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Alkaloids and phenylpropanoids from *Peganum nigellastrum*

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Abstract

Two canthin-6-one alkaloids, luotonins C and D, and two phenylpropanoids, dihydrosinapyl ferulate and dihydroconiferyl ferulate, were isolated from the aerial parts of *Peganum nigellastrum* along with four known alkaloids, harmine, 3-phenylquinoline, 3-(4-hydroxyphenyl)quinoline and 3-(1H-indol-3-yl)quinoline. Their structures were elucidated by spectroscopic techniques. The structures of luotonins C and D were also confirmed by chemical synthesis.

Keywords: *Peganum nigellastrum*; Zygophyllaceae; Alkaloids; Canthin-6-one; Luotonins C and D; Harmine; Quinoline; Phenylpropanoids

1. Introduction

The plant *Peganum nigellastrum* Bunge ('Luo-Tuo-Hao' in Chinese, Zygophyllaceae), commonly found in the northwest region of China, has been used in Chinese traditional medicine for the treatment of rheumatism, abscesses, and inflammatory diseases (Xiao, 1993). Pharmacological study of this plant revealed that the basic fraction showed potent anti-tumor activity (Xiao et al., 1988). On the other hand, the harmala alkaloids from *P. harmala* show CNS stimulant and hypotensive activities, and harmine and harmaline have been reported as hallucinogens (Bolle, Casini, Mazzanti, Palmery, Tita & Tucci, 1996; Siddiqui, Khan, Siddiqui & Faizi, 1987). In our survey of lead compounds for useful drugs, we have examined the chemical components of *P. nigellastrum* and have reported two new cytotoxic alkaloids (Ma, Hano, Nomura & Chen, 1997). In the present study we report the isolation of a β-carboline alkaloid, harmine (Duan, Zhou, Zhao, Wang & Che, 1998), two new canthin-6-one alkaloids, luotonins C (**1**) and D (**2**), and three quinoline alkaloids, 3-phenylquinoline (Sakamoto, Kondo, Murata & Yamanaka, 1993), 3-(4-hydroxyphenyl)quinoline (Cacchi, Fabrizi, Marinelli, Moro & Pace, 1996) and 3-(1H-indol-3-yl)quinoline (Maguire, Sheets, McVety, Spada & Zilberstein, 1994), together with two new phenylpropanoids, dihydrosinapyl ferulate (**3**) and dihydroconiferyl ferulate (**4**). The known alkaloids were identified by comparisons of their physical and spectral data with these reported in the literature. 3-Phenylquinoline, 3-(4-hydroxyphenyl)quinoline and 3-(1H-indol-3-yl)quinoline are isolated here for the first time from natural resources.

2. Results and discussion

Luotonin C (**1**) was obtained as pale yellow needles, mp 166–168°C, and gave an orange-red color with Dragendorff reagent on a tlc plate. The molecular formula $C_{16}H_{12}N_2O_2$ was determined by HRMS. The UV spectrum of **1** exhibited absorption maxima at 209, 265(sh), 273, 308, 346, 358, and 376(sh) nm. The IR spectrum showed absorption bands due to carbonyl group and aromatic ring at 1663, 1608, and 1517 cm^{-1}. The ^{1}H-NMR spectrum of **1** displayed a pair of *ortho*-coupled signals in a heterocyclic system at δ 7.81 (*d*, J = 5.0 Hz), 8.75 (*d*, J = 5.0 Hz), a set of ABX-

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Table 1
^{1}H- and ^{13}C-NMR data of **1** and **2** in $CDCl_3$

