESY spectrum of **2**

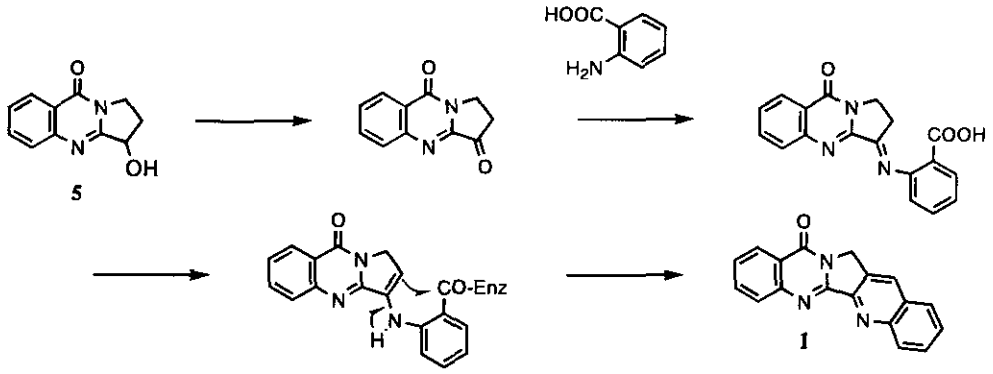



Figure 5 Hypothesis of the biosynthetic route to luotonin A (**1**) from vasicinone (**5**)

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) equiped with a SSC-3000B UV monitor. SSC Senshu Pack Silica 4251-N (10φ x 250 mm) and Aquasil SS-762N (20φ 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 succesively 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 needels, mp 252 °C (decomp). Dragendroff test: positive (orange). UV λmax (MeOH) nm (logε): 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 [$(M+H)^+$, $C_{18}H_{12}N_3O$, repuires 286.0980]

*Luotonin B (2)*

Compound (**2**) was obtained as an amorphous powder. Dragendorff test: positive (orange). $[\alpha]_D$ 0° (*c* = 0.01 in chloroform). UV λmax (MeOH) nm (logε): 212 (4.67), 249 (4.69), 296 (sh, 4.10), 322 (sh, 4.27), 337 (4.32), 352 (4.22). IR νmax (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*

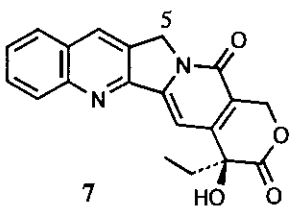
A chloroform solution (5 mL) of luotonin A (**1**, 10mg) was directly exposed to sunlight for two weeks. The product was purified by preparative TLC [chloroform - acetone (8 : 1)] to give compound (**8**, 0.5mg). The compound (**8**) was identified as **2** by direct comparison with **2**, including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

AKNOWLEDGEMENT

We are grateful to Prof. H. Itokawa and Dr. K. Takeya, Tokyo College of Pharmacy, for the bioassay of the cytotoxicity against mouse leukemia P-388 cells.

REFERENCES AND NOTES

1. P. -G. Xiao, 'A Pictorial Encyclopaedia of Chinese Medical Herbs' (Japanese edition), Vol. III, p. 125, Chuokoron-sha, Inc., Tokyo, 1992.
2. X. -H. Xiao, G. -L. Qou, H. -L. Wang, L. -S. Lui, Y. -L. Zheng, Z. -J. Jia, and Z. -B. Deng, *Chinese J. Pharmacol.Toxicol.*, 1988, 232.
3. D. Batsuren, M. V. Telezhenetskaya, and S. Yu. Yunusov, *Khim. Prir. Soedin.* 1978, 418.
4. D. Batsuren, M. V. Telezhenetskaya, and S. Yu. Yunusov, *Khim. Prir. Soedin.* 1980, 736.
5. R. R. Arndt, S. H. Egger, and A. Jordaan, *Tetrahedron*, 1967, **23**, 3521.
6. B. K. Chowdhury, *Ind. J. Chem.*, 1987, **26B**, 688.
7. K. L. Dhar, M.P. Jain, S. K. Koul, and C. K. Atal, *Phytochemistry*, 1981, **20**, 319.
8. A. AL-Shamma, S. Drake, D. L. Flynn, A. Mitscher, Y. H. Park, G. S. R. Rao, A. Simpson, J. K. Swayze, T. Veysoglu, and S. T. -S. Wu, *J. Nat. Prod.*, 1981, **44** , 745.
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.



7

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.
12. D. R. Liljegren, *Phytochemistry*, 1968, **7**, 1299.
13. K. Waiblinger, S. Johne, and D. Gröger, *Phytochemistry*, 1972, **11**, 2263.
14. M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail, and G. A. Sim, *J. Am. Chem. Soc.*, 1966, **88**, 3888.

Received, 3rd April, 1997