	1			**2**	
Position	^{13}C	^{1}H	HMBC[a]	^{13}C	^{1}H
1	114.6	7.81 *d* (5.0)	C-2, 12, 15	114.6	7.80 *d* (5.0)
2	145.8	8.75 *d* (5.0)	C-1, 14	145.3	8.73 *d* (5.0)
4	136.2	7.89 *q* (1.4)	C-6, 15, CH_3	134.1	7.87 *t* (1.4)
5	137.8			143.4	
6	160.7			160.1	
8	101.3	8.27 *d* (2.4)	C-10, 12, 13	101.2	8.24 *d* (2.4)
9	162.4			162.3	
10	114.2	7.08 *dd* (2.4, 8.6)	C-12	114.2	7.08 *dd* (2.4, 8.6)
11	123.5	7.97 *d* (8.6)	C-9, 13, 14	123.4	7.94 *d* (8.6)
12	117.7			117.5	
13	141.4			141.5	
14	130.1			130.1	
15	131.5			131.2	
16	135.8			135.7	
CH_2				24.4	2.83 *dq* (1.3, 7.4)
CH_3	17.8	2.44 *d* (1.4)	C-4, 5, 6	12.9	1.37 *t* (7.4)
OCH_3	55.9	3.99 *s*	C-9	56.0	3.98 *s*

[a] HMBC correlations of the proton; Coupling constant *J* is in Hz.

type signals in an aromatic ring at δ 7.08 (*dd*, J = 2.4 and 8.6 Hz), 7.97 (*d*, J = 8.6 Hz), 8.27 (*d*, J = 2.4 Hz), an olefinic signal at δ 7.89 (*q*, J = 1.4 Hz), a methoxyl signal at δ 3.99 (*s*), and a vinyl methyl signal at δ 2.44 (*d*, J = 1.4 Hz). The above signal pattern suggested **1** to be a canthin-6-one derivative similar to those given in the literature (Li, Koike & Ohmoto, 1993; Ohmoto & Koike, 1984). Detailed analysis of the ^{13}C-NMR spectral data of **1**, combined with ^{13}C–^{1}H COSY and HMBC spectra, revealed the presence of a β-carboline skeleton and a lactam carbonyl group in **1** (Table 1). Furthermore, the HMBC spectrum showed significant correlations of an olefinic proton at δ 7.89 with a lactam carbonyl group at δ 160.7 and a methyl carbon at δ 17.8, as well as of methyl protons at δ 2.44 with the lactam carbonyl group and the olefinic carbon at δ 136.2. The structure of luotonin C (**1**) is represented as 5-methyl-9-methoxycanthin-6-one.

Luotonin D (**2**) was obtained as pale yellow needles, mp 141–143°C, and showed positive Dragendorff reaction on a tlc plate. The molecular formula $C_{17}H_{14}N_2O_2$ was determined by HRMS. The IR and UV spectra were similar to **1**, suggesting **2** to be an analogue. The ^{1}H- and ^{13}C-NMR spectral data of **2** were also closely related to those of **1**, except for the presence of an ethyl group in **2**. By comparison of the ^{1}H- and ^{13}C-NMR spectral data of **2** and **1** (Table 1), the structure of luotonin D was characterized as 5-ethyl-9-methoxycanthin-6-one. Luotonins C (**1**) and D (**2**) are the first examples of canthin-6-one alkaloids isolated from plants of the genus *Peganum*.

Luotonins C (**1**) and D (**2**) both were prepared in two steps from harmine via 1-formyl-7-methoxy-β-carboline by slightly modified procedures of Del Giudice et al. (Del Giudice, Gatta & Settimj, 1990). A reaction of 1-formyl-7-methoxy-β-carboline with propionic anhydride afforded **1**, while a reaction with *n*-butyric anhydride gave **2**. Although neither reaction gave satisfactory yield, the structures of luotonins C (**1**) and D (**2**) were nonetheless unambiguously confirmed.

Table 2
^{1}H- and ^{13}C-NMR spectral data of **3** and **4** in $CDCl_3$

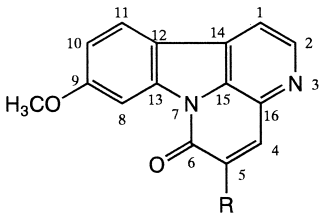
	3			**4**	
Position	^{13}C	^{1}H	HMBC[a]	^{13}C	^{1}H
1	127.0			127.0	
2	109.4	7.03 *d* (1.8)	C-4, 6, 7	109.4	7.03 *d* (1.8)
3	146.8			146.8	
4	148.0			148.0	
5	114.7	6.92 *d* (8.2)	C-1, 3	114.7	6.92 *d* (8.2)
6	123.0	7.08 *dd* (1.8, 8.2)	C-2, 4	123.0	7.08 *dd* (1.8, 8.2)
7	144.9	7.60 *d* (15.9)	C-2, 9	144.8	7.60 *d* (15.9)
8	115.5	6.30 *d* (15.9)	C-1	115.5	6.29 *d* (15.9)
9	167.3			167.3	
1′	132.4			133.0	
2′	105.5	6.43 *s*	C-3′, 4′, 7′	111.0	6.70 *d* (1.8)
3′	147.0			143.8	
4′	132.9			146.4	
5′	147.0			114.3	6.48 *d* (8.5)
6′	105.5	6.43 *s*	C-4′, 5′, 7′	121.0	6.71 *dd* (1.8, 8.5)
7′	32.5	2.67 *t* (7.6)	C-1′, 2′, 8′	32.0	2.67 *t* (7.6)
8′	30.6	2.02 *m*	C-7′	30.6	2.00 *m*
9′	63.8	4.23 *t* (6.5)	C-9, 7′	63.8	4.22 *t* (6.6)
3-OCH_3	56.0	3.93 *s*	C-3	55.9	3.94 *s*
3′-OCH_3	56.3	3.88 *s*	C-3′	56.0	3.87 *s*
5′-OCH_3	56.3	3.88 *s*	C-5′		
4-OH		5.86 *s*			5.86 *s*
4′-OH		5.38 *s*			5.47 *s*

[a] HMBC correlations of the proton; Coupling constant *J* is in Hz.

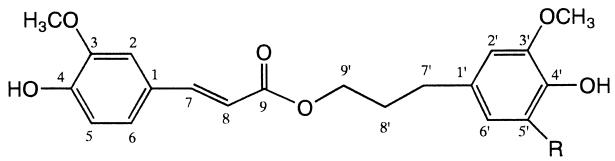
Compound **3** was obtained as a pale yellowish semi-solid. The molecular formula $C_{21}H_{24}O_7$ was established by HRMS. Absorption maxima at 206, 230(sh), 298(sh), and 325 nm in the UV spectrum indicated the presence of a feruloyl moiety in the molecule, supported by the presence of a fragment ion peak at m/z 177 in the mass spectrum (El-Gamal et al., 1994; Nakatani, Inatani & Fuwa, 1980). The IR spectrum showed characteristic absorption bands at 3421, 1698, and 1633 cm^{-1}, indicating the presence of a hydroxyl group and an α,β-unsaturated ester carbonyl group. The ^{1}H-NMR spectrum showed signals due to a set of ABX-type aromatic protons at δ 6.92 (*d*, J = 8.2 Hz), 7.03 (*d*, J = 1.8 Hz), 7.08 (*dd*, J = 1.8 and 8.2 Hz), two aromatic protons at δ 6.43 (2H, *s*), two olefinic protons each having *trans*-orientation at δ 6.30 and 7.60 (each 1H, *d*, J = 15.9 Hz), three methoxyl protons at δ 3.88 (6H, *s*) and 3.93 (3H, *s*), aliphatic protons due to a $-CH_2CH_2CH_2O-$ moiety at δ 2.02 (2H, *m*), 2.67 (2H, *t*), 4.23 (2H, *t*), and two hydroxyl pro-

tons at δ 5.38 (*s*) and 5.86 (*s*). The ^{13}C-NMR spectral data of **3** were analyzed and the ^{13}C–^{1}H connectivities in the molecule were confirmed with the aid of ^{13}C–^{1}H COSY and HMBC spectra as summarized in Table 2. The position of one of three methoxyl groups was also confirmed by an NOE difference experiment, in which irradiation of the methoxyl signal at δ 3.93 enhanced the signal of the aromatic proton at δ 7.03 by 15%. The above NMR spectral data revealed the presence of ferulic acid and dihydrosinapyl alcohol moieties in the molecule. Compound **3** was thus characterized as dihydrosinapyl ferulate.

Compound **4** was obtained as a pale yellow semi-solid. HRMS gave the molecular formula $C_{20}H_{22}O_6$. The UV spectrum of **4** exhibited absorption maxima at 203, 216(sh), 229, 289, and 325 nm, similar to **3**. The ^{1}H-NMR spectrum of **4** was also similar to that of **3** but was distinctive in that it showed the signals of two sets of ABX-type aromatic protons and of two methoxyl groups (Table 2). The ^{13}C-NMR spectrum supported that **4** is an analogue of **3** (Table 2), and the positions of the two methoxyl groups were confirmed by NOE difference spectra. Irradiation of the methoxyl signal at δ 3.87 caused an enhancement of the aromatic proton signal at δ 6.70 (18%), while the aromatic proton at δ 7.03 was enhanced 14% by irradiation of the methoxyl signal at δ 3.94. Compound **4** was therefore dihydroconiferyl ferulate.



1. R = CH_3
2. R = CH_2CH_3



3. R=OCH_3
4. R=H

3. Experimental

3.1. General

Mps: uncorr. UV spectra: Shimadzu 265 UV photometer. IR spectra: Jasco FTIR 300 spectrometer. NMR spectra: Jeol JNM 400 FTNMR spectrometer using TMS as internal standard. FAB MS: Jeol JMS DX-303 spectrometer. EI MS: Jeol JMS-AM II 50 spectrometer. HPLC: SSC E-3100 system (Senshu Scientific Tokyo) with UV detection using a Senshu Pack Silica 4251-N column (10ϕ × 250 mm diameter).

3.2. Plant material

The aerial parts of *P. nigellastrum* Bunge were collected in the Beishan area of Wuhai City, Inner Mongolia, China, in August 1994, and identified by professor Shi-rui/Xing. The voucher specimen (NM 940824-1) was deposited in the herbarium of Shenyang Pharmaceutical University.

3.3. Extraction and isolation

The dried aerial parts of *P. nigellastrum* (5 kg) were extracted three times with EtOH under reflux for 2 h, with the resulting EtOH solution evaporated in vacuo to give a residue (700 g). The EtOH extract (350 g) was fractionated over Amberlite XAD-2 by successive elution with *n*-hexane, C_6H_6, $CHCl_3$, Me_2CO, and MeOH. The *n*-hexane part (17 g) was chromatographed over silica gel (250 g) using *n*-hexane with increasing amount of EtOAc as eluent to afford 3-phenylquinoline (5 mg). The C_6H_6 (15 g) was subjected to silica gel (250 g) column chromatography using *n*-hexane with increasing amount of EtOAc as eluent. The fr. eluted with *n*-hexane-EtOAc (10:1–5:1) was purified by prep. TLC with $CHCl_3$–Me_2CO (5:1) to afford luotonin C (**1**, 2 mg), luotonin D (**2**, 1 mg) and 3-(4-hydroxylphenyl)quinoline (3 mg). The fr. eluted with *n*-hexane-EtOAc (3:1–1:1) was purified by prep. TLC with $CHCl_3$–MeOH (8:1) and HPLC with $CHCl_3$–EtOAc (2:1) to afford dihydrosinapyl ferulate (**3**, 4 mg), dihydroconiferyl ferulate (**4**, 5 mg) and 3-(1H-indol-3-yl)quinoline (3 mg). The $CHCl_3$ part (30 g) was chromatographed over silica gel (300 g) using C_6H_6 with increasing amount of Me_2CO as eluent to afford luotonin C (**1**, 1 mg) and harmine (348 mg).

3.4. Luotonin C (*1*)

Pale yellow needles (acetone), mp 166–168°C. Dragendorff test: positive (orange–red). UV (MeOH) λ_{max} nm (log ε): 209 (4.35), 265(sh, 3.90), 273 (4.02), 308 (3.66), 346 (3.71), 358 (3.70), 376 (sh, 3.47). IR (KBr) ν_{max} cm^{-1}: 2924, 2853, 1663, 1634, 1608, 1517, 1456, 1277, 1223. ^{1}H- and ^{13}C-NMR spectral data: Table 1. FABMS: *m/z* 265 $[M+H]^+$. HR-FABMS: *m/z* 265.0966 $[M+H]^+$ ($C_{16}H_{13}N_2O_2$, requires 265.0977).

3.5. Luotonin D (2)

Pale yellow needles (acetone), mp 141–143°C. Dragendorff test: positive (orange–red). UV (MeOH) λ_{max} nm (log ε): 210 (4.36), 266 (sh, 3.92), 273 (4.06), 310 (3.60), 347 (3.76), 358 (3.75), 376 (sh, 3.54). IR (KBr) ν_{max} cm^{-1}: 2923, 1662, 1637, 1609, 1498, 1458, 1279, 1223. ^1H- and ^{13}C-NMR spectral data: Table 1. EIMS 70 eV: m/z (rel. int.) 278 $[M]^+$ (100), 263 (51), 249 (10), 235 (22), 192 (26). HR-EIMS: m/z 278.1063 $[M]^+$ ($C_{17}H_{14}N_2O_2$, requires 278.1056).

3.6. 1-Formyl-7-methoxy-β-carboline

To a solution of harmine (100 mg) in 3 ml of EtOH was added freshly sublimed SeO_2 (100 mg) and the mixture was heated until reflux began, this being maintained for 3 h. The hot suspension was then eluted, filtered and the solution was evaporated to dryness with the residue purified by prep. TLC, with $CHCl_3$–Me_2CO (6:1) to afford 1-formyl-7-methoxy-β-carboline (28.3 mg). The physical and spectral data agreed with those reported in the literature (Li et al., 1993).

3.7. Luotonins C (1) and D (2)

A solution of 1-formyl-7-methoxy-β-carboline (20 mg) in triethylamine (0.4 ml) and either propionic anhydride or *n*-butyric anhydride (0.6 ml) was heated until boiling occured, this being maintained for 2 h. The solution was poured into saturated $NaHCO_3$ solution, extracted with CH_2Cl_2, with the resulting CH_2Cl_2 solubles dried (Na_2SO_4) and evaporated to dryness. The residue was purified by prep. TLC with C_6H_6–Me_2CO (5: 1) to afford **1** (3.5 mg) or **2** (3.1 mg).

3.8. Dihydrosinapyl ferulate (3)

Pale yellow semi-solid. UV (MeOH) λ_{max} nm (log ε): 206 (4.87), 230 (sh, 4.43), 298 (sh, 4.29), 325 (4.45). IR (KBr) ν_{max} cm^{-1}: 3421, 2941, 2838, 1698, 1633, 1602, 1517, 1457, 1428, 1270, 1159, 1116. ^1H- and ^{13}C-NMR spectral data: Table 2. FABMS: m/z 389 $[M+H]^+$, 388 $[M]^+$, 211, 177. HR-FABMS: m/z 388.1551 $[M]^+$ ($C_{21}H_{24}O_7$, requires 388.1522).

3.9. Dihydroconiferyl ferulate (4)

Pale yellow semi-solid. UV (MeOH) λ_{max} nm (log ε): 203 (4.70), 216 (sh, 4.40), 229 (4.34), 289 (4.25), 325 (4.39). IR (KBr) ν_{max} cm^{-1}: 3450, 2975, 2941, 2901, 2849, 1709, 1635, 1603, 1516, 1431, 1267, 1173, 1028. ^1H- and ^{13}C-NMR spectral data: Table 2. FABMS: m/z 359 $[M+H]^+$, 358 $[M]^+$. HR-FABMS: m/z 358.1479 $[M]^+$ ($C_{20}H_{22}O_6$, requires 358.1417).

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